EXHIBIT 1

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Vaccine Safety

Adjuvants help vaccines work better.

What is an adjuvant and why is it added to a vaccine?

An adjuvant is an ingredient used in some vaccines that helps create a stronger immune response in people receiving the vaccine. In other words, adjuvants help vaccines work better. Some vaccines that are made from weakened or killed germs contain naturally occurring adjuvants and help the body produce a strong protective immune response. However, most vaccines developed today include just small components of germs, such as their proteins, rather than the entire virus or bacteria. Adjuvants help the body to produce an immune response strong enough to protect the person from the disease he or she is being vaccinated against. Adjuvanted vaccines can cause more local reactions (such as redness, swelling, and pain at the injection site) and more systemic reactions (such as fever, chills and body aches) than non-adjuvanted vaccines.

Adjuvants have been used safely in vaccines for decades.

Aluminum salts, such as aluminum hydroxide, aluminum phosphate, and aluminum potassium sulfate have been used safely in vaccines for more than 70 years. Aluminum salts were initially used in the 1930s, 1940s, and 1950s with diphtheria and tetanus vaccines after it was found they strengthened the body's immune response to these vaccines.

Newer adjuvants have been developed to target specific components of the body's immune response, so that protection against disease is stronger and lasts longer.

In all cases, vaccines containing adjuvants are tested for safety and effectiveness in clinical trials before they are licensed for use in the United States, and they are continuously monitored by CDC and FDA once they are approved.

Several different adjuvants are used in U.S. vaccines.

Adjuvant	Composition	Vaccines
Aluminum	One or more of the	Anthrax, DT, DTaP (Daptacel), DTaP (Infanrix), DTaP-IPV (Kinrix), DTaP-IPV

	following: amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (Alum)	(Quadracel), DTaP-HepB-IPV (Pediarix), DTaP –IPV/Hib (Pentacel), Hep A (Havrix), Hep A (Vaqta), Hep B (Engerix-B), Hep B (Recombivax), HepA/Hep B (Twinrix), HIB (PedvaxHIB), HPV (Gardasil 9), Japanese encephalitis (Ixiaro), MenB (Bexsero, Trumenba), Pneumococcal (Prevnar 13), Td (Tenivac), Td (Mass Biologics), Tdap (Adacel), Tdap (Boostrix)
AS04	Monophosphoryl lipid A (MPL) + aluminum salt	Cervarix
MF59	Oil in water emulsion composed of squalene	Fluad
$AS01_B$	Monophosphoryl lipid A (MPL) and QS-21, a natural compound extracted from the Chilean soapbark tree, combined in a liposomal formulation	Shingrix
CpG 1018	Cytosine phosphoguanine (CpG), a synthetic form of DNA that mimics bacterial and viral genetic material	Heplisav-B
No adjuvant		ActHIB, chickenpox, live zoster (Zostavax), measles, mumps & rubella (MMR), meningococcal (Menactra, Menveo), rotavirus, seasonal influenza (except Fluad), single antigen polio (IPOL), yellow fever

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Aluminum

Aluminum-containing adjuvants are vaccine ingredients that have been used in vaccines since the 1930s. Small amounts of aluminum are added to help the body build stronger immunity against the germ in the vaccine. Aluminum is one of the most common metals found in nature and is present in air, food, and water. Scientific research has shown the amount of aluminum exposure in people who follow the recommended vaccine schedule is low and is not readily absorbed by the body. Read the research on aluminum exposure and vaccines. Also, see FDA's web page on common ingredients in U.S. licensed vaccines for more information.

AS04

Beginning in 2009, monophosphoryl lipid A (MPL) was used in one U.S. vaccine (Cervarix®); however, the vaccine is no longer available in the United States due to low market demand. This immune-boosting substance was isolated from the

surface of bacteria.

MF59

MF59 is the adjuvant contained in Fluad (an influenza vaccine licensed for adults aged 65 or older). MF59 is an oil-in-water emulsion composed of squalene, which is a naturally occurring oil found in many plant and animal cells, as well as in humans. MF59, used in flu vaccines in Europe since 1997 and in the United States since 2016, has been given to millions of people and has an excellent safety record.

$AS01_B$

$AS01_B$ is an adjuvant suspension used with the antigen component of Shingrix vaccine. Shingrix is the recombinant zoster vaccine recommended for persons aged 50 years or older. $AS01_B$ is made of up of monophosphoryl lipid A (MPL), an immune-boosting substance isolated from the surface of bacteria, and QS-21, a natural compound extracted from the Chilean soapbark tree (*Quillaja saponaria* Molina). In pre-licensure clinical trials, $AS01_B$ was associated with local and systemic reactions, but the overall safety profile was reassuring.

$AS01_B$ is also a component of vaccines currently being tested in clinical trials, including malaria and HIV vaccines. To date, these trials have included over 15,000 people.

CpG 1018

CpG 1018 is a recently developed adjuvant used in Heplisav-B vaccine. It is made up of cytosine phosphoguanine (CpG) motifs, which is a synthetic form of DNA that mimics bacterial and viral genetic material. When CpG 1018is included in a vaccine, it increases the body's immune response.

In pre-licensure clinical trials, adverse events after Heplisav-B were comparable to those observed after another U.S.-licensed, non-adjuvanted hepatitis B vaccine.

CDC and FDA closely monitor the safety of all vaccines.

CDC and the Food and Drug Administration (FDA) are committed to ensuring that vaccines provided to the public are safe and effective. Once vaccines are licensed in the United States, CDC and FDA continuously monitor them through several safety systems. Learn more about CDC's vaccine safety systems.

Related Links

Vaccine Additives

Vaccine Ingredients Sorted by Vaccine [PDF-182 KB]

Vaccine Ingredients

Page last reviewed: October 24, 2018

Content source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)

EXHIBIT 2

ScienceDirect

Alum

Alum is potassium aluminum sulfate that is used as the starting solution to precipitate antigens with either aluminum phosphate or aluminum hydroxide.

From: Introduction to Biomedical Engineering (Third Edition), 2012

Related terms:
Haematoxylin, Antigen, Antibody, Protein, Immunization, Immune Response, Vaccination

Vaccines

Alan R. Shaw, Mark B. Feinberg, in Clinical Immunology (Third Edition), 2008

Alum

Alum, the classical adjuvant most often used in vaccines in humans, includes a range of salts of aluminum precipitated under basic conditions, usually aluminum sulfate mixed with sodium or potassium hydroxide plus a variable amount of phosphate.[89] The relative proportions will determine the size, charge, and solubility of alum. The composition of alum used as an adjuvant varies in currently available vaccines and may influence vaccine immunogenicity. Alum is utilized as an adjuvant in many of the currently available vaccines composed of inactivated toxins or recombinant proteins (live attenuated vaccines do not include alum or other adjuvants).

Alum serves two main purposes as an adjuvant. First, it acts as an antigen depot. Vaccine antigens adsorb to alum and elute from it following injection into the host. Second, alum acts a mild irritant, causing the recruitment of leukocytes necessary for generation of an immune response to the site of injection. Adsorption of antigens on to alum routinely improves immunogenicity, particularly the antibody response. Alum does not typically enhance CD8 T-cell responses. Alum has been a component of many vaccines for decades and has an excellent safety record. As new adjuvants are developed, alum may remain as a component of combination adjuvant mixtures (as is the case with some newer adjuvants now approaching clinical use), or it may eventually be supplanted by other agents that more effectively provide favorable depot and local inflammatory responses to accentuate host immune responses.

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Exhibit 2

URL: https://www.sciencedirect.com/science/article/pii/B9780323044042100922

Adjuvants Targeting the DNA Sensing Pathways – Alum Based Adjuvants

Christophe J. Desmet, in Biological DNA Sensor, 2014

Alum Induces Signs of Cell Damage In Vivo

Alum is not usually considered as a cytotoxic adjuvant. It is indeed well-tolerated in a vast majority of cases and does not usually induce clinically visible inflammatory reactions in vaccinated subjects. However, this does not forcefully mean that alum is devoid of any cytotoxic activity. A few earlier studies actually reported some level of cytotoxicity of alum *in vivo*[75–77]. In an elegant more recent study, Philippa Marrack's group investigated the composition of alum nodules *in vivo*[14]. It is well known that alum quickly forms nodules at sites of injection and it has long been thought that these nodules play a role in the adjuvant activity of alum. Yet, the main conclusion of Philippa Marrack's team was that nodule formation is dispensable for the adjuvant activity of alum [14]. Interestingly, in addition to alum, nodules were shown to contain large quantities of fibrin derived from thrombin-cleaved fibrinogen. Fibrin deposition is often observed in inflammatory conditions such as foreign body reactions [78]. Alum nodules were furthermore shown to also contain histones and host DNA indicative of the presence of extracellular chromatin within alum nodules. Thus, such studies compellingly indicate that alum treatment leads to the release of intracellular molecules at sites of injection, some of which could potentially be recognized as DAMPs.

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URL: https://www.sciencedirect.com/science/article/pii/B9780124047327000125

Inflammatory/Noninflammatory Adjuvants and Nanotechnology—The Secret to Vaccine Design

K.L. Wilson, ... M. Plebanski, in Micro and Nanotechnology in Vaccine Development, 2017

6.4.1 Alum

Alum was first licenced for human use in the 1920s and for the next 70 years would be the only adjuvant on the market.[48] Vaccines containing alum as adjuvant component have a demonstrated safety profile of more than six decades of use, and it is uncommon for them to be associated with severe local reactions. The success of alum stems from its safety profile, cost effectiveness, and, most importantly, the fact that it enhances the response to a range of antigens. These reasons deemed it to be the adjuvant of choice for so long without the full understanding of its mode of action. While there are now many reports of how alum interacts with immune cells, the overall mechanism is still not fully definite. Initially it was proposed that alum

creates a depot effect at the site of injection, ensuring slow release of its adsorbed antigen and leading to long-lasting responses.[49,50] However, there have been challenges to this theory as studies have shown that excision of the injection site containing alum 2–24 hours post injection does not affect resulting immune responses, indicating that there is no depot effect.[51] Alum–antigen complexes can be cleared after a couple of weeks, and the long-lasting effects are most likely attributed to the innate cells that are attracted to the site and initially take up the antigen.[50] Alum enhances DC uptake of antigen, promotes sustained antigen–major histocompatibility complex (MHC)II expression,[52] and also interacts uniquely with the DC surface membrane.[53] In particular, in response to alum, inflammatory monocytes and DCs rapidly engulf antigen and migrate to local lymph nodes to present antigen to T cells, and while alum may not directly activate these migratory DCs, it induces high levels of the endogenous danger signal, e.g., uric acid, which increases the recruitment and activation of neutrophils and inflammatory monocytes, and induces differentiation of inflammatory DCs.[54] Alum has also been shown to induce cell death, which releases host cell DNA, thus acting as another DAMP.[55] The activation of these DAMP signals has been reported to promote alum's characteristic Th2-type immune response.[54] NACHT, LRR and PYD domains-containing protein 3 (NALP3) is an intracellular NLR which senses danger signals, such as DAMPs, and whose signaling initiates the assembly of the NALP3 inflammasome, resulting in activation of caspase 1 and subsequent induction of pro-IL-1β followed by the cytokine IL-1β.[56] Alum stimulates the release of cytokines such as IL-1β and IL-18 via caspase 1 activation in DCs,[57,58] mediated through NALP3 inflammasome signaling,[59,60] which can be activated by uric acid crystals.[54,61] Some studies have shown that mice deficient in caspase 1 or NALP3 fail to produce antibodies to alum adjuvants, whereas others question the importance of NALP3 signaling in the overall adjuvant activity of alum resulting in T and B cell responses.[62,63] Additionally, some researchers have suggested alum can result in responses independent of traditional TLR signaling, as antibody production was uninhibited in the TLR signaling molecules, myeloid differentiation primary-response protein 88 (MyD88), and Toll/IL-1 receptor (TIR) domain-containing adaptor inducing IFNβ (TRIF) deficient mice.[64] Other innate cells associated with the Th2 immunostimulatory effects of alum are a specific type of GR-1+ cell that secretes IL-4, as well as eosinophils that also secrete IL-4 to promote early B cell priming and class switching of IgM, and type 2 NKT cells produce IL-4, which is CD1d dependent.[65–67] Alum has also been shown to enhance upregulation of MHCII expression on monocytes, which is IL-4 dependent, and cells exposed to alum increase synthesis of IL-4 mRNA but not IFNγ mRNA.[68] IL-4 secretion is known to inhibit Th1 responses, potentially adding to the Th2 skewed responses seen with alum. Throughout the conflicting evidence and without a fully defined mechanism for alum's adjuvanticity, the general consensus is that alum increases recognition and uptake of antigen, recruits innate cells, promotes proinflammatory cytokines through danger signals, and skews the immune system toward a Th2 response (Table 6.1).[50,69] Although alum has been successfully used in many vaccine formulations, such as diphtheria, tetanus, pneumococcus, etc., and in combination adjuvant systems in hepatitis B virus (HBV) and human papilloma virus (HPV) vaccines,[70] it is not so successful against other diseases (such as cancer or complex diseases) where Th1 and/or CD8 T cell responses are required.

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URL: https://www.sciencedirect.com/science/article/pii/B9780323399814000063

The Role of Inflammasomes in Adjuvant-Driven Humoral and Cellular Immune Responses

N. Muñoz-Wolf, ... E.C. Lavelle, in Immunopotentiators in Modern Vaccines (Second Edition), 2017

Other Danger-Associated Molecular Patterns and Lysosomal Destabilization

Alum can trigger necrosis and release of several other DAMPs (i.e., ATP, DNA, IL-1α, and IL-33[50,52,59]), which can contribute to NLRP3 activation and/or Th2 polarization.

Alum salts promote necrosis and DNA release in vivo with consequent activation of IRF3, and enhanced migration of inflammatory monocytes to draining lymph nodes after IP injection.[50] Importantly, when intramuscular immunization of alum was performed, migration of antigen-loaded DCs to lymph nodes did not require DNA as treatment with DNase-I neither had impact nor did it affect DC's costimulatory molecule expression. Instead, DNase-I treatment affected the duration of the interaction between antigen-specific T cells and antigen-loaded DCs in the nodes by affecting the antigen-presenting capacity of DCs. The study suggested that alum facilitates host DNA cytoplasmic delivery into DCs activating DNA-sensing pathways involving the DNA-sensing adaptor Stimulator of Interferon Genes (STING) to increase antigen presentation to T cells in the nodes.[51] Phagocytosis of particulates including alum can lead to lysosomal rupture and release of enzymes into the cytosol, including cathepsin B, which can mediate pyroptosis, cell death associated with NLRP3 activation.[60]

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URL: https://www.sciencedirect.com/science/article/pii/B9780128040195000025

Discovery of Immune Potentiators as Vaccine Adjuvants

C. Buonsanti, U. D'Oro, in Immunopotentiators in Modern Vaccines (Second Edition), 2017

Pattern Recognition Receptor Agonists as Vaccine Immune Potentiators

Alum salts and emulsions were developed empirically and have been used in the clinic for many years without knowing their mechanism of action. However, the field of adjuvant discovery underwent a drastic change recently, when the molecular mechanisms of immune activation were elucidated and PRRs were discovered as key molecules that regulate innate immune signaling. Among them, Toll-like receptors (TLRs) and nucleotide-binding and oligomerization domain (NOD)-like receptors

(NLRs) are the most studied PRRs that control and modulate the cellular immune response[25,26] (Table 5.1).

Table 5.1. Pattern Recognition Receptors

TLR	Localization	Ligands	Signal Adaptor	Production
TLR1	Cell surface	Bacterial lipoproteins from *Mycobacteria* and *Neisseria*	MyD88	IC
TLR2	Cell surface	Triacylated lipoproteins (Pam3CSK4), yeast zymosan particles, peptidoglycans, lipoproteins, glycolipids, LPS		
TLR6	Cell surface	Yeast zymosan particles, lipoteichoic acid, lipopeptides from *Mycoplasma*	MyD88	IC
TLR3	Endosomes	Viral dsRNA (poly I:C)	TRIF	IC, type1 IFN
TLR4	Cell surface/endosomes	LPS, paclitaxel	TRIF, MyD88	IC, type1 IFN
TLR5	Cell surface	Bacterial flagellin	MyD88	IC
TLR7	Endosomes	ssRNA, imidazoquinolines (R848, R-837)	MyD88	IC, type1 IFN
TLR8	Endosomes	ssRNA, imidazoquinolines (R848)	MyD88	IC, type1 IFN
TLR9	Endosomes	CpG, CpG-containing oligonucleotides	MyD88	IC, type1 IFN
TLR10	Endosomes	Profilin-like proteins	MyD88	IC
NOD1	Cytoplasm	Diaminopimelate-containing	RIPK2	IL-1β

		muramyl tripeptide mostly found in gram-negative bacterial peptidoglycan		
NOD2	Cytoplasm	Muramyl dipeptide from gram-positive and gram-negative bacterial peptidoglycan	RIPK2	IL-1β
NLRP3	Cytoplasm	ATP, viral RNA, muramyl dipeptide, imidazoquinoline, uric acid crystals, silica, aluminum salts, chitosan, QuilA	Inflammasome	IL-1β, IL-18
MMR	Cell surface	Mannose, fucose	CRD domains	Antigen uptake
Type II C-type lectin receptors (dectin-1, mincle, DC-SIGN)	Cell surface	β-Glucan, virus	Src, Syk	T-cell interaction
RIG-1 like receptors (RIG-1, MDA5)	Cytoplasm	Viral dsRNA	IRF	IC, type1 IFN
STING	Cytoplasm	DNA	TBK1–IRF3	IFN-β

CRD, cysteine-rich domain; *DC-SIGN*, dendritic cell-specific intracellular adhesion melecule-3-grabbing non-integrin; *IC*, inflammatory cytokines; *IFN*, interferon; *IL*, interleukin; *MMR*, macrophage mannose receptor; *NOD*, nucleotide-binding and oligomerization domain; poly I:C, polyinosinic:polycytidilic acid; *RIPK*, receptor-interacting protein kinases; *ss*, single stranded; *STING*, stimulators of interferons genes; *TLR*, Toll-like receptor.

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URL: https://www.sciencedirect.com/science/article/pii/B9780128040195000050

Vaccine Adjuvantsa

Steven G. Reed PhD, ... Rhea N. Coler MSc, PhD, in The Vaccine Book (Second Edition), 2016

4 Mechanisms of action

Alum was originally thought to boost the immune response by slowing the release of the antigen from the immunization site thus prolonging antigen presentation (depot effect). However this hypothesis has recently been disproven, at least in animal models, as excision of the immunization site within hours of injection did not impair the adaptive immune response. Rather alum's adjuvant activity depends on the production of IL-1β downstream of the release of DAMPs such as uric acid and host DNA which are released by damaged cells and recognized by specific receptors.[23–26]

MF59 consists of an oil (squalene)-in-water nanoemulsion composed of <250 nm droplet; it is licensed Europe in influenza vaccines.[27] MF59 formulation has also been tested with herpes simplex virus (HSV), HBV, and HIV vaccine candidates. Overall, MF59 has an excellent safety profile, and with several antigens significant increase in antibody titers with reportedly more balanced Th1/Th2 responses than those obtained with alum. MF59 causes a local increase in extracellular ATP in the muscle which leads to recruitment of monocytes, macrophages, and granulocytes and production of cytokine and chemokine which shape the adaptive immune response.[28] Injection of apyrase to hydrolyze the ATP reduces the T cell and antibody responses to MF59 adjuvanted vaccination.[29]

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URL: https://www.sciencedirect.com/science/article/pii/B9780128021743000047

Vaccines and Clinical Immunization

Tak W. Mak, Mary E. Saunders, in The Immune Response, 2006

i) Adjuvants

The reader will recall from Chapter 6 that one of the key functions of an adjuvant is to induce local inflammation. Such inflammation recruits APCs and lymphocytes to the site of infection or inoculation, ensuring that antigens are presented to T cells in an activatory context. The precise mechanism underlying adjuvant activity is unknown and it is still not clear whether the adjuvant exerts its effects at the site of injection or in the local lymph node. What is clear is that the immunogen and the adjuvant must be injected together to achieve optimal immunogenicity: separate injections into the same animal are not enough. How then does an adjuvant work? One possibility is that the adjuvant promotes capture of the adjuvant-immunogen mixture by DCs in the tissues. These DCs become activated and transport the immunogen from the site of injection to the local lymph node and its resident naive T cells. Another hypothesis holds that an adjuvant represents "nonself" to the host innate immune system. In this context, the adjuvant works because it contains a molecule or sequence that binds to PRRs on innate immune system cells, initiating the inflammatory response. Alternatively, the adjuvant may cause sufficient damage to host cells at the injection site that stress molecules are released. In either case, the

presence of inflammatory mediators (particularly IL-1) induces APCs to upregulate their costimulatory molecules, meaning that antigen presentation will be more likely to activate than anergize T cells. The adaptive immune response is thus enhanced and the vaccination is a success. However, it remains puzzling why killed virus particles and bacteria, which retain the motifs recognized by PRRs, still require adjuvants to induce an effective immune response. Moreover, host cell debris injected as an adjuvant has no immunostimulatory activity, while lipid-containing particles, which are thought to be innocuous, do have adjuvant activity.

Another explanation for adjuvant activity may be the "depot effect." Injection of adjuvant material often induces the formation at the injection site of a granuloma that allows only a slow release of the vaccine antigen. The life of the antigen in the tissues is therefore prolonged, and degradation is avoided until the antigen is taken up by APCs. This protective effect means that use of an adjuvant can reduce the amount of antigen needed to induce an immune response, an important factor when vaccines containing low amounts of antigen, such as purified protein vaccines or DNA vaccines, are used. We will now examine a selection of clinical and experimental adjuvants (Table 23-5).

Table 23-5. Adjuvants and Delivery Vehicles

Adjuvants	Characteristics	Pros	Cons
*Alum [aluminum hydroxide: $Al(OH)_3$; aluminum phosphate: $AlPO_4$]	Favors Th2 responses	Few side effects Stable	Does not induce Tc activation Requires painful injection Can cause necrosis
Bacterial toxins (derivatives of exotoxins)	Effective mucosal adjuvant	Not toxic at low concentrations	Possible neurological effects at high concentration
CpG motifs (component of bacterial DNA)	Favor Th1 responses and induce IFNγ secretion by APCs, which promotes CTL expansion	Induces strong Ab and CTL responses in mice	Does not work as well in humans
Cytokines	Immune deviation	Promotes most effective immune response	Systemic toxicity

Delivery Vehicles	Mechanism	Pros	Cons
Liposomes (spherical phospholipid bilayer with aqueous center)	Phagocytosis by macrophages and uptake by other host cells induces Th, B and Tc responses	No immune response to liposome itself	Not very efficient, requires large amounts of Ag Possible toxic side effects at high liposome concentrations
Virosomes (IRIV) (liposome plus viral envelope glycoproteins)	Viral fusion to host cell receptors and cell entry like a virus provokes Tc response Phagocytosis by APCs provokes Th and B responses	Very effective and efficient with few side effects Can capture naked DNA, small peptides, and large proteins	
ISCOMs (soccer ball-like cage of cholesterol, phospholipid, and saponin surrounding Ag)	Presentation of multivalent antigen Phagocytosed by APCs to provoke strong Th1, B, and CTL responses Saponin stimulates APCs	Low amounts of Ag required Particularly good for lipid-bearing Ag Local and systemic responses Can be given by oral and intranasal routes Relatively stable	More difficult to make than IRIVs Possibly toxic if saponin impure
SMAAs	Phagocytosed by APCs so that Tc, B, and Th responses induced	Can coat beads simultaneously with T and B	Labor-intensive to construct

(vaccine epitope bound to specific mAbs bound to microbeads)		epitopes	
Biodegradables (polymeric chitosan microspheres)	Slow degradation releases Ag over time; induces prolonged Ab response	Increased persistence of Ag	Continuous release of Ag may inhibit memory generation
DCs (immature DCs that have phagocytosed Ag)	Ag-loaded DCs activate naive Th and Tc cells	Efficient Provokes systemic and mucosal responses	Autologous DCs must be isolated for use in humans
Intracellular bacteria (attenuated intracellular bacteria transfected with naked DNA vaccines)	Macrophages phagocytose and lyse DNA-loaded bacteria, releasing Ag DNA Transcription and translation of Ag DNA occurs within host cell so a Tc response is induced	Relatively easy to construct	Possible integration of bacterial DNA into host genome Possible reversion of attenuating mutation
Gene gun (high-pressure blasting of DNA directly into cells)	Induces Tc response	Avoids degradation by nucleases	Complex apparatus required

* Alum is currently the only adjuvant licensed for standard patient treatment.

- **Alum:** Alum, a gel composed of aluminum hydroxide or aluminum phosphate, was one of the first adjuvants ever tried and is still the only one licensed for routine use in humans. Despite its long history, it is still not understood exactly how alum enhances the immune response. There is some evidence that alum promotes Th2 responses at the expense of Th1 responses, resulting in a bias toward antibody production. This characteristic means that alum is not

particularly good at supporting cell-mediated responses. An advantage of alum over protein adjuvants is that alum is more stable when the vaccine cold chain is broken. A disadvantage of alum is that it must be injected deep into muscle and not subcutaneously, increasing vaccinee discomfort. The presence of alum in subcutaneous tissues can cause necrosis.

- **Bacterial toxins:** Although not yet routinely used in humans due to safety concerns, some bacterial toxins or their components have proved to be useful adjuvants for immunization studies in experimental animals. Modified versions of cholera toxin have long been employed as adjuvants. The B subunit of natural cholera toxin directs the toxin to the M cells in the intestine. Natural cholera toxin can be altered by point mutation, or the A subunit can be deleted, to produce a molecule that is no longer toxic but still directs associated antigens to the mucosae. *Procholeragenoid* (PCG) is an immunogenic derivative of cholera toxin that is less expensive and easier to produce than natural cholera toxin B subunit. Another relatively recent toxin-derived adjuvant is heat-labile *enterotoxin* (HLT), a product of *E. coli.* Both PCG and HLT have substantial adjuvant activity when administered systemically or mucosally, and, when used at low concentrations, are not toxic to mice. HLT is the more effective adjuvant for intranasal administration of a vaccine and induces the production of large quantities of pathogen-specific secretory IgA (as well as antibodies directed against HLT). One problem with extending the use of these adjuvants to humans is that there are few good animal models in which to investigate toxic side effects. For unknown reasons, animals tend to be much more resistant than humans to the neurological effects of these toxins. Nevertheless, clinical trials in 2000 demonstrated that small amounts of HLT could be used as an adjuvant for an intranasally administered influenza vaccine in humans without adverse consequences.
- **CpG motifs:** As mentioned previously, bacterial DNA often contains CpG motifs, short sequences of unmethylated Pu-Pu-CpG-Py-Py. These motifs are about 20-fold more common in bacterial DNA than in eukaryotic DNA, making them ideal targets for PRR-mediated recognition by the innate immune system. Indeed, NK cells encountering synthetic versions of such sequences are stimulated to secrete IFNγ, and activated B cells increase their proliferation and antibody secretion. Several lines of evidence suggest that the CpG motif can act as an effective adjuvant in mice. First, immune responses characterized by the secretion of IL-6, IL-12, TNF, and the IFNs are observed following immunization of mice with bacterial DNA containing CpG. Secondly, when an animal is immunized with CpG plus a purified protein antigen, antibody- and cell-mediated responses to the antigen are achieved that are equivalent to those observed when a DNA vaccine containing the gene for the same protein antigen is used. Thirdly, CpG is a powerful inducer of IFNα secretion by APCs, and IFNα is known to stimulate expansion of memory $CD8^+$ CTLs. CpG may thus indirectly enhance memory CTL responses. Unfortunately, synthetic CpG motifs that work well in mice are not very effective in humans. The design of a CpG sequence more stimulatory in humans, and the investigation of whether a CpG adjuvant effect applies in humans at all, are ongoing.

- **Cytokines:** We have learned in previous chapters that the presence of certain key cytokines can influence an immune response to take on either a Th1- or a Th2-like character. For some pathogens, it may be advantageous to encourage a Th2 response in place of a Th1 response, or vice versa. Co-administration of cytokines (particularly IL-12) with vaccine antigens can favorably skew the immune response in mice. While direct injection of cytokines into humans has toxic side effects, intranasal administration of cytokines such as IL-2, GM-CSF, and IL-12 in human volunteers has been shown to enhance the induction of mucosal immunity by an antigen. Work on defining safe conditions for the use of such adjuvants is under way.

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URL: https://www.sciencedirect.com/science/article/pii/B9780120884513500259

The hematoxylins and eosin

John D. Bancroft, Christopher Layton, in Bancroft's Theory and Practice of Histological Techniques (Eighth Edition), 2019

Disadvantages of alum hematoxylins

The major disadvantage of alum hematoxylin stains is their sensitivity to any subsequently applied acidic staining solutions, e.g. van Gieson and trichrome stains. The application of the picric acid-acid fuchsin mixture in van Gieson's stain removes most of the hematoxylin so that the nuclei are barely discernible. Satisfactory nuclear staining is achieved, in this case, by using an iron-mordanted hematoxylin such as Weigert's hematoxylin (see p. 132), which is more resistant to the effect of picric acid. A suitable alternative is the combination of a celestine blue staining solution with an alum hematoxylin. Celestine blue is resistant to the effects of acid, and the ferric salt in the prepared celestine blue solution strengthens the bond between the nucleus and the alum hematoxylin providing a strong nuclear stain more resistant to acid.

Celestine blue-alum hematoxylin procedure
Celestine blue solution

Celestine blue B	2.5 g
Ferric ammonium sulfate	25 g
Glycerin	70 ml
Distilled water	500 ml

The ferric ammonium sulfate is dissolved in cold distilled water with stirring, the celestine blue is added and the mixture is boiled for a few minutes. After

cooling, the stain is filtered and glycerin is added. The final stain should be usable for over 5 months. Filter before use.

Method

1. Dewax sections, rehydrate through descending grades of alcohol and take to water.
2. Stain in celestine blue solution for 5 minutes.
3. Rinse in distilled water.
4. Stain in an alum hematoxylin, e.g. Mayer's or Cole's for 5 minutes.
5. Wash in water until blue.
6. Proceed with required staining technique.

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URL: https://www.sciencedirect.com/science/article/pii/B9780702068645000104

Scientific Fundamentals of Biotechnology

K.K. Jain, in Comprehensive Biotechnology (Second Edition), 2011

1.45.5.3 Role of Nanobiotechnology in Vaccines

In contrast to alum, which is conventionally used as an adjuvant, antigens covalently coupled to nanobeads, measuring 40 nm in diameter, induce substantial cell-mediated responses along with moderate humoral responses. No adverse reactions have been noted at the site of immunization in experimental animals. Thus, nanobead adjuvants in veterinary species may be useful for the induction of immunity to viral pathogens, where a cell-mediated response is required. These vaccines have potential usefulness for intracellular pathogens in humans. Most adjuvants only stimulate antibodies against a particular disease. The nanobead technology gives the immune system a further boost, also producing T-cells, which are needed to eliminate viruses or cancer. The size of 40 nm is critical, as it is a size similar to many viruses, where the nanobeads are taken up abundantly by the immune system and tricked into producing high levels of many types of T-cells.

A targeted synthetic vaccine platform creates fully integrated synthetic nanoparticle vaccines engineered to mimic the properties of natural pathogens to elicit a maximal immune response. Antigens and adjuvants are delivered within the same biodegradable nanoparticle, directly to antigen-presenting cells. This approach maximizes the immune response, while minimizing undesirable off-target effects.

Read full chapter

URL: https://www.sciencedirect.com/science/article/pii/B9780080885049000684

Connecting the Innate and Adaptive Immune Responses

Tom P. Monie, in The Innate Immune System, 2017

5.5.2.1 Alum

The mechanism of action by which alum compounds function as vaccine adjuvants remains somewhat controversial. It is clear that their administration increases the uptake and presentation of antigen by dendritic cells and also improves the recruitment of monocytes to the site of inoculation. However, it is currently not entirely clear as exactly which PRRs are involved in helping to drive the adjuvant activity of alum. A number of studies proposed that its effects were mediated through the NLRP3 inflammasome. Other studies have suggested that this may well not be the case. Alum is not proposed to directly activate the NLRP3 inflammasome but may stimulate uric acid production or lead to the release of DNA from dying cells or from damaged mitochondria, which then acts as an immune stimulator. The role of DNA in alum-induced adjuvant activity is gaining in popularity and has been suggested to also function through STING-dependent signaling pathways.

Read full chapter

URL: https://www.sciencedirect.com/science/article/pii/B9780128044643000053

EXHIBIT 3

antibody An immunoglobulin produced by plasma cells, which has a specific amino acid sequence and specifically binds to the antigen(s) (e.g., foreign proteins, microbes or toxins) that induced its synthesis; antibodies may also bind to closely related antigens.

EXHIBIT 4

Medical Definition of Arthralgia

Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Arthralgia: Pain in a joint.

Reviewed on 12/4/2018

EXHIBIT 5

autoantibody [aw″to-an´tĭ-bod″e]

an antibody formed in response to, and reacting against, an antigenic constituent of the individual's own tissues.

EXHIBIT 6

Understanding and Managing Chronic Inflammation

Medically reviewed by Seunggu Han, M.D. — Written by Adrienne Santos-Longhurst — Updated on July 27, 2018

What is inflammation?

Inflammation refers to your body's process of fighting against things that harm it, such as infections, injuries, and toxins, in an attempt to heal itself. When something damages your cells, your body releases chemicals that trigger a response from your immune system.

This response includes the release of antibodies and proteins, as well as increased blood flow to the damaged area. The whole process usually lasts for a few hours or days in the case of acute inflammation.

Chronic inflammation happens when this response lingers, leaving your body in a constant state of alert. Over time, chronic inflammation may have a negative impact on your tissues and organs. Some research suggests that chronic inflammation could also play a role in a range of conditions, from cancer to asthma.

Read on to learn more about chronic inflammation, including common causes and foods that fight it.

EXHIBIT 7

comorbid [ko-mor´bid]

pertaining to a disease or other pathological process that occurs simultaneously with another.

EXHIBIT 8

Medical Definition of Cytotoxic

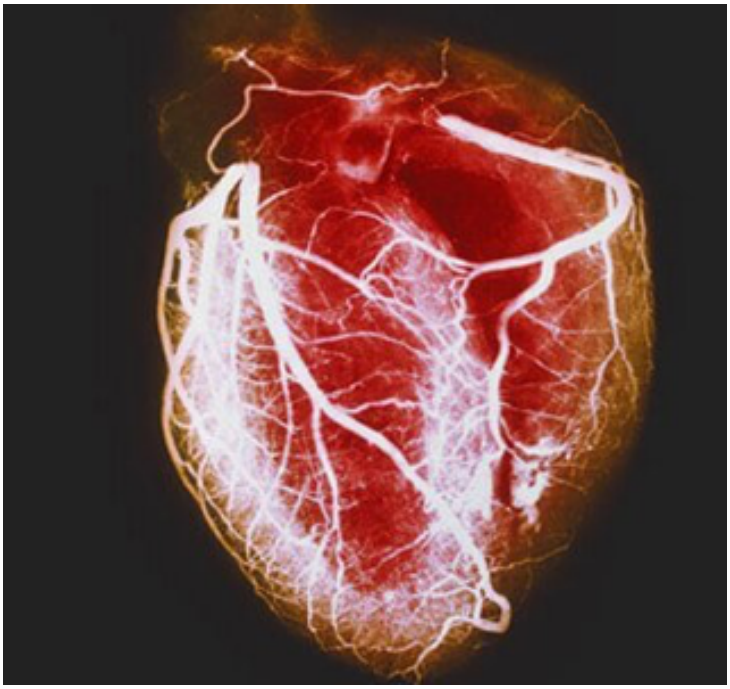
Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Cytotoxic: Toxic to cells, cell-toxic, cell-killing. Any agent or process that kills cells.

Chemotherapy and radiotherapy are forms of cytotoxic therapy. They kill cells.

The prefix cyto- denotes a cell. It comes from the Greek kytos meaning hollow, as a cell or container. Toxic is from the Greek toxikon = arrow poison.

CONTINUE SCROLLING OR **CLICK HERE** FOR RELATED SLIDESHOW



— SLIDESHOW —

Heart Disease: Causes of a Heart Attack

See Slideshow

EXHIBIT 9

Medical Definition of Demyelination

Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Demyelination: A degenerative process that erodes away the myelin sheath that normally protects nerve fibers. Demyelination exposes these fibers and appears to cause problems in nerve impulse conduction that may affect many physical systems. Demyelinization is seen in a number of diseases, particularly **multiple sclerosis**.

Diagnosis is by functional observation and by testing for myelin protein in the blood.

CONTINUE SCROLLING OR **CLICK HERE** FOR RELATED SLIDESHOW

SLIDESHOW

Brain Food Pictures: What to Eat to Boost Focus

See Slideshow

Exhibit 9

EXHIBIT 10

ep·i·dem·ic (ep'i-dem'ik),

The occurrence in a community or region of cases of an illness, specific health-related behavior, or other health-related events clearly in excess of normal expectancy; the word is also used to describe outbreaks of disease in animals or plants. Compare: **endemic**, **sporadic**. [epi- + G. *dēmos*, the people]

EXHIBIT 11

epitope (ĕp'ĭ-tōp')

n.

A localized region on the surface of an antigen capable of eliciting an immune response and of combining with a specific antibody to counter that response.

EXHIBIT 12

e·ti·ol·o·gy (ē'tē-ol'ŏ-jē), *Avoid the jargonistic substitution of this word for* cause *(of disease).*

1. The science and study of the causes of disease and their mode of operation. Compare: **pathogenesis**.

2. The science of causes, causality; in common usage, the cause itself.

[G. *aitia*, cause, + *logos*, treatise, discourse]

"CITE" Farlex Partner Medical Dictionary © Farlex 2012

Exhibit 12

EXHIBIT 13

his·to·pa·thol·o·gy (his'tō-pă-thol'ŏ-jē),

The science or study dealing with the cytologic and histologic structure of abnormal or diseased tissue.

Synonym(s): **pathologic histology**

EXHIBIT 14

Immune-Mediated Inflammatory Disorders (I.M.I.D.s): The Economic and Clinical Costs

John P. Williams, MD, MBA; Jonathan A. Meyers, FSA, MAAA

Abstract

Immune-mediated inflammatory disorders (I.M.I.D.s) are a group of diseases that involve an immune response that is inappropriate or excessive, and is caused, signified, or accompanied by dysregulation of the body's normal cytokine milieu. I.M.I.D.s cause acute or chronic inflammatory injury, sometimes severe, in any organ system. Despite strong evidence linking the pathophysiologies and treatments of the diseases that constitute the I.M.I.D. group, providers, payers, employers, and benefits consultants have been slow to adopt the I.M.I.D. concept. As a result, these stakeholders risk underestimating the significant clinical and economic burdens of the I.M.I.D. class. In this review we examine those burdens, specifically analyzing I.M.I.D. prevalence and cost data for a group of large employers. We also describe the scientific rationale for the I.M.I.D. paradigm, examine the cytokine dysregulation that many I.M.I.D.s share, and focus in detail on the pathophysiology of 3 I.M.I.D.s with high morbidity: rheumatoid arthritis, Crohn's disease, and type 1 diabetes mellitus. The review concludes with an evaluation of approved anticytokine I.M.I.D. therapies and those in development.

(Am J Manag Care. 2002;8:S664-S681)

The concept of autoimmune disease has existed for at least 100 years, and there has been biochemical evidence of these conditions' shared mechanisms for at least 15 years.[1,2] Dysregulation of cytokines, a group of small proteins produced ubiquitously in the body, has proven to be a key element in these shared mechanisms. In the last 5 years, anticytokine therapies have produced therapeutic breakthroughs in conditions ranging from rheumatoid arthritis (RA) to Crohn's disease (CD). Nevertheless, despite shared pathophysiology and therapy, autoimmune conditions are not typically seen as interrelated by payers, providers, employers, and benefits consultants (personal communication, P. Lopatka, July 2002). For example, RA is usually considered a rheumatological disorder and CD a gastrointestinal condition, even though the cytokine dysregulation and treatment of each illness may be similar.[3]

This review describes a new medical paradigm—the immune-mediated inflammatory disorders (I.M.I.D.s)—that will help healthcare stakeholders understand the economic and clinical burden of a group of diseases that together may affect as much as 5%-7% of the American population.[1,4] I.M.I.D.s involve an immune response that is inappropriate or excessive and is caused, signified, or accompanied by a dysregulation of the normal cytokine milieu. They also cause acute or chronic inflammatory injury, sometimes severe, in any organ system.

Many illnesses fulfill these criteria, from the relatively obscure, such as giant cell arteritis, to public health crises, such as type 1 diabetes mellitus (T1DM). For payers, it is helpful to focus on the conditions that have the highest per-member-

per-year (PMPY) cost, which takes into account prevalence of the disease and claims per patient. Before discussing I.M.I.D.s, however, it makes sense to briefly consider what exactly cytokines are and how their dysregulation can lead to pathophysiology.

What Are Cytokines and Why Do They Matter?

Cytokines are a family of proteins and polypeptides that are secreted by many different cell types and that are required for physiological immune function, inflammation, cell growth, and tissue repair.[3] Cytokine dysregulation, however, can lead to I.M.I.D.s.[5] In some cases cytokine overproduction may be associated with I.M.I.D. pathophysiology, as in rheumatoid arthritis and Crohn's disease.[4,5] Underproduction of cytokines may also be seen in certain I.M.I.D.s, as in systemic lupus erythematosus.[4] To make matters even more complicated, over- or underproduction of certain cytokines may have different effects, depending on where, when, and in what tissue microenvironments the dysregulation occurs.[5] Additionally, blocking or augmenting cytokine action through pharmacotherapy may reduce inflammation—or may have an unintended proinflammatory effect.[6]

As a general rule, it is helpful to think of cytokines as having proinflammatory or anti-inflammatory characteristics. The cytokines interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 12 (IL-12), interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α) are typically proinflammatory, whereas the cytokines interleukin 4 (IL-4), interleukin 10 (IL-10), and transforming growth factor β (TGF-β) are often anti-inflammatory.[3,5,7-14] These cytokines generate many of their pro- and anti-inflammatory effects through their actions on a group of immune cells known as helper or CD4+ T lymphocytes.

These helper T cells comprise 2 subsets: Th1 and Th2.[15] The Th1 cells have been associated with the development of I.M.I.D.s, and the Th2 cells may suppress their development. Cytokines steer developing helper T cells down the Th1 or Th2 paths, and cytokines produced by the Th1 subset tend to be proinflammatory, with Th2 cytokines typically being anti-inflammatory.[16] It should be noted, however, that recent investigations have shown that the Th1/Th2 paradigm may be incomplete. First, just as typically proinflammatory cytokines may sometimes be anti-inflammatory and anti-inflammatory cytokines may be proinflammatory, Th1 cells may sometimes have an anti-inflammatory effect, and Th2 cells a proinflammatory effect.[5] Second, a new class of helper T cells known as Th3 has recently been identified, and there may be other undescribed classes.[17]

Although the molecular biology of T cell differentiation is complex, it is worth examining, as many of the involved cytokines have offered and will offer important therapeutic targets. Simplified, the process works as follows: IL-12 causes differentiation of helper T cells into the Th1 proinflammatory subtype.[18] The Th1 cells then produce IL-2, which activates other T cells, and IFN-γ, which causes additional IL-12 production, which in turn leads to more Th1 differentiation.[14] Although not produced by T cells, IL-1 causes the production of IL-2 by T cells and the synthesis of TNF-α by many different cell types.[19,20] TNF-α causes the production of more IL-1 and the activation of more T cells, creating an inflammatory feedback loop.[20] As will be shown shortly, overproduction of these cytokines has been associated with a number of I.M.I.D.s.[4] In contrast, IL-4 and IL-10 lead to the differentiation of helper T cells into the Th2 anti-inflammatory subtype and to the suppression of Th1 proinflammatory development.[21] TGF-β, produced by a variety of cells, causes the production of IL-10, which further steers T cells into Th2 differentiation.[21]

Cytokines and I.M.I.D.s: What's the Connection?

As stated above, dysregulation of cytokines can lead to the tissue damage associated with I.M.I.D.s. But how exactly does it happen? This review will examine

the process in 3 I.M.I.D.s with significant clinical and economic burdens: rheumatoid arthritis, Crohn's disease, and type 1 diabetes mellitus.

RA: The Arche-typal I.M.I.D. RA is a chronic, destructive arthropathy that disables as many as 80% of its sufferers.[22] Small joint destruction proceeds at 2%-3% per year, and after 20 years of disease, 23% of patients have near-total joint destruction in their hands and feet.[23] The degenerative process, however, extends beyond the small joints, and RA may involve the cervical spine in approximately half of patients.[24]

Most important, RA is a systemic disease that causes substantially increased mortality, especially due to cardiac disease.[25,26] Specifically, it has been postulated that the inflammatory milieu of RA leads to increased atherosclerosis.[27]

RA's social costs are dramatic. As many as 10% of patients quit their jobs within 3 years of disease onset, and those patients with the most severe disease can expect a 60% decline in earnings during the first 6 years of illness.[28,29] In addition, the divorce rate of those with RA is said to be 70% higher than that of the general population.[29]

The pathogenesis of RA is not completely understood. For example, it is likely that RA's inflammatory process is initiated by an arthritogenic antigen, possibly of bacterial origin, but the antigen's identity is currently unknown.[30,31] That antigen activates helper T cells of the Th1 subtype, which subsequently secrete or cause to be secreted an array of cytokines that indirectly leads to joint inflammation and destruction.[22,32]

It was observed more than a decade ago that significant amounts of IL-1 and TNF-α are found in the joint spaces of RA patients, but not in those of normal controls.[33,34] In addition, an early transgenic mouse study showed that mice constitutively expressing TNF-α developed a chronic arthritis and that this arthritis could be stopped by treatment with antibodies against TNF-α.[35] It is now thought that T cells, once activated in RA, induce other cells to produce IL-1 and TNF-α, as well as an additional proinflammatory cytokine, interleukin-6 (IL-6).[36,37] Further, IL-1 and TNF-α further activate the T cell response, as well as lead to the secretion of destructive enzymes that erode the joint space.[22,38]

A number of cytokines, such as IL-4, IL-10, and TGF-β, may play an anti-inflammatory role in RA.[21] IL-10, for example, is one of the key cytokines that steers T cells toward the anti-inflammatory Th2 pathway, and it can be detected in the joint fluid of RA patients.[39] IL-4 and IL-10 lead to the formation of a naturally occurring IL-1 receptor antagonist that acts as a kind of molecular sponge for IL-1.[40] In addition, when IL-10 activity is blocked in patients with RA, secretion of proinflammatory cytokines TNF-α and IL-1 increases.[41]

These findings demonstrate that RA fits the I.M.I.D. paradigm. As defined in the beginning of the review, an I.M.I.D. involves an immune response that is inappropriate or excessive. Although the trigger for this immune response remains unknown, there can be no doubt that severe autoimmunity does occur in RA and involves the widespread activation of immune cells. In addition, with RA there are significant alterations in the physiological cytokine profile of the joint space that correlate with and may sometimes cause the immune response. Most importantly, this cytokine-driven immune response causes devastating injury to the host.

CD: Is It RA of the Gut? Crohn's disease (CD) is a chronic condition that involves severe, asymmetric inflammation throughout the gastrointestinal tract, as well as extragastrointestinal manifestations in the skin, eyes, and joints.[42] Like RA, CD is a systemic disease. One epidemiological study showed that after 20 years of disease, 26% of CD patients developed perianal fistulae, and 23% of those fistulae required bowel resection.[43] The incidence rates of liver/biliary tract and small bowel carcinoma in CD patients are, respectively, 5.2 and 17.4

times greater than the incidence rates of those cancers in the general population.[44] Not surprisingly, depression and anxiety are significantly more common in CD patients after diagnosis than before.[45]

Neither the cause nor the complete pathogenesis of CD is known, although a gene mutation on chromosome 16 is more than twice as likely to occur in CD patients as in controls.[46] Like RA, CD appears to be set in motion by an inappropriate or excessive immune response, potentially to intestinal bacteria. For example, mice deficient in the anti-inflammatory cytokine IL-10 develop a CD-like condition—unless they are maintained in a germ-free environment.[47]

In normal individuals, subpopulations of T cells, supported by IL-10, prevent an immune response against gut bacteria, but this regulation appears to be disrupted in CD.[48] In addition, the balance of proinflammatory and anti-inflammatory cytokines is disturbed, and as in RA, the production of IL-1 and TNF-α is increased.[49,50] This overproduction is significant because both of these cytokines cause the release of other inflammatory cytokines and proteolytic enzymes.

Clinical findings alone show little connection between RA and CD. The immunological and molecular evidence, however, suggest that CD and RA are both I.M.I.D.s. To review, I.M.I.D.s involve an immune response that is inappropriate or excessive and is caused, signified, or accompanied by a dysregulation of the normal cytokine milieu. They also cause acute or chronic inflammatory injury, sometimes severe, in any organ system.

CD likely involves an immune response to a currently unknown gut antigen, which is normally suppressed by appropriate cytokine regulation. Once in place, that aberrant immune response leads to a cytokine-driven inflammatory cascade that eventually causes major tissue damage throughout the gastrointestinal tract and elsewhere in the body. In summary, CD has cytokine dysregulation similar to that found in RA.

T1DM: The Most Morbid I.M.I.D. Just as RA has been considered a rheumatological condition and CD a gastrointestinal one, T1DM is typically viewed as an endocrine disease. The reason is obvious: destruction of the pancreas β cells in T1DM produces a hypoinsulinemia that, if untreated, invariably leads to death. The condition's only therapy to date has been insulin replacement, but even with insulin therapy, the risk of morbidity is high. One study showed that after 10 years of routine care, patients with T1DM have a 16% chance of developing sight-threatening retinopathy.[51] The risks of renal failure and lower extremity amputations are also greatly increased in T1DM.[52-54]

Findings of the last decade have established that almost all cases of T1DM have I.M.I.D. pathophysiology.[55-57] It is now thought that Th1 cells mediate the diabetic immune response, and in a T1DM mouse model, suppression of T cell activity prevents development of the disease.[56,58,59] As in the case of RA, the Th1-driven immune response in T1DM generates a series of proinflammatory cytokines, including IL-1, IL-2, IFN-γ, and TNF-α.[59] Certain cytokines such as IL-1 are indirectly cytotoxic to the β cells, causing free radicals to be generated that kill the insulin-producing cells.[60] In addition, these proinflammatory cytokines suppress β cell insulin secretion and also stimulate so-called cytotoxic T cells, which then attack and kill the β cells.[59,60] In contrast, the Th2 response and the cytokines that it elaborates have been shown to suppress the development of T1DM, potentially by inhibiting the pathogenic Th1 response, as in the case of IL-4.[57,61]

These findings strongly suggest that, like RA and CD, T1DM is an I.M.I.D. First, it involves an immune response that is excessive and inappropriate: total destruction of the pancreas's insulin-producing β cells. Second, it is caused or signified by a disruption in the normal cytokine milieu, specifically the overproduction of Th1 cytokines.[57] Third, the result of the immune-mediated

Table 1. Selected Immune-Mediated Inflammatory Disorders

Condition	Relative Overexpression of Cytokines	Relative Underexpression of Cytokines	Reference
Autoimmune hepatitis	TNF-α	IL-2, IL-4	62
Crohn's disease	TNF-α, IL-1, IL-2, IL-6, IL-8, IL-12, and IFN-γ	IL-3	4, 49, 50
Giant cell arteritis	IFN-γ, IL-1, IL-2, TNF-γ		63, 64
Type 1 diabetes mellitus	TNF-α, IL-1, IL-2, IL-12, IFN-γ		59, 65
Multiple sclerosis	IL-6, IL-12, TNF-α	IL-10	66, 67
Sarcoidosis	TNF-α, IL-1, IL-6, TGF-β		68, 69
Psoriatic arthritis	TNF-α, IL-1, IL-6, IL-8, IFN-γ		70
Psoriasis	TNF-α		71
Ankylosing spondylitis	TNF-α, IL-10	IFN-γ, IL-2	72
Rheumatoid arthritis	TNF-α, IL-1, IL-6		22
Systemic lupus erythematosus	IFN-γ	TNF-α	4
Systemic sclerosis	IL-4, TGF-β		73, 74
Ulcerative colitis	IL-1, IL-5, IL-6, IL-8, TNF-α	IL-3	49, 50, 65

IFN indicates interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

inflammatory destruction of β cells is catastrophic.

The Other I.M.I.D.s. Many other autoimmune diseases fit the I.M.I.D. profile, and **Table 1** offers an extensive but by no means complete list. It should be noted that similar cytokine dysregulation can cause different pathologies in different organ systems, depending on when, where, why, and how the dysregulation occurs. This is critical because the most efficacious treatment for apparently unrelated conditions may turn out to be a single drug or group of drugs, as in the case of anti-TNF-α therapy in RA and CD.

Giant cell (or temporal) arteritis and sarcoidosis present interesting examples of similar cytokine dysregulation in different tissue microenvironments causing different clinical findings. The former is an inflammatory condition involving upper extremity and particularly facial arteries, and results in headache, jaw pain, and even blindness, if untreated.[63] In contrast, sarcoidosis is a systemic inflammatory disorder whose clinical manifestations can range from asymptomatic chest X-ray abnormalities to multiorgan failure.[69]

Despite their clinical dissimilarities, the 2 conditions' cytokine pathophysiology is similar. In sarcoidosis, a condition in which granulomas are formed inappropriately and in absence of an infectious threat, high expression of IL-1 and TNF-α correlates with granuloma formation.[68,75] With giant cell arteritis, there are similar reports of cytokine elevation, specifically of IL-1 and TNF-α.[64,76]

Twenty years from now, a complete list of I.M.I.D.s may encompass not only the relatively rare diseases that we today consider to be of autoimmune origin but other, more prevalent conditions, including chronic obstructive pulmonary disease, asthma, and even atherosclerosis. Not all of these conditions will necessarily be treated or cured with pro- or anticytokine therapy. Cytokine dysregulation and immune-mediated inflammation,

however, may prove to be key drivers of these conditions' pathophysiologies.

The Clinical and Economic Burden of I.M.I.D.s for Large Employers and Managed Care Organizations

Although I.M.I.D.s may affect as much as 5% to 7% of the overall population and can cause severe morbidity and mortality, employers, managed care organizations (MCOs), and benefits consultants have not typically thought of I.M.I.D.s as a specific class of disease[1,4] (personal communication, P. Lopatka, July 2002). Major diagnostic categories (MDCs) group related diseases according to their *International Classification of Diseases, Ninth Revision* (ICD-9) codes, and there is an MDC called Endocrine, Metabolic, and Immunity Disorders.[77] The immunity disorders included in that MDC, however, typically involve reduction, not dysregulation, of immune function (eg, hypogammaglobulinemia).

Rather than being included in that MDC, most I.M.I.D.s fall into MDCs specific to the organs they affect. For example, RA falls into Diseases of the Musculoskeletal System and Connective Tissue, and multiple sclerosis is grouped with Diseases of the Nervous System and Sense Organs.[77] This type of organ-based classification somewhat mirrors the allocation of I.M.I.D.s to medical specialists. Although it is true that rheumatologists treat several kinds of I.M.I.D.s, including RA, systemic lupus erythematosus, and systemic sclerosis, many other I.M.I.D.s are treated by clinicians who specialize in the organs the I.M.I.D.s afflict.

Discussing the reasons why organ-based classification of the I.M.I.D.s still exists is beyond the scope of this review. It should be emphasized, however, that because I.M.I.D.s are usually not seen as a distinct disease class, their aggregate clinical and economic tolls are easily overlooked. Within the epidemiological literature, there are some estimates of the I.M.I.D.s' burden on the overall population, and the article will review those data. Yet for reasons described below, those data may not be relevant to MCOs, large employers, and benefits consultants. To understand the effect of I.M.I.D.s on the lives most pertinent to these groups, we will also examine I.M.I.D. claim prevalence and cost in a random sample of corporate workers and their dependents. To do so we will use the Medstat Group's MarketScan claims database.

I.M.I.D. Epidemiology and Cost in the Overall US Population. If autoimmune disease affects 5%-7% of Western populations, the prevalence of I.M.I.D.s at this time may be less, as cytokine dysregulation has not been convincingly implicated in all autoimmune conditions. Nevertheless, because autoimmune diseases involve immune-mediated inflammation—which almost by definition involves cytokine dysregulation—it can be argued that many additional autoimmune diseases will be classified as I.M.I.D.s once their molecular mechanisms are fully understood.

Table 2 lists the prevalence numbers for various I.M.I.D.s as cited in the literature. The prevalence rates vary dramatically, from a low of 16.9 per 100 000 for autoimmune hepatitis to 860 per 100 000 for RA. Although some I.M.I.D.s, such as CD, ulcerative colitis, and T1DM, have similar prevalence among men and women, many I.M.I.D.s, including RA, multiple sclerosis, and systemic lupus erythematosus, are much more prevalent among women.[78] There are at least 2 theories for the differential prevalence. First, female sex hormones may drive immune-mediated inflammation, and second, fetal cells may lodge within the organs of pregnant women during gestation (a phenomenon called microchimerism) and later cause I.M.I.D. pathophysiology.[79-81]

The I.M.I.D. costs reported in the literature similarly vary, both between different I.M.I.D.s and within the range of values for individual I.M.I.D.s. The latter variance results in part from conflicting methodologies used in the different studies to calculate cost.

Before considering these numbers, we will review the different types of costs. Direct costs are those generated in the

provision of care, and indirect costs are those nonmedical losses, such as lost productivity, that result from illness. Intangible costs are those losses, (eg, pain and suffering) that can be measured only subjectively. Direct costs can be divided into those that are medical (eg, surgeon's fees) and nonmedical (eg, parking at the hospital).[95]

Direct medical costs are the expenses most frequently considered in health economic studies, and those are the costs presented here, both from the literature and from MarketScan.[95] When only direct costs are considered, however, the true financial burden of a disease on patients, payers, employers, and society will likely be underestimated. This is especially true with the I.M.I.D.s because so many of them strike young people at the prime of their productivity and subject them to chronic disability.

Table 2. Prevalence of Immune-Mediated Inflammatory Disorders as Reported in the Literature

Condition	Prevalence Per 100 000	% Female	Reference
Autoimmune hepatitis	16.9	N/A	82
Crohn's disease	1.2-106	~50	83, 84, 85
Giant cell arteritis	27.9	N/A	86
Type 1 diabetes mellitus	192	47.8	78
Multiple sclerosis	120.5	64.2	87
Sarcoidosis	0.2-64	57.3	88
Psoriasis	537	N/A	89
Psoriatic arthritis*	101	N/A	90
Ankylosing spondylitis	100-200	~30	91, 92
Rheumatoid arthritis	860	74.8	78
Systemic lupus erythematosus	50.8	88.2	78, 93
Systemic sclerosis	37.5	92.2	78, 94
Ulcerative colitis	39-117	~50	83,84,85
All Prevalence-Listed I.M.I.D.s†	**2258 per 100 000**		

*Psoriatic arthritis prevalence is assumed to be captured within psoriasis prevalence.
†Range midpoints were used for total prevalence calculations.
I.M.I.D. indicates immune-mediated inflammatory disorder; NA, not available.

Of all I.M.I.D.s, RA offers the most extensive direct cost information. Inflated by the Medical Care consumer price index to year 2000 dollars, annual direct costs for the disease range from $2607 to $9644[96,97] (**Table 3**). For the other I.M.I.D.s, annual direct cost estimates range widely, from $791 for psoriasis to $15 733 for systemic lupus erythematosus. Most I.M.I.D.s, especially those that are debilitating, exhibit substantial cost skewness (ie, their costs are not evenly distributed among the patients suffering from the disease). With RA, for example, less than 25% of the patient population is responsible for between 43% and 75% of the disease's annual direct cost.[96] With CD and ulcerative colitis, the skewness is even more dramatic, with 2% of the patient population accounting for 34% and 39% of medical costs paid, respectively.[83] If the disease burden of these mostly severely afflicted individuals could be reduced, substantial cost savings might result.

I.M.I.D. Epidemiology and Cost in the Medstat MarketScan Population. There are 2 main reasons why the above data may not be relevant for MCOs, large employers, and benefits consultants. First, many of these cost and prevalence values are taken from the American population as a whole rather than from the population of workers/dependents to which large employers provide health insurance, and these 2 populations may not be comparable. For example, the overall population might include the sickest I.M.I.D. patients who have become so disabled that they are unable to work, causing the overall population's I.M.I.D. cost-severity mix to be higher than that of the large employer population. In contrast, I.M.I.D. prevalence might be higher in the large employer population than generally if I.M.I.D. patients or caregivers strive to work for large employers, knowing that health insurance and disability benefits are likely to be generous at those corporations.

Second, there is no consensus on how direct cost is calculated in the literature.

Are copayments included or not? Do medical costs reflect what providers charge or what they actually collect? The claims that providers make to payers (ie, charges) represent the price of the providers' services, but not necessarily the actual economic inputs required to offer those services. Alternatively, the amount actually paid to providers for care may be less than its true economic cost. Even if payment equals cost, it may be difficult to define what has been paid where. To make the data even more problematic, most of the literature cost studies were completed before the advent of anticytokine therapy.

Because of these potentially insoluble problems, large employers, MCOs, and benefits consultants need a different approach to understand the prevalence and cost of I.M.I.D.s in the corporate populations they represent. To that end, we calculate prevalence and cost using a method based on what employers designate as "eligible" (to be paid) claims for all inpatient and outpatient I.M.I.D. care. Rather than use a single employer or MCO, we use a group of employers, specifically those that are represented in the Medstat MarketScan database.

Table 3. Immune-Mediated Inflammatory Disorder Costs in the United States as Reported in the Literature

Disease	Direct Cost (Year 2000 $)	Reference
Ankylosing spondylitis	1838	91
Autoimmune hepatitis	N/A	
Crohn's disease	14 647	98
	14 302	83, 99
	5082	100
Type 1 diabetes mellitus	8800	101
Giant cell arteritis	N/A	
Multiple sclerosis	10 732	102
Psoriasis	791	89
Rheumatoid arthritis	9644	103
	9134	103
	7904	103
	7539	103
	6807	104
	6547	96
	6189	105
	5383	106
	4765	103
	2971	107
	2882	103
	2621	103
	2607	103
Sarcoidosis	N/A	
Systemic lupus erythematosus	15 733	108
	8002	109
Systemic sclerosis	5856	94
Ulcerative colitis	9609	83, 99

NA indicates not available.

Methods of Analysis

The Medstat Group, a subsidiary of the Thomson Corporation, is a health information company that maintains a multisource database of privately insured claim and encounter data known as MarketScan. The database includes 4 years of data (1997-2000), approximately 45 companies for each year of data, and approximately 2.5 million covered lives in each year. Individuals can be tracked via a unique identifier through all years for which MarketScan has data, and claims data capture inpatient admissions and services, outpatient services, and drug information. Patients are grouped by age, gender, employee classification, industry of employer, region, and insurance plan type.

Data runs were performed for 1999 and 2000. To calculate the prevalence numbers, the number of unique lives with at least 1 inpatient or outpatient claim indexed to 1 of the ICD-9 codes listed was summed in each plan year. Patient identification numbers were checked between the inpatient and outpatient claims lists to ensure there was no duplication. (Although it is possible that those patients suffering from milder I.M.I.D. cases might not make a single I.M.I.D.-related claim during a plan year, this was deemed to be unlikely. Nevertheless, it should be noted that for this reason, our methodology of prevalence calculation may slightly underestimate true I.M.I.D. prevalence.) The numbers of claimants for each

Table 4. Immune-Mediated Inflammatory Disorder Prevalence According to Medstat MarketScan, 1999-2000

Condition	Prevalence Per 100 000	% Female
Autoimmune hepatitis	15.2	48.2
Crohn's disease	118.6	54.9
Type 1 diabetes mellitus	700.3	49.5
Giant cell arteritis	9.5	72.2
Multiple sclerosis	130.4	74.3
Sarcoidosis	61.0	62.5
Psoriasis	329.5	50.8
Psoriatic arthritis	23.6*	48.1
Ankylosing spondylitis	24.2	36.1
Rheumatoid arthritis	335.8	73.7
Systemic lupus erythematosus	97.0	89.4
Systemic sclerosis	18.9	85.3
Ulcerative colitis	156.0	52.9
Sum prevalence of listed I.M.I.D.s	**2020 per 100 000**	

*Psoriatic arthritis prevalence is assumed to be captured within psoriasis prevalence
I.M.I.D. indicates immune-mediated inflammatory disorder.

I.M.I.D. ICD-9 codes were then divided by the total number of database participants to obtain prevalence rates per 100 000 lives. In addition, male and female distributions were determined. These operations were performed for both 1999 and 2000.

To generate I.M.I.D. cost data, we used the MarketScan's eligible claims field. For those male and female claimants for each ICD-9 codes, we summed **all** of their eligible claims. This calculation provided the total eligible healthcare claims for those people with I.M.I.D.s. For the people with each I.M.I.D., we then divided their total costs by the number of claimants to generate an average eligible claim cost for each I.M.I.D. patient; this operation was performed separately for both the male and female populations. Next, average eligible claim cost was generated for the overall MarketScan male and female populations, excluding those people with I.M.I.D. claims. Next, that value was subtracted from the average eligible claim cost for those patients with each I.M.I.D. In this way we removed what we estimated to be the medical cost for I.M.I.D. patients not associated with or secondary to I.M.I.D.s. In essence, the remaining value is the best possible valuation of medical claims directly or indirectly due to I.M.I.D. pathophysiology.

To generate the PMPY figure for each I.M.I.D., we multiplied the prevalence numbers per 100 000 lives by the average I.M.I.D. eligible claims cost (net of the baseline claims cost, as described above) and divided by 100 000. A query established that no claimant fell into multiple claims categories (eg, no patient with T1DM claims also had claims for CD). As a result, the PMPY figures generated are additive.

I.M.I.D. prevalence and gender distribution for the MarketScan population, averaged for the years 1999 and 2000, are listed in **Table 4**. The percentage-female values from MarketScan are similar to those found in the literature. For example, 49.5% of T1DM claimants in MarketScan are female, as compared to 47.8% in the literature. Likewise, the gender distribution for CD and ulcerative colitis is evenly split both in the literature and in MarketScan. For I.M.I.D.s that are typically more prevalent in the female population, there is also concurrence between MarketScan and the literature. The literature cites female percentages of 74.8% for RA and 88.2% for systemic lupus erythematosus, and from MarketScan the respective numbers are 73.7% and 89.4%.

There are, however, significant differences in the I.M.I.D. prevalence rates between the literature and MarketScan. In several cases, MarketScan I.M.I.D. prevalences are higher than those cited in the literature. For example, the MarketScan prevalences for CD, T1DM, and systemic lupus erythematosus are greater than the literature values by approximately 200% or more. In contrast, the MarketScan prevalence for RA is considerably less than that cited in the literature. Given the evidence that RA incidence has been progressively decreasing for at least 50 years, from 61.2 per 100 000 in 1955-

1964 to 32.7 per 100 000 in 1985-1994, the MarketScan figure may be more reflective of actual RA prevalence.[110,111]

As stated above, one might expect that I.M.I.D. prevalence would be lower in the MarketScan population than in the general population, as most of the I.M.I.D.s are chronic and many can be debilitating. For that reason, people with severe cases might be expected to quit their jobs and apply for Medicare/Social Security Insurance disability payments, thus exiting the MarketScan population. Large employers, however, typically provide benefits for employees and dependents with chronic illnesses that are better than those offered by Medicare.[112] The generosity of these benefits, combined with the disability coverage provided by large employers, might induce employees diagnosed with I.M.I.D.s to stay with their companies for as long as possible. Similarly, healthy workers with I.M.I.D.-afflicted dependents might be compelled to remain with their employers. Moreover, job seekers either suffering from I.M.I.D.s or with dependents who have the diseases might opt for employment at large corporations, especially given the limitations on pre-existing condition exclusions in effect since adoption of the Health Insurance Portability and Accountability Act. For these same reasons, it could be argued that I.M.I.D. patients in MarketScan might be more severely afflicted and thus more costly than those in the general population.

In fact, I.M.I.D. cost estimates are relatively similar in MarketScan and in the literature (**Table 5**). For example, MarketScan's average, annual, per patient eligible claims for RA are approximately $6204, as compared to literature citations of $2607 to $9694. For CD, the per patient eligible claims are $7420, as compared to literature reports of $5082 to $14 647.

Combining the prevalence and cost data from MarketScan offers perhaps the most important I.M.I.D. cost statistic for large employers, MCOs, and benefits consultants: PMPY eligible claims (**Table 6**). For each I.M.I.D., the PMPY cost represents the potential amount of disease-related cost divided by the total number of all workers and dependents in the insured population. Importantly, PMPY eligible claims reflect the average liability that employers can expect from I.M.I.D.s for claimants who have no other insurance policy primary to their employer-sponsored plan.

Table 5. Immune-Mediated Inflammatory Disorder Eligible Claim Cost According to Medstat MarketScan, 1999-2000

Condition	Eligible Claim Cost ($)
Autoimmune hepatitis	5151
Crohn's disease	7420
Type 1 diabetes mellitus	9476
Giant cell arteritis	8642
Multiple sclerosis	9382
Sarcoidosis	5388
Psoriasis	2126
Psoriatic arthritis	3638
Ankylosing spondylitis	4571
Rheumatoid arthritis	6204
Systemic lupus erythematosus	8141
Systemic sclerosis	9075
Ulcerative colitis	5775

Although some PMPY values listed seem small and potentially insignificant (eg, those for autoimmune hepatitis and giant cell arteritis), the sum PMPY expenses for the I.M.I.D.s listed total $139.84. To put this in perspective, the average per capita non-I.M.I.D. eligible claims cost—which represents the sum of all claims for those people without an I.M.I.D. diagnosis—averaged $2098.62 in 1999-2000. The per-capita eligible claim costs for the I.M.I.D.s listed are therefore 6.7% of the total per capita claim costs for non-I.M.I.D. patients in MarketScan.

It should be emphasized that many classical autoimmune diseases, such thyroiditis, autoimmune anemia, and myasthenia gravis, have not been included in the review because the role of cytokines in these conditions has yet to be elucidated. As the frontiers of molecular medicine expand, many of these autoimmune condi-

Table 6. Immune-Mediated Inflammatory Disorder Per Member Per Year Costs in Medstat MarketScan, 1999-2000

Condition	PMPY Eligible Claim Cost ($)
Type 1 diabetes mellitus	66.35
Rheumatoid arthritis	20.84
Multiple sclerosis	12.23
Ulcerative colitis	9.01
Crohn's disease	8.80
Systemic lupus erythematosus	7.90
Psoriasis	7.00
Sarcoidosis	3.29
Systemic sclerosis	1.71
Ankylosing spondylitis	1.11
Giant cell arteritis	0.82
Autoimmune hepatitis	0.78
Total	**139.84**

PMPY indicates per member per year.

tions may be determined to be cytokine driven, potentially allowing their inclusion in the I.M.I.D. group. For that reason, future studies could show that I.M.I.D.s represent a far greater share of large employers' total healthcare liability.

What Can Be Done—I.M.I.D. Therapy in the 21st Century

Before the 1940s, little could be done to treat most I.M.I.D.s. That changed in 1948 when American rheumatologist Phillip Hench administered a newly isolated compound called cortisone to patients with RA.[113] In many patients the results of treatment were dramatic, and word spread globally of a new miracle cure, with Hench and his colleagues winning the Nobel Prize for Medicine in 1950.[114] Unfortunately, the severe side effects of cortisone and other glucocorticoid compounds quickly became apparent. In addition, even with therapy, the disease often seemed to progress, despite the extremely high doses that were used in the early years of treatment.[114]

For other I.M.I.D.s, glucocorticoid therapy has achieved a much less controversial record and is often the standard of care. In CD, for example, prednisone is widely used for flares, and methylprednisone is standard therapy for multiple sclerosis relapses.[115,116] Nevertheless, even with the much lower doses used now than in Hench's time and careful tapers, prolonged glucocorticoid therapy remains fraught with side effects, including but not limited to osteoporosis, hypertension, hyperglycemia, and weight gain.[117] Cognizant of these side effects, clinicians have long wanted a new type of I.M.I.D. therapy with the same wide-reaching applicability and efficacy as glucocorticoids but with much greater safety.

Over the years, a wide range of immunosuppressant therapies, including azathioprine, cyclophosphamide, cyclosporine, leflunomide, methotrexate, and tacrolimus, have been used to treat I.M.I.D.s, often with great success.[118] Yet because many of these therapies involve serious systemic toxicity, the search continued for an effective I.M.I.D. therapy with moderate side effects.

The concept that proinflammatory cytokines might play a key role in the pathogenesis of not one but many I.M.I.D.s led investigators to theorize that blocking those cytokines might have therapeutic benefit. The hypothesis was correct, and currently, at least 5 anticytokine therapies have significantly advanced the treatment of several I.M.I.D.s, including RA and CD. In this section the review will examine the anticytokine therapies currently in use and those in development. *It is important to emphasize that anticytokine therapy is not appropriate for all patients with all I.M.I.D.s.* Nevertheless, the data presented below demonstrate the potential for significant therapeutic benefit in certain clinical situations.

TNF-α Blockade—The First Anticytokine Therapy. As mentioned above, several mouse studies suggested that TNF-α

blockade could be therapeutic in reducing the joint inflammation of RA.[35,119] These findings, as well as an overall appreciation of the inflammatory capabilities of TNF-α, presaged the development of several different TNF-α–blocking molecules, 2 of which are now available commercially. Anti-TNF-α therapy is not appropriate for all I.M.I.D. patients, but for certain patients there is substantial evidence of clinical benefit.

Infliximab. Infliximab, a mouse-human chimeric antibody against TNF-α, showed promise in early human clinical trials for RA. It significantly reduced a host of inflammatory signs, including joint swelling.[120] A larger trial tested infliximab with and without methotrexate, a standard therapy for RA. This trial of 101 patients showed that infliximab infusions of 10 mg/kg combined with methotrexate caused a 70%-90% reduction in the number of swollen joints and C-reactive protein, a marker for inflammation.[121] The efficacy of infliximab, however, was most convincingly demonstrated in the randomized, controlled Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT). Four hundred and twenty-eight patients receiving methotrexate were given infliximab, 3 mg/kg or 10 mg/kg, or placebo for 2 years. Infliximab and methotrexate were shown to be significantly better than methotrexate alone in reducing the signs and symptoms of RA. Most important, infliximab and methotrexate—but not methotrexate alone—were shown to halt the progression of joint damage.[122]

Infliximab's therapeutic value is not limited to RA—not surprising given the common cytokine-driven pathophysiology of many I.M.I.D.s. Infliximab, for example, has proven both safe and efficacious in the treatment of CD. An initial clinical trial of 108 patients showed that a single infusion of infliximab was an effective short-term treatment in patients with moderate-to-severe disease, with 33% of infliximab patients but only 4% of placebo controls achieving remission.[123] A subsequent study showed that infliximab enabled patients to attain remission and that repeated infliximab infusions could help recipients maintain it.[124]

Infliximab's use has not been limited to RA and CD. Open-label studies have shown it to be effective in the treatment of psoriasis and ankylosing spondylitis, and case reports have demonstrated its therapeutic value in sarcoidosis and giant cell arteritis.[71,125-127]

Etanercept. A fusion protein containing domains from the TNF-α receptor, etanercept is the other commercially available anti-TNF-α therapy.[3] A blinded trial of 180 RA patients showed those receiving etanercept had a 61% reduction in their number of swollen joints, as compared to a 25% reduction with placebo.[128] In a subsequent study in which RA patients received etanercept and methotrexate or methotrexate alone, 39% of the etanercept patients met ACR 50 criteria, as compared to 3% of those patients receiving methotrexate alone.[129] (The American College of Rheumatology [ACR] definition of improvement in clinical trials involves a) improvement in joint counts and b) improvement in at least 3 of the following: patient assessment, physician assessment, erythrocyte sedimentation rate, pain scale, and functional questionnaire. The ACR 20, ACR 50, and ACR 70 criteria reflect an improvement of 20%, 50%, or 70% in these metrics).[130] Etanercept has also been shown to be effective in reducing the signs and symptoms of juvenile RA.[131]

Like infliximab, etanercept has demonstrated efficacy in treating other I.M.I.D.s besides RA. A randomized, controlled trial of 60 patients with psoriasis and psoriatic arthritis showed that etanercept-treated patients achieved a 46% improvement in their psoriasis symptoms, as compared to a 9% improvement for placebo-treated patients.[132] In addition, 87% of etanercept-treated patients had significant improvement of their arthritic symptoms, as compared to 23% of patients treated with placebo.[132] A trial of 40 patients has demonstrated etanercept to be effective in the treatment of ankylosing spondylitis,

with 80% of patients in the treatment group achieving a clinical response as compared to 30% of patients in the placebo group.[133]

IL-1 and IL-2 Blockade—Another Anti-inflammatory Strategy. The above-mentioned studies confirmed what has long been suspected about TNF-α: it plays a proinflammatory role in several I.M.I.D.s. Similarly, a recently approved anti-IL-1 therapy, anakinra, and 2 recently approved anti-IL-2 therapies, daclizumab and basiliximab, highlight the importance of these 2 cytokines in the generation of I.M.I.D. pathophysiology.

Approved in 2001, anakinra is the recombinant version of a naturally occurring molecule known as the IL-1 receptor antagonist.[134] IL-1 plays a critical role in the inflammatory cascade of RA, and by blocking IL-1, anakinra has demonstrated clinical efficacy in the treatment of the disease.[135] In a randomized, controlled trial of 472 patients, 43% of those receiving anakinra at 150mg/day achieved the ACR 20 response, as compared to 27% of patients receiving placebo.[134]

Like IL-1, IL-2 plays a key part in T cell activation, an important event in the inflammatory process that leads to rejection of organ transplants in the absence of immunosuppressive therapy. By blocking IL-2, both daclizumab and basiliximab have been shown to reduce the rejection of kidney transplants at 6 months as compared to placebo, by 37% and 32%, respectively.[136,137] Given the widespread role of IL-2 in the inflammatory process, IL-2 blockade may prove to have wide-reaching therapeutic utility.

Anticytokine Therapy—Is it a Panacea? The new anticytokine therapies have dramatically improved the prognoses for several I.M.I.D.s, including RA and CD. The success of these therapies, however, raises the question of whether blocking cytokine activity might interfere with normal physiology. Unfor-tunately, anticytokine therapies may, in relatively rare instances, have that effect.[138] Both infliximab and etanercept have been associated anecdotally with systemic lupus erythematosus and more broadly with the induction of antinuclear antibodies in as many as 10% of treated patients.[139,140]

There is also evidence that inhibition of TNF-α may suppress normal immunity. For example, a retrospective study of 147 000 patients treated with infliximab suggests the rate of active tuberculosis infection is higher in those patients than in controls.[141] Similarly, a series of randomized, controlled trials with 771 patients showed those receiving infliximab had a 26% risk of any infection during 27 weeks of follow-up as compared to a 16% risk at 20 weeks for those receiving placebo. Despite this increased risk of infections in the infliximab population, there was no increased risk of serious infection.[142] In summary, none of the anticytokine therapies are without side effects, but these adverse events appear to be relatively rare.[142,143]

Anticytokine Therapy—The Next Generation. With the success of infliximab and etanercept, many new anticytokine therapies are in development, and several are in phase III trials. Like infliximab, many of these products are monoclonal antibodies. Because monoclonal antibodies in phase III trials have a 30% or higher chance of receiving final Food and Drug Administration approval, large employers, MCOs, and benefit consultants should consider these molecules in advance of their potential launches.[144]

Adalimumab, a human anti-TNF monoclonal antibody; CDP571, a "humanized" anti-TNF monoclonal antibody; and CDP870, a high affinity anti-TNF-α antibody fragment are each in phase III clinical trials.[145-147] Preliminary results from the Safety Trial of Adalimumab in Rheumatoid Arthritis (STAR) indicate a 30% achievement of ACR 50 criteria with adalimumab treatment, as compared to 11% with placebo.[148] With the success of anti-TNF-α therapy in the treatment of CD, trials of adalimumab for that condition are also scheduled.[149]

A phase III trial of CDP571 for CD has recently yielded preliminary results, which showed treated patients did not meet the study's primary end point.[147] The drug has, however, shown promise as a potential therapy for RA.[150] CDP870 is also undergoing phase III testing for RA and CD.[145] CDP870 is different from adalimumab and CDP571, however, in that it is PEGylated (ie, the drug formulation contains a polyethylene glycol [PEG] moiety that improves the drug's pharmacokinetics and allows it to stay in the circulation longer).[151,152] The PEGylation strategy is also being used with a new soluble version of the TNF-α receptor, currently in phase II trials.[153] In addition to these anti-TNF-α therapies, several molecules targeting other cytokines are also in development (**Table 7**).

Table 7. Other Anticytokine Therapies in Development

Condition	Product	Cytokine Targeted	Development Phase of Product
Crohn's disease	J-695	IL-12	Phase II
	SMART	IFN-γ	Phase II
Multiple sclerosis	J-695	IL-12	Phase II
Rheumatoid arthritis	J-695	IL-12	Phase II
	HuMax-IL15	IL-15	Phase II
Psoriasis	ABX-IL-8	IL-8	Phase II
Systemic sclerosis	CAT-192	TGF-β	Phase II

IFN indicates interferon; IL, interleukin; TGF, transforming growth factor.

Conclusion

I.M.I.D.s result from inappropriate or excessive immune responses. I.M.I.D.s are caused, signified, or accompanied by dysregulation of the normal cytokine milieu, and they lead to acute or chronic inflammatory injury that may be life-threatening. Healthcare stakeholders have been slow to embrace the I.M.I.D. concept, but the past decade's advances in molecular medicine have shown that many ostensibly unrelated diseases, including RA, CD, and T1DM, share I.M.I.D. pathophysiology. That anticytokine therapies have been effective in treating a number of I.M.I.D.s further emphasizes the legitimacy of the I.M.I.D. concept. For large employers, I.M.I.D.s represent more than 6% of total healthcare spending, and that number will likely grow as many diseases not currently classified as I.M.I.D.s are shown to share that pathophysiology. As a result, it becomes ever more critical that providers, payers, large employers, and benefits consultants understand the clinical and economic burden of I.M.I.D.s. Without that understanding, they risk underestimating what may be a key cost driver—both economically and clinically—for years to come.

···REFERENCES···

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EXHIBIT 15

im·mune sys·tem

an intricate complex of interrelated cellular, molecular, and genetic components that provides a defense, the immune response, against foreign organisms or substances and aberrant native cells.

EXHIBIT 16

Inflammation | definition of Inflammation by Medical dictionary

https://medical-dictionary.thefreedictionary.com/Inflammation

Inflammation

Also found in: **Dictionary**, **Thesaurus**, **Encyclopedia**, **Wikipedia**.

inflammation [in″flah-ma´shun]

a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent and the injured tissue. adj., *adj* inflam´matory.

The **inflammatory RESPONSE** can be provoked by physical, chemical, and biologic agents, including mechanical trauma, exposure to excessive amounts of sunlight, x-rays and radioactive materials, corrosive chemicals, extremes of heat and cold, or by infectious agents such as bacteria, viruses, and other pathogenic microorganisms. Although these infectious agents can produce inflammation, infection and inflammation are not synonymous.

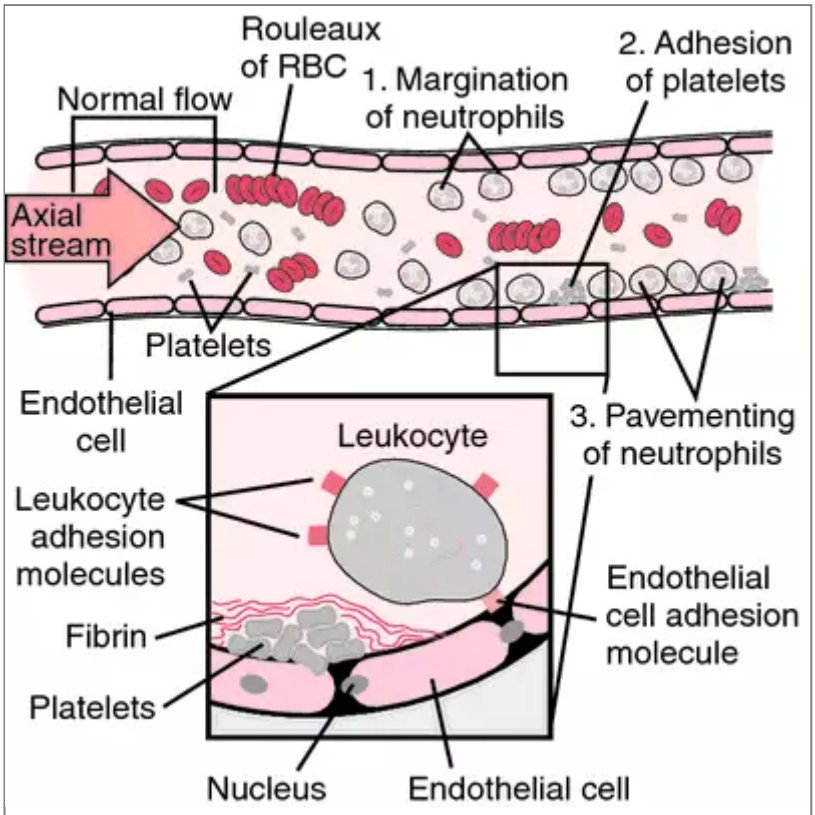
The classic signs of inflammation are *heat, redness, swelling, pain,* and *loss of function.* These are manifestations of the physiologic changes that occur during the inflammatory process. The three major components of this process are (1) changes in the caliber of blood vessels and the rate of blood flow through them (hemodynamic changes); (2) increased capillary permeability; and (3) leukocytic exudation.

Hemodynamic changes begin soon after injury and progress at varying rates, according to the extent of injury. They start with dilation of the arterioles and the opening of new capillaries and venular beds in the area. This causes an accelerated flow of blood, accounting for the signs of heat and redness. Next follows *increased permeability* of the microcirculation, which permits leakage of protein-rich fluid out of small blood vessels and into the extravascular fluid compartment, accounting for the inflammatory edema.

Leukocytic exudation occurs in the following sequence. First, the **LEUKOCYTES** move to the endothelial lining of the small blood vessels (*margination*) and line the endothelium in a tightly packed formation (*pavementing*). Eventually, these leukocytes move through the endothelial spaces and escape into the extravascular space (*emigration*). Once they are outside the blood vessels they are free to move and, by **CHEMOTAXIS**, are drawn to the site of injury. Accumulations of **NEUTROPHILS** and **MACROPHAGES** at the area of inflammation act to neutralize foreign particles by **PHAGOCYTOSIS**.

Chemical mediators of the inflammatory process include a variety of substances originating in the plasma and the cells of uninjured tissue, and possibly from the damaged tissue. The major kinds of mediators are (1) *vasoactive amines,* such as **HISTAMINE** and **SEROTONIN**; (2) *plasma **endopeptidases*** that comprise three interrelated systems, the kinin system that produces **BRADYKININ**, the complement system that produces proteins that interact with antigen--antibody complexes and mediate immunologic injury and inflammation, and the clotting system that increases vascular permeability and chemotactic activity for the leukocytes; (3) **PROSTAGLANDINS**, which can reproduce several aspects of the inflammatory process; (4) neutrophil products; (5) lymphocyte factors; and (6) other mediators, such as **slow-reacting SUBSTANCE of anaphylaxis** and **endogenous PYROGEN**.

Hormonal Response. Some hormones, such as **CORTISOL**, have an **ANTIINFLAMMATORY** action that limits inflammation to a local reaction while others are proinflammatory. Thus, the endocrine system has a regulatory effect on the process of inflammation so that it can be balanced and beneficial in the body's attempts to recover from injury.



Cellular changes in inflammation. *1,* Margination of neutrophils brings these inflammatory cells in close contact with the endothelium. *2,* Adhesion of platelets results in the release of mediators of inflammation and coagulation. Fibrin strands are the first signs of clot formation. *3,* Pavementing of leukocytes is mediated by adhesion molecules activated by the mediators of inflammation released from platelets and leukocytes. RBC, red blood cells. From Damjanov, 2000.

acute inflammation inflammation, usually of sudden onset, marked by the classical signs of heat, redness, swelling, pain, and loss of function, and in which vascular and exudative processes predominate.

catarrhal inflammation a form affecting mainly a mucous surface, marked by a copious discharge of mucus and epithelial debris.

chronic inflammation prolonged and persistent inflammation marked chiefly by new connective tissue formation; it may be a continuation of an acute form or a prolonged low-grade form.

exudative inflammation one in which the prominent feature is an exudate.

fibrinous inflammation one marked by an exudate of coagulated fibrin.

granulomatous inflammation a form, usually chronic, attended by formation of granulomas.

interstitial inflammation inflammation affecting chiefly the stroma of an organ.

parenchymatous inflammation inflammation affecting chiefly the essential tissue elements of an organ.

productive inflammation (**proliferative inflammation**) one leading to the production of new connective tissue fibers.

pseudomembranous inflammation an acute **inflammatory RESPONSE** to a powerful necrotizing toxin (such as**diphtheria TOXIN**), characterized by formation on a mucosal surface of a false membrane composed of

precipitated fibrin, necrotic epithelium, and inflammatory leukocytes.

purulent inflammation **suppurative inflammation**.

serous inflammation one producing a serous exudate.

subacute inflammation a condition intermediate between chronic and acute inflammation, exhibiting some of the characteristics of each.

suppurative inflammation one marked by pus formation.

toxic inflammation one due to a poison, e.g., a bacterial product.

traumatic inflammation one that follows a wound or injury.

ulcerative inflammation that in which necrosis on or near the surface leads to loss of tissue and creation of a local defect (ulcer).

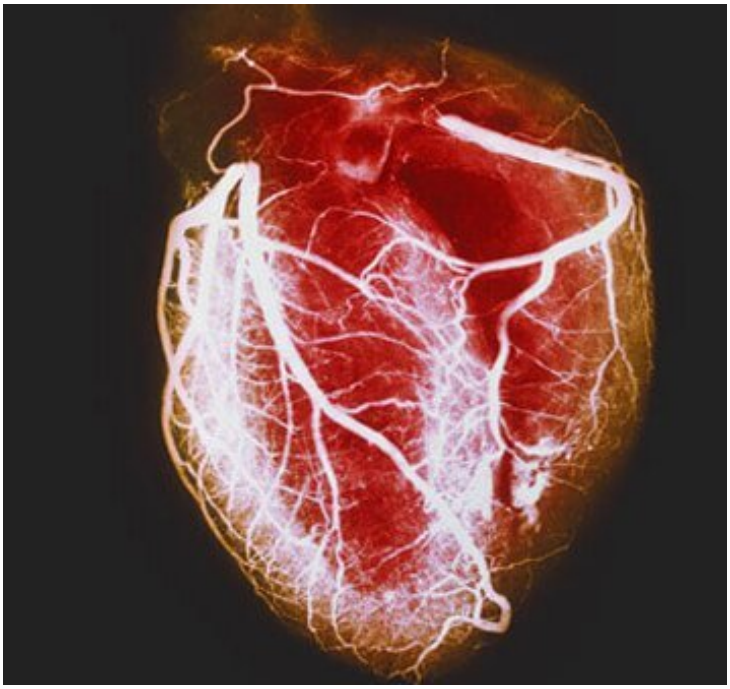
EXHIBIT 17

Medical Definition of Macrophage

Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Macrophage: A type of white blood cell that ingests foreign material. Macrophages are key players in the immune response to foreign invaders of the body, such as infectious microorganisms. They are normally found in the liver, spleen, and connective tissues of the body.

CONTINUE SCROLLING OR **CLICK HERE** FOR RELATED SLIDESHOW



— SLIDESHOW —

Heart Disease: Causes of a Heart Attack

See Slideshow

Reviewed on 12/12/2018

Exhibit 17

EXHIBIT 18

Medical emergency | definition of medical emergency by Medical dictionary

https://medical-dictionary.thefreedictionary.com/medical+emergency

medical emergency

Also found in: **Wikipedia**.

medical emergency A medical or behavioural condition, the onset of which is sudden and manifests itself by symptoms of sufficient severity, including severe pain, such that a prudent lay person could reasonably expect the absence of immediate medical attention to result in:
(1) placing the health of the afflicted person with such a condition in serious jeopardy;
(2) serious impairment to the person's bodily functions;
(3) serious dysfunction of any bodily organ or part; or
(4) serious disfugurement.

Examples
Severe chest pain, severe or multiple injuries, severe SOB, loss of consciousness, sudden change in mental status (e.g., disorientation), severe bleeding, acute pain, conditions requiring immediate attention (e.g., acute MI, appendicitis, poisoning, convulsions).

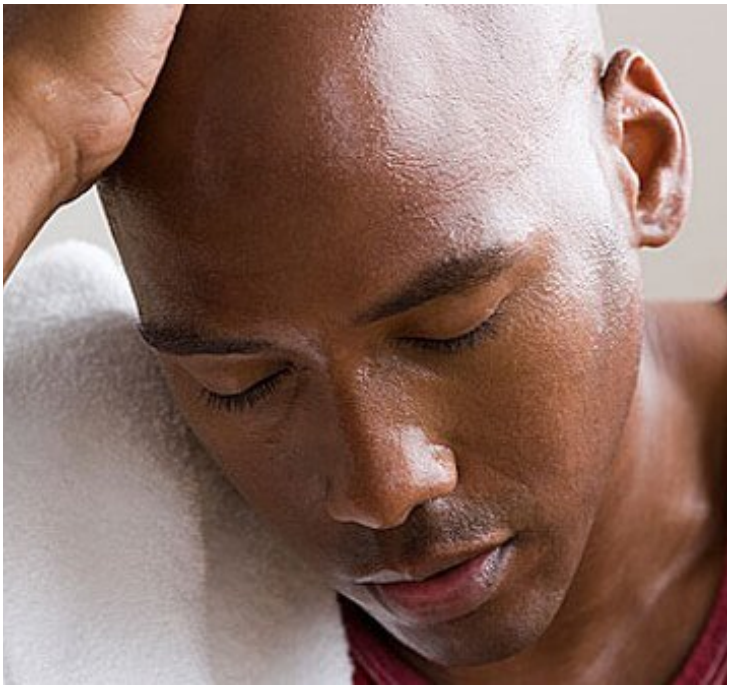
EXHIBIT 19

Medical Definition of Myalgia

Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Myalgia: Pain in the muscles or within muscle tissue.

CONTINUE SCROLLING OR **CLICK HERE** FOR RELATED SLIDESHOW



Reviewed on 12/11/2018

EXHIBIT 20

URL of this page: //medlineplus.gov/ency/article/002261.htm

Myelin

Myelin is an insulating layer, or sheath that forms around nerves, including those in the brain and spinal cord. It is made up of protein and fatty substances.

This myelin sheath allows electrical impulses to transmit quickly and efficiently along the nerve cells. If myelin is damaged, these impulses slow down. This can cause diseases such as multiple sclerosis.

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. Accessed June 11, 2017.

Review Date 5/13/2019

Updated by: Linda J. Vorvick, MD, Clinical Associate Professor, Department of Family Medicine, UW Medicine, School of Medicine, University of Washington, Seattle, WA. Also reviewed by David Zieve, MD, MHA, Medical Director, Brenda Conaway, Editorial Director, and the A.D.A.M. Editorial team.

EXHIBIT 21

pan·dem·ic (pan-dem'ik),

Denoting a disease affecting or attacking the population of an extensive region, country, continent, global; extensively epidemic.
[pan- + G. *dēmos*, the people]

EXHIBIT 22

path·o·gen·e·sis (path'ō-jen'ĕ-sis),

The pathologic, physiologic, or biochemical mechanism resulting in the development of a disease or morbid process. Compare: **etiology**.

[patho- + G. *genesis*, production]

EXHIBIT 23

permanent injury

Primary tabs

Definition from Nolo's Plain-English Law Dictionary

Physical or mental damage that will indefinitely restrict the employment or other normal activities of an individual. In a lawsuit to recover damages caused by the negligence or intentional wrongful act of another, a permanent injury can be a major element in an award of general damages.
Definition provided by Nolo's Plain-English Law Dictionary.

EXHIBIT 24

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(/)

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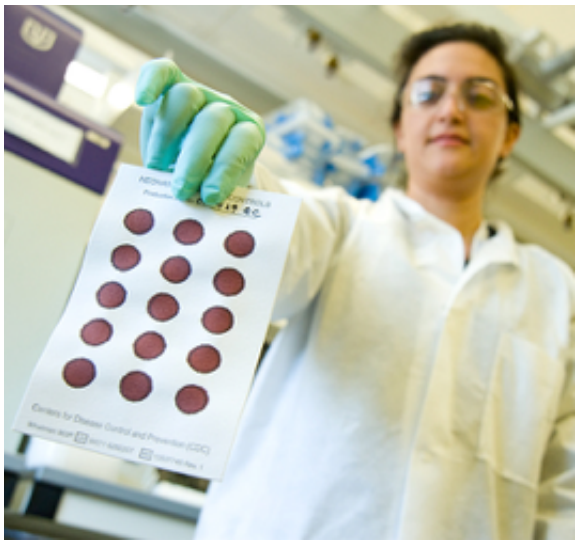

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EXHIBIT 25

progressive disease Medspeak

Any chronic or dread disease (e.g., cancer), which progresses with time in scope and/or severity.

Oncology
A term defined in the context of clinical trials as tumour growth of ≥ 20% since treatment began, including local or metastatic tumour mass.

EXHIBIT 26

regression (rĭ-grĕsh′ən)

n.

1. The process or an instance of regressing, as to a less perfect or less developed state.
2. *Psychology* Reversion to an earlier or less mature pattern of feeling or behavior.
3. *Medicine* Subsidence of the symptoms or process of a disease.
4. *Statistics* A technique for predicting the value of a dependent variable as a function of one or more independent variables in the presence of random error.

EXHIBIT 27

NCHS Data Brief ■ No. 328 ■ November 2018

Mortality in the United States, 2017

Sherry L. Murphy, B.S., Jiaquan Xu, M.D., Kenneth D. Kochanek, M.A., and Elizabeth Arias, Ph.D.

Key findings

Data from the National Vital Statistics System

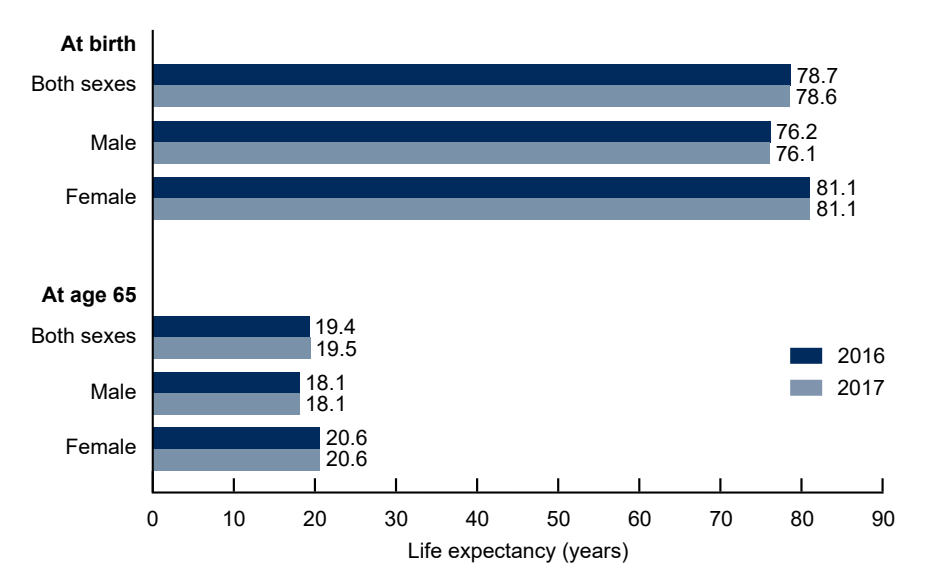
- Life expectancy for the U.S. population declined to 78.6 years in 2017.
- The age-adjusted death rate increased by 0.4% from 728.8 deaths per 100,000 standard population in 2016 to 731.9 in 2017.
- Age-specific death rates increased from 2016 to 2017 for age groups 25–34, 35–44, and 85 and over, and decreased for the age group 45–54.
- The 10 leading causes of death in 2017 remained the same as in 2016.
- The infant mortality rate of 579.3 infant deaths per 100,000 live births in 2017 was not significantly different from the 2016 rate.
- The 10 leading causes of infant death in 2017 remained the same as in 2016 although 4 causes changed ranks.

This report presents final 2017 U.S. mortality data on deaths and death rates by demographic and medical characteristics. These data provide information on mortality patterns among U.S. residents by variables such as sex, race and ethnicity, and cause of death. Life expectancy estimates, age-specific death rates, age-adjusted death rates by race and ethnicity and sex, 10 leading causes of death, and 10 leading causes of infant death were analyzed by comparing 2017 and 2016 final data (1).

How long can we expect to live?

In 2017, life expectancy at birth was 78.6 years for the total U.S. population—a decrease from 78.7 years in 2016 (Figure 1). For males, life expectancy changed from 76.2 in 2016 to 76.1 in 2017. For females, life expectancy remained the same at 81.1.

Figure 1. Life expectancy at selected ages, by sex: United States, 2016 and 2017



NOTES: Life expectancies for 2016 were revised using updated Medicare data; therefore, figures may differ from those previously published. Access data table for Figure 1 at: https://www.cdc.gov/nchs/data/databriefs/db328_tables-508.pdf#1.
SOURCE: NCHS, National Vital Statistics System, Mortality.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Center for Health Statistics

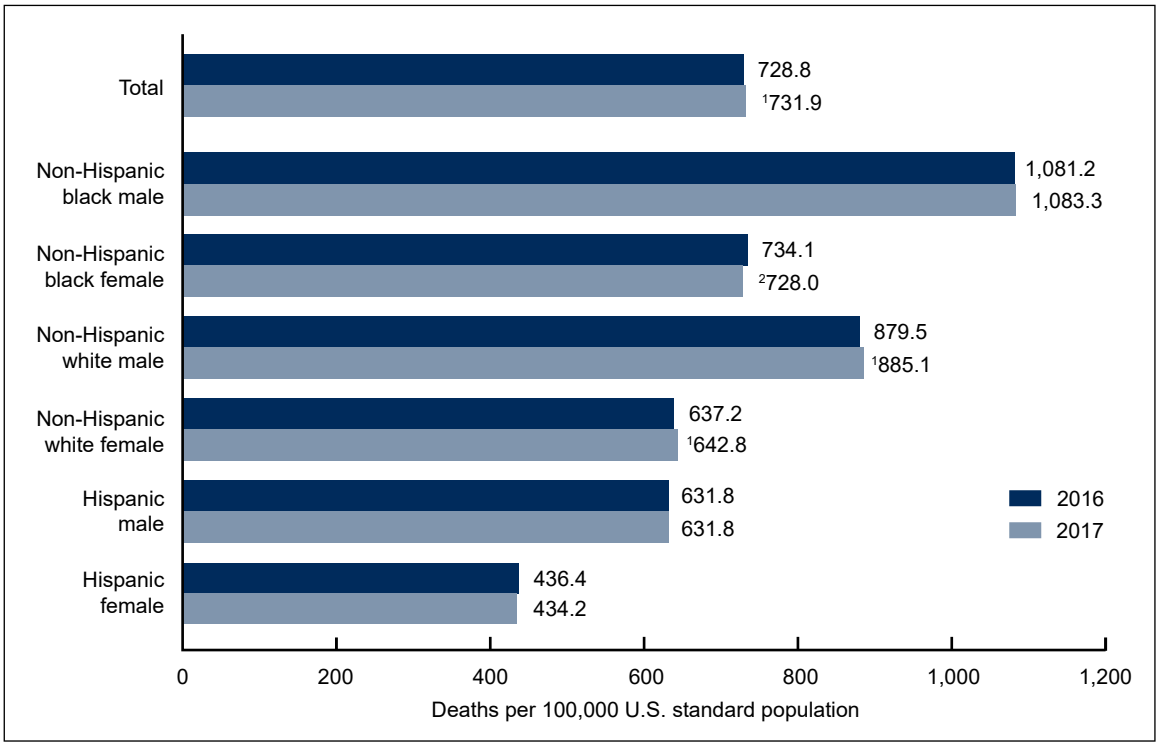
Life expectancy for females was consistently higher than it was for males. In 2017, the difference in life expectancy between females and males increased 0.1 year from 4.9 years in 2016 to 5.0 years in 2017.

In 2017, life expectancy at age 65 for the total population was 19.5 years, an increase of 0.1 year from 2016. Life expectancy at age 65 was 20.6 years for females and 18.1 years for males, both unchanged from 2016. The difference in life expectancy at age 65 between females and males was 2.5 years, unchanged from 2016.

What are the age-adjusted death rates for race–ethnicity–sex groups?

The age-adjusted death rate for the total population increased 0.4% from 728.8 per 100,000 standard population in 2016 to 731.9 in 2017 (Figure 2). Age-adjusted death rates increased in 2017 from 2016 for non-Hispanic white males (0.6%) and non-Hispanic white females (0.9%). The age-adjusted death rate decreased for non-Hispanic black females (0.8%). Rates did not change significantly for non-Hispanic black males, Hispanic males, and Hispanic females from 2016 to 2017.

Figure 2. Age-adjusted death rates, by race and ethnicity and sex: United States, 2016 and 2017



[1]Statistically significant increase in age-adjusted death rate from 2016 to 2017 ($p < 0.05$).
[2]Statistically significant decrease in age-adjusted death rate from 2016 to 2017 ($p < 0.05$).
NOTE: Access data table for Figure 2 at: https://www.cdc.gov/nchs/data/databriefs/db328_tables-508.pdf#2.
SOURCE: NCHS, National Vital Statistics System, Mortality.

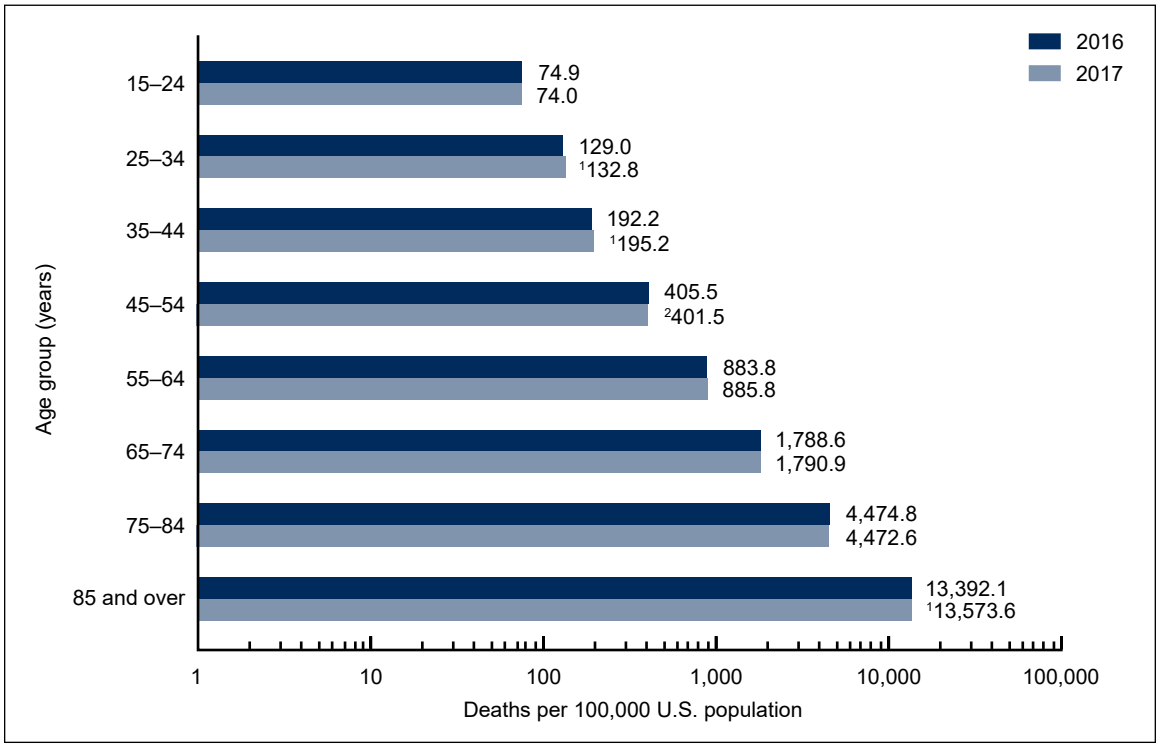
Did age-specific death rates change among those aged 15 years and over?

Death rates increased significantly between 2016 and 2017 for age groups 25–34 (2.9%), 35–44 (1.6%), and 85 and over (1.4%) (Figure 3).

The death rate decreased significantly for age group 45–54 (1.0%).

Rates for other age groups did not change significantly between 2016 and 2017.

Figure 3. Death rates for ages 15 years and over: United States, 2016 and 2017



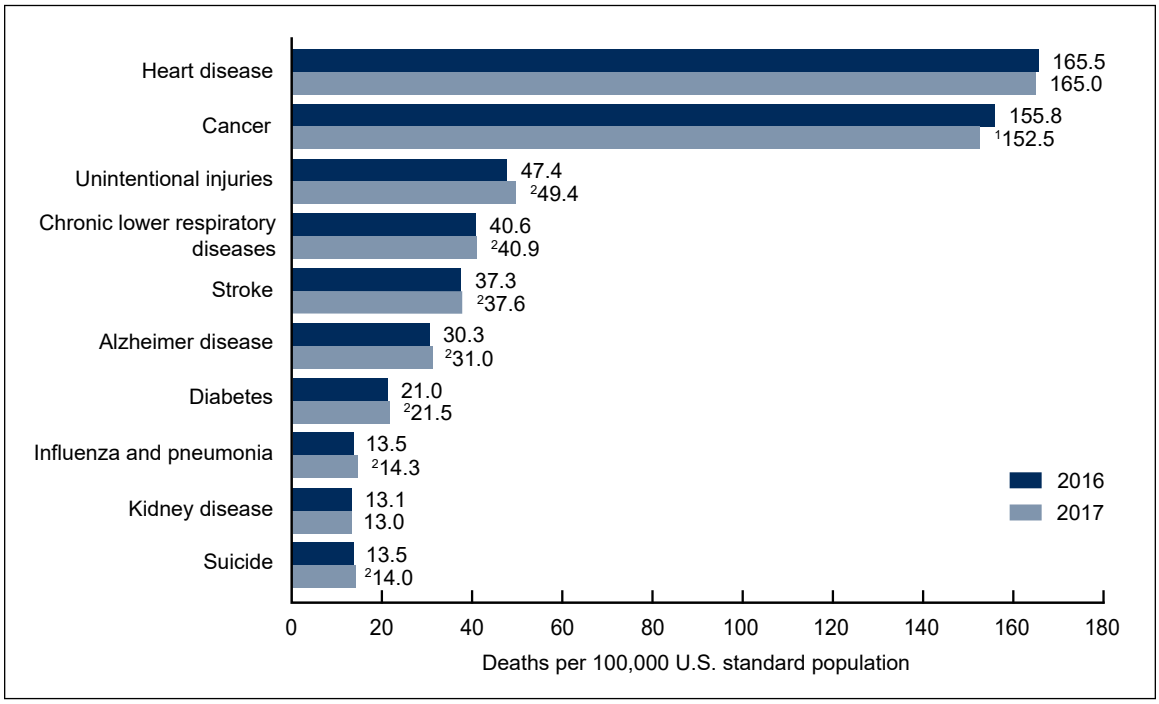
[1]Statistically significant increase in age-specific death rate from 2016 to 2017 ($p < 0.05$).
[2]Statistically significant decrease in age-specific death rate from 2016 to 2017 ($p < 0.05$).
NOTES: Rates are plotted on a logarithmic scale. Access data table for Figure 3 at: https://www.cdc.gov/nchs/data/databriefs/db328_tables-508.pdf#3.
SOURCE: NCHS, National Vital Statistics System, Mortality.

What are the leading causes of death?

In 2017, the 10 leading causes of death (heart disease, cancer, unintentional injuries, chronic lower respiratory diseases, stroke, Alzheimer disease, diabetes, influenza and pneumonia, kidney disease, and suicide) remained the same as in 2016. Causes of death are ranked according to number of deaths (1). The 10 leading causes accounted for 74.0% of all deaths in the United States in 2017.

From 2016 to 2017, age-adjusted death rates increased for 7 of 10 leading causes of death and decreased for 1 (Figure 4). The rate increased 4.2% for unintentional injuries, 0.7% for chronic lower respiratory diseases, 0.8% for stroke, 2.3% for Alzheimer disease, 2.4% for diabetes, 5.9% for influenza and pneumonia, and 3.7% for suicide. The rate decreased 2.1% for cancer. Rates for heart disease and kidney disease did not change significantly.

Figure 4. Age-adjusted death rates for the 10 leading causes of death: United States, 2016 and 2017



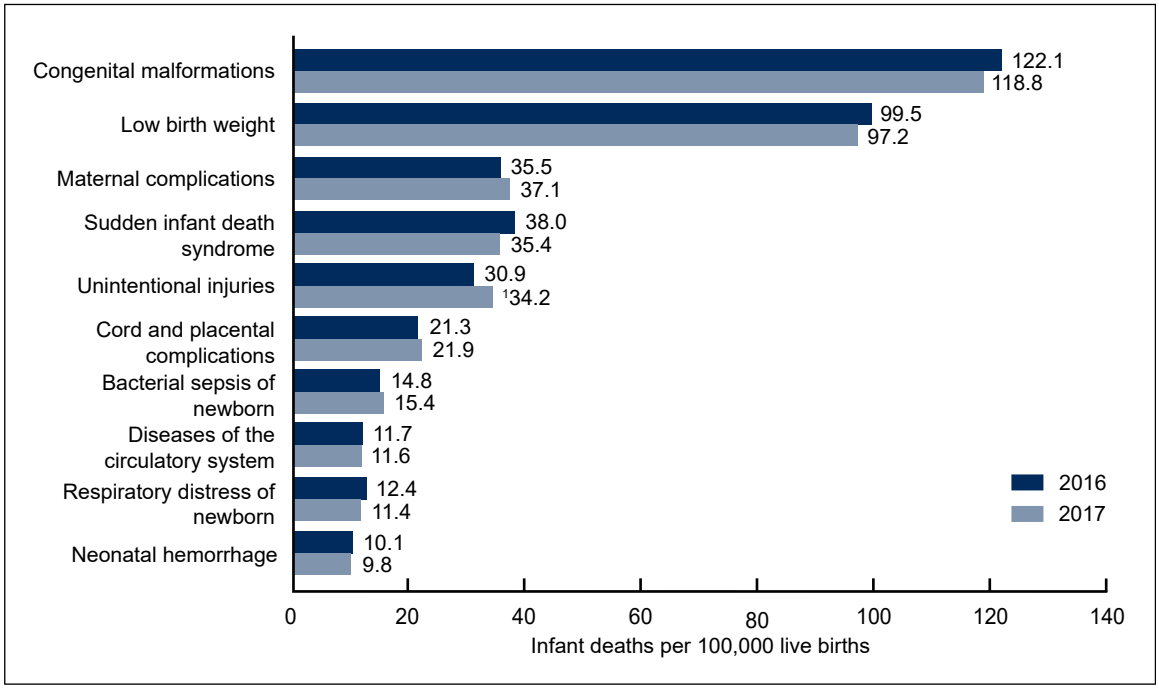
[1]Statistically significant decrease in age-adjusted death rate from 2016 to 2017 ($p < 0.05$).
[2]Statistically significant increase in age-adjusted death rate from 2016 to 2017 ($p < 0.05$).
NOTES: A total of 2,813,503 resident deaths were registered in the United States in 2017. The 10 leading causes accounted for 74.0% of all deaths in the United States in 2017. Causes of death are ranked according to number of deaths. Rankings for 2016 data are not shown. Data table for Figure 4 includes the number of deaths for leading causes. Access data table for Figure 4 at: https://www.cdc.gov/nchs/data/databriefs/db328_tables-508.pdf#4.
SOURCE: NCHS, National Vital Statistics System, Mortality.

What are the leading causes of infant death?

The infant mortality rate (IMR)—the ratio of infant deaths to live births in a given year—is generally regarded as a good indicator of the overall health of a population. IMR changed from 587.0 infant deaths per 100,000 live births in 2016 to 579.3 in 2017, but this change was not statistically significant.

The 10 leading causes of infant death in 2017 accounted for 67.8% of all infant deaths in the United States. The leading causes remained the same as in 2016 although maternal complications became the third leading cause while sudden infant death syndrome became the fourth, and diseases of the circulatory system became the eighth leading cause while respiratory distress of newborn became the ninth (Figure 5). Causes of infant death are ranked according to number of infant deaths (1). IMR for unintentional injuries increased 10.7% from 30.9 infant deaths per 100,000 live births in 2016 to 34.2 in 2017. Mortality rates for other leading causes of infant death did not change significantly.

Figure 5. Infant mortality rates for the 10 leading causes of infant death in 2017: United States, 2016 and 2017



[1]Statistically significant increase in mortality rate from 2016 to 2017 ($p < 0.05$).
NOTES: A total of 22,335 deaths occurred in children under age 1 year in the United States in 2017, with an infant mortality rate of 579.3 infant deaths per 100,000 live births. The 10 leading causes of infant death in 2017 accounted for 67.8% of all infant deaths in the United States. A total of 23,161 infant deaths occurred in 2016, with an infant mortality rate of 587.0 infant deaths per 100,000 live births. Causes of death are ranked according to number of deaths. Rankings for 2016 data are not shown. Data table for Figure 5 includes the number of deaths under age 1 year for leading causes of infant death. Access data table for Figure 5 at: https://www.cdc.gov/nchs/data/databriefs/db328_table-508.pdf#5.
SOURCE: NCHS, National Vital Statistics System, Mortality.

Summary

In 2017, a total of 2,813,503 resident deaths were registered in the United States—69,255 more deaths than in 2016. From 2016 to 2017, the age-adjusted death rate for the total population increased 0.4%, and life expectancy at birth decreased 0.1 year. Age-specific death rates between 2016 and 2017 increased for age groups 25–34, 35–44, and 85 and over, and decreased for age group 45–54. Age-adjusted death rates increased for non-Hispanic white males and non-Hispanic white females and decreased for non-Hispanic black females.

The 10 leading causes of death in 2017 remained the same as in 2016. Age-adjusted death rates increased for seven leading causes and decreased for one. Life expectancy at birth decreased 0.1 year from 78.7 years in 2016 to 78.6 in 2017, largely because of increases in mortality from unintentional injuries, suicide, diabetes, and influenza and pneumonia, with unintentional injuries making the largest contribution.

In 2017, a total of 22,335 deaths occurred among children under age 1 year, which was 826 fewer infant deaths than in 2016. The leading causes of infant death were the same in 2017 and 2016 although maternal complications became the third leading cause while sudden infant death syndrome became the fourth, and diseases of the circulatory system became the eighth leading cause while respiratory distress of newborn became the ninth. The only significant change among the 10 leading causes of infant death was a 10.7% increase in IMR for unintentional injuries.

Definitions

Cause of death: Based on medical information—including injury diagnoses and external causes of injury—entered on death certificates filed in the United States. This information is classified and coded in accordance with the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision* (2).

Death rates: For 2017, based on population estimates for July 1, 2017, that are consistent with the April 1, 2010, census. These population estimates (as well as population figures for the 2010 census) are available on the National Center for Health Statistics' (NCHS) website (3). Age-adjusted death rates are useful when comparing different populations because they remove the potential bias that can occur when the populations being compared have different age structures. NCHS uses the direct method of standardization; see Technical Notes of "Deaths: Final Data for 2016" (1) for more information.

Infant mortality rate (IMR): Computed by dividing the number of infant deaths in a calendar year by the number of live births registered for that same time period. IMR is the most widely used index for measuring the risk of dying during the first year of life.

Leading causes of death: Ranked according to the number of deaths assigned to rankable causes (4).

Life expectancy: The expected average number of years of life remaining at a given age. It is denoted by e_x, which means the average number of subsequent years of life for someone now aged x. Life expectancy estimates for 2017 are based on a methodology first implemented with 2008 final mortality data (5). Life expectancies for 2016 were revised using updated Medicare

data; therefore, figures may differ from those previously published (1). Life expectancies for 2017 may change slightly when updated Medicare data become available.

Data source and methods

The data shown in this report reflect information collected by NCHS for 2016 and 2017 from death certificates filed in all 50 states and the District of Columbia and compiled into national data known as the National Vital Statistics System. The standard presentation of life expectancy estimates are rounded to one decimal place. Changes in life expectancy, computed using figures rounded to one decimal, may slightly overestimate or underestimate the actual change. Changes in life expectancy from 2016 to 2017 using unrounded estimates were less than 0.1 year. Death rates shown in this report are calculated based on postcensal population estimates as of July 1, 2016, and July 1, 2017, which are consistent with the April 1, 2010, census. Differences between death rates were evaluated using a two-tailed *z* test.

About the authors

Sherry L. Murphy, Jiaquan Xu, Kenneth D. Kochanek, and Elizabeth Arias are with the National Center for Health Statistics, Division of Vital Statistics.

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Exhibit 27

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NCHS Data Brief ■ No. 328 ■ November 2018

Keywords: life expectancy • leading cause • National Vital Statistics System

Suggested citation

Murphy SL, Xu JQ, Kochanek KD, Arias E. Mortality in the United States, 2017. NCHS Data Brief, no 328. Hyattsville, MD: National Center for Health Statistics. 2018.

Copyright information

National Center for Health Statistics

ISSN 1941–4927 Print ed.
ISSN 1941–4935 Online ed.
DHHS Publication No. 2019–1209
CS298755

Exhibit 27

EXHIBIT 28

National Vital Statistics Reports

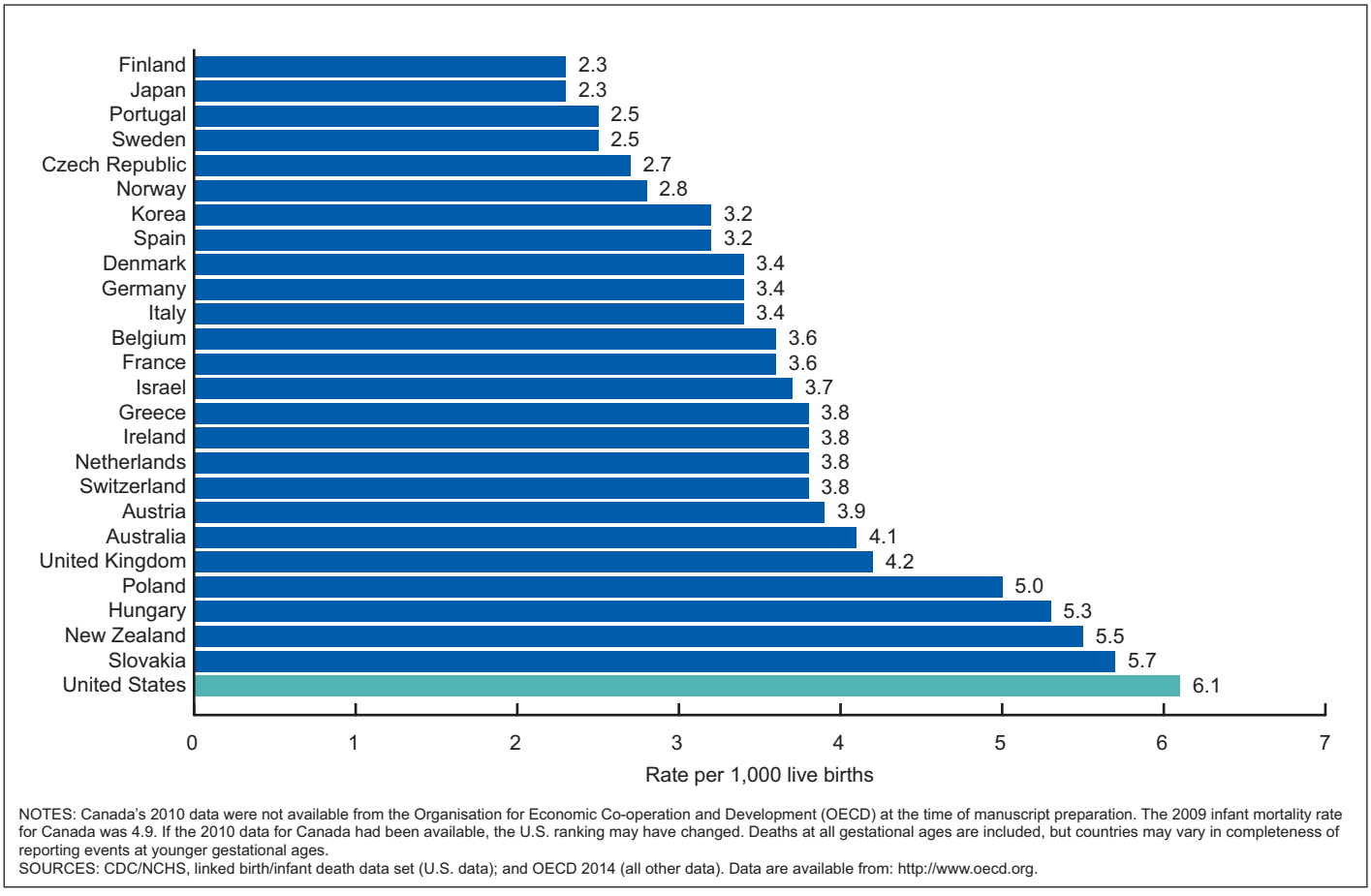
Volume 63, Number 5 September 24, 2014

International Comparisons of Infant Mortality and Related Factors: United States and Europe, 2010

by Marian F. MacDorman, Ph.D., and T.J. Mathews, M.S., National Center for Health Statistics; Ashna D. Mohangoo, Ph.D., TNO Child Health, Netherlands; and Jennifer Zeitlin, M.D., Inserm, France

Abstract

Objectives—This report investigates the reasons for the United States' high infant mortality rate when compared with European countries. Specifically, the report measures the impact on infant mortality differences of two major factors: the percentage of preterm births and gestational age-specific infant mortality rates.



NOTES: Canada's 2010 data were not available from the Organisation for Economic Co-operation and Development (OECD) at the time of manuscript preparation. The 2009 infant mortality rate for Canada was 4.9. If the 2010 data for Canada had been available, the U.S. ranking may have changed. Deaths at all gestational ages are included, but countries may vary in completeness of reporting events at younger gestational ages.
SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data); and OECD 2014 (all other data). Data are available from: http://www.oecd.org.

Figure 1. Infant mortality rates: Selected Organisation for Economic Co-operation and Development countries, 2010

Methods—Infant mortality and preterm birth data are compared between the United States and European countries. The percent contribution of the two factors to infant mortality differences is computed using the Kitagawa method, with Sweden as the reference country.

Results—In 2010, the U.S. infant mortality rate was 6.1 infant deaths per 1,000 live births, and the United States ranked 26th in infant mortality among Organisation for Economic Co-operation and Development countries. After excluding births at less than 24 weeks of gestation to ensure international comparability, the U.S. infant mortality rate was 4.2, still higher than for most European countries and about twice the rates for Finland, Sweden, and Denmark. U.S. infant mortality rates for very preterm infants (24–31 weeks of gestation) compared favorably with most European rates. However, the U.S. mortality rate for infants at 32–36 weeks was second-highest, and the rate for infants at 37 weeks of gestation or more was highest, among the countries studied. About 39% of the United States' higher infant mortality rate when compared with that of Sweden was due to a higher percentage of preterm births, while 47% was due to a higher infant mortality rate at 37 weeks of gestation or more. If the United States could reduce these two factors to Sweden's levels, the U.S. infant mortality rate would fall by 43%, with nearly 7,300 infant deaths averted annually.

Keywords: Euro-Peristat Project • preterm birth • gestational age-specific infant mortality rates

Introduction

Infant mortality is an important indicator of the health of a nation because it is associated with a variety of factors such as maternal health, quality and access to medical care, socioeconomic conditions, and public health practices (1–3). After a plateau from 2000 to 2005 (4), the U.S. infant mortality rate declined from 6.87 infant deaths per 1,000 live births in 2005 to 6.07 in 2011 (5,6). Yet, the United States' infant mortality rate remains higher than for most other developed countries (7). This report compares infant mortality rates between the United States and selected European countries and assesses the impact on infant mortality differences of the percentage of preterm births and gestational age-specific infant mortality rates.

Methods

U.S. data from the 2010 linked birth/infant death data set (latest available at the time of manuscript preparation) were compared with international data from two sources: the Organisation for Economic Co-operation and Development (OECD) database (7), and the second European Perinatal Health Report (EPHR) (8). However, these two sources differ in several important respects. For example, data from the OECD database were not limited by gestational age, and ranked the United Kingdom as a whole rather than by components (England and Wales, Scotland, and Northern Ireland). In contrast, EPHR showed data for England and Wales, Scotland, and Northern Ireland separately and excluded births at the lowest gestational ages to facilitate more valid international comparisons. The OECD database also included countries outside of Europe, whereas EPHR focused exclusively on European countries (7,8).

Data from the OECD database (Figure 1) are used to establish a baseline for international comparison. In keeping with *Health, United States* procedures for international infant mortality ranking, countries with less than 2.5 million population were excluded from the rankings (9). International rankings of infant mortality can vary depending on the availability of required data and other inclusion criteria (7,9).

European data shown in the remainder of the report are from EPHR and associated unpublished data (8). Data were included for countries listed in both EPHR and the OECD database. Among the European countries, not all countries were able to supply all requested data metrics. Thus, countries included in the Table and Figures 2–5 are those that supplied the requisite data. Because most European countries use the obstetric estimate of gestation to measure gestational age (8), U.S. data in this report are tabulated using the obstetric estimate of gestation to facilitate international comparisons.

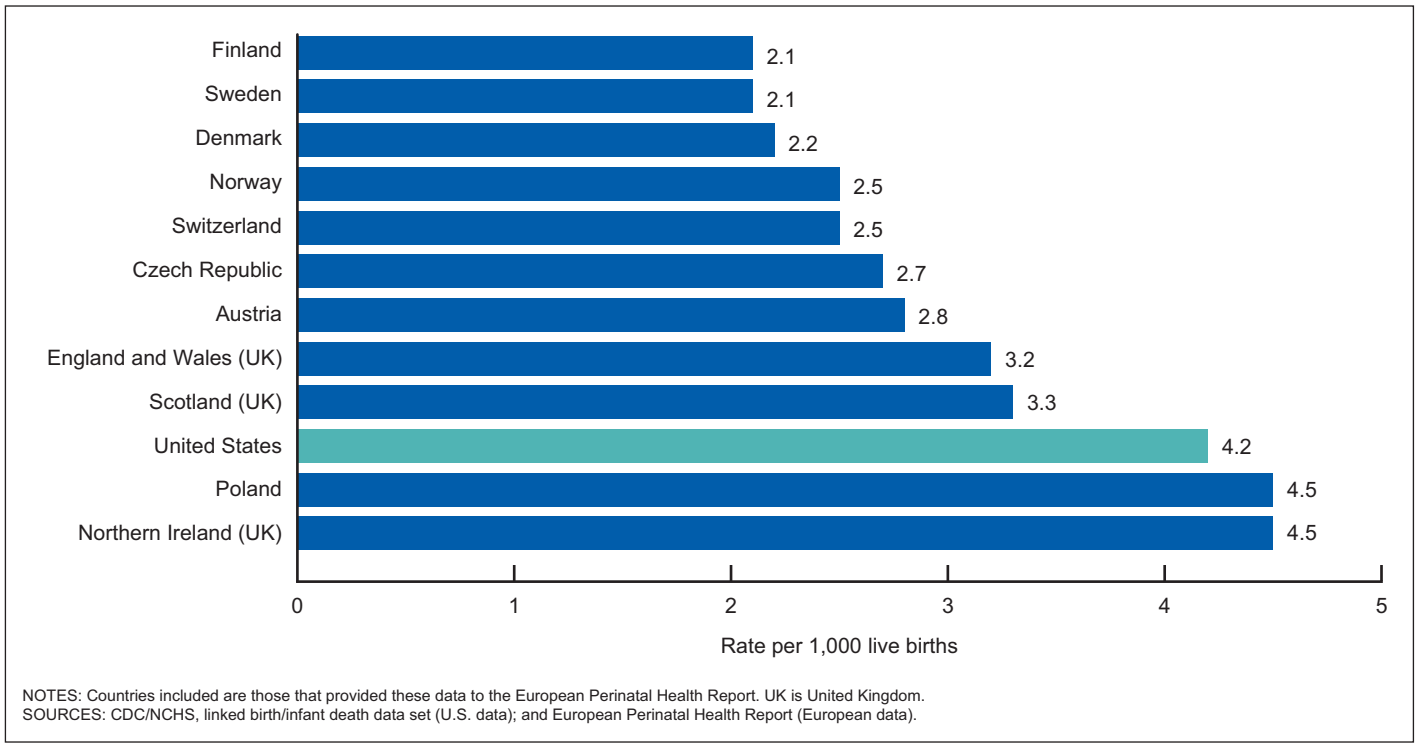
In the United States and most European countries, no gestational age or birthweight lower limit is placed on the reporting of live births or infant deaths, although a few countries do have lower limits for birth registration or reporting (7,8,10). Some studies have found variations between countries in the distribution of births and infant deaths at 22–23 weeks of gestation, suggesting the possibility of variations in reporting at these early gestational ages (11–13). Thus, events at less than 24 weeks of gestation were excluded from the analysis (except for Figure 1) to ensure international comparability. This is not meant to minimize the importance of these early infant deaths, which contribute substantially to the United States' overall infant mortality rate; rather, the approach recognizes that accurate international comparisons may not be possible for events at less than 24 weeks of gestation.

The Kitagawa method is a further development of direct standardization that more precisely quantifies the relative contribution of changes in variable-specific rates and in population composition to the total changes in rates in cases where both are changing simultaneously (14). In this report, the Kitagawa method is used to estimate the percent contribution of differences in the distribution of births by gestational age, and in gestational age-specific infant mortality rates to the overall difference in infant mortality rates between countries. It is also used to estimate the infant mortality rate that would have occurred, and the number of infant deaths that could have been averted, had different conditions been present.

Results

Despite recent declines in infant mortality (4), the United States ranked 26th among the 29 OECD countries in 2010 (9), behind most European countries as well as Japan, Korea, Israel, Australia, and New Zealand (Figure 1). The U.S. infant mortality rate of 6.1 infant deaths per 1,000 live births was more than twice that for Japan and Finland (both 2.3), the countries with the lowest rates. Twenty-one of the 26 OECD countries studied had infant mortality rates below 5.0. This pattern of high infant mortality rates in the United States when compared with other developed countries has persisted for many years (7,9).

When births at less than 24 weeks were excluded, the U.S. infant mortality rate dropped from 6.1 to 4.2 infant deaths per 1,000 live births in 2010 (Figure 2). The U.S. infant mortality rate (excluding births at less than 24 weeks) of 4.2 was about twice the rate for Finland, Sweden, and Denmark, the countries with the lowest rates. Compared with the U.S. rate, infant mortality rates were lower for 9 of the 11 European countries; the rate for both Poland and Northern Ireland was higher at 4.5.



NOTES: Countries included are those that provided these data to the European Perinatal Health Report. UK is United Kingdom.
SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data); and European Perinatal Health Report (European data).

Figure 2. Infant mortality rates excluding births at less than 24 weeks of gestation: United States and selected European countries, 2010

Gestational age-specific infant mortality rates are shown in the Table for the United States and the 11 European countries that supplied these data. The United States compares favorably with most European countries in infant mortality rates for very preterm infants (24–31 weeks of gestation), but the comparison becomes less favorable as gestational age increases. The U.S. mortality rate for infants born at 24–27 weeks of gestation was fifth lowest of 12 countries. Seven countries had higher rates, and only Denmark, Finland, Norway, and Sweden had lower rates than the United States. For infants born at 28–31 weeks of gestation, the U.S. rate was in the middle—fifth lowest of eight countries. For infants born at 32–36 weeks of gestation (the preterm category where most preterm births occur), the U.S. infant mortality rate was second highest among 11 countries; only Poland had a higher rate. However, for infants born at 37 weeks of gestation or more, the U.S. infant mortality rate was highest among the countries studied (2.20 per 1,000), and about twice the rates for Denmark, Finland, Norway, Sweden, and Switzerland.

Another important factor affecting infant mortality differences is the percentage of infants born preterm (i.e., before 37 completed weeks of gestation), because preterm infants have higher mortality rates than those born at 37 weeks of gestation or more (14). In 2010, after births at less than 24 weeks were excluded, 9.8% of U.S. births were preterm,

Table. Gestational age-specific infant mortality rates: United States and selected European countries, 2010

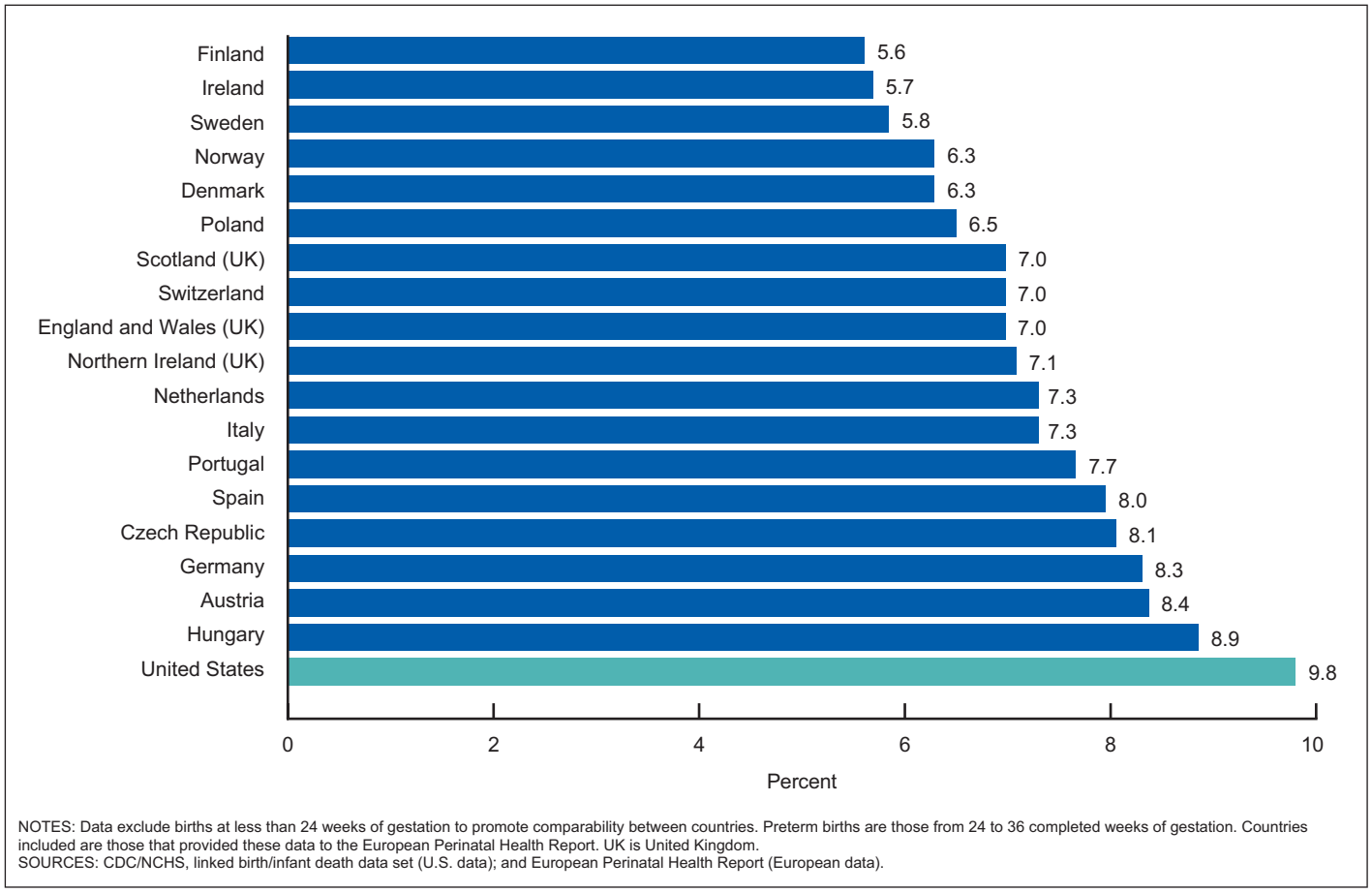
Country	Gestational age (weeks)			
	24–27	28–31	32–36	37 or more
Austria	217.39	34.38	8.01	1.24
Czech Republic	294.12	40.37	7.19	1.15
Denmark	198.80	*	7.95	1.11
England and Wales (UK)	245.17	48.29	8.73	1.60
Finland	203.25	*	9.74	0.97
Northern Ireland (UK)	325.00	*	*	1.96
Norway	189.78	*	9.87	1.06
Poland	429.32	98.98	15.43	1.95
Scotland (UK)	282.49	47.01	8.38	1.58
Sweden	165.08	35.14	8.33	1.10
Switzerland	308.41	41.75	6.77	1.12
United States	208.08	44.65	10.20	2.20

* Figure does not meet standards of reliability or precision; based on fewer than 20 deaths in the numerator.

NOTES: Infant mortality rates are per 1,000 live births in specified group. Countries included are those that provided these data to the European Perinatal Health Report. UK is United Kingdom.

SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data), and European Perinatal Health Report (European data).

Exhibit 28



NOTES: Data exclude births at less than 24 weeks of gestation to promote comparability between countries. Preterm births are those from 24 to 36 completed weeks of gestation. Countries included are those that provided these data to the European Perinatal Health Report. UK is United Kingdom.
SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data); and European Perinatal Health Report (European data).

Figure 3. Percentage of preterm births: United States and selected European countries, 2010

the highest among the 19 countries studied (Figure 3). The percentage of preterm births in the United States was 40% higher than in England and Wales, and 69%–75% higher than in Finland, Ireland, and Sweden.

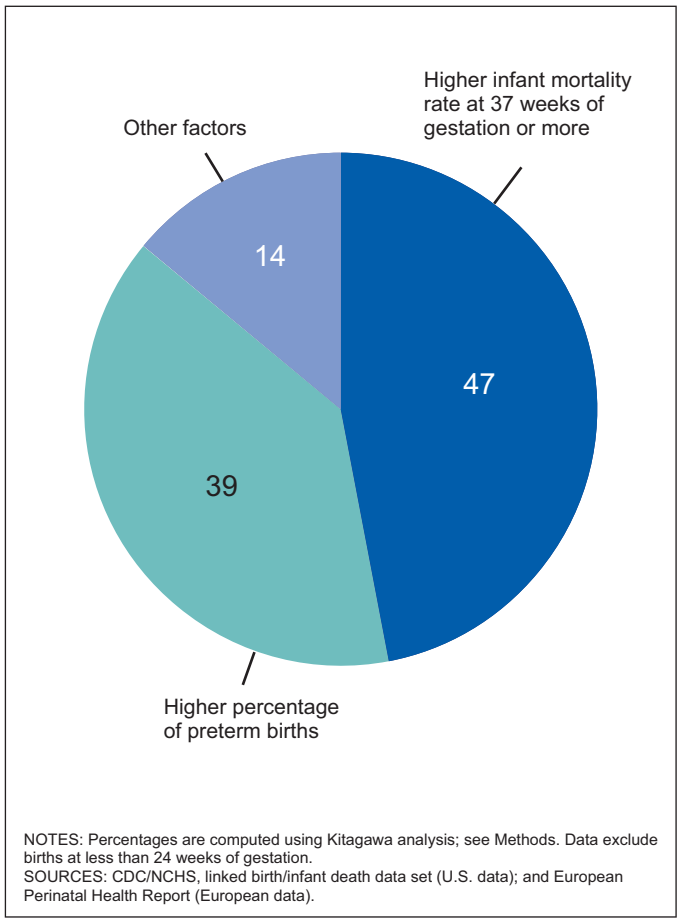
The Kitagawa method (14) was used to examine the contribution of preterm births and gestational age-specific infant mortality rates to the infant mortality difference between the United States and Sweden. About 39% of the United States' higher infant mortality rate when compared with that of Sweden was due to the higher U.S. percentage of preterm births, while 47% of the difference was due to the higher U.S. mortality rate for infants at 37 weeks of gestation or more (although Sweden had lower infant mortality rates at all gestational ages) (Figure 4). Other factors (such as infant mortality rates at 24–27, 28–31, and 32–36 weeks of gestation, and the percentage of births at 37 weeks or more) also contributed modestly to the differences; however, their influence was small when compared with these two main factors. For example, even though the U.S. infant mortality rate at 32–36 weeks of gestation was second-highest among the countries studied, the United States' higher gestational age-specific infant mortality rate at 32–36 weeks of gestation contributed only 6% to the overall difference.

If the United States could reduce its percentage of preterm births to Sweden's levels, the U.S. infant mortality rate (excluding events at less than 24 weeks of gestation) would decline by 19% to a rate of 3.4 (Figure 5). This would result in over 3,200 fewer infant deaths than actually occurred in 2010. Alternatively, if the United States could reduce its infant mortality rate for infants at 37 weeks of gestation or more to Swedish levels, the U.S. infant mortality rate (excluding events at less than 24 weeks) would decline by 24% to 3.2. This would avert nearly 4,100 infant deaths. If both factors could be reduced to the levels in Sweden, the U.S. infant mortality rate (excluding events at less than 24 weeks) would decline by 43% to 2.4, and nearly 7,300 infant deaths would be averted.

Discussion

Since 2005, the United States has made important progress in reducing both infant mortality and preterm birth (5,15,16). Because both the linked birth/infant death and EPHR data are available for the 2010 data year, the current analysis focused on 2010 data (8). However, more recent U.S. data on both infant mortality and preterm birth are available from other sources (6,16). The 2011 infant mortality rate of 6.07 infant deaths per 1,000 live births was 12% lower than the rate of 6.87 in 2005. The percentage of preterm births declined from a high of 12.80% in 2006 to 11.55% in 2012 (16). Despite these improvements, the U.S. international infant mortality ranking did not improve during this time frame (7,9), and the U.S. infant mortality rate remains higher than for most European countries, even after excluding births at less than 24 weeks of gestation. Furthermore, the United States still has the highest percentage of preterm births among the countries studied.

The United States compares favorably with most European countries in the survival of very preterm infants. However, the comparison becomes less favorable as gestational age increases. For example, the



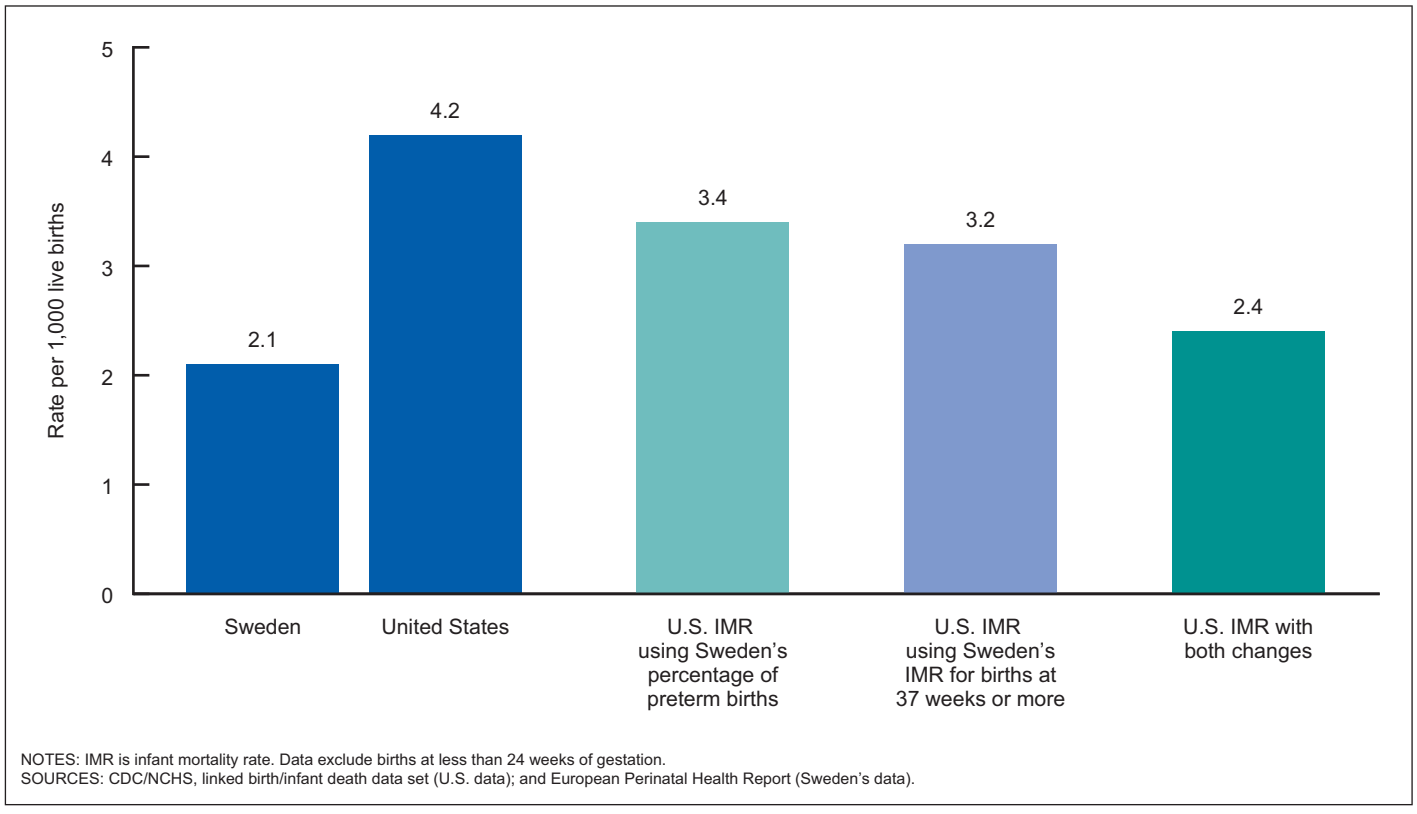
NOTES: Percentages are computed using Kitagawa analysis; see Methods. Data exclude births at less than 24 weeks of gestation.
SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data); and European Perinatal Health Report (European data).

Figure 4. Percent contribution of various factors to the higher U.S. infant mortality rate compared with that of Sweden

U.S. infant mortality rate at 37 weeks of gestation or more was highest among the countries studied, and about twice the rates for Denmark, Finland, Norway, Sweden, and Switzerland.

This study found that 39% of the United States' higher infant mortality rate, when compared with that of Sweden, was due to the higher U.S. percentage of preterm births, while 47% of the difference was due to the United States' higher infant mortality rate for infants at 37 weeks of gestation or more. A previous report found a larger effect for preterm birth (10), mostly due to the inclusion of births at 22–23 weeks of gestation in that report. Recent declines in the U.S. infant mortality rate and percentage of preterm births, and the use of the obstetric estimate to measure gestational age in the current report (compared with gestational age based on the last menstrual period used in the previous report), may have also contributed to the difference in findings between the two reports.

The findings from the current analysis suggest that declines in either the percentage of preterm births or in infant mortality rates at 37 weeks of gestation or more could have a substantial positive impact on the U.S. infant mortality rate. If both of these factors could be reduced to Sweden's levels, the U.S. infant mortality rate (excluding events at less than 24 weeks) would be reduced from 4.2 to 2.4—a decline of 43%. Such a decline would mean nearly 7,300 fewer infant deaths than actually occurred in the United States in 2010.



NOTES: IMR is infant mortality rate. Data exclude births at less than 24 weeks of gestation.
SOURCES: CDC/NCHS, linked birth/infant death data set (U.S. data); and European Perinatal Health Report (Sweden's data).

Figure 5. Infant mortality rates for United States and Sweden, and U.S. infant mortality rate under various modeling assumptions, 2010

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National Vital Statistics Reports, Vol. 63, No. 5, September 24, 2014

Contents

Acknowledgments

The following Euro-Peristat Scientific Committee members contributed data to this study: Gerald Haidinger (Austria), Petr Velebil (Czech Republic), Anne-Marie Nybo-Andersen (Denmark), Mika Gissler (Finland), Kari Klungsoyr (Norway), Katarzyna Szamotulska (Poland), Karin Gottvall (Sweden), Sylvie Berrut (Switzerland), and Alison Macfarlane (United Kingdom).

Suggested citation

MacDorman MF, Mathews TJ, Mohangoo AD, Zeitlin J. International comparisons of infant mortality and related factors: United States and Europe, 2010. National vital statistics reports; vol 63 no 5. Hyattsville, MD: National Center for Health Statistics. 2014.

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DHHS Publication No. 2014–1120 • CS250171

Exhibit 28

EXHIBIT 29

EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natural Experiment

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ARTICLE INFO

Article history:
Received 4 June 2016
Received in revised form 21 January 2017
Accepted 29 January 2017
Available online xxxx

Keywords:
Diphtheria-tetanus-pertussis vaccine
DTP
Measles vaccine
Non-specific effects of vaccines
Oral polio vaccine

ABSTRACT

Background: We examined the introduction of diphtheria-tetanus-pertussis (DTP) and oral polio vaccine (OPV) in an urban community in Guinea-Bissau in the early 1980s.
Methods: The child population had been followed with 3-monthly nutritional weighing sessions since 1978. From June 1981 DTP and OPV were offered from 3 months of age at these sessions. Due to the 3-monthly intervals between sessions, the children were allocated by birthday in a 'natural experiment' to receive vaccinations early or late between 3 and 5 months of age. We included children who were <6 months of age when vaccinations started and children born until the end of December 1983. We compared mortality between 3 and 5 months of age of DTP-vaccinated and not-yet-DTP-vaccinated children in Cox proportional hazard models.
Results: Among 3–5-month-old children, having received DTP (±OPV) was associated with a mortality hazard ratio (HR) of 5.00 (95% CI 1.53–16.3) compared with not-yet-DTP-vaccinated children. Differences in background factors did not explain the effect. The negative effect was particularly strong for children who had received DTP-only and no OPV (HR = 10.0 (2.61–38.6)). All-cause infant mortality after 3 months of age increased after the introduction of these vaccines (HR = 2.12 (1.07–4.19)).
Conclusion: DTP was associated with increased mortality; OPV may modify the effect of DTP.

1. Introduction

Individually randomized studies to measure impact on child survival of different vaccines were not conducted when the Expanded Program on Immunization (EPI) was introduced in low-income countries in the 1970s. The disease-protective effects were well documented, so the main issue was at which age to introduce the vaccine most effectively (The Expanded Programme on Immunization, 1982). Except for measles vaccine (MV), surprisingly few studies examined the introduction of vaccines and their impact on child survival (Aaby et al., 1983, 2003a; Holt et al., 1990; The Kasongo Project Team, 1981). One trial of measles-vaccinated and measles-unvaccinated communities in Congo showed a larger than expected reduction in child mortality (Aaby et al., 1981); this observation was subsequently corroborated by community "trials" and before-after studies in several countries (Aaby et al. 1984, 1993, 2003a; Holt et al., 1990; Kapoor and Reddaiah, 1991). Hence, a vaccine may have non-specific effects (NSEs) on susceptibility to other infections (Aaby et al., 1995). WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recently reviewed the potential NSEs of BCG, diphtheria-tetanus-pertussis (DTP) and MV and recommended further research (Higgins et al., 2014; Strategic Advisory Group of experts on Immunization, 2014).

Though protective against the target diseases, DTP may increase susceptibility to unrelated infections (Aaby et al., 2003b, 2004a, 2012) (Appendix A). The SAGE review noticed that the majority of studies found a detrimental effect of DTP (Higgins et al., 2014). However, SAGE considered the evidence inconsistent because two studies reported beneficial effects (Higgins et al., 2014) and that most studies underestimated the benefit of DTP because studies were conducted in situations with herd immunity. Furthermore, all studies gave DTP and OPV together, making it impossible to separate effects of DTP and OPV (SAGE non-specific effects of vaccines Working Group, 2014).

On the other hand, the "unvaccinated" children in these studies have usually been frail children too sick or malnourish to get vaccinated, and the studies may therefore have underestimated the negative effect of DTP. We therefore examined what happened when DTP and OPV were first introduced, but not always given together, in 1981–1983 in the capital of Guinea-Bissau. In this situation the children were allocated by birthday to receive vaccines early or late and the "unvaccinated" were therefore not frail children.

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http://dx.doi.org/10.1016/j.ebiom.2017.01.041

2. Methods

2.1. Background

Bandim Health Project (BHP) has followed an urban community with a demographic surveillance system since December 1978, and took part in the introduction of vaccines well before a full-fledged national program was implemented with UNICEF support in 1986 (Aaby et al., 1984, 2004a).

2.2. Demographic Surveillance

In 1978–1979, under-five mortality was nearly 500/1000. Since malnutrition was assumed to be the main cause, a study was initiated to determine why children were malnourished (Aaby et al., 1983). However, severe malnutrition was not evident, and to understand the high mortality we started a health and demographic surveillance system (HDSS). The area was mapped and a census conducted. Four health workers were employed to identify pregnant women, encourage women to attend ante-natal clinics, and to follow children with anthropometric measurements to assess growth patterns and detect malnourished children. Each health worker followed a population of 1500–2000 individuals. The health workers were supervised by an expatriate nurse.

For each sub-district in Bandim, the responsible health worker kept a list of children under three years of age. BHP had no computerized surveillance system until 1990 but kept an A5 card ("BHP card") for each child, where weights and vaccination dates were noted. The child's growth card was kept by the mother.

The Bandim population was very mobile. It was important to maintain contact with the natal village for ceremonial purposes and to secure rice. Furthermore, mothers were not supposed to have sexual relations during breastfeeding (Jakobsen et al., 2004). Breastfeeding was prolonged in Guinea-Bissau. Thus, many women stayed in the rural areas with their natal family while breastfeeding. These cultural traditions introduced variability in the participation in weighing and vaccination sessions.

2.3. Anthropometry

We arranged quarterly weighing sessions in each sub-district. The responsible health worker advised mothers the day before a community weighing. The following morning, the weight was measured and noted on the child's growth card and the BHP card. When the World Food Program provided supplementary feeding this was given to families with malnourished children.

2.4. Vaccinations

There was no community vaccination program in 1981 except that we had organized a few measles vaccination campaigns (Aaby et al., 1984). Mothers could take their children to the Mother and Child Health Program in town. However, this clinic was mainly attended by the urban elite. Few children were vaccinated before BHP organized vaccination sessions (Table 1).

In June 1981, BHP started to provide vaccinations at the quarterly weighing sessions. A health center nurse accompanied the weighing team and vaccinated eligible children. DTP and OPV were provided from 3 months and MV from 9 months of age. OPV-at-birth was not given then. The three DTP and OPV doses could be given with an interval of one month but since we only arranged weighing every three months, most children had longer intervals between doses. DTP was administered intramuscularly and OPV as an oral drop. When both vaccines were administered at the same session OPV was usually given first and then DTP; the children would usually start crying after DTP due to the pain of the injection and it would therefore have complicated the administration of OPV to give DTP first. There were several periods where either OPV or DTP was missing (Fig. 1). BCG was rarely provided at the weighing sessions since most nurses were not trained to administer intra-dermal vaccination. A total of 269 children may have been BCG vaccinated as they had a vaccination date on their card (N = 192) or were noted to have received BCG but no date given (N = 77).

The expatriate nurse sometimes organized additional vaccination sessions in which the children were not weighed. During these sessions,

Table 1
Median age of vaccination and coverage for BCG, DTP and OPV of study cohort.

	1980	1981	1982	1983	1981–1983
Median age in days (N vaccines)					
BCG	9 (4)	48.5 (50)	34 (46)	25 (68)	33 (164)
DTP1	97 (12)	127 (147)	121 (164)	117 (278)	121 (589)
OPV1	98 (12)	118 (185)	121.5 (170)	117 (225)	118 (580)
MV	181 (5)	141 (53)	157 (2)	110 (1)	141.5 (56)
Coverage at 6 months of age					
BCG	1.7% (5/289)	3.5% (12/342)	23.7% (72/304)	17.4% (57/327)	14.5% (141/973)
DTP1	4.2% (12/289)	31.3% (107/342)	61.2% (186/304)	73.1% (239/327)	54.7% (532/973)
DTP3	2.4% (7/289)	0.9% (3/342)	4.3% (13/304)	4.0% (13/327)	3.0% (29/973)
OPV1	4.2% (12/289)	43.0% (147/342)	62.5% (190/304)	69.7% (228/327)	58.1% (565/973)
OPV3	2.4% (7/289)	2.0% (7/342)	4.3% (13/304)	4.0% (13/327)	3.4% (33/973)
MV	2.8% (8/289)	15.2% (52/342)	0.7% (2/304)	0% (0/327)	5.5% (54/973)
Coverage at one year of age					
BCG	2.6% (3/116)	2.4% (7/294)	15.4% (51/332)	17.4% (46/264)	11.7% (104/890)
DTP1	2.6% (3/116)	32.7% (96/294)	71.1% (236/332)	83.0% (219/264)	61.9% (551/890)
DTP3	2.6% (3/116)	4.4% (13/294)	18.4% (61/332)	43.2% (114/264)	21.1% (188/890)
OPV1	2.6% (3/116)	37.4% (110/294)	77.4% (257/332)	84.8% (224/264)	66.4% (591/890)
OPV3	2.6% (3/116)	12.2% (36/294)	32.5% (108/332)	44.3% (117/264)	29.3% (261/890)
MV	15.5% (18/116)	68.0% (200/294)	34.0% (113/332)	51.1% (135/264)	50.3% (448/890)

Notes: The inclusion criteria for the cohort in Table 1 are the same as for our study cohort: weight examination after 15 days of age and contribute time between 91 and 183 days of age.
Median age: 'year' means the year the vaccination was given, and median age is the median age at time of vaccination with a given vaccine among children vaccinated before turning 6 months. E.g. the 4 BCG vaccines in the 1980 column were given in 1980 to children with a median age of 9 days.
Coverage: 'year' means the year when the child turned exactly 1 year (or 6 months) old and coverage was assessed. Only children surviving to 1 year (or 6 months) of age were assessed for coverage. Children turning 1 year in 1984 were thus not presented in the table.

Please cite this article as: Mogensen, S.W., et al., The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natur..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.01.041

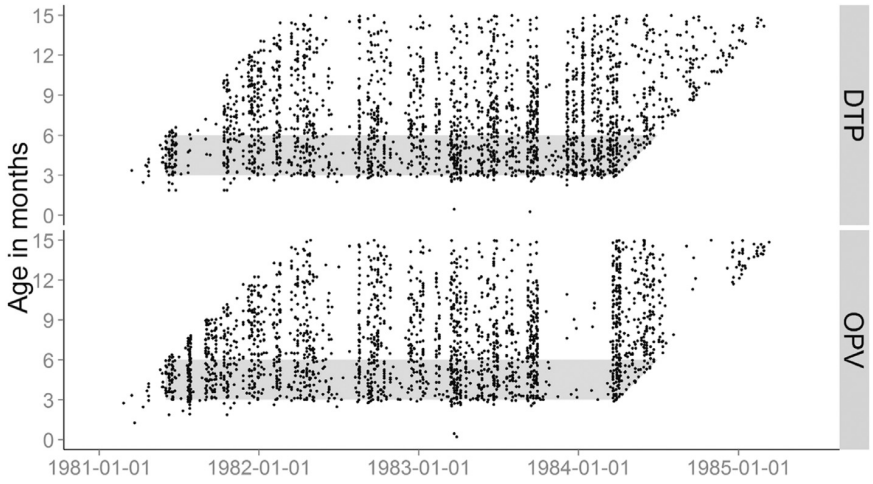

Fig. 1. Each vaccination of the specified type is plotted according age of the recipient and date of vaccination.

vaccinations were noted on the BHP cards. Both nurses and mothers thought that sick children should not be vaccinated; the BHP card often indicated that the child was 'sick', 'malnourished' or 'orphan' as an explanation of why an age-eligible child had not been vaccinated.

2.5. Data Control

When a computerized system became available in 1990–1991, weights and vaccinations from the BHP cards were entered. For the present analysis, all information on dates of visit, weights and vaccination dates was checked against the original cards. A few cards were not available or could no longer be found (Fig. 2).

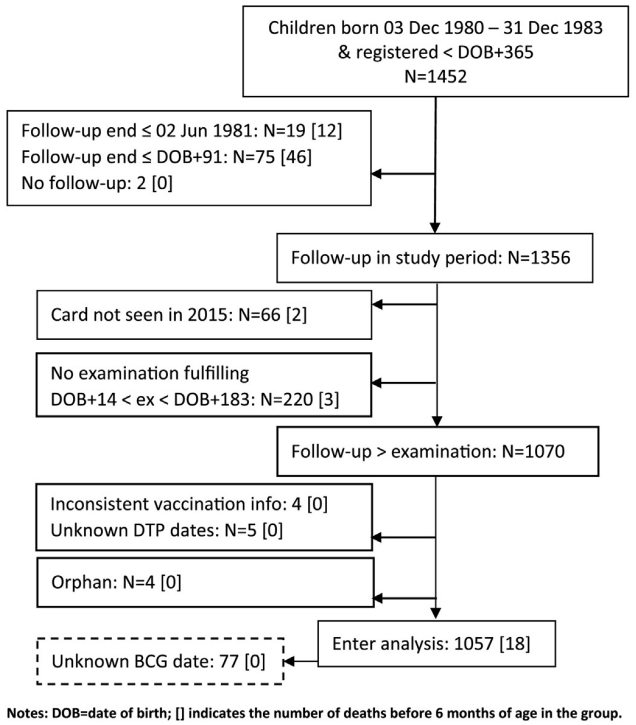

Fig. 2. Flowchart of study population and children included in the analyses. Notes: DOB = date of birth; [] indicates the number of deaths before 6 months of age in the group.

2.6. The Study Cohort

We included children born from December 3, 1980 as they would become eligible for vaccination before 6 months of age (Fig. 2). Few children were vaccinated with BCG (Table 1). Children who travelled and never attended any session were not included in the 'unvaccinated' group. Children weighed within a fortnight of their birth to obtain a birth weight were only included if they took part in a subsequent community weighing session. Furthermore, we excluded orphans since they were not breastfed and were likely to have different care. The cohort is depicted in Supplementary Fig. 1.

2.7. Natural Experiment for 3–5-month-old Children

Though not individually randomized, the present study is a natural experiment with limited bias in group allocation: With 3-monthly intervals between weighing sessions, children were allocated by their birthday to receive their first vaccinations early or late between 3 and 5 months of age (Fig. 3). We therefore compared 3–5-month-old children who had received DTP (±OPV) vaccinations early with children who had not yet received these vaccinations. Since there were no healthy "unvaccinated" children after 6 months of age unless they had travelled, we censored follow-up of all children at 6 months of age (Fig. 3).

Sick children were not vaccinated, in the main analysis we therefore censored 'unvaccinated' children who attended a weighing session but did not receive a vaccination (Fig. 3). Since the censoring of sick children could have introduced a bias, we also conducted an intention-to-treat analysis in which the censored children were transferred to the DTP group. Hence, in this analysis we compared the mortality of the intended-DTP-vaccinated group and the not yet DTP-vaccinated group.

Children were included from 91 days of age if they had been examined in a weighing session before 91 days; if they were only seen in a weighing session after 3 months of age they were only included from the day seen. DTP was not administered elsewhere and the follow-up time of children was therefore counted as DTP-unvaccinated time in the survival analysis until BHP provided the vaccine. Time as DTP-unvaccinated also came from children who did not turn up at the weighing sessions between 3 and 5 months of age but had been seen before 3 months of age and therefore were part of the community cohort (Fig. 3). Hence, the DTP-vaccinated and DTP-unvaccinated children were all children from the same cohort of children born in Bandim and their allocation depended on the timing of their birth date, the timing of the weighing sessions and their travelling pattern. We

Please cite this article as: Mogensen, S.W., et al., The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natur..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.01.041

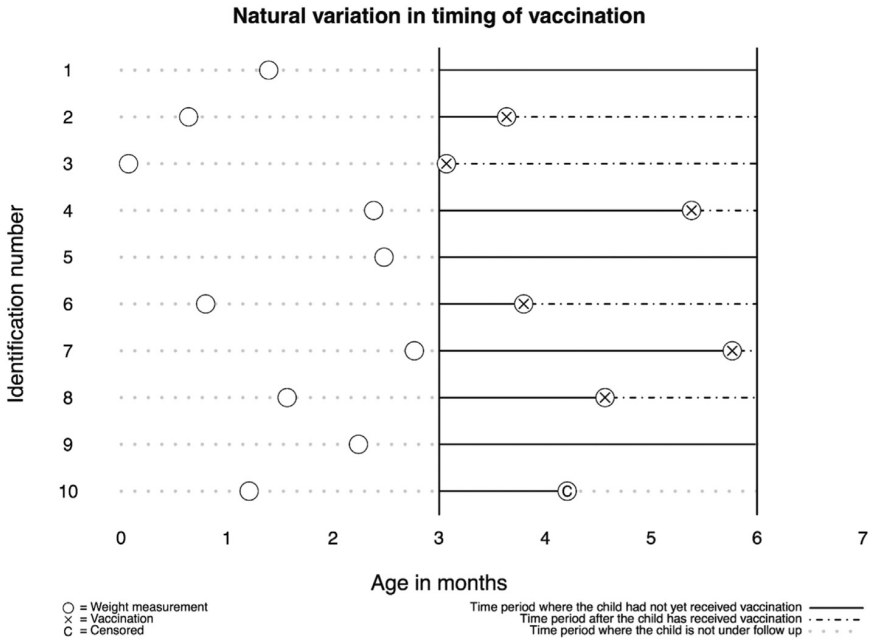

Fig. 3. Natural experiment study design. Note: Children were weighed every third month. After 3 months of age they received DTP and OPV on weighing days if they were healthy. Children who attended but were not vaccinated at a weighing session after 3 months of age were censored in the survival analysis comparing DTP-vaccinated and unvaccinated children.

compared the background factors for the children who were DTP vaccinated, attended a weighing session between 3 and 5 months but were not vaccinated and those who did not attend a weighing session (Table 2).

We also examined the mortality of children who due to logistic reasons had received DTP-only. Absences and travelling patterns are unlikely to differ between children who at their first vaccination had received DTP1 + OPV versus DTP1-only; these two groups were equally likely to receive subsequent vaccinations both with respect to timing of subsequent vaccinations and coverage (data available on request).

2.8. Statistical Methods

First possible enrolment date was June 2, 1981, when DTP and OPV vaccinations were introduced. Different vaccination groups were compared using a Cox proportional hazard model with age as underlying time.

Children were classified according to their most recent vaccination (Supplementary Table 1). We ignored BCG vaccinations in the main analysis because we gave few BCG vaccinations (Table 1) and some children had received BCG at the maternity ward without proper documentation as some children had a BCG scar but no vaccination card. To avoid survival bias, we used a landmark approach (Jensen et al., 2007); hence, a child's vaccination status was only updated from the day the information was collected. Due to the additional vaccination sessions organized by the expatriate nurse some "unvaccinated" children received a vaccine before the weighing session where they changed status to "vaccinated"; it is noted in the footnote to Table 3 how many had received such vaccinations. As a sensitivity analysis we also did an analysis including the additional vaccination sessions as landmarks. For the remainder of this paper, we will refer to these landmarks as vaccination-days-without-weighing.

The WHO z-score for weight-for-age was used to assess nutritional status. Control for sub-district, ethnic group and twinning did not change the results (data not shown). There was no obvious clustering

Table 2
Background factors children in the main analysis of vaccination and mortality between 3 and 5 months of age.

		DTP-vaccinated at 3–5 months	Attended weighing session at 3–5 months, not vaccinated	Did not attend weighing session at 3–5 months
Number		662	186	209
Male sex		52.1%	53.2%	54.1%
Twin		2.7%	2.2%	2.9%
Birth weight (SD)		3.23 (0.025)	3.28 (0.061)	3.22 (0.051)
Ethnic group				
• Pepel		46.8%	54.8%	45.0%
• Balanta		11.8%	13.4%	16.3%
• Other ethnic groups		41.4%	31.7%	38.8%
Mean weight-for-age z-score (SD) at examination before 3 months of age		−0.30 (0.037)	−0.34 (0.084)	−0.43 (0.066)
Follow-up time (person-years) between 3 and 5 months; [Median number of days of follow]	All time	135.5 [92]	36.8 [86]	47.4 [92]
	As DTP vaccinated	73.3	1.8	2.0
	As unvaccinated	62.2	35.1	45.4
Mean number (SD) of weighing sessions per year between 6 and 11 months of age		2.7 (0.03)	2.2 (0.07)	1.6 (0.08)

Table 3
Mortality rate and hazard rate (HR) for children from 3 months of age until first examination without vaccination or 6 months of age. Natural experiment.

Age group 3–5 months	Mortality rate (deaths/person-years)			HR (95% CI) for DTP vs unvaccinated
All				
Unvaccinated (N = 651)	4.5 (5/111.4)	DTP (±OPV) (N = 462)	17.4 (11/63.1)	5.00 (1.53–16.3)
		DTP only (N = 101)	35.2 (5/14.2)	10.0 (2.61–38.6)
		DTP + OPV (N = 361)	12.3 (6/48.9)	3.52 (0.96–12.9)
Girls				
Unvaccinated (N = 313)	1.9 (1/51.9)	DTP (±OPV) (N = 222)	13.3 (4/30.1)	9.98 (0.81–123.0)
		DTP only (N = 44)	16.2 (1/6.2)	12.0 (0.56–257.2)
		DTP + OPV (N = 178)	12.5 (3/23.9)	9.50 (0.73–124.0)
Boys				
Unvaccinated (N = 338)	6.7 (4/59.5)	DTP (±OPV) (N = 240)	21.2 (7/33.0)	3.93 (1.01–15.3)
		DTP only (N = 57)	49.8 (4/8.0)	8.93 (2.01–39.7)
		DTP + OPV (N = 183)	12.0 (3/24.9)	2.21 (0.44–11.0)

Notes: There were no deaths due accidents in this age group. BCG is disregarded in the analysis. Hence, the unvaccinated children have not received DTP, OPV or MV but may have received BCG. Of the 651 unvaccinated children, 219 received DTP and/or OPV before their first weighing examination. These children counted as 'unvaccinated' until their first weighing examination. Of the 462 children who received DTP (±OPV), 177 received an additional DTP or OPV before 6 months of age. The OPV-only is not presented in the table because there were no deaths and very little follow-up time in this age group.

of deaths and control for season and calendar time did not change estimates (data not shown).

There were 18 deaths between 3 and 5 months of age: 3 had cough and respiratory infections as the main symptom, 3 had fever (presumed malaria), 2 were due to diarrhea, 5 had diarrhea and vomiting, 1 was a sudden death, and 4 had no information on cause.

2.9. Ethics

The study of nutritional status was planned by SAREC (Swedish Agency for Research Collaboration with Developing Countries) and the Ministry of Health in Guinea-Bissau.

3. Results

Of 1356 children registered in Bandim and followed to 3 months of age (Fig. 2), 286 were never weighed, had no card or their card was lost. An additional 13 children had inconsistent information, vaccinations marked with a cross but without dates or were orphans. Hence, 1057 children were included in the study cohort. The median ages for DTP1 and OPV1 were 121 and 118 days, respectively (Table 1). The vaccination coverage at 6 months of age was 55% for DTP1; 3% got DTP3 (Table 1). Coverage for MV was only 6%. Of the DTP1, OPV1 and MV vaccinations noted on the BHP card 90–95% had been administered by the BHP.

For children examined after 91 days, a one-unit increase in w/a z-score was associated with an odds ratio of 1.07 (0.93–1.24) for receiving a vaccination at that weighing session.

3.1. Natural Experiment with 3–5-month-old Children

There were no marked differences in background factors for the three groups of children who were DTP vaccinated at 3–5 months of age, those who attended a weighing session but were not vaccinated, and those who did not attend a weighing session at 3–5 months of age (Table 2). Birth weight was similar in the three groups. Weight-for-age z-score before 3 months of age did not differ for the three groups (Table 2). Those who did not attend a weighing session at 3–5 months of age were significantly less likely to attend later weighing sessions during infancy, the mean number of visits being lower for those not attending than for those being DTP-vaccinated ($p < 0.001$) (Table 2); hence, they are likely to have travelled more than those who were DTP-vaccinated.

In the main experiment depicted in Fig. 3, DTP vaccination (±OPV) compared with 'DTP-unvaccinated' was associated with a HR of 5.00 (1.53–16.3) (Table 3); the HR was 9.98 (0.81–123) for girls and 3.93 (1.01–15.3) for boys. If we also included vaccinations given on vaccinations-days-without-weighing in the landmark analysis, DTP (±OPV) compared with unvaccinated was associated with a HR of 3.90 (1.20–12.3). When DTP had been given alone without OPV the HR was 10.0 (2.61–38.6) (Table 3). The difference between DTP-only children and DTP-plus-OPV does not reflect differences in follow-up and other vaccinations since the time to DTP2 and prevalence of DTP2 was the same for DTP-only and DTP-plus-OPV vaccinated children (data not shown). If we excluded the 269 children who may have been BCG vaccinated results were similar (Supplementary Table 2).

If the analysis was conducted as an intention-to-treat analysis in which the children weighed but not vaccinated were not censored but transferred to the DTP group, the intended-DTP-vaccinated group had a HR of 3.92 (1.20–12.8) compared with the not-yet vaccinated group (Supplementary Table 3).

3.2. Secondary Analyses

Since the introduction of DTP and OPV apparently was associated with increased mortality, we examined what happened to infant mortality from 3 to 12 months of age after the introduction of these vaccines. The mortality rate for all 3–11 months old children increased 2-fold (HR = 2.12 (1.07–4.19)) from 1980, before vaccinations, to 1982–1983, after the introduction of DTP and OPV (Table 4).

4. Discussion

4.1. Main Observations

DTP vaccinations were associated with increased infant mortality even though there was no vaccine-induced herd immunity. When unvaccinated controls were normal children who had not yet been eligible for vaccination, mortality was 5 times higher for DTP-vaccinated children. Co-administration of OPV with DTP may have reduced the negative effects of DTP.

4.2. Strength and Weaknesses

The present analysis assessed DTP and child survival in a "natural experiment" in which the children were allocated by the timing of their birth and community weighing sessions and the group allocation was therefore not influenced by the usual selection biases to the same extent as most other studies of DTP (Aaby et al., 2016). To assure that the censoring from the main analysis of children who were not vaccinated had not produced the unexpected strong result we made an intention-to-treat analysis but this did not change the result. If anything the unvaccinated children had slightly worse nutritional status before 3 months of age than the children who were subsequently DTP vaccinated ($p = 0.09$) (Table 2); the unvaccinated children travelled more than the DTP vaccinated children. These biases would tend to favor rather than increase mortality in the DTP group and the

Please cite this article as: Mogensen, S.W., et al., The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natur..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.01.041

Table 4
Mortality rates (deaths/100 person-years) between 3 and 11 months of age by study year.

Mortality rate	1980	1981	1982	1983	HR (95% CI) for 1982–1983 versus 1980
Children aged 3–11 months	4.7 (10/211.8) (N = 547)	7.2 (18/250.8) (N = 678)	8.0 (19/237.1) (N = 632)	12.1 (30/247.5) (N = 638)	2.12 (1.07–4.19)

Notes: Event recorded as accidents were not removed from this analysis.

estimates from the natural experiment may therefore still be conservative.

The estimated effects of DTP and OPV are unlikely to have been influenced by other vaccinations since very few had received other vaccines; if the children who may have received BCG were censored in the analysis the result was essentially the same (Supplementary Table 2).

The 3-monthly community examinations assured that we had follow-up information for all children and relatively accurate information on the time of death. Some children were excluded because a BHP card could not be found and we did not know whether they had been vaccinated or were travelling. Most likely, BHP cards may never have been made because the child was not coming for examination, or the card may have disappeared at community examinations, at the later handling of BHP cards by field workers or data entry clerks, or due to mice. However, the few missing cards are unlikely to have affected the main analysis as the mortality rate in this group was similar to the general mortality rate (Fig. 2).

To assure comparability of vaccinated and unvaccinated groups, also with respect to travelling, we included only children who had been weighed in Bandim in connection with the 3-monthly community examinations. This meant that children who mostly stayed outside the area were not included in the analysis; these children had no access to community vaccinations and they lived elsewhere where the mortality risk might have been quite different, e.g. due to a higher risk of malaria infection.

The present study was not a planned trial. The study would have been a cleaner natural experiment if vaccinations had only been administered at the weighing sessions. However, the expatriate nurse did organize additional vaccinations and some 'unvaccinated' children had therefore already received a vaccination before coming for a weighing session. These 'misclassifications' do not explain the increased mortality in the DTP group. The estimate for DTP-vaccinated (±OPV) compared with DTP-unvaccinated children was 4-fold higher mortality when we included these additional landmarks in the analysis.

4.3. Comparison with Previous Studies of DTP and OPV

There is only one other study of the introduction of DTP. In rural Guinea-Bissau, DTP (±OPV) was associated with 2-fold higher mortality (Aaby et al., 2004a). All studies that documented vaccination status and followed children prospectively indicate that DTP has negative effects; a meta-analysis of the eight studies found 2-fold higher mortality for DTP-vaccinated compared with DTP-unvaccinated, mostly BCG-vaccinated controls (Aaby et al., 2016) (Appendix A).

The negative effect of DTP was much worse in this natural experiment than has been reported in previous studies of DTP. This is presumably due to the "unvaccinated" control children in previous studies having been a frail subgroup too frail to get vaccinated. Previous studies have not been able to compare DTP-vaccinated children with "normal" controls. Hence, most previous studies have probably underestimated the negative effect of DTP.

The potentially differential effects of DTP and OPV have only been examined in few studies. However, we have recently been able to document marked beneficial NSEs of OPV. In an RCT, OPV at birth (OPV0) reduced infant mortality by 32% (0–57%) before the children received campaign-OPV (Lund et al., 2015). In Bissau campaign-OPV reduced the mortality rate by 19% (5–32%) (submitted). When DTP was missing for several months in Bissau, we showed that the all-cause case-fatality at the pediatric ward was 3-fold lower if the children had OPV-only as their most recent vaccination rather than the recommended combination of DTP and OPV (Aaby et al., 2004b). Thus, OPV may have modified the negative effect of DTP.

This pattern was also seen when DTP was first introduced in the rural areas of Guinea-Bissau in 1984 (Aaby et al., 2004a). OPV was not used the first year and the HR for DTP versus unvaccinated was 5.00 (0.63–39.7). In the period from 1985 to 1987, when DTP and OPV were nearly always administered together, the MRR was 1.90 (0.91–3.97). In the present study, the hazard ratio was 10.0 (2.61–38.6) for DTP-only but 3.52 (0.96–12.9) for children who received DTP and OPV simultaneously (Table 3). Based on these two studies of the introduction of DTP, the HR compared with DTP-unvaccinated children was significantly different for children who had received DTP-only (HR = 8.14 (2.63–15.2)) and for children who received both DTP and OPV (HR = 2.21 (1.16–4.19)) (test of interaction, p = 0.049). Hence, simultaneous administration of DTP and OPV may have alleviated the negative non-specific effect of DTP.

5. Conclusions

DTP was associated with 5-fold higher mortality than being unvaccinated. No prospective study has shown beneficial survival effects of DTP. Unfortunately, DTP is the most widely used vaccine, and the proportion who receives DTP3 is used globally as an indicator of the performance of national vaccination programs.

It should be of concern that the effect of routine vaccinations on all-cause mortality was not tested in randomized trials. All currently available evidence suggests that DTP vaccine may kill more children from other causes than it saves from diphtheria, tetanus or pertussis. Though a vaccine protects children against the target disease it may simultaneously increase susceptibility to unrelated infections.

The recently published SAGE review called for randomized trials of DTP (Higgins et al., 2014). However, at the same time the IVIR-AC committee to which SAGE delegated the follow-up studies of the NSEs of vaccines has indicated that it will not be possible to examine the effect of DTP in an unbiased way. If that decision by IVIR-AC remains unchallenged, the present study may remain the closest we will ever come to a RCT of the NSEs of DTP.

Funding

The present study and cleaning of the original data was supported by a common grant from DANIDA and the Novo Nordisk Foundation (FU-11-551). The work on non-specific effects of vaccines has been supported by the Danish Council for Development Research, Ministry of Foreign Affairs, Denmark [grant number 104.Dan.8.f.], Novo Nordisk Foundation and European Union FP7 support for OPTIMUNISE (grant: Health-F3-2011-261375). CSB held a starting grant from the ERC (ERC-2009-StG-243149). CVIVA is supported by a grant from the Danish National Research Foundation (DNRF108). PA held a research professorship grant from the Novo Nordisk Foundation.

Please cite this article as: Mogensen, S.W., et al., The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natur..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.01.041

Conflict of Interest

Nothing to declare

Contributions

CSB and PA proposed the study. PA collected the original data. AR is responsible for the demographic surveillance system. SWM and PA cleaned the data. SWM and AA conducted the statistical analyses. The first draft was written by PA; all authors contributed to the final version of the paper. PA and SWM will act as guarantors of the study.

Independence

The funding agencies had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

Data Sharing

Through request to the authors

Appendix A. The DTP Controversy

The issue of DTP vaccination and child mortality in high mortality areas was raised 15 years ago when a study from rural Guinea-Bissau showed 1.84-fold higher mortality for children who had received DTP1 vaccination (Aaby et al., 2016; Kristensen et al., 2000). All subsequent prospective studies have supported a negative effect (Aaby et al., 2016). Furthermore, DTP may have a negative effect when given simultaneously with or after MV (Aaby at el., 2003b, 2012). For example, the negative effect of high-titer measles vaccination (HTMV) in girls, which led to the global withdrawal of HTMV, was due to DTP being administered after MV because HTMV had been given early at 4–5 months of age (Aaby et al., 2003b).

DTP has not been shown to have beneficial effects in RCTs or natural experiments. The current policy for DTP has only been examined by reanalyses of existing data sets collected for other purposes. All such studies have had methodological problems related to different forms of frailty and survival bias (Aaby et al., 2012). These studies have updated follow-up time for DTP-vaccinated children who survived but children who died without their vaccination status being documented were classified as "unvaccinated". Such procedures give a misleading high mortality rate in the unvaccinated group, and the comparison of DTP-vaccinated survivors and "unvaccinated" children will therefore give a beneficial estimate for DTP (Aaby et al., 2016). If the mortality rate of unvaccinated children is unnaturally increased, the HR of unvaccinated children versus children who have received at least one vaccine may indicate how much bias there might be in the study, and we have called this HR the "bias-index". All studies with prospective follow-up have had a bias index below 2.0 (Aaby et al., 2016); in the present study the bias index was 0.41 (0.15–1.15) in the 3–5 months age group (Supplementary Table 2). In studies with survival bias and unnaturally high mortality in the unvaccinated group, the bias index has been 3–8 times higher (Aaby et al., 2016).

SAGE recently reviewed the potential NSEs of BCG, MV and DTP (Higgins et al., 2014; Strategic Advisory Group of experts on Immunization, 2014). The reviewers indicated that the majority of studies showed a deleterious effect of DTP but they concluded that the results were inconsistent because two studies showed a beneficial effect. The beneficial effect in these studies was not surprising because the mortality rate in the unvaccinated group was unnaturally high, and the bias index was 3.40 (2.93–3.95) and 7.52 (5.15–10.97), respectively (Aaby et al., 2016).

SAGE's working group on non-specific effects of vaccines further emphasized that the overall effect remains unclear because DTP has been given in combination with other vaccines and under circumstances where the burden of the target diseases has been reduced to a very low level. However, several previous studies have shown that the negative effect of DTP-plus-OPV was not due to OPV (Aaby et al., 2004a,b, 2012). OPV has probably reduced the overall negative effect of DTP. Previous studies have indicated that DTP (±OPV) was associated with a 2-fold higher mortality than DTP-unvaccinated children (Aaby et al., 2016). Since pertussis did not account for >5–6% of infant deaths in the only existing African study of the impact of pertussis on child mortality (Mahieu et al., 1978), it is not surprising that DTP is also associated with a strong negative effect prior to vaccine-induced herd immunity (Aaby et al., 2012).

Appendix B. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2017.01.041.

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Please cite this article as: Mogensen, S.W., et al., The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natur..., EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.01.041

EXHIBIT 30

VIEWPOINT

The non-specific and sex-differential effects of vaccines

Peter Aaby, Christine Stabell Benn, Katie L. Flanagan, Sabra L. Klein, Tobias R. Kollmann, David J. Lynn and Frank Shann

Abstract | The textbook view of vaccination is that it functions to induce immune memory of the specific pathogen components of the vaccine, leading to a quantitatively and qualitatively better response if the host is exposed to infection with the same pathogen. However, evidence accumulated over the past few decades increasingly suggests that vaccines can also have non-specific effects on unrelated infections and diseases, with important implications for childhood mortality particularly in low-income settings. Furthermore, many of these non-specific effects, as well as the pathogen-specific effects, of vaccines show differences between the sexes. Here, members of the Optimmunize consortium discuss the evidence for and potential mechanisms of non-specific and sex-differential effects of vaccines, as well as their potential policy implications. Given that the non-specific effects of some vaccines are now being tested for their ability to protect against COVID-19, the authors also comment on the broader implications of these trials.

For which vaccines and in which clinical contexts are non-specific effects of vaccines important? Are there differences between live and non-live vaccines? Can effects be both beneficial and harmful?

David J. Lynn. In my opinion, there is now substantial evidence that several different vaccines can have non-specific effects (NSEs; also known as heterologous effects) on immune responses, morbidity and mortality. A 2016 systematic review sponsored by the World Health Organization (WHO) concluded that the bacillus Calmette–Guérin (BCG) vaccine, diphtheria–tetanus–whole cell pertussis (DTPw) vaccine and measles vaccine were associated with effects on mortality that were "more than would be expected through their effects on the diseases they prevent"[1].

Katie L. Flanagan and Frank Shann. Theoretically, any vaccine can have NSEs because microbial antigens in vaccines stimulate an early innate immune response through pattern recognition receptors on immune cells. Some of the most compelling evidence for NSEs comes from randomized and observational studies in low-income settings[2,3], where live vaccines have been shown to have beneficial NSEs on all-cause childhood mortality but non-live vaccines might have detrimental NSEs. Combinations of live and non-live vaccines given at the same time have variable effects, with the NSEs being determined largely by the most recent vaccine administered[1,4].

Peter Aaby and Christine Stabell Benn. So far, all vaccines tested in epidemiological studies have shown important NSEs on child survival in low-income countries. Observations support a pattern whereby live vaccines (such as smallpox vaccine, BCG vaccine, measles vaccine and oral polio vaccine (OPV)) increase resistance to vaccine-unrelated infections, mainly pneumonia and sepsis, and therefore reduce overall mortality more than would be expected from preventing the vaccine-targeted infections. Hence, these live vaccines have a double benefit in that they prevent both target and non-target infections[4]. By contrast, non-live vaccines (such as DTP vaccine, the pentavalent vaccine for DTP, hepatitis B virus (HBV) and *Haemophilus influenzae* type b, inactivated polio vaccine, single HBV vaccine, the RTS,S/AS01 malaria vaccine, and the H1N1 influenza vaccine) seem to increase susceptibility to vaccine-unrelated infections, particularly in females[4]. Hence, non-live vaccines may have beneficial effects in preventing the target infection but negative effects by enhancing susceptibility to non-target infections. In epidemiological studies, the negative effects seem to be more pronounced than the beneficial effects, with the net effect being increased overall mortality for females. Fortunately, the most recent vaccine to be administered has the strongest NSEs, and so the negative effects of non-live vaccines can be at least partly abrogated by providing a live vaccine after the non-live vaccine.

K.L.F. and F. S. Evidence from multiple observational studies suggests that non-live vaccines, such as DTP vaccine, may increase all-cause mortality, especially in girls, because there is an increased number of deaths from pneumonia and sepsis that outweighs the reduction in deaths from diphtheria, tetanus and pertussis[5]. In a large randomized trial, the non-live RTS,S/AS01 malaria vaccine doubled all-cause mortality in girls in Africa[6].

Because of the potential for large effects on all-cause mortality, the NSEs of vaccines are most important in children younger than 5 years in high-mortality countries, where the vaccine schedule is for children to be immunized with live BCG vaccine and OPV at birth, non-live DTP vaccine at 6, 10 and 14 weeks of age and live measles vaccine at 9 months of age. DTP vaccine is often given with non-live inactivated polio vaccine, HBV vaccine, *H. influenzae* type b vaccine and pneumococcal conjugate vaccine, as well as live OPV and rotavirus vaccine.

D.J.L. More than ten studies have found that non-live vaccines (such as DTPw vaccine) are associated with increased all-cause mortality, particularly in girls[7]. It is important to note that the DTP vaccine is highly effective against the targeted diseases and, so far, the studies reporting deleterious NSEs have been observational and have been assessed to be at a high risk of bias. Perhaps understandably, in an age

of increasing vaccine hesitancy, many in the research community are resistant to considering that such deleterious NSEs could exist. However, if they do, there are potentially relatively easy solutions to mitigate these effects: substantial evidence is mounting that any deleterious effects of non-live vaccines can be mitigated by changing vaccine schedules so that a live vaccine is administered last[8].

Have there been similar observations in low-income and high-income settings?

K.L.F. and F.S. NSEs of vaccines have been described in both low-income and high-income settings. In low-income settings, as discussed already, much of the evidence relates to changes in all-cause mortality[2,3], although morbidity effects have also been widely described. In high-income countries, several studies from Europe and the USA suggest that admission to hospital for unrelated infections may be reduced by live measles vaccine and BCG vaccine but increased by non-live DTP vaccine[9,10]. Evidence from high-income countries also indicates that BCG vaccination may reduce the severity of allergy[11], malignancy[12–14], diabetes[15] and Alzheimer disease[16,17].

D.J.L. Most studies so far have been conducted in infants in low-income countries, although there have also been some studies in infants in high-income countries[18]. One study in Spain found that hospitalization rates owing to respiratory infections, not attributable to tuberculosis, were significantly lower in BCG-vaccinated children[18]. The hospitalization rate owing to sepsis in infants younger than 1 year was also significantly lower after BCG vaccination. Furthermore, three randomized controlled trials in low-birthweight neonates in Guinea-Bissau reported beneficial NSEs of BCG immunization[19]. However, another study, in Denmark, found that BCG vaccination at birth did not reduce the risk of hospitalization for somatic acquired disease (in other words, disease excluding injuries)[20]. It is not entirely clear why this study failed to detect a beneficial NSE of BCG vaccine, but it may relate to this measure of disease not being specific enough, differences in exposure between countries or differences in genetics or maternal immunity.

There have also been a small number of studies in adults in high-income countries. For example, a randomized placebo-controlled trial found that BCG vaccination protected Dutch adult volunteers against experimental infection 1 month later with an attenuated yellow fever virus vaccine strain[21], and BCG vaccination once a month for three consecutive months was found to significantly reduce acute upper respiratory tract infection in older individuals[22].

P.A. and C.S.B. We have been able to repeat the original observations from Guinea-Bissau of NSEs of vaccines in other low-income countries (such as Bangladesh, the Gambia, Ghana, India, Kenya, Malawi, Senegal and Sudan)[23] and also in a high-income setting in Denmark. In Denmark, child mortality is not a suitable outcome and we have therefore examined NSEs by testing the effect of vaccines on hospital admissions of children for non-target infections[9]. Furthermore, in historical studies of BCG and smallpox vaccines, we have been able to find associations between these live vaccines and decreased adult mortality[24]. The observations from other low-income countries seem to confirm the differential patterns of beneficial and harmful NSEs for live vaccines and non-live vaccines, respectively[4,23]. In Denmark, it has been easier to test the potential beneficial NSEs of live vaccines; so far, we have found beneficial NSEs of measles–mumps–rubella (MMR) vaccine, OPV, BCG vaccine and smallpox vaccine[9,24]. The MMR vaccine has been associated with decreased hospital admissions for respiratory infections also in the USA, Italy and the Netherlands[4]. The data from Denmark also indicate that giving the non-live DTP vaccine after the MMR vaccine may increase the risk of hospital admission by cancelling out the positive effect of the MMR vaccine[9].

Can you comment on the differences between males and females for these non-specific effects, as well as for vaccine responses generally?

Sabra L. Klein Let us begin with the vaccine-specific responses. Females of diverse ages typically develop a greater

The contributors

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antibody response (which is a primary correlate of protection) and report more adverse reactions to vaccines than do males[25]. For example, after vaccination against influenza, yellow fever, rubella, measles, mumps, hepatitis A, hepatitis B, herpes simplex type 2, rabies, smallpox and dengue viruses, protective antibody responses are twice as large in adult females as in adult males[25].

Regarding sex differences in NSEs, many studies have documented detrimental, female-biased effects. There is a growing body of literature showing that infant girls have increased mortality after receiving certain vaccines. For example, in the 1980s, when the high-titre measles vaccine (HTMV) was introduced to prevent measles infection in children younger than 9 months, there was a twofold increase in all-cause mortality in girls, but no increase in boys, in Guinea-Bissau[26]. It was subsequently determined that the increased mortality occurred only among girls who had received non-live DTP vaccine after HTMV, and not among girls who had received HTMV after their last dose of DTP vaccine[27]. The interaction between HTMV and DTP vaccine may have caused NSEs on all-cause mortality in girls, but not boys. Evidence from multiple studies of non-live vaccines, including DTP vaccine and the inactivated polio vaccine, shows that they have greater detrimental NSEs for girls than for boys[27,28]. More recently, increased female mortality after receipt of the RTS,S/AS01 malaria vaccine was reported in post hoc analyses of data[6]. The WHO has speculated that the increased mortality in girls was 'largely due to the low female mortality in the control arm' and 'could be due to chance', despite the *P* value of 0.0006 for girls, and a mortality after RTS,S/AS01 vaccination of 2.4% in girls compared with 1.8% in boys[29]. This vaccine has been rolled out into routine vaccination schedules without further studies to determine whether the RTS,S/AS01 vaccine does indeed increase mortality in girls. In keeping with NSEs disproportionately affecting females, data pertaining to the NSEs of BCG vaccine show that the beneficial effects on all-cause mortality are greater for girls than for boys younger than 5 years[3].

K.L.F. and F.S. Most studies have not analysed vaccine responses for sex differences but, when they have, sex differences in immunogenicity have been found for almost every licensed vaccine[30,31]. Generally, females have greater antibody responses to immunization, but sex differences in terms of cellular responses to immunization have rarely been investigated and are inconsistent[30]. Females also have more adverse events following immunization[25], perhaps because of biological sex differences, although reporting differences (a gender effect) may also have a role. Females are generally more susceptible than males to the NSEs of vaccines; for example, females benefit more from the beneficial NSEs of measles vaccine[32] but experience greater adverse NSEs following immunization with DTP vaccine[3,4,7].

P.A. and C.S.B. One of the first major discoveries in relation to NSEs was that HTMV was associated with twofold increased female mortality in Guinea-Bissau and Senegal. Even though HTMV protected against measles, the negative NSEs meant that there was an overall increase in female mortality[27]. Potential sex-differential effects of vaccines have therefore been a focus in the study of NSEs. In West Africa, where there were no major sex differences in terms of treatment or mortality in the prevaccine era, it has been a strong indication of NSEs when vaccines have sex-differential effects on overall mortality[4,23]. The live measles, BCG and smallpox vaccines have had stronger beneficial NSEs for females than for males. By contrast, the six non-live vaccines tested (DTP vaccine, the pentavalent vaccine for DTP, HBV and *H. influenzae* type b, inactivated polio vaccine, single HBV vaccine, the RTS,S/AS01 malaria vaccine and the H1N1 influenza vaccine) have all been associated with higher female mortality than male mortality[4]. This is a worrying finding, and nobody has been able to suggest a bias that might explain it. The effects are not trivial — data indicate that on the scale of Africa, hundreds of thousands of females per year may die owing to the negative NSEs of non-live vaccines.

Q *What do we know and not know about the mechanism of these non-specific and sex-differential effects?*

D.J.L. The mechanisms behind the NSEs of vaccines are incompletely understood but are likely to involve a combination of different effects on the innate and adaptive immune responses, heterologous T cell responses and influences on responses to other subsequent immunizations. One mechanism that we now know quite a lot about is trained immunity — a programme of innate immune memory that can be induced by vaccination, leading to the epigenetic and metabolic reprogramming of innate immune cells such as monocytes and natural killer cells[14]. However, most of what we know regarding trained immunity comes from studies of BCG vaccine and we know far less about whether other vaccines also induce trained immunity and, if so, whether they do so differently to BCG vaccine. Further research is therefore urgently needed to understand the potential immunological mechanisms that could explain the differential NSEs of live and non-live vaccines. This is an area of research that my laboratory is investigating.

P.A. and C.S.B. There are at least two mechanisms that have been documented: trained immunity and emergency granulopoesis (see the response from Tobias R. Kollmann)[33,34]. In addition, heterologous T cell reactivity may also have a role[33]. One very important new observation from epidemiological studies is that the beneficial NSEs of live vaccines such as measles vaccine and BCG vaccine become more pronounced if the vaccine is administered in the presence of pre-existing immunity[4,35], for example, if the mother was previously vaccinated with the same vaccine (vertical boosting) or if the child is vaccinated for a second time with a live vaccine (horizontal boosting)[36]. We are currently exploring in mechanistic studies why boosting results in amplification of the beneficial NSEs for the child.

Tobias R. Kollmann. We recently discovered one of the mechanisms by which BCG vaccine can protect newborns from infection[34]. Within hours of administration, BCG vaccine induces production of the growth factor granulocyte colony-stimulating factor (G-CSF), which in turn activates a process known as emergency granulopoiesis that increases the production of neutrophils ready to fight infectious threats. The kinetics of this rapid response to BCG vaccine fit perfectly with the epidemiological observations that BCG vaccine can protect newborns within just days of administration[19,37].

K.L.F. and F.S. Although the induction of vaccine-specific antibodies is generally considered to be a reliable surrogate marker of a protective vaccine response, we still do not understand the mechanisms for the specific effects of many vaccines. For example, we do not know how BCG vaccine protects against tuberculosis. However, we are beginning to understand the immunological mechanisms accounting for the NSEs of some vaccines. Human studies have shown that BCG vaccine enhances innate immunity

within days after immunization by epigenetic reprogramming that leads to trained immunity[14]. By contrast, immunization with DTP vaccine has been shown to suppress innate immunity and induce T cell anergy in female infants but not male infants, and this could explain how DTP vaccine might increase susceptibility to infections in females[38]. Theoretical biological mechanisms for sex differences in the NSEs include the opposing immunological effects of male and female sex hormones, the multiple X-linked (and some Y-linked) immune response genes and microRNAs, and sex differences in the microbiota, although causal links have not yet been confirmed[30,31].

S.L.K. So far, we do not know the mechanisms of sex differences in NSEs. We do, however, have data pertaining to sex differences in vaccine-specific immunity. Following vaccination with whole inactivated influenza virus, influenza trivalent inactivated vaccine or influenza quadrivalent inactivated vaccine, adult female mice generate greater quantity and quality of influenza-specific antibodies than do adult male mice[39–41]. Antibodies derived from vaccinated female mice are better at protecting naive mice (both male and female) than are antibodies from vaccinated male mice, and this protection is associated with increased antibody specificity and avidity for the H1N1 influenza virus[41]. The Toll-like receptor 7 gene (*Tlr7*) is encoded on the X chromosome and is expressed at a higher level by B cells from vaccinated female mice than by B cells from vaccinated male mice, which is associated with reduced DNA methylation in the *Tlr7* promoter region in female mice[41]. Data from humans and mice further show that the female bias of antibody responses to influenza vaccination is associated with circulating oestradiol levels, such that greater concentrations of oestradiol result in greater production of antibody to the vaccine antigen[42]. Taken together, both the expression of X-linked genes and increased levels of oestradiol are associated with improved vaccine-induced immunity in females. We now need to determine how to use this information to improve vaccine-induced immunity in males (for example, through the use of TLR7 adjuvants).

Should we consider changes to vaccine types, doses or schedules on the basis of these effects?

D.J.L. As discussed already, live vaccines have generally been associated with beneficial NSEs and non-live vaccines have been associated with potentially deleterious NSEs in some observational studies. There is also evidence that any deleterious effects of non-live vaccines can be mitigated by changing vaccine schedules such that a live vaccine is administered last[8]. In my opinion, however, it is too early to recommend any changes to schedules until further high-quality randomized controlled trials are conducted to assess the effects of such changes. Given the mounting data, these studies need to be urgently supported and conducted.

P.A. and C.S.B. We believe that there are several immediate considerations as a result of these NSEs. First, all children in Africa should receive the BCG vaccine at birth. This has been shown to reduce neonatal mortality by more than one-third[4], but currently less than 50% of children in Africa receive BCG vaccine in the first month of life. We should promote the use of BCG vaccine as a non-specific vaccine to boost the baby's immune system. Second, we should roll back the plan to phase out OPV. Vaccination campaigns with OPV have had a major role in reducing child mortality in low-income countries, with vaccination of only 68 children being needed to save the life of 1 child[43]. Hence, the benefits of OPV outweigh the minor risk of vaccine-derived polio infection. Third, we should ensure that children are given a live vaccine shortly after receiving non-live vaccines. For example, all studies show that the administration of DTP vaccine after measles vaccine is associated with higher female mortality than is administration of measles vaccine after DTP vaccine[23]. This might also explain why live HTMV had been associated with increased female mortality: HTMV was given so early in life that most children received DTP vaccine after HTMV[27].

F.S. There is already far greater evidence of harm from the NSEs of DTP vaccine in low-income countries than there was of harm from the rotavirus vaccine RotaShield when its use was suspended in the USA in 1999 after only 15 cases of intussusception. However, it is essential that we continue to immunize children against diphtheria, tetanus and pertussis. In high-mortality countries where BCG vaccine is given routinely at birth, DTP vaccine and other non-live vaccines could be given safely at 6, 10 and 14 weeks of age if a second dose of live BCG vaccine were to be given at the same time as the last priming dose of DTP vaccine at 14 weeks. After BCG vaccine administration at 14 weeks of age, I believe that no non-live vaccines should then be given to children younger than 5 years in high-mortality countries without robust evidence of safety from randomized trials. It has been estimated that this change in policy could save approximately 1 million lives every year[8].

What more do we need to know for these non-specific and sex-differential effects to be more widely accepted and the implications to be more widely considered?

D.J.L. There is still much to be understood regarding the NSEs and sex-differential effects of vaccines. We currently do not fully understand the mechanistic basis as to why non-live vaccines may be associated with deleterious NSEs, whereas live vaccines are associated with beneficial NSEs. We also do not understand the mechanisms through which changes to the order in which vaccines are administered may alter these NSEs. We also urgently need large, well-funded and international randomized controlled trials to confirm the data suggesting that non-live vaccines are associated with detrimental NSEs and to support any changes to vaccine schedules. Importantly, the international vaccine community should not be afraid to consider that there may be both beneficial and detrimental NSEs of certain vaccines. We are acutely aware of the potential for research in this area to be unintentionally or wilfully misinterpreted by individuals with antivaccine agendas. Understanding the NSEs of vaccines may result in exciting new beneficial interventions for unrelated infectious diseases and for non-infectious diseases such as allergy, asthma and even diabetes, while at the same time allowing us to mitigate against any potential risks.

P.A. and C.S.B. The argument can be split in two. First, do vaccines have only specific effects? The answer is clearly no! There is overwhelming evidence that vaccines affect the immune system more generally. Epidemiologically, we see effects of vaccines that cannot be explained by their specific effects[4]. Immunologically, we see that vaccines leave long-lasting imprints on the immune system that alter responses to heterologous challenges[33,34]. Second, can we predict the NSEs of any vaccine? We have seen repeatable patterns — for example, that live vaccines are associated with beneficial NSEs and non-live vaccines are associated with negative NSEs for females[4] — that made it possible to predict that the RTS,S/AS01 vaccine would have negative NSEs for

females[4]. This prediction indicates that we are on the right track with regard to our current thinking about NSEs. To move forward, we need to continue to make predictions and test them to refine our knowledge of the principles of NSEs.

F.S. We urgently need a trial in which children are randomized to receive or not receive a second dose of BCG vaccine with the last priming dose of DTP vaccine at 14 weeks of age in a high-mortality country, with a comparison of mortality from 14 weeks to 9 months of age (when measles vaccine is given)[8]. The same cohort of children should then be randomized again in the second year of life to receive or not receive the booster dose of DTP vaccine (which is recommended by the WHO but is not currently given in many countries, especially in countries in Africa, where it is not included in national vaccination schedules), with comparison of mortality from randomization to 3 years of age[8]. This would tell us whether DTP vaccine increases all-cause mortality and whether a second dose of BCG vaccine can ameliorate any harmful NSEs of DTP vaccine.

K.L.F. and F.S. We recommend using a systems vaccinology approach to elucidate the mechanisms of the NSEs of vaccines. Transcriptomics, epigenomics, proteomics and metabolomics will reveal the complex immunological pathways that are influenced by immunization[44]. This approach might identify the immunological correlates of both the beneficial NSEs and the harmful NSEs of vaccines and could be used to improve the design of future vaccines, adjuvants and immunomodulatory strategies.

S.L.K. We need more information on the mechanisms of NSEs. To address mechanisms, we need models, both animals and primary cell culture systems, that can be used to test the NSEs in both males and females. We also need more research into the sex differences in vaccine-specific immunity, with more data from diverse vaccine platforms. At the Optimmunize meeting held in February 2020, data on sex-differential effects were presented for vaccines that protect against influenza virus, rabies virus and even simian immunodeficiency virus.

T.R.K. The real hurdle before us is not so much the lack of knowledge but more so the willingness of the 'gatekeepers' of vaccination programmes around the world to even consider that vaccines might impact the host beyond the expected pathogen-specific effects. For example, the rapidity of BCG-mediated protection from newborn infection has been used to call into question the biological plausibility of NSEs of vaccines, but we now know that vaccines can have such rapid effects, for example, through emergency granulopoiesis[34]. The protective power of the immune system rests — at least in part — on its ability to take into account a multitude of signals, including non-specific, pathogen-agnostic effects, and to do so very, very fast.

Q At the time of going to press, with the COVID-19 pandemic dominating headlines around the globe, how do you think these non-specific and/or sex-differential effects of vaccines might be relevant to efforts to combat COVID-19?

D.J.L. The best hope for tackling the COVID-19 pandemic is a specific vaccine that provides direct protection against the virus. Efforts to develop such a vaccine are proceeding around the world at a breakneck pace, but an effective vaccine is still likely to be at least 12 months away. In the interim, several groups around the world have hypothesized that certain existing live vaccines, such as BCG vaccine, may provide non-specific protection against COVID-19 or reduce the severity of its symptoms. As discussed here, BCG vaccine is associated with non-specific protection against respiratory and viral infections in other contexts, and can non-specifically boost innate immune responses. Several epidemiological studies have also emerged as preprints suggesting that countries with routine BCG immunization programmes have lower case fatality rates for COVID-19 than countries that do not routinely use BCG vaccine[45–47]. However, it is important to note that such studies provide correlations not causation, and it is inherently difficult to account for all potential confounders, particularly given that the case statistics in each country are still highly uncertain. Nevertheless, several randomized controlled trials are now under way around the world to definitively assess whether BCG vaccine can provide non-specific protection against COVID-19. These studies include the BRACE trial ('BCG vaccination to reduce the impact of COVID-19 in Australian healthcare workers following coronavirus exposure'), which my laboratory is helping to roll out in South Australia. In this trial, thousands of health-care workers at hospitals around Australia are being randomized to receive BCG vaccine or not receive it, and we will assess whether those in the BCG vaccination arm are better protected against or have reduced severity of COVID-19. The world will be watching the outcome of these trials with significant interest, not only for an effect against COVID-19 but also as a demonstration of the potential importance of NSEs of vaccines more generally. Until then, however, BCG vaccine should be used only for its intended purpose of protecting infants globally against tuberculosis.

K.L.F. and F.S. Yellow fever virus, murine Mengo virus (also known as encephalomyocarditis virus) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are all single-stranded positive-sense RNA viruses. As the BCG vaccine has been shown to reduce the severity of infection with yellow fever virus and Mengo virus[21,48], this suggests that administration of BCG vaccine might reduce the severity of COVID-19 (REF.[21]). However, there is no evidence as yet of a protective effect from administration of a live vaccine during an infection with SARS-CoV-2, and it is unlikely that BCG vaccine given many decades ago will influence the response to SARS-CoV-2 now. If BCG vaccine is used, we think that it should be given with or after any non-live vaccine such as influenza vaccine[2,4]. If other live vaccines such as measles vaccine or OPV are used, they should be given at least 4 weeks after any non-live vaccine[32].

T.R.K. Given that there is no other form of intervention currently available, it would be foolish not to at least consider whether the fast and broadly protective NSEs of live vaccines (such as BCG vaccine, OPV and measles vaccine) might provide protection from severe COVID-19 (REF.[49]). But to be clear, there is currently no evidence I know of that shows that any of these live vaccines are in fact reducing the risk of either infection with SARS-CoV-2 or the resulting disease, COVID-19. So yes, they should be tested, but with the clear understanding that we currently simply do not know.

This is in fact going on already, as BCG vaccination is being tested in randomized clinical trials as a prophylactic vaccine in health-care workers in Australia (the BRACE trial mentioned earlier) and the Netherlands, and OPV is also being considered as an intervention. An additional advantage of this approach rests on the solid safety record of these live vaccines compared with many of the other interventions that are currently being considered for COVID-19, such as anti-HIV drugs. Importantly, even if a vaccine for COVID-19 were available

soon, this vaccine would have no record of either efficacy or safety — in other words, compared with the NSEs of our existing live vaccines, there is nothing we have available to use now that is as promising, as safe and as cheap.

One additional problematic issue to be aware of in this context is the already tenuous supply of BCG vaccine for newborns in the world, in whom it saves hundreds of thousands of lives every year through its pathogen-specific effects (targeting tuberculosis) as well as NSEs. If the story of hydroxycholoroquine as an unproven therapy for COVID-19 is any measure to anticipate what might happen, then any notion of BCG vaccine possibly protecting against COVID-19 might lead to a rush to buy up all existing BCG vaccine by higher-income countries. Unfortunately, this is already starting to happen. If this continues, without any internationally coordinated effort to rapidly expand production of BCG vaccine (which is necessary no matter what), then our response to SARS-CoV-2 could be responsible for many deaths in newborns from tuberculosis or sepsis[37]. This would be a severe violation of social justice.

K.L.F. and F.S. It is important that increased use of BCG vaccine in an attempt to reduce the severity of COVID-19 in rich countries does not cause a shortage of BCG vaccine in high-mortality, lower-income countries, where it is urgently needed to protect young children against tuberculosis[49].

S.L.K. With ongoing studies around the world, including by members of the Optimmunize consortium, evaluating the possible beneficial NSEs of BCG vaccine on outcomes of COVID-19, more consideration should be given to the sex of the trial participants as it may be that the beneficial NSEs are greater for females than for males. With the reports of male-biased severe outcomes of COVID-19 (REF.[50]), it is pertinent that we identify treatments that work equally well in males as they do in females. As we evaluate the efficacy of candidate COVID-19 vaccines, sex-differential antibody responses and protection should be considered in all vaccine trials. In a not-yet peer-reviewed study of 331 patients with confirmed SARS-CoV-2 infection in Wuhan, China, anti-SARS-CoV-2 IgG responses were measured and compared among patients with clinically diagnosed mild disease or severe disease[51]. Among patients with mild COVID-19, anti-SARS-CoV-2 IgG titres were similar between the sexes. By contrast, among patients with severe disease, females had greater antibody responses than males, with production of antibodies at earlier phases of disease, which suggests one possible immunological mechanism by which women might recover better from COVID-19 than men[51].

P.A. and C.S.B. We should also consider that the COVID-19 pandemic is likely to lead to delayed vaccinations and lower vaccine coverage worldwide. Missing the specific effects and particularly the NSEs of BCG vaccine, OPV and measles vaccine could easily result in far more deaths than the deaths caused by COVID-19. The COVID-19 pandemic is exposing our total reliance on the 'one-vaccine-to-one-disease' concept of health. The beneficial NSEs of some live vaccines clearly indicate that it is possible to train immunity to develop stronger resistance to unrelated infections. How to develop this capacity should become a major research priority. In the future, we will need a one-vaccine-to-many-diseases concept of health.

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https://doi.org/10.1038/s41577-020-0338-x

Published online: 27 May 2020

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Acknowledgements
T.R.K. is supported by the NIH National Institute of Allergy and Infectious Diseases (U19AI118608-02) and Telethon Kids and Perth Children's Hospital Foundation. D.J.L.'s work on the non-specific effects of vaccines is supported by the Flinders Foundation and the Australian National Health and Medical Research Council. The BRACE trial is supported by the Bill & Melinda Gates Foundation, Sarah and Lachlan Murdoch, the Royal Children's Hospital Foundation, the Minderoo Foundation, the South Australian government, the NAB Foundation, the Calvert-Jones Foundation and individual donors.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
K.L.F. has received consultation and lecture fees from Sanofi Pasteur, Seqirus and Pfizer in the past 5 years and is a member of the Australian Technical Advisory Group on Immunisation. The other authors declare no competing interests.

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EXHIBIT 31

Sudden infant death syndrome | definition of sudden infant death syndrome by Medical dictionary

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sudden infant death syndrome

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Related to sudden infant death syndrome: **shaken baby syndrome**

Sudden Infant Death Syndrome

Definition

Sudden infant **death** syndrome (SIDS) is the unexplained death without warning of an apparently healthy infant, usually during sleep.

Description

Also known as crib death, SIDS has baffled physicians and parents for years. In the 1990s, advances have been made in preventing the occurrence of SIDS, which killed more than 4,800 babies in 1992 and 3,279 infants in 1995. Education programs aimed at encouraging parents and caregivers to place babies on their backs and sides when putting them to bed have helped contribute to a lower mortality rate from SIDS.

In the United States, SIDS strikes one or two infants in every thousand, making it the leading cause of death in newborns. It accounts for about 10% of deaths occurring during the first year of life. SIDS most commonly affects babies between the ages of two months and six months; it almost never strikes infants younger than two weeks of age or older than eight months. Most SIDS deaths occur between midnight and 8 A.M.

Causes and symptoms

Risk factors for sids

The exact causes of SIDS are still unknown, although studies have shown that many of the infants had recently been under a doctor's care for a cold or other illness of the upper respiratory tract. Most SIDS deaths occur during the winter and early spring, which are the peak times for respiratory infections. The most common risk factors for SIDS include:

- sleeping on the stomach (in the prone position)
- mother who smokes during **pregnancy**; smokers are as much as three times more likely than nonsmokers to have a SIDS baby
- the presence of passive smoke in the household
- male sex; the male/female ratio in SIDS deaths is 3:2;
- belonging to an economically deprived or minority family
- mother under 20 years of age at pregnancy
- mother who abuses drugs
- mother with little or no prenatal care
- prematurity or low weight at birth
- family history of SIDS

Most of these risk factors are associated with significantly higher rates of SIDS; however, none of them are exact enough to be useful in predicting which specific children may die from SIDS.

Theories about sids

MEDICAL DISORDERS. Currently, it is not known whether the immediate cause of death from SIDS is a heart problem or a sudden interruption of breathing. The most consistent **autopsy** findings are pinpoint hemorrhages inside the baby's chest and mild inflammation or congestion of the nose, throat, and airway. Some doctors have thought that the children stop breathing because their upper airway gets blocked. Others have suggested that the children have an abnormally high blood level of the chemicals that transmit nerve impulses to the brain, or that there is too much fetal hemoglobin in the blood. A third theory concerns the possibility that SIDS infants have an underlying abnormality in the central nervous system. This suggestion is based on the assumption that normal infants sense when their air supply is inadequate and wake up. Babies with an abnormal nervous system, however, do not have the same alarm mechanism in their brains. Other theories about the cause of death in SIDS include immune system disorders that cause changes in the baby's heart rate and breathing patterns during sleep, or a metabolic disorder that causes a buildup of fatty acids in the baby's system.

PHYSICAL SURROUNDINGS. A recent theory proposes that SIDS is connected to the child's rebreathing of stale air trapped in soft bedding. In addition to the infant's sleeping in the prone position, pillows, sheepskins, and other soft items may contribute to trapping air around the baby's mouth and nose, which causes the baby to breathe in too much carbon dioxide and not enough oxygen. Wrapping a baby too warmly has also been proposed as a factor.

Diagnosis

The diagnosis of SIDS is primarily a diagnosis of exclusion. This means that it is given only after other possible causes of the baby's death have been ruled out. Known risk factors aid in the diagnosis. Unlike the pattern in other diseases, however, the diagnosis of SIDS can only be given post-mortem. It is recommended that all infants who die in their sleep receive an autopsy to determine the cause. Autopsies indicate a definite explanation in about 20% of cases of sudden infant death. In addition, an autopsy can often put to rest any doubts the parents may have. Investigation of the location of the death is also useful in determining the child's sleeping position, bedding, room temperature, and similar factors.

Treatment

There is no treatment for SIDS, only identification of risk factors and preventive measures. The baby's parents may benefit from referral to counseling and support groups for parents of SIDS victims.

Prevention

SIDS appears to be at least partly preventable, which has been shown by a substantial decrease in the case rate. The

following are recommended as preventive measures:

- Sleep position. The United States Department of Health and Human Services initiated a "Back-to-Sleep" campaign in 1994 to educate the public about sleep position. Prior to that time, an estimated 70% of infants slept on their stomachs, since parents had been taught that a "back down" position contributed to **choking** during sleep. There are some conditions for which doctors will recommend the prone position, but for normal infants, side or back (supine) positions are better. When placing an infant on his or her side, the parent should pull the child's lower arm forward so that he or she is less likely to roll over onto the stomach. When babies are awake and being observed, they should be placed on their stomachs frequently to aid in the development of the muscles and skills involved in lifting the head. Once a baby can roll over to his or her stomach, he or she has developed to the point where the risk of SIDS is minimal.
- Good prenatal care. Proper prenatal care can help prevent the abnormalities that put children at higher risk for SIDS. Mothers who do not receive prenatal care are also more likely to have premature and low birth-weight babies. Expectant mothers should also be warned about the risks of **smoking**, alcohol intake, and drug use during pregnancy.
- Proper bedding. Studies have shown that soft bedding, such as beanbags, waterbeds and soft mattresses, contributes to SIDS. Babies should sleep on firm mattresses with no soft or fluffy materials underneath or around them—including quilts, pillows, thick comforters or lambskin. Soft stuffed toys should not be placed in the crib while babies sleep.
- Room temperature. Although babies should be kept warm, they do not need to be any warmer than is comfortable for the caregiver. An overheated baby is more likely to sleep deeply, perhaps making it more difficult to wake when short of breath. Room temperature and wrapping should keep the baby warm and comfortable but not overheated.
- Diet. Some studies indicate that breastfed babies are at lower risk for SIDS. It is thought that the mother's milk may provide additional immunity to the infections that can trigger sudden death in infants.
- Bedsharing with parents. Opinions differ on whether or not bedsharing of infant and mother increases or decreases the risk of SIDS. Bedsharing may encourage breastfeeding or alter sleep patterns, which could lower the risk of SIDS. On the other hand, some studies suggest that bedsharing increases the risk of SIDS. In any case, mothers who choose to bring their babies to bed should observe the following cautions: Soft sleep surfaces, as well as quilts, blankets, comforters or pillows should not be placed under the baby. Parents who sleep with their infants should not smoke around the baby, or use alcohol or other drugs which might make them difficult to arouse. Parents should also be aware that adult beds are not built with the same safety features as infant cribs.
- Secondhand smoke. It is as important to keep the baby's environment smoke-free during infancy as it was when the mother was pregnant with the baby.
- Electronic monitoring. Electronic monitors are available for use in the home. These devices sound an alarm for the parents if the child stops breathing. There is no evidence, however, that these monitors prevent SIDS. In 1986, experts consulted by the National Institutes of Health (NIH) recommended monitors only for infants at risk. These infants include those who have had one or more episodes of breath stopping; premature infants with breathing difficulties; and babies with two or more older siblings that died of SIDS. Parents who use monitors should know how to use them properly and what to for the baby if the alarm goes off.
- Immunizations. There is no evidence that immunizations increase the risk of SIDS. In fact, babies who receive immunizations on schedule are less likely to die of SIDS.

Resources

Organizations

Association of SIDS and Infant Mortality Programs. MN SID Center, Children's Hospitals and Clinics, 2525 Chicago Ave.

So., Minneapolis, MN 55404. (612) 813-6285. **http://www.asip1.org**.

National Institute of Child Health and Human Development. Bldg 31, Room 2A32, MSC 2425, 31 Center Drive, Bethesda, MD 20892-2425. (800) 505-2742. **http://www.nichd.nih.gov/sids/sids.htm**.

National SIDS Resource Center. 2070 Chain Bridge Road, Suite 450, Vienna, VA 22181. (703) 821-8955. 〈http://www.circsol.com/SIDS/〉.

Sudden Infant Death Syndrome Alliance. 1314 Bedford Avenue, Suite 210, Baltimore, MD 21208. (800) 221-7437. **http://www.sidsalliance.org**.

sudden infant death syndrome (SIDS)

the sudden and unexpected death of an apparently healthy infant, not explained by careful postmortem studies. It typically occurs between birth and age 9 months, with the highest incidence at 3 to 5 months. Called also **crib death** or **cot death** because the infant often is found dead in the crib.

The incidence rate for SIDS in the United States is approximately 10,000 per year. After the first week of life it is the leading cause of death in one-year-olds, and is second only to accidents as a cause of death in children under the age of 15 years. The disorder occurs throughout the world, is more common in families in the lower socioeconomic classes, and affects males slightly more than females and non-Caucasians slightly more than Caucasians.

Children most at risk for SIDS are those who are premature, have a history of apnea from hyaline membrane disease or a seizure disorder, or have a family history of SIDS (especially among siblings) with or without a history of apnea.

There are many misconceptions about the cause of SIDS, most of which are likely to cause feelings of guilt or anger that only add to the heartache of parents whose children have died of the disorder. Among these misconceptions are the beliefs that the infant has suffocated under blankets or from aspirated vomitus, or that contraceptive pills, fluoridation, radioactive fallout, and even lack of breast-feeding have somehow contributed to the disorder.

Identification of infants at risk for SIDS includes determining whether the infant is subject to periods of apnea and if so, why. Diagnostic studies include pneumogram, chest x-ray, determination of chemoreceptor status, metabolic assessment, electrocardiogram, and cardiac and apnea monitoring.

Treatment and prevention of SIDS are necessarily aimed at identifying infants at high risk and instituting a program of apnea monitoring and resuscitation. If home monitoring is deemed necessary, the parents are taught how to place the electrodes over the baby's diaphragm, how to operate the monitoring equipment, and the basic maneuvers for cardiopulmonary resuscitation. While home monitoring does create problems and stress for family members, it usually is not required for more than a few months or at most a year. Most parents feel that the security it provides and the knowledge that their child can survive periods of apnea are worth the sacrifices necessary.

Through the efforts of the National Foundation for Sudden Infant Death, guilt and misunderstandings of the parents about the cause of their infant's death are being handled in a more sensitive and comforting way. Recent interest in research into

EXHIBIT 32

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Sudden Unexpected Infant Death and Sudden Infant Death Syndrome

About SUID and SIDS

Sudden unexpected infant death (SUID) is a term used to describe the sudden and unexpected death of a baby less than 1 year old in which the cause was not obvious before investigation. These deaths often happen during sleep or in the baby's sleep area. Learn more about the problem and CDC activities.

Understanding the Problem

About 3,600 babies in the United States die suddenly and unexpectedly each year. A thorough investigation is necessary to learn what caused these deaths. Sudden unexpected infant deaths include sudden infant death syndrome (SIDS), accidental suffocation in a sleeping environment, and other deaths from unknown causes. Although the SUID rate has declined since 1990s, significant racial and ethnic differences continue. See Data and Statistics for more information about trends and SUID by race and ethnicity.

Different practices in investigating and reporting SUID can affect the ability to reliably monitor SUID trends and risk factors at the state and national level. Additionally, because parents or caregivers do not usually see these deaths as they happen, investigators may not be able to get a clear description of the circumstances surrounding the death, which are necessary for determining the cause.

What Is CDC Doing About SUID and SIDS?

CDC's Division of Reproductive Health (DRH) provides scientific leadership in SUID by sharing the most up-to-date information about SUID rates and circumstances linked with SUID. CDC's Division of Reproductive Health also has SUID monitoring programs in 22 states and jurisdictions, covering about 1 in 3 SUID cases in the United States. Participating states and jurisdictions work to improve data quality on SUID cases. This effort leads to a better understanding of circumstances that may increase the risk of SUID. Program awardees also use data about SUID trends and circumstances to carry out strategies to reduce future deaths. In addition, CDC collaborated with a number of organizations and subject matter experts to develop training materials and a reporting form for investigators.

CDC supports the 2016 recommendations issued by the American Academy of Pediatrics (AAP) to reduce the risk of all sleep-related infant deaths. Caregivers can visit How to Keep Your Sleeping Baby Safe: AAP Policy Explained to find out more about these recommendations. CDC collaborates with the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development in its Safe to Sleep® campaign, formerly known as the Back to Sleep campaign. The Safe to Sleep® campaign has outreach activities to spread safe sleep messages and educational materials about ways to reduce the risk of SIDS and other sleep-related infant deaths. Learn more about CDC resources, publications, and activities to address SUID and SIDS.

Page last reviewed: April 29, 2020
Content source: Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion

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Duncan JR, Byard RW, editors. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU): University of Adelaide Press; 2018 May.

Chapter 32 Biomarkers of Sudden Infant Death Syndrome (SIDS) Risk and SIDS Death

Robin L Haynes, PhD.

▸ Author Information

Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant less than 1 year of age that remains unexplained after a complete autopsy and death scene investigation (1). Typically, SIDS is associated with a sleep period and with risk factors in the sleep environment — for example, prone/face-down sleep, bed sharing, soft bedding, and over-bundling (2-4). Despite national safe sleep campaigns, SIDS remains the leading cause of post-neonatal infant mortality in the United States, with an overall rate of 0.40 SIDS deaths per 1,000 live births (5).

SIDS is a complex heterogeneous disorder that presents in seemingly healthy infants as death — sudden and unexplained. For the family, it comes without warning, devastating all of those in and surrounding the family. For the medical examiner, it comes with the challenge of distinguishing the SIDS death from other sudden and unexpected deaths in infancy, those associated with accidental asphyxia (e.g. accidental suffocation while bed sharing), unidentified infection, or trauma. An ultimate goal in SIDS research is to identify specific biomarkers of SIDS risk which can be used to prevent a SIDS death from occurring (via successful intervention), thus alleviating the burden to the family; or, if the death does occur, to identify a readily accessible biomarker of SIDS death, thus alleviating the burden of the medical examiner adjudicating the death. In this chapter we will address the concept of biomarkers of SIDS, biomarkers of a SIDS death, and biomarkers of SIDS risk.

Biomarkers, defined as objective indicators of a pathologic process, medical condition, or medical state, can be presented in many different forms or types of measurements. They can be biochemical biomarkers with a distinct signature of a single metabolite or group of metabolites specific to a disease process, genomic biomarkers defined as a DNA or RNA characteristic associated with a pathogenic process, or biomarkers which utilize physiological tests (e.g. heart rate and blood pressure) to identify or predict a disease state or disease risk. There have been several studies reporting physiological biomarkers (apnea, cardiac rate abnormalities, and arousal deficits) in infants who subsequently died of SIDS (6-10). Likewise, there have been genetic studies reporting on the potential association of genetic alterations with SIDS death (11-25). In this chapter, however, we will focus solely on peripheral biochemical (metabolite or protein) markers taken in readily accessible fluid which have been reported on in SIDS and which have furthered our understanding of the processes underlying a SIDS death.

The discussion of biomarkers in this chapter is divided into two distinct categories: [1] post-mortem biomarkers which have provided insight into pathological mechanisms of SIDS death and which have potential to distinguish a SIDS death from other types of sudden and unexpected infant death at autopsy; and [2] maternal/infant biomarkers which have been identified as

potentially associated with a risk of SIDS death. It is important to note that different studies present conflicting data on the utility of certain biomarkers in SIDS. The biomarkers below are discussed with reference to the studies that both support and refute their use. It is also important to note that at this time there is no clinically available biochemical biomarker of SIDS. The intent of the discussion below, however, is to provide insight into the current landscape of research within this area.

Post-mortem Biomarkers of SIDS

Post-mortem biomarker related to serotonin neurotransmission

Serotonin (5-HT), a neurotransmitter produced from the essential amino acid tryptophan, mediates a large variety of functions both peripherally and centrally. Within the central nervous system (CNS), 5-HT is produced from the sequential reactions of tryptophan hydroxylase 2 (TPH2) and 5-hydroxyl-L-tryptophan decarboxylase (AADC). The production of CNS 5-HT is restricted to TPH2-expressing neurons within the brainstem, including within the pons and midbrain (the "rostral" 5-HT system) and the medulla oblongata (the "caudal" 5-HT system). While the rostral 5-HT system projects to the cortex, thalamus, hypothalamus, basal ganglia, hippocampus, and amygdala, and plays a significant role in cognition, waking, and mood, the caudal 5-HT system projects to sites within the brainstem and spinal cord and plays a significant role in homeostasis, respiratory, and autonomic regulation (26). Early in brain development, prior to the maturation of the 5-HT rostral and caudal systems, 5-HT plays a significant role as a trophic factor influencing processes such as cell division, differentiation, migration, and synaptogenesis (27, 28). Outside of the CNS, peripheral 5-HT is produced by tryptophan hydroxylase 1 (TPH1) expressing cells (enterochromaffin cells, EC) of the gut which are scattered within the gastrointestinal (GI) tract epithelium and produce approximately 90-95% of the total body 5-HT. Functions of peripheral 5-HT include platelet aggregation, GI motility, and metabolic homeostasis, including regulation of glucose homeostasis, gluconeogenesis, mobilization of free fatty acids, and browning of white adipose tissue (29-32).

Relative to SIDS, studies of 5-HT within the CNS specifically within the medullary (caudal) brainstem 5-HT network have shown multiple abnormalities in SIDS infants compared to autopsy controls (e.g. infant deaths of known cause). These abnormalities, along with evidence supporting the role of the brainstem in respiratory and autonomic regulation, sleep, and arousal (functions found to be subclinically defective in infants who subsequently die of SIDS (6-10)), provide rationale for the brainstem hypothesis in SIDS. This hypothesis suggests that the cause of death in a subset of SIDS cases relates to brainstem abnormalities in the neuroregulation of cardiorespiratory control (33, 34). The medullary 5-HT network is comprised of nuclei that contain 5-HT-producing neurons (raphe obscurus, magnus, and pallidus; extra-raphe: gigantocellularis, paragigantocellularis lateralis, and intermediate reticular zone) and nuclei that receive 5-HT projections and mediate respiratory and autonomic responses (e.g. hypoglossal nuclei, nucleus of the solitary tract, and dorsal motor nucleus of the vagus, the latter two nuclei directly part of the autonomic nervous system). In SIDS cases compared to controls, reported 5-HT defects include significantly decreased levels of 5-HT (~26%) in two of two 5-HT-producing nuclei (raphe obscurus and paragigantocellularis) sampled ($p<0.05$) (35), decreased TPH2 (~22%) in the medullary raphe (35) ($p=0.03$), and an increased density of 5-HT neurons in the raphe and extra-raphe nuclei (up to a 50% increase; $p<0.001$) (36). In addition, significant

decreases in $5HT_{1A}$ receptor binding, as determined by tissue autoradiography, have been reported in nuclei that contain 5-HT cell bodies and receive 5-HT cell projections within the medulla (up to a 50% decrease; $p<0.001$) (35-38). The finding of 5-HT receptor abnormalities is supported by immunocytochemistry showing a significant decrease in 5-HT receptor expression ($5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$) in medullary nuclei of SIDS infants compared to controls (39). Together, these data suggest CNS, specifically brainstem, abnormalities in 5-HT synthesis, 5-HT neuronal development, and 5-HT signaling (via 5-HT receptor binding) in a subset of SIDS infants.

In terms of biomarkers, recent progress has been made in identifying abnormalities in peripheral levels of 5-HT in SIDS cases. In a study of 61 SIDS cases and 15 non-SIDS autopsy controls, serum 5-HT levels, as measured by enzyme-linked immunosorbent assay (ELISA) and high-pressure liquid chromatography (HPLC), were found to be significantly elevated (average increase of 95%) in SIDS cases compared to controls (40) (Table 32.1, Figure 32.1A and B). These controls included cases dying acutely from accidental asphyxia (e.g. crib accidents), acquired lung disease, unsuspected congenital heart disease, and accidental head trauma. There was no statistically significant difference in the ratio of 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) (a breakdown product of 5-HT) between the two groups, suggesting that the increase in serum 5-HT in SIDS does not reflect a decreased breakdown of 5-HT itself (40). “High” serum 5-HT was defined post hoc as greater than two standard deviations above the mean of the controls, as determined by ELISA (≥211.8 ng/ml), and “normative” serum 5-HT as below this cut-off (<211.8 ng/ml). The percentage of SIDS cases with high serum 5-HT was 31% (19/61). There was no significant association of serum 5-HT levels with any known risk factor for SIDS (including male sex, prematurity, discovery in the prone position, and illness within 48 hours), or with the genotype of the serotonin transporter (5-HTT) promoter polymorphisms (5-HTTLPR) [SS, SL, or LL], or with the type of nutrition prior to death (breastmilk or formula-fed) (40). There was no effect of post-mortem interval or storage interval on levels of serum 5-HT (Figure 32.1C and D) (40).

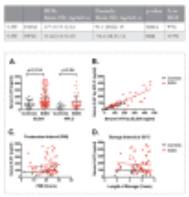
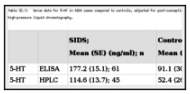


Figure 32.1:

Serum 5-HT levels in SIDS and non-SIDS autopsy controls. A. There is a significant increase in serum 5-HT levels in SIDS compared to controls as determined by ELISA ($p=0.014$) and HPLC ($p=0.04$). B. Serum 5-HT levels obtained by ELISA and HPLC show significant (more...)

While this study provides the first evidence of an abnormality in peripheral levels of 5-HT in SIDS cases, the source(s) of this increase is currently unknown. One possibility includes increased production of 5-HT by gut EC cells, potentially mediated by microbiota residing in the gut and known to influence host EC production of 5-HT (41, 42). Another possibility includes increased production of 5-HT by pulmonary neuroendocrine cells (PNEC) and neuroepithelial bodies (NEBs) of the lung, which function as airway oxygen/carbon dioxide sensors and release 5-HT in a dose-dependent manner in response to hypoxia (43, 44). Hyperplasia and hypertrophy of PNEC/NEB within the lungs of infants dying of SIDS have been reported, and secretory products of these PNEC/NEB cells have been proposed previously as a potential biological marker of SIDS (44). Also unknown are the relationship between the serum and brainstem 5-HT abnormalities in SIDS, and whether elevated serum 5-HT and specific brainstem 5-HT

abnormalities co-exist in a single subset of SIDS cases or whether two SIDS subsets exist, independent of the other, one with abnormalities in peripheral 5-HT and the other with abnormalities in central 5-HT. Despite these unanswered questions, measures of serum 5-HT may ultimately prove to be a forensic biomarker at autopsy to distinguish SIDS infants from other infants dying of sudden unexpected deaths, and to identify SIDS infants with central 5-HT abnormalities. Finally, these studies form the foundation of future research involving prospectively collected serum samples in apparently well infants to determine whether blood 5-HT levels can be used to predict the subsequent occurrence of SIDS.



		SIDS; Mean (SE) (ng/ml); n	Contro Mean (
5-HT	ELISA	177.2 (15.1); 61	91.1 (3(
5-HT	HPLC	114.6 (13.7); 45	52.4 (2(

Table 32.1:

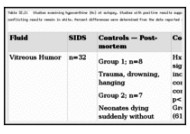
Serum data for 5-HT in SIDS cases compared to controls, adjusted for post-conceptional age (40). ELISA = enzyme-linked immunosorbent assay; HPLC = high-pressure liquid chromatography.

Post-mortem biomarkers related to hypoxia

Physiological evidence suggests that infants who subsequently die of SIDS have episodes of bradycardia and/or apnea days, or weeks, prior to their death (6, 45), thus implicating a role for hypoxia and/or chronic intermittent hypoxia in the pathogenesis of SIDS. Certain tissue changes further support a role for hypoxia in SIDS, i.e. brainstem gliosis, as originally reported by Naeye et al. (46), and the presence of hyperplasia of PNEC/NEB cells, as reported by Cutz et al. (44). In addition to tissue markers of hypoxia, metabolite and protein markers suggestive of hypoxia have also been identified in various fluids in SIDS cases at autopsy and are presented below as potential biomarkers of hypoxia in SIDS death.

Hypoxanthine

Hypoxanthine is a metabolite formed during purine catabolism and an intermediate in the purine salvage pathway. During hypoxia there is an accelerated breakdown of the purine adenosine monophosphate (AMP) as cells try to maintain energy state. As AMP breaks down during hypoxia, hypoxanthine accumulates. Increased hypoxanthine concentrations in plasma, urine, cerebrospinal fluid, amniotic fluid, and vitreous humor have been reported in various studies as significantly higher in hypoxic individuals, including hypoxic infants, compared to non-hypoxic individuals (as reviewed in (47)). Animal studies have confirmed the use of hypoxanthine as a biomarker of hypoxia (47), with increased significance in models of intermittent hypoxia as opposed to continuous hypoxia (48). In SIDS, this marker has been examined in multiple studies in the vitreous humor taken at autopsy. It was initially shown by Rognum et al. to be increased in the vitreous humor of 32 SIDS cases compared to 15 controls dying of various causes (49) (Table 32.2). This finding was replicated in a larger cohort of SIDS (n=73) and controls (n=23) with hypoxanthine levels corrected for potential post-mortem changes (50).



Fluid	SIDS	Controls -- Post-mortem	Co
Vitreous Humor	n=32	Group 1; n=8	Hx sig
		Trauma, drowning, hanging	inc cor cor
		Group 2; n=7	p<
		Neonates dying suddenly without	Gr 05

Table 32.2:

Studies examining hypoxanthine (Hx) at autopsy. Studies with positive results supporting the use of the biomarker are shaded. Studies with negative, conflicting results remain in

white. Percent differences were determined from the data reported in the (more...)

Other studies on hypoxanthine in SIDS, involving various ways of correcting for post-mortem changes, utilizing various groups of control infants, and analyzing hypoxanthine using different methods, have reported opposing results on the utility of hypoxanthine as a biomarker for hypoxia in SIDS infants (51, 52). In SIDS cases (n=50) compared to non-SIDS controls (n=41), Belonje et al. found no difference in vitreous humor levels of hypoxanthine (51), while Carpenter et al. found no difference in vitreous humor or cerebrospinal fluid levels of hypoxanthine in SIDS cases compared to cases dying of known causes of death (non-cardiac or pulmonary related causes of death) (SIDS, n=68 or 45, respectively; non-SIDS, n=38 or 21, respectively) (52) (Table 32.2).

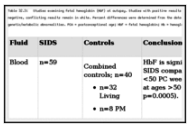
To avoid incorrectly correcting for post-mortem changes, and to investigate potential confounding factors such as prone sleep and age of the infant (and corresponding size of the eye), Opdal et al. undertook a large study examining different categories of SIDS (i.e. with or without resuscitation, with or without prior infection, prone vs supine sleep), infectious death controls, controls dying of heart/lung disease, and violent deaths (53). After matching all cases for post-mortem interval, hypoxanthine was confirmed to be increased in SIDS cases (n=82) compared to violent death infants (n=13) and infants dying from heart and lung disease (n=17) (53). There was no difference in SIDS cases compared to infectious death controls (n=22), nor were there differences among SIDS cases associated with time of death, infection, or sleep position (53) (Table 32.2). Taken together, these various studies suggest that hypoxanthine levels in the vitreous humor taken at autopsy may be utilized as a biomarker for intermittent hypoxic events prior to death in SIDS cases. They also suggest the potential use of hypoxanthine levels to differentiate a SIDS death from other infant deaths involving accidents and trauma.

Fetal hemoglobin

The protein hemoglobin is an iron-containing oxygen transport protein that is present in red blood cells and responsible for delivering oxygen to tissues throughout the body. In the adult, hemoglobin binds to oxygen in the lungs whereas in the fetus, fetal hemoglobin binds to oxygen in the placenta, specifically from the maternal circulation. The structure of hemoglobin is a heterotetramer protein consisting of two alpha chains and two non-alpha chains, with the non-alpha chains differing depending on whether the hemoglobin is the fetal form ($\alpha 2\gamma 2$) or the adult form ($\alpha 2\beta 2$). While the binding mechanism of the fetal and adult forms are the same, the affinity of the fetal form of hemoglobin for oxygen is much greater than that of the adult form, thus allowing fetal hemoglobin to more readily extract oxygen from the maternal circulation. After birth and through the first five to six months of life, the infant switches from the synthesis of fetal hemoglobin to adult hemoglobin (54, 55). However, under certain conditions, including chronic lung disease in infants (56), hypoxia in nonhuman primates (57), and hypoxemia in children with congenital cyanotic heart disease (54), increases in the amount of fetal hemoglobin have been reported and have been suggested as a marker of inadequate tissue oxygenation.

A number of studies have examined the potential of fetal hemoglobin at autopsy as a potential diagnostic post-mortem biomarker for SIDS but have reported conflicting results. In 1987, Giulian et al. reported a significant elevation of fetal hemoglobin in 59 SIDS cases compared to 40 non-SIDS controls, matched for post-conceptional age (58) (Table 32.3). This elevation was

postulated by Giulian to be due to a delay in the switch from fetal hemoglobin to adult hemoglobin (58). Supporting this original finding are two additional studies reporting elevated levels of fetal hemoglobin in SIDS infants compared to infants dying of known causes (59, 60) and compared to living, healthy controls (59) (Table 32.3). These data were suggested to provide evidence of an underlying condition in SIDS resulting from chronic hypoxemia (59). Refuting the observations above are studies showing no significant difference in fetal hemoglobin in SIDS compared to controls (61-64) (Table 32.3).



Fluid	SIDS	Controls	Conclusion
Blood	n=59	Combined controls; n=40 • n=32 Living • n=8 PM	HbF is signi SIDS compa <50 PC wer at ages >50 p=0.0005).

Table 32.3:

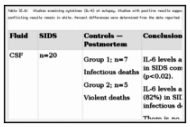
Studies examining fetal hemoglobin (HbF) at autopsy. Studies with positive results supporting the use of the biomarker are shaded. Studies with negative, conflicting results remain in white. Percent differences were determined from the data reported in (more...)

The conflicting results across the different studies are likely due to several factors including methodology of analysis, criteria for diagnosis of SIDS, different controls for comparison, and different analysis of age as a cofounding factor. Despite the disputed role of fetal hemoglobin as a post-mortem marker for SIDS, evidence from prospective studies of SIDS risk suggests an inverse relationship of adult hemoglobin levels measured at birth and a subsequent risk of SIDS death. These data will be discussed under the section “Infant Biomarkers of SIDS Risk”. It is important to note that levels of total hemoglobin cannot be accurately determined after death (65), and therefore the relationship between low total hemoglobin, anemia, and SIDS death cannot be quantitated. However, the fact that the peak incidence of SIDS co-incides with the nadir in the physiologic anemia of infancy (66) is possibly suggestive of such a relationship (65).

Post-mortem biomarker related to infection

Mild infection has been noted in approximately half of SIDS cases prior to death (67), with evidence of infection coming from post-mortem microbiology and autopsy indication of inflammatory reactions. The role of infection in SIDS is described elsewhere in this book and is therefore not emphasized here. However, with regard to biomarkers of SIDS, it is important to note the role of cytokines as a potential biomarker of immunologic activity and possible dysfunction. Cytokines are small proteins produced and released from various cell types including immune cells (i.e. mast cells, B lymphocytes, and T lymphocytes) and glial cells within the brain (astrocytes and microglia). While cytokines are important in host response to infection, they can also become dysregulated and can contribute to the pathogenesis of a disease process. One cytokine, interleukin-6 (IL-6), is of particular interest given its identification in the cerebrospinal fluid (CSF) of SIDS infants (68, 69). IL-6 acts as a pro-inflammatory cytokine and is an important mediator of fever, crossing the blood-brain barrier and affecting the body’s temperature set-point in the hypothalamus (70). Given that the IL-6 receptor shows widespread distribution throughout brainstem nuclei of the medulla (71), it likely plays a role in the co-ordination of brainstem responses to infectious challenges. Interestingly, IL-6 has also been shown to play a role in epigenetic modification of certain genes implicated in diseases such as anxiety (72) and cancer (73, 74). Vege et al. reported an overall increased level of IL-6 in the CSF of infants dying of SIDS (n=20) compared to infants dying from violent deaths (n=5), but

an overall decreased level of IL-6 in SIDS infants compared to infectious deaths (n=7) (68) (Table 32.4). Although there was no correlation between IL-6 levels and the presence, or absence, of clinical symptoms of infection prior to death, there was a subpopulation of SIDS cases that overlapped with the infectious death cases (68). In a larger dataset, Vege et al. confirmed the intermediate position of IL-6 levels in SIDS infants with SIDS infants (n=50) showing higher IL6 levels than violent deaths (n=8), but overall lower IL-6 levels than infectious deaths (n=18) and deaths due to heart/lung disease (n=22) (69) (Table 32.4). High levels of CSF IL-6 in SIDS infants were found to be associated with a peripheral immune response, as determined by IgA immunocytes in the laryngeal mucosa and epiglottis (75). Taken together, the high levels of CSF IL-6 may serve as a biomarker for a significant immunological activation in a subpopulation of SIDS infants. Given that this immunological response is often disproportionate with clinical symptoms of infection, high levels of CSF IL-6 in SIDS infants may also serve as a biomarker of over-activation of the immunologic response to moderate stimuli. Of note, the finding of increased IL-6 levels in CSF of SIDS infants, compared to other causes of death with and without infection, was not confirmed in a subsequent study by Vennemann et al., possibly due to heterogeneity of cases with infection and/or diagnostic criteria for SIDS classification (76) (Table 32.4).



Fluid	SIDS	Controls — Postmortem	Conclusion
CSF	n=20	Group 1: n=7 Infectious deaths	IL-6 levels a in SIDS con (p<0.02).
		Group 2: n=5 Violent deaths	IL-6 levels a (62%) in SI infectious d

Table 32.4:

Studies examining cytokines (IL-6) at autopsy. Studies with positive results supporting the use of the biomarker are shaded. Studies with negative, conflicting results remain in white. Percent differences were determined from the data reported in the (more...)

Post-mortem biomarker related to mast cell degranulation

Mast cells are a type of granulocyte residing in most tissue and preferentially located at the interfaces between host and environment (e.g. skin, mucosa of the GI tract, and lungs). They play a key role in the inflammatory process through the induced release of inflammatory mediators from their storage granules. These mediators include serine proteases (tryptase and chymase), monoamines (histamine and serotonin), proteoglycans (heparin and chondroitin sulfate proteoglycans), and lipid-derived signaling molecules (prostaglandins and leukotrienes). While mast cells are most highly recognized for their role in allergic reactions and anaphylaxis, they are increasingly being appreciated for their role in innate immune responses and in regulation of tissue homeostasis, the latter of which includes regulation of epithelial permeability, smooth muscle contraction and peristalsis, bronchoconstriction, blood flow, and wound healing (77). In an allergic reaction, mast cell activation is mediated via antigen-specific immunoglobulin E (IgE), which binds to its cell surface receptor, FceR1, and leads to crosslinking and aggregation of FceR1 on the surface of mast cells. This subsequently triggers the release of the biologically active compounds listed above (78). Under non-allergic inflammatory and physiological conditions, mast cell activation is mediated by many different non-IgE mechanisms, including binding of toll-like receptor (TLR) ligands to TLRs widely expressed on mast cells, receptor-mediated binding of neuropeptides (corticotrophin-releasing hormone, nerve growth factor, and brain-derived neurotrophic factor), cytokines, chemokines, and adenosine (79). Given the important role that mast cells have under pathological and physiological conditions, a marker by

which mast cell activation can be measured is crucial in understanding the involvement of mast cells under these different conditions. The protease tryptase is one such marker.

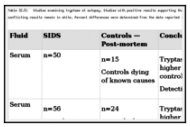
Tryptases are a family of proteases present in large amounts in mast cell secretory granules. Although basophils also contain and release tryptase, their levels of tryptase are approximately 100 times less than that of mast cells and therefore are not considered significant contributors to tryptase levels (80). There are two types of tryptases, α-tryptase, classified as αI and αII, and β-tryptase, classified as βI, βII, and βIII. The inactive α-protrypase is secreted constitutively from mast cells and is the major form found in the blood. The β-tryptase is stored in secretory granules and is secreted upon mast cell activation and degranulation (81). Tryptase levels in biological fluids are used as markers of mast cell number and activation, with β-tryptases being elevated in subjects with systemic anaphylaxis.

Studies have reported contradictory findings regarding serum levels of tryptase in SIDS (82-87), in part due to differences in detection method and antibody specificity. The α- and β-tryptase forms share 90% sequence identity, and detection of specific tryptase forms is difficult and highly dependent on the methodology used (81). Serum tryptase levels have been reported as elevated in SIDS in four different studies. Using a radioimmunoassay, Platt et al. examined serum levels of tryptase in SIDS cases (n=50) compared to control infants dying from known causes (n=15) and found significantly higher levels of tryptase in SIDS cases, with 40% of SIDS showing tryptase levels above the threshold chosen to indicate pre-mortem mast cell activation (82) (Table 32.5). In the study by Holgate et al. using a radioimmunoassay recognizing both α- and β-tryptase, elevated tryptase was reported in 82 unexplained deaths compared to 24 explained deaths (83) (Table 32.5).

The increases seen by Pratt and Holgate were suggested to support a previously proposed anaphylaxis hypothesis that SIDS infants develop an allergic sensitivity to cow's milk and that death occurs by regurgitation of recently ingested cow's milk into the airways (88-90). In 1999, Edston et al. examined predominately SIDS cases but divided them based on a low-tryptase group (<10 ug/ml) and a high-tryptase group (>10 ug/ml). High tryptase was reported in 40% of SIDS infants tested (n=16/40) (85). To address the anaphylaxis hypothesis, Edston et al also examined total IgE, an immunoglobin increased in the blood with allergic disease. While IgE was increased above clinical reference values in 33% (n=10/30) of the SIDS cases, there was no association between tryptase and IgE levels, and thus this study did not support the anaphylaxis hypothesis in SIDS death (85).

In 2001, Buckley et al. examined serum from SIDS and controls using two different methods, one recognizing predominately β-tryptase, the other with equal sensitivity to α- and β-tryptase (86) (Table 32.5). A significant increase in β-tryptase was reported in SIDS, supporting an increase in mast cell activation and degranulation in SIDS. There was no evidence of an increase in α-tryptase, the variant secreted constitutively from mast cells, suggesting that SIDS is not associated with mast cell hyperplasia (86). In contradiction to the studies described above are two studies by Nishio et al. and Hagan et al., which report no significant difference in tryptase between SIDS and controls (84, 87) (Table 32.5). It is important to note that Hagan et al. used frozen blood separated into serum after thaw, a potential confounder to the results.

Table 32.5:



Fluid	SIDS	Controls — Post-mortem	Conch
Serum	n=50	n=15 Controls dying of known causes	Tryptas higher control Detecti
Serum	n=56	n=24	Tryptas higher

Studies examining tryptase at autopsy. Studies with positive re the use of the biomarker are shaded. Studies with negative, co remain in white. Percent differences were determined from the the article. (more...)

In summary, of the results reported above, most available data on SIDS show increased levels of tryptase in a subpopulation of SIDS compared to controls with no associated increase in IgE. This supports a role for a non-IgE-mediated mast cell degranulation in SIDS, not related to anaphylaxis, but rather associated with other non-IgE mechanisms, i.e. potential activation via TLRs, neuropeptides, cytokines, and/or chemokines (79).

Biomarkers of SIDS Risk

Maternal biomarkers of SIDS risk

Maternal alpha-fetoprotein

Alpha-fetoprotein is a member of the albumin gene family and is thought to be the fetal form of serum albumin. While alpha-fetoprotein is postulated to serve roles similar to other members of the albumin family (e.g. transport of various ligands and oxygen free radical scavenging), its exact biological function remains relatively unclear. During fetal life, plasma levels of alpha-fetoprotein are highly abundant but then decrease rapidly after birth until adult levels are reached by two years of life (91). During pregnancy, alpha-fetoprotein produced by the fetus crosses the placenta and fetal membranes and appears in maternal serum (91). Prior to the now common use of fetal ultrasound investigation, maternal levels of alpha-fetoprotein in the serum and amniotic fluid were common screening methods for congenital anomalies, including neural tube defects, omphalocele, gastroschisis, and fetal bowel obstructions (91, 92). These defects commonly present with increased levels of maternal alpha-fetoprotein, while decreased levels of maternal alpha-fetoprotein are associated with an increased risk of Down Syndrome (93). High levels of maternal serum alpha-fetoprotein during the second trimester are also associated with the risk of stillbirth in normally formed infants and serve as a biochemical predictor of the risk of unexplained stillbirth (94, 95).

In 2004, Smith et al. sought to show a relationship between unexplained stillbirth and SIDS by examining the association of second-trimester maternal serum levels of alpha-fetoprotein and the subsequent risk of SIDS (96). In the study, a prenatal-screening database from western Scotland was linked to databases of maternity, perinatal death and birth, and death certification recorded from 1980 to 2001. Prenatal screening measurements of alpha-fetoprotein taken at 15 and 21 weeks of gestation were used and cases (n=214,532 women) were divided into quintiles based on increasing maternal levels of alpha-fetoprotein. The incidence of SIDS increased significantly across quintiles with an incidence of 2.7/10,000 births among the lowest quintile compared to 7.5/10,000 births among the highest quintile (p for trend <0.001) (Figure 32.2). After adjusting for birth weight and gestational age at delivery, each of which varies inversely with the risk of SIDS, a significant association between alpha-fetoprotein and risk for SIDS was determined with the odds ratio of SIDS death increasing significantly with increasing quintiles (p for trend = 0.01). The odds ratio of SIDS death was 2.2 (95% confidence interval, 1.1 to 4.3) at the highest quintile compared to 1.7 (95% confidence interval, 0.8 to 3.5) at the lowest quintile (96). The

association of high levels of alpha-fetoprotein with SIDS risk was cited by the authors to reflect the role of the intrauterine environment, specifically the role of placental dysfunction during pregnancy (96). This is based on the concept that elevated maternal serum levels of alpha-fetoprotein in the absence of fetal abnormalities indicate an increase in placental permeability and thus a defect in placental functions (94). Studies examining further the relationship between SIDS risk and maternal alpha-fetoprotein have yet to be reported.

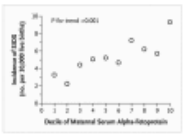


Figure 32.2:

Incidence of SIDS within a population of infants divided into 10 groups (deciles) based on maternal alpha-fetoprotein measured in the second trimester of pregnancy. There is a linear trend of increasing incidence of SIDS with increasing alpha-fetoprotein (more...)

Infant biomarkers of SIDS risk

Fetal hemoglobin

In the earlier section on post-mortem biomarkers of SIDS death, we describe studies examining blood levels of fetal hemoglobin in SIDS infants compared to control infants with various known causes of death. Although these studies show conflicting data on the use of fetal hemoglobin as a post-mortem biomarker for SIDS death, the concept of abnormalities in fetal versus adult levels of hemoglobin has been tested in the living infant in regard to risk of SIDS death. In 2003, Bard et al. prospectively examined levels of [γ/(γ+β)]-globin mRNA in normal healthy infants (n=37) compared to infants being monitored at home after an apparent life-threatening event (ALTE) and considered at risk for SIDS (n=35) (97). Levels of [γ/(γ+β)]-globin mRNA significantly correlate with levels of fetal hemoglobin synthesis (98), and thus represent an alternative means to examine the potential of fetal hemoglobin as a biomarker of SIDS risk. As previously stated, an increase in fetal hemoglobin in infancy is suggestive of hypoxia and/or hypoxemia (54, 56, 57), with the synthesis of fetal hemoglobin thought to be a result of an erythropoietic response to oxygen insufficiency (99). In the study, Bard et al. found significant increases in the [γ/(γ+β)]-globin mRNA levels in the ALTE group compared to the controls at two different age intervals, 42 to 45 post-conceptional weeks (55.2+/-17.4% compared to 42.6+/-13.7%, p=0.03) and 46 to 49 post-conceptional weeks (33.9+/-14% compared to 23.6+/- 9.8%, p=0.02). Other age intervals tested up to approximately 60 post-conceptional weeks were not significantly different. While the exact cause of the increased fetal hemoglobin in the ALTE infants is unknown, Bard et al. suggest a release of immature red cells into the circulation due to stress-induced erythropoiesis or possibly a decrease or delay in the switch from fetal to adult hemoglobin (97). Both could potentially result from repeated but undetected episodes of hypoxemia. Of note, chronic intermittent hypoxia is hypothesized to play a role in the pathogenesis of SIDS in some cases (34). Given that the study by Bard et al. does not prospectively follow the ALTE infants to look for associations between abnormal fetal hemoglobin and incidence of SIDS death, the significance of the increased fetal hemoglobin during the observed time frame is unknown. It does, however, support the role of hypoxia/hypoxemia in infants known to be at risk for SIDS death.

Adult hemoglobin

With regard to hemoglobin levels, the studies discussed thus far look at abnormally high levels of fetal hemoglobin and its relationship to SIDS death/risk. In 2004, Richardson et al. provided data supporting the concept of abnormal hemoglobin in SIDS, but did so by providing evidence of abnormally low levels of adult hemoglobin (100). In the population-based study, Richardson et al. followed up 3.2 million infants enrolled in California's Newborn Screening program from 1990 to 1997 in order to identify deaths attributed to SIDS, looking at the association between incidence of SIDS and adult levels of hemoglobin in the first hours after birth. Of the 3,242,606 infants, there were 2,425 deaths attributed to SIDS (74.8 per 100,000 live births). SIDS infants were categorized based on adult levels of hemoglobin at birth and put into quintiles. After adjusting for sex, race/ethnicity, maternal age, maternal education, maternal smoking, preeclampsia, intrauterine growth restriction, and gestational age, the risk for SIDS was estimated to be 2.15-fold greater for infants in the lowest quintile (i.e. the lowest levels of adult hemoglobin) (100). This decrease in adult hemoglobin represents a true decrease in the fraction of adult hemoglobin, rather than a decrease in total hemoglobin levels (100). The study by Richardson et al. suggests that the low levels of adult hemoglobin in SIDS infants reflect an underlying chronic pathological condition or developmental impairment in processes such as cardiorespiratory control. Alternatively, low adult hemoglobin may reflect adverse prenatal conditions, such as maternal smoking and/or drinking (100). Unknown in this study is the persistence of this decrease in adult hemoglobin after birth and closer to the time of death. However, the post-mortem studies described above suggesting increased fetal hemoglobin at autopsy support a persistence of the hemoglobin abnormality, at least in some SIDS cases (58-60).

Conclusions

As previously stated, there are no known biochemical biomarkers consistently used to identify SIDS infants at autopsy or to identify SIDS risk in living infants. While several of the biomarkers discussed above show potential, their use as forensic or clinical biomarkers requires critical steps, including validation in additional, oftentimes larger, datasets to access the reliability, sensitivity, and specificity of the biomarker. With regard to a forensic biomarker, this requirement proves difficult, given the relatively low incidence of SIDS death and the low availability of SIDS and non-SIDS autopsy tissue. This difficulty is exacerbated by the shift in nomenclature and the increasing movement away from calling a sudden and unexpected death a "SIDS death" to the increasingly used "sudden unexpected infant death (SUID)" or alternatively "cause unknown" (101). A death ruled as SIDS falls under the broader category of SUID, which includes deaths where evidence suggests a role for positional asphyxia or accidental overlay associated with bed sharing during a sleep period. Even after a thorough death scene investigation, the differentiation between a death due to SIDS and a death involving an asphyxia event is difficult, leaving a blurred distinction between the two and a possible misdiagnosis. A single biomarker specific to SIDS, or, more likely, a profile of multiple biomarkers specific to SIDS may allow for distinction between the two possible diagnoses. With regard to a biomarker of SIDS risk, validation requires very large prospective studies of living infants, collection of accessible fluids, and follow-up to determine incidence of SIDS death. Given that SIDS is likely a heterogeneous disorder due to multiple causes, a single biomarker will only identify a subset of infants at risk for SIDS, with this subset related to the biomarker-specific pathogenesis (e.g. SIDS related to 5-HT abnormalities). Despite the very difficult nature of biomarker studies in

SIDS, the importance lies in the potential ability to identify abnormalities in SIDS infants, to better define pathogenic mechanisms involved in SIDS, and to identify infants or a subset of infants who are at risk of SIDS death and who may benefit from preventative strategies.

Acknowledgments

The author thanks Dr Hannah C Kinney, Dr Eugene E Nattie, Dr Richard D Goldstein, Dr Alan D Michelson, and Jacob B Cotton for critical readings of this chapter.

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Bookshelf ID: NBK513404 PMID: 30035968

EXHIBIT 34

PLOS MEDICINE

RESEARCH ARTICLE

Causes of death and infant mortality rates among full-term births in the United States between 2010 and 2012: An observational study

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OPEN ACCESS

Citation: Bairoliya N, Fink G (2018) Causes of death and infant mortality rates among full-term births in the United States between 2010 and 2012: An observational study. PLoS Med 15(3): e1002531. https://doi.org/10.1371/journal.pmed.1002531

Academic Editor: Gordon C. Smith, Cambridge University, UNITED KINGDOM

Received: July 11, 2017

Accepted: February 13, 2018

Published: March 20, 2018

Data Availability Statement: The vital statistics data used in this project can be downloaded freely from the National Center for Health Statistics website: https://www.cdc.gov/nchs/data_access/vitalstatsonline.htm. The geocodes for these data are restricted by U.S. law to protect privacy and cannot be distributed by the authors according to the terms of our data use agreement with the NCHS. These data can be requested by submitting the form available at https://www.naphsis.org/research-requests. Aggregate geocoded data are provided here as Supporting Information in S2

Abstract

Background

While the high prevalence of preterm births and its impact on infant mortality in the US have been widely acknowledged, recent data suggest that even full-term births in the US face substantially higher mortality risks compared to European countries with low infant mortality rates. In this paper, we use the most recent birth records in the US to more closely analyze the primary causes underlying mortality rates among full-term births.

Methods and findings

Linked birth and death records for the period 2010–2012 were used to identify the state- and cause-specific burden of infant mortality among full-term infants (born at 37–42 weeks of gestation). Multivariable logistic models were used to assess the extent to which state-level differences in full-term infant mortality (FTIM) were attributable to observed differences in maternal and birth characteristics. Random effects models were used to assess the relative contribution of state-level variation to FTIM. Hypothetical mortality outcomes were computed under the assumption that all states could achieve the survival rates of the best-performing states. A total of 10,175,481 infants born full-term in the US between January 1, 2010, and December 31, 2012, were analyzed. FTIM rate (FTIMR) was 2.2 per 1,000 live births overall, and ranged between 1.29 (Connecticut, 95% CI 1.08, 1.53) and 3.77 (Mississippi, 95% CI 3.39, 4.19) at the state level. Zero states reached the rates reported in the 6 low-mortality European countries analyzed (FTIMR < 1.25), and 13 states had FTIMR > 2.75. Sudden unexpected death in infancy (SUDI) accounted for 43% of FTIM; congenital malformations and perinatal conditions accounted for 31% and 11.3% of FTIM, respectively. The largest mortality differentials between states with good and states with poor FTIMR were found for SUDI, with particularly large risk differentials for deaths due to sudden infant death syndrome (SIDS) (odds ratio [OR] 2.52, 95% CI 1.86, 3.42) and suffocation (OR 4.40, 95% CI 3.71, 5.21). Even though these mortality differences were partially explained by

Table. The European data underlying Fig 1 is available on the Euro-Peristat webpage at http://www.europeristat.com/our-indicators/euro-peristat-perinatal-health-indicators-2010.html.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: CDC, US Centers for Disease Control and Prevention; FTIM, full-term infant mortality; FTIMR, full-term infant mortality rate; NCHS, National Center for Health Statistics; OR, odds ratio; SIDS, sudden infant death syndrome; SUDI, sudden unexpected death in infancy.

state-level differences in maternal education, race, and maternal health, substantial state-level variation in infant mortality remained in fully adjusted models (SIDS OR 1.45, suffocation OR 2.92). The extent to which these state differentials are due to differential antenatal care standards as well as differential access to health services could not be determined due to data limitations. Overall, our estimates suggest that infant mortality could be reduced by 4,003 deaths (95% CI 2,284, 5,587) annually if all states were to achieve the mortality levels of the best-performing state in each cause-of-death category. Key limitations of the analysis are that information on termination rates at the state level was not available, and that causes of deaths may have been coded differentially across states.

Conclusions

More than 7,000 full-term infants die in the US each year. The results presented in this paper suggest that a substantial share of these deaths may be preventable. Potential improvements seem particularly large for SUDI, where very low rates have been achieved in a few states while average mortality rates remain high in most other areas. Given the high mortality burden due to SIDS and suffocation, policy efforts to promote compliance with recommended sleeping arrangements could be an effective first step in this direction.

Author summary

Why was this study done?

- High infant mortality rates in the US compared to other high-income countries have been well documented in the literature.
- Most of this literature primarily attributes high infant mortality in the US to the high rates of prematurity.
- Relatively little is known regarding the survival of infants born full-term.

What did the researchers do and find?

- We compared state-level mortality rates among full-term infants in the US to that of 6 European countries with low mortality rates.
- We showed that infants born full-term in the US face 50%–200% higher risks of infant mortality compared to these European countries.
- We found that the largest proportion of infant deaths among children born full-term in the US was due to sudden unexpected deaths of infants, which comprised both sudden infant death syndrome and other unexpected causes such as suffocation and violence.

What do these findings mean?

- Major improvements in full-term infant mortality (through increases in full-term infant survival and increases in pregnancy terminations) seem possible in the US.
- More research is needed to identify the most effective policies to achieve this objective.

Introduction

Despite some progress made in recent years, infant mortality rates in the US continue to be high compared to other high-income countries [1]. According to the latest estimates, the US currently ranks 44th among 199 countries of all income levels, with an infant mortality rate of 5.6 deaths per 1,000 live births in 2015, about 3 times the rate observed for countries at the very top of the ranking [1].

While the high rates of prematurity and prematurity-related mortality in the US have been well documented in the literature [2,3], the US performs comparably to other high-income countries when it comes to the survival of preterm infants. Fig 1 compares gestation-specific mortality rates in the US and 6 leading European countries (in terms of low infant mortality rates) with data available for 2010. On average, infant mortality appeared to be very similar for premature births in the US and in these European countries. The same was not true for children born after 36 weeks of gestation, where children born in the US faced more than twice the mortality risk of children in European countries with low infant mortality rates (odds ratio [OR] 2.02, 95% CI 1.84, 2.22). A recent US Centers for Disease Control and Prevention (CDC) report suggests that this mortality gap among full-term births now accounts for almost 50% of the infant mortality gap between Sweden and the US [4].

In this study, we used complete and geocoded birth records from the period 2010–2012 to better understand the high burden of mortality among full-term infants in the US. We identified the main causes underlying the high mortality rates among full-term infants overall in the aggregate data in a first step, and then explored differences in actual and potential birth outcomes across US states in a second step. By first reviewing the causes of death in this population, we could identify the main risk factors for infants in this generally low-risk population, and could clearly distinguish the relative importance of preexisting conditions such as malformations relative to perinatal and post-neonatal conditions (those arising in the 28–364 days after birth). In order to provide a better sense of feasible outcomes in this population, we estimated and compared cause-specific full-term mortality rates at the state level both unconditional and conditional on maternal characteristics. While these state-level comparisons did not allow us to identify the specific reasons why certain states have particularly high rates of mortality, they did allow us to identify areas where major improvements were possible in principle.

Methods

Study design

The study was designed as a cross-sectional study using birth and death records of all infants born in the US between January 1, 2010, and December 31, 2012. No pre-analysis plan was developed for this study. The main objective of the project was to identify the primary causes

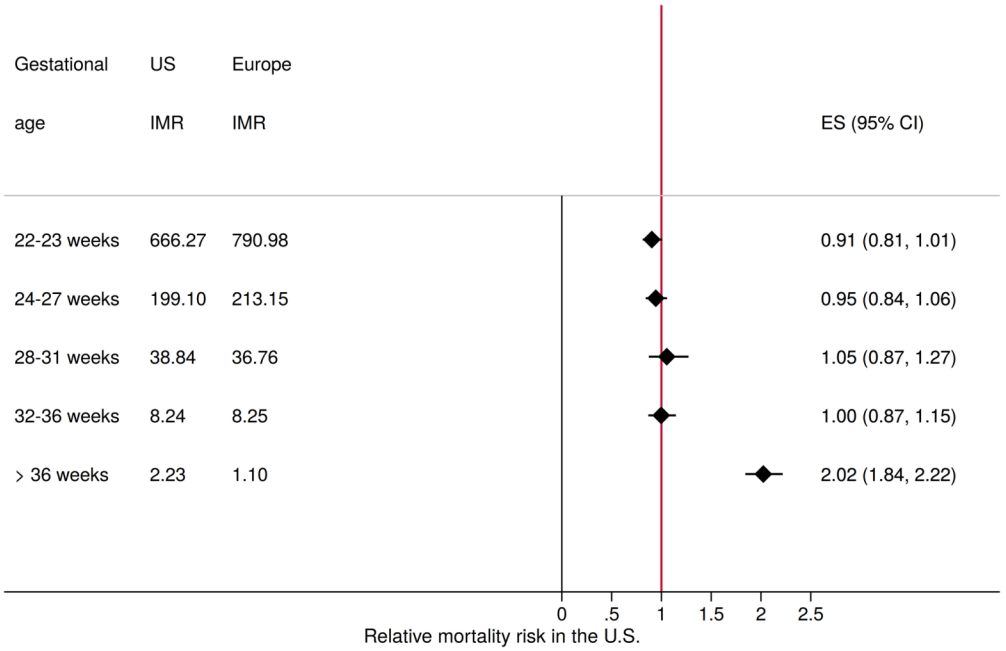

Fig 1. Relative mortality risk in the US and Europe by gestational age category. The figure shows infant mortality risk (IMR) in the US compared to the average rate observed in Austria, Denmark, Finland, Norway, Sweden, and Switzerland for the year 2010. Sources: Euro-Peristat, US birth and death records, author calculations. Gestational age in both the Euro-Peristat and US data is based on the best obstetrical estimate available, which in most cases corresponds to first trimester ultrasound. ES, effect size.

https://doi.org/10.1371/journal.pmed.1002531.g001

underlying the high infant mortality rates observed in the US nationally as well as at the state level.

Data sources

Linked birth and death records including restricted geographic identifiers were obtained from the National Center for Health Statistics (NCHS) for the years 2010 to 2012. All infants in the birth and death records could be directly linked to the geographic identifiers in these datasets (100% match rate). Additional data for Fig 1 were downloaded from the Euro-Peristat webpage at http://www.europeristat.com/our-indicators/euro-peristat-perinatal-health-indicators-2010.html.

Outcome measures

Our primary outcome measure of interest was the infant mortality rate among full-term births defined as the number of deaths per 1,000 children born alive between 37 and 42 weeks of gestation within the first year of their life. For the purpose of this study, we used the traditional definition of full-term, which includes early-term (37 and 38 weeks), full-term (39 and 40 weeks), late-term (41 weeks), and some post-term (42 weeks) births according to the more recent definition of the American College of Obstetricians and Gynecologists Committee on Obstetric Practice [5]. To adjust for differential outcomes in this relatively wide 5-week gestational window, we controlled for differences in gestational age by including binary indicators for gestational age category (37 weeks, 38 weeks, 41 weeks, 42 weeks) in our multivariable analysis, using the more narrow, revised full-term definition (39 and 40 weeks) as our reference group. Gestational age was computed by the NCHS based on last menstrual period reported

by the mother. To ensure gestational age was not measured differentially across states, we compared prematurity rates with rates of low birth weight in the full sample at the state level. The correlation of these measures at the state level was 0.97; the strong alignment between birth weight and reported gestational age is further supported by the descriptive statistics provided in S1 Table.

Causes of death for all children who died under the age of 1 year were based on death certificates, which are required to be completed by either a coroner or medical examiner in all US states, following CDC guidelines. Even though regulations vary by state, deaths due to violence or suspicious circumstances are further investigated and certified by a medical legal officer [6].

Death certificates were reviewed and coded following ICD-10 guidelines by the NCHS. For the purpose of this paper, we grouped reported causes of death into 4 main categories: (1) congenital malformations: ICD-10 codes Q00–Q99; (2) sudden unexpected death in infancy (SUDI): ICD-10 codes V01–Y89 and R00–R99; (3) perinatal conditions: ICD-10 codes P00–P96; and (4) all other causes: all other ICD-10 codes.

The SUDI grouping was chosen intentionally to minimize potential state-level differences in the attribution of unexplained deaths to sudden infant death syndrome (SIDS) versus "other unexplained causes" [7–9]. Some more disaggregated statistics for major causes of deaths (such as SIDS) were also computed as described further below.

Exclusion criteria

Children born prior to 37 or after 42 weeks of gestation were excluded from this study. All other children born alive in the US between January 1, 2010, and December 31, 2012, including multiple births and children born with malformations (not reported in the NCHS dataset), were analyzed in this study.

Covariates

In order to assess the extent to which state-level differences in infant mortality rates can be attributed to differences in maternal characteristics, we considered the following variables included in the original data file: mother's age, educational attainment, smoking behavior, diabetes, chronic hypertension, and eclampsia. We divided maternal age into 5 categories (<20, 20–34, 35–39, 40–44, and >44 years) and used age 20–34 as the reference group in our multivariable analysis. Similarly, we divided maternal educational attainment into 4 categories: less than high school, high school or some college credit without degree, associate or bachelor's degree, and master's degree or doctorate. In response to a reviewer request, we also added controls for mother's race: white, black, American Indian/Alaskan Native, and Asian/Pacific Islander. As for smoking, mothers reported the average number of cigarettes smoked per day during their first, second, and third trimesters. From this we constructed indicators for smoking (number of cigarettes per day > 0) or not for each trimester. We used indicators for previous diagnosis of diabetes, chronic hypertension, and eclampsia as provided in the dataset. All these variables were based on mother's self-report in the hospital around the time of delivery and were reported on the birth certificate. In addition, we included controls for the following birth characteristics: birth weight category (<1,500, 1,500–1,999, 2,000–2,499, 2,500–2,999, 3,000–3,499, 3,500–3,999, 4,000–4,499, and >4,499 g), multiple birth (1 if singleton, 2 if twin, 3 if triplet, 4 if quadruplet, and 5 if quintuplet or higher), infant sex, and gestational age (indicators for 37 weeks, 38 weeks, 41 weeks, and 42 weeks of gestation) in our empirical models. Further details of all these variables are provided in S1 Table.

Statistical methods

As a first step, we computed full-term infant mortality rates (FTIMRs) at the state level, and classified all US states into 5 groups: states with excellent FTIMR (FTIMR < 1.25—the European benchmark shown in Fig 1), states with good FTIMR ($1.25 \leq$ FTIMR < 1.75), states with average FTIMR ($1.75 \leq$ FTIMR < 2.25), states with fair FTIMR ($2.25 \leq$ FTIMR < 2.75), and finally states with poor FTIMR (FTIMR ≥ 2.75). The "excellent" group was chosen based on the FTIMRs observed in 6 European countries (Austria, Denmark, Finland, Norway, Sweden, and Switzerland), which ranged between 0.97 and 1.24, with a median FTIMR of 1.11. The remaining groups were defined by sequentially adding 0.5 deaths per 1,000 full-term live births (a 50% increase relative to the European average) to the cutoffs. In a second step, we decomposed mortality differences at the group level by cause of death. Third, we used multivariable regression models to assess the extent to which survival differences across states can be attributed to observable differences in maternal and birth characteristics. To do so, we first ran multivariable logistic models comparing infants born in the states with the highest mortality rates to infants born in the states with the lowest mortality rates. We estimated 3 separate models: a first model, where we did not adjust for any covariates; a second model, where we adjusted for maternal characteristics outlined in the covariates section above; and a third model (proposed by a reviewer), where we adjusted for maternal characteristics and birth characteristics (gestational age, infant sex, birth weight, and multiple birth). Model 2 was estimated to assess the extent to which state-level differences can be attributed to local variation in maternal characteristics such as age, education, race, and health status. Model 3 was estimated to assess the extent to which subsequent mortality differentials were explained by local variation in the prevalence of multiple births as well as differences in birth weight and the distribution of gestational age. In all 3 models, each observation corresponded to a child born full-term in the sample period. To assess the overall contribution of state-level characteristics to variation in FTIMR, we also estimated multilevel logistic models where we nested individual observations within states, and then estimated between-state variance in unconditional models as well as in models conditioning on maternal and birth characteristics. Lastly, we computed hypothetical mortality rates (which we refer to as "counterfactuals") under the assumptions that (i) all US states achieved the overall FTIMR of the best-performing states (good FTIMR group) and (ii) all US states achieved the specific FTIMRs of the best-performing state in each cause-of-death category.

Results

A total 10,175,481 children born full-term in the US between January 1, 2010, and December 31, 2012, were analyzed. FTIMR was 2.19 (95% CI 2.16, 2.22) per 1,000 full-term live births in the pooled sample. At the state level, estimated FTIMR ranged between 1.29 (95% CI 1.08, 1.53) in Connecticut and 3.77 (95% CI 3.39, 4.19) in Missouri. No state was classified as excellent in terms of their FTIMR; 10 states including Connecticut were classified as good, 17 as average, 11 as fair, and 13 as poor FTIMR (see Fig 2 and S2 Table for details).

Fig 3 compares early neonatal (death in the first 6 days after birth), late neonatal (death between 7 and 27 days after birth), and post-neonatal (death 28–364 days after birth) mortality rates across mortality groups. While only relatively minor differences were found with respect to early neonatal mortality, large absolute and relative differences were found for the post-neonatal period, with an average of 9.5 (95% CI 9.1, 9.9) deaths per 10,000 full-term births in states classified as having good FTIMR and a mortality rate of 20.9 (95% CI 20.1, 21.6) deaths per 10,000 full-term births in the states classified as having poor FTIMR.

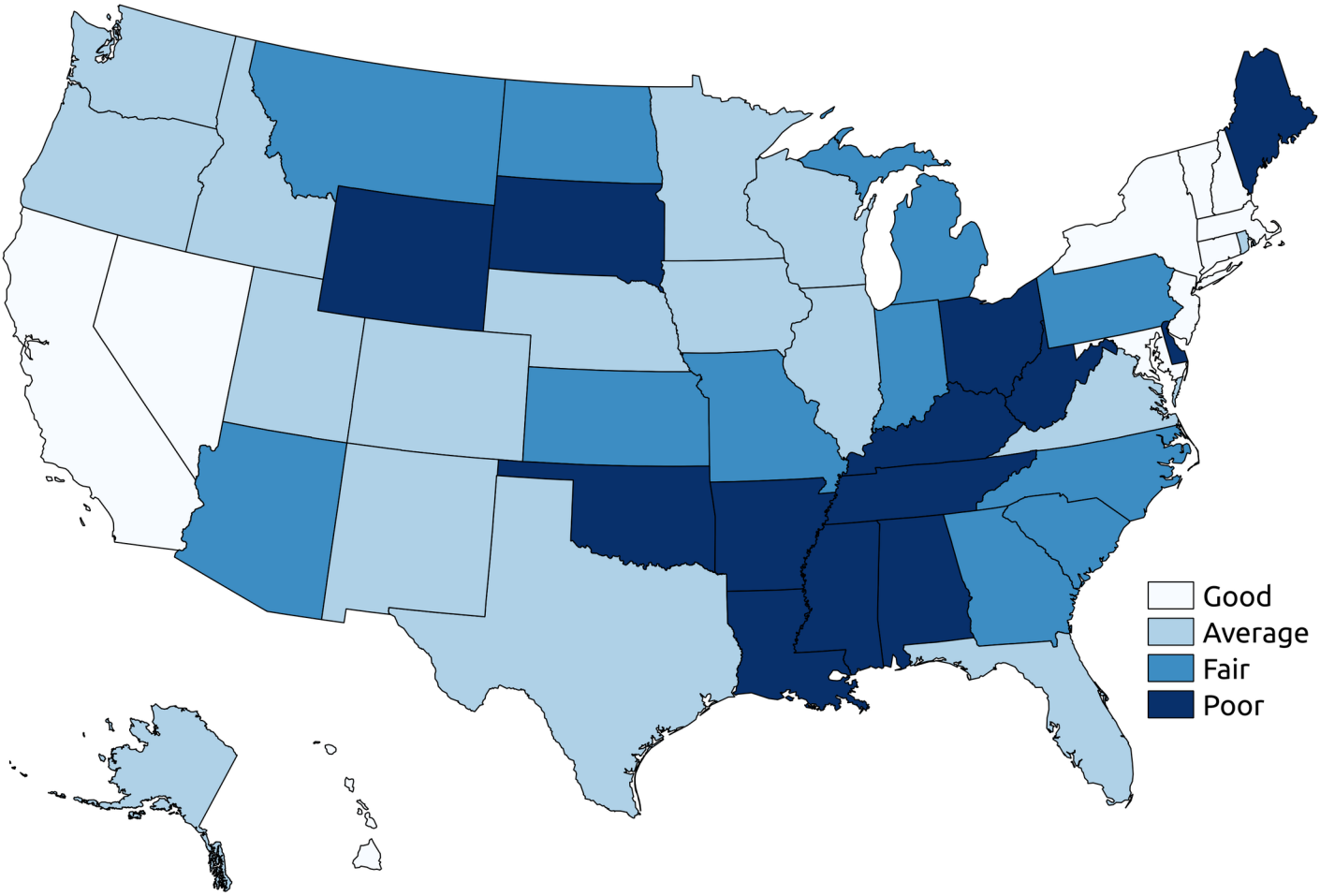

Fig 2. State-level FTIMR classification. The figure shows state level classification: states with good FTIMR ($1.25 \leq$ FTIMR < 1.75), states with average FTIMR ($1.75 \leq$ FTIMR < 2.25), states with fair FTIMR ($2.25 \leq$ FTIMR < 2.75), and states with poor FTIMR (FTIMR ≥ 2.75). All estimates are for full-term infants born in 2010–2012. FTIMR, full-term infant mortality rate.

https://doi.org/10.1371/journal.pmed.1002531.g002

Fig 4 summarizes the main causes of full-term infant mortality (FTIM). SUDI accounted for the largest proportion of deaths overall (43%), followed by congenital malformations (31%) and perinatal conditions (11%). The mortality risk due to congenital malformations increased from 5.6 deaths per 10,000 full-term live births in states with FTIMR < 2.75 to 8.4 deaths in states with poor FTIMR. The risk of SUDI was 5.6 in the states classified as having good FTIMR and 15.4 in the states classified as having poor FTIMR. Observed absolute mortality differences between FTIMR groups were smallest for perinatal conditions, with an estimated mortality rate of 2.1 in the states with good FTIMR and an estimated mortality of 2.8 in states with poor FTIMR.

In S1 and S2 Figs, we provide further details on the primary causes of congenital malformations. The 2 most common causes of deaths due to congenital malformation were Edwards syndrome and congenital malformations of the heart, which accounted for 10.9% and 14.6% of congenital malformation deaths, respectively.

In terms of the underlying causes of SUDI, 42.2% of SUDIs were due to SIDS (ICD-10: R95), followed by unknown and ill-defined causes (ICD-10: R99), which accounted for 20.6% of SUDIs, and accidental suffocation and strangulation (ICD-10: W75), which accounted for 16.1% of SUDIs. S3–S7 Figs provide further details on the spatial distribution of cause-specific SUDIs.

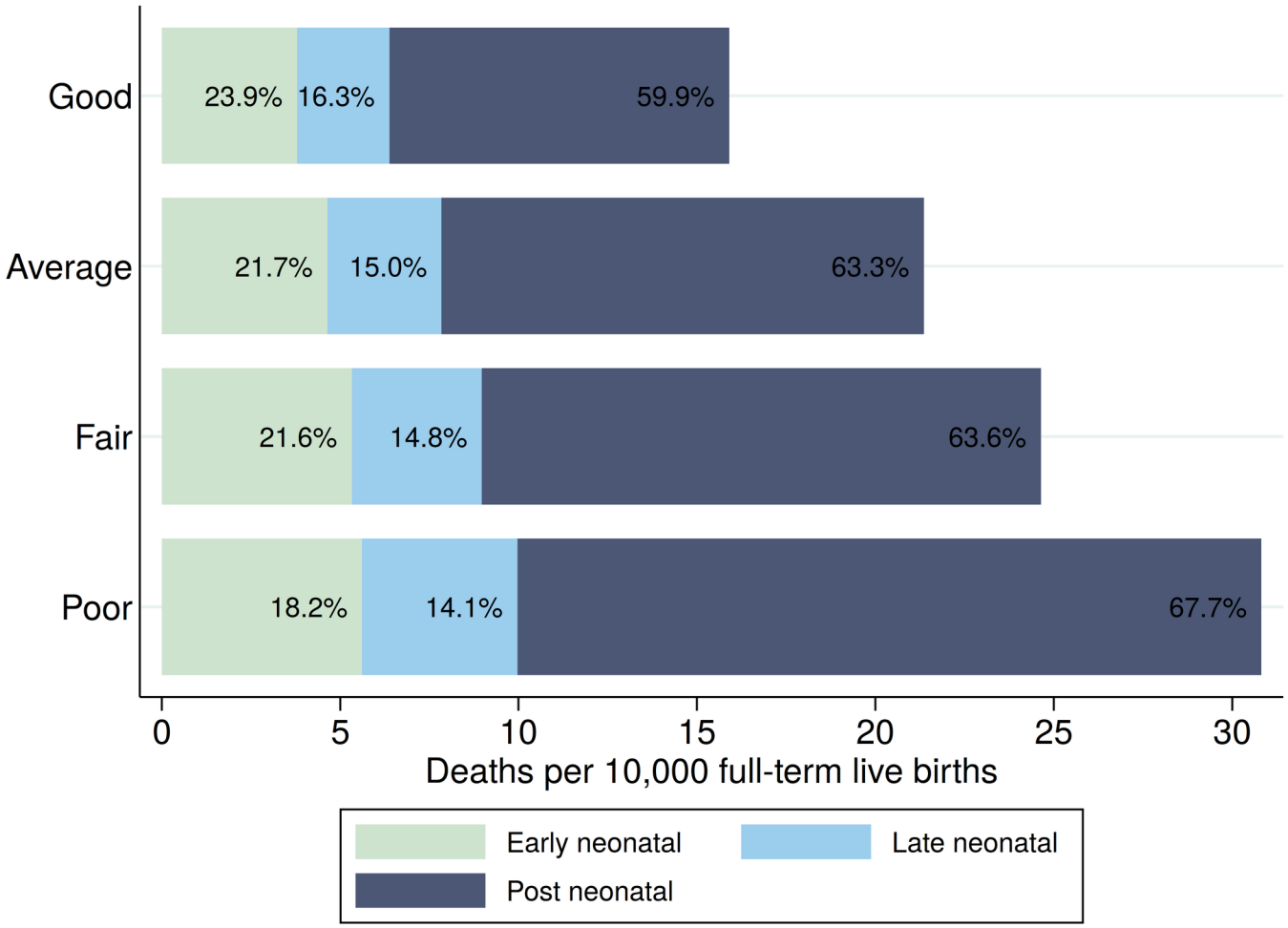

Fig 3. Group-specific mortality by age of death. The figure shows the number of infant deaths per 10,000 full-term births in the US by period and overall mortality group (states grouped on the basis of FTIMR: good, 1.25 ≤ FTIMR < 1.75; average, 1.75 ≤ FTIMR < 2.25; fair, 2.25 ≤ FTIMR < 2.75; and poor, FTIMR ≥ 2.75) for the years 2010 to 2012 as well as the percentage of deaths in each age category. Early neonatal mortality is defined as death in the first 6 days after birth. Late neonatal mortality is defined as deaths between 7 and 27 days after birth, and post-neonatal mortality is defined as death 28 to 364 days after birth. FTIMR, full-term infant mortality rate.

https://doi.org/10.1371/journal.pmed.1002531.g003

S8 Fig summarizes the relative importance of the 4 mortality groups in the neonatal, late neonatal, and post-neonatal periods. Congenital malformations accounted for 58.1% and 43.3% of overall mortality in the early neonatal and late neonatal periods, respectively. Perinatal conditions accounted for 31.7% and 22.8% of mortality in the same periods. In the post-neonatal period—which accounted for the majority of deaths overall (63.5%, as shown in Fig 3)—the large majority (60%) of deaths were due to SUDI.

Table 1 shows estimated OR for the group of states with poor FTIMR compared to the group of states with good FTIMR for the 4 main cause-of-death categories displayed in Fig 4. The table shows unadjusted OR estimates and OR estimates adjusted for the full set of covariates summarized in S1 Table. In unadjusted models, living in a state with poor FTIMR was associated with an increased odds of FTIM due to perinatal conditions of 35% (OR 1.35, 95% CI 1.17, 1.56) as well as an increased odds of death due to congenital malformations of 51% (1.51, 95% CI 1.24, 1.85). Risk differentials were largest for SUDI, with an estimated OR of 2.75 (95% CI 2.46, 3.07). When we adjusted for maternal age, education, race, and measures of

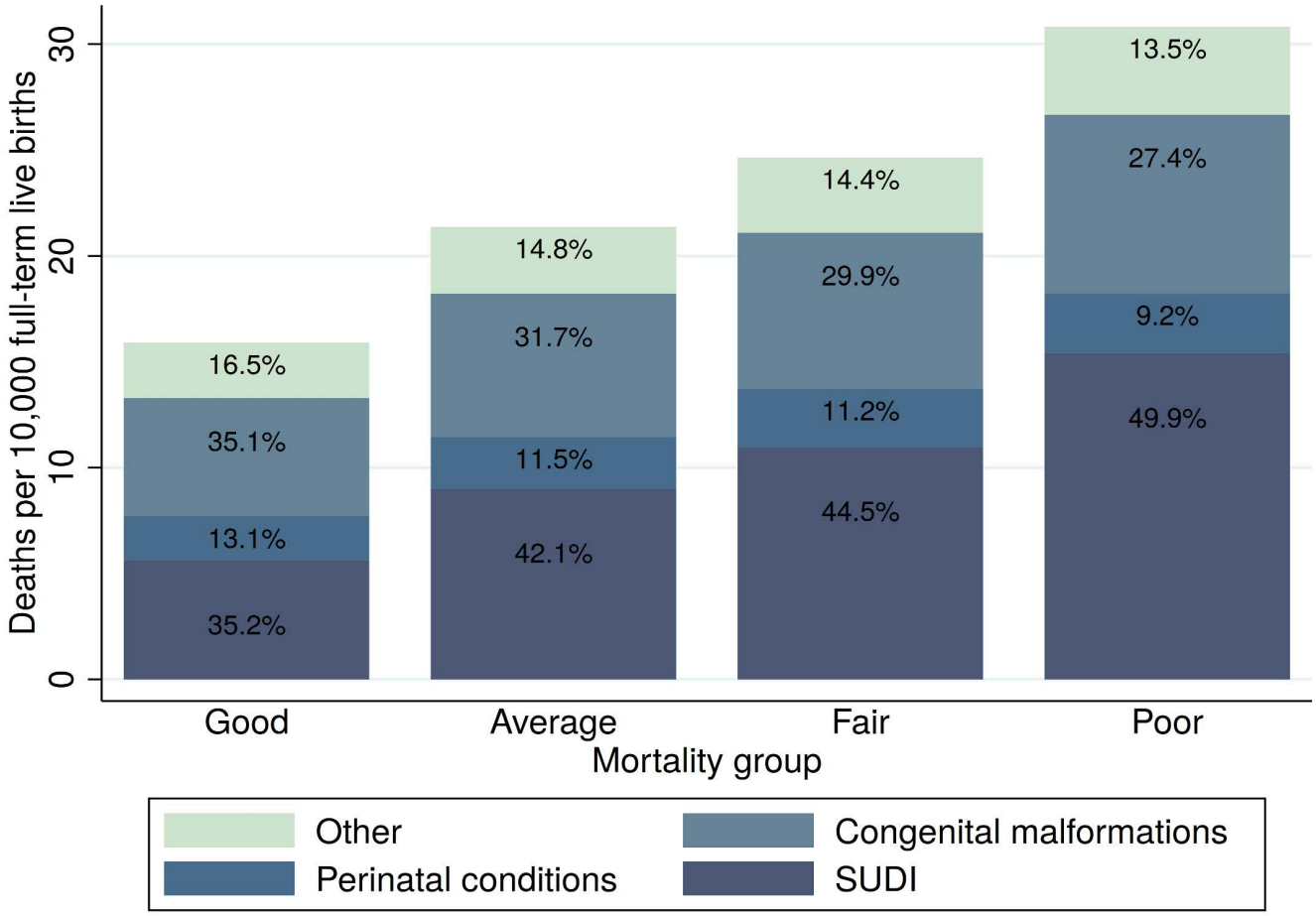

Fig 4. Cause-specific mortality rates. The figure shows the total number of deaths by FTIMR group for the years 2010–2012 as well as the percentage of deaths in each group in the different cause-of-death categories. The following ICD-10 causes of death were included: congenital malformations, Q00–Q99; SUDI, V01–Y89 and R00–R99; perinatal conditions, P00–P96; other, all other ICD-10 codes. Mortality group refers to states grouped on the basis of FTIMR: good ($1.25 \leq$ FTIMR < 1.75), average ($1.75 \leq$ FTIMR < 2.25), fair ($2.25 \leq$ FTIMR < 2.75), and poor (FTIMR ≥ 2.75). FTIMR, full-term infant mortality rate; SUDI, sudden unexpected death in infancy.

https://doi.org/10.1371/journal.pmed.1002531.g004

health status, estimated risk differentials declined for all risk factors, with the largest declines for SUDI, where estimated OR fell from 2.75 in unadjusted models to 1.70 in models adjusting for both maternal and birth characteristics. In general, differences between models 2 (adjusting for maternal characteristics only) and 3 (adjusting for maternal characteristics and birth characteristics) were small and not statistically significant.

In Table 2, we show estimated state variability in mortality outcomes based on multilevel logistic models. State-level variation was highest for SUDI (estimated state-level variance 0.118, 95% CI 0.068, 0.168) and congenital malformations (0.061, 95% CI 0.028, 0.095). These state-level differences were reduced substantially for all causes when we controlled for differences in maternal and birth characteristics, with particularly large reductions for SUDI, where estimated state variability dropped to 0.034 (95% CI 0.014, 0.054) when both maternal and birth characteristics were included in the model.

Table 3 shows estimated annual FTIM for our 2 hypothetical scenarios. Under the assumption that all states would achieve the survival outcomes of the 10 states with the lowest mortality

Table 1. Relative odds of cause-specific full-term infant mortality in states with poor FTIMR relative to states with good FTIMR.

Cause of death	Unadjusted odds		Adjusted odds			
	Model 1		Model 2		Model 3	
	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Congenital malformations	1.51**	1.24, 1.85	1.43**	1.28, 1.59	1.37**	1.19, 1.58
Perinatal conditions	1.35**	1.17, 1.56	1.19	1.01, 1.42	1.16	0.97, 1.38
SUDI	2.75**	2.46, 3.07	1.73**	1.51, 1.98	1.70**	1.48, 1.94
Other causes	1.58**	1.37, 1.82	1.40**	1.16, 1.67	1.37**	1.15, 1.64

Table shows relative odds of cause-specific mortality in states with overall poor FTIMR compared to states classified with good FTIMR. The states classified as having good FTIMR include CA, CT, HI, MA, MD, NH, NJ, NV, NY, and VT (total births 2,885,191; total deaths 4,589), and the states classified as having poor FTIMR include AL, AR, DE, KY, LA, ME, MS, OH, OK, SD, TN, WY, and WV (total births 1,476,604; total deaths 4,551). Model 2 adjusts for maternal characteristics including mother's age, education, race, and health status (smoking behavior, diabetes, chronic hypertension, and eclampsia). Model 3 adjusts for maternal characteristics and birth characteristics including gestational age, infant sex, birth weight, and multiple birth.

**$p < 0.01$.

FTIMR, full-term infant mortality rate; SUDI, sudden unexpected death in infancy.

https://doi.org/10.1371/journal.pmed.1002531.t001

outcomes overall (good FTIMR group), infant mortality would decline by an estimated 2,023 (95% CI 1,717, 2,329) deaths each year. Under the more ambitious counterfactual that all states could achieve the cause-specific mortality rates of the best-performing state in each cause-of-death category, infant mortality among full-term births would be reduced by 4,003 deaths (95% CI 2,284, 5,587) each year. Under both hypothetical scenarios, only about 10% of the potential improvements were related to perinatal conditions or other causes. More than 75% of the excess burden of mortality in both scenarios was due to congenital malformations and SUDI.

Discussion

The results presented in this paper show a large gap in the survival probabilities of full-term infants born in the US compared to European countries with low under-5 mortality rates. Pooling all available data between 2010 and 2012, we found that no single US state or territory achieved the full-term survival rates currently reported in leading European countries, with children born full-term in the 10 best-performing states facing about 50% higher risks of infant mortality, and children born in states with poor FTIMR facing almost 3 times the infant mortality risk of European countries with low infant mortality rates.

Given that survival rates among preterm infants in the US were found to be very similar to those of the same European countries (as illustrated in Fig 1), clinical care during or immediately

Table 2. Variation (on logit scale) in cause-specific mortality between US states estimated using the random intercept logistic model.

Model	Variance (95% CI) by cause of death			
	Congenital malformations	Perinatal conditions	SUDI	Other causes
Model 1	0.061** (0.028, 0.095)	0.021 (0.002, 0.039)	0.118** (0.068, 0.168)	0.033* (0.012, 0.054)
Model 2	0.032* (0.011, 0.054)	0.012 (0.000, 0.026)	0.035** (0.014, 0.056)	0.025 (0.003, 0.039)
Model 3	0.036* (0.013, 0.058)	0.012 (0.000, 0.020)	0.034** (0.014, 0.054)	0.025 (0.003, 0.039)

Estimates show state-level variation in mortality outcomes. Variances as well as 95% confidence intervals estimated using multivariable logistic model, where individuals (level 1) are nested into states (level 2). The results of the fully specified model are displayed in S4 Table.

*$p < 0.05$

**$p < 0.01$.

SUDI, sudden unexpected death in infancy.

https://doi.org/10.1371/journal.pmed.1002531.t002

Table 3. Estimated preventable deaths among full-term births.

Cause of death	Actual number of deaths 2010–2012	Counterfactual scenario			
		Mortality of good FTIMR group		Best US state	
		Predicted deaths (95% CI)	Mortality reduction	Predicted deaths (95% CI)	Mortality reduction
Congenital malformations	2,308	1,897 (1,805, 1,990)	411	683 (0, 1,456)	1,625
Perinatal conditions	839	711 (654, 767)	128	340 (0, 724)	499
SUDI	3,187	1,906 (1,813, 1,998)	1,281	1,752 (1,458, 2,046)	1,435
Other	1,098	894 (831, 958)	203	654 (387, 921)	444
All	7,431	5,408 (5,102, 5,714)	2,023	3,428 (1,844, 5,147)	4,003

Based on an estimated 3.4 million full-term live births per year. The best state estimates are from Vermont (congenital malformations, 2.01 deaths per 10,000 full-term births, 95% CI 0, 4.28), Rhode Island (perinatal conditions, 1.00 deaths per 10,000 full-term births, 95% CI 0, 2.13), New Jersey (SUDI, 5.15 deaths per 10,000 full-term births, 95% CI 4.29, 6.02), and Oregon (other causes, 1.92 deaths per 10,000 full-term births, 95% CI 1.14, 2.71). Good FTIMR group refers to states with $1.25 \leq \text{FTIMR} < 1.75$.

FTIMR, full-term infant mortality rate; SUDI, sudden unexpected death in infancy.

https://doi.org/10.1371/journal.pmed.1002531.t003

after delivery likely does not explain much of the mortality gap observed. In the sample analyzed, perinatal conditions—where healthcare quality likely matters most—accounted only for about 11% of total infant mortality among full-term births. In terms of the big picture, the high burden of FTIM in the US seemed to be mostly due to SUDI and congenital malformations, which accounted for 42.9% and 31.1% of the total infant mortality burden among full-term children, respectively, and for almost 80% of excess deaths in our counterfactual analysis. From a policy perspective, deaths due to malformations are quite different from deaths classified as SUDI. Malformations are in practice hard, if not impossible, to prevent; in most cases, the only way to "prevent" malformation-related infant mortality is to increase screening and early termination. In terms of the overall magnitude, we found malformation-specific FTIMRs of less than 3 per 10,000 live births in some states, such as Vermont and New Jersey, and rates 3 times higher in quite a few states in the Mississippi delta and surrounding states (see S2 Fig for details). Globally, WHO estimates suggest that 330,000 children die annually during the neonatal period due to congenital malformations [10,11], which corresponds to a risk of approximately 2.5 deaths per 10,000. Taking these global estimates as a benchmark suggests that children in the US face about 3 times the risk of death due to malformation in other countries. In practice, the extent to which these differences reflect differences in screening and termination policies rather than differences in medical care across states and countries is not clear; further research investigating the reach and effectiveness of early screening programs across countries and states will be needed to better understand these current gaps.

With respect to actual health improvements, the area with the most obvious and ample room for increasing the chances of child survival is SUDIs. Given that the attribution of deaths to SIDS versus "other unexplained causes" was not obvious in many cases [7,8], we mostly focused on the larger SUDI category in this paper. More than 3,000 infants died in the US each year between 2010 and 2012 due to causes that were—as the name suggests—not expected under normal conditions. This is perhaps most immediately obvious when it comes to accidental suffocation or strangulation in bed. Over 600 infants die in the US each year due to suffocation in bed; new strategies to convey optimal sleeping arrangements to parents will need to be developed and tested to prevent these deaths.

SUDI mortality in the best-performing states of the US (California and New York) was less than 6 deaths per 10,000 births; rates were more than twice as high (>12) in 12 states, including Ohio, South Dakota, and Tennessee. A large fraction of these deaths were attributed to SIDS,

which has previously been estimated to cause 6.4 deaths per 10,000 births [12]. Our results suggested SIDS incidence rates as low as 1.27 and 1.32 per 10,000 full-term live births in Nevada and New Mexico and as high as 13.33 and 8.75 in Arkansas and Mississippi. Evidence from European studies suggests that a large majority of SIDS deaths could historically be attributed to prone sleeping and maternal drug consumption [13]. Through active public health programs, the incidence of SIDS was lowered by 75% in Sweden [14] and Scotland [15]; general compliance with sleeping recommendations continues to be a challenge in the US, particularly among women with low socioeconomic status [16]. Empirically, a large proportion of the state-level differences in mortality due to both SIDS and the broader SUDI category could be attributed to state-level differences in maternal age and maternal education. As shown in the more detailed regression results in S4 Table, maternal characteristics were highly predictive of these mortality outcomes. We found that compared to children born to mothers with incomplete high school education, children of highly educated mothers (those with master's degree or doctorate) had 74% lower odds of SUDI, and that the risk of SUDI almost linearly declined with maternal age (conditional on all other factors). This suggests that mortality in this category is strongly influenced by maternal behavior and the early home environment, both of which should at least in principle be modifiable through targeted information and behavioral change interventions.

Our analysis is not without limitations. First, we have relatively little information on children's home environments, and thus cannot directly identify what is happening at children's homes or compare underlying risk factors. Second, it is possible that state-level estimates that we present may be biased if people move before or after birth. Empirically, for 97% of the observations, state of birth is the same as state of residence, which means that these biases should be small if they exist. Third, as mentioned above, we do not have information on termination rates at the state level, which are likely to (at least partially) explain differences in birth outcomes observed. According to the latest estimates available, approximately 700,000 legally induced abortions occurred in 2012 in the US [17], which corresponds to about 20% of the annual sample analyzed in this study. While it seems likely that infant mortality rates would be higher without these terminations, our data do not allow us to directly quantify these differences. Last, it seems likely that some of the less common causes of death (particularly in the ICD-10 R and W categories) are miscoded or coded differentially across states. To reduce this type of measurement error, we grouped all SUDIs together for most of our analyses.

Conclusion

More than 7,000 children born alive at full-term in the US each year die within their first year of life. The results presented in this paper suggest that a substantial proportion of these deaths are preventable, with particularly large improvements possible for SUDI.

Supporting information

S1 Fig. Primary causes of death due to malformation. The figure shows the FTIMR burden for the 7 most common causes of death due to malformation by mortality group among full-term infants born in 2010–2012.
(TIF)

S2 Fig. Spatial distribution of full-term infant mortality due to malformations. The figure shows the number of infant deaths per 10,000 full-term births due to malformations among full-term infants born in 2010–2012.
(TIF)

S3 Fig. Primary causes of deaths due to SUDI. The figure shows the FTIMR burden for the most common causes of death classified as SUDI by mortality group among full-term infants born in 2010–2012.
(TIF)

S4 Fig. Infant deaths per 10,000 full-term live births classified as SUDI. The figure shows the number of infant deaths per 10,000 full-term infants born in 2010–2012. Estimates include all deaths filed under ICD-10 codes V01–Y89 and R00–R99.
(TIF)

S5 Fig. Infant deaths per 10,000 full-term live births due to violence or assault. The figure shows the number of infant deaths per 10,000 full-term infants born in 2010–2012. Estimates include all deaths filed under ICD-10 codes Y079 (unspecified perpetrator of maltreatment and neglect) and Y09 (assault by unspecified means).
(TIF)

S6 Fig. Infant deaths per 10,000 full-term live births due to suffocation. The figure shows the number of deaths per 10,000 full-term births in 2010–2012 due to suffocation. Estimates include all deaths filed under ICD-10 codes W75 (accidental suffocation and strangulation in bed) and W84 (unspecified threat to breathing).
(TIF)

S7 Fig. Deaths per 10,000 full-term live births across states due to SIDS. The figure shows the number of deaths per 10,000 full-term births in 2010–2012 due to SIDS. Estimates include all deaths filed under ICD-10 code R95 (sudden infant death syndrome).
(TIF)

S8 Fig. Percentage of deaths in early, late, and post-neonatal periods due to specific causes. The figure shows the percentage of deaths occurring due to each cause of death in the early neonatal (1), late neonatal (2), and post-neonatal periods (3) in the years 2010–2012. Early neonatal mortality is defined as death in the first 6 days after birth. Late neonatal mortality is defined as deaths between 7 and 27 days after birth, and post-neonatal mortality is defined as deaths 28 to 364 days after birth.
(TIF)

S1 Table. Sample characteristics.
(DOCX)

S2 Table. State-specific mortality rates.
(DOCX)

S3 Table. Cause-specific mortality distribution by mortality group.
(DOCX)

S4 Table. Estimates from random intercept logistic model (odds ratio).
(DOCX)

S5 Table. Estimates from a logistic model using mortality groups (odds ratio).
(DOCX)

Acknowledgments

The authors would like to thank the National Center for Health Statistics as well as Euro-Peristat for the data provided to this project.

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EXHIBIT 35

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Vaccine Safety

Vaccines and Sudden Infant Death Syndrome (SIDS)

Vaccines have not been shown to cause sudden infant death syndrome (SIDS).

Babies receive multiple vaccines when they are between 2 to 4 months old. This age range is also the peak age for sudden infant death syndrome (SIDS). The timing of the 2 month and 4 month shots and SIDS has led some people to question whether they might be related. However, studies have found that vaccines do not cause and are not linked to SIDS.

Multiple research studies and safety reviews have looked at possible links between vaccines and SIDS. The evidence accumulated over many years do not show any links between childhood immunization and SIDS.

Placing healthy babies on their backs to sleep reduces the risk of SIDS.

SIDS is the sudden, unexpected death of a baby younger than 1 year of age that doesn't have a known cause even after a complete investigation. These deaths often happen during sleep or in the baby's sleep area.

In 1992, the American Academy of Pediatrics recommended that healthy babies be placed on their backs to sleep. That recommendation, along with the National Institute of Child Health and Human Development's 1994 "Back-to-Sleep" campaign (now known as the Safe to Sleep® campaign), encouraged caregivers to place infants on their backs to sleep, and coincided with a dramatic reduction in the SIDS rate in the United States. See the latest recommendations on a safe infant sleep environment from the American Academy of Pediatrics.

Learn more about SIDS

Learn more about CDC resources, publications, and activities to address SIDS.

Exhibit 35

Also, research has found the rate of SIDS declined dramatically following the 1994 "Back-to-Sleep" campaign, and then stabilized in the 2000s at a time when the number of infant immunizations was increasing. The findings provide strong evidence that immunization is not linked to SIDS. See the published article on SIDS rates.

CDC and FDA closely monitor the safety of all vaccines.

CDC and the Food and Drug Administration (FDA) are committed to ensuring that vaccines provided to the public are safe and effective. Once vaccines are licensed in the United States, CDC and FDA continuously monitor them through several safety systems. Learn more about CDC's vaccine safety monitoring and research.

Related Scientific Articles

Moro PL, Perez-Vilar S, Lewis P, Bryant-Genevier M, Kamiya H, Cano M. Safety Surveillance of Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) Vaccines . *Pediatrics*. 2018;142(1). pii: e20174171.

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Page last reviewed: October 16, 2018

Content source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)

Exhibit 35

EXHIBIT 36

Infanrix hexa and sudden death: a review of the periodic safety update reports submitted to the European Medicines Agency

Article *in* Indian Journal of Medical Ethics · September 2017

DOI: 10.20529/IJME.2017.079

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Exhibit 36

Indian Journal of Medical Ethics Online First Published September 5, 2017

COMMENT

Infanrix hexa and sudden death: a review of the periodic safety update reports submitted to the European Medicines Agency

JACOB PULIYEL, C SATHYAMALA

Abstract

There have been a number of spontaneous reports of sudden unexpected death soon after the administration of Infanrix hexa (combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliomyelitis and Haemophilus influenza type B vaccine). The manufacturer, GlaxoSmithKline (GSK), submits confidential periodic safety update reports (PSURs) on Infanrix hexa to the European Medicines Agency (EMA). The latest is the PSUR 19. Each PSUR contains an analysis of observed/expected sudden deaths, which shows that the number of observed deaths soon after immunisation is lower than that expected by chance.

This commentary focuses on that aspect of the PSUR which has a bearing on policy decisions. We analysed the data provided in the PSURs. It is apparent that the deaths acknowledged in the PSUR 16 were deleted from the PSUR 19. The number of observed deaths soon after vaccination among children older than one year was significantly higher than that expected by chance once the deleted deaths were restored and included in the analysis.

The manufacturer must explain the figures that have been submitted to the regulatory authorities. The procedures undertaken by the EMA to evaluate the manufacturer's claims in the PSUR need to be reviewed. The Drugs Controller General of India nearly automatically accepts drugs and vaccines approved by the EMA. There is a need to reappraise the reliance on due diligence by the EMA.

Introduction

On October 23, 2000, the marketing of two hexavalent vaccines, Infanrix hexa® (GlaxoSmithKline plc-GSK) and Hexavac® (Sanofi Pasteur MSD, SNC), which combine diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliomyelitis and Haemophilus influenza type B, was authorised in the European Union. Following authorisation, there were several spontaneous reports of sudden unexpected death soon after the administration of these hexavalent vaccines. In 2005, von Kries and colleagues (1) performed a detailed analysis in which they compared the observed deaths soon after vaccination with the deaths expected by chance. They found that the standardised mortality ratio (SMR) within two days of the Hexavac vaccination was significantly increased among children vaccinated in the second year of life. This was not the case with Infanrix hexa™. At the request of the marketing authorisation holder, Hexavac was withdrawn in 2005 and Infanrix hexa continued to be marketed in Europe (2).

According to European law, the European Medicines Agency (EMA) is accountable for the protection of public health through the evaluation of the medicines approved by it as the regulatory authority. The manufacturers are responsible for the efficacy, quality and safety of their drugs (3).

The Italian Court of Judge Nicola Di Leo made GlaxoSmithKline's confidential 15th and 16th periodic safety update reports (PSURs) from 2009 to 2011 available to the public (4). The PSUR 19 (incorporating PSURs 17, 18 and 19, dated January 15, 2015) was obtained by Dr Loretta Bolgan from the EMA under Article 3 of the EMA rules (EMA 110196/2006 of November 30, 2010) (5). Dr Bolgan sent this PSUR to the first author (JP), requesting him to write a report to be presented to the European Parliament. This commentary is based on all these PSURs. In the context of the safety aspect previously highlighted by von Kries (1), this commentary examines sudden deaths following the use of the Infanrix hexa vaccine. Other aspects dealt with in the PSURs are not examined.

PSUR 15 – clustering of deaths after vaccination

Most deaths occurring in the post-neonatal period are due to infections, congenital defects, malignancies or accidents. Seldom do babies die without any evident cause and such deaths are classified as (i) sudden infant death syndrome (SIDS), defined in the PSUR as death that occurs in the first year of life and remains unexplained after autopsy, or (ii) sudden unexpected death (SUD), defined as death which occurs in the first two years of life, and which remains unexplained after clinical and final event history, but without autopsy. Together, these two are considered sudden death (SD) in the PSUR 15.

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To cite: Puliyel J, Sathyamala C. Infanrix hexa and sudden death: a review of the periodic safety update reports submitted to the European Medicines Agency. *Indian J Med Ethics*. Published online on September 5, 2017. Corrected on September 9, 2017*. DOI: 10.20529/IJME.2017.079

Manuscript Editor: Mala Ramanathan

Exhibit 36

Indian Journal of Medical Ethics Online First Published September 5, 2017

A number of vaccines are administered on any given day to children under the age of 2 years; the number of children vaccinated all over the world is very large. It is possible that by chance, some vaccinated children might die of coincidental SIDS/SUD, events which might have occurred even if these children had not been vaccinated on that day. To ascertain if such a death was caused by vaccination or was a coincidental event, an observed/expected analysis of SD is performed. The analysis estimates if the number of deaths observed after vaccination exceeds that which can be expected by chance.

Sudden deaths: observed vs expected

The PSUR 15 explains how this analysis is performed (4:p 782): "The Company evaluated whether the number of sudden deaths reported in this age group exceeded the number one could expect to occur by coincidence. Since the distribution of the age at which subjects are vaccinated is unknown, the Company assumed that the proportion of adverse events by age is representative for the actual age distribution at vaccination. It can thus be estimated that 90.6% of all recipients of Infanrix hexa™ were in their first year of life, and 9.4% were in their second year of life. Therefore, the number of doses (since launch) was estimated to be 54,927,729 and 5,698,904, respectively. Given that Germany is the main country where Infanrix hexa doses are distributed (close to 30% only in Germany), it was assumed that the incidence of sudden death observed in Germany is representative of the entire population of Infanrix hexa™ recipients (German Federal Bureau of Statistics, Statistisches Bundesamt; incidence rate in first year of life: 0.454/1000 live births; second year: 0.062/1000 live births, data 2008)."

The PSUR documents the deaths reported within 20 days of vaccination.

The number of observed deaths was less than what was expected (Table 1). However, among the infants, there was a clustering of deaths immediately following vaccination, with 42 deaths taking place in the first three days after vaccination, and only 8 in the next 3 days. Among those below one year of age, 54 deaths (93%) occurred in the first 10 days, and 4 (7%) in the next 10 days. Had the deaths been "coincidental SIDS deaths", this disparity in the number of deaths in the two time periods would not have been observed. SIDS deaths would have been spread uniformly over the 20-day period. The fact that the rate of death decreases rapidly with the passage of time following immunisation suggests that the deaths could be related to vaccination.

Similarly, among children older than one year, 5 deaths (83.3%) occurred in the first 10 days and 1 death (17%) occurred in the next 10 days. The clustering of deaths reported in the PSUR 15 was noticed in the PSUR 16 as well, and this has been commented upon previously (6).

GlaxoSmithKline response

Responding to this criticism (7), the Chief Executive Officer (CEO) of GlaxoSmithKline (GSK), Sir Andrew Witty, through the company's Chief Medical Officer, Dr Norman Begg, suggested in a letter that reporters are much more likely to think about a potential causal association and thus, report an event to GSK if it occurs shortly after vaccination rather than if it occurs weeks later. He further wrote, "In light of the above, we remain confident in the conclusions previously reached by GSK and shared with regulatory agencies and public health authorities worldwide that the currently available data do not suggest an increased risk of sudden infant death following vaccination with Infanrix hexa. Should the available data and information change to suggest that there is such an increased risk, we

Table 1 (*Corrected)
PSUR 15: analysis of observed/expected sudden deaths

Time since vaccination	1st year			2nd year		
	Observed deaths	Cumulative observed deaths	Cumulative expected deaths	Observed deaths	Cumulative observed deaths	Cumulative expected deaths
Less than 1 day	10	10	54.7	1	1	0.8
1 day	10	20	109.3	1	2	1.5
2 days	13	33	164.0	1	3	2.3
3 days	9	42	218.6	0	3	3.1
4 days	7	49	273.3	0	3	3.9
5 days	1	50	327.9	0	3	4.6
6 days	0	50	382.6	0	3	5.4
7 days	1	51	437.3	1	4	6.2
8 days	1	52	491.9	1	5	7.0
9 days	2	54	546.6	0	5	7.7
13 days	0	54	765.2	1	6	10.8
15 days	1	55	874.5	0	6	12.4
16 days	1	56	929.2	0	6	13.2
18 days	1	57	1038.5	0	6	14.7
19 days	1	58	1093.1	0	6	15.5

(***Source:*** *Adapted from Table 24, The GlaxoSmithKline Biological Clinical Safety and Pharmacovigilance Report to Regulatory Authority, PSUR 15, p.783*)

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remain committed to promptly notify the authorities and to take the necessary actions to communicate such data and information to healthcare professionals."

This response contains a tacit admission that there was no active surveillance during the post-vaccination period and only deaths spontaneously reported to GSK were included under the heading of "observed deaths". This was likely to result in an underestimation of the deaths following vaccination. It is to be noted that for "expected deaths" the number of doses of vaccine distributed is utilised. The report acknowledges that all the doses of the vaccine distributed need not have been utilised. In this way, the figure for "expected deaths" may have been inflated.

However, in view of the CEO's explanation and assurance that GSK was committed to promptly notify the authorities and healthcare professionals of any increased risk with Infanrix hexa, the matter of the clustering of deaths was not pursued further.

PSUR 16: doubling of expected deaths

If all children who received the first dose of the vaccine go on to receive four doses and the last dose is in the second year of life, then it can be estimated that one-fourth (25%) of the doses are administered to children over the age of one year. This is the vaccine schedule recommended in Germany. However, some countries, such as Italy, advise only three doses, all in the first year and none in the second. Also, not all children receive all the doses recommended. So it is unlikely that 20%–25% of doses are used in the second year. In the PSUR 15, it was estimated that 90.6% of the doses sold were used for infants under one year of age and 9.4% for those above one year of age. In the PSUR 16, the estimate of doses received in the second year more than doubled (from 9.4% to 20%), and thus the estimate of expected deaths doubled. In spite of the doubling of expected deaths, the number of observed deaths in the second year was higher than expected in the first 3 days after vaccination (Table 36, p249). If the PSUR 15 estimate that 9.4 % of the doses are used in the second year is correct and holds true for the PSUR 16, observed deaths are higher than expected deaths in the first 7 days.

Table 2
PSUR 16: observed/expected deaths in 2nd year

Time since vaccination (days)	Cumulative observed (2nd year) PSUR 16	Cumulative expected deaths reported in PSUR 16 after doubling recipient numbers (20% doses in 2nd year)	Cumulative expected deaths if 9.4% doses were used in the 2nd year (as in PSUR 15)*
0	2	1.98	0.93
1	5	3.96	1.86
2	6	5.94	2.79
3	6	7.92	3.72
4	6	9.9	4.65
5	7	11.88	5.58
6	7	13.86	6.51
7	7	15.84	7.44

*Source: Adapted from PSUR 16, Table 36, p249 *Calculated by the authors*

PSUR 19: expected deaths weighted by country and yearly proportion of doses

In the PSUR 19, a weighted average of sudden deaths by calendar time of the German, French and Dutch incidence rates was calculated to arrive at the expected incidence of sudden deaths. In very simple terms, this means that if 60% of the doses were distributed in Germany in a given year, the SD rate in Germany was given a weightage of 60% when calculating the overall SD rate for that year; if 30% were distributed in France, the SD rate in France was given a weightage of 30% and 10% weightage was given to the Dutch SD rate. Finally, the overall SD rate was calculated for all the years together. The overall SD rate was calculated as 0.0102/1000 live births for the second year. This figure is one-sixth of the expected rate used in the PSURs 15 and 16 (which calculated expected sudden deaths at 0.062/1000 live births, using German data).

The Poisson 95% CI of the observed deaths in the second year is reported in Table 8 on p 447 of the PSUR 19. It is reported that for the second year of life, the number of observed deaths was higher, though not significantly, than that of expected deaths within a risk period of 1–4 days after vaccination.

Missing deaths in the PSUR 19

From the PSUR 16 to the PSUR 19, the total doses of the vaccine went up from 69 million to 112 million. According to the PSUR 19, 20.2% of the doses distributed were presumed to have been given to children in the second year of life (PSUR 19, pp 436–448). Cases of death in which the age of vaccination was not known, the time to death was not recorded, or the time to death exceeded 19 days, were excluded.

The PSUR 19 (deaths up to October 22, 2014) does not report the sudden deaths mentioned in the PSUR 16 (cases of death occurring up to October 22, 2011). It is of note that in the PSUR 16 the age of the child who died after vaccination and the time to death (within 14 days of vaccination) were both recorded. The cumulative deaths reported are lower in the PSUR 19 than in the PSUR 16. As for children over one year of age, the PSUR 19 records the occurrence of only 5 deaths in the first 19 days after vaccination, whereas the PSUR 16 reports 8. The numbers are not consistent with each other. We wonder why this is so.

Ten years after the publication of a Center for Disease Control paper examining the relationship between the measles, mumps and rubella (MMR) vaccine and autism (8), one of the authors, William Thompson, admitted that he and his co-authors omitted statistically significant information showing that African American males who received MMR before the age of 36 months were at increased risk of autism (9). The authors deleted the data of children who did not have Georgia birth certificates (10), thus disqualifying a disproportionate number of black children, and presented their data so that it

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showed that there was no increased risk. It is not clear whether the authors of the PSUR 19 similarly disqualified children documented to have died in the PSUR 16.

Table 3 presents the observed and expected deaths reported in the PSUR 19 and the observed deaths after restoring the deaths reported in the PSUR 16.

When the observed death figures from the PSUR 16 are used, the number of observed deaths is significantly higher than expected for the first four days after vaccination. It must be borne in mind, as explained earlier, that since the number of observed deaths is collected passively, it is likely to be underestimated. Expected deaths, on the other hand, are likely to be overestimated as they are calculated with the assumption that all the doses distributed have been used without any wastage and no vaccine has been discarded on account of exceeding its shelf life. GSK should have reported the statistically significant increased risk of death in the four-day period after vaccination to the regulatory authority and medical practitioners.

Table 3:
PSUR 19: Observed and expected deaths in the 2nd year

Time since vaccination (days)	Cumulative observed deaths according to PSUR 19	Cumulative observed deaths in PSUR 16* (Poisson 95% CI)	Cumulative expected deaths according to PSUR 19
0	0	2 (0.24-7.22)	0.54
1	2	5 (1.62-11.67)	1.08
2	3	6 (2.20-13.05)	1.62
3	3	6 (2.20-13.05)	2.16
4	3	6 (2.20-13.05)	2.70
5	3	7 (2.81-14.42)	3.24
6	3	7 (2.81-14.42)	3.77
7	3	7 (2.81-14.42)	4.31
8	4	7 (2.81-14.42)	4.85
9	4	7 (2.81-14.42)	5.39
10	4	7 (2.81-14.42)	5.93
11	4	7 (2.81-14.42)	6.47
12	4	7 (2.81-14.42)	7.01
13	5	8 (3.45-15.76)	7.55
14	5	8 (3.45-15.76)	8.09
15	5	8 (3.45-15.76)	8.63
16	5	8 (3.45-15.76)	9.17
17	5	8 (3.45-15.76)	9.71
18	5	8 (3.45-15.76)	10.24
19	5	8 (3.45-15.76)	10.78

Source: Data adapted from Table 8, PSUR 19, p 447
(**Data on deaths from the PSUR 16 from Table 36, p 249, with Poisson 95% CI added in*)

Doses used in the second year

The PSUR 19 assumes that 20.2% doses have been used in the second year. It states that since the distribution of the age at which subjects are vaccinated is unknown, the company assumed that the proportion of adverse events (including death) by age is representative of the actual age distribution at vaccination. Thus, as 20.2% of adverse events occurred among children above one year of age, the company assumed that 20.2% doses were used for this age group.

It is facile to estimate the number of doses used in the second year on the basis of the observed adverse events (including death), and then use this estimate of doses to calculate the number of expected deaths, and finally, to compare this number with that of observed deaths – given that the estimate of expected deaths is calculated from the observed adverse events (including death) in the first place.

Assuming that all deaths following vaccination are coincidental SIDS/SUD deaths and not causally related to the vaccine, and given that (according to the PSUR 19) the natural frequency of sudden deaths in the first year is 44 times higher than that in the second year (0.441/1000 in the first year and 0.0102/1000 in the second year), 44 times as many children have to be vaccinated in the second year to reach the same number of deaths as in the first year. In a cohort of 100 deaths, if 20% of sudden deaths occur in the second year and 80% in the first year, 880 children have to be vaccinated in the second year for every 80 vaccinated in the first year. In that case, it must be assumed that 91% of all doses of Infanrix hexa are used in the second year and only 9% are used in the first year (instead of it being the other way around). This reflects the absurdity of calculating dose distribution by age, on the basis of the age distribution of adverse events, as done in the GSK document.

The only way to estimate the number of doses used in the second year is to examine the vaccination schedules in different countries – looking at countries that advise the fourth dose in the second year and those that do not advise any doses in the second year. A weightage can be given for the number of doses distributed in these countries. The dropout rate (children dropping out of the vaccination programme after receiving the first doses) must also be factored into the final calculation of the proportion of doses used in the second year. It would seem that a reasonable estimate of doses used in the second year is probably 9.4% of the total doses, and this is the figure used in the PSUR 15.

The ethical dilemma – the trolley problem

This commentary does not attempt to examine if these excess deaths after vaccination (presumed to be caused by the vaccine) can be offset by the lives saved through disease prevention owing to the vaccine. In her classical thought experiment, called the "Trolley dilemma", Philippa Foot asks if it is ethical to redirect a runaway trolley from a track on which it would kill five persons to another track where only one would die (11). In a variation of the trolley dilemma, the single person on the alternative track is the child of the person who can switch the tracks. Judith Thomson assumes that five lives can be saved with organ transplants from one healthy

donor, and asks if it would be ethical to surreptitiously kill one person to save the other five (12). Ethicists argue that the end cannot justify the means. If one glosses over the deaths after vaccination, one can prevent/delay the evaluation of the vaccine's safety profile and this has the potential to result in more, unnecessary deaths, which is difficult to justify ethically.

Relevance to India

The regulatory authority of the Government of India is the Drug Controller General of India (DCGI). According to the DCGI's rules, drugs approved in one or more countries, such as the USA, the UK, Canada, Japan, Australia and the countries of the European Union, will be considered for approval in India (13). Only bridging studies for the evaluation of the impact of ethnic factors on the efficacy, safety, dosage and dose regimens of the drugs are required (14).

Recently, studies examining the immunogenicity and safety of the hexavalent combination in small trials have been published from India (15, 16). Also, *Indian Pediatrics* published an editorial entitled "Hexavalent vaccinations: The future of routine immunization?"(17), which suggested that this combined vaccine was being promoted for India. It is crucial that the regulatory authority in India is aware of the concerns raised in this commentary on the PSUR reports. This is especially so because surveillance systems in India are weak.

Summary and conclusion

von Kries (1) reported a statistically significant increase in the SMR in children in their second year of life, within two days of vaccination with Hexavac® (one of the two licensed hexavalent vaccines, now withdrawn).

In its periodic safety update reports, GSK, the company manufacturing Infanrix hexa, evaluates whether the number of sudden deaths reported after vaccination with their product exceeded the number that could be expected by chance. The clustering of deaths soon after immunisation suggests that the deaths could have been caused by the vaccine.

Furthermore, our analysis shows that the deaths acknowledged in the PSUR 16 have been deleted from the PSUR 19. The observed deaths are spontaneously reported to GSK and are likely to be underestimated. Adding in the deaths deleted from the PSUR 16, there is a statistically significant increased risk of death in the first four days after vaccination, compared to the expected deaths. The manufacturers will need to explain why these deaths were not included in the PSUR 19. The increased risk of death was not communicated to the regulatory authorities or to the health personnel administering this vaccine.

Given the above, it is difficult to understand how the EMA accepted the PSUR 19 at face value. It may be argued that due diligence was not exercised, as a result of which numerous children were unnecessarily exposed to the risk of death.

The DCGI must be made aware of these infirmities in the PSUR on Infanrix hexa™.

*__Corrections:__ *This paper was published online on September 5, 2017 and taken off the website for corrections by the authors on September 6, 2017. These corrections were:*

1) Table 1, Column 7: the entire column was replaced as figures had been taken from the wrong document. Corresponding changes were made in Column 1.

2) On the subsequent pages, corrections were made with regard to two numbers, column heads in both Tables 2 and 3; and References 8 to 14 which have been re-numbered.

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EXHIBIT 37

Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International 179 (2008) e25–e29
Forensic Science International
www.elsevier.com/locate/forsciint

Case report

β-Tryptase and quantitative mast-cell increase in a sudden infant death following hexavalent immunization

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Received 9 September 2007; received in revised form 18 April 2008; accepted 23 April 2008
Available online 6 June 2008

Abstract

The association between sudden infant death syndrome and immunization is frequently discussed. Serious adverse events following vaccination have generally been defined as those adverse events that result in permanent disability, hospitalization or prolongation of hospitalization, life threatening illness, congenital anomaly or death. They are generally referred to the inherent properties of the vaccine (vaccine reaction) or some error in the immunization process (programme error).The event could also be totally unrelated but only temporally linked to immunization (coincidental event). A fatal case of a 3-month-old female infant, who died within 24 h of vaccination with hexavalent vaccine is presented. Clinical data, post-mortem findings (acute pulmonary oedema, acute pulmonary emphysema), quali-quantitative data collected from immunohistochemical staining (degranulating mast cells) and laboratory analysis with a high level of β-tryptase in serum, 43.3 μg/l, allows us to conclude that acute respiratory failure likely due to post hexavalent immunization-related shock was the cause of death.

Keywords: Sudden infant death; Hexavalent vaccine; Mast-cell; β-Tryptase

1. Introduction

Combination vaccines for pediatric immunization are being developed to increase the acceptance of and compliance with vaccine recommendations. The reduction in the number of injections simplifies vaccination schedules, reduces pain reactions, exposure to excipients and adjuvants, the number of office visits and stress for parents and children, and will in the future simplify the introduction of new pediatric vaccines. While it is important from a public health perspective to develop combination vaccines, the safety and efficacy of the existent monovalent vaccines has to be maintained in combination vaccines [4]. Adverse events following immunization are defined as medical incidents which take place after an immunization [10]. Serious adverse events after vaccination have generally been defined as those adverse events that result in permanent disability, hospitalization or prolongation of hospitalization, life threatening illness, congenital anomaly or death. They are generally referred to the inherent properties of the vaccine (vaccine reaction) or some error in the immunization process (programme error). The event could also be totally unrelated but only temporally related to immunization (coincidental event) [5].

We present the fatal case of a 3-month-old female infant, who died within 24 h of vaccination with hexavalent vaccine (Infanrix Hexa).

2. Case report

2.1. Clinical history

The 3-month-old baby was a first born child, born at 41st week of gestation by caesarean delivery, with a birth weight of 3.400 g and an Apgar score of 9–10. The child's mother referred no significant family history, unremarkable pregnancy and good health of the baby, who was bottle-fed, until hexavalent immunization. She also referred that, in the morning, a few hours after immunization (at 11:00 a.m.) the baby presented feeding difficulty. Early in the afternoon the clinical conditions of the baby started getting worse with the onset of severe dyspnoea, so she was immediately taken to the

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doi:10.1016/j.forsciint.2008.04.018

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Emergency Department of the local hospital (7:46 p.m.). A state of shock with critical acute respiratory failure was diagnosed. The baby appeared pale and unresponsive. Inspiratory dyspnoea, triage and inspiration stridor signs were observed; systolic hypotension (50 mmHg) and tachycardia (180 bpm) were also detected (diastolic pressure was unappreciable). Laryngoscopy was unremarkable. Laboratory tests revealed the presence of hypereosinophilia (5%) and metabolic acidosis (pH 7.154) with blood desaturation (pO_2 75.9 mmHg at 8:48 p.m.) and compensatory hypercapnia. Adrenaline was repeatedly administered by aerosol; i.m and e.v. corticosteroids were also administered. The infant died in spite of resuscitation manoeuvres 2 h after hospital admission (10:50 p.m.).

2.2. Autoptic findings

A complete post mortem examination was performed two days after death. No putrefaction phenomena were evident. The autopsy was performed according to the Valdes Dapena method, including careful examination of the cardiac conduction system and of the central and peripheral autonomic nervous structures involved in cardiorespiratory reflexogenesis. The body was that of a 3 month old, well-developed and well-nourished, white infant with a body weight of 4930 g and body length of 55 cm. An immunization puncture mark was observed on the left thigh. Internal examination was unremarkable except for subpleural petechiae and heavy lungs presenting white foam on the main bronchi (Fig. 1).

2.3. Histological studies

The cardiac conduction system was removed in two blocks: The first included the sino-atrial node and the crista terminalis, while the second contained the atrioventricular node, from the His bundle down to the bifurcation and bundle branches. These two blocks were serially cut at intervals of 40 μm (levels) and stained alternately with haematoxylin–eosin and Azan. All tissue specimens were fixed in formalin and embedded in paraffin, then a routine hematoxilin and eosin stain was employed. An immunohistochemical technique was used to estimate mast-cell population, using the anti-tryptase antibody as a mast-cell specific marker on 5 μm thick paraffin sections. Enzyme pretreatment with proteinase K (0.01%; 37 °C) was necessary to facilitate antigen retrieval and to increase membrane permeability to antibodies. The primary anti-tryptase antibody (DAKO) was applied (in) at a 1:100 ratio and incubated overnight at 4 °C. The positive reaction was visualized by 3-diamino-9ethyl-carbazole (AEC) (Sigma). The sections were counterstained with Mayer's hematoxylin and mounted in Aquatex (Merck) and examined under a light microscope. We also proceeded to a quantitative analysis: in each histological section, 10 observations in different fields per slide equivalent to 70 observations were performed. The positive mast-cell count to the tryptase reaction was made at a magnification 10× using a light microscope coupled to a high resolution colour video camera. The video image was inputted to a computer programmed for quantitative morphometry. Afterwards a pulmonary area of 100 mm^2 was analyzed. We examined histological samples from a control group where the cause of death was clearly attributable to traumatic events (10 pediatric cases) [9].

Histological examination revealed polivisceral stasis, mild cerebral oedema. Acute pulmonary oedema mixed with areas of acute pulmonary emphysema were recorded. Myocardial interstitial oedema was also detected. Histological examination of the cardiac conduction system was unremarkable. Small

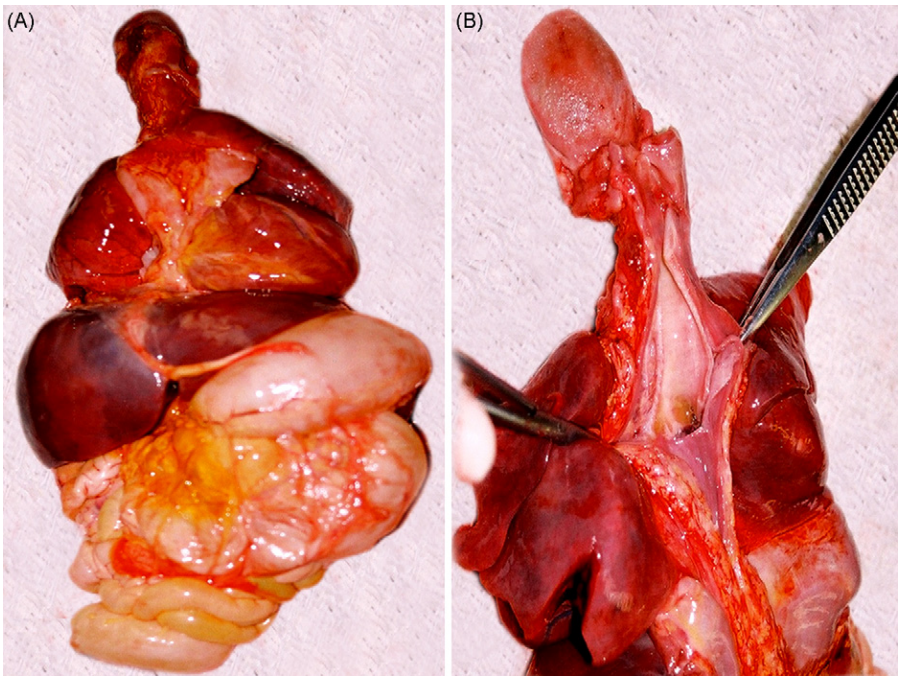

Fig. 1. Post-mortem section according to Valdes Dapena method: (A) sub-pleural petechiae and (B) white foam on the main bronchi.

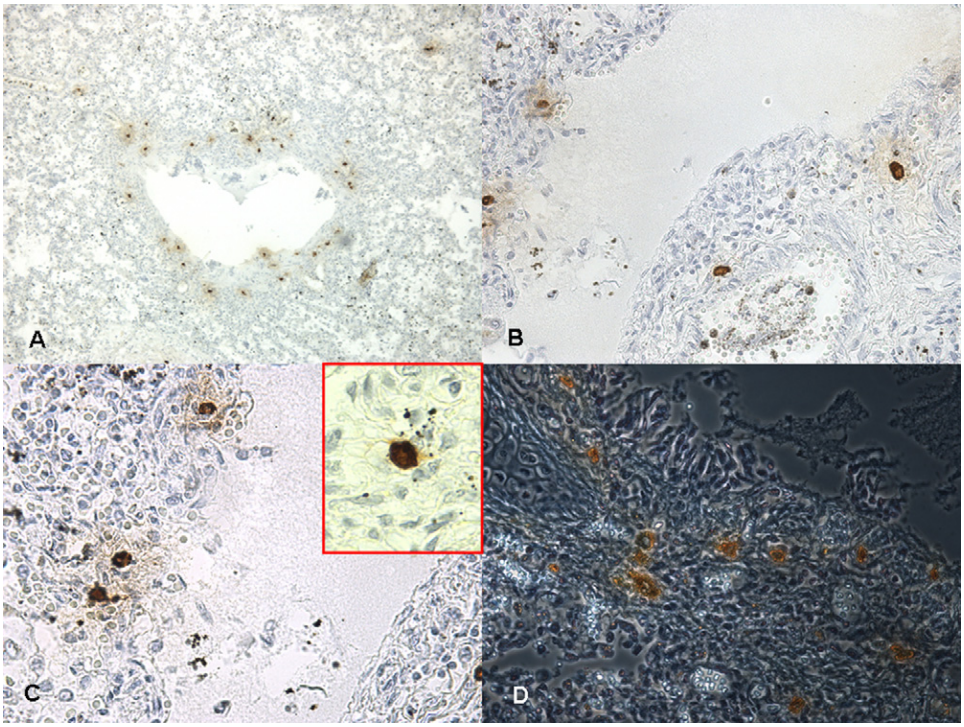

Fig. 2. Lung: degranulating mast cells (starry effect) with tryptase-positive material outside the cells was documented (A–B, Ab anti-tryptase, ×10, ×20). An halo (golden reaction) of tryptase positiveness around the mast cells (insert, ×100) reveals evidence of mast-cell degranulation (C, Ab anti-tryptase, ×40; D phase-contrast, Ab anti-tryptase, ×40).

intraparenchimal haemorrhages on spleen and adrenal glands were observed. Pulmonary mast cells were identified and quantified and a great number of degranulating mast cells with tryptase-positive material outside were observed (Fig. 2). Data resulting from quantitative analysis recorded a numerical increase in pulmonary mast cells in fatal anaphylactic shock (average mast-cell count 12471/100 mm^2) compared with that of the traumatic control group (traumatic death) whose average mast-cell count was 3657/100 mm^2.

2.4. Toxicological analysis

Toxicological analysis on blood and urine specimens for therapeutic and non therapeutic drugs were performed using gas chromatography–mass spectrometry and resulted negative.

2.5. Blood serum dosage

At the dissecting table, 10 cm^3 of femoral blood was extracted and immediately frozen at −20 °C. The tryptase was dosed utilizing an immunoenzymatic technique (Uni-CAP TRYPTASE Fluoroenzymeimmunoassay Pharmacia, AB Uppsala, Sweden). Tryptase level ≤10 μg/l was considered to indicate that mast cell had not occurred near the time of death. The lower limit of sensitivity for the assay was 10 μg/l. Serum dosage of mast cell β-tryptase from femoral blood detecting serum values of 43.3 μg/l (normal value ≤10 μg/l).

3. Discussion

Clinical data, post-mortem findings (acute pulmonary oedema, acute pulmonary emphysema), quali-quantitative data collected from immunoistochemical staining (degranulating mast cells) and laboratory analysis with a high level of β-tryptase in serum, 43.3 μg/l, lead to the conclusion that acute respiratory failure likely due to post hexavalent immunization-related shock was the cause of death.

The association between sudden infant death (SID) and immunization is frequently discussed. Because of the close temporal association between the first immunizations and the main peak of the SID incidence it has been speculated that immunization could cause SID. Many authors confirmed there was no increase in risk of SID from immunization and temporal association between vaccination and SID is coincidental and not causal [20]. A possible protective effect of immunization was also proposed [8]. Firstly, immunization protects against unrecognised whooping cough, which is a potential cause of sudden and unexpected death in this age group; secondly, there is cross-immunization with other bacteria or viruses, which protects infants at this vulnerable age. Many studies investigated the potential of the anaphylactic mechanism to death in infancy by determining relative levels of α and β tryptase and both total and allergen-specific IgE in sera from groups of infants whose deaths were attributed to SIDS or other causes. The comparison of the results of the specific immunoassay which detected both α and β forms with one which detects predominantly β-tryptase has suggested that

α-tryptase is secreted constitutively by mast cells (as it predominates in the serum of normal subjects and patients with systemic mastocitosis) and that β-like tryptase is released by mast cells upon anaphylaxis degranulation (as high concentrations have been found in the serum of patients with anaphylaxis) [3]. The influence of the post-mortem interval is not clear [6,7]. As artifactual values might occur, it is important to investigate possible confounding factors and to establish an upper cut-off value for tryptase measured post-mortem [6]. So several studies have established the usefulness of tryptase as a forensic marker in these conditions; tryptase levels >10 μg/l were considered to reflect substantial mast-cell activation and levels <10 μg/l were considered to indicate that mast-cell activation had not occurred near the time of death. The lower limit of sensitivity for the assay was 1 μg/l [2,9]. In the current study pulmonary mast cells were also identified and quantified using a immunohistochemical technique. Tryptase-positive mast cells were clearly stained and tryptase-positive material was found outside an otherwise intact-appearing mast cell, suggesting prior degranulation [9].

Vaccine associated anaphylaxis is a rare occurrence with only few cases despite of the million of doses administered, giving a risk of 0.65 cases per million doses. After administration of 7,644,049 vaccine doses only five potential cases of vaccine-associated anaphylaxis have been reported, none of which resulted in death. In addition to antigens vaccine contain several other components, including preservatives, adjuvants and manufacturing residuals; although it is not always clear which component might be responsible for anaphylaxis, gelatin and egg proteins are present in some vaccine in at sufficient level to induce hypersensitivity reactions [1]. Polyvalent vaccines were developed to increase acceptance of vaccinations by decreasing the number of injections necessary. Compared to their pentavalent predecessors, the hexavalent vaccines additionally contain hepatitis B serum. They are used for immunization against diphtheria, pertussis, tetanus, influenza, poliomyelitis and hepatitis B [22]. Hexavalent vaccines have been available on the European market since October 2000. A post marketing study demonstrated the safety and immunogenicity of hexavalent vaccine as an alternative to other licensed vaccines, so until April 2003, approximately 3 million children were vaccinated using this method and about 9 million doses were sold in the European union during this time. Children are to be vaccinated with these vaccines at the age of 2, 4, 6 and 12–14 months [23]. In 2003 the European Agency for the Evaluation of Medical Products (EMEA) through its scientific Committee for Proprietary Medicinal Products (CPMP) reviewed the safety of centrally authorized hexavalent vaccine investigating whether there might be a link between hexavalent vaccines and some cases of sudden infant deaths occurred after immunization and concluded that vaccination offers benefit to the individual child and to the general population and the causes of death of the five children dead within 24 h of vaccination with hexavalent vaccine remain unexplained, and it was impossible to establish a cause and effect association with hexavalent vaccines [17,18]. On the 21 September 2005, EMEA withdraw the marketing authorisation for an hexavalent vaccine, because of the low immunogenicity of the hepatitis B component and the variability in the long-term protection against hepatitis B. The Institute of Medicine accepts a causal relation of anaphylaxis with the hexavalent vaccine but the number of vaccine-associated cases seems to be small. It is not always clear which component of vaccine is involved in anaphylactic reactions: vaccine antigens (tetanus toxoid) [12], animal proteins (gelatine) [15] and antibiotics (neomycin) [11].

Very few cases of sudden unexpected death in infancy after hexavalent immunization have been described in literature and only three cases could be investigated with relation to increased serum levels of mast-cell tryptase, suggesting anaphylaxis as the main cause of death [23,13]. All cases of sudden unexpected death occurring in infancy and perinatal age, suggesting post-immunization-related shock as the cause of death, should always undergo a complete necropsy study [14,16,19,20]; immunohistochemical stainings and quantitative morphometry analysis are to be considered significant for the confirmation of suspects from the pathologists' point of view and toxicological analysis and tryptase serum detection represent important supporting evidence upon which diagnosis can be based [3,6,7,21].

Acknowledgment

Supported by a grant of Siniscalco Ceci Foundation, Banca del Monte of Foggia, Italy.

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EXHIBIT 38

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Sudden Infant Death Following Hexavalent Vaccination: A Neuropathologic Study

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Journal Name: Current Medicinal Chemistry

Volume 21 , Issue 7 , 2014

DOI : 10.2174/09298673113206660289 (https://doi.org/10.2174/09298673113206660289)

Journal Home (/node/602)

Abstract:

We examined a large number of sudden infant death syndrome victims in order to point out a possible causal relationship between a previous hexavalent vaccination and the sudden infant death. We selected 110 cases submitted to in-depth histological examination of the autonomic nervous system and provided with detailed clinical and environmental information. In 13 cases (11.8%) the death occurred in temporal association with administration of the hexavalent vaccine (from 1 to 7 days). In none of these victims congenital developmental alterations of the main nervous structures regulating the vital functions were observed. Only the hypoplasia of the arcuate nucleus was present in 5 cases. In one case in particular an acquired hyperacute encephalitis of the tractus solitarii nucleus was diagnosed in the brainstem. This study does not prove a causal relationship between the hexavalent vaccination and SIDS. However, we hypothesize that vaccine components could have a direct role in sparking off a lethal outcome in vulnerable babies. In conclusion, we sustain the need that deaths occurring in a short space of time after hexavalent vaccination are appropriately investigated and submitted to a post-mortem examination particularly of the autonomic nervous system by an expert pathologist to objectively evaluate the possible causative role of the vaccine in SIDS.

Keywords: Autonomic nervous system, brainstem, hexavalent vaccine, neuropathology, risk factors, SIDS.

Article Details

VOLUME: 21
ISSUE: 7
Year: 2014

Article Metrics

PDF: 43

EXHIBIT 39

OPEN

Sudden Infant Death After Vaccination

Survey of Forensic Autopsy Files

Motoki Osawa, MD, PhD, Ryoko Nagao, MD, PhD, Yu Kakimoto, MD, PhD, Yasuhiro Kakiuchi, MD, PhD, and Fumiko Satoh, MD, PhD

Abstract: Sudden infant deaths might be attributable to adverse reaction to vaccination, but separating them from coincidental occurrences is difficult. This study retrospectively investigated vaccination-related details and postmortem findings for 57 cases of sudden death in children 2 years or younger. Data were extracted from autopsy files at the Department of Forensic Medicine, Tokai University School of Medicine. Vaccination histories were available in 50 cases based on the maternity passbook. Of the 32 cases in which any vaccines were administered, 7 infants (21.9%) had received immunization within 7 days of death. The most frequent vaccine cited as the last immunization before death was *Haemophilus influenzae* B. Although a temporal association of vaccines with sudden death was present for two 3-month-old and one 14-month-old infants in whom death occurred within 3 days of receiving the *H. influenzae* type b and other vaccinations, a definitive relationship between the vaccine and death could not be identified. Histopathological examinations revealed pneumonia and upper respiratory infection as contributing to death in their cases. Moreover, all 3 cases showed hemophagocytosis in the spleen and lymph nodes, which are similar features to hemophagocytic lymphohistiocytosis. Judgment of the disorders as truly related to vaccination is difficult, but suspicious cases do exist. Forensic pathologists must devote more attention to vaccination in sudden infant death cases.

Key Words: Hib, *Streptococcus pneumoniae*, forensic autopsy, histopathology, hemophagocytosis

(*Am J Forensic Med Pathol* 2019;40: 232–237)

Sudden infant death (SID) usually occurs during the course of normal development and before revealing clinical symptoms, unlike cases in adults where the cause of death often can be inferred based on the clinical data and history.[1] Therefore, various disorders from abuse to congenital disease must be differentiated in SID cases.[2] To elucidate the etiological background, a sheet of more than 30 check points of settings has been used in Japan.[3] Vaccination history is included among the major points.

For unknown causes of SID, attributable factors have been sought from various approaches. For instance, forensic autopsy cases of unexpected simultaneous twin deaths have been investigated extensively.[4,5] Roberts[6] demonstrated such a twin death, which occurred a couple of hours after diphtheria, tetanus and pertussis (DTP) vaccination, speculating that the immunization potentially gives a clue for the attributable factors. Another case report described twins found dead simultaneously after combined vaccine including DTP.[7] Forensic autopsy has also revealed sudden death after DTP with mast cell increase as a relation to vaccination.[8] By contrast, several large-scale studies have revealed that increased DTP immunization coverage is associated with decreased sudden infant mortality.[9–14] Therefore, most SID syndrome (SIDS) cases are regarded as merely coincident, with no particular relation to DTP vaccination.[15]

Nevertheless, in 2011, 7 fatal incidents occurred in short order after combined immunization with *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* vaccines in Japan.[16] Because some relation was suspected, vaccination was discontinued temporarily. Based on the Preventive Vaccination Act, Japan has a system to notify the Pharmaceuticals and Medical Devices Agency when a critical side effect is suspected. Forensic pathologists receive detailed knowledge about the circumstances preceding death from police officers, but they usually do not devote much attention to the vaccination history unless parents claim adverse effects of vaccination. The number of licensed vaccines has increased to the present day, and they are administrated simultaneously.[17,18] It might be difficult to show evidence of adverse effect despite their potential incidence.[19] Presumably, most SIDS subjects younger than 6 months should inevitably receive vaccination. However, few data have been forthcoming related to how close to the time of death they were vaccinated and which kinds of vaccines were used.

We retrospectively extracted cases of medicolegal autopsy of death after vaccination from infant autopsy reports of the last 5 years. We first present the rate of death within 28 days after vaccination among SID cases, which include subjects younger than 2 years in the present study. In addition, 3 cases in which infants died within 3 days are described. Suspected adverse reactions are highlighted and explored.

MATERIALS AND METHODS

From autopsy cases conducted at the Department of Forensic Medicine, Tokai University School of Medicine, in 5 years (2013–2017), 57 cases of sudden death in infants younger than 2 years were reviewed, excluding deaths that were reasonably attributable to external causes such as abuse or burns. Data from clinical and laboratory examinations done in the emergency department, investigations conducted by police, the maternity record book, and other materials were used. In Japan, a record of received vaccines is noted in a maternity passbook kept by the mother, which includes all medical and welfare records of the mother and her baby. For this study, we obtained vaccination course information from these passbooks, which were available for all but 7 infants. This project was approved by the Ethics Committee of

Manuscript received January 16, 2019; accepted April 24, 2019.
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This work was supported in part by a Health and Labor Sciences Research Grant, Japan.
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ISSN: 0195-7910/19/4003–0232
DOI: 10.1097/PAF.0000000000000494

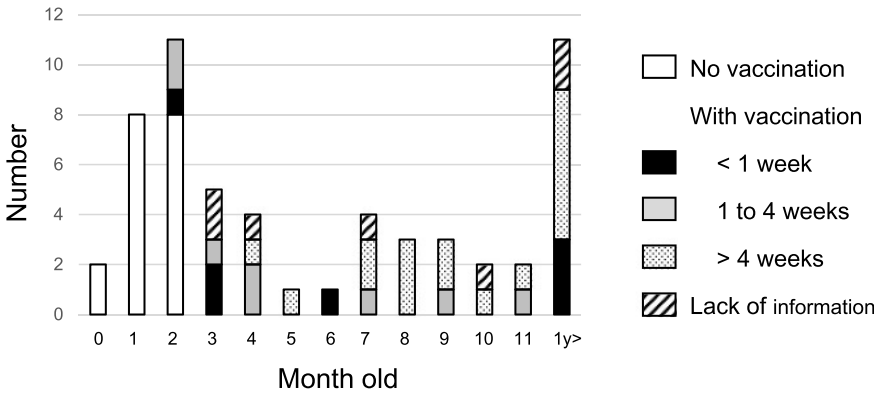

FIGURE 1. Age distribution and days after their last vaccine of 57 SID cases of autopsy for 5 years.

Tokai University School of Medicine as a retrospective clinical study (no. 16-281).

The series of autopsy was performed as reported previously.[20,21] Briefly, tissue examination was performed for formalin-fixed organs using hematoxylin and eosin staining and microscopy, with special staining, such as Gram staining, as appropriate. Biochemical laboratory tests were applied for markers that are stable after death. However, only a few test items could be tested because of the limited amount of samples obtained from autopsy. Viral antibody titer was measured in the serum for adenovirus, influenza A and B viruses, coxsackievirus B, and cytomegalovirus. Bacterial culture was performed using laryngeal swabs, blood in the heart, or a lung section.

RESULTS

Survey of SID Cases in Forensic Autopsy

Fifty-seven cases (33 male, 24 female) of SID, which were subjected to medicolegal autopsy, were extracted, excluding those from extrinsic causes such as abuse. The age distribution is presented in Figure 1. The cause of death, as diagnosed separately by 4 forensic pathologists, was regarded as SIDS suspected in 20 cases, infectious pulmonary disorders including pneumonia in 10 cases, viral encephalopathy in 3 cases, enteritis and ileus in 3 cases, congenital heart anomaly in 2 cases, potential asphyxia in 2 cases, and unknown cause in 17 cases.

Their vaccination history was confirmed in 50 cases based on passbook records, but such data were not available for 7. Eighteen infants younger than 2 months received no immunization. For the remaining 32 cases, the vaccination history was available. Of these, 7 infants (21.9%) received immunization within 7 days of death, and 8 (25.0%) received immunization from 8 to 28 days before death (Table 1). Of 11 infants younger than 6 months who received vaccination, 4 (36.4%) had received some vaccinations within 7 days.

Table 2 summarizes vaccines that infants were administered within the last 7 days and last 28 days before death. It was characterized that the most frequently administered vaccine in single or combined immunizations was Hib, occurring in 5 (71%) of the 7 infants who received vaccines within 3 days of death and 12 (80%) of 15 infants who received vaccines within 28 days of death. Among these cases, a temporal relationship to vaccination was considered at the time of autopsy for 3 infants (cases 1–3) because their death occurred within 3 days after vaccination. Moreover, they all had received Hib as the last vaccination. All exhibited similar courses as described hereinafter.

Course of 3 Suspicious Cases

The time course of vaccination before deaths within 3 days is summarized in Table 3. Parents pointed out that upper airway infectious symptoms occurred within the administered day in all cases except for the last one. Therefore, its causal relation to the

TABLE 1. Immunization Period Preceding Death

Days Immunized Before Death	Cases, n (%)	No.
1 d	0 (0)	
2 d	1 (3.1)	1
3 d	2 (6.3)	2, 3
4 d	2 (6.3)	
5 d	0 (0)	
6 d	1 (3.1)	
7 d	1 (3.1)	
2 wk	4 (12.5)	
3 wk	1 (3.1)	
4 wk	3 (9.4)	
>4 wk	17 (53.1)	
Immunized subtotal	32 (100.0)	
Not immunized	18	
Unknown	7	
Total	57	

TABLE 2. Vaccines in Single and Combined Immunization Given up to 7 Days (n = 7) and up to 28 Days (n = 15) Before Death

	No.	
Vaccines	<7 d (n = 7)	<28 d (n = 15)
Hib	5	12
S. pneumoniae (PCV13)	4	9
Quadruple vaccination for DTP-IPV	3	7
Rotavirus	1	3
Hepatitis B virus	1	3
Varicella	1	1
Influenza virus	0	1
Total	14	36

PCV indicates pneumococcal conjugate vaccine.

TABLE 3. Vaccination Course in 3 Cases With Sudden Death Occurring Within 3 Days

No.	Day After Birth	Received Vaccines
1	63	Hib, PCV7, and rotavirus (initial)
	91	DTP-IPV (initial)
	98	Hib, PCV7, and rotavirus (second)
	100	Sudden death
2	63	Hib, PCV13, HBV, and rotavirus (initial)
	107	Hib, PCV13, HBV, and rotavirus (second) and DTP-IPV (initial)
	110	Sudden death
3	3 mo	Hib, PCV13, and HBV (initial)
	4 mo	Hib, PCV13, and HBV (second), DTP-IPV (initial), BCG
	5 mo	Hib, PCV13 (third), and DTP-IPV (second)
	6 mo	DTP-IPV (third)
	10 mo	HBV (third)
	12 mo	PCV13 (fourth), VZV
	422 (14 mo)	Hib (fourth)
	435	Sudden death

BCG indicates *Bacillus* Calmette-Guérin vaccine; HBV, hepatitis B virus; PCV, pneumococcal conjugate vaccine; VZV, varicella-zoster virus.

deaths came into question. Their respective clinical courses and related postmortem examinations are explained hereinafter.

A 3-month-old female baby (case 1) developed cold symptoms on the day after the second combined immunization for Hib, *S. pneumoniae*, and rotavirus. In addition, the baby had received quadruple vaccination for diphtheria, pertussis, tetanus, and polio (DTP-IPV) 1 week prior. The infant was found limp in the evening, and then was transported by ambulance. The infant was in a state of shallow breathing at arrival, but she was died after 12 hours with little response to resuscitation. Leukocytosis of 23,000/μL and an elevated ferritin level of 16,380 ng/mL were observed in the emergency department.

Another 3-month-old male baby (case 2) received the second combined vaccination of Hib, *S. pneumoniae*, hepatitis B virus, and rotavirus, and simultaneously quadruple DTP-IPV vaccination. The infant showed cold-like symptoms continuously from the immunized day. He was found dead in sleep in the early morning of the third day.

A 1-year, 2-month-old male baby (case 3) received the fourth combined vaccination of Hib. He showed mild cold-like symptoms and high fever of more than 38°C from the following day. He was found dead in sleep in the early morning of the third day.

In these 3 cases, no swelling or callosity was observed on the injected skin, but autopsy showed some characteristically common histopathological findings as described hereinafter.

Histopathological Findings

In all 3 cases, mild inflammatory cell infiltration including neutrophils was visible around the tracheae and the bronchi, indicating tracheitis and bronchitis. In case 1, whole lungs were congested, accompanied by partial patchy pulmonary edema. Inflammatory cell infiltration was observed in alveolar walls and interlobular septa, showing interstitial pneumonia. Moderate inflammation was composed predominantly of mononuclear cells with no cells with intranuclear viral inclusion and antibody reactivity to influenza virus.

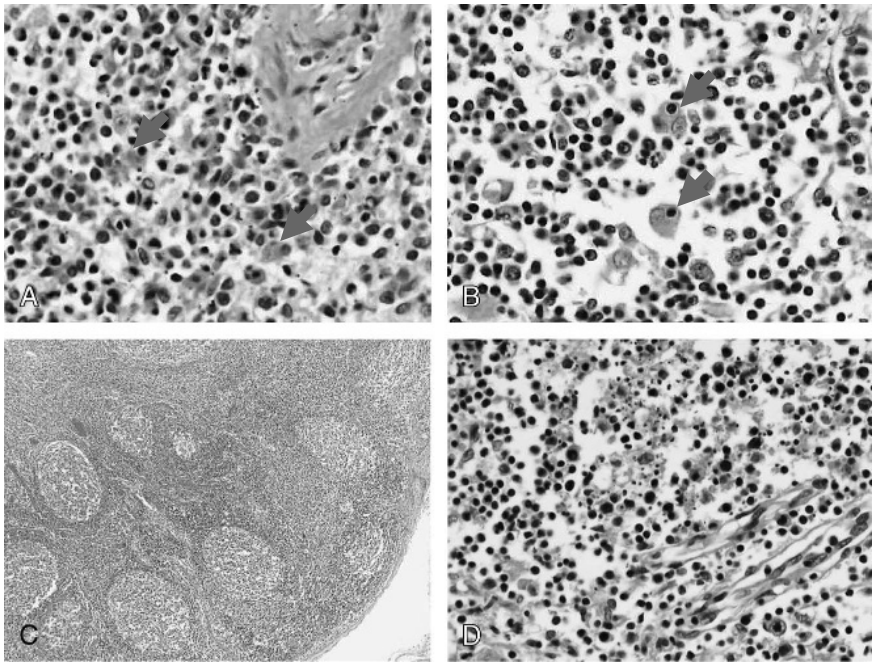

FIGURE 2. Hematoxylin and eosin–stained sections. A, The spleen of case 1 (original magnification ×400). B, The lymph node of case 2 (×400). C, The lymph node of case 3 (×20). D, The lymph node of case 1 (×400). In panels A and B, the hemophagocytosis was observed, as erythrocytes, leukocytes, and platelets were engulfed by activated macrophages in tissues of the spleen and the lymph node (arrows). In panel C, the cortex of lymph node expands with an increase of the number and size of secondary follicles. The follicles vary in size, but they exhibit polarized germinal centers including tingible body macrophages. In panel D, the apoptotic lymphocytes are evident as small, basophilic, and pyknotic (or fragmented) nuclei. Macrophages are present with engulfed cytoplasmic apoptotic bodies.

All 3 cases showed acute splenitis characterized by infiltration of neutrophils and congestion within the red pulp of the spleen. Moreover, extensive hemophagocytosis was visible in the spleen, liver, and lymph nodes (Fig. 2, A and B). In case 3, swollen lymph nodes were visible in the whole body such as cervical lymph nodes and mesentery lymph nodes. Histopathology showed reactive follicular hyperplasia (Fig. 2C). In case 1, numerous instances of lymphocyte apoptosis and abundant nuclear debris were found in the lymphoid tissue of the whole body, such as the lymph nodes, white pulp of the spleen, gut-associated lymphoid tissue, and bronchus-associated lymphoid tissue. Similar findings were visible in the lymph nodes and bronchus-associated lymphoid tissue of case 2 (Fig. 2D).

Laboratory Data

Influenza A virus was detected in our postmortem examination of case 1. Otherwise, no vital infectious sign was evident in other cases. Biochemistry indicated no increased C-reactive protein at postmortem. The cause of death was judged as pneumonia or upper respiratory infection at autopsy for all 3 cases.

Tryptase was measured for cases 2 and 3 at postmortem. Those levels were 5.8 and 2.8 μg/L, indicating no anaphylactic reaction potentially related to the SID cases.

H. influenzae and *S. pneumoniae* were not detected in the bacterial culture. However, α-hemolytic *Streptococcus* was detected in the lung section or in the blood in cases 1 and 2, respectively.

Other Similar Cases

Lymphadenopathy, splenomegaly, and pneumonia similar to these cases were found in 2 other cases in a review of extracted data for the other 54 cases. Severe infection and sudden death occurred in 1 case of a 4-month-old infant at 27 days after first immunization for Hib and *S. pneumoniae*. A 1-year, 9-month-old infant child died 4 months after the last *Bacillus* Calmette-Guérin vaccine, suggesting little or no relation.

DISCUSSION

In this study, we examined vaccination course in SID cases of children younger than 2 years. Among the 32 cases for which vaccination, vaccine, and timing data were available, 7 infants were immunized within 7 days of their death. During postmortem investigation, the relation to vaccination was assessed for 3 cases in which infants received immunization within 3 days of their death.

The antemortem point that is common among these 3 infant cases is that the condition immediately before death was associated with mild cold-like symptoms accompanied by fever. Their parents inferred some relation of the infant death with vaccination because cold-like symptoms appeared during the day after the vaccination. Moreover, their condition deteriorated suddenly at home, which consequently shows no apparent differences from the general situation of discovery of SIDS.[22]

All 3 deaths occurred after vaccination of Hib and *S. pneumoniae.* Four repetitions of combined immunization are recommended in Japan.[18] The safety of that recommendation has been confirmed etiologically.[23] Moreover, case 2 received 8 vaccines in 1 day. Simultaneous immunization of many sorts might affect the physical condition, as Ottaviani et al[24] and von Kries et al[19] reported cases of fatality after combined immunization of 6 vaccines.

Among the 3 cases reported here, features at postmortem examinations were heterogeneous, but similar findings related to the immune system were observed to some degree. Particularly, neutrophil infiltration in the spleen was evident, suggesting that the subjects were affected by hypercytokinemia deriving from an immunological reaction by some infection. The pathological features in lymphoid tissue and spleen demonstrated the presence of uncontrolled activated lymphocytes, histocytes, and macrophages. Blood culture developed the *Streptococcus* in 2 cases, but identifying the pathogenic bacteria of infection is generally difficult at the postmortem phase.[25] The important question of whether the disorders are truly related to vaccination remains.

Common features such as splenitis and hemophagocytosis were also evident among the 3 cases. The uncontrollable immune overreaction mainly caused by the activated lymphocytes and histiocytes/macrophages reminded us of hemophagocytic lymphohistiocytosis (HLH), which is clinically similar to macrophage activation syndrome (MAS). Actually, MAS/HLH is characterized by an overwhelming inflammatory reaction attributable to dysfunction of the immune system, accompanied by the continual activation and expression of T lymphocytes and macrophages.[26,27] This activation and expression leads to hypersecretion of proinflammatory cytokines, so-called cytokine storm, which might create unfavorable immunological conditions in infants who are affected by inflammation.[28] The case reports of MAS/HLH after immunization were limited, but some have been published.[29] As another instance, Otagiri et al[30] reported that a 19-month-old infant died of HLH after measles vaccination. The cause of death was determined as pneumonia or upper respiratory infection for the presented cases, but we thought that their fatality might be also attributable to MAS/HLH in some degree.

Concerning deaths after vaccination, estimation of coincident timing was performed based on epidemiological data obtained over a long period.[6] Brotherton et al[15] simulated the probability of death coincident with vaccination using vaccination-encountered age in a population of vaccination resisters and the age distribution of SIDS deaths in Australia. They estimated that 1.3% and 2.6% of the infant victims would be expected, by chance, to have some vaccination during the prior 24 and 48 hours, respectively. In the present study, 3 infants (9.4%) were found to have died within 3 days among 32 cases, and 7 (21.9%) were within 7 days, for whom a history of similar repetitive vaccinations was confirmed. We are not sure whether the present frequency is significantly different from their estimation. It is, anyhow, difficult to ascertain whether these were merely coincidental.

One cannot determine which vaccination affected the body adversely or in what way because the infants had received many vaccinations. This study found that the most frequent vaccine used before death was Hib. However, combined immunization of Hib and *S. pneumoniae* is recommend on 4 occasions before 2 years of age. More opportunities for exposure might affect the results. In Japan, fatal incidents occurred after combined immunization with Hib and *S. pneumoniae* vaccines in 2011.[16] Because some relation was suspected, vaccination was discontinued temporarily but was restarted soon thereafter. As described in the overview of the cases reported at that time, the cases are similar to the present cases: death occurred within 3 days after vaccination.

However, we concluded that the causal relationship of vaccination to the SID subjects was unknown in the reports because of the unclear mechanism how the 2 nonactive vaccines affect the mortality of infants and because of the difficulty to exclude potential coincidental occurrence. It is therefore necessary to consider it carefully at postmortem, along with the circumstances of death and autopsy findings, as many forensic pathologists may overlook this potential contributing factor.

The safety of Hib vaccine combined with other vaccines has been confirmed in general.[23,31,32] Moro et al[33] comprehensively analyzed numerous Hib vaccination cases including autopsy reports and death certificates for a total of 749 death cases. They

conclude that the review did not identify any new or unexpected safety concern for Hib vaccines. However, death certificates are usually written within a day of autopsy because of administrative purposes like burial. Detailed examinations should be completed within a couple of months. Therefore, we regard surveys based solely on death certificates as unproductive. In this point of view, reports of extensive analysis in forensic pathology are expected to be valuable.

Disorder after repetitive immunization, such as the second time in cases 1 and 2 and the fourth time in case 3, suggests the possibility of anaphylaxis. Vaccine-associated anaphylaxis is a rare event, with only a few cases reported despite the millions of doses administered, representing incidence of 0.65 cases per million doses.[34] By contrast, elevated tryptase activities were demonstrated in a part of forensic SIDS autopsy cases, in which tryptase is known as the most valuable marker at postmortem.[35,36] D'Errico et al[9] reported the fatal case of a 3-month-old infant who died within 24 hours of vaccination with hexavalent vaccine with postmortem findings such as acute pulmonary edema and a high level of β-tryptase, 43 mg/L, in serum. Similar cases have been reported for which anaphylaxis after immunization was suspected.[23] In this study, 2 of 3 cases showed no elevation of tryptase level at postmortem, in accordance with no relation of SIDS cases to tryptase elevation reported by Nishio and Suzuki.[37]

An adverse reaction to vaccination was suspected only because the parents had pointed it out. The role of infection and inflammation in SIDS cases has been investigated for a long time.[38] It is otherwise unlikely for a forensic pathologist to consider the vaccination history seriously. In fact, another case of sudden death (case 3) was found in which similar observations and adverse reactions should be suspected from reviewing other cases in the past. The possibility that the death resulted from vaccination effects should have received more attention.

A collaboration between forensic pathologists and pediatricians is important. In cases 1 and 2, we had contact with the pediatricians who administered vaccinations to the infants. The effects of vaccination were not considered at all for case 3 before the present survey. However, under the present circumstances, it takes 2 or 3 months at least before all autopsy test results are available. That period invariably leads physicians to hesitate to reconsider a case at that time and judge if the case should be notified to the agency. Although the autopsy rate in cases of infant death in Kanagawa Prefecture is higher than 80% at present, no child death review by experts has yet been performed in Japan. It is hoped that future studies will include general multiscriptual reviews conducted for acute infant death.[39]

In conclusion, there were a couple of SID cases in which the relations to vaccination was suspicious. Particularly, such a relation was observed after combined vaccination of Hib and *S. pneumoniae*, exhibiting histopathological features similar to MAS/HLH. However, it cannot be stated conclusively that they are related or coincidental deaths. We expect extensive postmortem examinations for SID cases to assess vaccination effects in infants.

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EXHIBIT 40

Medical Definition of Chronic disease

Medical Author: **William C. Shiel Jr., MD, FACP, FACR**

Chronic disease: A disease that persists for a long time. A chronic disease is one lasting 3 months or more, by the definition of the U.S. National Center for Health Statistics. Chronic diseases generally cannot be prevented by **vaccines** or cured by medication, nor do they just disappear. Eighty-eight percent of Americans over 65 years of age have at least one chronic health condition (as of 1998). Health damaging behaviors - particularly tobacco use, lack of physical activity, and poor eating habits - are major contributors to the leading chronic diseases.

Chronic diseases tend to become more common with age. The leading chronic diseases in developed countries include (in alphabetical order) **arthritis**, **cardiovascular disease** such as **heart attacks** and **stroke**, **cancer** such as breast and **colon cancer**, **diabetes**, **epilepsy** and **seizures**, **obesity**, and **oral health** problems. Each of these conditions **plague** older adults in the US (and other developed nations).

Arthritis and related conditions are the leading cause of disability in the US affecting nearly 43 million Americans. Although cost-effective interventions are available to reduce the burden of arthritis, they are underused. Regular, moderate **exercise** offers a host of benefits to people with arthritis by reducing **joint pain** and **stiffness**, building strong muscle around the joints, and increasing flexibility and endurance.

Cardiovascular disease is a growing concern in the US. **Heart disease** is the nation's leading cause of death. Three health-related behaviors--tobacco use, lack of physical activity, and poor **nutrition**--contribute markedly to **heart disease**. Modifying these behaviors is critical for both preventing and controlling **heart disease**. Modest changes in one or more of these risk factors among the population could have a profound public health impact.

Cancer is the second most common cause of death in the US. **Cancer** is largely controllable through **prevention**, early detection, and treatment. Reducing the nation's cancer burden requires reducing the prevalence of the behavioral and environmental factors that increase cancer risk. It also requires ensuring that **cancer screening** services and high-quality treatment are available and accessible, particularly to medically underserved populations.

- **Colorectal cancer** is the second leading cause of cancer-related deaths in the US, accounting for 10% of all cancer deaths. The risk of developing **colorectal cancer** increases with advancing age. Lack of physical activity, low fruit and vegetable intake, a low-**fiber diet**, obesity, **alcohol** consumption, and tobacco use may contribute to the risk for **colorectal** cancer.

  Three screening tools **flexible sigmoidoscopy**, **colonoscopy**, and the fecal occult blood test (FOBT) are widely accepted and used to detect colorectal cancer in its earliest stages, when treatment is most effective. In 1999, 66% of Americans aged 50 years or older reported not having had a **sigmoidoscopy** or colonoscopy within the last five years, and 79% reported not having had a **fecal occult blood test** within the last year.

- **Breast cancer** is best detected in its earliest, most treatable stage by **mammography**. Seventy-six percent of all diagnosed cases of breast cancer are among women aged 50 years or older.

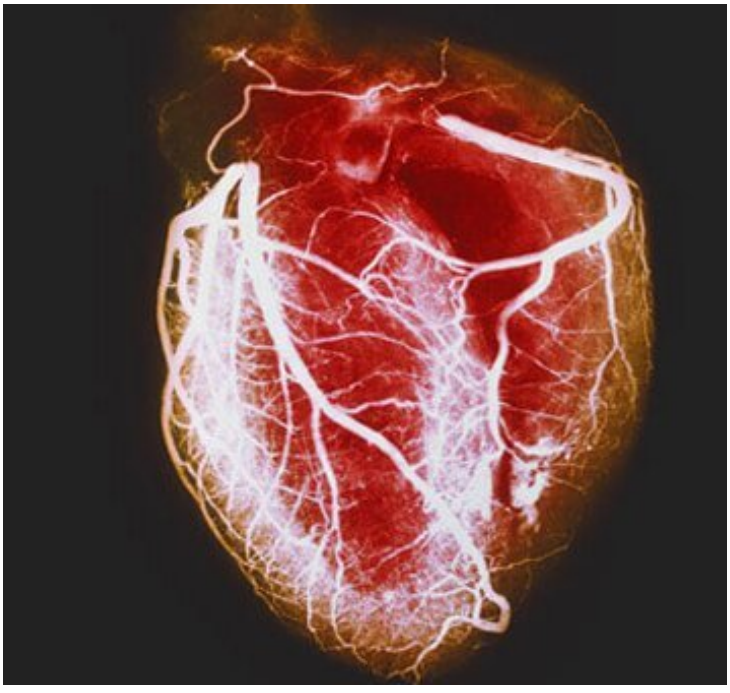
Diabetes is a serious, costly, and increasingly common chronic disease. Early detection, improved delivery of care, and better self-management are the key strategies for preventing much of the burden of **diabetes**. Seven million persons aged 65 years or older (20.1% of all people in this age group) have diabetes, most of them **type 2 diabetes**.

Epilepsy and seizures affect about 2.3 million Americans, and result in an estimated $12.5 billion in medical costs and lost or reduced earnings and production annually in the US. People of all ages are affected, but particularly the very young and the elderly. About 10% of Americans will experience a **seizure**, and about 3% will have or will have had a diagnosis of **epilepsy** by age 80.

Obesity has reached epidemic proportions among Americans in all age groups. Obesity among adults has doubled since 1980. People who are **obese** or **overweight** are at increased risk for **heart** disease, **high blood pressure**, diabetes, arthritis-related disabilities, and some **cancers**.

Oral health problems are an important and often overlooked component of an older adult's general health and well-being. Oral health problems can cause **pain** and suffering as well as difficulty in speaking, chewing, swallowing, and maintaining a nutritious **diet**.

CONTINUE SCROLLING OR **CLICK HERE** FOR RELATED SLIDESHOW



SLIDESHOW

Heart Disease: Causes of a Heart Attack

See Slideshow

Reviewed on 12/21/2018

EXHIBIT 41

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP)

About Chronic Diseases

Chronic diseases are defined broadly as conditions that last 1 year or more and require ongoing medical attention or limit activities of daily living or both. Chronic diseases such as heart disease, cancer, and diabetes are the leading causes of death and disability in the United States. They are also leading drivers of the nation's $3.5 trillion in annual health care costs.

Many chronic diseases are caused by a short list of risk behaviors:

- Tobacco use and exposure to secondhand smoke.
- Poor nutrition, including diets low in fruits and vegetables and high in sodium and saturated fats.
- Lack of physical activity.
- Excessive alcohol use.

Health and Economic Costs of Chronic Diseases

How You Can Prevent Chronic Diseases

Major Chronic Diseases

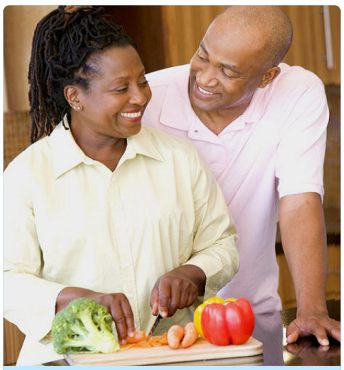
Heart Disease and Stroke

Cancer

Diabetes

Major Risk Factors

Excessive Alcohol Use



Poor Nutrition

Lack of Physical Activity

Tobacco Use

Page last reviewed: October 23, 2019
Content source: National Center for Chronic Disease Prevention and Health Promotion

EXHIBIT 42

A National and State Profile of Leading Health Problems and Health Care Quality for US Children: Key Insurance Disparities and Across-State Variations

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The views expressed in this report are those of the authors and do not necessarily represent those of the Department of Health and Human Services, the Agency for Healthcare Research and Quality, or the Centers for Medicare & Medicaid Services.
Please see Acknowledgments section for conflicts of interest information.
Publication of this article was supported by the US Department of Health and Human Services or the Agency for Healthcare Research and Quality.
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Received for publication March 1, 2010; accepted August 27, 2010.

ABSTRACT

BACKGROUND: Parent/consumer–reported data is valuable and necessary for population-based assessment of many key child health and health care quality measures relevant to both the Children's Health Insurance Program Reauthorization Act (CHIPRA) of 2009 and the Patient Protection and Affordable Care Act of 2010 (ACA).

OBJECTIVES: The aim of this study was to evaluate national and state prevalence of health problems and special health care needs in US children; to estimate health care quality related to adequacy and consistency of insurance coverage, access to specialist, mental health and preventive medical and dental care, developmental screening, and whether children meet criteria for having a medical home, including care coordination and family centeredness; and to assess differences in health and health care quality for children by insurance type, special health care needs status, race/ethnicity, and/or state of residence.

METHODS: National and state level estimates were derived from the 2007 National Survey of Children's Health (N = 91 642; children aged 0–17 years). Variations between children with public versus private sector health insurance, special health care needs, specific conditions, race/ethnicity, and across states were evaluated using multivariate logistic regression and/or standardized statistical tests.

RESULTS: An estimated 43% of US children (32 million) currently have at least 1 of 20 chronic health conditions assessed, increasing to 54.1% when overweight, obesity, or being at risk for developmental delays are included; 19.2% (14.2 million) have conditions resulting in a special health care need, a 1.6 point increase since 2003. Compared with privately insured children, the prevalence, complexity, and severity of health problems were systematically greater for the 29.1% of all children who are publicly insured children after adjusting for variations in demographic and socioeconomic factors. Forty-five percent of all children in the United States scored positively on a minimal quality composite measure: 1) adequate insurance, 2) preventive care visit, and 3) medical home. A 22.2 point difference existed across states and there were wide variations by health condition (autism, 22.8, to asthma, 39.4). After adjustment for demographic and health status differences, quality of care varied between children with public versus private health insurance on all but the following 3 measures: not receiving needed mental health services, care coordination, and performance on the minimal quality composite. A 4.60 fold (gaps in insurance) to 1.27 fold (preventive dental and medical care visits) difference in quality scores was observed across states. Notable disparities were observed among publicly insured children according to race/ethnicity and across all children by special needs status and household income.

CONCLUSIONS: Findings emphasize the importance of health care insurance duration and adequacy, health care access, chronic condition management, and other quality of care goals reflected in the 2009 CHIPRA legislation and the ACA. Despite disparities, similarities for public and privately insured children speak to the pervasive nature of availability, coverage, and access issues for mental health services in the United States, as well as the system-wide problem of care coordination and accessing specialist care for all children. Variations across states in key areas amenable to state policy and program management support cross-state learning and improvement efforts.

KEYWORDS: children's health insurance; children's health services; chronic conditions in childhood; CSHCN medical home; national survey of children's health (NSCH); quality of care

ACADEMIC PEDIATRICS 2011;11:S22–S33

THE CHILDREN'S HEALTH Insurance Program Reauthorization Act (CHIPRA) of 2009 (Public Law 111-3) seeks to improve access to and quality of care for children enrolled in the Children's Health Insurance Program (CHIP) and Medicaid.[1] The CHIPRA legislation mandates the implementation and evaluation of quality of care assessment efforts in CHIP and Medicaid programs. The more recent Patient Protection and Affordable Care Act of 2010 (ACA) further emphasizes CHIPRA insurance coverage and quality priorities and includes additional provisions related to ensuring coverage for children with special health care needs (CSHCN) and the provision of a full range of preventive services. Implementation of CHIPRA and ACA can benefit from a baseline, population-based assessment of the prevalence, severity, and complexity of health problems in US children, as well as a summary of what we currently know about health care quality and system performance through assessing the reported experiences of families. The 2007 National Survey of Children's Health (NSCH, released May 2009) is the most recent national and state-specific representative sample of children that allows identification of a range of health problems in children, as well as assessment of several quality of care indicators valid and meaningful to assess using parent-reported methods. NSCH allows for comparisons across children with public versus private sector insurance in addition to numerous demographic and socioeconomic factors. This paper summarizes findings from the 2007 NSCH beyond the high-level findings previously reported,[2] with a focus on comparing children with public versus private insurance as well as variations across states and for different racial, ethnic, and socioeconomic status groups.

Past studies have documented the most common health conditions for which children use health services and have delineated the availability of existing quality measures for these conditions.[3–5] Prior studies have also examined selected health problems and specific insurance, access, and quality of care topics for children by using the earlier 2003 NSCH.[6–20] A recently published study used the 2007 NSCH to explore in more depth state variations and disparities in overweight and obesity in children[21] and another used the 2007 NSCH to evaluate national findings on insurance adequacy, or underinsurance, in US children.[22] Others have summarized evidence of quality based on documentation of the provision of indicated care for specific health care needs in children as recorded in medical charts.[23] These reports of children's health problems and health care quality vary in the extent to which they examine a range of topics at the child level and the degree to which they provide information by insurance, race, ethnicity, special health care need, and specific conditions. This study further leverages the 2007 NSCH data to provide a more recent and comprehensive national and across-states profile of health problems and quality of care for all children and separately for public and privately insured children, comparing across a range of child demographic and health characteristics using data collection methods that were standardized across all children and all states.

METHODS

DATA SOURCE AND KEY ANALYTIC VARIABLES

Data for this study were drawn from the 2007 NSCH public use data files prepared by the Data Resource Center for Child and Adolescent Health.[24,25] NSCH data were collected between April 2007 and July 2008. The NSCH is directed and funded by the Maternal and Child Health Bureau of the Health Resources and Services Administration and is administered by the National Center for Health Statistics in conjunction with the National Immunization Survey using the State and Local Area Integrated Telephone Survey mechanism led by the National Center for Health Statistics.[24] The survey sample represented at least 1700 children from each state and the District of Columbia (1725–1932 cases across states). Respondents were a parent or other adult in the household who knew the child and the child's health and health history the best. All estimates from the NSCH data are adjusted for nonresponse bias and weighted to represent the noninstitutionalized population of children aged 0 to 17 years in each state, resulting in an estimated 73.76 million children in the United States. Table 1 summarizes characteristics of children represented by the NSCH for all US children aged 0 to 17 years; separate data are provided for children with public or private sector health insurance, including their age, sex, race/ethnicity, household language, household income, and special health care needs status.

Measures of children's health conditions and problems derived from the 2007 NSCH are described in more detail in Appendix A, Table A1, as well as in publicly available variable codebooks.[26] To summarize, those measures reported here include the following:

1. prevalence and service need complexity of CSHCN based on parent responses to the CSHCN Screener[27,28]
2. prevalence, multiplicity, and parent-assessed severity of 20 chronic medical, mental, behavioral, or developmental health conditions or problems. Respondents were asked if a doctor ever (or in the last 12 months) told them their child had each condition or problem. If they said "yes," they were then asked if the child currently had this condition (some exceptions exist, see Appendix A, Table A1). Parents were also asked if their child's condition was "moderate or severe" (versus mild). This approach reflects studies showing a general positivity bias in parent reports of children's functioning, often resulting in "moderate" ratings for conditions with notable health impacts.[29]
3. prevalence of 2 key risk factors: a) overweight or obesity for children aged 10 to 17 years, defined as ≥85% of body-mass-index (BMI; population-based estimates using parent report reliable for older children[30]) and b) moderate or high risk for developmental or behavioral problems based on responses to Parents Evaluation of Developmental Status items included in the NSCH.[31]

Several measures aligned with CHIPRA definitions of quality of care were derived from specific items in the 2007 NSCH (Figure 2 and Appendix A, Table A1). To summarize, the following indicators are reported here:

Table 1. Characteristics of Children Represented in the 2007 National Survey of Children's Health*

Child Characteristic†	All Children Aged 0–17 Years, % (N = 91 642)	Publicly Insured Children (n = 19 748)	Privately Insured Children (n = 64 165)	Uninsured Children (n = 6808)
Percentage (estimated number of children aged 0–17 years)	100 (73.76 million)	29.1 (21.46 million)	61.8 (45.58 million)	9.2 (6.78 million)
Age ($P \leq .001$ for differences across types of insurance)‡				
0–5 years (n = 27 566)	33.2	39.8	30.9	28.6
6–11 years (n = 27 792)	32.4	31.7	32.5	33.9
12–17 years (n = 36 284)	34.4	28.6	36.6	37.5
Sex ($P = .564$ for differences across types of insurance)‡				
Female (n = 43 997)	48.9	49.4	48.8	47.6
Male (n = 47 535)	51.1	50.6	51.2	52.4
Race ($P \leq .001$ for differences across types of insurance)‡				
White NH§ (n = 61 377)	56.2	34.5	69.4	37.6
Black NH (n = 8873)	14.2	25.1	9.0	13.7
Asian NH (n = 2312)	3.6	2.0	4.4	1.8
Hispanic (n = 11 523)	20.5	31.7	12.0	42.4
Multi/other NH (n = 6011)	5.5	6.6	5.2	4.5
Ethnicity/language ($P \leq .001$ for differences across types of insurance)‡				
Hispanic, English household language (n = 6554)	9.8	11.0	9.2	10.2
Hispanic, Spanish (n = 4937)	10.6	20.7	2.7	32.2
Non-Hispanic (n = 78 558)	79.6	68.3	88.1	57.7
Household income ($P \leq .001$ for differences across types of insurance)‡				
0%–99% FPL‖ (n = 10 971)	18.6	45.4	3.9	30.6
100%–199% FPL (n = 15 591)	21.0	34.4	13.0	32.1
200%–399% FPL (n = 30 792)	31.1	16.2	38.8	26.3
≥400% FPL (n = 34 288)	29.3	3.9	44.3	11.0
Geographic area ($P \leq .001$ for differences across types of insurance)‡				
Urban (n = 56 863)	71.5	68.5	73.0	69.9
Suburban (n = 9239)	10.2	8.1	11.3	9.5
Large town (n = 11 557)	9.1	11.2	7.9	10.6
Small town, rural (n = 13 551)	9.2	12.2	7.7	9.9
CSHCN¶ status ($P \leq .001$ for differences across types of insurance)‡				
Met CSHCN criteria (n = 18 352)	19.2	23.6	18.1	12.8
Non-CSHCN (n = 73 290)	80.8	76.4	81.9	87.2

*By insurance status and type. Weighted to represent the noninstitutionalized population of children aged 0–17 years in the United States.
†n = actual/raw number of children sampled. Numbers do not add to 100% of full sample size due to missing values on some variables and/or rounding. Insurance type was possible to calculate for 19 748 sampled children (98.9% of total sample).
‡Assessed using a chi-square test of differences across these three groups.
§NH = non-Hispanic.
¶CSHCN = children with special health care needs status.
‖FPL = federal poverty level.

- gaps in health insurance for children with either public (Medicaid or CHIP) or private health insurance, based on responses to a series of questions: if there was a positive response to questions asking if the child had any kind of health insurance coverage they were further asked if the child was insured by/through Medicaid or CHIP (previous research has found that respondents find it difficult to distinguish between Medicaid and CHIP, so these categories were combined); finally, respondents were asked if during the past 12 months, or since the child's birth (if under 12 months of age), whether there was any time when the child was not covered by health insurance
- adequacy of insurance is evaluated based on parent responses to questions about the extent to which the child's health insurance offers benefits or covers services that usually or always meet the child's needs, usually or always allow him or her to see needed health care providers, and whether any costs paid by the family beyond health insurance premiums or costs covered by insurance are usually or always reasonable
- three preventive care measures included the following: a) whether the child had a preventive medical care visit, b) whether the child had a preventive dental care visit, or c) whether the child had received a developmental screening using standardized parent-completed tools[32] (at <6 years of age) during the past 12 months
- two specialized services measures asked parents: a) whether children who needed specialist care had problems getting specialist care and b) whether children reported to have a mental, emotional, or behavioral health problem requiring treatment or counseling received any needed mental health services in the past 12 months[33]
- a multi-part medical home composite measure was used to assess medical home based on whether children: a) had a personal doctor or nurse and a usual source of care b) whether services received were family centered, and c) whether children had a problem accessing needed referrals and received needed care coordination[34]
- a "minimal quality of care index" consists of a composite measure assessing whether a child experienced each of 3

positive systems–level quality of care attributes: adequate insurance, at least 1 preventive care visit in the past year, and receipt of care in a medical home (as defined above).

Each of the individual quality of care measures used in this study (not including the index of 3 measures) have been reviewed and endorsed for voluntary use by the National Quality Forum (NQF). Measures of Medical Home and Insurance Adequacy have proceeded to final ratification by the NQF Board. The remaining measures are endorsed pending public final comment and Board ratification in Summer 2011. For more information, contact lead paper author.

Statistical Analysis

National and state-by-state population prevalence for all health and quality of care variables were weighted to represent the population of noninstitutionalized children aged 0 to 17 years in the United States. The statistical significance of differences observed between children with public versus private sector health insurance in the prevalence, severity, and complexity of health problems and the key quality indicators were assessed in 2 ways. For bivariate (unadjusted) analyses, standard t tests or chi-square tests of statistical differences (as appropriate) were used, employing a $P < .05$ level of significance. Nested t tests were used to compare each state's prevalence to the nation. Adjustments to standard errors to account for weighting, clustering, stratification, and increased variability that result from the complex sampling design of the NSCH were made by using the SPSS Complex Sample Module (SPSS Inc, Chicago, Ill). Multivariate regression analyses included type of health insurance (public, private, uninsured) in a series of logistic regression analyses using "privately insured" as the reference variable and controlling for child's age, sex, race, ethnicity, primary household language, and household income. For quality of care variables, the special health care needs status of the child was also included in regression models. SPSS version 15.0 (SPSS Inc, Chicago, Ill) was used. Regression results for each variable included in these models is available, although not fully reported here due to space limitations.

Finally, a test for the presence of statistical outliers in state distributions of prevalence of health problems and quality of care scores was conducted to assess the degree to which national rates and ranges across states might be impacted by extreme values (Grubbs test). This test was run for each of the 28 health variables assessed (20 chronic conditions, 2 health risks, 6 health summary variables) and each quality of care variable, for all children and separately for children with public or private sector health insurance.

Results

Prevalence of Health Problems

Prevalence and Complexity of Special Health Care Needs

Based on CSHCN screener results, 14.16 million (19.2%) of the estimated 73.76 million children in the United States have 1 or more ongoing health condition that results in greater need for or use of health services of a type or amount than is required by children generally.[27] CSHCN prevalence ranges from 14.5% to 24.4% across the 50 states and the District of Columbia for all children and is significantly higher for children with public health insurance (5.06 million [23.6%]) compared with those with private health insurance (8.25 million [18.1%]; $P < .05$). This difference (Table 2) remained after adjustment for other factors (adjusted odds ratio [AOR] 1.72, 95% confidence interval [CI], 1.49–2.00). The prevalence of CSHCN increased from 17.6% in the 2003 NSCH.[25] This increase was observed for both publicly insured (21.8% to 23.6%) and privately insured (16.9% to 18.1%) children between 2003 and 2007 ($P < .05$).

Need for or use of prescription drugs for an ongoing health condition is one reason for categorization as a CSHCN. However, the majority (60.3%) of CSHCN have more complex needs that result in an above-routine number of health care encounters, multidisciplinary care teams, and/or specialized services (Table 2). Publicly insured CSHCN (73.4%) have higher adjusted odds of requiring services that go beyond routine need or use of prescription medications (AOR 2.27, 95% CI, 1.84–2.80) compared with privately insured CSHCN (52.2%, Table 2).

Prevalence of Specific Chronic Health Conditions

Overall, 43% of all children were reported to currently have at least 1 of the 20 chronic health conditions assessed in the NSCH. Prevalence across all children aged 0 to 17 years ranged from a low of 0.1% for Tourette's syndrome to a high of 24.4% for environmental and skin allergies. Patterns in highest to lowest prevalence rates across health conditions was similar for children with either public or private health insurance (Figure 1). Publicly insured children had a 1.39 greater adjusted odds (Table 2) of experiencing 1 or more of the 20 chronic health conditions compared with privately insured children (47.4% vs 42.3%; 95% CI, 1.25–1.54). Publicly insured children are also significantly more likely than privately insured children to currently experience nearly each of the individual conditions assessed ($P < .05$; Figure 1 and Appendix B, Table B1).

The Prevalence of All Conditions Varied Substantially Across States

Prevalence rates varied from nearly 2 times to over 3 times across states for attention-deficit/hyperactivity disorder (3.4%–11.0%; $P < .001$), asthma (5.2%–14.4%; $P < .001$), chronic ear infections (4.1%–10.4%; $P < .001$), depression (1.2%–3.8%; $P < .05$), and overweight or obese (23.1%–44.4%; $P < .001$). In addition to the 20 chronic health conditions assessed, an even larger group of children aged 10 to 17 years were identified as being overweight or obese (31.6%), and 26.4% of children under age 6 years were estimated as having a moderate to high risk of chronic developmental, social and behavioral delays based on the Parents Evaluation of Developmental Status items included in the 2007 NSCH (26.4%). See

Table 2. Prevalence of Special Health Care Needs, Chronic Health Problems, and Key Health Risks for All Children Aged 0 to 17 Years, by Type of Health Insurance Coverage*

	All Children Aged 0–17 Years (N = 91 642) % (Quartiles)	Publicly Insured Children (n = 19 748) % (Quartiles)	Privately Insured Children (n = 64 165) % (Quartiles)	Adjusted OR (95% CI)†
CSHCN‡: has ongoing health conditions resulting in above routine and/or special health care need (CSHCN)	19.2 (14.5; 17.9; 22.7; 24.4)	23.6 (12.5; 22.9; 30.5; 37.1)	18.1 (13.2; 16.6; 19.8; 23.0)	1.72 (1.49–2.00)
Chronic condition: currently has ≥1 of 20 chronic conditions (see Appendix B for list of conditions; 90.2% of CSHCN had ≥1 from list)	43.0 (33.5; 41.2; 47.0; 53.3)	47.4 (28.4; 47.4; 55.8; 61.7)	42.3 (33.8; 39.6; 45.1; 49.5)	1.39 (1.25–1.54)
Multiple conditions: has ≥2 of 20 conditions assessed (among children with at least 1 condition) (See Appendix B for condition-specific results)	45.0 (37.1; 43.2; 48.7; 51.1)	52.7 (40.9; 49.7; 59.3; 72.3)	42.1 (35.5; 39.8; 44.1; 48.4)	1.44 (1.24–1.68)
Moderate or severe: parent-rated condition as greater than mild	49.9 (44.0; 47.8; 52.1; 55.3)	57.5 (45.6; 55.4; 62.3; 66.8)	45.6 (40.1; 43.6; 48.3; 52.1)	1.55 (1.35–1.78)
Service need complexity: CSHCN with ≥1 of 20 conditions assessed who require multiple types of special services, beyond primarily prescription medication management	60.3 (52.0; 57.9; 65.0; 77.2)	73.4 (58.1; 67.5; 80.1; 91.5)	52.2 (41.6; 49.2; 56.8; 67.7)	2.27 (1.84–2.80)
Health risks/BMI§: meets criteria for being overweight or obese (aged 10–17 years only)	31.6 (23.1; 28.4; 33.9; 44.4)	43.2 (27.2; 37.2; 45.3; 52.5)	27.3 (18.4; 23.9; 29.8; 37.8)	1.25 (1.03–1.52)
Health risks/development: meets criteria for being at risk for developmental, social or behavioral delays (aged <6 years)	26.4 (18.6; 22.7; 27.7; 35.2)	32.7 (16.8; 26.6; 36.7; 44.2)	22.1 (14.7; 19.5; 24.2; 26.3)	1.19 (0.96–1.48)

*State-specific findings can be found in Appendices C1, C2, and C3. Statistical analysis showed no significant outliers in the distribution across states (Grubbs test). State distribution quartiles are shown in parentheses (0% lowest across states, 25%, 75%, and 100% highest across states).
†Adjusted for child's age, sex, race/ethnicity, and household income using logistic regression analysis. OR = odds ratio; CI = confidence interval.
‡CSHCN = children with special health care needs.
§BMI = body mass index.

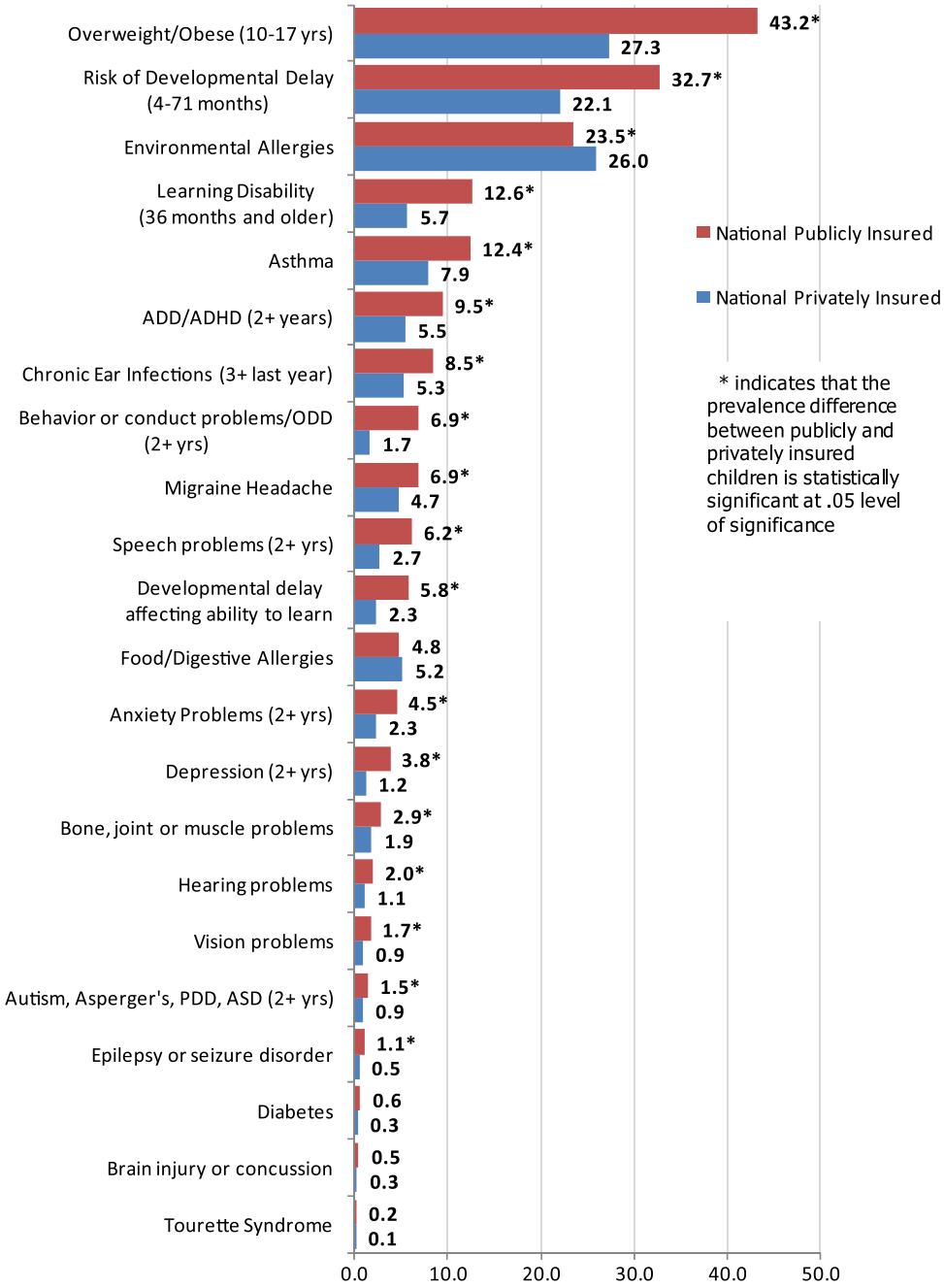

Figure 1. *Prevalence of current chronic health problems and key health risks for children with public versus private sector health insurance (aged 0–17 years unless otherwise noted).*

Appendix B, Table B1, for prevalence results for all 20 conditions.

Severity of Health Problems

Half of all children (49.9%), 57.5% of publicly insured children, and 45.6% of privately insured children who experienced the health conditions evaluated had parents who described these conditions as being moderate or severe (vs mild, as is expected for many conditions, especially if appropriate health care is received). The adjusted odds that parents of publicly insured children would rate their children's conditions as moderate or severe (vs mild) was 1.55 greater than for parents of privately insured children (95% CI, 1.35–1.78, Table 2). Appendix B, Table B1, includes data on the conditions most commonly rated as moderate/severe by parents of publicly insured children and statistical difference from privately insured children. Children with conditions who also met criteria for having a special health care need were much more likely to have their conditions rated as moderate or severe versus mild (Appendix B, Table B1). For instance, 38.6% of children who currently have asthma had parents reporting their condition was moderate or severe, compared with 65.1% of children with asthma who also met criteria for having a special health care need.

Prevalence of Multiple Conditions

Forty-five percent of children with any 1 of the 20 chronic health conditions assessed had more than 1 condition, meaning that nearly 1 in 5 (19.6%) of all children aged

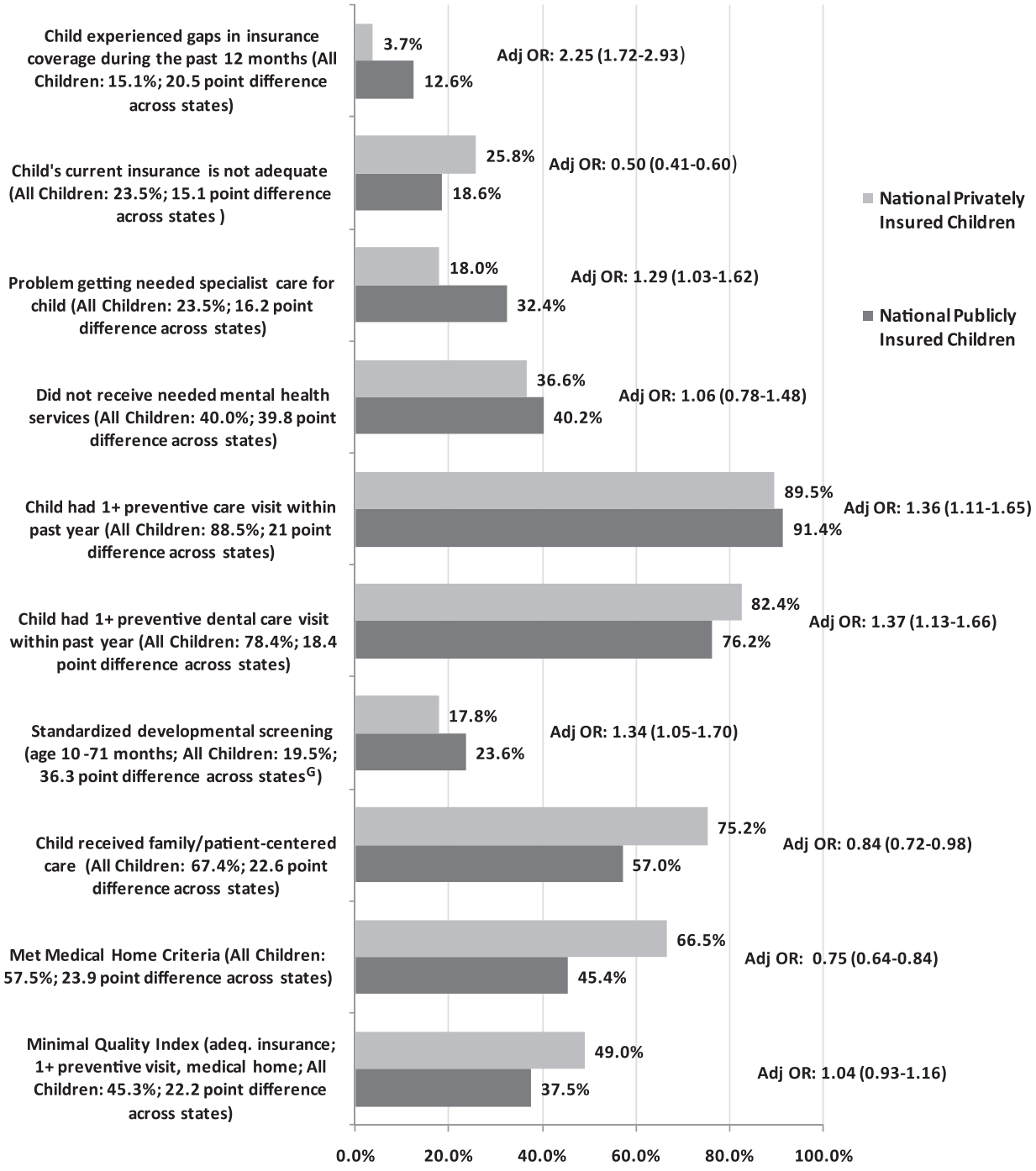

Figure 2. *Healthcare quality and system performance measure scores comparing children with private and public sector health insurance. *Adjusted odds ratio compares public versus privately insured children, with adjustment for age, sex, race/ethnicity, income, and children with special health care needs status using logistic regression. [(G)] State distribution has outliers. State findings are in Appendix C, Tables C1 through C3. OR indicates odds ratio.*

0 to 17 years currently experience at least 2 of the conditions assessed. More than half of all publicly insured children (52.7%) and over three fourths (78.6%) of these publicly insured who also qualified as having a special health care need (CSHCN) had 2 or more of the 20 health conditions assessed, rates substantially higher than for their privately insured counterparts. As shown in Table 2, publicly insured children had a 1.44 greater adjusted odds of having multiple conditions compared with privately insured children (95% CI, 1.24–1.68).

Although evaluated and available upon request, space limitations prevent an in-depth summary of variations observed in condition prevalence, severity, and complexity across states and according to a child's race/ethnicity, household income, or household primary language. To briefly summarize race/ethnicity and household income variations for publicly insured children, a consistent finding generally included a pattern of lower rates of prevalence of special needs and specific health conditions for Asian children and for Hispanic children living in households with Spanish

as their primary household language. This is a pattern consistent with prior research. White, black, and Hispanic children living in English-speaking households were more similar, although some variations were observed depending upon the health condition evaluated. Although prevalence rates varied by race/ethnicity, once identified as having a health condition or special health care need, groups were more similar. For example, although publicly insured Hispanic children were less likely to have parents who reported a current health condition among the 20 assessed (Hispanic, 30.6%; white, 50.4%; black, 51.3%), Hispanic children were similarly likely to have their conditions rated moderate or severe (vs mild; Hispanic, 46.4%; white, 49.7%; black, 43.9%). For publicly insured children, overall prevalence of having a health condition was generally similar across household income groups, although it was more variable on a condition by condition basis.

HEALTH CARE QUALITY AND SYSTEM PERFORMANCE

INSURANCE CONSISTENCY

Approximately 1 in 6 (15.1%), or 11.1 million children aged 0 to 17 years, experienced gaps in health insurance coverage during the past 12 months, including the 9.2% of children who were estimated to be uninsured. This varied nearly fivefold across states, from 5.7% in Massachusetts to 26.2% in Texas. Adjusted results revealed that publicly insured children had more than double the odds of experiencing a gap in coverage compared with privately insured children (AOR 2.25, 95% CI, 1.72–2.93; Figure 2). Among publicly insured children, gaps in insurance coverage were highest for Hispanic children (15.2%) and lowest for Asian children (6.7%; $P < .05$). Publicly insured children from poorer households (<200% federal poverty level) were more likely than publicly insured children from higher income households (>400% federal poverty level) to have gaps in insurance coverage (13% vs 6.0%; $P < .001$).

ADEQUACY OF INSURANCE

Among children with current health insurance, 15.7 million (23.5%) had parents who reported their coverage was never or only sometimes adequate in terms of coverage, access to, and costs of needed health care for their child. Reports of insurance inadequacy ranged from 16.2% in Hawaii to 31.3% in Minnesota. Publicly insured children had a lower reported frequency of inadequate insurance compared with privately insured children (18.6% vs 25.8%; Figure 2).

Among publicly insured children, whites had the lowest frequency of reported inadequate insurance (15%), a rate statistically different from that of Hispanics (20.6%), black children (20.3%), and Asian children (26.9%; $P < .05$). Notably, although the least likely to be uninsured or have gaps in health insurance, Asian children were most likely to be reported to have inadequate insurance (26.9%). Children with brain injury (40.4%), vision problems not corrected by glasses (37.2%), and migraine headaches (34.2%) were most likely to have parents who reported that their insurance was not adequate. Children with diabetes (25.1%), asthma (26.5%), and environmental or skin allergies (26.2%) were least likely.

PREVENTIVE MEDICAL CARE VISITS

Most parents reported that their children had at least 1 preventive health care visit during the past 12 months (88.5%), although this was less commonly reported for older children (96.0% for children aged 0–5 years and 84.8% for children aged 6–17 years; $P < .05$). After accounting for variations in age distribution and other factors, overall, publicly insured children had 1.36 greater odds of having a preventive care visit in the last year compared with privately insured children (95% CI, 1.11–1.65); Figure 2). Prevalence of preventive visits was similar across race/ethnicity groups for children aged under 6 years and was higher for black children aged 6 to 17 years ($P < .05$). Only small differences were observed according to a child's household income. Prevalence differences across states were particularly large for children aged 6 to 17 years (69.1%–96.9%; $P < .05$). Children with diabetes (98.2%), those aged under 6 years at risk for developmental or behavioral problems (95.2%), and children aged 2 to 17 years with developmental delay (93.8%) were most likely to have at least 1 preventive care visit. Children aged 10 to 17 years who were overweight or obese (84.3%) or with migraine headaches (86.8%) were least likely. It is important to note that publicly insured CSHCN were more likely to have had at least 1 preventive visit in the past year compared with those who did not meet criteria for having a special health care need (AOR 1.65, 95% CI, 1.34–2.04).

DEVELOPMENTAL SCREENING FOR YOUNG CHILDREN

Nearly 20% of children aged 10 to 76 months were reported by their parents to have been screened for development, social or behavioral delays using standardized parent-completed tools, which is one primary assessment method recommended by the American Academy of Pediatrics.[32] Publicly insured children had a higher frequency of parents reporting such screening compared with privately insured children (23.6% vs 17.8%; $P < .001$). Adjusted results revealed that publicly insured children had 1.34 greater odds of receiving a screening compared with privately insured children under age 6 (95% CI, 1.05–1.70; Figure 2). Among publicly insured children, screening rates were highest for black children (30.6%) and lowest for Asian children (20.2%). The range across states in the prevalence of screening was substantial (10.7%–47.0%).

PREVENTIVE DENTAL CARE VISITS

Results show that 78.4% of children had at least 1 preventive dental care visit in the past year. Prevalence was somewhat higher for children with private health insurance compared with publicly insured children (82.4% vs 76.2%). However, adjusted results revealed that publicly insured children had 1.37 greater odds of having parents report that their child had attended a preventive dental visit compared with parents of privately insured

children (95% CI, 1.13–1.66; Figure 2) Among publicly insured children, Asian children were least likely to have parents reporting a preventive dental visit (81.4%). Although less pronounced than for preventive medical care visits, substantial variation was observed across states (68.5%–86.9%; $P < .05$).

Access to Specialists and Mental Health Services

Nearly one third of publicly insured children whose parents reported needed specialist care also had problems accessing this specialist care (32.4%). This is substantially higher than for privately insured children (18.0%; $P < .05$), with adjusted odds of 1.29 (95% CI, 1.03–1.62; Figure 2). Problems accessing needed specialist care were most likely for publicly insured Asian children (41.5%), followed by Hispanic and black children (36.9% and 36.3%, respectively). Specialist care access problems were equally likely for lower income children, regardless of their source of insurance.

Rates of not obtaining mental health services for conditions that required such treatment or counseling were similar for public (40.2%) and privately insured (36.6%) children after adjustment for other factors (AOR 1.06, 95% CI, 0.78–1.48; Figure 2). Wide ranges were observed across states for both publicly insured children (14.4%–66.3%; $P < .05$) and privately insured children (15.2%–58.5%; $P < .05$). Among publicly insured children, Hispanic, Asian, and black children more often experienced not receiving needed mental health services compared with white children (51.3%, 45.5%, 44.2%, and 34.9%, respectively). Publicly insured children living in households with incomes above 400% of the federal poverty level were significantly less likely (31.0%) to fail to receive needed mental health services compared with lower income publicly insured children ($P < .05$).

Medical Home

Fewer than half of publicly insured children (45.4%) and two thirds of privately insured children (66.5%) met the multipart medical home measurement criteria, with publicly insured children having 0.75 the odds after adjusting for other factors (95% CI, 0.64–0.86; Figure 2). The difference observed between children with public versus private health insurance was least notable in terms of differences in having a personal doctor or nurse (90.8% vs 95.4%) and usual source of care (90.5% vs 96.3%), and most notable for receipt of family-centered care (57.0% vs 75.2%). Although the likelihood of meeting the threshold measure of care coordination included in the medical home composite measure was lower for publicly insured children (62.7% vs 73.8%), the adjusted odds ratio comparing public to privately insured children was not significant (AOR 0.84, 95% CI, 0.71–1.00). Publicly insured Hispanic (32.9%) and Asian (33.3%) children were least likely to have a medical home, followed by publicly insured black children (40.3%). A 30-point range was observed across states in the proportion of publicly insured children meeting criteria for having a medical home (32.9%–62.6%; $P < .05$; Figure 2).

Minimal Quality of Care Composite Index

Fewer than half of all US children (45.3%) met criteria for the minimal quality indicator (insurance usually or always adequate, at least 1 preventive care visit, and meeting medical home criteria), with wide variations across states, ranging from 35.7% to 57.9% (Figure 3). Older children (40.9% for children aged 12–17 years), CSHCN (38.2%), and publicly insured children (37.5%) scored positively on this minimal quality of care composite measure less frequently than younger, healthy, and privately insured children. Among health conditions, children with autism were least likely (22.8%) to score positively on this minimal quality index, and children with chronic ear infections, asthma and either food, environmental, or skin allergies were most likely to score positively among all chronic conditions assessed in this study (39.9%, 39.4%, 40.6%, and 45.0%, respectively).

Additional variations on quality of care across subgroups of all children, and specifically for children with public sector health insurance, are available. For more information on these findings please contact the first author.

Summary of Differences Observed Across US States

Highlights of differences across US states (including the District of Columbia) in the prevalence of health problems and scores on health care quality measures have been referenced throughout this paper, and more state-specific findings are available in Appendix C, Tables C1 through C3. In addition, state-specific profiles for most of the measures assessed in this study can be downloaded from http://www.childhealthdata.org or obtained from the first author.

As noted earlier, tests for statistical outliers in distributions across states were conducted for all of the 28 measures of health problems (20 conditions, 2 health risks, 6 summary measures) and each quality of care/system performance measure. These tests confirmed that neither the mean prevalence rates in health and quality of care measures across states (or national average) nor the often wide range in prevalence and performance between the states with the highest and lowest prevalence/scores were unduly impacted by or reflective of the presence of extreme values or outliers. This was true when assessed for all children, or separately for publicly insured and privately insured children. Among all measures assessed, there were 5 that did show the presence of extreme values/outliers for 1 more of these population groups: 1) prevalence of anxiety, 2) prevalence of Tourette's syndrome, 3) prevalence of overweight or obesity, 4) prevalence of children experiencing 1 or more of the 20 health conditions assessed (publicly insured children only), and 5) prevalence of developmental screening for young children (for all, public, and privately insured). Appendix C, Tables C1 through C3 provide further details on results of tests for state outliers for key health and quality variables.

Among the health condition complexity, severity, and service needs measures summarized in Table 2, a 1.26 (condition severity) to 1.87 (multiple conditions) times

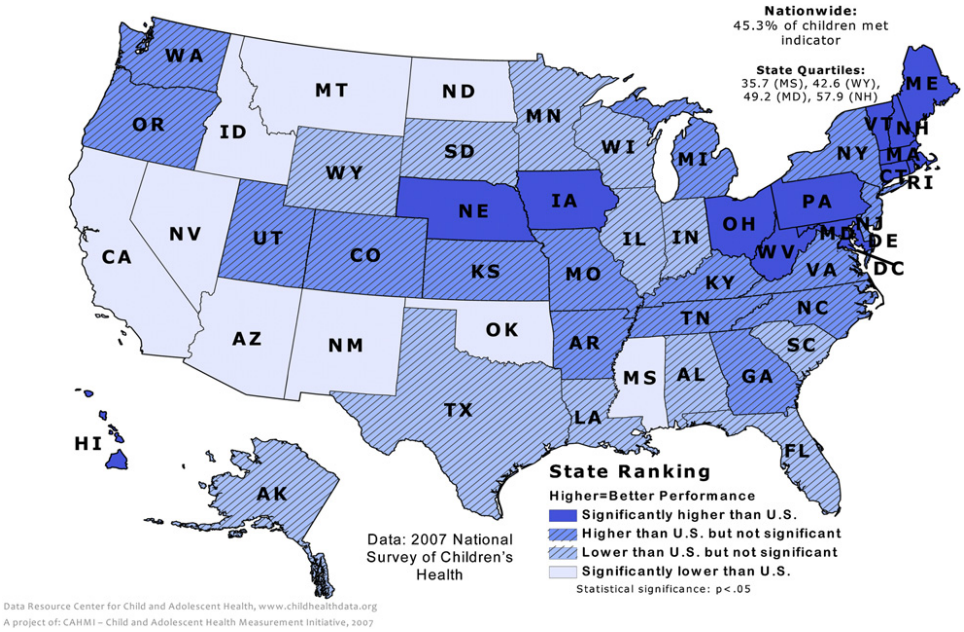

Figure 3. *United States map comparing states to the national prevalence on a minimum quality index (assesses the percentage of children who met medical home criteria, had ≥1 preventive care visits, and adequate insurance coverage). State-specific prevalences are in Appendix C, Tables C1 through C3.*

difference was observed between the state with the highest versus the lowest prevalence (privately insured across state ratios, 1.30 to 1.81; publicly insured across state ratios, 1.46 to 2.97). Excluding the developmental screening measure, which did include state outliers, a 4.60 fold (gaps in insurance) to 1.27 fold (preventive dental and medical care visits) difference was observed across states for quality of care measures (privately insured ratios, 6.92 for insurance gaps to 1.19 for preventive dental care; publicly insured ratios, 5.19 for gaps in insurance to 1.16 for preventive medical care). Overall, the 4-system performance measures with the greatest across-state variation were as follows: 1) gaps in health insurance coverage, 2) not receiving needed mental health care, 3) receipt of developmental screening using standardized parent-completed tools, and 4) problems with specialist care access. See Appendix C, Tables C1 through C3 for more details on state variations.

DISCUSSION AND CONCLUSIONS

This study supports CHIPRA and ACA emphasis on improving healthcare system performance in areas of insurance duration and adequacy, health care access, chronic condition management, and health promotion and disease prevention. Results are especially supportive of legislative goals focused on consistency of insurance, access to mental health and specialist care, preventive and developmental services, care coordination, family-centered care, and medical home and most integrated care setting. Study findings document the presence of a chronic health condition or substantial health risk for the majority of US children, highlighting the importance of the ACA provisions to eliminate preexisting condition exclusive practices and the emphasis in both CHIPRA and ACA on health promotion and primary prevention services for all children with a focus on promoting healthy development and preventing chronic health problems later in life.

Although the prevalence and complexity of health conditions are systematically and notably greater among publicly insured children, the majority of children with chronic conditions in the US are nonetheless privately insured. This poses an important consideration in the design and implementation of efforts to improve health and health care quality for all children and suggests the need for public and private sector partnership and alignment in quality measurement and improvement efforts. Study findings are unique in identifying prevalence rates for specific conditions and CSHCN by source of insurance—information that can help target priorities for quality measurement development and enhancement, and quality improvement. The higher prevalence of chronic conditions highlights the importance of provisions to extend dependent coverage to young adults aged up to 26 years set forth in the ACA.

Examining condition-specific clinical quality of care was beyond the scope of the NSCH. However, the survey provides clinically relevant health care quality information pertaining to all conditions (eg, medical home) and to several CHIPRA-required measurement topics. For example, the inclusion of body mass index documentation as a preventive measure in the initial core set[1] is well supported by the finding that 43.2% of publicly insured children aged 10 to 17 years are overweight or obese. Similarly, the preventive measure of use of standardized screening tools for assessing developmental, social or behavioral delays as well as the adolescent well-visit measure will help reveal substantial additional numbers of children and youth in need. Future measures should address prevention and early and appropriate diagnosis and treatment of health risks and problems

essential to maximizing the lifecourse health trajectories of all children. Given the prevalence of specific chronic conditions in children, consideration of additional quality measures for asthma, attention-deficit/hyperactivity disorder, chronic ear infections, and those for learning disabilities is supportable. In addition, as put forth by the Subcommittee on Quality Measures for Children's Healthcare in Medicaid and CHIP,[1] increased attention to measures of specialty care and care coordination are indicated. Data suggest that prime areas for quality measure development or enhancement include care for allergies, behavior and conduct problems, migraine headaches, speech problems, anxiety, and depression. All condition-specific quality measure development should keep in mind the high proportion of children with multiple conditions, the many common quality of care and system performance needs of children regardless of their conditions, and the wide variation in severity and health care needs among children with any single health condition.

Compared with privately insured children, those with public insurance experienced lower quality of care, including gaps in health insurance and problems accessing specialist care and on a multipart medical home composite measure. Exceptions include that publicly insured children scored better on "insurance adequacy," receipt of a standardized developmental screening, and having preventive care visits. After adjustment, these children were similar to privately insured children on rates of not receiving needed mental health services, the care coordination subdomain measure within the medical home measure, having problems accessing specialist care, and meeting a minimal quality of care index. Such similarities speak to the pervasive nature of availability, coverage, and access issues for mental health services in the United States, as well as the system-wide problem of care coordination and accessing specialist care for all children. Findings showing the substantially higher severity and complexity of conditions among children who also meet criteria for having a special health care need support stratifying CHIPRA's emphasis on quality measure scores for this important subgroup of children (CSHCN). Similarly, consistent race/ethnicity disparities in quality of care measures confirm consideration of quality of care separately for race/ethnicity subgroups.

This study documents the wide variation across states in the prevalence of health problems and in system performance and access to care. Consideration should be given to these findings in the implementation and evaluation of the CHIPRA legislation and in identifying promising practices and cross-state learning opportunities to improve system performance and quality of care for all US children. Given its broad scope and ability to reliably measure in a standardized manner a wide range of health problems and critical aspects of system performance at the child level, as well as by source of insurance, race, ethnicity, income, and CSHCN, the NSCH and similar national- and state-level surveys might be considered a platform for quality measurement from the family perspective.

Although national surveys have been a standard resource for estimating the prevalence of health conditions in the US population, there are, nonetheless, limitations to the use of surveys such as the NSCH. A comprehensive assessment of health needs and quality of care also requires the use of medical record and administrative data. One limitation of surveys such as the NSCH is the inability to ask about all possible health conditions in children or their detailed clinical presentation. Specific to the NSCH, with the exception of chronic ear infections, the NSCH does not ask about many common acute conditions in childhood that lead to use of health services (eg, upper respiratory infections, urinary tract infections) and for which quality measurement is also important. Another limitation of the NSCH is its general lack of condition-specific clinical quality measures. Despite these limitations and the distinct sources of quality information provided through other types of data (eg, medical charts, administrative data), overall conclusions on the leading health problems and quality of care set forth here are consistent with prior studies that used medical chart and clinical administrative data.[3,23,35,36] This lends confidence to our conclusions that at least half of all children in the United States today currently experience 1 or more health conditions and/or have a substantial risk to their health, and that fewer than 50% of children receive health care that meets a basic level of quality of care.

ACKNOWLEDGMENTS

This paper is based on a report prepared for the Agency for Healthcare Research and Quality through purchase order HHSP233200900801P by the US Department of Health and Human Services (HHS), with funds provided by the HHS Centers for Medicare & Medicaid Services. This study was also supported by The Child and Adolescent Health Measurement Initiative's Data Resource Center for Child and Adolescent Health, which is funded by the Maternal and Child Health Bureau through Cooperative Agreement 1-U59-MC06980-01.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.acap.2010.08.011.

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EXHIBIT 43

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PREVENTING CHRONIC DISEASE
PUBLIC HEALTH RESEARCH, PRACTICE, AND POLICY

Multiple Chronic Conditions Among Outpatient Pediatric Patients, Southeastern Michigan, 2008–2013

BRIEF — Volume 12 — February 12, 2015

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Suggested citation for this article: Rezaee ME, Pollock M. Multiple Chronic Conditions Among Outpatient Pediatric Patients, Southeastern Michigan, 2008–2013. Prev Chronic Dis 2015;12:140397. DOI: http://dx.doi.org/10.5888/pcd12.140397 .

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Abstract

Studies investigating the prevalence of multiple chronic conditions (MCCs) and their associated health care cost and use among pediatric populations have been limited. Among 14,404 pediatric patients receiving outpatient care in southeastern Michigan from 2008 through 2013, 82.1% had 0 chronic conditions, 16.2% had 1 chronic condition, and 1.6% had 2 or more chronic conditions. Greater numbers of chronic conditions significantly predicted outpatient cost (β = 581.7, $P < .001$), visit frequency (β = 9.1, $P < .001$), and days between appointments (β = −33.9, $P < .001$). Further study of MCCs among pediatric patients is needed given their increasing prevalence and their associated health care cost and use.

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Objective

Recent research concerning the epidemiology and cost implications of multiple chronic conditions (MCCs) has primarily centered on adult patients (1,2). The strong association of MCCs with age, health care use and cost, and reduced quality of life is an immense concern for the US health care system, which is preparing to care for a vast and aging baby-boomer population. Chronic disease is not unique to adults, however. Approximately 27% of children in the United States have a chronic condition and 1 in 15 have MCCs (3). Moreover, research indicates that the prevalence of chronic conditions is on the rise among pediatric patients (4,5). Studies investigating health care use and cost in this population have been limited. Most research has been conducted on children with special health care needs, which are often considered to include MCCs. However, research into children with special health care needs has primarily focused on children with disabilities, rather than broader pediatric populations with MCCs. The purpose of our study was to determine the prevalence and effect of MCCs among an outpatient population of children in Southeastern Michigan.

Methods

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We analyzed outpatient evaluation and management claims for patients under age 18 years and insured by the Beaumont Employee Health Plan (BEHP) to study MCC prevalence, cost, and use from 2008 through 2013. The BEHP is a regional health insurance provider serving Beaumont Health System employees (eg, physicians, nurses, clerical and facilities staff) and their families (spouses and children) in Southeastern Michigan. Beaumont Health System comprises 3 primary health care campuses (Royal Oak, Troy, Gross Pointe) and several satellite clinics and facilities in metropolitan Detroit. The BEHP provides health insurance coverage to approximately 30,000 people annually of whom about 7,000 are pediatric patients. The Beaumont Health System Research Institute for Human Investigation Committee (HIC) granted approval for this study (HIC no. 2014–051).

To determine MCC prevalence, the following 10 chronic conditions were identified by using *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM) codes assigned to patients' primary and secondary diagnoses on outpatient claims only: attention deficit disorder (ADD), asthma, autism, cancer, depression, diabetes, hyperlipidemia, hypertension, obesity, and substance abuse. Chronic conditions selected for this analysis represent a subset of prevalent and potentially preventable diseases specified by the Office of the Assistant Secretary for Health of the US Department of Health and Human Services for the purpose of standardizing MCC research initiatives (6). The conditions selected for the subset were those diseases that were deemed relevant to pediatric populations on the basis of the authors' clinical judgment. Patients were organized into the following chronic condition categories: 0 chronic conditions, 1 chronic condition, and 2 or more chronic conditions (ie, MCCs).

Average outpatient cost, number of outpatient visits, and days between appointments were calculated for each chronic condition category for each year of the study. Cost was defined as the total dollar amount paid for outpatient services by BEHP for each patient during the study period. We examined the crude and adjusted relationship between MCCs and outpatient cost, visit frequency, and days between appointments by using linear regression, adjusted for age and sex. Using the year 2008 as a reference, adjusted odds ratios for MCC occurrence were calculated for each year of the study period using multiple logistic regression. Ten pediatric patients (<0.01%) were dropped from the analysis because of missing demographic or outcomes information.

Results

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The study population consisted of 14,404 pediatric patients with an average age of 7.6 years (standard deviation, 6.0 y). Among these patients, 82.1% (n = 9,709) had 0 chronic conditions, 16.2% (n = 2,333) had 1 chronic condition, and 1.6% (n = 230) had 2 or more chronic conditions (Table 1). Outpatient health care costs for pediatric patients with MCCs were almost 4 times that of pediatric patients with no chronic conditions ($1,682.80 vs $460.60). Similarly, pediatric MCC patients had significantly more outpatient visits (26.1 vs 8.3) and fewer days between appointments (90.2 vs 153.1) than patients without chronic conditions. Adjusted regression analyses revealed that greater numbers of chronic conditions significantly predicted outpatient cost ($\beta = 581.7$, $P < .001$), outpatient visit frequency ($\beta = 9.1$, $P < .001$), and days between appointments ($\beta = -33.9$, $P < .001$). Asthma (9%), ADD (5.4%), the combination of asthma and ADD (1%), depression (0.6%), and diabetes (0.4%) were the most prevalent chronic conditions in this population (Table 2). These chronic conditions accounted for 30% of the BEHP's total pediatric outpatient cost.

From 2008 through 2013, the proportion of pediatric patients with chronic conditions rose from 9.8% to 13.8% in this population. Compared with 2008, the adjusted odds of having a chronic condition in 2013 were 1.5 (95% confidence interval, 1.3–1.6). Despite rising chronic condition prevalence, average outpatient cost, visit frequency, and days between appointments did not grow significantly over time. However, total outpatient cost and visits increased for chronic

condition patients during the study period, and total days between appointments decreased. Throughout the study period asthma was consistently a highly prevalent chronic condition, affecting approximately 6% of the population each year. The most growth in prevalence occurred among patients with diagnosed ADD (3.2% to 6.8%) and depression (0.3% to 1.0%). By 2013 ADD was the most prevalent chronic condition among pediatric patients.

Discussion

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Our study demonstrates that prevalence of MCCs among pediatric patients is increasing along with associated health care costs and office visits, whereas time between outpatient appointments is decreasing. We found that the top 5 most prevalent chronic conditions accounted for over 30% of outpatient costs attributable to pediatric patients.

An inpatient study by Zhong and colleagues found that more than 40% of pediatric patients had 1 chronic condition, whereas 17% had MCCs (7). Our investigation yielded much lower estimates because we studied an outpatient pediatric population; outpatients are less likely than hospitalized patients to have chronic diseases. However, as in our analysis, Zhong et al found that health care costs increased substantially with increasing numbers of chronic conditions (7). MCCs among pediatric patients have also been associated with increased use of in-hospital resources (8). Although we did not study costs associated with specific outpatient procedures, as Simon et al did (8), we found that pediatric MCC patients had significantly more outpatient visits than patients with no chronic conditions.

Chronic conditions can affect a child's emotional, physical, and social development and often have lasting health and health care consequences (9,10). Unlike adults with MCCs, children with MCCs face unique challenges to treatment adherence, disease acceptance, lifestyle modification, care coordination, reduction of exposure to chronic condition risk factors, and transitioning to adult health care settings (11). Recently published MCC research does not identify children as a population requiring future investigation (12,13). Given the adverse personal burden experienced by children with chronic conditions and the effect these conditions have on health care use and cost as demonstrated by this study, this population warrants more in-depth examination, especially given that these patients are likely to develop additional health problems and grow even more clinically complex as they age.

An important limitation of this study is that our results may not be applicable to children whose parents are unemployed. Our analysis captures data on pediatric patients whose parents have diverse occupations in terms of salary (eg, clinicians, laboratory technicians, clerical staff). However, it would be inappropriate to apply our results to uninsured, unemployed, or Medicaid populations. We know that people at the greatest risk for acquiring chronic conditions are those from low social and economic classes (14), who were not represented in our study. Thus, our results probably underestimate the prevalence and burden of MCCs among children in Southeastern, Michigan. Another limitation of this study is the use of ICD-9-CM codes to identify chronic conditions. The validity of diagnosis codes could have been improved if additional clinical or laboratory data had been available. Additionally, our results probably underestimate the prevalence of chronic conditions overall in this population because we did not identify additional chronic illnesses, including cerebral palsy, cystic fibrosis, disorders of malnutrition, consequences of low birth weight, or congenital defects. The greater the number of chronic conditions considered in MCC studies, the higher the MCC prevalence found (15). Lastly, important covariates associated with chronic conditions, such as socioeconomic status, ethnicity/race, and exposure to tobacco smoke, were not available for adjusted analyses, which may have resulted in overestimation of regression results.

Our investigation helps to demonstrate the importance of studying MCCs among pediatric patients, because MCCs are growing in prevalence and are associated with increased health care use and cost. Asthma, ADD, depression, and diabetes are prevalent and costly chronic conditions among children and are appropriate targets for intervention.

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Tables

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Table 1. Prevalence and Outcomes by Number of Chronic Conditions for Children Under 18 Years of Age (n = 14,404) in the Beaumont Employee Health Plan, Southeastern Michigan, 2008–2013

Return

No. of Chronic Conditions	Percentage of Pediatric Population With Conditions[a]	Average Outpatient Cost, $	Average No. of Outpatient Visits	Average No. Days Between Appointments
0	82.1	460.5	8.3	153.1
1	16.2	1,000.2	16.9	122.6
≥2	1.6	1,682.8	26.1	90.2

[a] Percentages do not add to 100 because of rounding.

Table 2. Prevalence and Associated Outpatient Cost for the Top 5 Chronic Conditions Among Children Under 18 Years of Age (n = 14,404) in the Beaumont Employee Health Plan, Southeastern Michigan, 2008–2013

Return

Chronic Condition	No. of Pediatric Patients	Percentage of Pediatric Population With Conditions	Total Outpatient Cost, $	Total Outpatient Cost, %
Asthma	1,298	9.0	1,394,594	17.0
ADD	780	5.4	671,444	8.2
Asthma and ADD	148	1.0	250,451	3.1
Depression	87	0.6	71,531	0.8
Diabetes	60	0.4	65,234	0.7

Abbreviation: ADD, attention deficit disorder.

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Page last reviewed: February 5, 2015
Content source: National Center for Chronic Disease Prevention and Health Promotion

EXHIBIT 44

ORIGINAL CONTRIBUTION

Dynamics of Obesity and Chronic Health Conditions Among Children and Youth

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James M. Perrin, MD

Context Rates of obesity and other childhood chronic conditions have increased over recent decades. Patterns of how conditions change over time have not been widely examined.

Objective To evaluate change in prevalence of obesity and other chronic conditions in US children, including incidence, remission, and prevalence.

Design, Setting, and Participants Prospective study using the National Longitudinal Survey of Youth–Child Cohort (1988-2006) of 3 nationally representative cohorts of children. Children were aged 2 through 8 years at the beginning of each study period, and cohorts were followed up for 6 years, from 1988 to 1994 (cohort 1, n=2337), 1994 to 2000 (cohort 2, n=1759), and 2000 to 2006 (n=905).

Main Outcome Measures Parent report of a child having a health condition that limited activities or schooling or required medicine, special equipment, or specialized health services and that lasted at least 12 months. Obesity was defined as a body mass index at or above the 95th percentile for age. Chronic conditions were grouped into 4 categories: obesity, asthma, other physical conditions, and behavior/learning problems.

Results The end-study prevalence of any chronic health condition was 12.8% (95% confidence interval [CI], 11.2%-14.5%) for cohort 1 in 1994, 25.1% (95% CI, 22.7%-27.6%) for cohort 2 in 2000, and 26.6% (95% CI, 23.5%-29.9%) for cohort 3 in 2006. There was substantial turnover in chronic conditions: 7.4% (95% CI, 6.5%-8.3%) of participants in all cohorts had a chronic condition at the beginning of the study that persisted to the end, 9.3% (95% CI, 8.3%-10.3%) reported conditions at the beginning that resolved within 6 years, and 13.4% (95% CI, 12.3%-14.6%) had new conditions that arose during the 6-year study period. The prevalence of having a chronic condition during any part of the 6-year study period was highest for cohort 3 (51.5%; 95% CI, 47.3%-55.0%), and there were higher rates among male (adjusted odds ratio [AOR], 1.24; 95% CI, 1.07-1.42), Hispanic (AOR, 1.36; 95% CI, 1.11-1.67), and black (AOR, 1.60; 95% CI, 1.35-1.90) youth.

Conclusions Prevalence of chronic conditions among children and youth increased from 1988 to 2006. However, presence of these conditions was dynamic over each 6-year cohort.

JAMA. 2010;303(7):623-630 www.jama.com

OVER THE PAST 30 YEARS, THE prevalence of chronic conditions in children and adolescents has increased,[1] particularly for asthma,[2] obesity,[3] and behavior/learning problems (eg, attention-deficit/hyperactivity disorder).[4] There have also been changes in rates of rarer conditions, such as sequelae of prematurity,[5] neonatal human immunodeficiency virus 1 infections,[6] and Down syndrome,[7] due to advances in treatment and prenatal care. Children with cystic fibrosis and sickle cell anemia now survive longer.[8] These increases raise important questions concerning the course of chronic conditions over time: what are the collective incidence, persistence, and remission rates?

In this analysis, we examined fluctuations in having a chronic health condition over time. The phrase *chronic condition* might imply permanence. Yet conditions change over time because of new treatments, environmental factors, and a child's development, in addition to the nature of the condition itself. Understanding prevalence and dynamics of chronic conditions on a national scale is important when designing health policy, making accurate clinical predictions, and targeting interventions to prevent chronic conditions. Because demographic variables are associated with prevalence of many conditions, as well as mitigating or causal factors (eg, health care access and environmental exposures),[1] understanding these changes among population subgroups can lead to intervention strategies to reduce disparities.

One previous study, using data from the 1960s to examine changes in having a chronic health condition over time, found that half of children with a chronic condition at the end of the study had been classified as having the condition at the beginning, and vice versa.[9] Since then, the epidemiology of chronic conditions in children has changed considerably, with a rise in overweight/obesity and mental health conditions. Furthermore, advances since 1960 in diagnosis and treatment

See also p 665 and Patient Page.

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Box. Specific Chronic Conditions Categorized as Behavior/Learning Problems and Other Physical Conditions

Behavior and learning problems:
- Learning disability
- Minimal brain dysfunction
- Minimal cerebral dysfunction
- Attention-deficit disorder
- Hyperkinesis
- Hyperactivity
- Mental retardation
- Serious emotional disturbance
- Chronic nervous disorder

Other physical conditions:
- Respiratory disorder (other than asthma)/sinus infections
- Speech impairment
- Serious difficulty hearing
- Serious difficulty seeing
- Allergic condition
- Crippled, orthopedic handicap
- Heart trouble
- Chronic ear problems/ear infections
- Blood disorder or immune deficiency
- Epilepsy or seizures
- Other condition

of other conditions may affect current persistence and remission rates. We therefore update this work with recent, nationally representative data and include obesity and behavior/learning problems. We estimated changes in prevalence, incidence, and rates of remission of broad categories of conditions using 3 consecutive cohorts of children. We also examined prevalence of having a condition during any part of the 6-year study period among these cohorts.

We asked the following questions: did prevalence of chronic conditions increase or decrease among cohorts over time and between same-aged cohorts measured 6 years apart? To what degree do chronic conditions persist, remit, and develop over time? Did the prevalence of chronic conditions during any part of the 6-year study period vary with sex, race/ethnicity, poverty, maternal educational attainment, or maternal obesity?

METHODS

We analyzed 3 cohorts of children born to women in the National Longitudinal Survey of Labor Market Experience, Youth Cohort (NLSY) (http://www.bls.gov/nls/nlsy79.htm). This ongoing survey collects annual data from a national probability sample of 12 686 youth aged 14 through 21 years in 1979 regarding their health, education, and employment, oversampling for racial/ethnic minority and economically disadvantaged white subjects. Of the original sample, 90% were interviewed in 1988 and 81% in 2000, and 56% of the original sample completed every annual survey from 1978 through 2006. In 1986, data collection extended to children of female participants, collected every 2 years. Children's eligibility required that they lived primarily with their mothers, and their mothers were interviewed in the same year. Until 2006, child-centered interviews were completed separately from mothers' interviews. The response rates for the child-centered interviews varied from 90% of eligible children in 1998 to 99% in 2006.

Interviews were typically conducted in the home by trained field staff. Verbal consent was obtained from mothers at each interview. The institutional review board at the Harvard School of Public Health deemed this study exempt from review.

This study focuses on 3 cohorts of children born to women in the NLSY. Cohort 1 includes children aged 2 through 8 years in 1988; cohort 2 includes children aged 2 through 8 years in 1994; and cohort 3 includes children aged 2 through 8 years in 2000. Each cohort was followed up for 6 years until ages 8 through 14 years in 1994 (cohort 1), 2000 (cohort 2), and 2006 (cohort 3). We included only children with complete health and measurement data for all biennial surveys during each study period (68% of all children surveyed during the first year of each study period). No child was in more than 1 cohort. Since all children had mothers who were aged 14 through 21 years in 1979, mothers of children in later cohorts were older.

Chronic Conditions

At each biennial interview, mothers were asked whether children had any physical, emotional, or mental condition that prevented him or her from attending school regularly, doing regular school work, or doing usual childhood activities or that required frequent attention or treatment from a doctor or other health professional, regular use of any medication, or use of special equipment. Mothers were then asked what conditions the child had and how long (number of years, or less than 1 year) the child had had the condition. Conditions were recorded verbatim and coded by the interviewer.

Because some conditions are rare, we categorized conditions into 4 groups: asthma, obesity, other physical conditions, and behavior/learning problems (BOX). Categories were not mutually exclusive: if a child had both asthma and seizure disorder, then she or he would be categorized as having asthma and other physical condition (seizure disorder). A condition was considered chronic if it lasted for at least 12 months. Obesity was defined as a body mass index (BMI), which was calculated as weight in kilograms divided by height in meters squared, at or above the age- and sex-specific 95th percentile.[10] Measurements were usually obtained by in-home interviewers with a scale and tape measure (eg, 83% of heights, 73% of weights in 1990; 74% of heights, 68% of weights in 2000); for others, parents reported the measurement. We also created a variable that identified children having a condition in any of the 4 subgroups.

Other Variables

We included several socioeconomic and demographic variables that we hypoth-

esized may be related to rates of chronic conditions and obesity, based on previous work.[11-13] We included child age, sex, and maternal education (≤12 years or >12 years of school). Although child race/ethnicity was unavailable, we used mother's race/ethnicity (black, Hispanic, or non-Hispanic white, assigned by surveyors based on the 1978 household screening data) as a proxy. Poverty level was defined as family income at the beginning of each study period (<100% or ≥100% of the federal poverty level)[14] and was missing for 16% of participants. Maternal obesity was defined as BMI at or above 30, derived from self-reported height and weight at the beginning of each cohort period.

Data Analysis

We used NLSY-provided weights to calculate means and proportions to represent children aged 2 through 8 years born to women who were aged 14 through 21 years in 1979 in the United States. We used a unique maternal identifier as the primary sampling unit to take into account clustering of observations within families. Data analysis was performed using SAS version 6 (SAS Institute, Cary, North Carolina) and Stata version 10 (StataCorp, College Station, Texas).

We calculated prevalence of any chronic condition and of conditions in the 4 subgroups (asthma, other physical condition, behavior/learning problem, and obesity) in the first and last year for all cohorts grouped together and for each cohort individually. Next, for any chronic condition and subgroups, we calculated incidence, persistence (proportion of children initially with a chronic condition who also had the condition at end of the study period), and "new cases" (proportion of conditions reported in the final year of each study period that were not present at the beginning). Estimations of behavior/learning problems were performed only for all cohorts combined because of small cell sizes for individual cohorts. Using data from each biennial data collection during the study periods, we then calculated the prevalence of having a chronic condition during any part of the 6-year study period for any chronic condition and subcategories of conditions for all cohorts.

We used χ^2 tests to compare differences in prevalence, incidence, persistence, new cases, and prevalence of having a chronic condition during any part of the 6-year study period between consecutive cohorts. A McNemar test (paired χ^2 test using the Yates correction) was used to estimate significance when evaluating changes in prevalence over time within cohorts. Finally, we examined the association between sociodemographic variables (child age, sex, race/ethnicity, maternal obesity, maternal education, poverty) and prevalence of having a chronic condition during any part of the 6-year study period in multivariate logistic regression models that included all participants. To account for missing poverty data, we used UVIS (univariate imputation sampling) in Stata version 10,[15] which imputes a variable using logit regression with sociodemographic variables having significant statistical association with nonmissing poverty data (child age, maternal obesity, maternal education, and race/ethnicity). All *P* values are 2-tailed. To account for multiple comparisons, *P* values of ≤.01 were considered significant. To account for cohort effects, we included a variable that designated the cohort in these models.

For sensitivity analyses, we separately performed the described analysis including only those with objectively measured height and weight and including only those with nonmissing poverty data.

Table 1. Baseline Characteristics of Children and Youth Aged 2 Through 8 Years in Longitudinal Cohorts in 1988, 1994, and 2000[a]

	Cohort 1 (n = 2337)[b]	Cohort 2 (n = 1759)[b]	Cohort 3 (n = 905)[b]
Age of child, mean (SD), y[c]	4.40 (1.83)	4.51 (1.60)	4.94 (1.49)
Age of mother, mean (SD), y[c]	27.6 (2.5)	32.9 (2.2)	38.3 (1.8)
Female sex, % (95% CI)	50.3 (47.8-52.7) n = 1156	48.4 (45.7-51.0) n = 902	49.0 (45.6-52.4) n = 451
Ethnicity, % (95% CI)			
Non-Hispanic white	72.6 (70.4-74.7) n = 1020	84.0 (82.2-85.7) n = 1028	83.8 (81.3-86.1) n = 589
Black	18.6 (16.9-20.4) n = 789	11.0 (9.6-12.5) n = 444	10.6 (8.9-12.7) n = 190
Hispanic	8.8 (7.8-10.1) n = 528	5.0 (4.2-5.9) n = 287	5.6 (4.3-7.2) n = 126
Mothers with >12 y of education, % (95% CI)[c]	28.4 (25.7-31.3) n = 610	49.9 (46.5-53.2) n = 806	62.9 (58.6-67.0) n = 530
Household poverty (<100% FPL), % (95% CI)[c]	25.1 (22.7-27.6) n = 791	13.1 (11.2-15.2) n = 342	12.0 (9.6-14.8) n = 152
Maternal obesity, % (95% CI)[c]	15.6 (13.6-17.9) n = 426	22.0 (19.4-24.9) n = 455	24.9 (21.4-28.6) n = 251

Abbreviations: CI, confidence interval; FPL, federal poverty level.
[a] All estimates weighted to nationally represent US children born to mothers who were 14 through 21 years old in 1979. Numbers are unweighted samples.
[b] Children were aged 2 through 8 years in their respective study periods: 1988 for cohort 1, 1994 for cohort 2, and 2000 for cohort 3.
[c] Measured in the first year of the cohort study (in 1988 for cohort 1, 1994 for cohort 2, 2000 for cohort 3).

RESULTS

Data were available for 2337 children in cohort 1, 1759 children in cohort 2, and 905 children in cohort 3 and their mothers (TABLE 1). Differences in race and poverty status among the cohorts largely reflect the age shift of mothers of the NLSY such that mothers of the children in cohorts 2 and 3 were progressively older than those in cohort 1. Rates of maternal obesity increased with each cohort (cohort 1, 15.6%; 95% confidence interval [CI], 13.6%-17.9%; cohort 2, 22.0%; 95% CI, 19.4%-24.9%; cohort 3, 24.9%; 95% CI, 21.4%-28.6%).

Prevalence, Incidence, Persistence, and New Cases

Prevalence of any chronic condition, including obesity, increased with subsequent cohorts (TABLE 2). The baseline prevalence for cohort 2 (16.6%; 95% CI, 14.6%-18.8%) and cohort 3 (25.2%; 95% CI, 22.0%-28.7%) was higher compared with cohort 1 (11.2%; 95% CI, 9.7%-12.8%; $P<.001$). Within-cohort differences between baseline and end-study prevalence of having any chronic condition were seen for cohort 1 (baseline, 11.2%; 95% CI, 9.7%-12.8%; end-study, 12.8%; 95% CI, 11.2%-14.5%; $P=.01$) and cohort 2 (baseline, 16.6%; 95% CI, 14.6%-18.8%; end-study, 25.1%; 95% CI, 22.7%-27.6%; $P<.001$) but not for cohort 3 (baseline, 25.2%; 95% CI, 22.0%-28.7%; end-study, 26.6%; 95% CI, 23.5%-29.9%; $P=.44$).

Having a chronic condition was dynamic over time. Combining all cohorts, 16.6% (95% CI, 15.3%-18.0%) of children had any chronic condition at baseline. At the end of the study period, 20.8% (95% CI, 19.4%-22.3%) reported a chronic condition. However, only 7.4% (95% CI, 6.5%-8.3%) of all children reported a chronic condition both at baseline and at the end of the study period; 13.4% (95% CI, 12.3%-14.6%) of participants represented new cases. For 9.3% of children (95% CI, 8.3%-10.3%), a chronic condition was

Table 2. Weighted Prevalence, Incidence, Percentage of New Cases, and Persistence of Chronic Conditions[a]

Cohort/Chronic Condition	% (95% Confidence Interval)											
	BL Prevalence	*P* Value[b]	ES Prevalence	*P* Value[b]	*P* Value vs BL of Same Cohort	Incidence During Study	*P* Value[b]	New Cases[c]	*P* Value[b]	Persisting Conditions[d]	*P* Value[b]
All cohorts (n = 5001)											
Chronic condition (any)	16.6 (15.3-18.0) n = 858		20.8 (19.4-22.3) n = 1069		<.001	16.1 (14.7-17.5) n = 667		77.3 (74.5-80.1) n = 884		37.6 (33.8-41.6) n = 351	
Asthma	2.0 (1.6-2.6) n = 119		3.6 (3.1-4.3) n = 195		<.001	2.9 (2.3-3.5) n = 145		76.7 (68.4-85.4) n = 145		42.4 (31.6-54.0) n = 50	
Other physical condition	3.9 (3.3-4.7) n = 170		5.7 (4.9-6.6) n = 235		<.001	4.6 (3.9-5.4) n = 184		77.3 (70.7-82.8) n = 184		32.9 (25.4-41.4) n = 51	
Obesity	11.9 (10.8-13.1) n = 611		13.3 (12.1-14.5) n = 721		<.001	10.1 (9.0-11.2) n = 467		66.7 (62.4-70.7) n = 467		37.2 (32.7-42.0) n = 254	
Behavior/learning problem[e]	1.0 (0.7-1.4) n = 48		4.7 (4.0-5.4) n = 221		<.001	4.2 (3.6-5.0) n = 202		89.9 (83.9-93.8) n = 202		45.5 (28.9-62.1) n = 19	
Cohort 1 (n = 2337)[f]											
Chronic condition (any)	11.2 (9.7-12.8) n = 285		12.8 (11.2-14.5) n = 334		.01	9.7 (8.2-11.5) n = 216		79.8 (75.1-84.6) n = 312		32.1 (25.9-38.9) n = 106	
Asthma	1.6 (1.1-2.3) n = 49		3.1 (2.3-4.1) n = 80		.002	2.5 (1.8-3.4) n = 61		78.0 (64.1-87.5) n = 61		42.3 (25.7-60.9) n = 19	
Other physical condition	3.1 (2.3-4.1) n = 63		2.3 (1.6-3.1) n = 43		.31	1.6 (1.0-2.4) n = 35		67.2 (50.1-80.6) n = 35		24.2 (14.0-38.5) n = 18	
Obesity	7.0 (5.9-8.3) n = 187		8.3 (7.0-9.7) n = 225		.03	6.5 (5.3-7.9) n = 157		73.2 (65.4-79.8) n = 157		31.5 (24.0-40.1) n = 68	
Cohort 2 (n = 1759)[f]											
Chronic condition (any)	16.6 (14.6-18.8) n = 324	<.001	25.1 (22.7-27.6) n = 475	<.001	<.001	20.4 (18.1-23.0) n = 306	<.001	79.7 (75.8-83.6) n = 393	.97	42.1 (35.8-48.7) n = 151	.04
Asthma	1.8 (1.2-2.7) n = 43	.65	4.5 (3.5-5.8) n = 82	.05	<.001	3.7 (2.8-4.9) n = 61	.06	80.6 (68.0-89.0) n = 61	.75	47.9 (29.5-66.9) n = 21	.69
Other physical condition	4.1 (3.1-5.4) n = 63	.13	7.7 (6.3-9.4) n = 118	<.001	<.001	6.3 (5.0-7.9) n = 97	<.001	78.6 (64.9-76.2) n = 97	.18	40.1 (27.3-54.3) n = 21	.12
Obesity	12.3 (10.6-14.3) n = 241	<.001	16.9 (14.9-19.2) n = 335	<.001	<.001	13.7 (11.7-15.4) n = 224	<.001	70.8 (64.9-76.2) n = 224	.42	40.1 (32.8-47.8) n = 111	.13
Cohort 3 (n = 905)[f]											
Chronic condition (any)	25.2 (22.0-28.7) n = 249	<.001	26.6 (23.5-29.9) n = 260	.44	.54	20.4 (17.2-24.0) n = 145	.98	71.3 (65.1-77.5) n = 179	.02	36.7 (30.2-43.9) n = 94	.26
Asthma	2.9 (1.8-4.6) n = 27	.10	3.1 (2.1-4.6) n = 33	.11	.43	2.1 (1.4-3.3) n = 23	.03	66.1 (45.4-81.9) n = 23	.26	37.0 (20.6-57.2) n = 10	.44
Other physical condition	5.0 (3.6-6.9) n = 44	.38	8.0 (6.2-10.3) n = 64	.81	.04	6.7 (5.0-8.9) n = 52	.81	79.9 (67.3-88.4) n = 52	.82	32.2 (18.8-49.3) n = 12	.46
Obesity	19.0 (16.2-22.3) n = 183	<.001	15.8 (13.2-18.9) n = 161	.43	.13	10.6 (8.3-13.4) n = 86	.06	54.4 (45.3-63.1) n = 86	.003	37.8 (30.3-46.2) n = 75	.69

Abbreviations: BL, baseline; ES, end study.
[a] Percentages will not necessarily sum to 100 because of differing denominators.
[b] *P* value vs previous cohort.
[c] Percentage of end-study new cases is the number of children who had the condition at the end of the study period who did not report the condition at study entry divided by the total number of children who reported a condition at the end of the study period.
[d] Percentage of conditions present at baseline that persisted is the number of children who reported the condition at study entry who also reported condition at the end of the study period divided by the total number of children with the condition at study entry.
[e] For individual cohorts, analysis of behavior/learning problems was not performed because of small cell sizes.
[f] Children were aged 2 through 8 years in their respective study periods: 1988 for cohort 1, 1994 for cohort 2, and 2000 for cohort 3.

reported at baseline but remitted by the study's end.

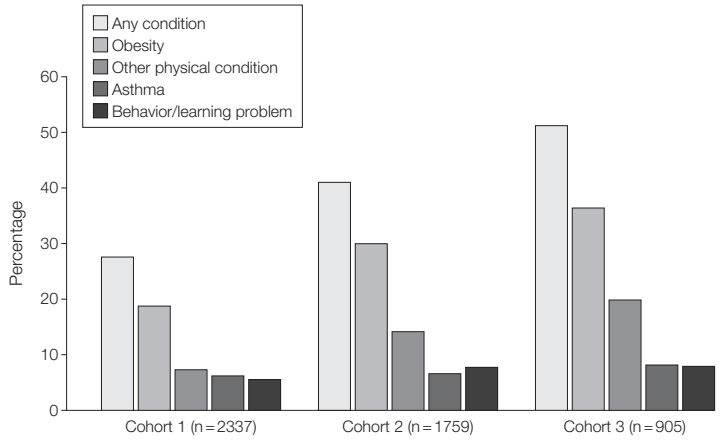
Similar to patterns for all chronic conditions, there was substantial change within individuals having or not having subcategory conditions (Table 2). The prevalence of asthma and behavior/learning problems was higher at the end of the study periods compared with baseline for all cohorts combined (asthma: baseline, 2.0%; 95% CI, 1.6%-2.6%; end-study, 3.6%; 95% CI, 3.1%-4.3%; behavior/learning problems: baseline, 1.0%; 95% CI, 0.7%-1.4%; end-study, 4.7%; 95% CI, 4.0%-5.4%; $P<.001$). For all cohorts, 42.4% (95% CI, 31.6%-54.0%) of children with asthma and 45.5% (95% CI, 28.9%-62.1%) of children with behavior/learning problems at the beginning of the study reported them 6 years later.

For obesity, the baseline prevalence increased substantially over time, with cohort 2 (12.3%; 95% CI, 10.6%-14.3%) and cohort 3 (19.0%; 95% CI, 16.2%-22.3%) higher compared with cohort 1 (7.0%; 95% CI, 5.9%-8.3%; $P<.001$). Also, prevalence increased over time in cohort 2 (end-study, 16.9%; 95% CI, 14.9%-19.2%; $P<.001$) but not in cohort 3 (end-study, 15.8%; 95% CI, 13.2%-18.9%, $P=.13$). Among-cohort differences in prevalence at the end of the study, compared with the previous cohort, were seen in cohort 2 ($P<.001$) but not in cohort 3 ($P=.44$). For all cohorts, 37.2% (95% CI, 32.7%-42.0%) of children with obesity at the beginning of the study were so classified 6 years later.

Although no significant change was found over time in the prevalence of other physical conditions within cohort 1 (baseline, 3.1%; 95% CI, 2.3%-4.1%; end-study, 2.3%, 95% CI, 1.6%-3.1%; $P=.31$), rates increased over time within cohort 2 (baseline, 4.1%, 95% CI, 3.1%-5.4%; end-study, 7.7%, 95% CI, 6.3%-9.4%; $P<.001$).

The prevalence of having a chronic condition during any part of the 6-year study period increased approximately 10% with each cohort, with 51.5% (95% CI, 47.3%-55.0%) of cohort 3 reporting a chronic condition during the most recent study period (FIGURE). Increases in obesity and other physical conditions largely drove this increase across the 3 cohorts.

Figure. Prevalence of Any Chronic Condition and Subgroups of Conditions During Any Part of the 6-Year Study Period for Cohorts 1, 2, and 3



For the prevalence of any condition and for the subgroups obesity and other physical condition, $P<.001$ for group comparison among cohorts. For asthma, $P=.01$ for group comparison among cohorts. For behavior/learning problem, $P=.07$ for group comparison among cohorts. Other physical conditions included respiratory disorders (other than asthma) and sinus infections, speech impairments, serious difficulties hearing, serious difficulties seeing, allergic conditions, crippled or orthopedic handicaps, heart trouble, chronic ear problems or ear infections, blood disorders or immune deficiency, epilepsy or seizures, or other conditions.

Association With Sociodemographic Characteristics

Greater odds of the prevalence of having a chronic condition during any part of the 6-year study period were found among black children (46.6%; 95% CI, 43.6%-49.7%) and Hispanic children (42.3%; 95% CI, 38.4%-46.3%) compared with non-Hispanic white children (36.8%; 95% CI, 34.7%-38.9%) (adjusted odds ratio [AOR], 1.60; 95% CI, 1.35-1.90, and AOR, 1.36; 95% CI, 1.11-1.67, respectively) (TABLE 3). The higher odds of prevalence of asthma and obesity among ethnic minority children contributed to these differences, although ethnic minority children were less likely to have reported other physical conditions and behavior/learning problems. We found associations between maternal obesity and having any chronic condition and all subcategories of conditions; this association was strongest for child obesity (42.1%; 95% CI, 38.2%-46.1%, vs 23.3%; 95% CI, 21.6%-25.1%, of children with mothers who were not obese) (AOR, 2.07; 95% CI, 1.70-2.51). There was also an association between male sex and prevalence of having a condition during any part of the 6-year study period for all conditions except obesity.

Sensitivity analyses with objective height and weight data and nonmissing poverty data were consistent with the main findings (eTable 1 and eTable 2, available at http://www.jama.com).

COMMENT

In our analysis of 3 nationally representative cohorts of children, we examined changes in the incidence, rates of remission, and prevalence of obesity and other chronic conditions at any time in 6 years. We offer 3 key findings. First, there was a high prevalence of having a chronic condition during any part of the 6-year study period. Second, this prevalence increased with each subsequent cohort. Third, the presence of a chronic condition was dynamic over time, with much variation in the persistence of conditions.

This study complements recent work documenting the increasing inci-

dence and prevalence of chronic conditions, especially asthma[2] and overweight/obesity.[3,16] Our study is among the first to examine increasing prevalence of chronic conditions in a cohort over time in the United States and to document the patterns of change in chronic conditions in different cohorts over several years. It also is congruent with work by Jessop and Stein,[9] who analyzed survey data from 1963 to 1970, and Neff et al,[17] who analyzed claims data from a large health insurer. Both studies found similar patterns of remission of conditions over time.

We found that prevalence of a chronic condition at any point during the study period was very high and increased over time. Among cohort 3, 51.5% of 8- through 14-year-olds at one point in the 6-year study period reported a chronic condition compared with 27.8% in cohort 1. Others report similar changes in prevalence over the past 2 decades in childhood obesity,[3,16] asthma,[2,18] and diagnoses of neurobehavioral disorders,[4] especially autism.[19]

Many factors may have contributed, including environmental changes, which may affect rates of chronic respiratory conditions[46] and obesity,[47] better survival rates of conditions such as prematurity,[5] and the development of "late effects" of some treatments, such as chemotherapy.[34] Medicaid expansions and the State Children's Health Insurance Plan (S-CHIP) increased access to health care during the time this study was conducted,[48,49] and children in later cohorts would have had greater opportunities for diagnosis and ongoing treatment of their chronic conditions. This may be especially true for less severe conditions that rarely flare to the point of needing emergent care. The push for increased surveillance for behavior/learning problems in children may have identified cases that would have previously gone undiagnosed. For some behavior/learning problems, patients qualify for therapies only with a diagnosis; thus, diagnosis may be influenced by pursuit of treatment.

A surprising finding is that many children with a reported chronic condition at ages 2 through 8 years did not have the condition 6 years later. Additionally, most chronic conditions at the end of each study period represented new conditions that developed in the previous 6 years. This dynamism challenges the notion that chronic conditions persist without change. Although having a chronic condition in childhood is a risk factor for having the same chronic condition later, many chronic conditions appear to remit for a significant period before relapsing or resolve completely. After cancer treatment, a child may no longer fit criteria for having a chronic condition, although late effects can result in other conditions.[34] Many young children with developmental delay receive therapy during critical years before catching up.[35,36] A child's natural development helps resolve conditions such as chronic constipation. For conditions where symptoms wax and wane, mild cases may be more common and likelier to remit, while severe cases may persist.[17] This cycling is distinct from patterns of chronic conditions in adults, where conditions present later in life and persist, and represents in part differences in epidemiology and development in children compared with adults.[20]

Our finding of limited persistence of asthma complements findings from earlier studies. In a study following up children from birth to puberty, more than 50% with wheezing before age 4 years

Table 3. Weighted Adjusted Odds Ratios of Prevalence of Having a Chronic Condition or Subcategory of Condition During Any Part of the 6-Year Study Period[a]

	Prevalence During Any Part of the 6-y Study Period, AOR (95% CI)[a]				
Cohorts 1, 2, and 3 (n = 5001)	Any Condition (n = 1959)	Asthma (n = 362)	Other Physical Condition (n = 548)	Behavior/Learning Problem (n = 317)	Obesity (n = 1429)
Age, continuous	0.95 (0.91-0.99)	1.02 (0.94-1.11)	1.15 (1.08-1.22)	1.13 (1.04-1.23)	0.85 (0.81-0.89)
Male sex	1.24 (1.07-1.42) n = 1025	1.59 (1.23-2.05) n = 218	1.52 (1.23-1.87) n = 324	2.96 (2.18-4.02) n = 238	1.06 (0.91-1.24) n = 712
Race/ethnicity					
Black	1.60 (1.35-1.90) n = 628	1.59 (1.17-2.17) n = 132	0.59 (0.45-0.77) n = 117	0.74 (0.42-0.95) n = 84	2.04 (1.69-2.46) n = 504
Hispanic	1.36 (1.11-1.67) n = 379	1.46 (1.02-2.01) n = 77	0.73 (0.53-0.99) n = 78	0.63 (0.49-0.95) n = 51	1.58 (1.27-1.97) n = 289
Maternal BMI ≥30	1.96 (1.63-2.36) n = 611	1.46 (1.07-1.99) n = 118	1.56 (1.20-2.03) n = 160	1.74 (1.27-2.40) n = 106	2.07 (1.70-2.51) n = 485
Maternal education >12 y	0.85 (0.71-1.00) n = 789	0.93 (0.68-1.27) n = 142	1.15 (0.91-1.46) n = 261	0.67 (0.49-0.91) n = 98	0.75 (0.62-0.91) n = 550
Household poverty <100% FPL	1.00 (0.82-1.20) n = 513	1.12 (0.81-1.56) n = 111	0.98 (0.73-1.32) n = 111	1.64 (1.15-2.32) n = 96	0.99 (0.80-1.22) n = 391

Abbreviations: AOR, adjusted odds ratio; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; FPL, federal poverty level.
[a] Adjusted for age per 1-year increase, sex (reference category, female), race/ethnicity (reference, non-Hispanic white), maternal obesity (reference, maternal BMI <30), maternal education (reference, ≤12 years), poverty status (reference, ≥100% FPL), and cohort group using logistic regression, taking into account sample weights and clustering of observations within families.

had no wheezing at age 6 years[2]; among cases of persistent asthma before puberty, 40% remitted following puberty.[21] In other longitudinal, population-based studies, more than half of cases of mild asthma resolved.[22,23]

Fluctuations over time for obesity are also noteworthy. Although past reports emphasized that obesity in childhood predicts obesity later in life,[24,25] recent studies highlight individual variability of obesity during childhood. Robbins et al[26] followed up children aged 3 through 7 years in Philadelphia health centers, and although prevalence of obesity did not change after 2 years, a substantial minority changed classification. Studies of older children found less movement.[27,28] Notably, in our study, prevalence of obesity did not change from 2000 to 2006. This is likely due to the decrease in new cases at the end of the study among children in cohort 3 compared with cohort 2 and is consistent with previous reports of flattening childhood obesity rates in recent years.[29]

Previous longitudinal studies of children with attention-deficit/hyperactivity disorder demonstrated a higher degree of persistence than what we found among children with behavior/learning problems. One review estimated persistence of 69% to 79% at ages 10 through 21 years[30]; however, most subjects were patients referred to specialists or diagnosed by standardized research criteria with likely greater severity that is less apt to resolve. Patients with conduct disorder demonstrated a persistence of only 50% after 1 year, but many patients with remitted conditions met diagnostic criteria again in subsequent years.[31] In contrast, studies of patients with autism and Asperger syndrome reveal that it rarely resolves.[32,33] As behavior/learning problems often present in middle childhood, higher prevalence at the end of the study period is not surprising.

The prevalence of any chronic condition during any part of the 6-year study period was associated with male sex, minority race/ethnicity, and maternal obesity. The association between maternal obesity and offspring chronic conditions may be driven by the association between maternal weight and child weight. However, children of obese mothers were more likely to have other conditions as well. The association of maternal obesity during gestation and chronic conditions in children is beginning to be explored,[37,38] and previous studies alluded to an increased rate of health problems generally in caregivers of children with disabilities.[39,40] Associations between male sex and poverty and behavior/learning problems are congruent with other studies.[41-43] The association of minority race/ethnicity with asthma and obesity and the inverse relationship of minority race/ethnicity with other physical conditions and behavior/learning problems are consistent with previous studies.[12,43-45]

Limitations

Children's information was parent-reported and subject to recall bias. Except for obesity, the NLSY did not use objective criteria for diagnoses. Some children may have been overdiagnosed, which may affect perception of remission. The NLSY definition of chronic conditions differs from other surveys and methods, and rates cannot be directly compared.[50] We could not examine associations between disease severity and resolution. Some conditions are more common among children of older mothers, and older, more educated mothers may have different health care–seeking behaviors and access to services, which may affect prevalence of some conditions. If a child had a condition that resolved but then developed another, separate condition within the same subcategory, we categorized this child as having a persistent condition; however, this potential misclassification would bias toward the null hypothesis. Categories of behavior/learning problems and other chronic conditions were heterogeneous, and we could not make conclusions about specific conditions.

Implications

Chronic conditions in childhood are common and dynamic, underscoring the benefits of continuous, comprehensive health services for all children to adjust treatment of chronic conditions, promote remission, and prevent onset of new conditions. Future research should examine etiological differences between persistent and remitted cases.

Author Contributions: Dr Van Cleave had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Gortmaker, Perrin.
Acquisition of data: Gortmaker.
Analysis and interpretation of data: Van Cleave, Gortmaker, Perrin.
Drafting of the manuscript: Van Cleave, Gortmaker, Perrin.
Critical revision of the manuscript for important intellectual content: Gortmaker, Perrin.
Statistical analysis: Van Cleave, Gortmaker, Perrin.
Obtained funding: Gortmaker, Perrin.
Administrative, technical, or material support: Perrin.
Study supervision: Gortmaker, Perrin.
Financial Disclosures: None reported.
Funding/Support: Preparation of this work was supported by a Robert Wood Johnson Foundation Investigator Award in Health Policy Research (grant 033659), the Centers for Disease Control and Prevention (Prevention Research Centers grant U48DP00064), and a cooperative agreement with the Maternal and Child Health Bureau (U53MC04773).
Role of the Sponsor: The funding agencies had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.
Disclaimer: This work is solely the responsibility of the authors and does not represent the official views of the Centers for Disease Control and Prevention or other granting institutions.
Online-Only Material: eTables 1 and 2 are available at http://www.jama.com.
Additional Contributions: Arthur Sobol, MS, Harvard School of Public Health, helped acquire and analyze a portion of the data presented here. He did not receive compensation for his contribution separate from his supporting grant.

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EXHIBIT 45

By James M. Perrin, L. Elizabeth Anderson, and Jeanne Van Cleave

The Rise In Chronic Conditions Among Infants, Children, And Youth Can Be Met With Continued Health System Innovations

DOI: 10.1377/hlthaff.2014.0832
HEALTH AFFAIRS 33,
NO. 12 (2014): 2099–2105

ABSTRACT **Since the early twentieth century, medical and public health innovations have led to dramatic changes in the epidemiology of health conditions among infants, children, and youth. Infectious diseases have substantially diminished, and survival rates for children with cancer, congenital heart disease, leukemia, and other conditions have greatly improved. However, over the past fifty years chronic health conditions and disabilities among children and youth have steadily risen, primarily from four classes of common conditions: asthma, obesity, mental health conditions, and neurodevelopmental disorders. In this article we describe the epidemiological shift among infants, children, and youth and examine sociodemographic and other factors contributing to it. We describe how health systems are responding by reorganizing and innovating. For children with rare complex conditions, concentrating subspecialty care at regional centers has been effective. For the much larger numbers of children with common chronic conditions, primary care providers have expanded diagnosis, treatment, and management options in promising ways.**

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The incidence and impact of many serious childhood infectious diseases have declined markedly over the past century. This decrease reflects public health efforts to improve nutrition and sanitation and, in more recent decades, childhood immunization programs that have eliminated or greatly diminished the incidence of infectious conditions that have high morbidity and mortality, including tuberculosis, bacterial meningitis, and measles.[1,2] Newer vaccines have decreased severe morbidity in populations at higher risk, such as children with compromised immune systems.[3,4]

While infectious diseases have diminished, many relatively rare conditions, which had high mortality in the mid-twentieth century, have experienced great improvements in survival rates. Numerous childhood cancers, congenital heart disease, spina bifida, cystic fibrosis, and sickle cell disease with high early-life mortality rates declined rapidly over the latter half of the past century. For example, leukemia was rapidly fatal in 1960. Today, five-year survival exceeds 95 percent, with similar dramatic improvements seen in most of these other conditions. Increased survival largely reflects medical advances, in both treating the primary condition (such as advancements in surgical techniques for congenital heart conditions) and improved management of serious complications common across conditions (such as improved enteral nutrition methods to supplement poor growth). Much of this improvement in mortality had occurred by about 1980, with some additional mortality gains in recent years.[5] With few exceptions, prevalence of these previously fatal conditions was stable for the latter third of the last century.

Concurrent growth in technology and clinical

sophistication in care of sick newborns has led to the development of regionalized programs of specialized neonatal intensive care. These advances have contributed to improvements in the long-term survival of smaller premature babies who would have died in a previous era.[6–8] Accompanying these neonatal care improvements has been a substantial decline in long-term morbidity from pulmonary disease, blindness, and neurological conditions following premature birth. With improving survival rates, children with extremely low birthweight experience persistently high rates of long-term chronic conditions.[9] However, premature infants of higher birthweight experience much less long-term morbidity than in years past, and the absolute numbers of extremely low-birthweight infants are small (less than 1 percent of all births). Advances in prenatal and newborn screening for genetic and infectious conditions have also prevented a significant proportion of intellectual disabilities.[10]

A few new conditions may have contributed to rates of disabilities among children, such as those following poor intrauterine brain and somatic growth because of a mother's substance abuse or use of particular prescription drugs before or during pregnancy. Although it has been difficult to determine the prevalence of such intrauterine exposures and their contribution to long-term childhood illness and disability, they are associated with preterm birth and neurocognitive problems.[11–13] HIV infection, both perinatal and acquired, has arisen as a new condition, with adolescents being a relatively small, but increasing, proportion of new infections. Congenitally acquired HIV is now very rare—about one hundred cases per year in the United States—as a result of better maternal screening and prevention over the past decade. The total number of children and youth age twenty-four or younger affected by HIV is less than 50,000. Exhibit 1 describes the changing epidemiology of childhood chronic conditions, their drivers, and changes in service organization.

Growth Of Common Chronic Conditions

Despite decreases in infectious diseases and trends of initial survival improvement in generally rare and complex conditions followed by mainly stable rates of these conditions, total rates of chronic health conditions and disability among children and youth continued to rise steadily over the past half-century. In 1960, 1.8 percent of children were reported to have a health condition severe enough to interfere with usual daily activities. In 2010, more than 8 percent of children had a health condition that interfered with daily activities—an increase of more than 400 percent in fifty years.[14–16] Much of the growth has come from four classes of more common, usually less complex conditions that have not had substantial mortality associated with them: asthma,[17] obesity,[18] mental health conditions,[19,20] and neurodevelopmental disorders, none of which reflect changes in survival.[21] In contrast to rare and complex conditions, much of the growth in these conditions, and thus rates of chronic conditions in general, reflect an increase in prevalence. The prevalence of all of these conditions grew substantially in the 1980s and 1990s, although rates of obesity and asthma may have stabilized since the turn of the twenty-first century. For other conditions, such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders, rates of diagnosis continue to increase as a result of better awareness of the conditions rather than actual growth

EXHIBIT 1

Changing Epidemiology Of Childhood Chronic Conditions, Their Drivers, And Changes In Service Organization

Main condition groups	Health condition examples	Drivers	Change in services
Individually rare, usually serious conditions	Childhood cancer, cystic fibrosis, congenital heart disease, and complications from premature births	High mortality drops in mid-twentieth century Highly specialized, technology-enhanced care has led to lower mortality with varying morbidity	Complexity of care is beyond the scope of primary care physicians for these rare conditions Care has become multidisciplinary and concentrated in specialty centers
Common chronic health conditions and developmental and mental health conditions	Asthma, overweight and obesity, attention deficit hyperactivity disorder, and autism spectrum disorders	Dramatic growth in prevalence has risen since the 1980s Likely environmental changes, particularly social environments and potentially environmental toxins; some genetic basis; greater emphasis on screening and identification	Increasing prevalence has overwhelmed the supply of pediatric specialists Care has become decentralized, focused in pediatric primary care; also, some community-based care through schools and regional early-intervention programs

SOURCE Authors' analysis.

Exhibit 45

In the 1990s children's poverty status increasingly correlated with prevalence of chronic health conditions.

in their prevalence.

What has caused such substantial growth in common conditions in recent decades? Increasingly, evidence points to genetic bases for these conditions, but genetic drift—changes in the gene pool of reproductive-age adolescents and young adults—alone cannot explain this rapid growth. Genetic susceptibility typically interacts with environmental triggers or exposures to cause these conditions, and a number of in utero and postnatal exposures could explain some growth, although research on potential perinatal toxins has been limited. Some growth likely reflects changes in children's social environments, with changing patterns of how children spend their time. Greater variation in adult supervision of young children, along with changes in children's diets, level of physical activity, and media exposure (such as television, mobile technology, and connectedness via social media) have been implicated in growth of these four major condition groups.[22] Additionally, growing public awareness of these conditions coupled with advances in screening in health care and school settings may identify mildly affected children who in previous years may have gone undiagnosed, accounting for some of the rapid increase in overall prevalence.

Sociodemographic Factors

What sociodemographic factors put a child at increased risk to have a chronic condition, and how do they relate to the changing epidemiology of these conditions? Data from the late 1980s indicate that children living in poverty were less likely than middle-class children to have a chronic physical condition.[23] However, in the 1990s children's poverty status increasingly correlated with prevalence of chronic health conditions. Recent data show that children living at or below the federal poverty level have a one-third increased risk of a chronic health care need.[24] Other studies document the correlation between poverty and special health care needs and the association of poverty with asthma, overweight status, and mental health conditions.[25,26]

Additional sociodemographic factors are also associated with some children's having a chronic health condition. Publicly insured children have more chronic conditions than children who have private health insurance (24 percent versus 18 percent).[27] Male children have 50 percent higher rates of chronic health conditions,[26] as well as increased prevalence of asthma, autism spectrum disorders, physical disability, behavior or learning problems, and medical complexity, compared to female children. Race has a less straightforward relationship with special health care needs. While some studies report that non-Hispanic white children are most likely to have a chronic health condition,[15] others report higher rates among non-Hispanic black and Hispanic children.[16]

Persistent racial and ethnic disparities in asthma and obesity include higher rates of asthma among non-Hispanic black children than non-Hispanic white children, across all income levels. American Indian and Alaska Native children have 25 percent higher asthma rates compared to non-Hispanic white children, and Puerto Rican children have a 140 percent higher prevalence, compared to non-Hispanic white children.[17] Hispanic children in general have comparable rates of asthma diagnosis with non-Hispanic white children, yet Hispanic and non-Hispanic black children have more hospitalizations for asthma than white children. Persistent and distinct racial disparities in childhood obesity remain. Fourteen percent of white children and adolescents were obese in 2009–10, compared to 24 percent and 21 percent of black and Hispanic children, respectively.[18]

Mental health and neurodevelopmental disparities present a more complicated picture. Children from racial and ethnic minorities have less frequent parent-recognized or medically diagnosed mental health conditions, although not for every mental health condition. While black children are less likely than whites to be diagnosed with anxiety or depression, they have 150 percent increased odds of having a diagnosis of a behavioral or conduct disorder.[28] Prevalence rates of ADHD among non-Hispanics are twice those of Hispanic prevalence rates, and over time, white and black children have increasingly accounted for a greater proportion of ADHD diagnoses.[19] Mental health racial disparities partly reflect differences in the use of services. White children and adolescents use mental health services and are prescribed psychotropic medications more commonly than black and Hispanic

children, with widening disparities in mental health care expenditures between white and Hispanic children in recent years.[29] Children with neurodevelopmental disorders, and in particular autism spectrum disorders, show similar patterns. While white children were typically diagnosed with autism spectrum disorders one and a half years before black children in the 1990s,[30] disparities in the age of diagnosis have decreased, and the median age of diagnosis of autism spectrum disorders is no longer significantly different among whites, blacks, and Hispanics. However, the prevalence of autism spectrum disorders shows a racial disparity: 15.8 per 1,000 for whites, 12.3 per 1,000 for blacks, and 10.8 per 1,000 for Hispanics.[31]

Language at home is also associated with different rates of childhood chronic conditions. Children from homes where English is not the primary language are more likely than others to be overweight, although they have decreased odds of numerous conditions, including asthma, ADHD, depression and anxiety, and developmental delay.[32] Hispanic children from homes where English is not the primary language are more commonly reported in fair or poor health than Hispanic children with English as primary language or white children, in addition to having 50 percent increased odds of being overweight.[32] They also are less frequently diagnosed with asthma when compared to Hispanic children for whom English is the primary language at home.[33] The fact that children from homes where English is not the primary language, and especially Hispanic children in this subgroup, are less frequently insured may explain some of the disparity, along with issues of cultural differences and linguistic barriers to care.

Organizing Health Care Delivery For Children

REGIONALIZATION FOR COMPLEX CONDITIONS

The distinction between relatively rare conditions with stable rates and very common conditions with major growth has implications for health care delivery. The rare conditions almost always require subspecialists to manage much of the ongoing patient care, and the small numbers of children with such conditions make it unlikely that primary care and smaller specialty practices can have the specialized competence needed to manage the conditions. For example, in the United States, care for nearly all of the 25,000 patients with cystic fibrosis, which is considered a rare condition, is delivered within an organized network of about 110 subspecialty centers that provide multidisciplinary services and are accredited by the Cystic Fibrosis Foundation.[34]

The sheer volume of children and youth with more common conditions makes focusing their care in regionalized subspecialty centers unrealistic.

Such organization facilitates data gathering, center-by-center quality comparisons, and rapid dissemination of new research findings into clinical settings. For other conditions, subspecialty units and primary care providers may collaborate on more aspects of care, depending on the condition. Essentially, though, all children with rare conditions are best served through high-quality service programs that have sufficient patient volume to support the clinical expertise and ancillary services necessary to address their specialized needs.[35] The variety of these conditions has led to the development of regionalized services available only in children's hospitals and related institutions having organized multispecialty pediatric programs.

Studies vary regarding experiences accessing subspecialty care by families of children with complex conditions, despite the growth of accomplished, organized, multidisciplinary pediatric subspecialty programs,[36] especially in children's hospitals. For severe chronic conditions, such as cystic fibrosis and childhood cancers, most children appear to see subspecialists whether they have private or public (or in some cases, no) insurance, although children insured by Medicaid may face barriers to some subspecialty care,[37] and several studies have indicated that many children with some of these chronic conditions do not regularly see pediatric subspecialists.[38]

Given the high rates of Medicaid coverage of children, including those with complex chronic conditions,[39] hospitals and other providers for these children often rely on Medicaid reimbursement, with its accompanying financial burden. Although many hospitals have been able to negotiate better rates from their state Medicaid agencies, providers where these arrangements

The diseases that infants, children, and youth experience have changed dramatically over the past half century.

do not exist (often for outpatient care) are reimbursed at about 65 percent of Medicare rates for comparable services. This low reimbursement, coupled with a growing proportion of Medicaid patients presenting with complex conditions, means that hospitals must supplement their patient revenues by other sources, such as philanthropy.

In addition, regionalization of many complex subspecialty programs has led to service areas that extend beyond state boundaries, and providers often see children from neighboring states. States typically have very different Medicaid payment and coverage policies, which raises issues relating to licensure and support for out-of-state services and treatments. Limited Medicaid reimbursement and problems in maintaining complex subspecialty units also make prospects for new physicians to join these practices more daunting, thereby limiting the pipeline of needed pediatric subspecialists.

Recognizing the importance of ensuring the quality of subspecialty care, several professional organizations have developed standards for numbers and types of staff and levels of training needed to support appropriate care for children with complex needs. For example, the American Academy of Pediatrics Committee on Fetus and Newborn has defined four levels of service for newborn intensive care, indicating needed staff and equipment and aligning those requirements with the specific types of care (and newborn conditions) that each level can provide or treat.[40] These standards take into account the varied distribution of services across the country, including both well-resourced facilities located in urban centers and less well-resourced facilities located in sparsely populated rural communities. Recent work by the pediatric surgical community has defined similar standards for different types of pediatric surgical care,[41] focused on assuring high quality of care and best outcomes for children with complex conditions while preserving healthier patients' ability to obtain low-risk procedures locally. Such standards and organization of services may increase health system efficiency by ensuring that patients' needs and institutional resources are optimally matched.

DECENTRALIZATION FOR COMMON CONDITIONS

The sheer volume of children and youth with more common conditions—obesity, asthma, mental health conditions, and neurodevelopmental disorders—makes focusing their care mainly in regionalized subspecialty centers unrealistic. Although severe cases may benefit from referral to a specialty care program for intense care needs (such as a child with morbid obesity being referred to a hospital-based multidisciplinary weight management program), the vast majority of children with these conditions will continue to receive most of their chronic care in primary care settings. Families usually prefer this arrangement, most primary care physicians see this degree of chronic disease management as within their scope of care, and primary care practices see a large enough volume of children with these conditions to build clinical expertise and systematize office workflow to attend to the various aspects of managing chronic conditions (such as performing a reminder or recall procedure for annual influenza vaccines for children with asthma).

Several innovations in care support primary care diagnosis, treatment, and management of these four condition groups. In mental health, for example, a severe shortage of child psychiatrists and geographic maldistribution means that pediatric primary care physicians are pressed to provide much of the mental health care for US children. In response, about half of the states have developed some form of telephone advice service for primary care physicians, who can call when they have questions about adjusting psychopharmacologic treatment or proper diagnosis. Physicians using these programs report improved ability to meet patients' needs for mental health care.[42] In other communities, primary care providers have arranged to co-locate mental health practitioners in their practices—allowing both referral to a mental health specialist and training and feedback to the primary care physicians.

Many practices have also expanded team care as part of implementing medical home programs. Nonphysician care coordinators assist with the ongoing management and monitoring of other chronic conditions, while ensuring access to and arranging appropriate services, including coordination with schools.[43] Such personnel help with using patient registries, arranging periodic chronic care visits, and referral to

and communication with community-based resources.

Current payment arrangements, still predominantly fee-for-service, provide only limited incentives to support these practice innovations. Many pediatric practices have acquired public and private grants to support additional staff for care coordination or mental health co-location. The Affordable Care Act, with its emphasis on enhanced payment arrangements for medical home services and chronic care management, offers substantial promise and early support for practice transformation.[44] Its continued implementation with proper incentives could speed change in practice arrangements. Bundled payment allows more prospective budgeting and supports the efforts of primary care providers to develop team-based arrangements. A number of state experiments, funded mainly through Medicaid, have also changed payment arrangements and encouraged the transformation of primary care to team-based practice.[45]

Recent and emerging technologies—especially mobile devices, patient portals, and other web-based technologies—can facilitate the monitoring of patients' symptoms, encourage patients to adhere to treatments, connect patients to others with similar problems for peer-based support, and notify patients of needed changes in treatment. Telemedicine provides new ways to extend specialized services (such as treatment for autism spectrum disorders) to rural communities. Although socioeconomic status influences the use of the Internet and other technologies, most new methods do not require substantial expenditures or sophistication by consumers. These technologies may add substantially to practices' ability to deliver care for chronic conditions. For example, instead of requiring specified return visits for asthma or ADHD, patients or parents could provide routine information about a child's status daily or weekly, allowing office personnel to review the data in close to real time or in aggregate, decide about needed treatment changes, and determine more rationally the need for office visits. Here, too, a move away from fee-for-service payment to bundled or capitated arrangements with incentives to adopt and use such technologies will lessen the need for inefficient but billed face-to-face visits to ensure adequate practice income.

Conclusion

The diseases that infants, children, and youth experience, including those responsible for substantial hospitalization, have changed dramatically over the past half century. A wider array of vaccines has markedly decreased many previously fatal infections as well as others that caused enough morbidity to lead to hospitalization. With advances in medical care, mortality rates began stabilizing for many severe, rare conditions around 1980, although since then other more common conditions have greatly increased in prevalence, out of proportion to changes in the occurrence of rare conditions. The differential epidemiology in these groups of conditions calls for a system of regionalized care for rare, complex conditions, based mainly in pediatric hospitals, and decentralized care for the common conditions, with the bulk of care delivered and received in primary care settings. ■

L. Elizabeth Anderson's time was supported by a grant to the American Pediatric Society/Society for Pediatric Research (Grant No. T35 HD 7446). This work is solely the responsibility of the authors and does not represent the official views of the American Pediatric Society/Society for Pediatric Research.

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Exhibit 45

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Exhibit 45

EXHIBIT 46

Prescription Medication Use Among Children and Adolescents in the United States

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abstract

BACKGROUND AND OBJECTIVES: Information on the use of prescription medications among children and adolescents in the United States is lacking. We estimate the prevalence of prescription medication use, concurrent use, and potential major drug–drug interactions (DDIs) in this population.

METHODS: We conducted descriptive analyses using nationally representative data for people ≤19 years old from NHANES. Data were derived from a medication log administered by direct observation during in-home interviews. Acute medications were used for ≤30 days. Concurrent use was defined as use of ≥2 prescription medications. Micromedex was used to identify potentially major DDIs.

RESULTS: During 2013–2014, 19.8% of children and adolescents used at least 1 prescription medication, and 7.1% used acute medications. Concurrent use of prescription medications was 7.5% overall and was highest among boys 6 to 12 years old (12%) and among boys and girls ages 13 to 19 years old (10% for both). Using pooled 2009–2014 data, we found that 8.2% of concurrent users of prescription medications were at risk for a potentially major DDI. The vast majority of interacting regimens involved antidepressants and were more common among adolescent girls than boys (18.1% vs 6.6%; $P < .05$), driven largely by greater rates of use of acute medications.

CONCLUSIONS: Many US children and adolescents use prescription medications with nearly 1 in 12 concurrent users of prescription medications potentially at risk for a major DDI. Efforts to prevent adverse drug events in children and adolescents should consider the role of interacting drug combinations, especially among adolescent girls.

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DOI: https://doi.org/10.1542/peds.2018-1042

Accepted for publication Jun 28, 2018

WHAT'S KNOWN ON THIS SUBJECT: Adverse drug events remain a leading cause of death among children and adolescents in the United States. Current information on concurrent use of prescription medications, however, is lacking and can be used to better guide efforts to improve their safe use.

WHAT THIS STUDY ADDS: One-fifth of children and adolescents regularly use prescription medications with nearly 1 in 12 concurrent users of prescription medications at risk for a major drug–drug interaction. Efforts to prevent adverse drug events should consider the role of interacting combinations.

To cite: Qato DM, Alexander GC, Guadamuz JS, et al. Prescription Medication Use Among Children and Adolescents in the United States. *Pediatrics.* 2018;142(3):e20181042

Prescription medications are often indicated for the treatment of common pediatric and adolescent chronic conditions, such as depression and attention-deficit/hyperactivity disorder,[1–3] and acute ailments, such as respiratory tract and sexually transmitted infections.[4,5] In addition to known clinical benefits, many of these medications are associated with rare but serious adverse effects, such as serotonin syndrome, suicidal ideation, and sudden death.[6–12] Despite the implementation of national programs focusing on medication safety in young children, adverse drug events remain a leading cause of injuries and death among both children and adolescents in the United States.[13–16] Current data on the use and concurrent use of prescription medications can be used to better guide efforts to reduce the burden of adverse drug events in this vulnerable population.

Authors of several national studies have evaluated the use of prescription medications in younger populations.[17,18] Despite important insights from these studies, however, they have several limitations. First, these previous studies have not been used to describe the concurrent use of specific types of acute and chronic prescription medications. This information is important because the use of multiple medications is associated with an increased risk for adverse drug events.[19] Second, the authors of these studies do not provide information on the use of prescription medications among boys and girls separately for children and adolescents. This is important because of known differences in the use of specific drug classes between children and adolescents and between boys and girls. Finally, authors of previous studies do not examine the concurrent use of interacting drug regimens.

We used nationally representative, directly observed data from the most recent 6 cycles of the NHANES to examine the use of prescription medications among children and adolescents in the United States overall and stratified by age group and gender. We also examined the prevalence of potentially contraindicated or major drug–drug interactions (DDIs).

METHODS

Participants

NHANES is a nationally representative survey sampled from the US civilian, noninstitutionalized population conducted by the National Center for Health Statistics. Sampling methods are described elsewhere.[20] We restricted our sample to children and adolescents ages 0 to 19 years who responded to the prescription medication questionnaires. A parent or caregiver provided information for survey participants who were <16 years of age and for those who could not answer themselves. We used the 6 most recent NHANES cycles (2003–2004, 2005–2006, 2007–2008, 2009–2010, 2011–2012, and 2013–2014). A total of 23 179 children and adolescent participants ages 0 to 19 years were sampled over the 10-year period examined. Twenty-seven participants were excluded because of missing medication information, yielding an analytic subsample of 23 152 participants.

Prescription Medication Data

During the household interview participants were asked whether they had taken a prescription medication in the past 30 days.[21] Those who answered "yes" were asked to show the interviewer the medication containers for all the medications used. If no container was available, the participant was asked to name the prescription medication they used. Prescription medication names and therapeutic classes were coded by using Lexicon Plus (Cerner Multum, Inc, Kansas City, MO), a proprietary database of Cerner Multum, Inc.

We differentiated acute versus chronic prescription medication use on the basis of duration of use. For each medication, participants were asked "How long was medication taken?" We defined acute use as the use of a prescription medicine for ≤30 days and all other use as chronic use. For prescription medications used chronically, approximately three-fourths (73%) were used for >365 days. We defined concurrent use as the simultaneous use of at least 2 prescription medications during the past 30 days. We also identified therapeutic classes most commonly used together, overall and by age group and gender.

We used Micromedex to identify potential DDIs for all prescription medications used in children and adolescents during 2013–2014. Micromedex defines contraindicated combinations as "drugs [that] are contraindicated for concurrent use" and major DDIs as "the interaction may be life threatening, require medical intervention to minimize or prevent serious adverse events, or both."

Analyses

We used descriptive statistics to estimate the prevalence of prescription medication and concurrent use for each of the 6 NHANES cycles examined. For analyses stratified by age group and gender, we focused on the most recent cycle (2013–2014); to increase sample size, however, we pooled the most recent 3 cycles (2009–2010, 2011–2012, and 2013–2014) for the analyses of commonly used combinations of therapeutic drug classes and DDIs. We used Taylor linearization methods to incorporate sample weights to adjust for the complex sampling methods in NHANES for estimate prevalence and conduct statistical tests. For most analyses, we used logistic regression

TABLE 1 Prevalence of Prescription Medication Use in Previous 30 Days Among Children and Adolescents in the United States (2013–2014)

	Participants		Prevalence of Use, % (95% CI)		
	n	% (95% CI)	Any Medication	Acute Medication, ≤30 d	Chronic Medication, >30 d
No. participants			786	299	528
Overall	4404		19.8 (17.2–22.8)	7.1 (5.9–8.5)	13.9 (11.7–16.6)
Age group, y					
0–5	1603	25.9 (23.5–28.4)	14.7 (12.4–17.4)**	9.1 (7.6–10.8)*	6.4 (4.8–8.4)**
6–12	1563	37.6 (35.0–40.3)	21.0 (17.7–24.9)	5.5 (4.2–7.2)	16.5 (13.7–19.7)
13–19	1238	36.5 (33.6–39.4)	22.8 (18.0–28.4)	7.1 (5.0–10.0)	17.6 (13.5–22.7)
Gender					
Girl	2161	48.9 (46.3–51.4)	19.3 (16.0–23.2)	8.0 (6.0–10.6)	12.6 (9.7–16.4)
Boy	2243	51.1 (48.6–53.7)	20.3 (17.5–23.4)	6.3 (5.0–7.7)	15.2 (12.9–17.7)
Asthma					
Yes	413	9.7 (8.6–11.0)	58.5 (51.4–65.3)**	11.7 (8.1–16.6)*	51.9 (44.3–59.4)**
No	3577	90.3 (89.0–91.4)	15.9 (13.3–18.8)	6.4 (5.3–7.8)	10.3 (8.2–12.9)
General health condition					
Excellent	1882	43.9 (40.9–46.9)	12.8 (10.5–15.6)**	5.3 (4.1–6.8)*	7.9 (6.0–10.4)**
Very good	1209	29.0 (26.6–31.5)	21.1 (18.0–24.6)	7.5 (6.0–9.2)	14.9 (11.8–18.5)
Good	1054	21.8 (18.7–25.3)	27.1 (21.8–33.2)	8.7 (6.4–11.8)	20.9 (16.3–26.3)
Fair	226	4.6 (3.5–6.0)	39.0 (26.5–53.2)	14.0 (8.9–21.3)	27.4 (16.6–41.8)
Poor	33	0.7 (0.4–1.2)	56.1 (33.2–76.7)	11.5 (3.7–30.4)	50.3 (29.0–71.6)
Race and/or ethnicity					
Non-Hispanic white	1201	53.1 (42.6–63.4)	22.7 (18.2–27.8)*	8.0 (6.1–10.3)	16.3 (12.4–21.1)*
Non-Hispanic African American	1090	13.8 (9.8–19.1)	18.9 (15.1–23.5)	7.3 (5.2–10.1)	13.1 (10.5–16.2)
Hispanic or Latino	1414	23.0 (16.5–31.3)	16.2 (12.2–21.2)	6.3 (4.6–8.6)	10.6 (7.5–14.9)
Other	699	10.0 (8.1–12.4)	14.8 (11.6–18.7)	4.2 (2.7–6.4)	10.9 (8.5–13.9)
Family income-to-poverty ratio, %					
<130	2014	38.3 (31.2–45.9)	21.0 (17.4–25.2)	7.6 (5.8–10.1)	14.3 (11.8–17.3)
130–349	1257	33.8 (29.3–38.7)	21.3 (18.2–24.8)	7.5 (5.5–10.2)	14.9 (11.3–17.3)
>349	803	27.9 (21.2–35.7)	18.2 (15.3–21.6)	6.9 (5.2–9.2)	12.9 (10.6–17.3)
Usual source of care					
Yes	4138	94.0 (92.8–95.0)	20.5 (17.8–23.4)*	7.3 (6.1–8.8)	14.4 (12.0–17.2)**
No	266	6.0 (5.0–7.2)	10.1 (5.3–18.4)	3.4 (1.4–8.3)	6.7 (3.2–13.4)
Insurance status					
No insurance	350	8.1 (6.6–9.8)	6.5 (3.4–11.9)**	2.3 (1.0–5.2)**	4.5 (2.1–9.3)**
Public insurance only	2200	39.6 (33.3–46.2)	21.3 (18.0–25.0)	8.0 (5.9–10.7)	14.8 (12.1–17.9)
Any private insurance	1799	52.3 (45.5–59.1)	20.8 (17.5–24.6)	7.2 (5.9–8.7)	14.7 (11.6–18.6)

Estimates are weighted to account for differential probabilities of selection and differential nonresponse. Acute and chronic medication use are not mutually exclusive (eg, participants can use both types of prescription medications).

** $P < .01$; * $P < .05$ by using χ^2 to test for differences across respondent characteristics.

to test for significance of differences by gender within age groups; to examine gender differences in the concurrent use of therapeutic drug classes and DDIs, the Pearson's χ^2 test was used. We tested for the statistical significance of trends across cycles using logistic regression. We used Stata (Stata Corp, College Station, TX) version 14 to perform all analyses. All *P* values reported are 2-sided. The study was considered exempt by a University of Illinois at Chicago Institutional Review Board.

RESULTS

Table 1 reports prevalence of the use of prescription medications overall and by population characteristics for 2013–2014. Nearly one-fifth (19.8%) of children and adolescents ≤19 years old used at least 1 prescription medication in the previous 30 days; 13.9% used chronic medications, and 7.1% used acute medications. Prescription medication use increased with age, ranging from 14.7% in children ages 0 to 5 years to 22.8% among adolescents (13–19 years). Acute medication use was highest in younger children (9.1%), whereas the use of chronic medications was lowest (6.4%).

Figure 1 depicts the prevalence of the use of prescription medications over time (Fig 1A) and by age group and gender (Fig 1B). The most commonly used prescription medications included respiratory agents, especially bronchodilators (the most common type was albuterol) and psychotherapeutic agents, especially central nervous system (CNS) stimulants (methylphenidate was most common) and antidepressants (fluoxetine was most common) (Table 2). There was a notable decline in the use of acute medications between 2003–2004 and 2013–2014 (10.9% [95% confidence interval (CI), 9.3%–12.9%] to 7.1% [CI, 6.0%–8.4%]; $P < .01$), driven largely by a decrease in the use of antibiotics (7.9% [CI, 6.6%–9.5%] to 4.8% [CI, 3.7%–6.1%]; $P < .01$; Supplemental Table 5).

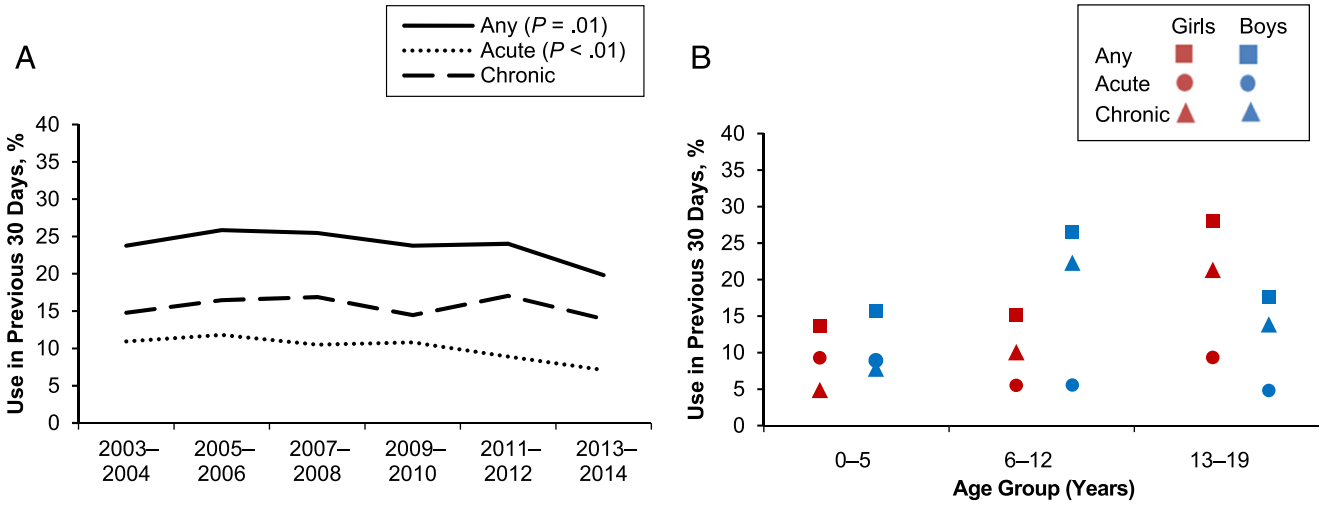

FIGURE 1

Weighted prevalence estimates of prescription medication use over time and by gender and age group among children and adolescents in the United States. Estimates are weighted to account for differential probabilities of selection and differential nonresponse. A, Trends in medication use (2003–2014). B, Medication use by gender and age (2013–2014). Boys ages 6 to 12 years report higher use of any prescription medication and chronic medications than girls ($P < .01$ and $P < .01$, respectively). Adolescent girls (13–19 years) report higher use of any prescription medication, including acute and chronic medications than adolescent boys ($P < .01$, $P < .05$, and $P < .05$, respectively).

The use of prescription medications was highest among adolescent girls (28.0% [CI, 21.3%–35.8%]) and boys ages 6 to 12 (26.5% [CI, 22.5%–30.9%]; Fig 1). Gender differences were also most pronounced in these 2 age groups. Among children ages 6 to 12, boys were nearly twice as likely to use prescription medications compared with girls (26.5% [CI, 22.5%–30.9%] vs 15.1% [CI, 11.8%–19.0%]; $P < .01$); this difference was primarily due to a higher rate of chronic medication use (22.3% [CI, 19.1%–25.9%] vs 10.1% [CI, 7.7%–13.0%]; $P < .01$), especially CNS stimulants (9.4% [CI, 7.2%–12.0%] vs 2.2% [CI, 1.2%–4.1%]; $P < .01$), α-adrenergic agonists (3.3% [CI, 1.9%–5.7%] vs 1.1% [CI, 0.3%–3.5%]; $P < .01$), and leukotriene modifiers (4.0% [CI, 2.5%–6.4%] vs 0.9% [CI, 0.4%–1.9%], respectively; $P < .01$) (Table 2).

During adolescence, however, girls were more likely to use prescription medications than boys; use was 28.0% (CI, 21.3%–35.8%) among girls versus 17.6% (CI, 13.3%–22.9%) among boys ($P < .01$). This difference was due to a higher rate of both acute (9.3% [CI, 5.9%–14.4%] vs 4.8% [CI, 3.2%–7.2%]; $P < .05$) and chronic (21.3% [CI, 15.0%–29.4%] vs 13.8% [CI, 10.2%–18.5%]; $P < .05$) medication use, specifically antidepressants (3.8% [CI, 1.9%–7.6%] vs 1.3% [CI, 0.5%–3.7%]; $P < .01$), antibiotics (6.6% [CI, 3.6%–11.7%] vs 3.5% [CI, 2.0%–6.0%]; $P < .01$), analgesics (3.2% [CI, 1.6%–6.4%] vs 1.2% [CI, 0.6%–2.3%]; $P < .01$), and antiemetics (1.6% [CI, 0.8%–3.3%] vs 0.1% [CI, 0.0%–1.2%]; $P = .06$) (Table 2). Rates of CNS stimulant use, in contrast, were lower in adolescent girls than in boys (1.6% [CI, 0.9%–3.0%] vs 4.3% [CI, 2.3%–7.9%]; $P < .01$).

Concurrent Use of Prescription Medications

Figure 2 depicts the prevalence of the use of concurrent prescription medications over time (Fig 2A) and by age group and gender (Fig 2B). During 2013 and 2014, 7.5% (CI, 6.6%–8.6%) of children and adolescents concurrently used prescription medications, a decrease from 2003–2004 (9.9% [CI, 9.1%–10.8%]; $P < .01$). The concurrent use of prescription medications varied across age groups for both boys and girls and was substantially more common among boys ages 6 to 12 than among girls in this age group (11.9% [CI, 8.8%–15.8%] vs 4.7% [CI, 3.4%–6.7%]; $P < .01$).

Among concurrent users of prescription medications, combinations that involved respiratory agents, such as bronchodilators and leukotriene modifiers, and psychotropic medications, including antidepressants, atypical antipsychotics, and CNS stimulants, were the most prevalent (Table 3). More than 20% (23.7% [CI, 11.1%–43.8%]) of boys ages 6 to 12 and more than half (57.9% [CI, 35.1%–77.7%]) of adolescent boys who used CNS stimulants concurrently used ≥2 other psychotropic medications. Combinations that involved antidepressants were most prevalent among adolescent girls; among these antidepressant users, 51.2% [CI, 19.4%–82.6%] were concurrently using at least 2 psychotropic medications.

Potential Major DDIs

Among concurrent users of ≥2 prescription medications during 2013–2014 ($N = 301$), a total of 156

TABLE 2 Weighted Prevalence in the Use of Prescription Medications in the Previous 30 Days by Therapeutic Drug Class Among Children and Adolescents in the United States, Overall and by Gender and Age Group (2013–2014)

	Prevalence of Use, % (95% CI)						
	Overall, *n* = 4404	0–5 y		6–12 y		13–19 y	
		Girls (*n* = 775)	Boys (*n* = 828)	Girls (*n* = 746)	Boys (*n* = 817)	Girls (*n* = 640)	Boys (*n* = 598)
Respiratory agents	6.8 (5.8–8.0)	3.9 (2.3–6.6)	5.1 (3.3–7.8)**	5.7 (4.1–8.0)	11.5 (8.9–14.7)*	6.4 (4.2–9.7)	7.0 (4.9–9.9)
Bronchodilators	3.5 (3.0–4.1)	2.7 (1.5–4.7)	2.7 (1.5–4.8)	3.0 (1.7–5.5)	5.5 (4.3–6.9)*	3.4 (1.7–7.0)[a]	3.2 (2.1–5.0)
Albuterol	3.0 (2.5–3.6)	2.6 (1.5–4.5)	2.5 (1.4–4.5)	2.6 (1.4–4.8)	4.5 (3.1–6.6)	3.1 (1.4–6.9)[a]	2.6 (1.8–3.6)
Levalbuterol	0.3 (0.1–0.6)[a]	0.2 (0.0–1.5)[a]	0.2 (0.0–0.8)[a]	0.4 (0.1–2.5)[a]	0.4 (0.2–1.0)[a]	0.1 (0.0–1.0)[a]	0.2 (0.1–1.1)[a]
Fluticasone and salmeterol	0.1 (0.1–0.3)[a]	0.0	0.1 (0.0–0.8)[a]	0.0	0.4 (0.2–0.8)[a]	0.3 (0.1–1.5)[a]	0.0
Antihistamines	1.8 (1.3–2.6)	0.9 (0.3–2.6)[a]	1.4 (0.7–2.7)[a]	2.1 (1.1–4.0)[a]	3.2 (2.1–5.0)	1.0 (0.7–1.7)	2.0 (0.8–5.0)[a]
Cetirizine	1.0 (0.6–1.6)	0.9 (0.3–2.6)[a]	0.8 (0.5–1.4)	0.9 (0.4–2.3)[a]	1.3 (0.7–2.4)	0.7 (0.4–1.4)[a]	1.2 (0.3–4.2)[a]
Loratadine	0.4 (0.3–0.7)	0.0	0.1 (0.0–0.7)[a]	0.3 (0.1–1.4)[a]	1.2 (0.7–2.0)	0.1 (0.0–0.6)[a]	0.7 (0.2–2.8)[a]
Cyproheptadine	0.2 (0.1–0.6)[a]	0.0	0.1 (0.0–0.9)[a]	0.0	0.8 (0.2–2.7)[a]	0.1 (0.0–1.0)[a]	0.0
Leukotriene modifiers	1.7 (1.3–2.2)	0.3 (0.1–1.0)[a]	0.8 (0.4–1.7)[a]**	0.9 (0.4–1.9)[a]	4.0 (2.5–6.4)**	1.9 (1.1–3.1)	1.8 (0.8–3.7)[a]
Montelukast	1.7 (1.3–2.2)	0.3 (0.1–1.0)[a]	0.8 (0.4–1.7)[a]**	0.9 (0.4–1.9)[a]	4.0 (2.5–6.4)**	1.9 (1.1–3.1)	1.8 (0.8–3.7)[a]
Respiratory inhalant products	1.3 (0.8–1.9)	0.4 (0.1–1.5)[a]	1.3 (0.6–3.0)[a]	1.0 (0.4–2.4)[a]	3.1 (2.1–4.5)	0.2 (0.0–1.0)[a]	1.4 (0.5–3.7)[a]
Beclomethasone	0.5 (0.3–0.9)	0.1 (0.0–0.6)[a]	0.7 (0.2–2.4)[a]	0.4 (0.1–1.1)[a]	1.2 (0.5–2.4)[a]*	0.1 (0.0–1.0)[a]	0.6 (0.1–3.1)[a]
Fluticasone	0.7 (0.4–1.2)	0.3 (0.1–1.1)[a]	0.6 (0.2–1.9)[a]	0.6 (0.2–1.6)[a]	1.6 (0.9–2.7)	0.1 (0.0–0.7)[a]	0.7 (0.2–2.8)[a]
Psychotherapeutic agents[b]	5.0 (4.0–6.3)	0.1 (0.0–0.6)[a]	1.5 (0.5–5.0)[a]	3.5 (1.8–6.7)[a]	10.9 (8.7–13.6)**	6.4 (4.1–9.9)	5.7 (3.4–9.5)
CNS stimulants	3.3 (2.5–4.4)	0.1 (0.0–0.6)[a]	1.1 (0.3–4.3)[a]	2.2 (1.2–4.1)	9.4 (7.2–12.0)**	1.6 (0.9–3.0)	4.3 (2.3–7.9)**
Methylphenidate	1.6 (1.0–2.4)	0.1 (0.0–0.6)[a]	0.2 (0.0–1.5)[a]	0.5 (0.1–2.5)[a]	5.2 (3.2–8.3)**	0.8 (0.2–2.9)[a]	1.8 (0.8–3.9)[a]
Lisdexamfetamine	0.7 (0.5–1.0)	0.0	0.0	0.8 (0.2–2.8)[a]	1.7 (1.0–3.2)	0.1 (0.0–1.0)[a]	1.1 (0.4–2.8)[a]
Amphetamine and dextroamphetamine	0.4 (0.2–0.9)[a]	0.0	0.6 (0.1–5.1)[a]	0.3 (0.1–1.5)[a]	0.9 (0.4–2.0)[a]	0.3 (0.1–1.0)[a]	0.4 (0.1–2.1)[a]
Antidepressants	1.2 (0.7–2.0)	0.0	0.0	0.1 (0.0–0.7)[a]	1.3 (0.5–3.5)[a]*	3.8 (1.9–7.6)[a]	1.3 (0.5–3.7)[a]**
Fluoxetine	0.3 (0.2–0.6)	0.0	0.0	0.0	0.5 (0.2–1.6)[a]	0.7 (0.2–2.1)[a]	0.5 (0.2–1.6)[a]
Escitalopram	0.2 (0.1–0.9)[a]	0.0	0.0	0.0	0.0	0.8 (0.1–4.9)[a]	0.5 (0.1–3.9)[a]
Trazodone	0.2 (0.0–0.8)[a]	0.0	0.0	0.1 (0.0–0.7)[a]	0.0	0.9 (0.2–4.6)[a]	0.0
Antipsychotics	0.9 (0.7–1.1)	0.0	0.0	0.8 (0.3–2.7)[a]	1.9 (1.1–3.3)*	1.2 (0.6–2.5)[a]	1.1 (0.3–3.3)[a]
Aripiprazole	0.2 (0.1–0.5)[a]	0.0	0.0	0.0	0.5 (0.3–0.7)	0.4 (0.1–1.7)[a]	0.4 (0.1–3.3)[a]
Risperidone	0.4 (0.3–0.7)	0.0	0.0	0.8 (0.3–2.7)[a]	0.9 (0.3–3.1)[a]	0.1 (0.0–0.9)[a]	0.6 (0.2–2.5)[a]
Quetiapine	0.2 (0.1–0.4)	0.0	0.0	0.0	0.7 (0.4–1.1)	0.5 (0.2–1.9)[a]	0.0
Anticonvulsants	0.8 (0.3–1.9)[a]	0.0	0.2 (0.0–1.5)[a]	1.0 (0.3–3.6)[a]	0.4 (0.1–1.4)[a]	1.7 (0.5–5.6)[a]	1.2 (0.4–3.8)[a]
Topiramate	0.3 (0.1–1.1)[a]	0.0	0.2 (0.0–1.5)[a]	0.3 (0.2–0.5)	0.0	1.1 (0.2–6.7)[a]	0.3 (0.1–1.5)[a]
Lamotrigine	0.3 (0.1–0.9)[a]	0.0	0.0	0.0	0.0	0.9 (0.2–4.6)[a]	0.6 (0.1–3.4)[a]
Divalproex sodium	0.1 (0.0–0.5)[a]	0.0	0.0	0.0	0.2 (0.0–1.7)[a]	0.2 (0.0–1.3)[a]	0.2 (0.0–1.1)[a]
Anxiolytics, sedatives, and hypnotics	0.3 (0.2–0.4)	0.0	0.3 (0.0–2.4)[a]	0.2 (0.1–0.4)[a]	0.1 (0.0–0.4)[a]	0.8 (0.2–2.9)[a]	0.2 (0.0–1.2)[a]**
Hydroxyzine	0.2 (0.1–0.4)	0.0	0.3 (0.0–2.4)[a]	0.2 (0.1–0.4)[a]	0.1 (0.0–0.4)[a]	0.7 (0.2–3.1)[a]	0.1 (0.0–0.9)[a]
Antibiotics	4.8 (3.7–6.2)	7.0 (5.2–9.4)	5.8 (4.5–7.4)	3.0 (1.6–5.6)	3.5 (2.0–6.1)*	6.6 (3.6–11.7)	3.5 (2.0–6.0)**
Penicillins	2.2 (1.6–3.0)	4.5 (3.3–6.1)	3.1 (2.2–4.4)	1.1 (0.5–2.6)[a]	1.4 (0.7–2.8)[a]	2.6 (1.1–6.0)[a]	1.1 (0.3–3.8)[a]**
Amoxicillin	1.8 (1.4–2.4)	4.4 (3.2–6.1)	2.9 (2.0–4.3)	0.6 (0.2–1.6)[a]	1.4 (0.7–2.8)[a]	1.1 (0.6–2.0)	1.1 (0.3–3.8)[a]
Macrolide derivatives	0.8 (0.5–1.4)	0.2 (0.0–1.0)[a]	1.0 (0.4–2.3)[a]	1.2 (0.4–3.8)[a]	1.4 (0.5–3.6)[a]	0.9 (0.3–2.8)[a]	0.2 (0.0–1.0)[a]*
Azithromycin	0.8 (0.5–1.2)	0.2 (0.0–1.0)[a]	0.9 (0.4–2.1)[a]	1.2 (0.4–3.8)[a]	1.4 (0.5–3.6)[a]	0.5 (0.1–2.9)[a]	0.2 (0.0–1.0)[a]
Cephalosporins	0.5 (0.2–1.3)[a]	0.7 (0.2–3.1)[a]	0.9 (0.3–2.7)[a]	0.2 (0.0–1.1)[a]	0.3 (0.0–2.3)[a]	0.6 (0.1–4.9)[a]	0.5 (0.1–2.8)[a]
Cefdinir	0.2 (0.1–0.6)[a]	0.2 (0.0–1.3)[a]	0.2 (0.1–0.8)[a]	0.1 (0.0–1.2)[a]	0.3 (0.0–2.3)[a]	0.0	0.4 (0.1–3.3)[a]
Topical agents	3.0 (2.5–3.5)	1.2 (0.6–2.3)	1.7 (1.0–2.9)	1.9 (1.0–3.5)	4.9 (3.2–7.5)	3.7 (1.8–7.4)[a]	3.7 (1.7–8.0)[a]
Nasal preparations	1.5 (1.0–2.3)	0.1 (0.0–1.3)[a]	0.7 (0.3–1.5)[a]	0.5 (0.2–1.3)[a]	4.2 (2.5–7.1)**	0.8 (0.2–3.1)[a]	2.0 (0.7–5.5)[a]
Fluticasone nasal	1.0 (0.6–1.7)	0.0	0.3 (0.1–1.6)[a]	0.3 (0.1–1.2)[a]	2.4 (1.1–4.9)[a]*	0.6 (0.1–3.0)[a]	2.0 (0.7–5.5)[a]
Mometasone nasal	0.5 (0.2–0.9)[a]	0.1 (0.0–1.3)[a]	0.2 (0.0–1.4)[a]	0.1 (0.0–0.7)[a]	1.7 (0.7–4.3)[a]*	0.2 (0.0–2.0)[a]	0.2 (0.0–0.7)[a]
Dermatological agents	1.2 (0.7–1.9)	0.5 (0.2–1.2)[a]	0.6 (0.2–1.5)[a]	1.4 (0.7–3.0)[a]	0.7 (0.3–1.7)[a]**	2.8 (1.1–6.9)[a]	0.9 (0.2–3.1)[a]
Tretinoin topical	0.2 (0.1–0.7)[a]	0.0	0.0	0.1 (0.0–1.0)[a]	0.0	0.7 (0.1–4.4)[a]	0.3 (0.0–2.6)[a]
Hormones or hormone modifiers	2.1 (1.5–2.8)	1.0 (0.5–2.1)[a]	1.1 (0.5–2.6)[a]**	1.2 (0.5–2.5)[a]	0.9 (0.2–3.3)[a]	7.2 (5.3–9.5)	0.7 (0.2–2.1)[a]**
Contraceptives[c]	2.5 (1.8–3.5)	0.0	0.0	0.0	0.0	7.0 (5.2–9.3)	0.0
Ethinyl estradiol–norethindrone[c]	0.9 (0.4–2.1)[a]	0.0	0.0	0.0	0.0	2.5 (1.1–5.6)[a]	0.0
Adrenal cortical steroids	0.6 (0.4–1.0)	1.0 (0.5–2.1)[a]	0.9 (0.4–2.2)[a]**	0.7 (0.2–2.4)[a]	0.4 (0.1–1.8)[a]	0.2 (0.0–1.0)[a]	0.6 (0.2–2.0)[a]**
Budesonide	0.3 (0.2–0.7)[a]	0.5 (0.2–1.6)[a]	0.4 (0.2–1.2)[a]	0.5 (0.1–2.6)[a]	0.4 (0.1–1.9)[a]	0.1 (0.0–1.0)[a]	0.1 (0.0–1.0)[a]

TABLE 2 Continued

	Prevalence of Use, % (95% CI)						
	Overall, *n* = 4404	0–5 y		6–12 y		13–19 y	
		Girls (*n* = 775)	Boys (*n* = 828)	Girls (*n* = 746)	Boys (*n* = 817)	Girls (*n* = 640)	Boys (*n* = 598)
Gastrointestinal agents	1.5 (0.8–2.6)	1.7 (1.0–3.0)	1.6 (0.9–2.6)	1.0 (0.4–2.7)[a]	1.4 (0.5–3.9)[a]	2.0 (0.8–4.9)[a]	1.3 (0.4–3.7)[a**]
PPIs	0.6 (0.3–1.3)[a]	0.2 (0.0–0.9)[a]	0.5 (0.2–1.3)[a]	0.1 (0.0–0.7)[a]	0.6 (0.1–2.8)[a]	1.1 (0.4–3.1)[a]	1.0 (0.2–4.6)[a]
Omeprazole	0.3 (0.1–1.2)[a]	0.1 (0.0–1.0)[a]	0.1 (0.0–0.6)[a]	0.1 (0.0–0.7)[a]	0.6 (0.1–2.8)[a]	0.1 (0.0–0.8)[a]	0.9 (0.2–4.8)[a]
H_2 antagonist	0.5 (0.3–1.0)[a]	1.4 (0.7–3.1)[a]	1.0 (0.6–1.9)	0.5 (0.1–2.1)[a]	0.3 (0.1–1.2)[a]	0.2 (0.0–1.3)[a]	0.1 (0.0–0.8)[a**]
Ranitidine	0.5 (0.3–1.0)[a]	1.4 (0.6–3.1)[a]	1.0 (0.6–1.9)	0.5 (0.1–2.1)[a]	0.3 (0.1–1.2)[a]	0.2 (0.0–1.3)[a]	0.1 (0.0–0.8)[a]
Antiadrenergic agents, centrally acting	1.1 (0.8–1.6)	0.0	0.2 (0.0–1.3)[a]	1.1 (0.3–3.5)[a]	3.3 (1.9–5.7)**	0.2 (0.0–1.9)[a]	1.4 (0.5–4.1)[a**]
Clonidine	0.8 (0.5–1.3)	0.0	0.2 (0.0–1.3)[a]	1.0 (0.3–3.5)[a]	2.1 (0.8–5.0)[a]	0.2 (0.0–1.9)[a]	0.9 (0.2–4.5)[a]
Guanfacine	0.4 (0.2–0.9)[a]	0.0	0.0	0.2 (0.0–0.8)[a]	1.5 (0.6–3.6)[a**]	0.0	0.5 (0.1–2.3)[a]
Analgesics	1.1 (0.7–1.6)	0.2 (0.0–0.8)[a]	1.0 (0.4–2.3)[a]	0.6 (0.2–1.9)[a]	0.2 (0.0–0.7)[a]	3.2 (1.6–6.4)[a]	1.2 (0.6–2.3)[a*]
Nonsteroidal anti-inflammatory agents	0.7 (0.4–1.2)	0.2 (0.0–0.8)[a]	0.9 (0.4–2.2)[a*]	0.3 (0.1–1.1)[a]	0.0	1.8 (0.8–4.2)[a]	1.0 (0.5–2.2)[a]
Diclofenac	0.1 (0.0–0.8)[a]	0.0	0.0	0.0	0.0	0.6 (0.1–4.9)[a]	0.1 (0.0–0.7)[a]
Narcotics	0.4 (0.2–0.8)	0.0	0.1 (0.0–0.6)[a*]	0.5 (0.1–1.8)[a]	0.2 (0.0–0.7)[a]	1.5 (0.7–3.5)[a]	0.2 (0.0–0.9)[a**]
Hydrocodone	0.3 (0.2–0.7)[a]	0.0	0.1 (0.0–0.6)[a]	0.2 (0.0–1.0)[a]	0.1 (0.0–0.8)[a]	1.3 (0.5–3.4)[a]	0.2 (0.0–0.9)[a]
Oxycodone	0.2 (0.1–0.5)[a]	0.0	0.0	0.3 (0.0–2.2)[a]	0.0	0.8 (0.3–2.6)[a]	0.0
Antiemetic agents	0.7 (0.5–1.1)	0.5 (0.1–1.6)[a]	1.1 (0.3–3.3)[a]	0.8 (0.3–2.4)[a]	0.2 (0.1–0.6)[a]	1.6 (0.8–3.3)[a]	0.1 (0.0–1.2)[a]
Ondansetron	0.5 (0.3–0.8)	0.1 (0.0–1.1)[a]	0.7 (0.2–2.8)[a]	0.5 (0.1–2.3)[a]	0.1 (0.0–0.5)[a]	1.1 (0.4–3.2)[a]	0.1 (0.0–1.2)[a]
Diphenhydramine	0.2 (0.1–0.3)[a]	0.2 (0.0–0.8)[a]	0.0	0.1 (0.0–1.2)[a]	0.2 (0.0–0.8)[a]	0.5 (0.2–1.6)[a]	0.0
Promethazine	0.1 (0.0–0.4)[a]	0.1 (0.0–0.9)[a]	0.4 (0.1–1.8)[a*]	0.1 (0.0–1.0)[a]	0.0	0.0	0.0
Antidiabetic agents	0.5 (0.2–1.2)[a]	0.0	0.0	0.1 (0.0–0.7)[a]	1.3 (0.3–5.2)[a]	1.4 (0.5–3.7)[a]	0.1 (0.0–1.0)[a]
Metformin	0.2 (0.1–0.6)[a]	0.0	0.0	0.0	0.1 (0.0–0.4)[a]	1.1 (0.3–3.7)[a]	0.0
Insulin	0.3 (0.1–0.8)[a]	0.0	0.0	0.1 (0.0–0.7)[a]	1.2 (0.3–5.3)[a*]	0.3 (0.1–0.6)[a]	0.1 (0.0–1.0)[a]

Estimates are weighted to account for differential probabilities of selection and differential nonresponse. H_2, histamine$_2$; PPI, proton pump inhibitors.

[a] Estimates are unreliable; relative SE >30%.

[b] Derived from Multum classification of psychotherapeutic agents, CNS stimulants, anticonvulsants, and anxiolytics, sedatives, and hypnotics.

[c] Among girls only.

** $P < .01$; * $P < .05$ by using logistic regressions to test differences between girls and boys.

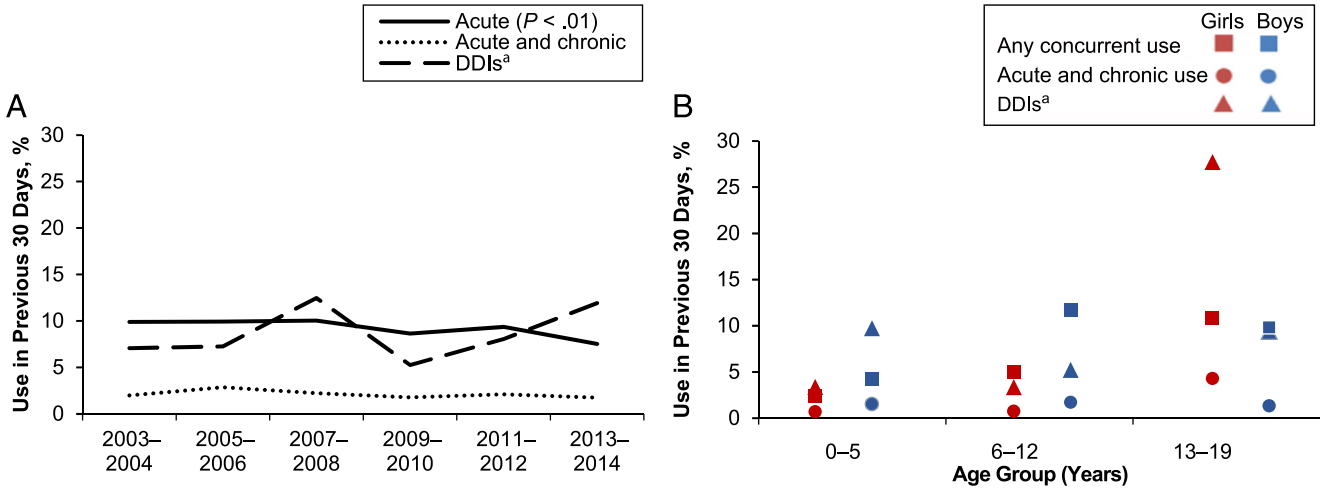

FIGURE 2

Weighted prevalence estimates of concurrent (≥2) prescription medication use over time and by gender and age group among children and adolescents in the United States. Estimates are weighted to account for differential probabilities of selection and differential nonresponse. A, Trends in concurrent medication use (2013–2014). B, Concurrent use by gender and age group (2013–2014). Boys ages 0 to 5 and 6 to 12 years report higher prevalence of concurrent medication use than girls ($P = .05$ and $P < .05$, respectively). [a] Prevalence of potential DDIs restricted to children who concurrently use ≥2 prescription medications; because of the small number of DDI cases, trend estimates are unstable.

unique prescription medications were used (Supplemental Table 6). Using Micromedex, a total of 826 potential DDIs (contraindicated [$n = 29$] or potentially major [$n = 797$]) for these 156 medications were identified. In the pooled 2003–2014 sample, 101 unique combinations of these potential DDIs were used by 158 people (Supplemental Table 7). In the pooled 2009–2014 sample, 63 unique DDI combinations were used

TABLE 3 Most Common Prescription Medication Combinations Used Among Children and Adolescents Concurrently Using Prescription Medications Overall and by Gender and Age Group in the United States (2009–2014)

	Prevalence of Combinations, No. (%) [95% CI]						
	Overall, *n* = 993	0–5 y		6–12 y		13–19 y	
		Girls (*n* = 112)	Boys (*n* = 159)	Girls (*n* = 156)	Boys (*n* = 244)	Girls (*n* = 170)	Boys (*n* = 152)
Bronchodilators–respiratory inhalants	123 (10.5) [7.9–13.7]	12 (10.1) [5.1–19.3][a]	21 (21.4) [13.0–33.1]*	24 (13.8) [6.6–26.6][a]	38 (12.0) [8.1–17.4]	11 (3.5) [1.7–7.0][a]	17 (9.2) [5.1–15.9]**
Bronchodilators–leukotriene modifiers	99 (8.2) [6.3–10.4]	6 (4.6) [2.1–10.0][a]	12 (6.6) [3.0–13.8][a]	20 (8.9) [5.6–13.8]	36 (12.7) [8.4–18.6]	11 (6.6) [2.6–15.8][a]	14 (6.7) [3.5–12.6][a]
Bronchodilators–adrenal cortical steroids	96 (6.9) [5.1–9.2]	24 (15.8) [10.1–23.9]	29 (13.8) [8.0–22.7]	14 (10.4) [4.6–21.8][a]	18 (4.4) [2.7–7.2]**	6 (3.1) [1.1–8.3][a]	5 (5.1) [2.0–12.1][a]
CNS stimulants–α adrenergic blockers	54 (5.4) [3.7–7.7]	0 (0.0)	1 (0.4) [0.1–3.4][a]	8 (4.1) [1.8–9.2][a]	33 (13.8) [8.5–21.5]***	0 (0.0)	12 (8.7) [4.5–16.1][a]***
Bronchodilators–nasal preparations	56 (5.3) [3.6–7.7]	1 (0.9) [0.1–6.5][a]	7 (4.6) [1.8–11.5][a]*	9 (3.5) [1.6–7.6][a]	25 (9.1) [5.4–14.9]*	5 (3.2) [0.7–13.0][a]	9 (6.7) [3.2–13.7][a]
Leukotriene modifiers–nasal preparations	47 (4.9) [3.2–7.3]	0 (0.0)	4 (2.4) [0.9–6.3][a]	11 (6.8) [3.1–14.3][a]	21 (8.9) [5.5–14.1]	2 (2.5) [0.4–14.0][a]	9 (5.2) [2.3–11.6][a]
Bronchodilators–antihistamines	50 (3.7) [2.6–5.1]	5 (4.2) [1.6–10.6][a]	4 (2.0) [0.7–6.0][a]	12 (6.1) [3.6–10.1]	18 (5.7) [3.2–9.9]	5 (2.3) [0.8–6.3][a]	6 (2.5) [1.2–5.0][a]
CNS stimulants–atypical antipsychotics	38 (3.5) [2.3–5.2]	0 (0.0)	0 (0.0)	5 (4.8) [2.1–10.4][a]	24 (8.2) [4.8–13.9]	2 (0.6) [0.1–2.5][a]	7 (4.5) [1.8–10.6][a]***
Nasal preparations–antihistamines	35 (3.4) [2.3–5.1]	0 (0.0)	0 (0.0)	6 (3.0) [1.5–5.7][a]	20 (8.2) [4.8–13.6]	2 (0.6) [0.1–2.8][a]	7 (5.3) [2.5–11.0][a]***
CNS stimulants–antidepressants	33 (3.4) [2.1–5.6]	0 (0.0)	0 (0.0)	7 (3.6) [1.4–8.5][a]	13 (5.4) [2.8–10.1][a]	6 (2.5) [0.7–8.3][a]	7 (5.6) [2.0–14.8][a]
Leukotriene modifiers–respiratory inhalants	42 (3.0) [2.1–4.4]	3 (2.1) [0.6–6.8][a]	5 (2.7) [1.1–6.5][a]	6 (2.4) [0.9–6.2][a]	19 (6.9) [3.8–12.5]**	4 (1.0) [0.3–2.8][a]	5 (2.3) [0.9–5.5][a]
Atypical antipsychotic–α adrenergic blockers	23 (2.6) [1.7–4.1]	0 (0.0)	0 (0.0)	2 (2.3) [0.5–10.8][a]	15 (7.2) [4.0–12.5]	1 (0.7) [0.1–4.7][a]	5 (2.9) [1.0–8.0][a]
CNS stimulants–antihistamines	24 (2.5) [1.3–4.6][a]	0 (0.0)	0 (0.0)	7 (4.2) [1.7–10.2][a]	13 (7.5) [3.5–15.4][a]	2 (0.4) [0.1–3.1][a]	2 (0.6) [0.1–2.9][a]
Bronchodilators–penicillins	38 (2.4) [1.6–3.5]	16 (12.0) [7.2–19.4]	12 (6.0) [3.2–10.9][a]*	5 (1.9) [0.7–5.6][a]	3 (0.7) [0.2–2.2][a]	1 (1.3) [0.2–9.1][a]	1 (0.4) [0.1–3.2][a]
Bronchodilators–CNS stimulants	26 (2.3) [1.4–3.9]	0 (0.0)	0 (0.0)	8 (4.2) [1.7–9.8][a]	12 (3.2) [1.9–5.4]	1 (1.9) [0.3–12.9][a]	5 (2.8) [0.8–8.9][a]
Antidepressants–anticonvulsants	11 (1.8) [0.8–4.4][a]	0 (0.0)	0 (0.0)	2 (3.0) [0.7–12.3][a]	2 (0.5) [0.1–2.1][a]*	5 (3.5) [0.8–14.0][a]	2 (2.1) [0.4–9.6][a]
Respiratory inhalants–adrenal cortical steroids	14 (1.7) [0.8–3.6][a]	2 (1.7) [0.4–7.1][a]	2 (1.7) [0.4–7.1][a]	4 (4.6) [1.0–18.7][a]	3 (0.9) [0.3–2.9][a]*	2 (0.9) [0.2–4.1][a]	1 (1.6) [0.2–11.0][a]
CNS stimulants–penicillins	22 (1.6) [0.9–3.0]	6 (4.9) [1.8–12.4][a]	5 (2.3) [0.9–5.8][a]	4 (1.9) [0.7–5.1][a]	3 (1.3) [0.4–4.0][a]	3 (1.7) [0.4–7.4][a]	1 (0.4) [0.0–2.9][a]
Narcotic analgesics–NSAIDs	13 (1.6) [0.7–3.6][a]	0 (0.0)	1 (0.2) [0.0–1.7][a]	2 (1.0) [0.2–4.4][a]	0 (0.0)	5 (2.4) [0.7–7.7][a]	5 (4.2) [1.3–13.1][a]
Contraceptives–antidepressants[b]	6 (2.9) [1.2–7.0][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (5.3) [2.2–12.0][a]	0 (0.0)
Adrenal cortical steroids–leukotriene modifiers	22 (1.3) [0.8–2.2]	3 (1.9) [0.6–5.8][a]	7 (3.9) [1.5–9.6][a]	3 (1.1) [0.3–3.4][a]	5 (1.3) [0.4–3.5][a]	3 (0.9) [0.3–3.1][a]	1 (0.3) [0.0–2.5][a]
Bronchodilators–antidepressants	8 (1.3) [0.5–3.1][a]	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.6) [0.2–2.1][a]	4 (4.0) [1.3–11.2][a]	1 (0.6) [0.1–4.7][a]*
Narcotic analgesics–penicillins	11 (1.2) [0.6–2.5][a]	0 (0.0)	0 (0.0)	1 (0.4) [0.0–2.6][a]	2 (0.7) [0.2–3.0][a]	6 (3.3) [1.2–8.8][a]	2 (0.8) [0.2–3.2][a]*
Adrenal cortical steroids–penicillins	12 (1.1) [0.5–2.3][a]	2 (1.0) [0.2–4.0][a]	5 (3.6) [1.4–9.4][a]	1 (0.3) [0.0–2.1][a]	2 (0.7) [0.2–2.8][a]	2 (1.6) [0.3–8.3][a]	0 (0.0)
Bronchodilators–NSAIDs	13 (1.1) [0.6–1.9]	1 (1.4) [0.2–9.3][a]	5 (3.0) [0.9–9.5][a]	1 (0.6) [0.1–4.8][a]	0 (0.0)	3 (1.3) [0.4–4.7][a]	3 (1.0) [0.3–3.3][a]
Penicillins–macrolides	5 (1.0) [0.2–4.2][a]	2 (6.0) [0.9–30.5][a]	1 (2.7) [0.4–16.7][a]***	1 (1.1) [0.1–8.3][a]	0 (0.0)	1 (0.5) [0.1–3.2][a]	0 (0.0)
Antidepressants–PPIs	5 (1.0) [0.3–3.1][a]	0 (0.0)	0 (0.0)	1 (0.7) [0.1–5.0][a]	1 (0.2) [0.0–1.5][a]	3 (3.3) [0.9–11.4][a]	0 (0.0)
Contraceptives–dermatological agents[b]	3 (2.1) [0.6–7.0][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.7) [1.1–12.0][a]	0 (0.0)
Penicillins–nasal preparations	6 (0.8) [0.3–2.4][a]	1 (5.6) [0.7–31.8][a]	0 (0.0)	3 (2.0) [0.4–8.5][a]	2 (0.8) [0.2–3.2][a]	0 (0.0)	0 (0.0)
Macrolides–nasal preparations	5 (0.6) [0.2–2.3][a]	1 (5.6) [0.7–31.8][a]	1 (0.3) [0.0–2.4][a]**	0 (0.0)	2 (0.5) [0.1–2.0][a]	1 (0.4) [0.0–2.8][a]	0 (0.0)
Bronchodilators–cephalosporins	6 (0.6) [0.2–1.5][a]	1 (1.0) [0.1–7.6][a]	4 (3.9) [1.2–11.8][a]	0 (0.0)	1 (0.2) [0.0–1.6][a]	0 (0.0)	0 (0.0)
Penicillins–cephalosporins	4 (0.5) [0.2–1.5][a]	2 (3.6) [0.6–18.5][a]	2 (2.1) [0.5–8.0][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Estimates are restricted to children who report the use of ≥2 prescription medications.

[a] Estimates are unreliable; relative SE >30%.

[b] Estimates are restricted to girls.

*** $P < .01$; ** $P < .05$; * $P < .10$ by using χ^2 tests to determine differences between girls and boys.

by 78 people, making the overall rate 0.7% (CI, 0.5%–1.0%). We found only 1 individual using a contraindicated DDI (aripiprazole–metoclopramide).

Among concurrent users of prescription medications, 8.2% [CI, 6.0%–11.1%] were at risk for potential major DDIs (Table 4). Nearly half of interacting regimens used involved psychotropic agents, primarily antidepressants, the most common adverse interaction effect being QT prolongation. The majority (68.0% [CI, 55.9%–78.0%]) of these interacting regimens involved the use of at least 1 acute medication.

The use of combinations with potential major DDIs was higher among adolescent girls (18.1% [CI, 11.0%–28.4%]) than among boys (6.6% [CI, 3.6%–11.9%], $P < .05$). This difference was largely due to the disproportionate use of tricyclic antidepressants in interacting combinations that involved acute medications, specifically nonsteroidal anti-inflammatory drugs (NSAIDs) (most commonly diclofenac–amitriptyline), antiemetics (ondansetron–nortriptyline), and albuterol (albuterol–amitriptyline) by adolescent girls. Atypical antipsychotics accounted for the vast majority of interacting drug combinations in boys ages 6 to 12, whereas antidepressants accounted for the majority in adolescent boys.

Whereas overall prevalence of potential major DDIs among concurrent users of prescription medications is similar to that reported during 2003–2008 (8.7% [95% CI, 6.5%–11.5%]), there was a significant increase among adolescent girls (11.8% [CI, 6.9%–19.7%] during 2003–2008 vs 18.1% [CI, 11.0%–28.4%] during 2009–2014; $P < .05$; Supplemental Table 8).

DISCUSSION

We used directly observed medication data from nationally representative samples to examine use and concurrent use of prescription medications among children and adolescents in the United States. During 2013–2014, one-fifth of children and adolescents used at least 1 prescription medication and ~1 in 10 concurrently used ≥2 prescription medications. Among children and adolescents concurrently using ≥2 prescription medications, 1 in 12 was at risk for a major DDI. Our findings are important because prescription medications are commonly used by children and adolescents in the United States, and population-level monitoring is needed to detect modifiable risk and optimize safe use.

Since 2003–2004, we observed a decline in the use of acute medications, corroborating findings from a previous analysis of pharmacy claims.[22] This decline was mainly attributable to lower rates of antibiotic use, which was also observed in previous research. We also found, however, that acute medications were commonly used in potentially life-threatening drug combinations, particularly among adolescent girls. More than three-quarters of potential major DDIs we identified involved prescription medications used acutely (eg, macrolide antibiotics, antiemetics, and albuterol), and nearly half of these interacting combinations also involved an antidepressant. The potential for QT prolongation is especially noteworthy given that it is often asymptomatic and unpredictable yet can develop quickly into a serious arrhythmia or sudden cardiac death,[23,24] a serious yet underreported problem in children and adolescents.[25,26] Instability in insurance coverage for children and fragmentation of health care (eg, use of retail clinics and emergency departments for episodic care) may increase the risk that a physician prescribing medication for an acute illness is unaware of a child's chronic medication regimen. Systemic strategies, including patient and provider access to accurate medication use data, are needed to reduce the risk of such major DDIs among children.

Highlighted in our findings is also an important opportunity to improve the safe use of medications among adolescent girls given that nearly 1 in 5 adolescent girls who concurrently used prescription medications was at risk for a DDI. The threefold higher prevalence of these interacting drug regimens among adolescent girls, when compared with adolescent boys, was largely due to a higher rate of tricyclic antidepressant use in combination with acute medications, most commonly macrolide antibiotics, antiemetics, NSAIDs, and proton pump inhibitors (PPIs). These acute medications may be prescribed to treat sexually transmitted infections (eg, azithromycin) or gastrointestinal symptoms (eg, ondansetron, omeprazole) from eating disorders.[27,28] Some are also available over-the-counter (OTC), specifically NSAIDs and PPIs. Product labeling for OTC medications does not always include comprehensive information on adverse effects, including DDIs. For example, in the labeling for OTC omeprazole, the DDI between citalopram and omeprazole is not mentioned.[29] Many adolescent girls and their parents may therefore not be aware of the cardiovascular risks associated with use.

We also found that prescription medications associated with an increased risk of suicidality are commonly used in children and adolescents and are often used together. For example, more than half of adolescent girls taking antidepressants concurrently use at least 2 additional psychotropic medications or hormonal contraceptives. Although there is some evidence that the combined use of these drugs may increase the onset and severity of suicidal thoughts and behavior,[19,30] we found no cases of DDIs associated with suicidality. This finding is expected because suicidality, in contrast to serotonin

TABLE 4 Potentially Major DDIs Among Children and Adolescents Concurrently Using Prescription Medications Overall and by Gender and Age Group in the United States (2009–2014)

	Prevalence of DDIs, No. (%) [95% CI]								
	Overall, n = 993	0–5 y		6–12 y		13–19 y		Adverse Interaction Effect
		Girls (n = 112)	Boys (n = 159)	Girls (n = 156)	Boys (n = 244)	Girls (n = 170)	Boys (n = 152)	
Any major DDI	78 (8.2) [6.0–11.1]	4 (3.0) [1.0–8.2][a]	6 (3.0) [1.0–8.6][a]	7 (3.0) [1.2–7.0][a]	19 (5.7) [3.3–9.6]	30 (18.1) [11.0–28.4]	12 (6.6) [3.6–30.2]	
Psychotherapeutic agents	48 (5.6) [3.6–8.6]	0 (0.0)	0 (0.0)	5 (2.2) [0.9–5.0][a]	14 (4.4) [2.3–8.2][a]	20 (12.5) [6.2–23.6][a]	9 (5.4) [2.5–37.0][a]*	
Antidepressants	33 (3.9) [2.5–6.1]	0 (0.0)	0 (0.0)	5 (2.2) [0.9–5.0][a]	7 (2.2) [1.0–5.2][a]	15 (9.1) [4.9–16.1]	6 (3.9) [1.5–46.5][a]	
SSRIs	21 (2.3) [1.4–3.9]	0 (0.0)	0 (0.0)	3 (1.5) [0.4–3.0][a]	2 (0.7) [0.2–2.8][a]	11 (4.9) [2.2–10.6][a]	5 (3.7) [1.3–49.4][a]*	
Citalopram–omeprazole[b]	1 (0.4) [0.1–3.2][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7) [0.2–11.5][a]	0 (0.0)	QT prolongation
Fluoxetine–trazodone	4 (0.4) [0.2–1.0][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4) [0.1–3.0][a]	2 (0.8) [0.2–3.2][a]	1 (0.5) [0.1–96.4][a]	QTprolongation and/or serotonin syndrome
Escitalopram–amphetamines	1 (0.3) [0.0–2.3][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6) [0.2–98.0][a]	Serotonin syndrome
Sertraline–amphetamines	3 (0.3) [0.1–0.9][a]	0 (0.0)	0 (0.0)	1 (0.4) [0.1–1.0][a]	0 (0.0)	1 (0.4) [0.1–2.7][a]	1 (0.7) [0.1–100.0][a]	Serotonin syndrome
Escitalopram–hydroxyzine[b]	2 (0.2) [0.1–1.1][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3) [0.0–2.0][a]	1 (0.7) [0.1–4.7][a]	0 (0.0)	QT prolongation
Sertraline–trazodone	2 (0.1) [0.0–0.5][a]	0 (0.0)	0 (0.0)	2 (0.8) [0.2–2.0][a]	0 (0.0)*	0 (0.0)	0 (0.0)	Serotonin syndrome
Fluoxetine–amphetamines	1 (0.1) [0.0–0.7][a]	0 (0.0)	0 (0.0)	1 (0.7) [0.1–1.0][a]	0 (0.0)	0 (0.0)	0 (0.0)	Serotonin syndrome
Tricyclics	8 (1.4) [0.6–3.3][a]	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.0) [0.2–4.3][a]	5 (4.5) [1.6–11.8][a]	0 (0.0)*	
Amitriptyline–albuterol[b]	1 (0.3) [0.0–2.5][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3) [0.2–9.1][a]	0 (0.0)	Cardiovascular effects
Nortriptyline–ondansetron[b]	1 (0.3) [0.0–1.9][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0) [0.1–6.9][a]	0 (0.0)	QT prolongation
Imipramine–quetiapine	1 (0.2) [0.0–1.1][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7) [0.1–5.1][a]	0 (0.0)	0 (0.0)	QT prolongation
Atypical antipsychotics	21 (1.8) [1.1–3.1]	0 (0.0)	0 (0.0)	0 (0.0)	9 (3.3) [1.4–7.4][a]*	8 (2.8) [1.3–5.9][a]	4 (1.9) [0.7–49.2][a]	
Quetiapine–aripiprazole	3 (0.3) [0.1–1.3][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8) [0.2–3.4][a]	1 (0.4) [0.1–98.0][a]	QT prolongation
Risperidone–lithium	3 (0.3) [0.1–0.9][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3) [0.0–2.3][a]	0 (0.0)	2 (1.1) [0.3–69.8][a]	Extrapyramidal symptoms
Quetiapine–trazodone	3 (0.2) [0.1–0.8][a]	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5) [0.1–2.2][a]	1 (0.5) [0.1–3.5][a]	0 (0.0)	QT prolongation
Quetiapine–risperidone	2 (0.2) [0.0–0.7][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5) [0.1–3.2][a]	1 (0.3) [0.0–2.1][a]	0 (0.0)	QT prolongation
Risperidone–hydroxyzine[b]	2 (0.1) [0.0–0.7][a]	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.4) [0.1–3.1][a]	0 (0.0)	0 (0.0)	QT prolongation
CNS stimulants	14 (1.7) [0.8–3.5][a]	0 (0.0)	0 (0.0)	4 (1.8) [0.6–4.0][a]	3 (1.0) [0.3–3.2][a]	4 (2.8) [0.7–10.8][a]	3 (2.5) [0.6–67.9][a]	
Atomoxetine–albuterol[b]	2 (0.6) [0.1–3.2][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5) [0.1–3.2][a]	1 (1.9) [0.3–12.9][a]	0 (0.0)	Cardiovascular effects
Amphetamines–trazodone	4 (0.2) [0.1–0.6][a]	0 (0.0)	0 (0.0)	2 (0.7) [0.2–2.0][a]	2 (0.5) [0.1–2.2][a]	0 (0.0)	0 (0.0)	Serotonin syndrome
Methylphenidate–bupropion	3 (0.2) [0.0–0.5][a]	0 (0.0)	0 (0.0)	1 (0.4) [0.1–1.0][a]	0 (0.0)	1 (0.2) [0.0–1.5][a]	1 (0.2) [0.0–99.2][a]	Seizures
Analgesics	11 (1.4) [0.5–3.4][a]	0 (0.0)	2 (0.6) [0.1–2.4][a]	0 (0.0)	1 (0.2) [0.0–1.3][a]	8 (4.9) [1.8–12.6][a]	0 (0.0)*	
Narcotics	7 (0.8) [0.3–1.9][a]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2) [0.0–1.3][a]	6 (3.0) [1.2–7.2][a]	0 (0.0)*	
Hydrocodone[b]–oxycodone[b]	2 (0.4) [0.1–1.7][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5) [0.4–6.5][a]	0 (0.0)	CNS depression
Hydrocodone[b]–cyclobenzaprine[b]	2 (0.2) [0.1–0.9][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.9) [0.2–3.5][a]	0 (0.0)	CNS depression

TABLE 4 Continued

	Prevalence of DDIs, No. (%) [95% CI]								
	Overall, $n = 993$	0–5 y		6–12 y		13–19 y		Adverse Interaction Effect
		Girls ($n = 112$)	Boys ($n = 159$)	Girls ($n = 156$)	Boys ($n = 244$)	Girls ($n = 170$)	Boys ($n = 152$)	
NSAIDs	4 (0.5) [0.1–2.5][a]	0 (0.0)	2 (0.6) [0.1–2.4][a]	0 (0.0)	0 (0.0)	2 (1.9) [0.3–9.6][a]	0 (0.0)	
Naproxen[b]–amitriptyline	1 (0.1) [0.0–0.6][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3) [0.0–2.3][a]	0 (0.0)	Bleeding
Antiemetic or antibiotics	14 (1.6) [0.9–2.8]	0 (0.0)	1 (1.1) [0.2–7.9][a]	1 (0.4) [0.1–1.0][a]	2 (0.5) [0.1–2.0][a]	7 (4.0) [1.7–9.4][a]	3 (1.2) [0.4–58.8][a]	
Promethazine[b]–azithromycin[b]	3 (0.3) [0.1–1.1][a]	0 (0.0)	1 (1.1) [0.2–7.9][a]	1 (0.4) [0.1–1.0][a]	0 (0.0)	0 (0.0)	1 (0.3) [0.0–99.4][a]	QT prolongation
Ondansetron[b]–erythromycin	2 (0.2) [0.0–0.7][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6) [0.1–2.6][a]	0 (0.0)	QT prolongation
Ondansetron[b]–clarithromycin	1 (0.1) [0.0–0.9][a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5) [0.1–3.2][a]	0 (0.0)	QT prolongation

Estimates are weighted to account for differential probabilities of selection and differential nonresponse. Micromedex was used to determine potential contraindicated and major DDIs among all medications used by children or adolescents who report using ≥2 medications during 2013–2014 (with Supplemental Table 6, we provide a list of these medications, and with Supplemental Table 7 , we provide a list of all DDIs found). Aripiprazole–metoclopramide is considered a contraindicated DDI; all other DDIs were considered potentially major DDIs. DDI, contraindicated or major DDI; SSRI, selective serotonin reuptake inhibitor.

[a] Estimates are unreliable; relative SE >30%.

[b] Acute medications (used ≤30 days).

** $P < .05$; * $P < .10$ by using χ^2 tests to determine differences between girls and boys.

syndrome and QT prolongation, is not captured as a potential DDI in existing drug interaction software,[31] including Micromedex. Therefore, health care professionals, including psychiatrists, may not be fully aware of the suicidal risks associated with the concurrent use of prescription medications in the evaluation and treatment of depressive symptoms in younger patients.

With these findings, it is suggested that preventive efforts used to improve the safe use of medications by children should promote public and health professional awareness of the increased risks associated with concurrent use of prescription and OTC medications, particularly suicidality and QT prolongation. Such efforts may include incorporation of a list of commonly used medications and interacting combinations associated with increases in QT prolongation and suicidal risks in treatment guidelines and screening tools for depression. Efforts should be used to target both consumers and health professionals, including those involved in acute or episodic care. Such efforts are particularly important among adolescents considering suicide and cardiovascular events, as well as unintentional drug overdoses, are persistently leading causes of death.[16,32] Nonmedical use of prescription drugs, including opioids, is also more prevalent in adolescents than in younger children[33] and is known to increase risk for both suicide and sudden death.

This study has several limitations. We examined the potential for DDIs rather than actual adverse drug events. Prescribers might have been aware of the risks and benefits of coprescribing the medications, established an appropriate monitoring plan, and determined in discussion with the patient and/or caregiver that the potentially interacting regimen may be the best option for the patient.

Second, our medication data were limited to prescription medications and were not used to capture information on OTC products. Therefore, we likely underestimate the rates of use, and potential DDIs, for several therapeutic classes (eg, antihistamines, PPIs, NSAIDs) that are widely available without a prescription. Moreover, OTC products, including acetaminophen and cough and cold medicines, are key contributors to adverse drug events in children.[34] Third, we used the Micromedex drug interaction software to identify potentially major DDIs in our sample, and other drug interaction software may yield different estimates. However, the accuracy, including sensitivity and specificity, comprehensiveness, and usefulness of Micromedex has been previously established.[35,36]For example, authors of a study in which the various software for DDIs were evaluated ranked Micromedex the highest in accuracy.[35] Fourth, the estimates for DDIs are for 30-day point prevalence. Therefore, it is possible that over a longer period of time, the concurrent use of potential DDIs is greater than our estimates.

In addition, the distinction between acute and chronic is not absolute; some medications initiated <30 days before data collection, yet intended for chronic use, may have been misclassified as acute. Last, for children <16 years old, a parent or guardian answered questions on their child's use of prescription medications. Therefore, underreporting is possible, particularly for prescription medications surreptitiously used by the child. Finally, NHANES may be underpowered to examine the prevalence of medication use, resulting in unreliable nationwide estimates.

CONCLUSIONS

Using nationally representative data, we found that many children and adolescents use and concurrently use prescription medications in the United States. Among concurrent users of prescription medications, nearly 1 in 12 was at risk for a major DDI. Largely because of their higher rate of acute medication use, adolescent girls were at a higher risk of using interacting drug regimens than other subgroups. Treatment and prevention efforts to reduce the burden of adverse drug events in younger populations should be used to consider the role of interacting drug combinations, especially among these individuals.

ABBREVIATIONS

CI: confidence interval
CNS: central nervous system
DDI: drug–drug interaction
NSAID: nonsteroidal anti-inflammatory drug
OTC: over-the-counter
PPI: proton pump inhibitor

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: Dr Qato serves as a paid consultant for Public Citizen's Health Research Group; Dr Lindau is the founder and co-owner of NowPow, LLC; and the other authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Dr Qato was supported in part by the Robert Wood Johnson Foundation as part of the Clinical Scholars Leadership program. Ms Guadamuz was supported in part by the Robert Wood Johnson Foundation as part of the Health Policy Research Scholar program. The views expressed here do not necessarily reflect the views of the Robert Wood Johnson Foundation.

POTENTIAL CONFLICT OF INTEREST: Dr Alexander is chair of the Food and Drug Administration's Peripheral and Central Nervous System Advisory Committee; serves as a paid advisor to IQVIA; serves on the advisory board of MesaRx Innovations; holds equity in Monument Analytics, a health care consultancy whose clients include the life sciences industry as well as plaintiffs in opioid litigation; and serves as a member of OptumRx's Pharmacy and Therapeutics Committee. This arrangement has been reviewed and approved by Johns Hopkins University in accordance with its conflict of interest policies; and the other authors have indicated they have no potential conflicts of interest to disclose.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2018-2023.

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Prescription Medication Use Among Children and Adolescents in the United States

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Pediatrics 2018;142;
DOI: 10.1542/peds.2018-1042 originally published online August 27, 2018;

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EXHIBIT 47

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP)

Chronic Diseases in America

CDC's National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP)

CHRONIC DISEASES IN AMERICA

6 IN 10
Adults in the US have a **chronic disease**

4 IN 10
Adults in the US have **two or more**

THE LEADING CAUSES OF DEATH AND DISABILITY
and Leading Drivers of the Nation's **$3.5 Trillion** in Annual Health Care Costs

THE KEY LIFESTYLE RISKS FOR CHRONIC DISEASE

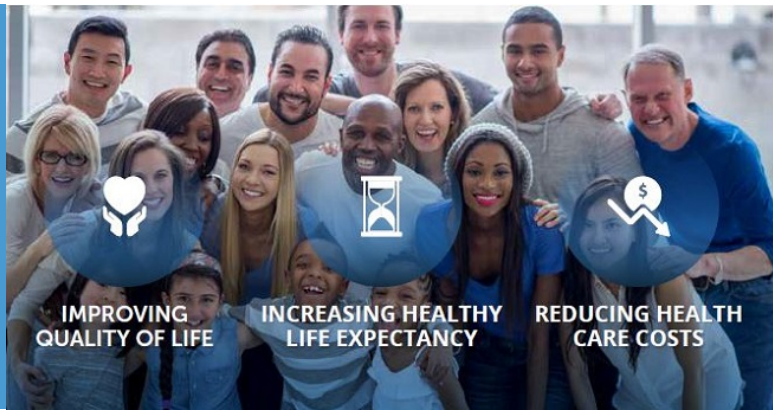
TOBACCO USE

POOR NUTRITION

LACK OF PHYSICAL ACTIVITY

EXCESSIVE ALCOHOL USE

NCCDPHP PREVENTS CHRONIC DISEASE AND PROMOTES HEALTH FOR PEOPLE OF ALL AGES



WE WORK TO IMPROVE HEALTH ACROSS THE LIFE SPAN

Where People Live, Learn, Work, and Play

Infants
Reduce the leading causes of infant death and illness.

Children and Adolescents
Help support healthy communities, child care programs, and schools so children can eat well, stay active, and avoid risky behaviors.

Adults
Help adults lead healthy and active lives and increase the use of preventive services like cancer screenings.

Older Adults
Promote quality of life and independence for people as they age.

WHAT WE DO

Find out how chronic diseases affect populations in the United States.

Study interventions to find out what works best to prevent and control chronic diseases.

Fund and guide states, territories, cities, and tribes to use interventions that work.

Share information to help all Americans understand the risk factors for chronic diseases and how to reduce them.

HOW WE DO IT

Measure
how many Americans have chronic diseases or chronic disease risk factors.

Improve
environments to make it easier for people to make healthy choices.

Strengthen
health care systems to deliver prevention services that keep people well and diagnose diseases early.

Connect
clinical services to community programs that help people prevent and manage their chronic diseases and conditions.

OUR IMPACT

Since 2012, the Tips From Former Smokers® campaign has motivated over 500,000 cigarette smokers to quit for good.

The percentage of adults meeting the national guideline for aerobic physical activity increased from 44% in 2008 to 54% in 2017.

The percentage of adults who have their high blood pressure under control increased from 43.3% in 2005-2006 to 48.5% in 2015-2016.

Teen birth rates fell 55% from 2007 to 2017 —an all-time low.

From 1999-2004 to 2011-2016, the percentage of low-income children with dental sealants increased 75%.

Over 297,000 people have participated in the National Diabetes Prevention Program lifestyle change program.

Since 1991, the National Breast and Cervical Cancer Early Detection Program has served more than 5.4 million women and found 65,879 cases of breast cancer and 207,727 precancerous cervical lesions.

From 2000 to 2014, 30% more schools offered at least 2 vegetables at lunch.

Chronic Diseases in America [PDF – 3 MB, HTML]

Page last reviewed: October 23, 2019, 12:00 AM
Content source: National Center for Chronic Disease Prevention and Health Promotion

EXHIBIT 48

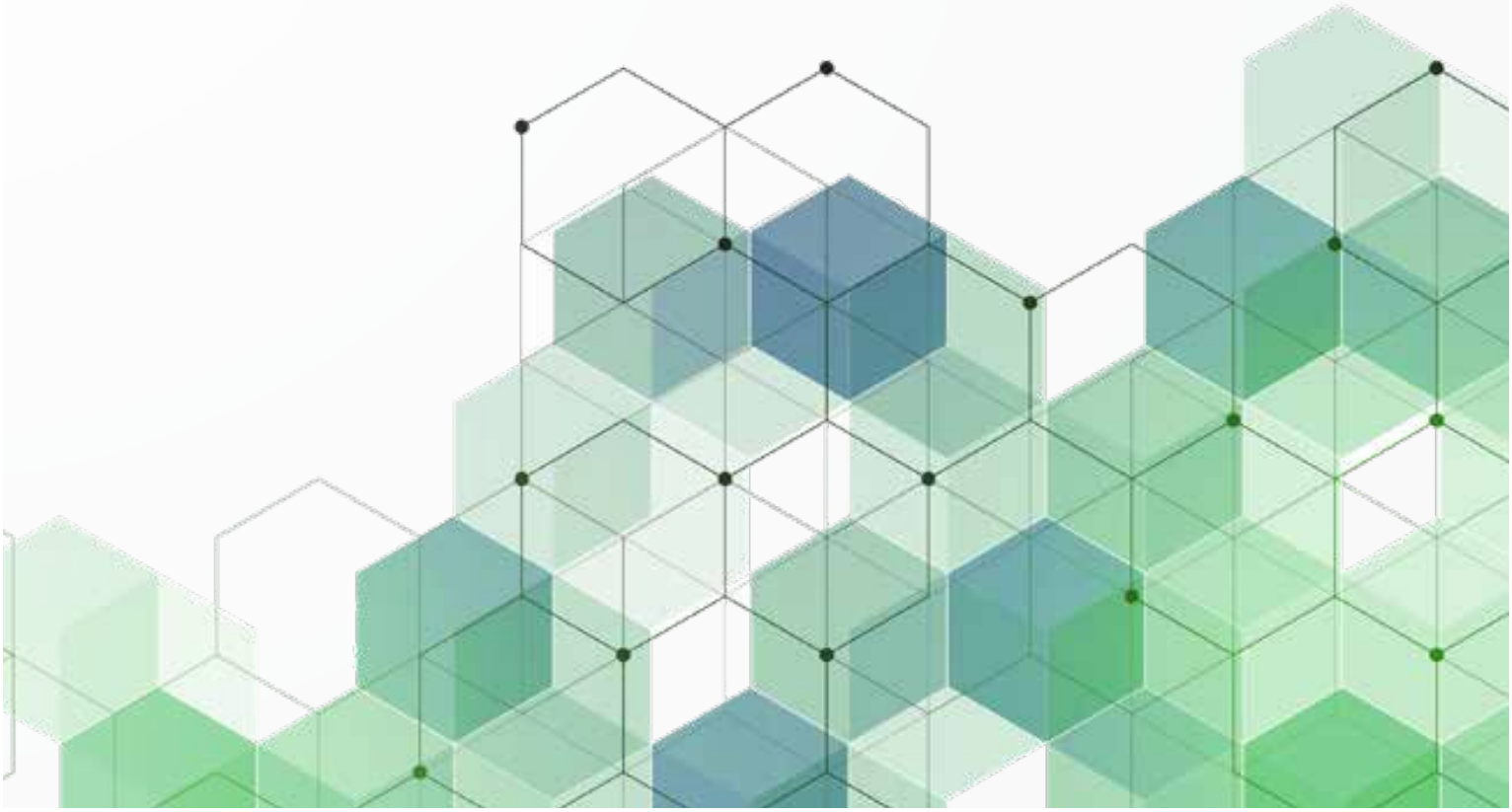
REDUCING THE BURDEN OF CHRONIC DISEASE

A Report of the Aspen Health Strategy Group

THE ASPEN INSTITUTE HEALTH STRATEGY GROUP

Foreword by Kathleen Sebelius and Tommy G. Thompson

Edited by Alan R. Weil and Rachel Dolan



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funding for this report provided by:

Robert Wood Johnson Foundation

Laurie M. Tisch ILLUMINATION FUND

The mission of the **Aspen Health Strategy Group** is to promote improvements in policy and practice by providing leadership on important and complex health issues. The group is comprised of 24 senior leaders across influential sectors including health, business, media, and technology, and is part of the Health, Medicine and Society Program at the Aspen Institute. Co-chaired by Kathleen Sebelius and Tommy G. Thompson, both former governors and former US Secretaries of Health and Human Services, the Aspen Health Strategy Group tackles one health issue annually through a year-long, in-depth study. This book is a collection of papers on the group's third subject: prevention of chronic disease. The papers address topics related to the financing, services and ethical issues related to chronic disease, and includes a final consensus report based on the group's work.

Aspen Health Strategy Group. (2019). *Reducing the Burden of Chronic Disease*. Washington DC: The Aspen Institute. http://aspeninstitute.org/AHSGreport2019

The Aspen Institute
2300 N Street, NW
Suite 700
Washington, DC 20037

Published in the United States of America in 2019 by The Aspen Institute

Printed in the United States of America
Wye Publication Number: 19/004

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Five Big Ideas to Reduce the Burden of Chronic Disease

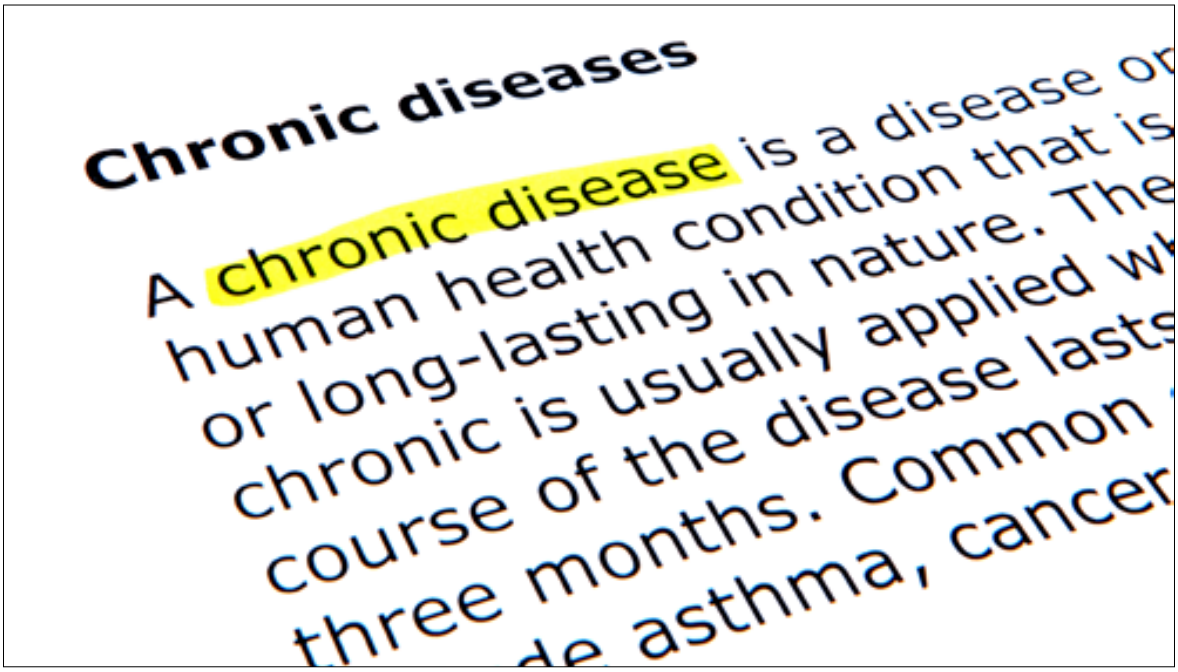
Introduction

Chronic diseases, generally defined as conditions that last one year or more and require ongoing medical attention or limit daily activities, are the leading causes of death and disability in the United States. Common chronic diseases include hypertension, heart disease, and diabetes. Most chronic diseases cannot be cured, but most can be managed in ways that reduce the daily burden of the disease and/or the likelihood it will progress to more serious symptoms.

Source: CDC, National Center for Chronic Disease Prevention and Health Promotion

More than half of adults ages 18 and older have at least one chronic condition; more than one-quarter have at least two. Eighty-six percent of the nation's $2.7 trillion annual health care expenditures in 2014 were on behalf of people with chronic diseases and mental health conditions. Seven of the top ten causes of death are associated with chronic diseases including heart disease, cancer, chronic respiratory disease, stroke, Alzheimer's, diabetes and chronic liver disease.

The Aspen Health Strategy Group selected prevention of chronic disease as its topic for discussion in 2018, its third year. This group of leaders in and outside health care spent three days considering the topic with the assistance of subject matter experts who prepared four background papers to frame the conversation. In our discussions the group quickly came to the conclusion that addressing chronic diseases means taking on obesity – because so many diseases are directly associated with that condition. The group emerged with five big ideas to tackle obesity in order to reduce the burden of chronic disease.



The Aspen Health Strategy Group's goal is to promote improvements in policy and practice by providing leadership on important and complex health issues. Co-chaired by Kathleen Sebelius and Tommy Thompson, both former governors and former US Secretaries of Health and Human Services, the group is composed of 24 senior leaders across sectors including health, business, media, and technology. (More information about the Aspen Health Strategy Group can be found at www.aspeninstitute.org/health.) This report captures the deliberations of the group, but no specific proposal or statement in the report should be considered to represent the opinion of any individual member of the group.

Background

"Several factors, most of which are outside the traditional health care system, affect chronic disease prevalence, morbidity and mortality rates. These determinants of health include environmental factors, socio-economic status, transportation, genetics, lifestyle and behavioral factors, social services and education," says Kenneth Thorpe in "Understanding and Preventing Chronic Disease."

The prevalence of chronic disease and the number of patients with multiple chronic conditions have increased markedly over the past two decades. Compared to 8% in 1995, 18% of adults were treated for five or more chronic diseases in 2015. The costs of treating chronic diseases are also high: As of 2016, chronic diseases accounted for more than $2 trillion in health care spending per year and about six out of every seven dollars spent on health care.

REDUCING THE BURDEN OF CHRONIC DISEASE

A Report of the Aspen Health Strategy Group

The mission of the Aspen Health Strategy Group is to promote improvements in policy and practice by providing leadership on important and complex health issues. The group is comprised of 24 senior leaders across influential sectors including health, business, media, and technology, and is part of the Health, Medicine and Society Program at the Aspen Institute. Co-chaired by Kathleen Sebelius and Tommy G. Thompson, both former governors and former US Secretaries of Health and Human Services, the Aspen Health Strategy Group tackles one health issue annually through a year-long, in-depth study. This book is a collection of papers on the group's third subject: prevention of chronic disease. The papers address topics related to the financing, services and ethical issues related to chronic disease, and includes a final consensus report based on the group's work.

THE ASPEN INSTITUTE HEALTH STRATEGY GROUP

THE ASPEN INSTITUTE
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funding for this report provided by:

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19/004

EXHIBIT 49

Centers for Disease Control and Prevention
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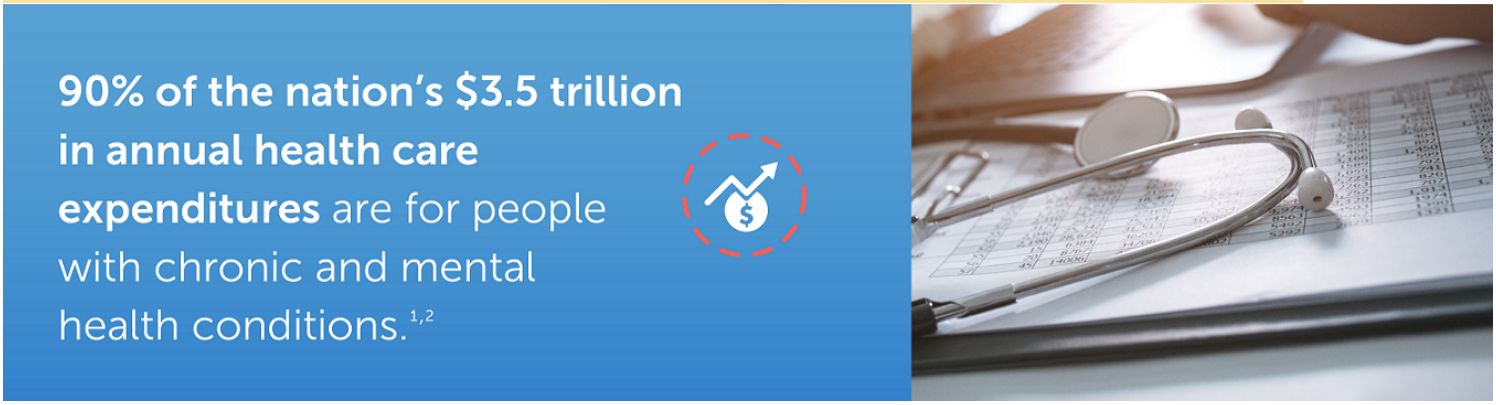
National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP)

Health and Economic Costs of Chronic Diseases



Chronic diseases have significant health and economic costs in the United States. Preventing chronic diseases, or managing symptoms when prevention is not possible, can reduce these costs.

Diseases

Heart Disease and Stroke

Nothing kills more Americans than heart disease and stroke. More than 859,000 Americans die of heart disease or stroke every year—that's one-third of all deaths. These diseases take an economic toll, as well, costing our health care system $199 billion per year and causing $131 billion in lost productivity on the job.[3]

Cancer

Each year in the United States, more than 1.6 million people are diagnosed with cancer, and almost 600,000 die from it, making it the second leading cause of death. The cost of cancer care continues to rise and is expected to reach almost $174 billion by 2020."[4]

Diabetes

More than 34.2 million Americans have diabetes, and another 88 million adults in the United States have a condition called prediabetes, which puts them at risk for type 2 diabetes. Diabetes can cause serious complications, including heart disease, kidney failure, and blindness. In 2017, the total estimated cost of diagnosed diabetes was $327 billion in medical costs and lost productivity.[5]

Obesity

Obesity affects almost 1 in 5 children and 1 in 3 adults, putting people at risk for chronic diseases such as diabetes, heart disease, and some cancers. Over a quarter of all Americans 17 to 24 years are too heavy to join the military. Obesity costs the US health care system $147 billion a year.[6]

Arthritis

Arthritis affects 54.4 million adults in the United States, which is about 1 in 4 adults. It is a leading cause of work disability in the United States, one of the most common chronic conditions, and a common cause of chronic pain. The total cost attributable to arthritis and related conditions was about $304 billion in 2013. Of this amount, nearly $140 billion was for medical costs and $164 billion was for indirect costs associated with lost earnings.[7]

Alzheimer's Disease

Alzheimer's disease, a type of dementia, is an irreversible, progressive brain disease that affects about 5.7 million Americans. It is the sixth leading cause of death among all adults and the fifth leading cause for those aged 65 or older. In 2010 , the costs of treating Alzheimer's disease were estimated to fall between $159 billion and $215 billion.[8] By 2040, these costs are projected to jump to between $379 billion and $500 billion annually.

Epilepsy

In the United States, about 3 million adults and 470,000 children and teens younger than 18 have active epilepsy—meaning that they have been diagnosed by a doctor, had a recent seizure, or both. Adults with epilepsy report worse mental health, more cognitive impairment, and barriers in social participation compared to adults without epilepsy. Average direct health care costs for a person with epilepsy range from $10,200 to $47,900 per year (in 2013 dollars).[9]

Tooth Decay

Cavities (also called tooth decay) are one of the most common chronic diseases in the United States. One in five children aged 6 to 11 years and one in four adults have untreated cavities. Untreated cavities can cause pain and infections that may lead to problems eating, speaking and learning. On average, 34 million school hours are lost each year because of unplanned (emergency) dental care, and over $45 billion is lost in productivity due to dental disease.[10,11]

Risk Factors

Cigarette Smoking

Cigarette smoking is the leading cause of preventable death and disease in the United States. More than 16 million Americans have at least one disease caused by smoking. This amounts to $170 billion in direct medical costs that could be saved every year if we could prevent youth from starting to smoke and help every person who smokes quit.[12]

Lack of Physical Activity

Not getting enough physical activity comes with high health and financial costs. It can lead to heart disease, type 2 diabetes, some cancers, and obesity. In addition, lack of physical activity costs the nation $117 billion annually for related health care.[13]

Excessive Alcohol Use

Excessive alcohol use is responsible for 88,000 deaths in the United States each year, including 1 in 10 deaths among working-age adults. [14,15] In 2010, excessive alcohol use cost the US economy $249 billion, or $2.05 a drink, and $2 of every $5 of these costs were paid by the public. Binge drinking is responsible for over half the deaths and three-quarters of the costs due to excessive alcohol use.[16]

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Page last reviewed: March 23, 2020, 12:00 AM
Content source: National Center for Chronic Disease Prevention and Health Promotion

EXHIBIT 50

Published in final edited form as:
Annu Rev Public Health. 2019 April 01; 40: 127–146. doi:10.1146/annurev-publhealth-040218-044008.

Interventions to Support Behavioral Self-management of Chronic Diseases

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Abstract

A majority of the U.S. adult population has one or more chronic conditions that require medical intervention and long-term self-management. Such conditions are among the 10 leading causes of mortality; an estimated 86 % of the nation's $2.7 trillion in annual health care expenditures goes toward their treatment and management. Patient self-management of chronic diseases is increasingly essential to improve health behaviors, health outcomes, and quality of life and, in some cases, has demonstrated effectiveness for reducing health care utilization and the societal cost burden of chronic conditions. This review synthesizes the current state of the science of chronic disease self-management interventions and the evidence of their effectiveness, especially when applied with a systematic application of theories or models that account for a wide range of influences on behavior. Our analysis of selected outcomes from randomized controlled trials of chronic disease self-management interventions contained in 10 Cochrane Systematic Reviews provides additional evidence demonstrating that self-management can improve quality of life and reduce utilization across several conditions.

Keywords

Chronic diseases; disease management; disease self-management; health behavior; health care utilization; health outcomes

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

INTRODUCTION

Chronic diseases[1] impose an enormous and growing burden on individuals, families, and society, as well as on health care systems in the United States and globally (37, 44, 58). They account for most deaths and are major contributors to disability and health care costs (19, 20, 30, 56). Overall U.S. costs of chronic disease are projected to accumulate by 2030 to more than $42 trillion, with medical outlays and productivity losses costing $8,600 per person (98). Although innovation in biomedical research has produced clinical medical treatments that can slow progression and mitigate the impact of many chronic conditions, the management of these conditions increasingly involves partnering with patients to support efforts to undertake long-term adherence to a preventive or therapeutic regimen that can improve functional status and health outcomes (13, 14). This typically includes patients adopting and maintaining multiple lifestyle behavioral changes in dietary practices, exercise, and the use of prescribed medications, as well as managing complex communications with family and health care providers and systems. As a consequence, the development of intervention programs that can educate and assist people in adopting and maintaining long-term health behavior change, in their efforts to prevent further progression of disease and improve quality of life, is a continuing need (11, 99).

The traditional medical model, which historically has focused on managing a specific disease condition, as opposed to managing the patient, has proven to be both expensive and ineffective in the treatment of chronic diseases because many people have more than one chronic condition and competing life circumstances that impair patients' capacity to self-manage their conditions. The limitations of the medical model have resulted in a new and evolving chronic disease treatment paradigm that requires a patient-provider partnership involving collaborative care and education in chronic disease self-management to ensure the best possible health outcomes for the patient (2, 12, 50, 57, 80, 101). A significant proportion of the unnecessary health care utilization costs and poor health outcomes associated with the treatment of chronic diseases result, in large part, from the failure of patients to effectively self-manage their condition in response to recommended medical therapy (13). Thus, if the management of chronic diseases is to be advanced, health care providers and systems of care need to organize patient self-management into an integrated system of chronic illness care that can increase the capacity of patients by providing knowledge, resources, and skills necessary to perform the multiple tasks necessary to self-manage their conditions better (114). This approach requires building on and tailoring what is already known to be effective and disseminating evidence-based programs and practices beyond the clinical setting to enable and support people in the context of their homes and diverse communities.

This review is organized into several parts. First, we examine the concept, theories, and intervention methods that underlie chronic disease self-management programs. Second, we summarize selected chronic disease self-management programs that have been tested—and in some cases scaled beyond clinical settings to population-level use—in high-prevalence

[1]Arthritis, asthma, cancer, chronic obstructive pulmonary disease, heart disease, HIV/AIDS, hypertension, stroke, type 2 diabetes, and obesity, as well as mental illness and other conditions.

chronic diseases. Finally, we synthesize the evidence for effectiveness and report the results of our own meta-analysis of selected outcomes from randomized controlled trials (RCTs) of chronic disease self-management interventions contained in 10 Cochrane Systematic Reviews.

CHRONIC DISEASE SELF-MANAGEMENT: CONCEPT, THEORY, AND INTERVENTION METHODS

Research to develop and evaluate disease self-management programs dates back over 40 years. While early chronic disease-management programs were oriented largely with respect to the role of the health provider, initial efforts to develop disease self-management for patients were designed to provide disease-specific information and improve compliance with prescribed medication. Since then, chronic disease self-management has matured and evolved to support and enable patients to develop a broad range of behavioral skills and other capacities that the available evidence shows can be effective in helping people to navigate a variety of disease-management tasks across a range of chronic conditions.

Concept and Definition

Disease self-management has been variously defined (7) and is distinct from related concepts such as self-care (104), patient activation (55, 65), and patient-centered care (67, 92). Grady & Gough (50) have defined self-management "as the day-to-day management of chronic conditions by individuals over the course of an illness." (p. e26) According to Lorig & Holman (80), for the patient, self-management involves three separate but related sets of tasks: medical or behavioral management of the disease, role management, and emotional management. Bandura(6) has proposed that "[s]elf-management operates through a set of psychological sub functions. People have to learn to monitor their health behavior and the circumstances under which it occurs, and how to use proximal goals to motivate themselves and guide their behavior" (p. 151). Moreover, because managing a chronic condition constitutes a problem-based endeavor, six self-management skills—problem solving, decision making, resource utilization, the formation and long-term maintenance of a patient-provider partnership, action planning, and self-tailoring—are central to the successful self-management of chronic conditions (80). Healthy People 2020 (54) recommends that those with chronic conditions engage in disease self-management as a means by which to cope with problems and challenges.

Theories and Mechanisms of Self-Management

Two theoretical perspectives from psychology have dominated chronic disease self-management intervention: self-regulation theory and social cognitive theory.

Self-regulation theory.—In self-regulation theory, a person is motivated to self-regulate by a desired goal or behavioral endpoint. The power of the goal is associated with a value that the goal represents for the individual. The more salient the goal is, the more the more the person will engage in self-regulation behavior. The model also posits that engaging in any disease management action (e.g., changing a behavior such as taking medication, diet, or physical activity) will be influenced by both internal and external factors. The self-regulation

theory of disease management views individual self-regulation of health-related behavior as central to achieving the desired outcomes of treatment (27). One of the first theory-based models of chronic disease self-management was Clark & Starr-Schneidkraut's (29) use of self-regulation theory in asthma control.

Social cognitive theory.—Social cognitive theory (4) is a cornerstone of effective disease self-management interventions. According to Bandura (6), "This theory posits a multifaceted causal structure in which self-efficacy beliefs operate together with goals, outcome expectations, and perceived environmental impediments and facilitators in the regulation of human motivation, behavior, and well-being" (p.143). The construct of self-efficacy describes one's confidence or personal agency to exercise control and is believed to be the common pathway through which psychosocial factors influence health functioning (5). Thus, self-efficacy is a core belief that underlies each of the basic processes of personal behavioral change: the extent to which one considers changing health habits, whether one mobilizes the motivation and perseverance required to succeed, whether one has the ability to overcome temporary setbacks and relapses, and the extent to which one is able to maintain new behavior (6). Self-efficacy is a significant predictor of psychological well-being, adherence to prescribed treatments, and pain coping mechanisms in arthritis (3).

Intervention Methods

Three principal methods of intervention delivery characterize chronic disease self-management programs: small-group meetings, Internet-based and mHealth technologies, and printed materials.

Small-group meetings.—Peer-led, small-group meetings comprise the basic intervention method of chronic disease self-management programs and have been used successfully across a wide spectrum of chronic conditions (80). This format provides for face-to-face engagement between and among participants as well as for individual attention, and it facilitates peer interaction, discussion, and social support, as well as an economy of scale in the delivery of educational programs. Program attendance and participation tend to be high, and small-group meetings have been widely evaluated for feasibility, acceptability, and impact on health care outcomes; however, attendance rates and completion may suffer owing to the need for patients to attend scheduled group sessions.

Internet-based and mHealth technologies.—New information and communication technologies can reach large numbers of the population with disease self-management programs and permit standardizing and tailoring of health-related messages. In addition, the technology is mobile, offers privacy and anonymity, and usability can be made graphically engaging (95). Internet-based self-management has been implemented and evaluated in arthritis and fibromyalgia (83), osteoarthritis (111), and other chronic conditions (76). Mobile phone applications have been of recent interest; however, further research is needed to assess the acceptability, risks, and long-term cost-effectiveness (35).

Printed materials.—Not all patients are able or willing to participate in small-group meetings or Internet-based chronic disease self-management programs, and thus printed

materials that are distributed either through mail or in person present a feasible alternative intervention method. When mailed, such materials also offer some of the same advantages of delivery and use as do Internet-based formats and have demonstrated promise in improving health indicators while reducing physician visits among patients with arthritis and/or depression and among African Americans (74). Moreover, such materials provide reinforcement or clarification of valuable health information.

CHRONIC DISEASE SELF-MANAGEMENT PROGRAMS

Substantial evidence of effectiveness has accumulated for several disease self-management programs. Interventions have sought to influence a broad range of outcomes, including health behaviors, medication adherence, health status, disease progression, quality of life, utilization of health services, and health care costs. In the following section, we describe selected chronic disease self-management programs that have been evaluated and, in some cases, scaled and disseminated to population-level implementation.

Disease-Specific Programs

Chronic disease self-management has focused largely on four prevalent disease-specific conditions where adherence to recommended medical regimens and behavioral change are essential to improving health outcomes and quality of life: arthritis, asthma, cardiovascular disease, and diabetes.

Arthritis.—The Arthritis Self-Management Program (ASMP) was originally developed by Lorig (79), who is widely recognized as having codified and disseminated the first application of a disease-specific model of behavioral self-management in chronic disease to arthritis (51). Grounded in Bandura's Social Cognitive Theory (4) and focused on developing patients' self-efficacy, the original ASMP comprised a six-week interactive program, consisting of weekly two-hour sessions guided by two trained instructors, that was designed to assist people with arthritis in learning how to manage their condition (70). The ASMP covers topics and techniques to deal with problems associated with arthritis, appropriate exercise, appropriate use of medications, and effective communication with family, friends, and health care professionals. In addition, the program teaches pain management techniques, nutrition, and evaluation of new treatments (70, 91). The ASMP has been evaluated extensively and has demonstrated clinically significant outcomes showing that disease self-management in patients with arthritis yields sustained benefits while reducing health care costs (81). A 12-year review of RCTs of the ASMP concluded that the program improves behaviors, self-efficacy, and aspects of health status (81). In addition, it showed that the effects and long-term outcomes of the ASMP persist for as long as four years without formal reinforcement, with clinical improvement gains that produce cost savings.

Asthma.—Asthma self-management programs date back to the 1970s (25, 26, 28) and have been recommended by asthma guidelines for both pediatric and adult care. Asthma self-management programs for children and adolescents and their families have demonstrated effectiveness in improving lung function and self-control, while reducing school

absenteeism, number of days with restricted activity, number of emergency department visits, and number of disturbed nights (52). Self-management for asthma can reduce unscheduled care and improve asthma control, can be delivered effectively to diverse demographic and cultural groups, is applicable to a broad range of clinical settings, and does not significantly increase total health care costs (103). Two evidence-based adaptations have been scaled for use at the population level: the American Lung Association Open Airways For Schools® (1), a school-based curriculum that has been designed as an interactive education program for children to promote asthma self-management; and the National Heart, Lung, and Blood Institute Asthma Action Plan (93), which provides information on how to self-manage asthma on a daily basis. Dissemination of asthma self-management as an evidence-based practice also has been incorporated into the US National Asthma Education and Prevention Program's Guidelines for the Diagnosis and Management of Asthma (94) since 2007, as well as the Global Initiative for Asthma (45), and is one of the goals of the Merck Childhood Asthma Network (113).

Cardiovascular disease (CVD).—Coronary heart disease is the leading cause of death attributable to cardiovascular disease (CVD), followed by stroke, hypertension, and heart failure (9, 21). Nearly 80% of CVD deaths could be prevented through optimal management of risk factors, including smoking cessation and physical activity (117). Adults 20–39 years of age comprise the largest segment of the untreated adult population with poor to intermediate CVD risk profiles in the United States (106); thus, the American Heart Association (AHA) has adopted a life course approach to CVD risk factor-management, emphasizing both primary prevention of CVD risk factors beginning in childhood and secondary prevention including provider and patient self-management in people with established CVD. The AHA has endorsed patient self-management of CVD as an effective means by which to manage the condition and improve outcomes (8), including patients with coronary heart disease (100), hypertension (17), and heart failure (115).

Diabetes.—Numerous studies have demonstrated the impact of diabetes self-management on improving health status in people with type 2 diabetes. In a meta-analysis of 11 RCTs designed to evaluate the effectiveness of diabetes self-management education interventions delivered in conjunction with primary care among Hispanic adults with type 2 diabetes, Ferguson et al. (39) reported that primary care and self-management together were effective in improving glycemic control in Hispanic adults. In addition, two programs—the American Association of Diabetes Educators' (AADE) Diabetes Self-Management Education and Training (DSME/T) program and the Stanford Diabetes Self-Management Program—are considered evidence-based programs that have demonstrated effectiveness in helping patients with diabetes lower A1C and improve overall health status (73, 75, 77, 78). The Community Preventive Services Task Force recommends the use of diabetes self-management mobile phone applications, when implemented in health care systems, to improve blood glucose levels among patients with type 2 diabetes (31).

The Chronic Disease Self-Management Program (CDSMP)

The Chronic Disease Self-Management Program (CDSMP) is perhaps the most well-known program to assist people with a broad range of chronic conditions (82, 84, 85); (https://

www.selfmanagementresource.com/). This program was developed at Stanford University and is based on the original ASMP. The CDSMP is an effective self-management education program that teaches a range of skills useful for managing a variety of chronic conditions. The program has been endorsed by *Healthy People 2020* (54) as an evidence-based approached that helps people with chronic conditions learn how to manage and improve their own health, while reducing health care costs. The CDSMP focuses on problems that are frequently encountered by individuals with any chronic condition, including pain management, diet and nutrition, exercise, and medication use, as well as coping with emotions and communicating with health care providers and family members. The six-week program is led by a pair of trained facilitators who have learned to live with chronic disease themselves. The workshops are offered to 10–20 participants in a group setting, and cover 17 hours of material that focuses on imparting and building the skills that people with chronic disease need to manage their conditions, sharing experiences, and providing mutual peer support.

The CDSMP has produced significant measurable improvements in both health outcomes and quality of life. The CDSMP significantly improves exercise capacity, cognitive symptom management and communication with physicians, as well as measures of health status at one year (84); it also significantly lowers health distress and improves disease-specific self-efficacy at two years (82). In addition, the CDSMP has been shown to reduce health care expenditures and pay for itself within the first year. Cost savings include significantly fewer emergency room visits at one year (84), and significantly lower inpatient and outpatient visits, fewer hospitalizations, and lower health care costs at two years (82). One study (49) found that in patients with arthritis and multiple comorbid conditions, the CDSMP may be more cost-effective than the Arthritis Self-Help Course. The CDSMP has also demonstrated effectiveness across cultural groups and regions. A community-based Spanish-language version of the CDSMP—Tomando Control de su Salud—assists Latinos with managing chronic illness (https://www.selfmanagementresource.com/programs/small-group-spanish/tomando-control-de-su-salud), and a version of the CDSMP has been implemented in China across multiple chronic conditions and was found to improve health behavior, self-efficacy, and health status while reducing the number of hospitalizations six months after program participation (43). The CDSMP has been replicated in other diverse populations, both inside and outside the United States. It is estimated to have reached over 100,000 Americans (96), having been disseminated widely across regions, including rural areas (107, 108), and across community settings, including the workplace (109). The CDSMP also has been adapted for online use in the Better Choices, Better Health® program of the National Council on Aging (https://www.canaryhealth.com/bcbh-better-choices-better-health/).

WHAT IS THE EVIDENCE FOR EFFECTIVENESS OF SELF-MANAGEMENT INTERVENTIONS?

Evidence for the effectiveness of disease self-management has grown steadily in recent decades. The earliest evidence for effectiveness came from the Stanford Patient Education Research Center, which developed and evaluated several disease-specific programs in a

series of RCTs and follow-up longitudinal studies. These include the ASMP (72, 81), the Spanish ASMP (71), and the CDSMP (84, 85)

Systematic Reviews and Meta-Analyses

Numerous reviews have examined the conceptual and theoretical basis for self-management intervention (23, 24, 50). Previous systematic reviews and meta-analyses have examined medication adherence and self-management interventions (32, 53, 67, 105) and patient compliance with treatment across a range of conditions and outcomes. These have included self-management in arthritis (90), asthma (52, 102), chronic low back pain (38), cancer-related fatigue (10), chronic obstructive pulmonary disease (18, 88), diabetes (39), heart failure (22, 97), hypertension (33, 34), osteoarthritis (66), and other conditions (7, 89). Other reviews have sought to assess self-management programs in relation to behavior change theory (88), quality of life (40), impact of self-monitoring on health care utilization (86), new technologies (95), the effectiveness of lay leaders (41), methodological issues in evaluating self-management intervention programs (116), and the effectiveness of various characteristics of self-management programs (16, 61–63). Although some reviews have produced inconsistent findings, the bulk of reviews have found that disease self-management has the potential to produce modest but clinically significant improvements in patient self-efficacy, health behaviors, health status, and quality of life. In addition, reductions in unnecessary health care utilization, hospitalizations, and health care costs have also been reported.

The *Cochrane Database of Systematic Reviews* contains numerous reviews that have assessed the quality of evidence for the effectiveness of self-management interventions across a range of disease conditions (**see** Table 1). We assessed the evidence for effectiveness contained in 10 of 35 eligible reviews that focused on disease-specific self-management programs, or broader programs of disease management that included patient self-management. The range of outcomes of interest across studies of self-management interventions for multiple chronic conditions included health behaviors, health status, quality of life, and utilization of health care services. The quality of evidence for effectiveness ranged from low to moderate, and in several cases the evidence was insufficient or equivocal.

While systematic reviews and meta-analyses can illuminate the collective effectiveness of interventions, the results should be viewed with some caution. The studies contained in such reviews frequently suffer from a number of methodologic weaknesses. These include lack of (or inadequate) behavioral theory, failure to implement the interventions with fidelity to original design specifications, and short-term follow-up. Thus, effect sizes are modest and may not be necessarily be indicative of true intervention impact. In addition, most studies included in systematic reviews and meta-analyses comprise RCTs, which focus on internal validity rather than external validity. Pragmatic trials and use of evaluative frameworks that emphasize external validity can provide greater insight into the effectiveness of self-management interventions and their clinical value. For example, several evaluations using the RE-AIM (reach, effectiveness, adoption, implementation, maintenance) framework have

evaluated the reach and effectiveness of disease self-management programs in several areas (46–48, 59, 69, 112).

Quality of Life and Health Care Utilization: Evidence of Effectiveness

We conducted meta-analyses for reviews across the Airways Cochrane editorial group (Table 1) that compare a self-management intervention to a control for the primary outcomes of health-related quality of life (HRQoL) and number of all-cause hospitalization days. The systematic reviews we used are deemed to be of sufficiently high quality to be included.

Two statistical models were employed in our meta-analyses. The fixed-effects model assumes identical treatment effects in the studies (homogeneity of the true treatment effect) and the variances around each mean effect depend primarily on the size of each study (15). The random-effects model includes between-study differences in treatment effects in the calculation of the variances, leading to wider confidence intervals when a given level of heterogeneity in treatment effect is observed (36). We can also employ the meta-analysis methodology as a cumulative meta-analysis by updating the pooled estimate of the intervention effect each time the results of a new trial are published. In cumulative meta-analysis, the experiments are accumulated from the earliest to the latest, where each successive experiment includes a synthesis of all previous experiments. This chronological combining of the experiments will show if there is a consistency in the results of consecutive studies and indicate the point at which no further studies are necessary because the results continually favor one intervention.

Fixed effect meta-analyses for HRQoL are presented in Figures 1a,b. The meta-analysis uses the studies that include HRQoL from the systematic reviews across the Cochrane Airways editorial group that compare a self-management intervention to a control based on the St.George's Respiratory Questionnaire (SGRQ Total) (60) quality of life measure.[2] The meta-analyses of SGRQ Total revealed minimal heterogeneity ($I^2=0.0\%, p=0.789$). Systematic reviews with other measures of HRQoL, such as the Chronic Respiratory Disease Questionnaire, are meta-analyzed in Table 1, but we combined only the common measure of SGRQ Total in the Airways group to ensure homogeneity. Figure 1a shows a significant increase in the HRQoL for the self-management intervention as compared to usual care (the pooled 95% confidence interval (CI) for the mean difference in the SGRQ is [−5.182, −1.875; $p=0.000$]). The cumulative meta-analysis in Figure 1b reveals that this significant increase in the HRQoL for the self-management intervention appeared in the literature in 2009 and has remained stable in subsequent years.

Random effects meta-analyses for all-cause hospitalization days are presented in Figures 2a,b. This analysis uses systematic reviews across the Cochrane Airways editorial group that compare a self-management intervention to a control based on all-cause hospitalization days.[3] Systematic reviews with other measures of hospital utilization are provided in Table 1; however, we combined the common measure all-cause hospitalization days, rather than change from baseline or respiratory-related hospitalization days, or number of admissions, to

[2]See Related Resources for a listing of the studies included in the HRQoL analysis.
[3]See Related Resources for a listing of the studies included in the hospital days analysis.

ensure homogeneity. The meta-analyses of all-cause hospitalization days had significant heterogeneity (I^2=61%, p=0.009) so a random effects approach is used. From Figure 2a, there is a marginally significant decrease in all-cause hospitalization days for the self-management intervention as compared to usual care (the pooled 95% CI for the mean difference in the all-cause hospitalization days is −2.575, 0.201; p=0.094). The cumulative meta-analysis in Figure 2b reveals that this marginal significant reduction in the all-cause hospitalization days for the self-management intervention as compared with usual care appeared in the literature in 2010 and has remained stable in subsequent years. Individual systematic reviews from Table 1 found no statistically significant difference between self-management interventions and a control group.

CONCLUSION

Over the last 50 years, considerable progress has been made in chronic disease self-management. Much of the empirical research and reviews that have been conducted on the reach and effectiveness of interventions such as the CDSMP and other programs have demonstrated small to moderate effects for changes in health behaviors, health status, and health care utilization for selected chronic conditions, with estimates of their cost-benefit and their cost-effectiveness. Because published trials that have been included in most systematic reviews, to date, suffer from publication bias and a range of methodological limitations, future trials of self-management for chronic conditions would benefit from better descriptions of the intervention under study, common and standardized measures of outcome, and mixed-method designs. However, the current evidence for effectiveness suggests that chronic disease self-management is a mature science and can yield important benefits to patients, including improvements in quality of life and reductions in utilization of health care resources. Identifying the most effective methods by which self-management programs can be delivered and scaled for use at the population level should continue to be a priority.

ACKNOWLEDGEMENTS

The authors acknowledge the following awards and sources of partial support for this work: Dr. Allegrante, National Institutes of Health (NIH) (NHLBI R25HL116378); Dr. Wells, National Science Foundation (DMS 1611893) and the NIH (U19AI111143); Dr. Peterson, Paul B. Beeson Award from the National Institute on Aging, the American Federation for Aging Research, The John A. Hartford Foundation, and The Atlantic Philanthropies (K23AG042869). We gratefully acknowledge the helpful comments and edits of Lawrence W. Green and his support of this manuscript.

RELATED RESOURCES

Studies in the HRQoL Analysis

From Kelly et al.(64)

Greening NJ, Williams JEA, Hussain SF, Harvey-Dunstan TC, Bankart MJ, et al. 2014. An early rehabilitation intervention to enhance recovery during hospital admission for an exacerbation of chronic respiratory disease: randomised controlled trial. *BMJ (Clinical research ed.)*. 349:4315.

From Lenferink et al.(68)

Hernández C, Alonso A, Garcia-Aymerich J, Serra I, Marti D, et al. 2015. Effectiveness of community-based integrated care in frail COPD patients: a randomized controlled trial. *NPJ Prim Care Respir Med.* 25: 1–6.

From McCabe et al.(87)

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From Zwerink et al.(118)

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Studies in the Hospital Days Analysis

From Kelly et al. (64)

Greening NJ, Williams JEA, Hussain SF, Harvey-Dunstan TC, Bankart MJ, et al. 2014. An early rehabilitation intervention to enhance recovery during hospital admission for an exacerbation of chronic respiratory disease: randomised controlled trial. *BMJ (Clinical research ed.).* 349:4315.

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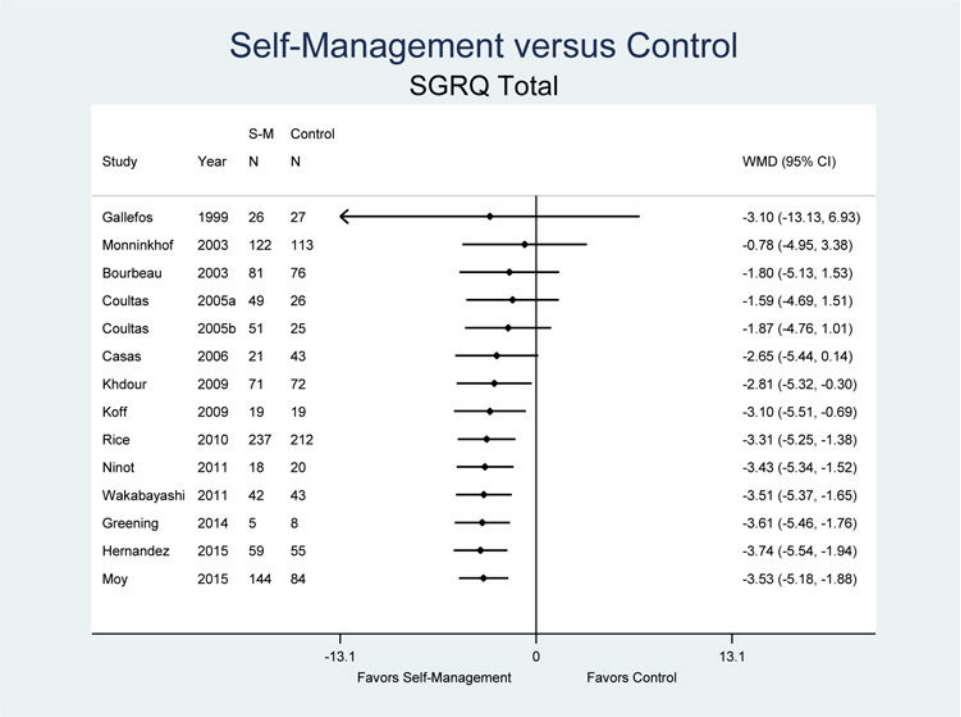
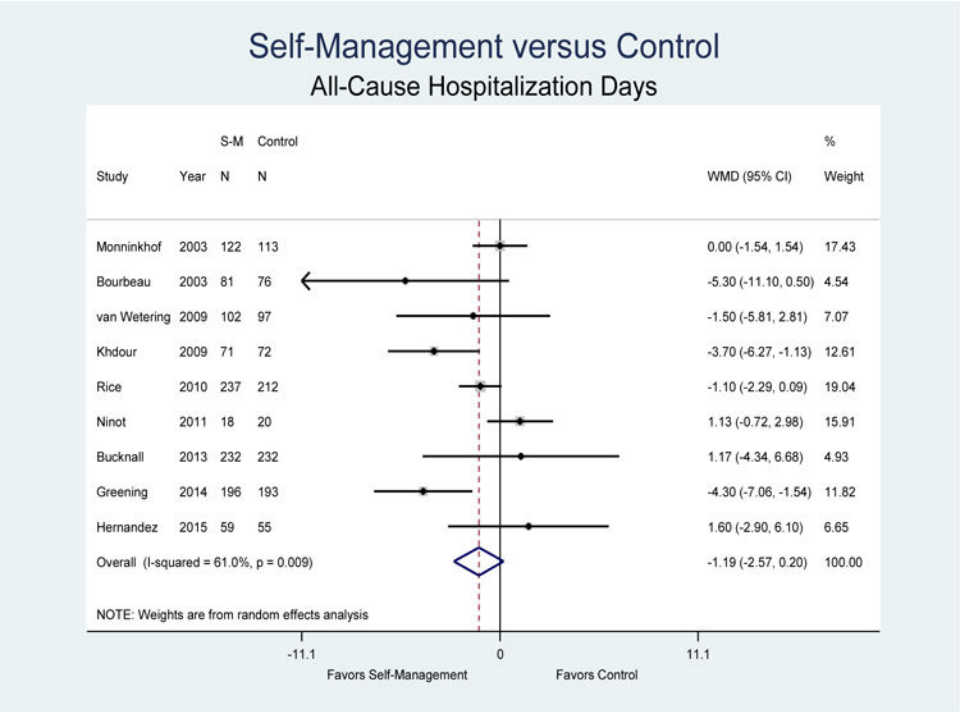


Figure 1.

(a) Fixed effects meta-analyses and (b) fixed effects cumulative meta-analyses for self-management (SM) intervention versus a control from the systematic reviews from the Cochrane Airways group in Table 1, which includes the primary outcome health-related quality of life (HRQoL) assessed by St George's Respiratory Questionnaire (SGRQ). Panel

a shows the significant increase in the HRQoL for the SM intervention as compared with usual care. Panel *b* reveals that this significant increase in the HRQoL for the SM intervention appeared in the literature in 2009 and has remained stable in subsequent years. Data from Reference 61. Other abbreviations: CI, confidence interval; WMD, weighted mean difference.



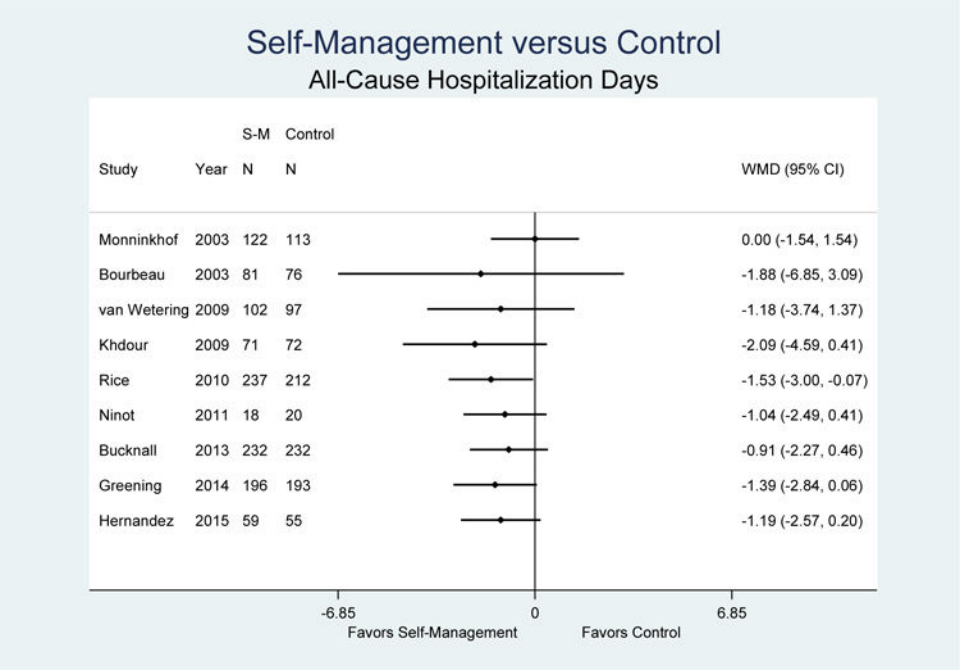


Figure 2.

(a) Random effects meta-analyses and (*b*) Random effects cumulative meta-analyses for a self-management (SM) intervention versus a control from the systematic reviews from the Cochrane Airways group in Table 1, which includes the primary outcome all-cause hospitalization days. Panel *a* shows a marginally significant decrease in all-cause hospitalization days for the SM intervention as compared with usual care. Panel *b* reveals

that this marginal significant reduction in the all-cause hospitalization days for the SM intervention appeared in the literature in 2010 and has remained stable in subsequent years. Other abbreviations: CI, confidence interval; WMD, weighted mean difference.

Table 1

Characteristics and quality of evidence from systematic reviews of intervention studies of chronic disease self-management contained in the Cochrane Database of Systematic Reviews[a]

Review investigators and reference	Cochrane editorial group	Search date[b]	Disease condition and/or focus[c]	Study designs included	N of studies	N of participants	Intervention	Primary outcomes of interest	Quality of evidence[d]
de Jongh et al. (35)	Consumers and communication	June 2009	Mobile phone messaging for facilitating self-management of long-term illnesses	RCTs, QRCTs, CBAs, or ITSs with at least three time points before and after the intervention	4	182	Mobile phone messaging applications designed to facilitate self-management of long-term illnesses	Health outcomes and patients' capacity to self-manage their condition	Low to moderate
Foster et al. (41)	Consumers and communication	July 2006	Self-management education programs led by lay leaders for people with chronic health conditions[e]	RCTs comparing structured lay-led self-management education programs for chronic conditions against no intervention or clinician-led programs	17	7,442	Patient self-management and education	Health status, health behaviors, and healthcare utilization	Insufficient or equivocal evidence
Fryer et al., (42)	Stroke	August 2016	Self-management programmes for people living with the long-term effects of stroke	RCTs of adults with stroke living in the community who received self-management interventions	14	1,863	Self-management interventions (including more than one component of self-management or targeted more than a single domain of change, or both	QoL, self-efficacy, activity or participation levels, impairments, health service usage, health behaviors (such as medication adherence or lifestyle behaviors), cost, participant satisfaction, or adverse events.	Moderate evidence
Kelly et al. (64)	Airways	December 2017	Self-management for non-cystic fibrosis bronchiectasis	RCTs of any duration that included adults or children with a diagnosis of non-cystic fibrosis bronchiectasis assessing self-management interventions delivered in any form	2	84	Patient self-management for airway clearance, medication, exercise, and action plans for children and adults	QoL, hospital admissions	Insufficient or equivocal evidence
Kroon et al. (66)	Musculoskeletal	January 2103	Self-management education programs for osteoarthritis	RCTs of self-management education programs in people with osteoarthritis	29	6,753	Patient self-management education	Self-management skills, pain, osteoarthritis symptoms and function	Low to moderate
Lenferink et al. (68)	Airways	May 2016	Self-management interventions including action plans for patients with COPD	RCTs evaluating a self-management intervention for people with COPD published since 1995	22	3,854	Patient action plans	QoL, hospital admissions	Moderate to high
McCabe et al. (87)	Airways	November 2016	Smart technology for self-management of COPD	RCTs that measured effects of remote and Web 2.0-based interventions defined as technologies including PCs and applications for mobile technology, such as iPad, Android tablets, smart phones, and Skype, on behavioral change towards self-management of COPD	3	557	Smart technology to support self-management, and digital information and education about self-management	HRQoL	Insufficient evidence
Peytremann-Bridevaux, et al. (102)	Effective practice and organization of care	June 2014	Chronic disease management for asthma	Individual or CRCTs, NRCTs, and CBAs comparing chronic disease management programs with usual care in adults over 16 years of age with a diagnosis of asthma	20	81,746	Various chronic disease management, including patient self-management education	Asthma-specific QoL, asthma severity, and lung function	Low to Moderate
Smith et al. (110)	Effective practice and organization of care	September 2015	Improving outcomes for people with multiple chronic conditions[e]	RCTs, NRCTs, CBAs, and ITSs evaluating interventions to improve outcomes for people with multimorbidity in primary care and community settings	18	NA	Interventions that involved changes to the organization of care delivery and patient-focused interventions	Clinical outcomes, health service use, medication adherence, patient-related health behaviors, health professional behaviors, and costs	Low to moderate

Review investigators and reference	Cochrane editorial group	Search date[b]	Disease condition and/or focus[c]	Study designs included	N of studies	N of participants	Intervention	Primary outcomes of interest	Quality of evidence[d]
Zwerink et al. (118)	Airways	August 2011	Self-management for patients with COPD	RCTs and NRCTs published after 1994, assessing the efficacy of self-management interventions for individuals with COPD	29	3,189	Patient self-management training	QoL, hospital admissions, and improvement in dyspnea	Insufficient evidence

Abbreviations: CBA, Controlled Before-After; COPD, chronic obstructive pulmonary disease; CRCT, cluster-randomized controlled trial; HRQoL, health-related quality of life; ITS, interrupted time series; NRCT, nonrandomized controlled trial; PC, personal computers; QoL, quality of life; QRCT, quasi-randomized controlled trial; RCT, randomized controlled trial; NA, not available.

[a] Reviews contained in the table were identified by searching the Cochrane Database for Systematic Reviews using the search term, "chronic disease self-management". Of the 35 reviews identified as of May 15, 2018, 10 assessed the evidence for effectiveness of disease-specific self-management programs or broader programs of disease management that included patient self-management.

[b] The date up to which studies were captured in the review.

[c] Statement is drawn from the Cochrane authors' "plain language summary" of the abstract.

[d] Based on the Cochrane Editorial Group's quality rating system. High quality: Further research is very unlikely to change confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate. Very low quality: Very uncertain about the estimate. Where a clear rating of the quality of evidence was not provided by authors, we have indicated the quality of evidence as insufficient or equivocal.

[e] Conditions included arthritis, diabetes, hypertension, and chronic pain.

EXHIBIT 51

The Growing Crisis of Chronic Disease in the United States

Chronic Diseases: What Are They?

Chronic diseases are ongoing, generally incurable illnesses or conditions, such as heart disease, asthma, cancer, and diabetes. These diseases are often preventable, and frequently manageable through early detection, improved diet, exercise, and treatment therapy.

Chronic Diseases: Costing Lives, Reducing Quality of Life

Chronic diseases are the leading cause of death and disability in the United States.

- 133 million Americans – 45% of the population – have at least one chronic disease.[1]
- Chronic diseases are responsible for seven out of every 10 deaths in the U.S., killing more than 1.7 million Americans every year.[2]
- Chronic diseases can be disabling and reduce a person's quality of life, especially if left undiagnosed or untreated. For example, every 30 seconds a lower limb is amputated as a consequence of diabetes.[3]

Chronic Diseases: Increasing Demand for Health Care and Driving Up Costs

People with chronic conditions are the most frequent users of health care in the U.S.

- They account for 81% of hospital admissions; 91% of all prescriptions filled; and 76% of all physician visits.[4]

Chronic diseases also account for the vast majority of health spending. In the U.S., total spending on public and private health care amounted to approximately $2 trillion during 2005.[5]

- Of that amount, more than 75% went toward treatment of chronic disease.[6]
- That is equivalent to $5,000 worth of spending per person on treatment of chronic disease[7] – more than double what the average American spends on gasoline in a year.[8]
- In publicly funded health programs, spending on chronic disease represents an even greater proportion of total spending: more than 99% in Medicare and 83% in Medicaid.[9]

*Because the data used for these calculations only refers to the non-institutionalized population, it is likely that actual spending on chronically ill beneficiaries is higher since the rate of chronic illness is higher among the institutionalized population.

Chronic Diseases: Costing U.S. Employers and Employees

U.S. employers and employees are paying for the high costs of chronic disease through the increase in health costs associated with greater demand for and use of health care services.

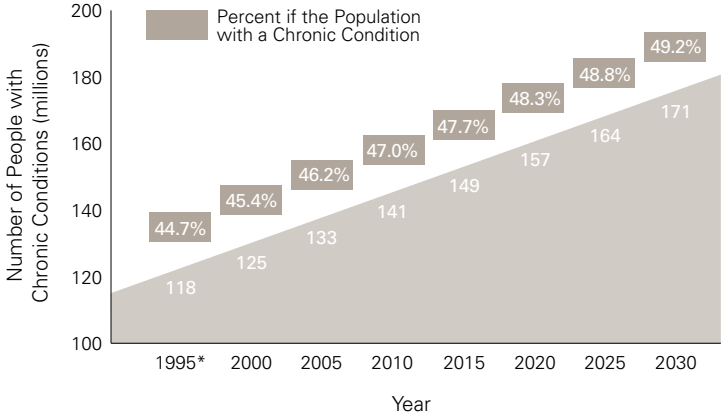
- Health care premiums for employer-sponsored family coverage have increased by 87% since 2000.[10]
- Health care coverage costs for people with a chronic condition average $6,032 annually – five times higher than for those without such a condition.[11]
- The total cost of obesity to U.S. companies is estimated at $13 billion annually. This includes the "extra" cost of health insurance ($8 billion), sick leave ($2.4 billion), life insurance ($1.8 billion), and disability insurance ($1 billion) associated with obesity.[12]

Chronic Diseases: Costing Our Future

While today's situation is grave, the chronic disease crisis looms even larger tomorrow.

- By 2025, chronic diseases will affect an estimated 164 million Americans – nearly half (49%) of the population (see Chart 1).[13]

Chart 1: The Number of People with Chronic Conditions is Rapidly Increasing



Source: Wu, Shin-Yi, and Green, Anthony. *Projection of Chronic Illness Prevalence and Cost Inflation.* RAND Corporation, October 2000.

Exhibit 51

- Overweight rates have been climbing over the past few decades among children. About 9 million (or roughly one in six kids ages 6–19) were overweight in 2004 – more than triple the number of overweight children in 1980.[14]
- Given current trends, one in three children born in 2000 will develop diabetes over the course of a lifetime.[15]

Chronic Diseases: Often Preventable, Frequently Manageable

Many chronic diseases could be prevented, delayed, or alleviated, through simple lifestyle changes.

- The U.S. Centers for Disease Control and Prevention (CDC)[16] estimates that eliminating three risk factors – poor diet, inactivity, and smoking – would prevent:
  - 80% of heart disease and stroke;
  - 80% of type 2 diabetes; and,
  - 40% of cancer.

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PARTNERSHIP TO FIGHT CHRONIC DISEASE

A VISION FOR A HEALTHIER FUTURE

EXHIBIT 52

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Developmental Disabilities

Developmental disabilities are a group of conditions due to an impairment in physical, learning, language, or behavior areas. About one in six children in the U.S. have one or more developmental disabilities or other developmental delays.

Facts
Milestones, screening, causes and risk factors, and living with a disability.

Research & Tracking
What we've learned and CDC's work.

Resource Center
Print or order free materials.

Links to Other Websites
Find information from other organizations.

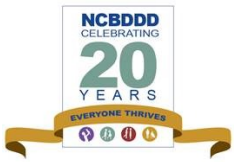
Specific Conditions
Information about specific types of developmental disabilities.

Articles
Scientific articles.

Multimedia & Tools
Videos, podcasts, and widgets.

About Us
Overview of CDC's work.

View events in NCBDDD's history »



Developmental Milestones

Children reach milestones in how they play, learn, speak, behave, and move. CDC has checklists to help parents and others track a child's development.

Page last reviewed: September 26, 2019
Content source: National Center on Birth Defects and Developmental Disabilities , Centers for Disease Control and Prevention

EXHIBIT 53

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Developmental Disabilities

Facts About Developmental Disabilities

Developmental disabilities are a group of conditions due to an impairment in physical, learning, language, or behavior areas. These conditions begin during the developmental period, may impact day-to-day functioning, and usually last throughout a person's lifetime.[1]

Developmental Milestones

Skills such as taking a first step, smiling for the first time, and waving "bye-bye" are called developmental milestones. Children reach milestones in how they play, learn, speak, behave, and move (for example, crawling and walking).

Children develop at their own pace, so it's impossible to tell exactly when a child will learn a given skill. However, the developmental milestones give a general idea of the changes to expect as a child gets older.

As a parent, you know your child best. If your child is not meeting the milestones for his or her age, or if you think there could be a problem with the way your child plays, learns, speaks, acts, and moves talk to your child's doctor and share your concerns. Don't wait. Acting early can make a real difference!

Milestones children should reach »

What to do if you're concerned »

Developmental Monitoring and Screening

A child's growth and development are followed through a partnership between parents and health care professionals. At each well-child visit, the doctor looks for developmental delays or problems and talks with the parents about any concerns the parents might have. This is called *developmental monitoring*.

Any problems noticed during developmental monitoring should be followed up with *developmental screening.* Developmental screening is a short test to tell if a child is learning basic skills when he or she should, or if there are delays.

If a child has a developmental delay, it is important to get help as soon as possible. Early identification and intervention can have a significant impact on a child's ability to learn new skills, as well as reduce the need for costly interventions over time.

Developmental monitoring and screening »

Causes and Risk Factors

Developmental disabilities begin anytime during the developmental period and usually last throughout a person's lifetime. Most developmental disabilities begin before a baby is born, but some can happen after birth because of injury, infection, or other factors.

Most developmental disabilities are thought to be caused by a complex mix of factors. These factors include genetics; parental health and behaviors (such as smoking and drinking) during pregnancy; complications during birth; infections the mother might have during pregnancy or the baby might have very early in life; and exposure of the mother or child to high levels of environmental toxins, such as lead. For some developmental disabilities, such as fetal alcohol syndrome, which is caused by drinking alcohol during pregnancy, we know the cause. But for most, we don't.

Following are some examples of what we know about specific developmental disabilities:

- At least 25% of hearing loss among babies is due to maternal infections during pregnancy, such as cytomegalovirus (CMV) infection; complications after birth; and head trauma.
- Some of the most common known causes of intellectual disability include fetal alcohol syndrome; genetic and chromosomal conditions, such as Down syndrome and fragile X syndrome; and certain infections during pregnancy.
- Children who have a sibling with autism are at a higher risk of also having autism spectrum disorder.
- Low birthweight**,** premature birth, multiple birth, and infections during pregnancy are associated with an increased risk for many developmental disabilities.
- Untreated newborn jaundice (high levels of bilirubin in the blood during the first few days after birth) can cause a type of brain damage known as kernicterus. Children with kernicterus are more likely to have cerebral palsy, hearing and vision problems, and problems with their teeth. Early detection and treatment of newborn jaundice can prevent kernicterus.

The Study to Explore Early Development (SEED) is a multiyear study funded by CDC. It is currently the largest study in the United States to help identify factors that may put children at risk for autism spectrum disorders and other developmental disabilities.

Learn more about SEED »

Who Is Affected

Developmental disabilities occur among all racial, ethnic, and socioeconomic groups. Recent estimates in the United States show that about one in six, or about 17%, of children aged 3 through 17 years have one or more developmental disabilities, such as:

- ADHD,
- autism spectrum disorder,
- cerebral palsy,
- hearing loss,

- intellectual disability [271 KB, 2 Pages, 508],
- learning disability,
- vision impairment [304 KB, 2 Pages, 508],
- and other developmental delays.[2]

Learn more about the number of children in the U.S. with developmental disabilities »

For over a decade, CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network has been tracking the number and characteristics of children with autism spectrum disorder, cerebral palsy, and intellectual disability in several diverse communities throughout the United States.

CDC's tracking of the number and characteristics of children with autism spectrum disorder »
CDC's tracking of the number and characteristics of children with cerebral palsy »

Living With a Developmental Disability

Children and adults with disabilities need health care and health programs for the same reasons anyone else does—to stay well, active, and a part of the community.

Having a disability does not mean a person is not healthy or that he or she cannot be healthy. Being healthy means the same thing for all of us—getting and staying well so we can lead full, active lives. That includes having the tools and information to make healthy choices and knowing how to prevent illness. Some health conditions, such as asthma, gastrointestinal symptoms, eczema and skin allergies, and migraine headaches, have been found to be more common among children with developmental disabilities. Thus, it is especially important for children with developmental disabilities to see a health care provider regularly.

Learn more about healthy living »

CDC does not study education or treatment programs for people with developmental disabilities, nor does it provide direct services to people with developmental disabilities or to their families. However, CDC has put together a list of resources for people affected by developmental disabilities.

List of developmental disabilities resources »

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Page last reviewed: September 26, 2019
Content source: National Center on Birth Defects and Developmental Disabilities , Centers for Disease Control and Prevention

EXHIBIT 54

neurodevelopmental disorder (no͝or′ō-dī-vĕl′əp-mĕn′tl, nyo͝or′ō-)

n.

Any of various conditions, including attention deficit hyperactivity disorder, autism, intellectual disability, and learning disabilities, that begin in childhood and involve impairments in neurological functioning that affect behavior, cognition, or motor skills.

EXHIBIT 55

Neurobehavioural effects of developmental toxicity

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Lancet Neurol 2014; 13: 330–38

Published Online
February 15, 2014
http://dx.doi.org/10.1016/S1474-4422(13)70278-3

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Neurodevelopmental disabilities, including autism, attention-deficit hyperactivity disorder, dyslexia, and other cognitive impairments, affect millions of children worldwide, and some diagnoses seem to be increasing in frequency. Industrial chemicals that injure the developing brain are among the known causes for this rise in prevalence. In 2006, we did a systematic review and identified five industrial chemicals as developmental neurotoxicants: lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene. Since 2006, epidemiological studies have documented six additional developmental neurotoxicants—manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and the polybrominated diphenyl ethers. We postulate that even more neurotoxicants remain undiscovered. To control the pandemic of developmental neurotoxicity, we propose a global prevention strategy. Untested chemicals should not be presumed to be safe to brain development, and chemicals in existing use and all new chemicals must therefore be tested for developmental neurotoxicity. To coordinate these efforts and to accelerate translation of science into prevention, we propose the urgent formation of a new international clearinghouse.

Introduction

Disorders of neurobehavioural development affect 10–15% of all births,[1] and prevalence rates of autism spectrum disorder and attention-deficit hyperactivity disorder seem to be increasing worldwide.[2] Subclinical decrements in brain function are even more common than these neurobehavioural developmental disorders. All these disabilities can have severe consequences[3]—they diminish quality of life, reduce academic achievement, and disturb behaviour, with profound consequences for the welfare and productivity of entire societies.[4]

The root causes of the present global pandemic of neurodevelopmental disorders are only partly understood. Although genetic factors have a role,[5] they cannot explain recent increases in reported prevalence, and none of the genes discovered so far seem to be responsible for more than a small proportion of cases.[5] Overall, genetic factors seem to account for no more than perhaps 30–40% of all cases of neurodevelopmental disorders. Thus, non-genetic, environmental exposures are involved in causation, in some cases probably by interacting with genetically inherited predispositions.

Strong evidence exists that industrial chemicals widely disseminated in the environment are important contributors to what we have called the global, silent pandemic of neurodevelopmental toxicity.[6,7] The developing human brain is uniquely vulnerable to toxic chemical exposures, and major windows of developmental vulnerability occur in utero and during infancy and early childhood.[8] During these sensitive life stages, chemicals can cause permanent brain injury at low levels of exposure that would have little or no adverse effect in an adult.

In 2006, we did a systematic review of the published clinical and epidemiological studies into the neurotoxicity of industrial chemicals, with a focus on developmental neurotoxicity.[6] We identified five industrial chemicals that could be reliably classified as developmental neurotoxicants: lead, methylmercury, arsenic, polychlorinated biphenyls, and toluene. We also noted 201 chemicals that had been reported to cause injury to the nervous system in adults, mostly in connection with occupational exposures, poisoning incidents, or suicide attempts. Additionally, more than 1000 chemicals have been reported to be neurotoxic in animals in laboratory studies.

We noted that recognition of the risks of industrial chemicals to brain development has historically needed decades of research and scrutiny, as shown in the cases of lead and methylmercury.[9,10] In most cases, discovery began with clinical diagnosis of poisoning in workers and episodes of high-dose exposure. More sophisticated epidemiological studies typically began only much later. Results from such studies documented developmental neurotoxicity at much lower exposure levels than had previously been thought to be safe. Thus, recognition of widespread subclinical toxicity often did not occur until decades after the initial evidence of neurotoxicity. A recurring theme was that early warnings of subclinical neurotoxicity were often ignored or even dismissed.[11] David P Rall, former Director of the US National Institute of Environmental Health Sciences, once noted that "if thalidomide had caused a ten-point loss of intelligence quotient (IQ) instead of obvious birth defects of the limbs, it would probably still be on the market".[12] Many industrial chemicals marketed at present probably cause IQ deficits of far fewer than ten points and have therefore eluded detection so far, but their combined effects could have enormous consequences.

In our 2006 review,[6] we expressed concern that additional developmental neurotoxicants might lurk undiscovered among the 201 chemicals then known to be neurotoxic to adult human beings and among the many thousands of pesticides, solvents, and other industrial chemicals in widespread use that had never been tested for neurodevelopmental toxicity. Since our previous review, new data have emerged about the vulnerability of the developing brain and the neurotoxicity of industrial chemicals. Particularly important new evidence derives from prospective epidemiological birth cohort studies.

In this Review, we consider recent information about the developmental neurotoxicity of industrial chemicals

to update our previous report.[6] Additionally, we propose strategies to counter this pandemic and to prevent the spread of neurological disease and disability in children worldwide.

Unique vulnerability of the developing brain

The fetus is not well protected against industrial chemicals. The placenta does not block the passage of many environmental toxicants from the maternal to the fetal circulation,[13] and more than 200 foreign chemicals have been detected in umbilical cord blood.[14] Additionally, many environmental chemicals are transferred to the infant through human breastmilk.[13] During fetal life and early infancy, the blood–brain barrier provides only partial protection against the entry of chemicals into the CNS.[15]

Moreover, the developing human brain is exceptionally sensitive to injury caused by toxic chemicals,[6] and several developmental processes have been shown to be highly vulnerable to chemical toxicity. For example, in-vitro studies suggest that neural stem cells are very sensitive to neurotoxic substances such as methylmercury.[16] Some pesticides inhibit cholinesterase function in the developing brain,[17] thereby affecting the crucial regulatory role of acetylcholine before synapse formation.[18] Early-life epigenetic changes are also known to affect subsequent gene expression in the brain.[19] In summary, industrial chemicals known or suspected to be neurotoxic to adults are also likely to present risks to the developing brain.

Figure 1 shows the unique vulnerability of the brain during early life and indicates how developmental exposures to toxic chemicals are particularly likely to lead to functional deficits and disease later in life.

New findings about known hazards

Recent research on well-documented neurotoxicants has generated important new insights into the neurodevelopmental consequences of early exposures to these industrial chemicals.

Joint analyses that gathered data for lead-associated IQ deficits from seven international studies[20,21] support the conclusion that no safe level of exposure to lead exists.[22] Cognitive deficits in adults who had previously shown lead-associated developmental delays at school age suggest that the effects of lead neurotoxicity are probably permanent.[23] Brain imaging of young adults who had raised lead concentrations in their blood during childhood showed exposure-related decreases in brain volume.[24] Lead exposure in early childhood is associated with reduced school performance[25] and with delinquent behaviour later in life.[26,27]

Developmental neurotoxicity due to methylmercury occurs at much lower exposures than the concentrations that affect adult brain function.[28] Deficits at 7 years of age that were linked to low-level prenatal exposures to methylmercury were still detectable at the age of 14 years.[29] Some common genetic polymorphisms seem to increase the vulnerability of the developing brain to methylmercury toxicity.[30] Functional MRI scans of people exposed prenatally to excess amounts of methylmercury showed abnormally expanded activation of brain regions in response to sensory stimulation and motor tasks (figure 2).[31] Because some adverse effects might be counterbalanced by essential fatty acids from seafood, statistical adjustment for maternal diet during pregnancy results in stronger methylmercury effects.[32,33]

Prenatal and early postnatal exposures to inorganic arsenic from drinking water are associated with cognitive deficits that are apparent at school age.[34,35] Infants who survived the Morinaga milk arsenic poisoning incident had highly raised risks of neurological disease during adult life.[36]

The developmental neurotoxicity of polychlorinated biphenyls has been consolidated and strengthened by recent findings.[37] Although little new information has been published about the developmental neurotoxicity of toluene, much has been learned about the developmental neurotoxicity of another common solvent, ethanol, through research on fetal alcohol exposure. Maternal consumption of alcohol during pregnancy, even in very small quantities, has been linked to a range of neurobehavioural adverse effects in offspring, including reduced IQ, impaired executive function and social judgment, delinquent behaviour, seizures, other neurological signs, and sensory problems.[38]

Newly recognised developmental neurotoxicants

Prospective epidemiological birth cohort studies make it possible to measure maternal or fetal exposures in real time during pregnancy as these exposures actually occur, thus generating unbiased information about the degree and timing of prenatal exposures. Children in these prospective studies are followed longitudinally and assessed with age-appropriate tests to show delayed or deranged neurobehavioural development. These powerful epidemiological methods have enabled the discovery of additional developmental neurotoxicants.

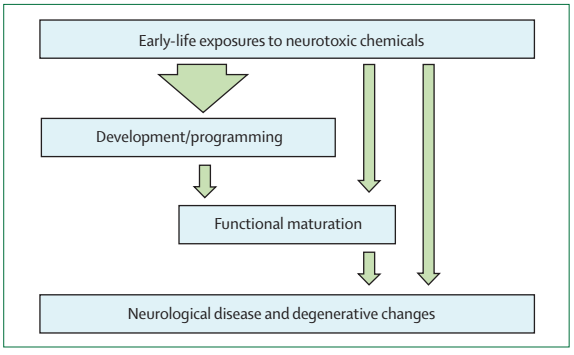

Figure 1: **Effect of neurotoxicants during early brain development**
Exposures in early life to neurotoxic chemicals can cause a wide range of adverse effects on brain development and maturation that can manifest as functional impairments or disease at any point in the human lifespan, from early infancy to very old age.

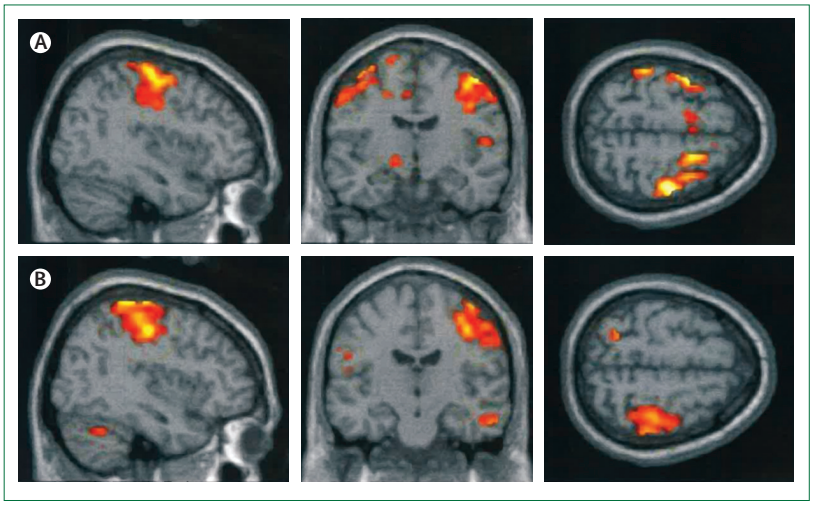

Figure 2: **Functional MRI scans show abnormal activation in the brain**
Average activation during finger tapping with the left hand in three adolescents with increased prenatal methylmercury exposure (A) and three control adolescents (B). The control participants activate the premotor and motor cortices on the right, whereas participants exposed to methylmercury activate these areas bilaterally.[31]

Cross-sectional data from Bangladesh show that exposure to manganese from drinking water is associated with reduced mathematics achievement scores in school children.[39] A study in Quebec, Canada, showed a strong correlation between manganese concentrations in hair and hyperactivity.[40] School-aged children living near manganese mining and processing facilities have shown associations between airborne manganese concentrations and diminished intellectual function[41] and with impaired motor skills and reduced olfactory function.[42] These results are supported by experimental findings in mice.[43]

A meta-analysis of 27 cross-sectional studies of children exposed to fluoride in drinking water, mainly from China, suggests an average IQ decrement of about seven points in children exposed to raised fluoride concentrations.[44] Confounding from other substances seemed unlikely in most of these studies. Further characterisation of the dose–response association would be desirable.

The occupational health literature[45] suggests that solvents can act as neurotoxicants, but the identification of individual responsible compounds is hampered by the complexity of exposures. In a French cohort study of 3000 children, investigators linked maternal occupational solvent exposure during pregnancy to deficits in behavioural assessment at 2 years of age.[46] The data showed dose-related increased risks for hyperactivity and aggressive behaviour. One in every five mothers in this cohort reported solvent exposures in common jobs, such as nurse or other hospital employee, chemist, cleaner, hairdresser, and beautician. In Massachusetts, USA, follow-up of a well-defined population with prenatal and early childhood exposure to the solvent tetrachloroethylene (also called perchlorethylene) in drinking water showed a tendency towards deficient neurological function and increased risk of psychiatric diagnoses.[47]

Acute pesticide poisoning occurs frequently in children worldwide, and subclinical pesticide toxicity is also widespread. Clinical data suggest that acute pesticide poisoning during childhood might lead to lasting neurobehavioural deficits.[48,49] Highly toxic and bio-accumulative pesticides are now banned in high-income nations, but are still used in many low-income and middle-income countries. In particular, the organochlorine compounds dichlorodiphenyltrichloroethane (DDT), its metabolite dichlorodiphenyldichloroethylene (DDE), and chlordecone (Kepone), tend to be highly persistent and remain widespread in the environment and in people's bodies in high-use regions. Recent studies have shown inverse correlations between serum concentrations of DDT or DDE (which indicate accumulated exposures), and neurodevelopmental performance.[50,51]

Organophosphate pesticides are eliminated from the human body much more rapidly than are organochlorines, and exposure assessment is therefore inherently less precise. Nonetheless, three prospective epidemiological birth cohort studies provide new evidence that prenatal exposure to organophosphate pesticides can cause developmental neurotoxicity. In these studies, prenatal organophosphate exposure was assessed by measurement of maternal urinary excretion of pesticide metabolites during pregnancy. Dose-related correlations were recorded between maternal exposures to chlorpyrifos or other organophosphates and small head circumference at birth—which is an indication of slowed brain growth in utero—and with neurobehavioural deficits that have persisted to at least 7 years of age.[52–54] In a subgroup study, MRI of the brain showed that prenatal chlorpyrifos exposure was associated with structural abnormalities that included thinning of the cerebral cortex.[55]

Herbicides and fungicides might also have neurotoxic potential.[56] Propoxur,[57] a carbamate pesticide, and permethrine,[58] a member of the pyrethroid class of pesticides, have recently been linked to neurodevelopmental deficits in children.

The group of compounds known as polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants and are structurally very similar to the polychlorinated biphenyls. Experimental evidence now suggests that the PBDEs might also be neurotoxic.[59] Epidemiological studies in Europe and the USA have shown neurodevelopmental deficits in children with increased prenatal exposures to these compounds.[60–62] Thus, the PBDEs should be regarded as hazards to human neurobehavioural development, although attribution of relative toxic potentials to individual PBDE congeners is not yet possible.

Other suspected developmental neurotoxicants

A serious difficulty that complicates many epidemiological studies of neurodevelopmental toxicity in children is the problem of mixed exposures. Most populations are exposed to more than one neurotoxicant at a time, and yet

most studies have only a finite amount of power and precision in exposure assessment to discern the possible effects of even single neurotoxicants. A further problem in many epidemiological studies of non-persistent toxicants is that imprecise assessment of exposure tends to obscure associations that might actually be present.[63] Guidance from experimental neurotoxicity studies is therefore crucial. In the assessment of potential developmental neurotoxicants, we have used a strength of evidence approach similar to that used by the International Agency for Research on Cancer for assessing epidemiological and experimental studies.

Phthalates and bisphenol A are added to many different types of plastics, cosmetics, and other consumer products. Since they are eliminated rapidly in urine, exposure assessment is complicated, and such imprecision might lead to underestimation of the true risk of neurotoxicity. The best-documented effects of early-life exposure to phthalates are the consequence of disruption of endocrine signalling.[64] Thus, prenatal exposures to phthalates have been linked to both neurodevelopmental deficits and to behavioural abnormalities characterised by shortened attention span and impaired social interactions.[65] The neurobehavioural toxicity of these compounds seems to affect mainly boys and could therefore relate to endocrine disruption in the developing brain.[66] In regard to bisphenol A, a prospective study showed that point estimates of exposure during gestation were linked to abnormalities in behaviour and executive function in children at 3 years of age.[67]

Exposure to air pollution can cause neurodevelopmental delays and disorders of behavioural functions.[68,69] Of the individual components of air pollution, carbon monoxide is a well-documented neurotoxicant, and indoor exposure to this substance has now been linked to deficient neurobehavioural performance in children.[70] Less clear is the reported contribution of nitrogen oxides to neurodevelopmental deficits,[71] since these compounds often co-occur with carbon monoxide as part of complex emissions. Tobacco smoke is a complex mixture of hundreds of chemical compounds and is now a well-documented cause of developmental neurotoxicity.[72] Infants exposed prenatally to polycyclic aromatic hydrocarbons from traffic exhausts at 5 years of age showed greater cognitive impairment and lower IQ than those exposed to lower levels of these compounds.[68]

Perfluorinated compounds, such as perfluorooctanoic acid and perfluorooctane sulphonate, are highly persistent in the environment and in the human body, and seem to be neurotoxic.[73] Emerging epidemiological evidence suggests that these compounds might indeed impede neurobehavioural development.[74]

Developmental neurotoxicity and clinical neurology

Exposures in early life to developmental neurotoxicants are now being linked to specific clinical syndromes in children. For example, an increased risk of attention-deficit hyperactivity disorder has been linked to prenatal exposures to manganese, organophosphates,[75] and phthalates.[76] Phthalates have also been linked to behaviours that resemble components of autism spectrum disorder.[77] Prenatal exposure to automotive air pollution in California, USA, has been linked to an increased risk for autism spectrum disorder.[78]

The persistent decrements in intelligence documented in children, adolescents, and young adults exposed in early life to neurotoxicants could presage the development of neurodegenerative disease later in life. Thus, accumulated exposure to lead is associated with cognitive decline in the elderly.[79] Manganese exposure may lead to parkinsonism, and experimental studies have reported Parkinson's disease as a result of developmental exposures to the insecticide rotenone, the herbicides paraquat and maneb, and the solvent trichloroethylene.[80] Any environmental exposure that increases the risk of neurodegenerative disorders in later life (figure 1) requires urgent investigation as the world's population continues to age.[81]

The expanding complement of neurotoxicants

In our 2006 review,[6] we expressed concern that additional developmental neurotoxicants might lie undiscovered in the 201 chemicals that were then known to be neurotoxic to human adults, in the roughly 1000 chemicals known to be neurotoxic in animal species, and in the many thousands of industrial chemicals and pesticides that have never been tested for neurotoxicity. Exposure to neurotoxic chemicals is not rare, since almost half of the 201 known human neurotoxicants are regarded as high production volume chemicals.

Our updated literature review shows that since 2006 the list of recognised human neurotoxicants has expanded by 12 chemicals, from 202 (including ethanol) to 214 (table 1 and appendix)—that is, by about two substances per year. Many of these chemicals are widely used and disseminated extensively in the global environment. Of the newly identified neurodevelopmental toxicants, pesticides constitute the largest group, as was already the case in

See Online for appendix

	Number known in 2006	Number known in 2013	Identified since 2006
Metals and inorganic compounds	25	26	Hydrogen phosphide[82]
Organic solvents	39*	40	Ethyl chloride[83]
Pesticides	92	101	Acetamiprid,[84] amitraz,[85] avermectin,[86] emamectin,[87] fipronil (Termidor),[88] glyphosate,[89] hexaconazole,[90] imidacloprid,[91] tetramethylenedisulfotetramine[92]
Other organic compounds	46	47	1,3-butadiene[93]
Total	202*	214	12 new substances

*Including ethanol.

Table 1: **Industrial chemicals known to be toxic to the human nervous system in 2006 and 2013, according to chemical group**

	Known in 2006	Newly identified
Metals and inorganic compounds	Arsenic and arsenic compounds, lead, and methylmercury	Fluoride and manganese
Organic solvents	(Ethanol) toluene	Tetrachloroethylene
Pesticides	None	Chlorpyrifos and DDT/DDE
Other organic compounds	Polychlorinated biphenyls	Brominated diphenyl ethers
Total	6*	6

DDT=dichlorodiphenyltrichloroethane. DDE=dichlorodiphenyldichloroethylene. *Including ethanol.

Table 2: **Industrial chemicals known to cause developmental neurotoxicity in human beings in 2006 and 2013, according to chemical group**

	Number of IQ points lost
Major medical and neurodevelopmental disorders	
Preterm birth	34 031 025
Autism spectrum disorders	7 109 899
Paediatric bipolar disorder	8 164 080
Attention-deficit hyperactivity disorder	16 799 400
Postnatal traumatic brain injury	5 827 300
Environmental chemical exposures	
Lead	22 947 450
Methylmercury	1 590 000*
Organophosphate pesticides	16 899 488
Other neurotoxicants	Unknown

IQ=intelligence quotient. Data from from Bellinger.[94] *From Grandjean and colleagues.[95]

Table 3: **Total losses of IQ points in US children 0–5 years of age associated with major risk factors, including developmental exposure to industrial chemicals that cause neurotoxicity**

2006. In the same 7-year period, the number of known developmental neurotoxicants has doubled from six to 12 (table 2). Although the pace of scientific discovery of new neurodevelopmental hazards is more rapid today than in the past, it is still slower than the identification of adult neurotoxicants.

The gap that exists between the number of substances known to be toxic to the adult brain and the smaller number known to be toxic to the much more vulnerable developing brain is unlikely to close in the near future. This discrepancy is attributable to the fact that toxicity to the adult brain is usually discovered as a result of acute poisoning incidents, typically with a clear and immediate association between causative exposure and adverse effects, as occurs for workplace exposures or suicide attempts. By contrast, the recognition of developmental neurotoxicity relies on two sets of evidence collected at two different points in time: exposure data (often obtained from the mother during pregnancy), and data for the child's postnatal neurobehavioural development (often obtained 5–10 years later). Because brain functions develop sequentially, the full effects of early neurotoxic damage might not become apparent until school age or beyond. The most reliable evidence of developmental neurotoxicity is obtained through prospective studies that include real-time recording of information about exposure in early life followed by serial clinical assessments of the child. Such research is inherently slow and is hampered by the difficulty of reliable assessment of exposures to individual toxicants in complex mixtures.

Consequences of developmental neurotoxicity

Developmental neurotoxicity causes brain damage that is too often untreatable and frequently permanent. The consequence of such brain damage is impaired CNS function that lasts a lifetime and might result in reduced intelligence, as expressed in terms of lost IQ points, or disruption in behaviour. A recent study compared the estimated total IQ losses from major paediatric causes and showed that the magnitude of losses attributable to lead, pesticides, and other neurotoxicants was in the same range as, or even greater than, the losses associated with medical events such as preterm birth, traumatic brain injury, brain tumours, and congenital heart disease (table 3).[94]

Loss of cognitive skills reduces children's academic and economic attainments and has substantial long-term economic effects on societies.[4] Thus, each loss of one IQ point has been estimated to decrease average lifetime earnings capacity by about €12 000 or US$18 000 in 2008 currencies.[96] The most recent estimates from the USA indicate that the annual costs of childhood lead poisoning are about US$50 billion and that the annual costs of methylmercury toxicity are roughly US$5 billion.[97] In the European Union, methylmercury exposure is estimated to cause a loss of about 600 000 IQ points every year, corresponding to an annual economic loss of close to €10 billion. In France alone, lead exposure is associated with IQ losses that correspond to annual costs that might exceed €20 billion.[98] Since IQ losses represent only one aspect of developmental neurotoxicity, the total costs are surely even higher.

Evidence from worldwide sources indicates that average national IQ scores are associated with gross domestic product (GDP)—a correlation that might be causal in both directions.[99] Thus, poverty can cause low IQ, but the opposite is also true. In view of the widespread exposures to lead, pesticides, and other neurotoxicants in developing countries, where chemical controls might be ineffective compared with those in more developed countries,[100,101] developmental exposures to industrial chemicals could contribute substantially to the recorded correlation between IQ and GDP. If this theory is true, developing countries could take decades to emerge from poverty. Consequently, pollution abatement might then be delayed, and a vicious circle can result.

The antisocial behaviour, criminal behaviour, violence, and substance abuse that seem to result from early-life exposures to some neurotoxic chemicals result in increased needs for special educational services, institutionalisation, and even incarceration. In the USA, the murder rate fell sharply 20 years after the removal of lead from petrol,[102] a finding consistent with the idea that

exposure to lead in early life is a powerful determinant of behaviour decades later. Although poorly quantified, such behavioural and social consequences of neurodevelopmental toxicity are potentially very costly.[76]

Prevention of developmental neurotoxicity caused by industrial chemicals is highly cost effective. A study that quantified the gains resulting from the phase-out of lead additives from petrol reported that in the USA alone, the introduction of lead-free petrol has generated an economic benefit of $200 billion in each annual birth cohort since 1980,[103] an aggregate benefit in the past 30 years of over $3 trillion. This success has since been repeated in more than 150 countries, resulting in vast additional savings. Every US$1 spent to reduce lead hazards is estimated to produce a benefit of US$17–220, which represents a cost-benefit ratio that is even better than that for vaccines.[4] Furthermore, the costs associated with the late-life consequences of developmental neurotoxicity are enormous, and the benefits from prevention of degenerative brain disorders could be very substantial.

New methods to identify developmental neurotoxicants

New toxicological methods now allow a rational strategy for the identification of developmental neurotoxicants based on a multidisciplinary approach.[104] A new guideline has been approved as a standardised approach for the identification of developmental neurotoxicants.[105] However, completion of such tests is expensive and requires the use of many laboratory animals, and reliance on mammals for chemicals testing purposes needs to be reduced.[106] US governmental agencies have established the National Center for Computational Toxicology and an initiative—the Tox 21 Program—to promote the evolution of toxicology from a mainly observational science to a predominantly predictive science.[107]

In-vitro methods have now reached a level of predictive validity that means they can be applied to neurotoxicity testing.[108] Some of these tests are based on neural stem cells. Although these cell systems do not have a blood–brain barrier and particular metabolising enzymes, these approaches are highly promising. As a further option, data for protein links and protein–protein interactions can now be used to explore potential neurotoxicity in silico,[109] thus showing that existing computational methods might predict potential toxic effects.[110]

In summary, use of the whole range of approaches along with clinical and epidemiological evidence, when available, should enable the integration of information for use in at least a tentative risk assessment. With these methods, we anticipate that the pace of scientific discovery in developmental neurotoxicology will accelerate further in the years ahead.

Conclusions and recommendations

The updated findings presented in this Review confirm and extend our 2006 conclusions.[6] During the 7 years since our previous report, the number of industrial chemicals recognised to be developmental neurotoxicants has doubled. Exposures to these industrial chemicals in the environment contribute to the pandemic of developmental neurotoxicity.

Two major obstacles impede efforts to control the global pandemic of developmental neurotoxicity. These barriers, which we noted in our previous review[6] and were recently underlined by the US National Research Council,[111] are: large gaps in the testing of chemicals for developmental neurotoxicity, which results in a paucity of systematic data to guide prevention; and the huge amount of proof needed for regulation. Thus, very few chemicals have been regulated as a result of developmental neurotoxicity.

The presumption that new chemicals and technologies are safe until proven otherwise is a fundamental problem.[111] Classic examples of new chemicals that were introduced because they conveyed certain benefits, but were later shown to cause great harm, include several neurotoxicants, asbestos, thalidomide, diethylstilboestrol, and the chlorofluorocarbons.[112] A recurring theme in each of these cases was that commercial introduction and wide dissemination of the chemicals preceded any systematic effort to assess potential toxicity. Particularly absent were advance efforts to study possible effects on children's health or the potential of exposures in early life to disrupt early development. Similar challenges have been confronted in other public health disasters, such as those caused by tobacco smoking, alcohol use, and refined foods. These problems have been recently termed industrial epidemics.[113]

To control the pandemic of developmental neurotoxicity, we propose a coordinated international strategy (panel). Mandatory and transparent assessment of evidence for neurotoxicity is the foundation of this strategy. Assessment of toxicity must be followed by governmental regulation and market intervention. Voluntary controls seem to be of little value.[11]

***Panel:* Recommendations for an international clearinghouse on neurotoxicity**

The main purpose of this agency would be to promote optimum brain health, not just avoidance of neurological disease, by inspiring, facilitating, and coordinating research and public policies that aim to protect brain development during the most sensitive life stages. The main efforts would aim to:

- Screen industrial chemicals present in human exposures for neurotoxic effects so that hazardous substances can be identified for tighter control
- Stimulate and coordinate new research to understand how toxic chemicals interfere with brain development and how best to prevent long-term dysfunctions and deficits
- Function as a clearinghouse for research data and strategies by gathering and assessing documentation about brain toxicity and stimulating international collaboration on research and prevention
- Promote policy development aimed at protecting vulnerable populations against chemicals that are toxic to the brain without needing unrealistic amounts of scientific proof

The three pillars of our proposed strategy are: legally mandated testing of existing industrial chemicals and pesticides already in commerce, with prioritisation of those with the most widespread use, and incorporation of new assessment technologies; legally mandated premarket evaluation of new chemicals before they enter markets, with use of precautionary approaches for chemical testing that recognise the unique vulnerability of the developing brain; and the formation of a new clearinghouse for neurotoxicity as a parallel to the International Agency for Research on Cancer. This new agency will assess industrial chemicals for developmental neurotoxicity with a precautionary approach that emphasises prevention and does not require absolute proof of toxicity. It will facilitate and coordinate epidemiological and toxicological studies and will lead the urgently needed global programmes for prevention.

These new approaches must reverse the dangerous presumption that new chemicals and technologies are safe until proven otherwise. They must also overcome the existing requirement to produce absolute proof of toxicity before action can be started to protect children against neurotoxic substances. Precautionary interpretation of data about developmental neurotoxicity should take into account the very large individual and societal costs that result from failure to act on available documentation to prevent disease in children.[114] Academic research has often favoured scepticism and required extensive replication before acceptance of a hypothesis,[114] thereby adding to the inertia in toxicology and environmental health research and the consequent disregard of many other potential neurotoxicants.[115] Additionally, the strength of evidence that is needed to constitute "proof" should be analysed in a societal perspective, so that the implications of ignoring a developmental neurotoxicant and of failing to act on the basis of available data are also taken into account.

Finally, we emphasise that the total number of neurotoxic substances now recognised almost certainly represents an underestimate of the true number of developmental neurotoxicants that have been released into the global environment. Our very great concern is that children worldwide are being exposed to unrecognised toxic chemicals that are silently eroding intelligence, disrupting behaviours, truncating future achievements, and damaging societies, perhaps most seriously in developing countries. A new framework of action is needed.

Search strategy and selection criteria

We identified studies published since 2006 on the neurotoxic effects of industrial chemicals in human beings by using the search terms "neurotoxicity syndromes"[MeSH], "neurotoxic", "neurologic", or "neuro*", combined with "exposure" and "poisoning" in PubMed, from 2006 to the end of 2012. For developmental neurotoxicity, the search terms were "prenatal exposure delayed effects"[MeSH], "maternal exposure" or "maternal fetal exchange", "developmental disabilities/chemically induced" and "neurotoxins", all of which were searched for with the limiters "All Child: 0–18 years, Human". We also used references cited in the publications retrieved.

Contributors

Both authors did the literature review, wrote and revised the report, and approved the final version.

Conflicts of interest

PG has provided paid expert testimony about mercury toxicology for the US Department of Justice. PJL has provided paid expert testimony in cases of childhood lead poisoning. We declare that we have no other conflicts of interest.

Acknowledgments

This work was supported by the National Institutes of Health, National Institute for Environmental Health Sciences (ES09584, ES09797, and ES11687). The funding source had no role in the literature review, interpretation of data, writing of this Review, or in the decision to submit for publication. The contents of this paper are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health. We thank Mary S Wolff (Icahn School of Medicine at Mount Sinai, New York, NY, USA) and Linda S Birnbaum (US National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA) for their critical reading of the report.

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EXHIBIT 56

Autism | definition of autism by Medical dictionary

autism

Also found in: **Dictionary**, **Thesaurus**, **Acronyms**, **Encyclopedia**, **Wikipedia**.

Autism

Definition

Autism is a complex developmental disorder distinguished by difficulties with social interaction, verbal and nonverbal communication, and behavioral problems, including repetitive behaviors and narrow focus of interest.

Description

Classic autism is one of several disorders categorized as autism spectrum disorders (ASD). Other ASDs include Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder. According to the National Institutes of Health (NIH), three to six out of every 1,000 children in the United States have autism. Autism is four times more likely to be diagnosed in males. Autism is a disorder that is also prevalent worldwide. In the United Kingdom, one out of every 100 children have autism, with over half a million total diagnosed in the United Kingdom as of 2007. In China, one in every 1,000 children is diagnosed with autism. In India, the rate of incidence is 1 in every 250 children. In Mexico, two to six in every 1,000 children are autistic. Autism is not specific to any one socio-economic, ethnic, or racial group.

Autism usually manifests before a child is three years old and it continues throughout his/her lifetime. The degree of impairment varies from mild to severe. Autism is treatable and, with early diagnosis and treatment, autistic children have the ability to lead healthy, full lives.

Causes and symptoms

Researchers know that autism is a complex brain disorder that affects the way the brain uses or transmits information. Studies have implicated several causes for the disorder but still more investigation is needed. Studies have found abnormalities in several parts of the brain that are believed to have occurred during fetal development. The problem may be centered in the parts of the brain responsible for processing language and information from the senses.

There also appears to be a strong genetic basis for autism. Family studies have shown that identical twins are more likely to both be diagnosed with autism than twins who are fraternal (not genetically identical). In a family with one autistic child, the chance of having another child with autism is about one in 20 or approximately 5%, much higher than in the general population.

The severity of the condition varies between individuals, ranging from the most severe (extremely unusual, repetitive, self-injurious, and aggressive behavior) to very mild. No one autistic child is alike in the manifestation of their symptoms so treatment options must be devised to treat each autistic child individually.

Autistic children have different ways of learning and experiencing the world around them. Often autistic children have more acute reactions to sensory stimulation such as sound and touch. This results in avoidance of eye contact, physical contact, and oftentimes an aversion to music and other sounds. It is perhaps the way autistic children experience their world that causes difficulties with social interaction, language, and nonverbal communication.

Profound problems with social interaction are the most common symptoms of autism and the most visible. Human beings are social and social interaction is present from birth onward. Children with autism have difficulty making social connections. A developmental milestone is when an infant can follow an object or person with his/her gaze. Autistic children tend to avoid eye contact altogether. They do not actively cuddle or hug but rather they passively accept physical contact or they shy away from it. They may become rigid or flaccid when they are held, cry when picked up, and show little interest in human contact. Such a child does not lift his/her arms in anticipation of being picked up. The child may appear to have formed no attachment to his/her parents, and does not learn typical childhood games, such as "peek-a-boo."
Autistic children do not readily learn social cues. They do not know when or how to react to specific social situations or exchanges. Because of this, autistic children tend to look at and respond to different situations similarly. They do not understand that others have different perspectives and, therefore, autistic children seem to lack empathy.
Because of their problems socially and the inability to translate social interactions appropriately, autistic children seem to have uncontrolled emotional outbursts, expressing themselves in a manner that does not suit the specific social situation of the moment.

Language problems

Verbal communication problems vary greatly for autistic children. Some children do not speak at all. Some will only use one or two words at a time. Some autistic children may develop vocabulary only to loose it. Other autistic children may develop an extensive vocabulary; however, they have difficulty sustaining a natural, "back-and-forth" conversation. Autistic children tend to talk in a sing-song voice or more robotically without emotional inflections. Often autistic children do not take body language into consideration and they take what is being said quite literally. Because of their impinged language skills and the inability to express their needs, autistic children seem to act inappropriately to get what they need. They may grab something without asking or blurt out statements.

Restricted interests and activity

Language and social problems inhibit social play for autistic children. Autistic children do not engage in imaginative play and role playing. They focus on repetition, some focusing on a subject of interest very intensely.
Autistic children often stick to a rigid daily routine. Any variance to the routine may be upsetting to them and result in an extreme emotional response. Repetitive physical behaviors such as rocking, spinning, and arm flapping are also characteristic of autism. The repetitive behaviors are often self-soothing responses to sensory stimulation from the outside world.

Sensory problems

The sensory world poses a real problem to many autistic children, who seem overwhelmed by their own senses. A child with autism may ignore objects or become obsessed with them, continually watching the object or the movement of his or her fingers over it. Some children with autism may react to sounds by banging their head or flapping their fingers. Some high-functioning autistic adults who have written books about their childhood experiences report that sounds were often excruciatingly painful to them, forcing them to withdraw from their environment or try to cope by withdrawing into their own world of sensation and movement.

Diagnosis

There is no medical test for diagnosing autism. Diagnosis is made after careful observation and screening by parents, caregivers, and physicians. Early diagnosis is beneficial in treating the symptoms of autism. Some early warning signs are:

- avoiding eye contact
- avoiding physical contact such as hugs

- inability to play make-believe
- not pointing out interesting objects
- not responding to conversation directed at him/her
- practicing excessively repetitive behaviors
- repeating words or phrases
- loosing skills and/or language after learning them

Once parents feel there is a problem or their pediatrician has identified developmental problems during well-baby check-ups, they can seek out a developmental pediatrician for further diagnosis. There are several screening tests used. They are:

- Childhood Autism Rating Scale (CARS)-a test based on a 15 point scale where specific behaviors are observed by the physician.
- Checklist for Autism in Toddlers (CHAT)-a test to detect autism in 18-month olds that utilizes questionnaires filled out by both the parents and the pediatrician.
- Autism Screening Questionnaire-a 40-item questionnaire for diagnosing children four and older.
- Screening Test for Autism in Two-Year Olds-a direct observation of three skill areas including play, motor imitation, and joint attention.

Some children have a few of the symptoms of autism, but not enough to be diagnosed with the "classical" form of the condition. Children who have autistic behavior but no problems with language may be diagnosed with Asperger syndrome by using the Autism Spectrum Screening Questionnaire, the Australian Scale for Asperger's Syndrome, or the Childhood Asperger Syndrome Test. Children who have no initial symptoms but who begin to show autistic behavior as they get older might be diagnosed with "childhood disintegrative disorder" (CDD), another autistic spectrum disorder. It is also important to rule out other problems that seem similar to autism.

Treatment

Because the symptoms of autism can vary greatly from one person to the next, there is not a single treatment that works for every person. A spectrum of interventions including behavioral and educational training, diet and nutrition, alternative medicine and therapies, and medication should be utilized and fine-tuned to treat the individual. The most strongly recommended treatment option is behavioral and educational training. Early intervention and treatment is key to helping autistic children grow into productive adults.

Educational and behavioral treatment

Several educational and behavioral treatments are:

- Applied Behavior Analysis (ABA)
- speech therapy
- occupational therapy, including sensory integration therapy
- social skills therapy, including play therapy

Typically, behavioral techniques are used to help the child respond and decrease symptoms. This might include positive reinforcement to boost language and social skills. This training includes structured, skill-oriented instruction designed to improve social and language abilities. Training needs to begin as early as possible, since early intervention appears to positively influence brain development.
Most autistic children respond to intervention at home as well as at school. Schools focus on areas where the child may be delayed, such as in speech or socialization. As autistic children grow and move to different phases of childhood and adolescence, parents in collaboration with educators and physicians need to adapt the treatment to best suit the needs of their autistic child.

Medication

No single medication treats symptoms of autism; however, some medications have been used to combat specific needs in autistic children. Drugs can control epilepsy, which afflicts up to 20% of people with autism. Medication can also treat anxiety, depression, and hyperactivity.
Five types of drugs are sometimes prescribed to help the behavior problems of people with autism are:

- stimulants, such as methylphenidate (Ritalin)
- antidepressants, such as fluroxamine (Luvox)
- opiate blockers, such as naltrexone (ReVia)
- antipsychotics
- tranquilizers

Alternative treatment

Many parents report success with megavitamin therapy. Some studies have shown that vitamin B_6 with magnesium improves eye contact and speech and lessens tantrum behavior. Vitamin B_6 causes fewer side effects than other medications and is considered safe when used in appropriate doses. However, not many health practitioners advocate its use in the treatment of autism, citing that the studies showing its benefit were flawed.

DMG (dimethylglycine)

This compound, available in many health food stores, is legally classified as a food, not a vitamin or drug. Some researchers claim that it improves speech in children with autism. Those who respond to this treatment will usually do so within a week. Again, many doctors do not feel that the studies are adequate to promote this treatment.

Diet

Many parents have seen beneficial affects from a gluten-free and casein-free diet. Gluten is a substance found in the seeds of cereal plants such as wheat, barley, oats, and rye. Casein is a protein found in milk. Often people have allergies to these substances without realizing it. Many foods have these substances as an ingredient; however, there is a growing number of gluten-free and casein-free foods available for people that would like to eliminate them from their diets.

Exercise

One researcher found that vigorous exercise (20 minutes or longer, three or four days a week) seems to decrease hyperactivity, aggression, self-injury and other autistic symptoms.

Prognosis

Autism is treatable but not curable. With appropriate treatments adjusted to suit the autistic child as he/she grows up, the symptoms of autism improve. Today, parents and caregivers are focused on providing the best therapies possible in order for autistic children to develop to their highest potential. Because the incidence of autism seems to be increasing at a rapid rate worldwide, enough so that the CDC has voiced concern about its prevalence, there is more awareness of autism and more ongoing research efforts. People with autism have a normal life expectancy and with proper intervention they can lead full lives.

Prevention

Until the cause of autism is discovered, prevention is not possible.

Key Terms

Antidepressants
A type of medication that is used to treat depression; it is also sometimes used to treat autism.

Asperger syndrome
Children who have autistic behavior but no problems with language and no clinically significant cognitive delay.

Encephalitis
A rare inflammation of the brain caused by a viral infection. It has been linked to the development of autism.

Fragile X syndrome
A genetic condition related to the X chromosome that affects mental, physical and sensory development.

Major tranquilizers
The family of drugs that includes the psychotropic or neuroleptic drugs, sometimes used to help autistic people. They carry significant risk of side effects, including Parkinsonism and movement disorders, and should be prescribed with caution.

Opiate blockers
A type of drug that blocks the effects of natural opiates in the system. This makes some people, including some people with autism, appear more responsive to their environment.

Phenylketonuria (PKU)
An enzyme deficiency present at birth that disrupts metabolism and causes brain damage. This rare inherited defect may be linked to the development of autism.

Rubella
Also known as German measles. When a woman contracts rubella during pregnancy, her developing infant may be damaged. One of the problems that may result is autism.

Stimulants
A class of drugs, including Ritalin, used to treat people with autism. They may make children calmer and better able to concentrate, but they also may limit growth or have other side effects.

Tuberous sclerosis

A genetic disease that causes skin problems, seizures, and mental retardation. Autism occurs more often in individuals with tuberous sclerosis.

For Your Information

Resources

Books

- Tuchman, Roberto and Isabelle Rapin, eds.*Autism: A Neurological Disorder of Early Brain Development.* London: MacKeith Press for the International Child Neurology Association, 2006.
- Brock, Stephen E., Shane R. Jimerson, and Robin L. Hansen.*Identifying, Assessing, and Treating Autism at School.* New York: Springer, 2006.

Organizations

- Autism Network International. PO Box 35448, Syracuse, NY 13235.
- Autism Research Institute. (866) 366-3361. <http://www.autism.com>.
- Autism Society of America. 7910 Woodmont Avenue, Suite 300, Bethesda, Maryland 20814-3067. (800) 328-8476. <http://www.autism- society.org>.
- Autism Speaks. 2 Park Avenue, 11th Floor, New York, NY 10016. (212) 252-8584. <http://www.autismspeaks.org>.
- National Fragile X Foundation. PO Box 190488, San Francisco, CA 94119. (800) 688-8765. <http://www.nfxf.org.>
- National Institute of Neurological Disorders and Stroke. PO Box 5801, Bethesda, MD 20824. (800) 352-9424. <http://www.ninds.nih.gov/index.htm.>

Autistic children

Autism

EXHIBIT 57

autism spectrum disorder

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A neurodevelopmental disorder starting in early childhood, characterized by impairments in social interaction and communication and by restricted or repetitive patterns of behavior, with symptoms varying from mild to severe.

EXHIBIT 58

The Prevalence of Parent-Reported Autism Spectrum Disorder Among US Children

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abstract

OBJECTIVES: To estimate the national prevalence of parent-reported autism spectrum disorder (ASD) diagnosis among US children aged 3 to 17 years as well as their treatment and health care experiences using the 2016 National Survey of Children's Health (NSCH).

METHODS: The 2016 NSCH is a nationally representative survey of 50 212 children focused on the health and well-being of children aged 0 to 17 years. The NSCH collected parent-reported information on whether children ever received an ASD diagnosis by a care provider, current ASD status, health care use, access and challenges, and methods of treatment. We calculated weighted prevalence estimates of ASD, compared health care experiences of children with ASD to other children, and examined factors associated with increased likelihood of medication and behavioral treatment.

RESULTS: Parents of an estimated 1.5 million US children aged 3 to 17 years (2.50%) reported that their child had ever received an ASD diagnosis and currently had the condition. Children with parent-reported ASD diagnosis were more likely to have greater health care needs and difficulties accessing health care than children with other emotional or behavioral disorders (attention-deficit/hyperactivity disorder, anxiety, behavioral or conduct problems, depression, developmental delay, Down syndrome, intellectual disability, learning disability, Tourette syndrome) and children without these conditions. Of children with current ASD, 27% were taking medication for ASD-related symptoms, whereas 64% received behavioral treatments in the last 12 months, with variations by sociodemographic characteristics and co-occurring conditions.

CONCLUSIONS: The estimated prevalence of US children with a parent-reported ASD diagnosis is now 1 in 40, with rates of ASD-specific treatment usage varying by children's sociodemographic and co-occurring conditions.

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Dr Kogan conceptualized and designed the study and drafted most of the initial manuscript; Dr Vladutiu conducted the data analyses and assisted with drafting of the initial manuscript; Dr Schieve assisted with drafting of the initial manuscript and provided critical review of subsequent manuscript drafts; Drs Ghandour, Blumberg, Zablotsky, Perrin, Shattuck, Kuhlthau, Harwood, and Lu provided critical reviews on all manuscript drafts; and all authors approved the final manuscript as submitted.

WHAT'S KNOWN ON THIS SUBJECT: Previous studies over the last 20 years have shown an increasing prevalence of autism spectrum disorder (ASD) among US children. Moreover, families of children with ASD have reported greater health care needs and challenges compared with children with other emotional or behavioral conditions.

WHAT THIS STUDY ADDS: In this study, we present new nationally representative data on the prevalence of ASD, reported health care challenges, and estimates on ASD-specific behavioral and medication treatments. The estimated prevalence of US children with parent-reported diagnosis of ASD is now 1 in 40.

To cite: Kogan MD, Vladutiu CJ, Schieve LA, et al. The Prevalence of Parent-Reported Autism Spectrum Disorder Among US Children. *Pediatrics.* 2018;142(6):e20174161

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by persistent deficits in social communication and interactions and restricted, repetitive patterns of behaviors or interests.[1] The prevalence of diagnosed ASD has increased in the United States and globally within the last 30 to 40 years.[2–17] Although not fully understood, this increase likely results from multiple factors including broadening diagnostic criteria, increased provider ascertainment at earlier ages, increased parent awareness, and an increase in some risk factors such as births to older parents.[18–20]

The challenges of ASD are many and varied. Compared with the general population, children with ASD experience an increased prevalence of co-occurring conditions, such as asthma, eczema, gastrointestinal disturbances, and seizures.[21] In addition, 83% of children with ASD have a co-occurrence of ≥1 non-ASD developmental diagnosis.[22] Children with ASD have greater health services needs, including therapy,[23] emergency department care,[24] physician visits, and hospitalizations.[25] Increased and unpredictable needs for health care visits can also affect parents' employment,[26] increase financial and time burdens,[27] and disrupt family routines.[28] Few national studies have compared the disparities in health services and challenges for families having a child with ASD.[27] Although ASD currently has no known cure, the most common treatments to ameliorate the symptoms include behavioral, language, speech, physical, and occupational therapies.[29] Pharmacological agents have been Food and Drug Administration–approved to treat irritability associated with ASD symptoms.[30] The costs of caring for a child with ASD in the United States, including health care and non–health care services, was estimated at $17 081 per year beyond the costs of caring for a child without ASD, with total societal costs of caring for children with ASD estimated at $11.5 billion in 2011.[31]

The 2016 National Survey of Children's Health (NSCH), a nationally and state-representative survey of 50 212 children, ages 0 to 17 years, offers the opportunity to address certain gaps in our knowledge. This study provides the most recent nationally representative estimate on children with ASD. Although the National Health Interview Survey (NHIS) can provide ASD prevalence estimates,[9] the NSCH has a larger sample, and can explore other aspects of the child's condition, including ASD-specific treatments, plus an in-depth examination of their health care needs and experiences. The 2016 NSCH also offers the first opportunity to provide national estimates on ASD-specific drug and behavioral treatments.

METHODS

The 2016 NSCH was designed, directed, and funded by the Health Resources and Services Administration's Maternal and Child Health Bureau. The survey was conducted by the US Census Bureau, which used a national address-based sample for data collection by either Web or mail. The NSCH provides information on the health and well-being of children on the basis of information from their parents or other caregivers (hereafter referred to as "parents"). Questionnaires were available in English or Spanish. The 2016 NSCH used a 2-phased data collection approach: (1) an initial household screener assessed the presence of children, their basic demographic characteristics, and special health care need status and (2) a substantive, age-specific topical questionnaire completed by parents of one randomly selected child per household. Children with special health care needs had a higher probability of selection compared with other children to increase the sample size among this population.

From June 2016 to February 2017, topical questionnaires were completed. The proportion of households known to include children that completed the topical questionnaire was 69.7%. The overall weighted response rate, which includes nonresponse to the screener to identify whether households include children, was 40.7%. Additional details about the survey methodology are available elsewhere.[32] This study was a secondary analysis of publicly available data. The data were collected under Title 13, US Code, Section 8(b). All data products are reviewed for adherence to privacy protection and disclosure avoidance guidelines by the Census Bureau's Disclosure Review Board.

Analyses for this study were limited to 43 283 children aged 3 to 17 years and excluded children ≤2 years (n = 6929). Parents were asked if a doctor or other health care provider had ever told them that their child had "Autism or Autism Spectrum Disorder, including diagnoses of Asperger's Disorder or Pervasive Developmental Disorder." Parents who responded "Yes" were subsequently asked if their child currently has the condition (see Fig 1). For this analysis, children identified as having ASD were those with a parent report of (1) ever told by a doctor and/or other health care provider that their child had ASD and (2) child currently has ASD. In

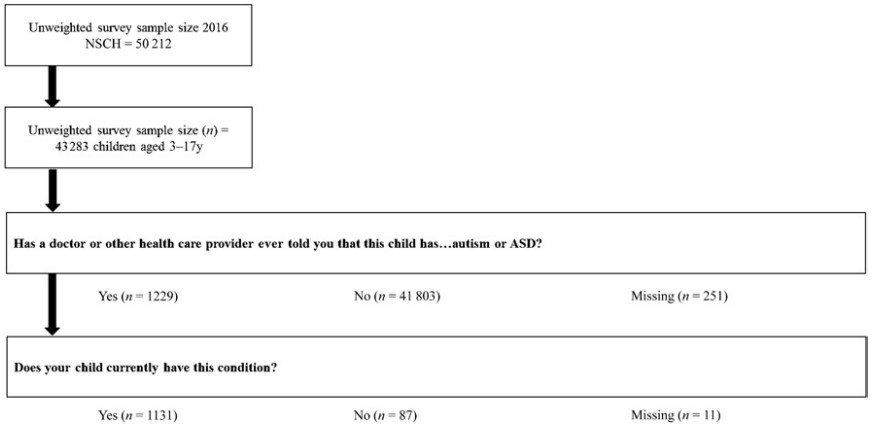

FIGURE 1
Flow diagram of survey-participant progress through the autism and ASD questions, NSCH, 2016.

87 cases, the parent reported that the child was ever diagnosed with ASD but did not currently have the condition (7.1%). These cases were not considered as having ASD for this analysis. Children whose parents did not know or refused to answer either of the ASD questions were excluded from analyses (n = 262; 0.6%)

The prevalence of ASD was estimated overall and by selected demographic, socioeconomic, and childbirth characteristics in Table 1. We also provided unadjusted and adjusted prevalence rate ratios (PRRs). In Table 2 we examined (1) health care service use including medical and behavioral treatments for ASD symptoms and receipt of specialist care other than mental health treatment and/or counseling; and (2) parental frustration, access to treatment, and quality of care received including receipt of care in a medical home. We defined medical home using the American Academy of Pediatrics framework, specifically whether[33] (1) the child had a personal physician or nurse; (2) the child had a usual place for sick care; (3) the family reported no problems obtaining needed referrals; (4) the family reported receipt of family centered care; and (5) the family reported receipt of effective care coordination, if needed. All 5 criteria needed to be met for a child to have a medical home.

We additionally explored how the health care experiences of children with ASD and their families differed from children with other diagnosed emotional, behavioral, and developmental conditions (EBDs) (attention-deficit/hyperactivity disorder [ADHD], anxiety, behavioral and/or conduct problems, depression, developmental delay, Down syndrome, intellectual disability, learning disability, Tourette syndrome), and those without ASD or other EBDs. EBDs were identified analogously to ASD: affirmative responses to both questions of whether the parent was "ever told child had condition" and whether "child currently has the condition." Health care experiences included services used, difficulties accessing needed care, and current receipt of services under a special education or early intervention plan. We estimated weighted percentages, PRRs, and 95% confidence intervals (CIs) comparing children with ASD to children with other EBDs but not ASD, and all other children without EBDs or ASD. The PRRs were adjusted for child age, sex, and race and/or ethnicity.

Finally, we examined factors associated with the likelihood of receiving medication and/or behavioral treatment of ASD-related symptoms among children with ASD and health insurance using multivariable logistic regression. Covariates included sociodemographic characteristics, years since diagnosis (calculated by subtracting age at diagnosis from child's current age), provider type for ASD diagnosis, and presence of co-occurring conditions. (Supplemental Table 4 provides prevalence estimates of co-occurring conditions.) Additionally, the medication model included behavioral treatment, whereas the behavioral treatment model included medication. Adjusted PRRs and 95% CIs are reported.

Missing data for child sex, race, and ethnicity were imputed by using hot deck methods during the weighting process, whereas the household income-to-poverty ratio was imputed by using regression methods. All estimates were weighted by using survey sampling weights available from the Census Bureau to produce estimates that are representative of the US noninstitutionalized child population aged 3 to 17 years. These weights reflect the inverse of the probability that the address was selected for the survey and were adjusted to account for the higher likelihood that children with special health care needs would be selected for the topical interview, as well as nonresponse. Weighted analyses were conducted by using SAS 9.4 (SAS Institute, Inc, Cary, NC) and SAS-callable SUDAAN 11.0 (RTI International, Research Triangle Park, NC).

RESULTS

From these nationally representative data, we estimated the point-prevalence of parent-reported ASD diagnosis in 2016

TABLE 1 Point Prevalence of Parent-Reported Current ASD Among Children Aged 3–17 Years According to Selected Demographic, Socioeconomic, and Birth Characteristics, United States, 2016

Characteristics	No. in Sample (Unweighted)	No. With ASD (Unweighted)	No. With ASD (Weighted) in Thousands	Weighted Prevalence of ASD, %	95% CI	Unadjusted PRR (95% CI)	Adjusted PRR[a] (95% CI)
All children aged 3–17 y	43 021	1131	1529	2.5	2.23–2.81	—	—
Age, y							
3–5	7530	123	235	1.97	1.41–2.74	0.74 (0.51–1.07)	0.81 (0.56–1.18)
6–11	14 925	422	641	2.61	2.15–3.15	0.98 (0.77–1.26)	1.02 (0.79–1.30)
12–17	20 566	586	653	2.65	2.27–3.10	1.00 (referent)	1.00 (referent)
Sex							
Boys	22 064	917	1208	3.88	3.41–4.41	3.63 (2.71–4.85)[b]	3.46 (2.55–4.69)[b]
Girls	20 957	214	321	1.07	0.82–1.39	1.00 (referent)	1.00 (referent)
Race and/or ethnicity							
Hispanic	4734	125	368	2.43	1.76–3.35	0.94 (0.67–1.34)	0.93 (0.64–1.36)
Non-Hispanic white	30 215	790	815	2.57	2.26–2.93	1.00 (referent)	1.00 (referent)
Non-Hispanic African American	2542	80	220	2.79	2.03–3.81	1.08 (0.77–1.52)	0.81 (0.54–1.21)
Non-Hispanic other and/or multiracial	5530	136	126	1.96	1.52–2.51	0.76 (0.57–1.01)	0.75 (0.55–1.03)
Highest level of education by parent in household							
Less than high school	973	27	146	2.56	1.56–4.16	0.94 (0.64–1.80)	0.73 (0.38–1.40)
High school, GED, vocational	5377	160	350	2.98	2.19–4.06	1.26 (0.89–1.77)	0.88 (0.58–1.31)
Some college and/or technical school	9630	289	323	2.42	1.96–2.98	1.02 (0.79–1.31)	0.80 (0.59–1.09)
College degree or higher	26 012	632	672	2.37	2.05–2.74	1.00 (referent)	1.00 (referent)
Family structure[c]							
2 parents, married	31 108	752	832	2.12	1.85–2.43	1.00 (referent)	1.00 (referent)
2 parents, unmarried	2649	86	167	3.36	2.08–5.37	1.58 (0.97–2.59)	1.49 (0.91–2.45)
Single mother	5446	196	378	3.86	3.02–4.93	1.82 (1.37–2.41)[b]	1.47 (1.05–2.05)[b]
Other	3092	84	128	2.29	1.46–3.58	1.08 (0.68–1.72)	1.02 (0.58–1.80)
Household poverty							
<100% FPL	4230	165	455	3.51	2.64–4.66	1.79 (1.29–2.47)[b]	2.06 (1.31–3.22)[b]
100%–199% FPL	6910	219	338	2.48	1.84–3.34	1.27 (0.85–1.88)	1.38 (0.91–2.07)
200%–399% FPL	13 172	333	378	2.3	1.87–2.84	1.17 (0.87–1.59)	1.27 (0.94–1.70)
≥400% FPL	18 709	415	357	1.97	1.62–2.39	1.00 (referent)	1.00 (referent)
Region							
Northeast	8148	246	267	2.74	2.19–3.42	1.23 (0.83–1.82)	1.20 (0.80–1.80)
Midwest	11 266	283	353	2.71	2.22–3.31	1.22 (0.83–1.78)	1.14 (0.79–1.64)
South	12 640	343	577	2.46	2.04–2.95	1.10 (0.76–1.60)	0.99 (0.68–1.43)
West	10 967	259	332	2.23	1.61–3.07	1.00 (referent)	1.00 (referent)
Nativity							
US born	41 230	1101	1495	2.58	2.30–2.91	2.08 (1.25–3.47)[b]	2.34 (1.30–4.21)[b]
Foreign born	1530	30	34	1.24	0.75–2.03	1.00 (referent)	1.00 (referent)
Child born preterm							
Yes	4633	187	279	4.03	3.10–5.23	1.78 (1.33–2.39)[b]	1.71 (1.26–2.31)[b]
No	37 757	923	1206	2.26	1.99–2.58	1.00 (referent)	1.00 (referent)
Age of mother at child's birth, y							
18–24	7394	228	383	2.8	2.24–3.50	1.00 (referent)	1.00 (referent)
25–29	10 340	287	353	2.35	1.87–2.95	0.84 (0.61–1.16)	0.96 (0.68–1.36)
30–34	12 726	298	358	2.21	1.72–2.84	0.79 (0.61–1.16)	0.95 (0.64–1.39)
35–39	7923	185	269	2.84	2.11–3.82	1.01 (0.70–1.47)	1.20 (0.81–1.79)
≥40	2178	64	61	2.4	1.52–3.75	0.85 (0.52–1.41)	1.03 (0.61–1.75)

Note: n = 262 are missing ASD status. Data source: 2016 NSCH. GED, General Educational Development; —, not applicable.

[a] Adjusted for all other demographic, sociodemographic, and birth characteristics shown.

[b] Significant value.

[c] Categories are mutually exclusive.

to be 2.50 per 100 children. This represents an estimated 1.5 million children aged 3 to 17 years (Table 1). After adjustment for selected demographic, socioeconomic, and birth characteristics, ASD prevalence in boys was 3.46 times higher than in girls. Additionally, ASD prevalence was 47% higher for children with single mothers than children in 2-parent, married households, 2.06 times as high for children from households <100% of the federal poverty level (FPL) compared with children from households ≥400% of

TABLE 2 PRRs of Having Selected Health Care Characteristics According to Parent-Reported ASD Status and Other EBDs, United States, 2016

	Children With ASD (n = 1131), Weighted %	Children With Other EBDs[a] (n = 7795), Weighted %	All Other Children (n = 33 014), Weighted %	Children With ASD Versus Children With Other EBDs, [a] Adjusted PRR (95% CI)[b]	Children With ASD Versus All Other Children, Adjusted PRR (95% CI)[b]
Insurance status[c]					
Public only	46.9	40.2	28.4	1.12 (0.98–1.28)	1.68 (1.47–1.91)[d]
Private only	39.9	46.6	59.8	0.88 (0.77–1.02)	0.66 (0.57–0.75)[d]
Other	11.0	9.1	5.4	1.22 (0.88–1.70)	2.10 (1.53–2.87)[d]
Uninsured	2.1	4.1	6.4	0.53 (0.30–0.93)[d]	0.35 (0.20–0.60)[d]
Visited health care provider in past 12 mo	93.9	89.6	83.6	1.05 (1.01–1.08)[d]	1.12 (1.09–1.15)[d]
Had a usual place for preventive care	94.8	94.7	91.3	1.00 (0.95–1.05)	1.04 (0.99–1.09)
Any dental visit in past 12 mo	84.3	88.8	87.1	0.96 (0.91–1.02)	0.96 (0.90–1.01)
Received treatment or counseling from mental health provider	45.9	39.6	3.0	1.28 (1.13–1.45)[d]	15.18 (12.76–18.05)[d]
Big problem to get mental health treatment or counseling (among those who needed to see a mental health professional)	22.9	15.9	8.5	1.44 (1.04–1.98)[d]	2.71 (1.46–5.05)[d]
Used medication because of emotions or behavior	42.1	37.2	0.4	1.18 (1.02–1.36)[d]	84.56 (59.19–120.81)[d]
Saw specialist other than mental health professional	35.0	27.2	11.7	1.32 (1.11–1.57)[d]	2.97 (2.52–3.50)[d]
Big problem to get the specialist care needed (among those who needed to see a specialist other than mental health professional)	18.4	12.0	4.3	1.40 (0.86–2.29)	3.61 (2.03–6.45)[d]
Used alternative health care or treatment	15.5	11.4	5.4	1.43 (1.07–1.92)[d]	2.83 (2.15–3.71)[d]
Did not get needed health care	10.8	8.3	2.1	1.37 (0.93–2.02)	5.63 (3.86–8.21)[d]
Type of care not received (among those who did not get needed care)					
Medical and/or dental	32.1	63.2	86.0	0.56 (0.36–0.88)[d]	0.41 (0.26–0.64)[d]
Hearing and/or vision	24.5[e]	27.7	31.7	0.83 (0.39–1.77)	0.69 (0.32–1.52)
Mental health	63.1	42.4	9.5[e]	1.46 (1.07–1.98)[d]	6.82 (3.45–13.51)[d]
Other	27.6	20.4	5.8[e]	1.27 (0.64–2.53)	4.02 (1.74–9.28)[d]
Usually or always parents frustrated in efforts to get services	14.9	7.4	1.5	2.02 (1.45–2.82)[d]	10.53 (7.51–14.77)[d]
Child had a special education or early intervention plan	87.5	41.3	6.1	2.15 (1.99–2.32)[d]	13.83 (12.37–15.45)[d]
Age at time of first plan, y (among those with a special education or early intervention plan)					
0–2	20.2	15.7	24.2	1.12 (0.84–1.49)	0.83 (0.62–1.11)
3–5	57.7	31.3	39.8	1.77 (1.53–2.05)[d]	1.46 (1.23–1.73)[d]
6–8	14.1	33.5	22.5	0.45 (0.34–0.60)[d]	0.61 (0.44–0.85)[d]
≥9	8.0	19.6	13.5	0.50 (0.34–0.72)[d]	0.65 (0.42–1.01)
Currently receiving services under plan (among those with a special education or early intervention plan)	84.3	78.0	37.3	1.07 (1.01–1.14)[d]	2.27 (1.97–2.61)[d]
Ever received special services to meet developmental needs	89.0	40.0	9.3	2.22 (2.05–2.41)[d]	9.32 (8.52–10.18)[d]
Age when began receiving these services, y (among those who received special services)					
0–2	27.8	22.7	25.8	1.14 (0.92–1.42)	1.15 (0.93–1.42)
3–5	52.0	35.8	48.9	1.42 (1.22–1.66)[d]	1.06 (0.91–1.22)
6–8	12.7	29.1	19.6	0.46 (0.35–0.62)[d]	0.62 (0.45–0.85)[d]
≥9	7.6	12.4	5.7	0.66 (0.41–1.06)	1.25 (0.72–2.17)
Currently receiving special services for developmental needs (among those who received special services)	76.2	59.2	23.7	1.27 (1.17–1.37)[d]	3.30 (2.82–3.87)[d]
Had a medical home	31.6	41.5	50.0	0.77 (0.65–0.91)[d]	0.63 (0.54–0.73)[d]
Had at least 1 personal doctor or nurse	78.4	76.7	71.4	1.03 (0.95–1.11)	1.10 (1.02–1.18)[d]
Had usual sources for sick care	87.6	80.7	78.7	1.09 (1.03–1.15)[d]	1.11 (1.05–1.17)[d]
Received family-centered care (among those with a health care visit in the past 12 mo)	72.6	82.7	87.2	0.89 (0.82–0.96)[d]	0.84 (0.77–0.91)[d]
Had no problems getting referrals when needed (among those who needed a referral)	66.5	71.5	81.2	0.93 (0.80–1.08)	0.81 (0.71–0.93)[d]
Received all needed components of care coordination (among those who needed care coordination)	46.0	60.2	76.3	0.76 (0.65–0.88)[d]	0.59 (0.51–0.68)[d]

Note: n = 1343 are missing data on EBD status (n = 262 are missing data on autism, and the rest are missing data on at least 1 of the other EBDs we assessed). Data source: 2016 NSCH.

[a] EBDs include ADHD, anxiety, behavioral or conduct problems, depression, developmental delay, Down syndrome, intellectual disability, learning disability, and Tourette syndrome. In addition, 92.3% of children with ASD were reported to have other EBDs.

[b] Adjusted for age, sex, and race and/or ethnicity.

[c] Other insurance includes private, public, and unspecified.

[d] Significant value.

[e] Estimates are considered unreliable and should be used with caution. Data have a relative SE >30%.

the FPL and 71% higher in children born preterm than term. Prevalence was 2.34 times as high for US-born than foreign-born children. The same variables were also significant when unadjusted.

Children with a parent-reported ASD diagnosis (including those who had other EBDs) had more needs and difficulties regarding health care access and use compared with children without an ASD diagnosis but who had other EBDs, and children with parent-reported ASD also had more needs and difficulties compared with other children without ASD or EBDs (Table 2). Compared with children with other EBDs, children with ASD were more likely in the past 12 months to have received mental health counseling, seen a specialist besides a mental health professional, used alternative health care or treatments, ever have a special education or early intervention plan, and currently receive special services for developmental needs. However, children with ASD were also more likely to have parents who reported difficulties with health care. They were 44% more likely to report problems getting mental health treatment, 46% more likely to report not receiving needed mental health care, 2.02 times more likely to report being usually or always frustrated in getting services, 23% less likely to have a medical home, and 24% less likely to receive needed care coordination. Children with ASD also had greater needs and difficulties compared with all other children (without other EBDs).

Of children with a parent-reported ASD diagnosis, ~27% were taking medication for ASD-related symptoms. Children with ASD from households where the highest level of education was less than high school were 74% more likely to be currently taking medication for ASD symptoms compared with children from households with at least a college degree (Table 3). In addition, current medication use varied regionally and was higher among children who were diagnosed >7 years ago, who had attention-deficit disorder (ADD) and/or ADHD or behavioral and/or conduct problems, and who received behavioral treatment of their ASD symptoms in the past 12 months. Children in 2-parent, unmarried households were less likely than children in 2-parent, married households to be taking medication for ASD.

Approximately 64% of children with a parent-reported ASD diagnosis received behavioral treatment in the past 12 months. Children with behavioral and/or conduct problems had a higher prevalence of behavioral treatment, as well as those currently taking medications for ASD-related symptoms. US-born children or those diagnosed with ADD and/or ADHD were less likely to have received behavioral treatment.

DISCUSSION

We used the recently released 2016 NSCH to estimate a nationally representative prevalence for children with a parent-reported ASD diagnosis of 2.50%. This is the fourth ASD prevalence report from the NSCH; however, because of several notable updates to the NSCH data collection, comparisons of the ASD prevalence estimates presented here with previously published NSCH estimates must be done cautiously because we cannot tell what proportion was explained by internal survey changes rather than external factors. The 2016 NSCH was conducted by using an address-based sample. Families responded by either mail or the Internet, whereas previous surveys were administered by telephone. In addition, question wording was changed twice. In the 2007 survey, the wording was expanded to add specificity and align with the Diagnostic and Statistical Manual of Mental Disorders fourth edition.[1] Also, beginning with the 2007 survey, the ASD case definition was more strictly defined on the basis of parents' affirmative responses to 2 questions ("ever diagnosed with ASD" and "currently has ASD") to exclude diagnoses that may have been lost because of maturation, treatment, or new information.[34] Questions were also modified slightly in the 2016 NSCH to reflect changes in the most recent American Psychiatric Association criteria.[1]

In addition to the NSCH, population-based ASD prevalence estimates for US children have been reported from the NHIS, a nationally representative in-person household survey that includes ASD questions similar to those in the NSCH,[5,9,17] and the Autism and Developmental Disabilities Monitoring Network (ADDM), an ongoing surveillance system in local population-based areas in which ASD cases are identified through education and health records review.[35] The most recent published NHIS ASD prevalence estimate (2.76%) is higher than that reported here but is based solely on ever receiving a diagnosis.[36] The estimate of current ASD from the 2016 NHIS is 2.47%, which virtually matches the NSCH estimate reported here.[37]

The most recent composite ADDM prevalence estimate (1.68%), using 2014 data, was higher than the previous estimate of 1.46% using 2012 data, although this was still lower than the ASD estimate from the 2016 NSCH.[35,38] Any conclusions from this latter comparison must be tempered given that estimates from these different systems reflect different years, populations (11 local US populations versus the entire United States), and ages (children aged 8 years in ADDM vs 3–17 years

TABLE 3 Percentage of Children Aged 3–17 Years Who Took Medication for ASD or Received Behavioral Treatment of ASD, Among Children Aged 3–17 Years Who Currently Have ASD and are Insured, United States, 2016

	Medication for ASD (n = 323)			Behavioral Treatment of ASD (n = 686)		
	Weighted %	Adjusted PRR[a]	95% CI	Weighted %	Adjusted PRR[a]	95% CI
Overall	27.4	—	—	64.2	—	—
Sex						
Boys	30.1	1.30	0.89–1.89	63.1	0.96	0.81–1.14
Girls	17.4	1.00	Referent	68.4	1.00	Referent
Race and/or ethnicity						
Hispanic	15.5	0.89	0.60–1.31	67.7	1.19	0.99–1.42
Non-Hispanic white	30.1	1.00	Referent	62.5	1.00	Referent
Non-Hispanic African American	41.2	1.12	0.79–1.58	67.2	1.10	0.89–1.34
Non-Hispanic other and/or multiracial	21.1	0.90	0.58–1.39	59.9	0.92	0.71–1.19
Highest level of education by parent in household						
Less than high school	55.5[b]	1.74[c]	1.17–2.59[c]	49.5[b]	0.64	0.39–1.05
High school, GED, vocational	23.4	0.99	0.67–1.47	75.0	1.10	0.91–1.34
Some college and/or technical school	23.8	0.88	0.61–1.26	63.5	1.00	0.83–1.20
College degree or higher	24.3	1.00	Referent	63.6	1.00	Referent
Family structure						
2 parents, married	24.5	1.00	Referent	64.8	1.00	Referent
2 parents, unmarried	19.9[b]	0.40[c]	0.19–0.82[c]	71.7	1.13	0.91–1.40
Single mother	37.7	0.88	0.63–1.22	56.1	0.80	0.63–1.02
Other	26.9	0.76	0.49–1.19	80.1	1.21	0.98–1.49
Household poverty						
<100% FPL	27.6	1.03	0.57–1.84	68.7	1.08	0.81–1.43
100%–199% FPL	34.3	1.33	0.77–2.27	58.6	0.98	0.71–1.35
200%–399% FPL	22.5	1.09	0.73–1.63	64.9	1.00	0.82–1.24
≥400% FPL	26.2	1.00	Referent	63.1	1.00	Referent
Insurance status[d]						
Public only	32.4	1.08	0.73–1.60	67.3	0.98	0.80–1.19
Private only	22.4	1.00	Referent	64.4	1.00	Referent
Other	24.6	1.03	0.71–1.49	50.2	0.80	0.62–1.03
Region						
Northeast	26.3	1.41	0.89–2.24	67.5	1.11	0.88–1.41
Midwest	34.4	1.95[c]	1.26–3.00[c]	61.0	1.03	0.81–1.31
South	32.4	1.48	0.99–2.23	66.0	1.11	0.88–1.41
West	12.4	1.00	Referent	61.9	1.00	Referent
Nativity						
US born	27.2	0.73	0.42–1.27	64.0	0.77[c]	0.63–0.93[c]
Foreign born	39.4[b]	1.00	Referent	76.1[b]	1.00	Referent
Years since first diagnosis[e]						
0–2	18.1	1.00	Referent	68.9	1.00	Referent
3–6	22.5	1.12	0.76–1.65	65.1	0.98	0.81–1.18
≥7	42.9	1.66[c]	1.19–2.32[c]	62.8	0.92	0.77–1.12
Type of health care provider to give diagnosis						
Primary care provider	28.7	1.00	Referent	55.7	1.00	Referent
Specialist	24.4	0.82	0.61–1.11	65.7	1.15	0.89–1.49
Psychologist (school and nonschool)	23.6	0.70	0.49–1.01	59.7	1.16	0.90–1.51
Psychiatrist	35.2	1.03	0.74–1.44	69.7	1.18	0.89–1.57
Other or don't know	34.0	0.70	0.45–1.10	75.3	1.42[c]	1.10–1.84[c]
Co-occurring conditions						
ADD and/or ADHD (ref = no ADD and/or ADHD)	47.5	3.30[c]	2.14–5.07[c]	62.8	0.84[c]	0.70–0.99[c]
Anxiety problems (ref = no anxiety problems)	39.9	1.16	0.89–1.51	68.0	1.05	0.89–1.24
Developmental delay (ref = no developmental delay)	32.1	1.30	0.98–1.73	68.2	1.08	0.93–1.26
Behavioral or conduct problems (ref = no behavioral or conduct problems)	39.2	1.72[c]	1.26–2.33[c]	72.0	1.39[c]	1.16–1.66[c]
Currently taking medication for ASD	—	—	—	75.3	1.31[c]	1.13–1.52[c]
Received behavioral treatment of ASD	32.0	1.64[c]	1.18–2.28[c]	—	—	—

Data source: 2016 NSCH. GED, General Educational Development; ref, referent; —, not applicable.

[a] Adjusted for all other demographic, sociodemographic, and birth characteristics shown.

[b] Estimates are considered unreliable and should be used with caution. Data have a relative SE >30% or unweighted denominator n < 30.

[c] Significant value.

[d] Other insurance includes private, public, and unspecified.

[e] The analyses included those with missing information on age at diagnosis (n = 95).

in NSCH).[35] The estimates derived from previous versions of the NSCH and ADDM are similar when comparing overlapping years.[7,39] In addition, the estimate from the NSCH falls within the range of estimates (1.31%–2.93%) from the 11 ADDM sites, and sites able to access both health and education records had higher prevalence estimates compared with sites accessing only health records.

Because there is no biological marker, ASD is a particularly challenging condition to track; thus, multiple systems with different case ascertainment strategies and supplemental data collection for children with ASD are useful in developing a full picture of ASD prevalence. Findings from the NHIS, NSCH, and ADDM each contribute unique information that, when combined, helps form a comprehensive picture of ASD among children in the United States.

Estimates reported here indicate less variation in prevalence rates across child age, race and/or ethnicity, or socioeconomic groups than observed in earlier ASD prevalence studies.[7,40] Policy changes such as the 2007 AAP recommendations for universal screening by 18 to 24 months may have helped to increase ASD diagnosis among young children, thus reducing the prevalence disparity by age.[41] These types of recommendations might have also had broader impacts by increasing provider and parent awareness of ASD generally, possibly contributing to increased diagnoses in traditionally underserved racial-ethnic groups. In addition, among the 87 cases of ever but not current autism, we do not know if the children achieved optimal outcomes or were initially misdiagnosed.[42]

The current study demonstrates that families face challenges in accessing healthcare services. Because children with ASD are likely to need multiple types of services and accompanying care coordination,[43–46] the challenge of achieving a medical home appears evident among children with ASD in that they were less likely to meet the medical home criteria than other children. Although children with ASD were more likely to have a personal doctor or nurse and have a usual source for sick care, they were less likely to get needed referrals for specialty care. Consistent with previous studies, we found that children with ASD also had high rates of co-occurring mental health conditions or EBD diagnoses.[21,22,35] We were not able to determine if some of the co-occurring EBD diagnoses were precursor diagnoses made as part of the diagnostic trajectory of a child receiving an ASD diagnosis versus being distinct co-occurring conditions. Nonetheless, our findings indicate that children with ASD face many developmental challenges; they particularly need referrals and care coordination.

Treatments for ASD-related symptoms have primarily been focused on behavior change and/or skill building.[19] Psychotropic medications have been used to relieve some emotional and behavioral symptoms, such as challenging behaviors, irritability, anxiety, and hyperactivity.[47] However, there are no pharmacologic options for treating the core deficits of ASD.[48] In the current study, we found that 27% of US children with ASD took medication for ASD-related symptoms and 64% received behavioral treatment. Children with ASD who had reported diagnoses of behavioral and/or conduct problems were significantly more likely to have their ASD symptoms treated with medication and receive behavioral treatment. Children with ASD who were also reported to be diagnosed with ADD and/or ADHD were 3.30 times as likely to be treated with medication for their ASD symptoms, whereas they were only 84% as likely to receive behavioral therapy. Other studies have revealed a higher rate of psychotropic medication use for children with ASD when there was a comorbid diagnosis of ADHD.[49]

Our study has several strengths, including its large, nationally representative sample, which enabled examination by subgroups, and the comprehensive set of questions on ASD, co-occurring conditions, and health care experiences. However, there are also limitations. The data are cross-sectional and based on parent report of diagnoses. Undiagnosed ASD could not be assessed, so the prevalence of ASD may be underestimated, especially for younger children. Parents' reports of diagnoses were not clinically validated; however, studies have revealed a high concordance rate (93%–98%) between parent reports of their child receiving a definite ASD diagnosis and clinician's diagnosis in verbal children.[50,51] An ASD registry in the United Kingdom revealed the reliability of parent-reported ASD diagnoses of children was 96% when compared with clinical reports.[52] In addition, there was a high level of agreement between parents and clinicians on ASD-related behaviors at 12 months of age.[53] However, family characteristics may affect the degree of parent-clinician agreement.[54] Studies have also revealed a high test-retest reliability on maternal report of their child's mental health conditions.[55] Other studies found good agreement between parental report and pediatricians' records,[56] and fair-to-excellent agreement between parents and day care providers on recall of early symptoms associated with ASD.[57] Although the specific autism diagnosis question from NHIS and NSCH has not been

externally validated, the consistency of results from the NSCH, NHIS, and ADDM when compared from the same approximate period suggests a degree of reliability of these estimates.[5] There is also the potential for nonresponse bias. The Census Bureau applied a nonresponse weighting adjustment that significantly mitigated any identified differential nonresponse.[58] However, there could be additional sources of bias, such as selection bias, that are not controlled by the weighting adjustments. In addition, the NSCH applied poststratification adjustments to ensure that sociodemographic subgroups were appropriately represented in the estimates.

From the 2016 NSCH data, we estimated that 1 in 40 children in the United States have a parent-reported ASD diagnosis. Because ASD is a lifelong condition for most children, an important area of future research would be to study life course development and understand what factors influence health and well-being in young adulthood and beyond for these children.

ACKNOWLEDGMENT

We thank Alicia A. Livinski, MPH, MLS, at the National Institutes of Health Library, for her dedication and thorough library research work.

ABBREVIATIONS

ADD: attention-deficit disorder
ADDM: Autism and Developmental Disabilities Monitoring Network
ADHD: attention-deficit/hyperactivity disorder
ASD: autism spectrum disorder
CI: confidence interval
EBD: emotional, behavioral, and developmental condition
FPL: federal poverty level
NHIS: National Health Interview Survey
NSCH: National Survey of Children's Health
PRR: prevalence rate ratio

DOI: https://doi.org/10.1542/peds.2017-4161

Accepted for publication Aug 27, 2018

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Dr Shattuck is supported by grant R01MH104423 from the National Institute of Mental Health. Funded by the National Institutes of Health (NIH).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2018-2950.

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EXHIBIT 59

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Morbidity and Mortality Weekly Report (*MMWR*)

Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016

Surveillance Summaries / March 27, 2020 / 69(4);1–12

Please note:. *This report has been corrected. An erratum has been published..*

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View suggested citation

Abstract

Problem/Condition: Autism spectrum disorder (ASD).

Period Covered: 2016.

Description of System: The Autism and Developmental Disabilities Monitoring (ADDM) Network is an active surveillance program that provides estimates of the prevalence of ASD among children aged 8 years whose parents or guardians live in 11 ADDM Network sites in the United States (Arizona, Arkansas, Colorado, Georgia, Maryland, Minnesota, Missouri, New Jersey, North Carolina, Tennessee, and Wisconsin). Surveillance is conducted in two phases. The first phase involves review and abstraction of comprehensive evaluations that were completed by medical and educational service providers in the community. In the second phase, experienced clinicians who systematically review all abstracted information determine ASD case status. The case definition is based on ASD criteria described in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*.

Results: For 2016, across all 11 sites, ASD prevalence was 18.5 per 1,000 (one in 54) children aged 8 years, and ASD was 4.3 times as prevalent among boys as among girls. ASD prevalence varied by site, ranging from 13.1 (Colorado) to 31.4 (New Jersey). Prevalence estimates were approximately identical for non-Hispanic white (white), non-Hispanic black (black), and Asian/Pacific Islander children (18.5, 18.3, and 17.9, respectively) but lower for Hispanic children (15.4). Among children with ASD for whom data on intellectual or cognitive functioning were available, 33% were classified as having intellectual disability (intelligence quotient [IQ] ≤70); this percentage was higher among girls than boys (39% versus 32%) and among black and Hispanic than white children (47%, 36%, and 27%, respectively). Black children with ASD were less likely to have a first evaluation by age 36 months than were white children with ASD (40% versus 45%). The overall median age at earliest known ASD diagnosis (51 months) was similar by sex and racial and ethnic groups; however, black children with IQ ≤70 had a later median age at ASD diagnosis than white children with IQ ≤70 (48 months versus 42 months).

Interpretation: The prevalence of ASD varied considerably across sites and was higher than previous estimates since 2014. Although no overall difference in ASD prevalence between black and white children aged 8 years was observed, the disparities for black children persisted in early evaluation and diagnosis of ASD. Hispanic children also continue to be identified as having ASD less frequently than white or black children.

Public Health Action: These findings highlight the variability in the evaluation and detection of ASD across communities and between sociodemographic groups. Continued efforts are needed for early and equitable identification of ASD and timely enrollment in services.

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Introduction

Autism spectrum disorder (ASD) is a developmental disability characterized by persistent impairments in social interaction and the presence of restricted, repetitive patterns of behaviors, interests, or activities (*1*). CDC has been tracking the prevalence of ASD since 1996, beginning with children in metropolitan Atlanta, Georgia (*2*). Subsequently, CDC established the Autism and Developmental Disabilities Monitoring (ADDM) Network, which has reported ASD prevalence in multiple communities in even-numbered years since 2000.

The previous ADDM Network ASD prevalence estimate was 16.8 per 1,000 (one in 59) children aged 8 years in 2014 (*3*). This is approximately 2.5 times higher than the first ADDM Network ASD prevalence estimates of 6.7 (one in 150) from 2000 and 2002 (*4–7*). Findings from each surveillance year between 2000 and 2014 have included variability and disparities in the prevalence of ASD (*3–5*,*8–11*). In contrast to other developmental disabilities (*12–15*), the ADDM Network reported higher ASD prevalence among more socioeconomically advantaged groups and among children classified as non-Hispanic white (white) than among other groups (*16*,*17*). Overall, the magnitude of prevalence differences by race and ethnicity has declined in recent years (*3*,*17*). Reduction of these disparities might indicate progress toward enhanced detection of ASD among all children.

Timely evaluation and identification of ASD among young children continue to be important public health goals (*18*,*19*) because evidence links early treatment and services for ASD with improved outcomes (*20*–*23*). Although greater numbers of children are identified as having ASD over time, previous ADDM Network findings suggest little overall change in the median age at ASD diagnosis (range: 50–56 months), and fewer than half of children with ASD had a record of a developmental evaluation by age 36 months (*3*–*5*,*8*–*11*). However, considerable variability has been reported between communities in both ASD prevalence and the ages at which ASD is diagnosed.

This report provides the latest available data on ASD prevalence among children aged 8 years living in ADDM Network sites in 2016, including variations in prevalence by site and demographic characteristics, median ages when children are evaluated and ASD is diagnosed, and co-occurrence of intellectual disability. Pediatric health care providers, educators, researchers, service providers, and policymakers can use these data to anticipate service needs in their communities and help develop policies that ensure early and comprehensive identification of ASD.

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Methods

Surveillance Sites and Procedures

The ADDM Network was composed of 11 sites for surveillance year 2016 (Arizona, Arkansas, Colorado, Georgia, Maryland, Minnesota, Missouri, New Jersey, North Carolina, Tennessee, and Wisconsin). Children included in ADDM surveillance year 2016 were born in 2008 and had a parent or guardian who lived in one of 11 surveillance sites in 2016. Each site selected a portion of its state (except Arkansas, which included the entire state) to monitor ASD among children aged 8 years in 2016. All sites functioned as public health authorities under the Health Insurance Portability and Accountability Act of 1996 Privacy Rule and met applicable local institutional review board, privacy, and confidentiality requirements under 45 CFR 46 (*24*). The racial and ethnic composition of populations in ADDM Network sites is provided (Supplementary Table 1, https://stacks.cdc.gov/view/cdc/85386).

Case Ascertainment and Surveillance Case Definition

The ADDM Network uses a multiple-source, records-based surveillance methodology developed by CDC's Metropolitan Atlanta Developmental Disabilities Surveillance Program (*2*,*24*). The ADDM Network ASD surveillance methodology is a two-phase process that has been described previously (*3*). In brief, in the first phase, ADDM Network staff review records from medical, education, and service providers (e.g., autism specialty clinics or intervention providers) in the community after requesting records that include various billing codes from the *International Classification of Disease, Ninth Revision* (ICD-9) or *International Classification of Diseases, Tenth Revision* (ICD-10) or special education exceptionalities (Supplementary Table 2, https://stacks.cdc.gov/view/cdc/85386). If any record contains an indication of ASD, the child's evaluations and other information (e.g., intelligence quotient [IQ] tests) are abstracted and compiled from all available sources in the community. Although all ADDM Network sites use records from medical and service providers, not all sites have complete access to education records.

In the second phase, an ADDM Network clinician reviews the deidentified, compiled record for each child to determine ASD case status. The ADDM Network ASD case definition is based on the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5), and the process for scoring the features of the surveillance case definition have been described previously (*3*,*25*,*26*). ADDM Network clinicians might assign ASD case status if documented evidence satisfies the behavioral criteria for the ASD case definition, or if the child has an established ASD diagnosis. ADDM Network clinicians might decide a child who otherwise meets ASD surveillance criteria should not be included as a case because of insufficient or conflicting information or if other conditions better account for the child's symptoms. Another clinician performs a secondary review if the first reviewer indicates uncertainty. To monitor interrater reliability, 10% of records

were randomly selected for an independent review (ASD case status kappa = 0.89) (Supplementary Table 3, https://stacks.cdc.gov/view/cdc/85386). At most ADDM Network sites, clinicians also applied the previous ASD case definition based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR) for at least a portion of the children with abstracted information.

Additional Data Sources and Variable Definitions

Population denominators were obtained from the National Center for Health Statistics vintage 2018 bridged-race postcensal population estimates for 2016 (*27*). For study areas comprising subcounty school districts, a standardization process using public school enrollment counts was used to adjust the population estimates (Supplementary Methods, https://stacks.cdc.gov/view/cdc/85386). Each site linked each child to birth certificate information from their state. When successful, this linkage indicates which children were born in the state that they lived in at age 8 years and provides additional demographic information. Information about race and ethnicity came from information abstracted from the medical or education records, which was augmented by data from birth certificates and data from administrative or billing information. Children with race coded as “other” or “multiracial” were excluded from race-specific estimates, as were American Indian/Alaskan Native children because of small numbers.

Age at first developmental evaluation on record was based on each child’s abstracted evaluation information and restricted to children born in the state (or ADDM Network surveillance area in Minnesota) where the ADDM Network site is located. Age at first ASD diagnosis was based on the age of a child when an examiner recorded an ASD diagnostic statement or noted the child’s age when another provider previously diagnosed ASD. Intellectual disability status was based on IQ scores ≤70 on a child’s most recent test available through 2016. A child without an IQ score also could be classified as having intellectual disability on the basis of an examiner’s statement of intellectual disability in a developmental evaluation. Children were considered to have community-identified ASD if their records contained any of the following: 1) a diagnostic statement from a qualified professional of autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), Asperger disorder, or ASD; 2) any ASD ICD billing code at any time from birth through 2016; or 3) receipt of (or met eligibility for) special education services under the autism classification in public school.

Analytic Methods

Prevalence was calculated as the number of children with ASD per 1,000 children aged 8 years in the defined population or subgroup. Overall prevalence estimates included all children identified with ASD. Results for the combined (overall) total include data from all sites unless otherwise noted. Ninety-five percent confidence intervals (CIs) for prevalence, proportions, and prevalence ratios were calculated using the Wilson score method. Pearson chi-square tests were performed for comparison of proportions, and the Mantel-Haenszel (Woolf) test of homogeneity was used to compare prevalence ratios across sites. Permutation tests were conducted to test differences in medians. Statistical tests with p values <0.05 were considered statistically significant, as were 95% CIs that excluded 1.0 for prevalence ratios. Cumulative incidence of ASD diagnoses was calculated as the total children with ASD diagnosed during or before a given month of age, divided by the total population of children aged 8 years in the surveillance area. R software (version 3.5.3; R Foundation) and additional packages were used to conduct analyses. Additional information about the statistical software is available (Supplementary Table 4, https://stacks.cdc.gov/view/cdc/85386).

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Results

ASD Prevalence

The combined ASD prevalence, with data from all 11 sites, was 18.5 per 1,000 (one in 54) children aged 8 years. ASD prevalence ranged from 13.1 per 1,000 children aged 8 years (one in 76) in Colorado to 31.4 per 1,000 children aged 8 years (one in 32) in New Jersey (Table 1). The estimate for New Jersey was higher than for every other ADDM Network site. Two sites with limited or no access to education records had the lowest ASD prevalence estimates (Colorado [13.1] and Missouri [13.6]). ASD prevalence among boys was higher than among girls (29.7 versus 6.9). The combined male-to-female prevalence ratio was 4.3:1; site-specific ratios ranged from 3.4:1 to 4.7:1, with little evidence of heterogeneity by site.

Comparisons between surveillance years 2014 and 2016 are available (Supplementary Table 5, https://stacks.cdc.gov/view/cdc/85386). Among five sites that used the DSM-5–based case definition within the same geographic areas in 2014 and 2016, Maryland and Tennessee reported similar ASD prevalence in both years, whereas Arkansas, New Jersey, and Wisconsin reported increases. In the subset of children whose records also were reviewed using ADDM Network DSM-IV-TR ASD criteria, the DSM-IV-TR criteria classified 2% more ASD cases than DSM-5 criteria (Supplementary Table 6, https://stacks.cdc.gov/view/cdc/85386).

Overall ASD prevalence per 1,000 children aged 8 years was similar among white and non-Hispanic black (black) children (18.5 and 18.3, respectively) (Table 2). The white-to-black ASD prevalence ratio for the combined ADDM Network was 1.0; however, the prevalence ratio for white to black children was >1.0 in two ADDM Network sites (Arkansas and New Jersey). ASD prevalence among Asian/Pacific Islander children was 17.9 and similar to that among white and black children. ASD prevalence among Hispanic children was 15.4, which was lower than the prevalence among white and black children (white-to-Hispanic prevalence ratio and black-to-Hispanic prevalence ratio: 1.2). Numerator and denominator counts by race and ethnicity are available (Supplementary Table 7, https://stacks.cdc.gov/view/cdc/85386).

Co-Occurring Intellectual Disability

Ten of the 11 ADDM Network sites collected information on intellectual functioning for at least 60% of children meeting the ASD case definition (range: 65% [Maryland and Wisconsin] to 96% [Arkansas]). Similar proportions of cases among boys and girls had information on intellectual ability (80% versus 78%), as did white and black children (81% versus 79%). Across states, greater absolute variability was reported in the prevalence of ASD without intellectual disability than ASD with intellectual disability at the 10 sites (Figure 1).

Among children meeting ASD case status who had IQ information, 33% were classified as having intellectual disability (IQ ≤70) at their most recent test or examination, 24% had an IQ in the borderline range (IQ 71–85), and 42% had an IQ in the average or higher range (IQ >85) (Table 3). The percentage of children with co-occurring intellectual disability varied by site (range: 25% [New Jersey] to 42% [Georgia]). Overall, a higher percentage of girls than boys was classified as having intellectual disability (40% versus 32%), and black and Hispanic children were more likely than white children to be classified as having intellectual disability (47%, 36%, and 27%, respectively) (Supplementary Figures, https://stacks.cdc.gov/view/cdc/85386).

Age at First Evaluation and ASD Diagnosis

Among 3,981 children aged 8 years with ASD who were born in the state of residence, 44% were evaluated by age 36 months, with wide variation across ADDM Network sites (range: 33% [Arkansas] to 62% [North Carolina]) (Table 4). The median age at first evaluation ranged from 29 months (North Carolina) to 46 months (Arkansas). A higher percentage of girls was evaluated by age 36 months than boys (48% versus 43%) (Table 4). The majority of children with ASD and IQ ≤70

(58%) were evaluated by age 36 months, compared with 38% of children with IQ >70. The percentage of children with ASD evaluated by age 36 months varied by race and ethnicity: 45% among white children, 43% among Hispanic children, and 40% among black children.

Of the 5,108 children with ASD, 3,764 (74%) had an evaluation containing a statement of a clinical ASD diagnosis. Among those 3,764 children, the median age at ASD diagnosis was 51 months (range: 38 months [North Carolina] to 57 months [Arizona]) (Table 5). Children with ASD and IQ ≤70 had a median age at diagnosis of 44 months, whereas children with IQ >70 had a median age at diagnosis of 57 months. Among children with ASD and IQ ≤70, black children had an older median age at diagnosis than white children (48 versus 42 months). The cumulative incidence of ASD diagnoses indicates that community providers in New Jersey diagnosed more ASD cases by age 3 years than any other ADDM Network site, although the median age at diagnosis in New Jersey was the same as that of the overall ADDM Network (51 months) (Figure 2). The overall cumulative incidence of ASD diagnoses was 13.2 per 1,000 children by the time they turned age 8 years.

Comparison with Prevalence of Community-Identified ASD

In addition to ASD diagnoses written in developmental evaluations, many children received an ASD classification in school (Supplementary Table 8, https://stacks.cdc.gov/view/cdc/85386), and certain children's medical records contained ICD billing codes indicating ASD. The prevalence of all children with an ASD diagnosis, education classification, or ICD code was 17.2 per 1,000 children aged 8 years, approximately 7% lower than the ADDM Network estimate of 18.5 per 1,000 children aged 8 years (Figure 3). Although community-identified ASD prevalence was similar to ASD prevalence on the basis of the ADDM Network case definition, ADDM sites with higher prevalence also ascertained more children without an ASD diagnosis.

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Discussion

The latest ASD prevalence estimate, as measured by the ADDM Network, is 18.5 per 1,000 children aged 8 years in 2016. This is approximately 10% higher than the 16.8 prevalence estimate the ADDM Network reported in 2014 (*3*) and approximately 175% higher than (2.8 times) the first estimates reported by the ADDM Network in 2000 and 2002 (*4*,*5*). These changes could reflect differences in community practices for identifying ASD, changes in the data available to the surveillance system, or other unknown factors.

As with previous reports, observed ASD prevalence varies among ADDM Network sites. Community-level differences related to ASD diagnosis or classification for services correlate with ASD prevalence estimates; ASD prevalence and rank order of estimates across sites were similar to the prevalence of community-identified ASD. Previous analyses from the ADDM Network have shown a positive association between neighborhood socioeconomic status (SES) and ASD prevalence, which suggests ASD might be more readily identified in high-SES communities or among populations with good access to services (*16*). In this sense, the ADDM Network prevalence estimates could be used to support efforts to improve ASD diagnosis for lower-SES groups in the community.

Timely Evaluations and Age at First ASD Diagnosis

For the first time since ADDM began, no statistically significant difference was found in the overall ASD prevalence among black and white children. This diminishing disparity in ASD prevalence might signify progress toward earlier and more equitable identification of ASD. Although black children with ASD were more likely than white children to have an intellectual disability and children with intellectual disability were more likely to be evaluated early, black children were still less likely than white children to be evaluated by age 36 months. In addition, among children with intellectual

disability, the median age at ASD diagnosis was 6 months later for black than for white children. Further study is needed to identify community-level barriers to timely evaluation and diagnosis of ASD so that treatments can be delivered as early as possible. Examining differences between communities with earlier and later ASD identification might reveal successful practices or policies that could be implemented in other communities. CDC's "Learn the Signs. Act Early." initiative works with Act Early Ambassadors who support state and territorial or national efforts to improve early identification of developmental disabilities and promote the integration of developmental monitoring in systems that serve children and their families (https://www.cdc.gov/ncbddd/actearly).

Although early diagnosis of ASD is a major public health goal and one of the *Healthy People 2020* objectives (https://www.healthypeople.gov/2020/default), the median age at first ASD diagnosis has changed little over the course of ADDM Network reporting. However, this metric might not fully capture community progress toward early identification and could mask improvement. For instance, the median age at ASD diagnosis will increase if the community begins diagnosing more ASD among children at older ages who in previous years would not have received an ASD diagnosis by age 8 years. An absolute metric, such as cumulative incidence, might reveal advances in early identification over previous cohorts, as shown in the Early ADDM Network report (*28*).

Comparison with Other Autism Data Systems

The National Survey of Children's Health (NSCH) and the National Health Interview Survey (NHIS) are two U.S. nationally representative surveys that measure ASD prevalence by asking parents and caregivers if a doctor or health professional told them that their child has ASD. The 2016 NSCH and the 2015–2017 NHIS both estimated ASD prevalence at 25 per 1,000 children aged 3–17 years (*13*,*29*). Important differences exist between the national surveys and the ADDM Network that warrant consideration when comparing prevalence estimates. The surveys rely on parent-reported ASD diagnoses among children aged 3–17 years in a nationally representative sample, whereas the ADDM Network uses documented information from qualified professionals and monitors ASD among children aged 8 years in participating communities. These data sources might be used in complementary ways; for instance, most ADDM Network sites indicate lower ASD prevalence than the survey estimates, which might indicate a need for improved ASD identification in those communities. To facilitate comparisons between different data systems, CDC developed an interactive website that presents U.S. state-based ASD prevalence data from four data systems (ADDM Network, NSCH, Medicaid, and special education) (https://www.cdc.gov/ncbddd/autism/data/index.html).

ASD prevalence reports from other countries provide information about aspects of ASD that are not measured in the United States and allow for comparisons with the ADDM Network. In 2018, Canada released the first National Autism Surveillance System (NASS) report (*30*). NASS monitored ASD among 1.9 million children aged 5–17 years across all Canadian provinces in 2015 and relied on existing ASD diagnoses to ascertain cases. The overall ASD prevalence was 15.2 per 1,000 children with province estimates ranging from 8.0 to 17.5 per 1,000, which was slightly lower than estimates from the ADDM Network. NASS data indicated that 28% of children with ASD received the diagnosis after age 8 years. A study using linked registry data from Denmark revealed an even greater proportion of children with ASD diagnosed after age 8 years (*31*). Danish children born in 2008 had a cumulative incidence of diagnosed ASD of approximately 12 per 1,000 children by age 8 years (comparable to the cumulative incidence of ASD diagnoses among children aged 8 years in the ADDM Network) (Figure 2); among older Danish cohorts, cumulative incidence was as high as 28 per 1,000 by age 15–16 years.

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Limitations

The findings in this report are subject to at least four limitations. First, the ADDM Network methods rely on the quality and completeness of existing documents to ascertain cases. Sites without access to education records for large portions of their population might not be ascertaining children (particularly black or Hispanic children) (*32*) who only receive services for ASD at school. Second, record completeness is also important for documenting when ASD was first diagnosed in a child, whether the child had IQ testing, and when a child was first evaluated. Reduced access to records, incomplete records, or both, could lead to an underestimate of the number of children identified as having ASD. Third, sites participating in the ADDM Network are funded through a competitive process and are encouraged to include diverse communities; however, the resulting sites are not nationally representative and do not generate nationally representative ASD prevalence estimates. Finally, geographic coverage of the ADDM Network has changed over time, complicating interpretation of temporal trends.

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Future Directions

The ADDM Network will continue data collection for the 2018 and 2020 surveillance years. Sites in 11 states will monitor ASD prevalence among children aged 4 and 8 years. Five sites have initiated a new activity to describe outcomes for children aged 16 years who were initially ascertained by the ADDM Network at age 8 years. To provide the most comprehensive information on how communities identify, serve, and support persons with ASD, the ADDM Network increasingly will focus on community indicators of ASD identification, disparities in service use, and co-occurring conditions among persons with ASD.

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Conclusion

These findings from the 2016 ADDM Network indicate considerable variability in ASD prevalence across communities and higher ASD prevalence than previous estimates from the ADDM Network. For the first time, no overall difference in ASD prevalence between black and white children was reported, although disparities in early intervention and identification persist for black children. ASD prevalence among Hispanic children continues to be lower than among white or black children. Black and Hispanic children with ASD were evaluated at older ages than white children and were more likely to have intellectual disability. Black children with intellectual disability and ASD also received diagnoses at older ages than did white children with intellectual disability and ASD, which might limit opportunities to receive services that could improve their outcomes and quality of life. ASD continues to be a public health concern; the latest data from the ADDM Network underscore the ongoing need for timely and accessible developmental assessments, educational supports, and services for persons with ASD and their families.

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Acknowledgments

Project management, data collection, and abstraction: Kristen Clancy Mancilla, Arizona; Michelle Cantrell Kelley and Sandra Walker, Arkansas; MJ Erickson-Eger, Leanne Glenn, Julia Group, Leovi Madera, Jean Menconi, and Cynthia Vogel, Colorado; Kate Sidwell, Cindy Cruz Alvarez, Michael Verile, and Yuriy Levin, New Jersey; Julie Rusyniak, North Carolina; Madeline Santulli, Tennessee; Lynn Peterson, Lynn Boelter, Robert Rettammel, and Madison Carey, Wisconsin. Data management and programming support: Susan Williams, CDC; Julie Nick, Arkansas; Bill Vertees, Colorado; Adam Ryer, Maryland; Paul Zumoff, New Jersey; Carrie Arneson, Wisconsin. Clinician review activities: Stefanie Jernigan, Arkansas; Terry Hall, Patricia LaVesser, Judy Reaven, and Nuri Reyes, Colorado; Rebecca Born, Cassondra Gayman, Michael Morrier, Catherine Rice, and Daniel Wright, Georgia; Rebecca Born and Mary Schlaak, Maryland; Catherine Burrows, Minnesota; Carolyn Skowyra, Rebecca Born, and Michael Bunis, Missouri; Audrey Mars, Mildred Waale, Arline Fusco, Tara Gleeson,

and Gail Burack, New Jersey; Jessica Rivers, Julie Rusyniak, and Tamara Watson, North Carolina; Evon Batey Lee, Amy Swanson, and Amy S. Weitlauf, Tennessee; Madison Carey, Mary Schlaak, and Maia Piccagli, Wisconsin. Project staff including data abstractors, epidemiologists, and others. Ongoing ADDM Network support: Bruce Heath, CDC.

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Conflicts of Interest

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. Zachary Warren reports personal fees from Hoffman La Roche, grants and personal fees from Adaptive Technology Consulting, grants from Autism Speaks, grants from Cognoa, and grants from Simons Foundation; all fees and grants received are outside the submitted work. John Constantino reports a grant from Western Psychological Services outside the submitted work.

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TABLE 1. Prevalence* of autism spectrum disorder among children aged 8 years, overall and by sex — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016

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Site	Overall†				Male	Female	Male-to-female prevalence ratio (95% CI)§
	Description of surveillance area	No. with ASD	Total population	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	
Arizona	Part of one county in metropolitan Phoenix†	282	17,656	16.0 (14.2–17.9)	25.4 (22.4–28.9)	6.0 (4.6–7.9)	4.2 (3.1–5.7)
Arkansas	All 75 counties in Arkansas	606	40,225	15.1 (13.9–16.3)	24.3 (22.3–26.5)	5.4 (4.5–6.5)	4.5 (3.7–5.5)
Colorado	Seven counties in metropolitan Denver	537	40,874	13.1 (12.1–14.3)	21.2 (19.3–23.2)	4.7 (3.9–5.8)	4.5 (3.6–5.6)
Georgia	Two counties in metropolitan Atlanta	456	24,113	18.9 (17.3–20.7)	30.4 (27.5–33.6)	7.1 (5.7–8.7)	4.3 (3.4–5.4)

Maryland	One county in metropolitan Baltimore	192	9,993	19.2 (16.7–22.1)	30.1 (25.8–35.2)	7.8 (5.7–10.7)	3.9 (2.7–5.5)
Minnesota	Parts of two counties including Minneapolis–St. Paul	313	13,728	22.8 (20.4–25.4)	36.3 (32.1–41.0)	9.2 (7.2–11.8)	3.9 (3.0–5.2)
Missouri	Two counties in metropolitan St. Louis	213	15,635	13.6 (11.9–15.6)	21.1 (18.1–24.5)	6.2 (4.6–8.1)	3.4 (2.5–4.7)
New Jersey	Four counties including metropolitan Newark	1,036	33,031	31.4 (29.5–33.3)	50.0 (46.8–53.4)	12.0 (10.4–13.8)	4.2 (3.6–4.9)
North Carolina	Four counties in central North Carolina	489	19,291	25.3 (23.2–27.7)	41.2 (37.5–45.3)	8.7 (7.0–10.8)	4.7 (3.7–6.0)
Tennessee	11 counties in middle Tennessee	405	25,839	15.7 (14.2–17.3)	25.5 (22.9–28.3)	5.5 (4.4–7.0)	4.6 (3.6–6.0)
Wisconsin	10 counties in southeastern Wisconsin	579	35,034	16.5 (15.2–17.9)	26.3 (24.0–28.7)	6.3 (5.2–7.6)	4.2 (3.4–5.2)
Total		**5,108**	**275,419**	**18.5 (18.0–19.1)**	**29.7 (28.8–30.6)**	**6.9 (6.5–7.4)**	**4.3 (4.0–4.6)**

Abbreviations: ASD = autism spectrum disorder; CI = confidence interval.
* Per 1,000 children aged 8 years.
† All children are included in the total regardless of sex or race/ethnicity.
§ Wilson score 95% CIs exclude 1.0 in all sites, indicating significantly higher prevalence among males than among females.

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TABLE 2. Prevalence* of autism spectrum disorder among children aged 8 years, by race/ethnicity — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016

	Non-Hispanic white	Non-Hispanic black	Hispanic	Asian/Pacific Islander†	Prevalence ratios		
Site	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	Prevalence (95% CI)	Non-Hispanic white to non-Hispanic black (95% CI)	Non-Hispanic white to Hispanic (95% CI)	Non-Hispanic black to Hispanic (95% CI)
Arizona	18.8 (16.0–22.0)	12.3 (7.6–19.9)	12.4 (10.1–15.3)	11.1 (5.6–21.8)	1.5 (0.9–2.5)	1.5 (1.2–2.0)§	1.0 (0.6–1.7)
Arkansas	16.6 (15.1–18.2)	12.1 (9.9–14.8)	9.6 (7.3–12.7)	18.6 (11.8–29.1)	1.4 (1.1–1.7)§	1.7 (1.3–2.3)§	1.3 (0.9–1.8)
Colorado	13.0 (11.6–14.6)	15.4 (11.5–20.7)	10.3 (8.8–12.1)	8.3 (5.2–13.3)	0.8 (0.6–1.2)	1.3 (1.0–1.5)§	1.5 (1.1–2.1)§
Georgia	18.9 (15.9–22.5)	19.7 (17.1–22.6)	11.3 (8.9–14.4)	23.0 (17.3–30.6)	1.0 (0.8–1.2)	1.7 (1.2–2.3)§	1.7 (1.3–2.3)§
Maryland	16.8 (13.5–20.8)	19.6 (15.5–24.7)	13.6 (7.8–23.6)	17.9 (10.5–30.4)	0.9 (0.6–1.2)	1.2 (0.7–2.2)	1.4 (0.8–2.6)
Minnesota	24.6 (20.9–28.9)	25.8 (21.2–31.5)	17.6 (12.8–24.3)	16.3 (11.7–22.6)	1.0 (0.7–1.2)	1.4 (1.0–2.0)	1.5 (1.0–2.1)§
Missouri	15.2 (12.8–18.2)	11.6 (9.2–14.6)	3.8 (1.3–11.1)	4.2 (1.4–12.4)	1.3 (1.0–1.8)	4.0 (1.4–11.9)§	3.1 (1.0–9.2)§
New Jersey	33.4 (30.5–36.6)	26.3 (22.8–30.2)	29.8 (26.7–33.2)	26.9 (20.7–35.0)	1.3 (1.1–1.5)§	1.1 (1.0–1.3)	0.9 (0.7–1.1)
North Carolina	23.3 (20.6–26.3)	27.9 (23.2–33.4)	19.3 (15.1–24.6)	28.2 (20.8–38.2)	0.8 (0.7–1.0)	1.2 (0.9–1.6)	1.4 (1.1–2.0)§
Tennessee	15.6 (13.8–17.6)	17.4 (14.2–21.3)	11.1 (8.1–15.1)	14.9 (8.7–25.3)	0.9 (0.7–1.1)	1.4 (1.0–2.0)§	1.6 (1.1–2.3)§
Wisconsin	17.0 (15.3–18.9)	13.8 (11.3–16.9)	15.4 (12.6–18.7)	15.5 (10.5–22.8)	1.2 (1.0–1.5)	1.1 (0.9–1.4)	0.9 (0.7–1.2)
Total	**18.5 (17.9–**	**18.3 (17.2–**	**15.4 (14.4–**	**17.9 (15.9–**	**1.0 (0.9–**	**1.2 (1.1–**	**1.2 (1.1–**

	19.3)	19.4)	16.4)	20.1)	1.1)	1.3)[§]	1.3)[§]

Abbreviation: CI = confidence interval.

* Per 1,000 children aged 8 years.

† Because of small sample size relative to other groups, prevalence ratios are not shown for Asians/Pacific Islanders. Overall, the Hispanic-to-Asian/Pacific Islander prevalence ratio is statistically significant (prevalence ratio: 0.9; Wilson score: 95% CI = 0.8–0.99), but the non-Hispanic white-to-Asian/Pacific Islander and non-Hispanic black-to-Asian/Pacific Islander prevalence ratios are not statistically different from 1.0. Counts for the numerator and denominator are available (Supplementary Table 7, [https://stacks.cdc.gov/view/cdc/85386]).

§ Wilson score 95% CIs exclude 1.0, indicating significantly different prevalence between groups.

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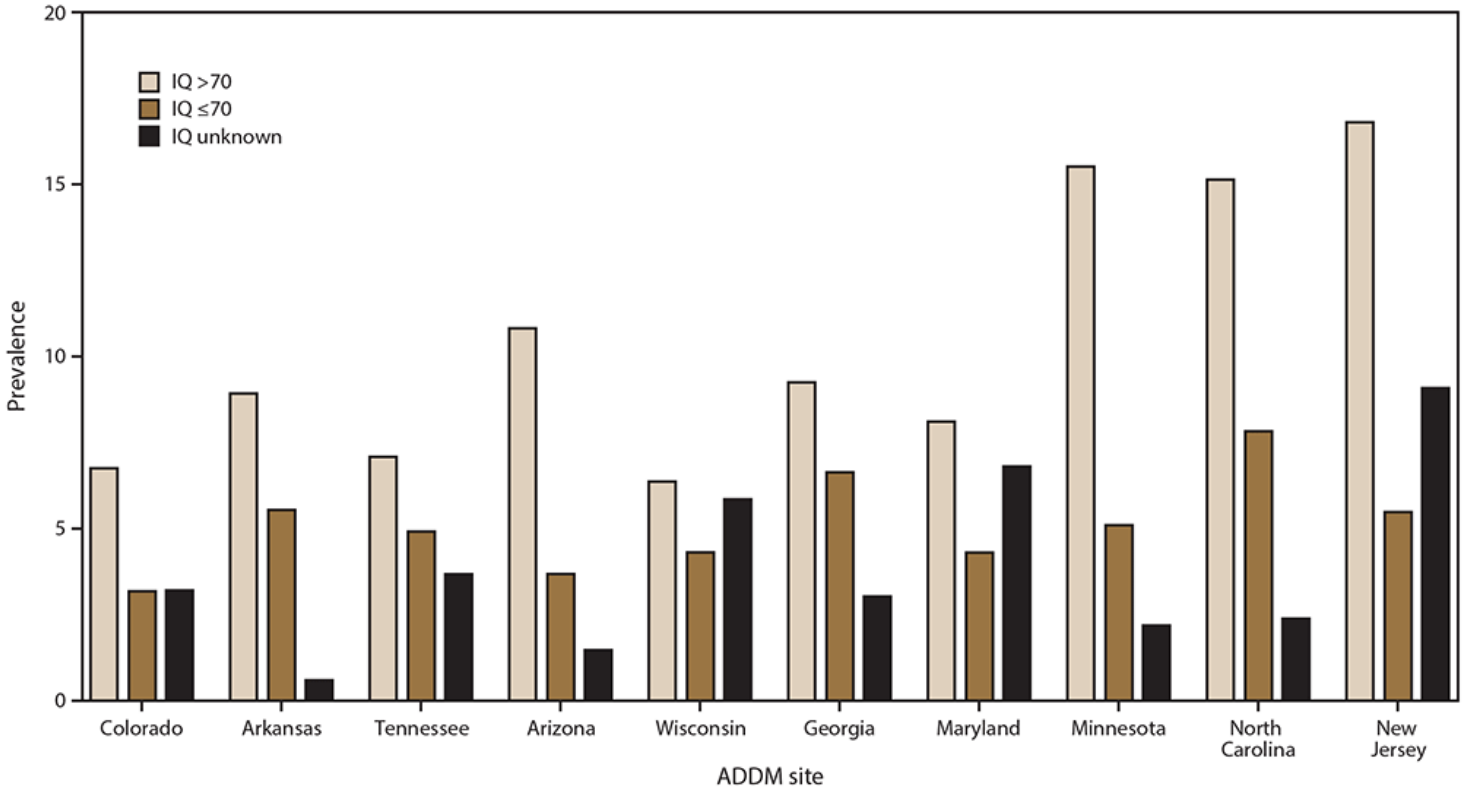
FIGURE 1. Prevalence* of autism spectrum disorder among children aged 8 years, by most recent intelligence quotient score and site — Autism and Developmental Disabilities Monitoring Network, 10 sites,† United States, 2016



Abbreviations: ADDM = Autism and Developmental Disabilities Monitoring Network; IQ = intelligence quotient.

* Per 1,000 children aged 8 years.

† Missouri is not included because it did not collect IQ information on at least 60% of children with ASD. The total numbers of children with ASD, by site: n = 537 (Colorado), n = 606 (Arkansas), n = 405 (Tennessee), n = 282 (Arizona), n = 579 (Wisconsin), n = 456 (Georgia), n = 192 (Maryland), n = 313 (Minnesota), n = 489 (North Carolina), and n = 1,036 (New Jersey).

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TABLE 3. Availability and distribution of intelligence quotient scores among children aged 8 years with autism spectrum disorder — Autism and Developmental Disabilities Monitoring Network, 10 sites,† United States, 2016

Monitoring Network, 10 sites,* United States, 2016

Site	Total no. with ASD	With IQ information	Cognitive level*		
		No. (%)	IQ ≤70 (%)	IQ 71–85 (%)	IQ >85 (%)
Arizona	282	256 (90.8)	25.4	30.1	44.5
Arkansas	606	582 (96.0)	38.3	23.0	38.1
Colorado	537	406 (75.6)	32.0	22.2	45.8
Georgia	456	383 (84.0)	41.8	20.9	37.1
Maryland	192	124 (64.6)	34.7	25.8	38.7
Minnesota	313	283 (90.4)	24.7	19.8	54.1
New Jersey	1,036	736 (71.0)	24.6	27.9	47.4
North Carolina	489	443 (90.6)	34.1	20.8	44.9
Tennessee	405	310 (76.5)	41.0	30.3	27.7
Wisconsin	579	374 (64.6)	40.4	21.7	38.0
Total	**4,895**	**3,897 (79.6)**	**33.4**	**24.1**	**42.1**

Abbreviation: ASD = autism spectrum disorder; IQ = intelligence quotient.

* Levels of intellectual functioning might not always sum to exactly 100% because 15 children (three in Arkansas, one in Georgia, one in Maryland, four in Minnesota, one in North Carolina, one in New Jersey, and three in Tennessee) had evidence of IQ >70 (i.e., examiner statement) but without sufficient detail to be assigned to either the IQ 71–85 or IQ >85 categories.

† Missouri is not included because it did not collect IQ information on at least 60% of children with ASD.

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TABLE 4. Number and percentage of children aged 8 years with autism spectrum disorder who received a comprehensive evaluation by a qualified professional at age ≤36 months, 37–48 months, or >48 months and the median age at first evaluation, by site and selected characteristics — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016

		Youngest age when child first received a comprehensive evaluation	

Site/Characteristic	Total no. of children with ASD and a linked birth certificate	≤36 mos	37–48 mos	>48 mos	Median age (mos) at first evaluation
		No. (%)	No. (%)	No. (%)	
Site					
Arizona	218	83 (38.1)	53 (24.3)	82 (37.6)	43.0
Arkansas	481	157 (32.6)	105 (21.8)	219 (45.5)	46.0
Colorado	388	186 (47.9)	60 (15.5)	142 (36.6)	37.5
Georgia	337	125 (37.1)	79 (23.4)	133 (39.5)	43.0
Maryland	172	83 (48.3)	37 (21.5)	52 (30.2)	38.0
Minnesota	246	96 (39.0)	48 (19.5)	102 (41.5)	44.5
Missouri	147	58 (39.5)	34 (23.1)	55 (37.4)	41.0
New Jersey	816	355 (43.5)	163 (20.0)	298 (36.5)	39.5
North Carolina	371	231 (62.3)	42 (11.3)	98 (26.4)	29.0
Tennessee	314	113 (36.0)	69 (22.0)	132 (42.0)	45.0
Wisconsin	491	247 (50.3)	87 (17.7)	157 (32.0)	36.0
Characteristic					
Sex					
Female	730	352 (48.2)	119 (16.3)	259 (35.5)	38.0
Male	3,251	1,382 (42.5)	658 (20.2)	1,211 (37.3)	41.0
Intellectual disability status					
IQ >70	2,038	787 (38.6)	398 (19.5)	853 (41.9)	43.0

IQ ≤70	1,057	617 (58.4)	188 (17.8)	252 (23.8)	34.0
IQ unknown	886	330 (37.2)	191 (21.6)	365 (41.2)	43.5
Race/Ethnicity					
Non-Hispanic white	2,063	935 (45.3)	399 (19.3)	729 (35.3)	39.0
Non-Hispanic black	859	342 (39.8)	196 (22.8)	321 (37.4)	42.0
Hispanic	730	313 (42.9)	129 (17.7)	288 (39.5)	40.0
Total	**3,981**	**1,734 (43.6)**	**777 (19.5)**	**1,470 (36.9)**	**40.0**

Abbreviations: ASD = autism spectrum disorder; IQ = intelligence quotient.

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TABLE 5. Median age at earliest known autism spectrum disorder diagnosis, by intellectual disability status — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016

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Site/Characteristic	No. with ASD	All children with an ASD diagnosis*		Children with an ASD diagnosis and IQ ≤70		Children with an ASD diagnosis and IQ >70	
		No.	Median age (mos) at diagnosis	No.	Median age (mos) at diagnosis	No.	Median age (mos) at diagnosis
Site							
Arizona	282	193	57.0	45	51.0	127	59.0
Arkansas	606	489	56.0	204	49.0	267	63.0
Colorado	537	362	48.5	105	43.0	187	54.0
Georgia	456	306	55.0	116	50.5	138	60.0

Maryland	192	150	47.5	39	37.0	60	53.0
Minnesota	313	170	56.0	40	51.5	112	60.0
Missouri	213	194	56.0	13	68.0	37	71.0
New Jersey	1,036	844	51.0	162	44.0	431	53.0
North Carolina	489	280	38.0	95	30.0	156	49.0
Tennessee	405	307	51.0	112	41.5	142	59.0
Wisconsin	579	469	49.0	128	42.0	173	59.0
Characteristic							
Sex							
Female	938	649	51.0	211	43.0	281	56.0
Male	4,170	3,115	51.0	848	45.0	1,549	57.0
Race/Ethnicity							
Non-Hispanic white	2,613	1,950	51.0	468	42.0	1,064	58.0
Non-Hispanic black	1,070	781	53.0	295	48.0	283	57.0
Hispanic	909	674	51.0	196	44.0	310	55.0
Total	**5,108**	**3,764**	**51.0**	**1,059**	**44.0**	**1,830**	**57.0**

Abbreviations: ASD = autism spectrum disorder; IQ = intelligence quotient.

* Children with unknown IQ are included in the “all children with an ASD diagnosis” category but not the IQ-specific strata.

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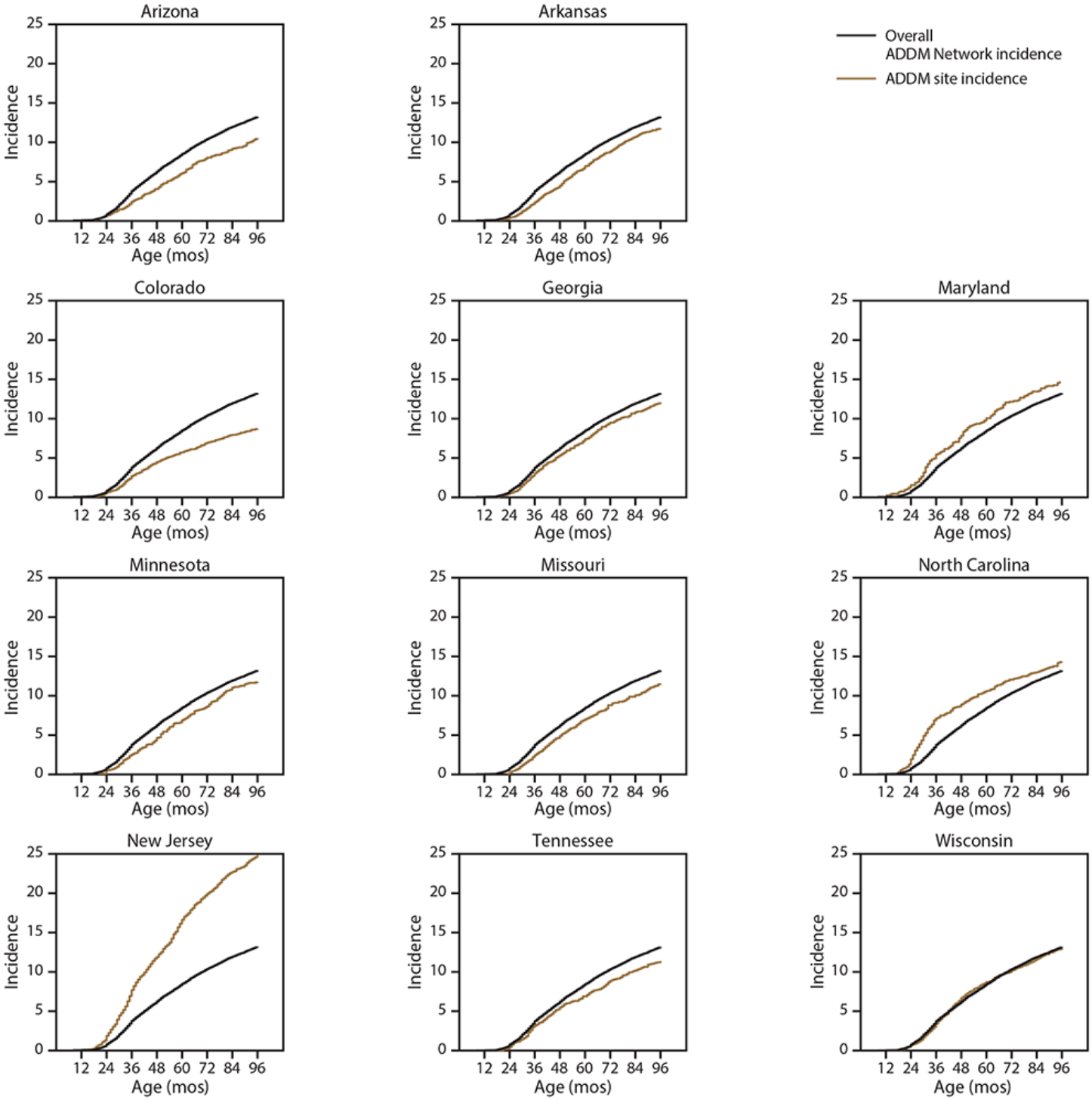
FIGURE 2. Cumulative incidence* of autism spectrum disorder diagnoses,† by age and site — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016



Abbreviations: ADDM = Autism and Developmental Disabilities Monitoring Network; ASD = autism spectrum disorder; ICD = International Classification of Diseases.

* Per 1,000 children aged 8 years.

† These data only include the portion of ADDM Network ASD cases with a documented diagnostic statement of ASD in the record. Children counted as ADDM Network cases without a documented ASD diagnosis (i.e., does not consider special education classifications or ICD codes) are not included.

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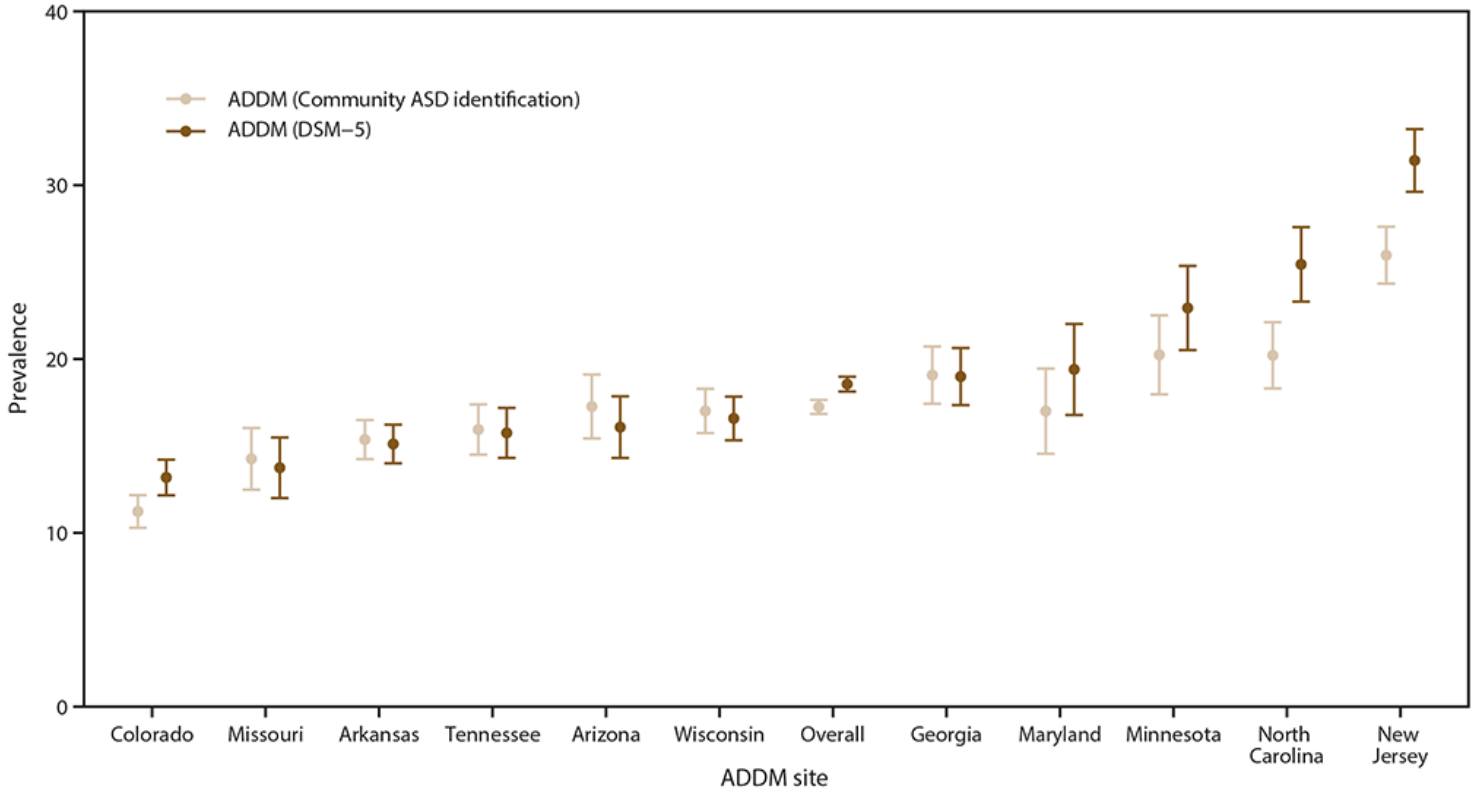
FIGURE 3. Comparison of autism spectrum disorder prevalence*,† on the basis of *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*, criteria and community-identified§ autism spectrum disorder prevalence, by site and overall — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016



Abbreviations: ADDM = Autism and Developmental Disabilities Monitoring Network; ASD = autism spectrum disorder; DSM-5 = *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*; ICD = International Classification of Diseases.

* Per 1,000 children aged 8 years.

† The dots represent prevalence estimates and the vertical lines bounded by bars represent the corresponding 95% confidence intervals.

§ Children are considered to be identified as having ASD by the community if they have an autism diagnosis stated in an evaluation, have been determined to meet autism eligibility in special education, or have been assigned an autism ICD code. Missouri, Colorado, and Wisconsin had limited access to information from educational records.

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Suggested citation for this article: Maenner MJ, Shaw KA, Baio J, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. MMWR Surveill Summ 2020;69(No. SS-4):1–12. DOI: http://dx.doi.org/10.15585/mmwr.ss6904a1 .

EXHIBIT 60

Journal of Autism and Developmental Disorders
https://doi.org/10.1007/s10803-018-3670-2

ORIGINAL PAPER

California Autism Prevalence Trends from 1931 to 2014 and Comparison to National ASD Data from IDEA and ADDM

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Abstract
Time trends in U.S. autism prevalence from three ongoing datasets [Individuals with Disabilities Education Act, Autism and Developmental Disabilities Monitoring Network, and California Department of Developmental Services (CDDS)] are calculated using two different methods: (1) constant-age tracking of 8 year-olds and (2) age-resolved snapshots. The data are consistent across methods in showing a strong upward trend over time. The prevalence of autism in the CDDS dataset, the longest of the three data records, increased from 0.001% in the cohort born in 1931 to 1.2% among 5 year-olds born in 2012. This increase began around ~ 1940 at a rate that has gradually accelerated over time, including notable change points around birth years 1980, 1990 and, most recently, 2007.

Keywords Autistic disorder · Autism spectrum disorder · ASD prevalence · Time trends · ADDM · IDEA · CDDS

Introduction

Autism was first described in the 1940s as a childhood psychiatric disorder characterized by early expressed impairment in social interaction and communication and repetitive or circumscribed interests or behavior (Kanner 1943). Kanner's original term for the condition was *early infantile autism* or *infantile autism*. While originally attributed by some to bad parenting (i.e., "refrigerator mothers") (Bettelheim 1967), today autism is widely recognized as a complex developmental disorder triggered by environmental factors acting on a genetically-susceptible population, in which inflammation may interfere with early brain synapse formation and pruning (Pardo et al. 2005; Goines and Ashwood 2012; Bilbo et al. 2015). Autism frequently co-occurs with other neurological and behavioral conditions (Van Der Meer et al. 2012) and is often accompanied by elevated levels of cellular oxidative stress, mitochondrial dysfunction, and/or immune and gastrointestinal disorder (James et al. 2009; Chaidez et al. 2013; Frye and James 2014).

Autism is diagnosed by confirmation of behaviors by experts, as there are no valid biomarkers or determinative tests. Autism diagnostic criteria were formalized for the first time in the 3rd Edition of the American Psychiatric Association's (APA) *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-III*) (APA 1980) to clarify the difference between *infantile autism* and childhood schizophrenia. In a subsequent revision to *DSM-IV*, published in 1994 (APA 1994), three autism subtypes were described: *autistic disorder* (*AD*), *pervasive developmental disorder-not otherwise specified* (*PDD-NOS*) and *Asperger's syndrome*. These latter subtypes represent milder, variant forms, while *AD* is the most severe expression of autism. By definition, *AD* is in place by age 3, although it typically is not diagnosed until a median age of 4 years (MacFarlane and Kanaya 2009; CDC 2016).

DSM-5, published in November, 2013, formally defined the term *autism spectrum disorder* (*ASD*), which encompasses but no longer distinguishes between *AD, PDD-NOS* and *Asperger's syndrome*, based on the rationale that the clinical distinction between the subtypes is not well

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10803-018-3670-2) contains supplementary material, which is available to authorized users.

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Published online: 05 July 2018

defined (APA 2013). Indeed the CDC Autism and Developmental Disabilities Monitoring (ADDM) Network reports a wide range of variability among individual states in the proportion of ASD cases assigned to each subtype. Among 8 year-olds surveyed across 11 states in 2012, AD accounted for 26–74% (46% on average) of ASD cases, PDD-NOS accounted for 15–58% (44% on average) and Asperger's for 2–19% (10% on average) (CDC 2016). Under DSM-5, in place of the old subtypes, clinicians rate the severity of deficits in two principal domains of (1) social communication and interaction and (2) restrictive and repetitive patterns of behavior (Gibbs et al. 2012; Volkmar and Reichow 2013).

Epidemiologic estimates of autism prevalence in the United States were in the range of 1 in 2500 prior to 1985, but increased to 1/150 among 8 year-olds born in 1992 and again to 1/68 for 8 year-olds born in 2002 (McDonald and Paul 2010; CDC 2016). Despite the rapid and broad rise in the reported ASD prevalence, a number of analyses have concluded that much of the apparent rise may not reflect a real increase in ASD cases. Rather, these studies have argued that diagnostic substitution for intellectual disability, the expansion of diagnostic criteria, and improved awareness of the condition have played an important role in explaining the increase in ASD (Croen et al. 2002; Gurney et al. 2003; Fombonne 2009; Keyes et al. 2012; Polyak et al. 2015). The epidemiologic literature also has suggested that the large variation in reported ASD prevalence from different geographic regions (e.g., the more than threefold differences among states in ADDM surveys) indicates a level of inconsistency in the data that precludes drawing conclusions about time trends (Fombonne 2009).

A recent analysis of data from the U.S. Department of Education Individuals with Disabilities Education Act (IDEA) offered a different view, finding that the large majority (75–80%) of the increase in ASD prevalence, over the period from 1988 to 2002, was due to a true increase in the condition rather than to better and expanded diagnosis (Nevison 2014). That investigation, which focused on IDEA data, compared two independent methods for calculating the trend slope of ASD prevalence versus birth year and found that both methods gave largely consistent results. The methods involved (1) tracking prevalence at a constant age over multiple, successive years of reports, and (2) examining an age-resolved snapshot from the most recent individual year's report. The conceptual distinction between methods 1 and 2 was also important in demonstrating that diagnostic substitution for intellectual disability is an unsatisfactory explanation for the rise in ASD in most states, including California (Blaxill et al. 2003; Croen and Grether 2003; Nevison and Blaxill 2017).

The "constant-age tracking" method is the approach adopted by the ADDM network, which tracks ASD prevalence among 8 year-olds in successive biannual reports (CDC 2016). Two other data systems compile successive annual reports with separate counts for each age from early childhood to adulthood. These include IDEA and the California Department of Developmental Services (CDDS). The successive annual reports from these networks not only allow for constant-age tracking of any age cohort, but also effectively provide the opportunity to compute a prevalence snapshot, resolved by age, for any given report year. Using simple algebra, a prevalence versus birth year curve can be constructed from any of the individual age-resolved reports, thereby providing an independent, alternative approach to constant-age tracking for estimating the time trend in autism prevalence.

This alternative approach is referred to here as the "age-resolved snapshot" method. A key advantage is that the time trend derived from an age-resolved snapshot is substantially insulated from the biasing influences of better and/or expanded diagnosis, since these influences potentially may affect all age cohorts in the snapshot equally, provided the cohorts are old enough to be full ascertained. The age of full ascertainment for ASD commonly (although perhaps inappropriately) has been assumed to be about 8 (CDC 2016). Thus, in principle if ASD is truly a constant prevalence condition, a snapshot-based prevalence versus birth year plot, beginning around age 8 and extending back in time to older birth cohorts, should be a flat line with a slope of 0. In practice, children of different ages are not necessarily equally likely to be evaluated for ASD in a given year, and adults are even less likely to be evaluated. However, the IDEA law includes the Child Find mandate, which requires that all U.S. school districts locate and evaluate all children with disabilities from birth through age 21, suggesting there is an ongoing legal mandate to identify children with ASD throughout their school years (Wright and Wright 2007).

Earlier studies using "age-period-cohort" approaches to understand the interaction of age, cohort and report year effects (Gurney et al. 2003; Newschaffer et al. 2007; Keyes et al. 2012) have employed some of the same concepts involved in the age-resolved snapshot versus constant-age tracking method. Those studies have focused largely on following specific birth cohorts as they age and have noted a tendency toward an ongoing increase in prevalence within a given cohort well beyond age 8, as well as an overall increase in prevalence among younger versus older birth cohorts. However, the previous studies generally have not plotted or defined the time trend in autism per se, visualized as a simple graph of prevalence versus birth year.

In this paper, we apply the age-resolved snapshot and constant-age tracking method to a set of 13 CDDS annual reports, which date as far back as birth year 1931 and extend through birth year 2014. The *DSM-IV* definitions were used for most of these reports, although the two most recent reports were transitioning to *DSM-5*. For the older data, we

use the *DSM-5* term ASD to refer to the sum of AD, PDD-NOS and Asperger's syndrome. We compare the CDDS trends to trends in ASD from the California IDEA dataset. In addition, we calculate the IDEA ASD trend slopes in the 15 states surveyed by the ADDM Network, which allows for comparison of 8 year-old constant-age tracking trends between IDEA and ADDM. Our primary goal is to quantify and characterize the time trend in U.S. autism prevalence as well as possible using the best available data. Two secondary goals are (1) to test the hypothesis that the trend slopes derived from the most recently available age-resolved snapshots for the CDDS and IDEA datasets are significantly greater than zero (i.e., not flat lines), and (2) to examine reasons for the large variations in reported ASD prevalence among different states and data networks.

Methods

Autism Prevalence Data

Table 1 summarizes the three main sources of ASD prevalence data used in this paper. Each dataset is described in greater detail below and the complete datasets are provided in Supplementary Files S1–4. Since all relevant information had been de-identified prior to our activities and since the datasets were aggregated by age at the state level, this project did not require institutional review and approval.

California Department of Developmental Services (CDDS)

CDDS provides services to eligible individuals living in California who meet the DSM diagnostic criteria for autism. To qualify for CDDS services, these individuals also must have a level of impairment that rises to the level of a "developmental disability," where the latter is defined as a non-physical, substantial disability that is expected to continue indefinitely (Autism Society San Francisco Bay Area 2015). Historically, the CDDS screening system has reserved the name "autism" for "full syndrome" cases, which have a modal age of 3 at diagnosis (Fountain and Bearman 2011) and are almost always diagnosed with AD. Official CDDS publications have focused on this more severely affected population (CDDS 2003). Milder subtypes such as Asperger's syndrome and PDD-NOS have not been eligible for services unless they have another qualifying disability (Fountain and Bearman 2011). In addition to an autism diagnosis, individuals applying for CDDS services must demonstrate significant functional disability in 3 out of 7 life challenges, which include self-care, language, learning, mobility, self-direction, capacity for independent living and economic self-sufficiency (Autism Society San Francisco Bay Area 2015).

For the current study, CDDS autism counts were obtained as a set of 10 consecutive annual reports for 1997–2006 and 3 additional reports for 2014, 2016 and 2017. Each of the annual reports provides an age-resolved snapshot for that year of the number of individuals receiving services for autism. The counts are listed back to birth year 1970 for the 2016 report and back to birth year 1931 for all other reports. The 2014 snapshot was obtained from a published report (Autism Society San Francisco Bay Area 2015) while all the other reports were obtained through direct requests to CDDS. The data include only individuals who are "active" in the system and are classified under Code 1 on their Client Development Evaluation Report (CDER).

The definition of Code 1 has changed several times over the years within the CDDS system (http://www.dds.ca.gov/CDER/Index.cfm). In May, 2007 (but not implemented until November 2008), Code 1 was revised from its historic name, "autism, full syndrome," to "autistic disorder (AD)."

Table 1 Summary of ASD datasets

Dataset	CDDS	IDEA	ADDM
Regions covered	California	All 50 states + D.C.	Selected counties in up to 15 states, varying by report
Age of autism counts	3–83	3–21	8
Denominator used to estimate prevalence	California birth data 1931–2014	NCES public school populations K-12 (age 5–17)	U.S. Census data
ASD types included	Code 1 autism (mainly AD)	Varies by state. Some may include only AD, others some or all ASD types	All ASD types, including Asperger's syndrome
Report years	1997–2006, annually, 2014, 2016, 2017	1991–2011, annually	2000–2012, biannually
Sponsoring agency	Calif. Department of Developmental Services	U.S. Department of Education	U.S. Centers for Disease Control and Prevention
Supplementary data files	S1	S2, S3	S4

The two terms are similar (CDDS 2003), but the latter was adopted when CDDS began using separate codes 3 and 4 for Asperger's syndrome and PDD-NOS, respectively, under the DSM-IV nomenclature. In November 2014, Code 1 was revised again to "autism spectrum disorder" as CDDS began shifting to the DSM-5 framework. The revised definitions initially apply only to new cases entering the system, while older cases retain their original classifications. However, most consumers are on an annual diagnosis update schedule (a minority are on a triannual schedule) with the result that 98% are transitioned within 2 years and 99% within 3 years to the new codes (Paul Choate, personal comm. 1/30/18). The chronology is such that the original definition of Code 1 (autism, full syndrome) applies to the 1997–2006 snapshots, the DSM-IV definition "autistic disorder" applies to the 2014 snapshot, and the new DSM-5 definition of Code 1 applies to the 2016 and 2017 snapshots.

The Code 1 autism counts were converted to prevalence in % for birth years 1970–2014, using California live birth data as denominators (http://www.dof.ca.gov/research/demographic/reports/projections/births/), consistent with the methodology used by CDDS (2003). The 1931–1969 counts were converted into prevalence using estimated California births from http://www.dof.ca.gov/Forecasting/Demographics/Estimates/E-7/. These birth estimates extend to the present day and, in the overlapping range, agree well with the 1970–2014 live birth estimates, to within a standard deviation of ±9000. The full set of 13 age-resolved annual reports (1997–2006, 2014, 2016 and 2017) is available in Supplementary File S1.

Individuals with Disabilities Education Act (IDEA)

The Individuals with Disabilities Education Act (IDEA) requires the collection of special education enrollment counts for 13 specific disability categories. IDEA is federally mandated and regulated under the U.S. Department of Education, but allows individual states discretion in determining special education categories, without reference to DSM or other diagnostic criteria. Rather, the determination of whether a student qualifies for autism services is made by district-level professionals in concert with the student's parents and teachers (MacFarlane and Kanaya 2009).

ASD counts were obtained from the IDEA database for each of the 50 United States (http://www.ideadata.org). For report years through 2011, ASD counts for children age 6 through 17 are available in age-resolved annual reports beginning in 1991, while counts for 5 year-olds are available beginning in the 2000 report. ASD prevalence was calculated by dividing the IDEA counts by total statewide public school populations from the National Center for Education Statistics (NCES) (http://nces.ed.gov/ccd/elsi/). The NCES data are resolved by grade from kindergarten (age 5) to 12th grade (age 17) and are available in annual reports from 1991 to 2011. While additional IDEA reports have been published beyond 2011, from the 2012 report onward the findings were reformulated such that ASD counts are no longer available in age-resolved format, but rather are aggregated into broad age categories. These more recent reports do not provide the age-resolved data that our methodology requires.

The full datasets of IDEA ASD counts and computed IDEA/NCES ASD prevalence are available in Supplementary Files S2 and S3, respectively. Both datasets are presented for all 50 United States, although this paper focuses on the 15 states in which ADDM data are available, and on California, where CDDS data are available. We also compute the overall U.S. ASD prevalence by summing all (non-blank) data from all 50 states plus Washington, D.C. and dividing by the sum of the NCES public school populations in those states. In the early 1990s, only about half of states provided ASD counts, but data are available from at least 48 states for every year thereafter.

Autism and Developmental Disabilities Monitoring (ADDM) Network

The Autism and Developmental Disabilities Monitoring (ADDM) Network is a surveillance system conducted in selected regions of the United States that was established by the Centers for Disease Control (CDC) in 2000 to provide estimates of autism prevalence among 8 year-old children. Reports are available biannually for birth years from 1992 to 2004, for a total of 7 reports (CDC 2007a, b, 2009a, b, 2012, 2014, 2016). ADDM ASD cases are determined by systematic review and abstraction of information contained in existing evaluations conducted for developmental health and special education purposes, followed by independent scoring and analysis by experienced clinicians to determine which children satisfy the DSM-IV-based definitions of ASD. (Note, in some states ADDM researchers have access only to health records and not education records.) ADDM uses U.S. Census-based data for the age cohort denominators needed to compute prevalence. ADDM data cover all ASD subtypes, including AD, PDD-NOS and Asperger's disorder. While ADDM, over the lifetime of the Network, covers parts of 15 different states, the states surveyed are not consistent from report to report and the number of counties referenced in each successive report is also somewhat variable. These differences, along with the ADDM prevalence in each of the seven reports, are presented in Supplementary File S4. ADDM also computes an overall U.S. ASD prevalence estimate by tabulating all the cases in the participating states from a given report year and dividing by the total 8 year-old population.

Quantifying ASD Trend Slopes

Constant-Age Tracking Versus Age-Resolved Snapshots

The CDDS, IDEA and ADDM reports described above were used to construct the temporal trend in ASD, which was plotted as prevalence versus birth year. Birth year was calculated according to Eq. (1).

$$Birth\ Year = Report\ Year - Age, \quad (1)$$

Here we note that Eq. 1 corresponds closely to the Cohort = Period − Age equation used in previous age–period–cohort analyses (Gurney et al. 2003; Keyes et al. 2012). For CDDS and IDEA, which provide a series of annual reports, each of which contains data across a range of ages, the prevalence versus birth year curve can be constructed using two independent approaches: "constant-age tracking" and "age-resolved snapshot" (Nevison 2014). For constant-age tracking, *Report Year* is varied while *Age* is held constant. For the age-resolved snapshot, *Age* is varied while *Report Year* is held constant, using the most recently available report. For ADDM, constant-age tracking is the only approach that can be used, since the ADDM biannual reports provide ASD prevalence estimates only for 8 year-old children.

Least Squares Linear Regression and the Null Hypothesis

The slopes of the ASD prevalence versus birth year curves were quantified by least squares linear regression using Matlab R2015b software. With ASD prevalence on the Y-axis and birth year on the X-axis, these slopes reflect the time trend in ASD prevalence, approximated as a linear fit to the data. Hereafter, the terms b_{snap} and b_{track} are used to refer to the ASD trend slopes for the age-resolved snapshot and constant-age tracking curves, respectively. The linear regression approach assumes that the ASD prevalence versus birth year relationship can be represented as a linear change over short intervals of data. The errors in the trend slopes were taken from the covariance matrix of the regression.

In analyzing the trend slopes, a key hypothesis tested was the null hypothesis for the age-resolved snapshot slope: is b_{snap} significantly indistinguishable from 0, i.e., is ASD prevalence independent of birth year? The alternative to the null hypothesis is that b_{snap} is significantly different from 0, which would indicate a real change in ASD prevalence over time. A t statistic was calculated as the ratio of the slope/slope error. Significance was evaluated from a table of critical values of the t distribution, with a chosen confidence level of $p < 0.01$ (Walpole and Myers 1985). The same evaluation was performed for all constant-age tracking slopes b_{track}.

The trend slopes were calculated over selected birth year intervals, as summarized in the results below. These calculations focus on age 8 as the constant age tracked, consistent with ADDM (CDC 2016). The CDDS calculations included the continuous set of 1997–2006 reports plus the 2014, 2016 and 2017 reports, with the latter serving as the most recently available age-resolved snapshot.

For IDEA data, the trend calculations were based on 8 year-old b_{track} slopes calculated over the 1994–2003 birth year interval. The b_{snap} slopes were calculated over this same interval from the most recent available IDEA age-resolved snapshot in 2011. Since the IDEA/NCES snapshots have an upper age limit of 17, and we set the lower age limit at the tracking age (i.e., 8), these upper and lower bounds define the 1994–2003 birth year interval of the analysis. The lower age limit is needed to avoid the non-linear rollover that typically occurs at the younger end of an age-resolved snapshot due to underascertainment in very young children (Nevison 2014).

Results

California Department of Developmental Services (CDDS) Data Analysis

Long-Term Trend Since 1931

Figure 1 shows an apparent ~ 1000-fold increase in CDDS autism prevalence between birth year 1931, when prevalence was only ~ 0.001%, and birth year 2012, when prevalence had increased to 1.18% among 5 year-olds born in

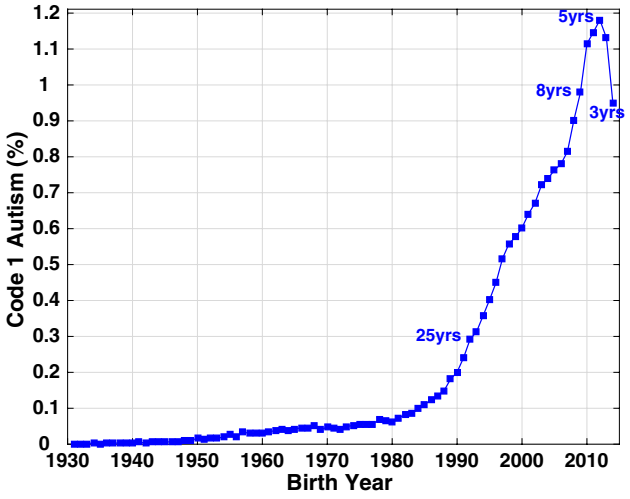

Fig. 1 Age-resolved snapshot for 2017, showing the growth in California Department of Developmental Services (CDDS) Code 1 autism prevalence from 0.001% in birth year 1931 to 1.18% in birth year 2012. (Color figure online)

Table 2 ASD prevalence slopes: ADDM and IDEA snapshot versus tracking

State	Trend slope ± slope error (per 10,000 per year)[a]			IDEA slope ratio[e] b_{snap}/b_{track}	Recent 8 year-old prevalence (%)	
	ADDM[b,c] b_{track}	IDEA[d] b_{snap}	IDEA[d] b_{track}		ADDM (BY 2002 or 2004)[f]	IDEA (BY 2003)
Alabama		4.0 ± 0.9	5.4 ± 0.2	0.74	0.57	0.66
Arizona	9.1 ± 1.5	5.9 ± 0.3	8.0 ± 0.3	**0.74**	1.52	0.96
Arkansas		4.1 ± 0.6	5.8 ± 0.8	0.71	1.20	0.84
California	N/A	7.6 ± 0.2	9.7 ± 0.4	**0.78**	N/A	1.25
Colorado		3.4 ± 0.3	5.2 ± 0.4	**0.67**	1.08	0.61
Florida		6.6 ± 0.5	7.6 ± 0.7	**0.87**	N/A	0.98
Georgia	8.2 ± 0.8	3.4 ± 0.8	4.8 ± 0.4	0.70	1.55	0.80
Maryland	7.4 ± 2.0	5.0 ± 0.9	8.2 ± 0.5	0.61	1.16	1.15
Missouri		5.0 ± 0.6	7.5 ± 0.4	0.67	1.15	1.03
New Jersey	13 ± 0.9	9.8 ± 0.6	10.4 ± 0.6	**0.94**	2.46	1.46
No. Carolina	12 ± 1.3	5.0 ± 0.4	7.5 ± 0.4	**0.67**	1.69	1.04
Pennsylvania		9.6 ± 0.5	12.6 ± 0.5	**0.76**	N/A	1.53
S. Carolina	6.1 ± 1.4	4.0 ± 0.3	5.1 ± 0.5	**0.78**	1.24	0.74
Utah		2.0 ± 0.3	5.6 ± 0.4	0.36	1.73	0.76
W. Virginia		4.3 ± 0.7	5.2 ± 0.4	0.82	N/A	0.76
Wisconsin	4.7 ± 0.9	6.0 ± 0.5	8.1 ± 0.3	**0.74**	1.08	1.22

[a]To convert to %/year, divide by 100

[b]The ADDM 8 year-old tracking slope is reported only when the least squares linear regression slope b_{track} is statistically different from 0 at a confidence level of $p < 0.01$ or better

[c]For ADDM data, the birth year span ranges from as early as 1992 to as late as 2004. See Fig. 5 and Supplementary File S4 for individual state details

[d]For all IDEA data, the birth year span is 1994–2003, the tracking age is 8 years old and the 2011 IDEA snapshot age range is 8–17 years old

[e]b_{snap}/b_{track} slope ratios are shown in bold face, indicating smaller uncertainty, when the slope error is ≤ 10% of the regression slope for both b_{track} and b_{snap}

[f]Birth Year (BY) 2004 prevalence shown if available, otherwise BY 2002

that year. Figure 1 plots the 2017 age-resolved snapshot, which is based on the DSM-5 criteria for ASD with additional CDDS requirements for functional disability in 3 out 7 life challenges.

Snapshot and Tracking Slopes

The snapshot versus tracking slope analysis is focused on the most recent decades of the CDDS data from 1989-present. This period covers school-age children, who are more likely than adults to be re-evaluated periodically for ASD, given the Child Find legal mandate (Wright and Wright 2007) and parental motivation of publicly-funded services. The availability of successive annual reports in these recent decades also allows for intercomparison of the age-resolved snapshot and constant-age tracking methods for estimating time trends. These recent time trends can be approximated with linear fits and thus used to quantify trend slopes. For the birth year 1989–2009 interval, the 2017 b_{snap} trend slope (3.8 ± 0.08 per 10,000 per year) is significantly greater than 0 at a high confidence level ($p \ll 0.01$), suggesting that the data are inconsistent with the null hypothesis that Code 1 autism is a constant prevalence condition (Fig. 2). The constant-age tracking slope b_{track} (4.3 ± 0.15 per 10,000 per year) also differs significantly from 0 at a high confidence level ($p \ll 0.01$).

The CDDS b_{snap} slope is somewhat flatter than the b_{track} slope computed over the same 1989–2009 birth year interval. The flatter slope is the result of upward revision over time in Code 1 autism prevalence for the earlier birth cohorts in the snapshot. For example, in the 1997 CDDS report, prevalence among 8 year-olds born in 1989 was 0.08%, but had been revised upward to 0.18% in the 2017 CDDS report for this same 1989 birth cohort. Regardless of which of these starting points is used, the growth to a prevalence of 0.98% among 8 year-olds in the 2009 birth cohort represents a substantial increase. The b_{snap}:b_{track} slope ratio over the 1989–2009 birth year interval is 0.87, suggesting the trend slopes are largely consistent across the constant-age tracking and age-resolved snapshot methods (Fig. 2).

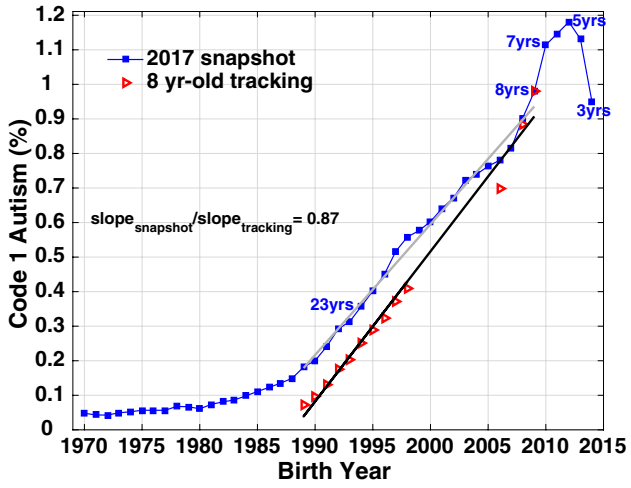

Fig. 2 CDDS data from 1997 to 2006, 2014, 2016 and 2017 reports, comparing 8 year-old tracking (red triangles) to 2017 age-resolved snapshot (blue squares) slopes over birth year interval 1989–2009. The b_{snap}:b_{track} slope ratio, representing the ratio of the grey:black slopes, is 0.87. Selected ages are labeled on the blue age-resolved snapshot curve, indicating the age of each birth cohort in 2017. (Color figure online)

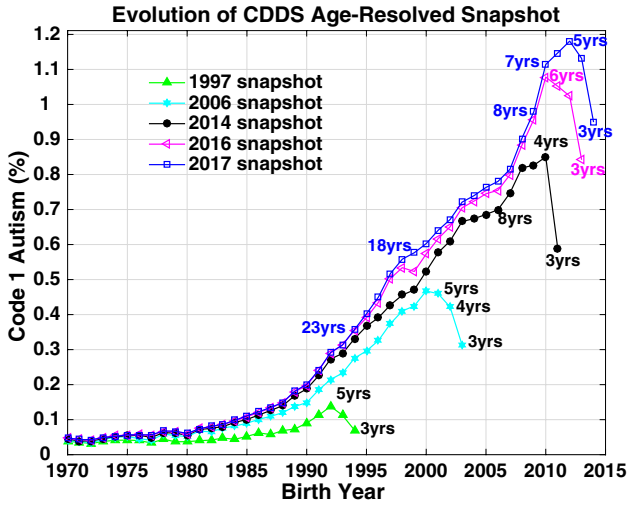

Fig. 3 CDDS Code 1 autism data comparing 1997 (green triangles), 2006 (cyan squares), 2014 (black circles), 2016 (magenta triangles) and 2017 (blue squares) age-resolved snapshots. Selected ages are labeled on each snapshot curve, indicating the age of each birth cohort at the time each respective CDDS report was compiled. (Color figure online)

Evolution of the CDDS Snapshot

Code 1 autism prevalence in the CDDS age-resolved snapshots has evolved substantially over time from the earliest report in 1997 to the most recent report in 2017 (Fig. 3). The 1997 age-resolved snapshot is relatively flat through the 1970s but prevalence starts trending upward around the mid to late 1980s, reaching a high of 0.14% among 5 year-olds born in 1992. Moving ahead 9 years, prevalence has increased overall in the 2006 snapshot from about birth year 1975 onward and reaches a high of 0.47% among 6 year-olds born in 2000. Another 8 years later, prevalence has increased again in the 2014 snapshot, with proportionally more growth for the more recent (after 1985) birth cohorts than the older birth cohorts, and reaches a high of 0.86% among 4 year-olds born in 2010. Additional increases occur in the 2016 snapshot, mainly after birth year 1995, with peak prevalence (1.08%) still occurring among the 2010 birth cohort, now age 6. One year later in the 2017 snapshot, peak prevalence (1.18%) shifts to 5 year-olds born in 2012.

Visual inspection of the complete 2017 snapshot (Fig. 1) suggests that Code 1 autism prevalence has been creeping slowly upward from near-zero levels since at least 1940, reaching 0.06% in 1980, at which point the numbers started rising more quickly, tripling to 0.18% by 1989. The change point at which the even more rapid increase of the 1990s and 2000s began is debatable, but appears to have occurred between 1988 and 1990. The 2014, 2016 and 2017 snapshots all suggest a slower rate of growth in the late 1990s and mid 2000s (Figs. 1, 3). However, all three snapshots show that the rate of growth accelerated again after 2006 until prevalence had reached an all-time high of 1.18% of 5 year-old children born in 2012.

Cohort Analysis

A conventional cohort–period–age plot (Gurney et al. 2003) provides an alternative, complementary way to examine how prevalence among selected birth cohorts has evolved as they age. Figure 4, which follows 11 CDDS birth cohorts over time, shows an upward revision with age starting among the birth cohorts of the late 1980s. Prevalence increases rapidly between ages 2 and 8. After age 8, a flatter but still ongoing upward revision continues throughout the teenage years and into adulthood. For example, prevalence in the 1997 birth cohort rises by 0.15% from age 8 to 20, while prevalence in the 1989 birth cohort rises by 0.11% from age 8 to 28. However, the cohort–period–age plot suggests little upward revision with age among the birth cohorts of the 1970s and early 1980s, consistent with Fig. 3.

Comparison of CDDS, IDEA and ADDM 8 Year-Old Prevalence in 16 States

Expanding the analysis to the IDEA and ADDM datasets allows examination of ASD trends in states beyond California and among different networks that often include a larger share of milder ASD than CDDS. Here, in order to compare all 3 networks, we show 8 year-old tracking trends, rather

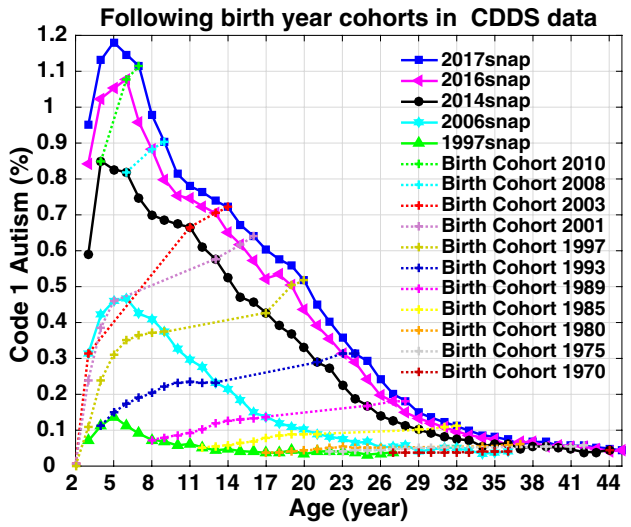

Fig. 4 CDDS prevalence by age, period, and year of birth. Dotted lines represent children born in the denoted year as they age over time. The period curves use the same data as the age-resolved snapshots for 1997, 2006, 2014, 2016 and 2017 in Fig. 3, but are plotted versus age (i.e., in reverse order along the X-axis) rather than versus birth year. (Color figure online)

than age-resolved snapshot trends, because only the former are available for ADDM. Figure 5 and Table 2 show that ASD prevalence varies among states, and also between different datasets within a given state. For IDEA, ASD prevalence among 8 year-olds born in 2003 (the most recent birth cohort available) varies over more than a factor of 2 among the 16 states examined, ranging from 0.61% in Colorado to 1.53% in Pennsylvania. For ADDM, ASD prevalence among 8 year-olds in the most recently available 2004 birth cohort also varies by more than a factor of 2, ranging from 1.08% in Wisconsin to 2.46% in New Jersey. Moreover, the New Jersey prevalence is more than 4 times larger than the Alabama prevalence of 0.57% (reported most recently for the 2002 birth cohort) (CDC 2014). In California, both absolute prevalence and the rate of increase in prevalence are greater in IDEA than in CDDS. In the remaining states, ADDM prevalence exceeds IDEA prevalence in 11 of 15 overlapping states and is comparable in 4 states.

All 2011 IDEA b_{snap} trend slopes presented in Table 2 are significantly >0, suggesting the IDEA data are inconsistent with the null hypothesis that ASD is a constant prevalence condition over time. The IDEA b_{snap} slope errors are considerably larger than for the CDDS data (ranging from 3 to 25% of b_{snap} with a median of 9%), but the t statistic still indicates that IDEA b_{snap} is non-zero at the $p<0.01$ confidence level for all 16 states in Table 2. Similarly, all IDEA 8 year-old tracking slopes b_{track} are significantly >0 ($p<0.01$) in all 16 states, with slope errors ranging from 4 to 14% (median 6%) of b_{track}. Interestingly, however, the ADDM b_{track} slopes differ significantly from 0 in only 7 out of the 15 ADDM states in Table 2.

The IDEA b_{snap} trend slopes are invariably flatter than the b_{track} slopes in all 16 states, with b_{snap}:b_{track} slope ratios ranging from 0.36 in Utah to 0.94 in New Jersey (Fig. 6). Excluding Utah, which is an outlier compared to the other states, the mean b_{snap}:b_{track} slope ratio is 0.75 ± 0.085. In California, the ratio is 0.78.

Nationwide ASD Prevalence Trends Among 8 Year-Olds

The 8 year-old tracking curves for United States nationwide ASD prevalence differ across networks, just as they did among the 16 individual states (Fig. 7). Nationwide ADDM prevalence is a factor of 1.5–2.5 higher than IDEA over the overlapping 1992–2003 birth year interval. The two datasets follow a similar trend slope over most of this interval, with notable exceptions between 1992–1994 and 2002–2004. ADDM suggests a flat trend over these periods, while IDEA 8 year-old prevalence climbs from 0.27 to 0.36% for 1992–1994 and from 0.92% in 2002 to 1.03% in 2003. CDDS data, which are plotted in Fig. 7 for comparison although they are for California only, also increase from 1992 to 1994, with a trend slope similar to that of IDEA. Although there is a data gap in the CDDS 8 year-old tracking data from birth year 1998–2006, the 2014, 2016 and 2017 CDDS reports suggest that prevalence continues to increase over those gap years.

Discussion

California Department of Developmental Services (CDDS)

Overview of Trends and Current Prevalence

The CDDS data featured in Figs. 1, 2, 3, 4 are widely considered the most reliable long-term record of autism prevalence trends in the United States (McDonald and Paul 2010; Autism Society San Francisco Bay Area 2015). The prevalence versus birth year curves suggest a dramatic increase over time, especially when viewed in the context of the full span of data extending back to birth year 1931, when the reported prevalence was only ~0.001% (Fig. 1). While a substantial fraction of the 1931 birth cohort was likely deceased by the time of the 2017 snapshot, this cohort had the same low prevalence at the time of the 1997 snapshot, when it was only in its 60s (Supplementary File S1). The increase from ~0.001 to 1.18% in the 2012 birth cohort has occurred gradually, with a slow upward creep starting as far back as the 1940s, but with several change points along the way,

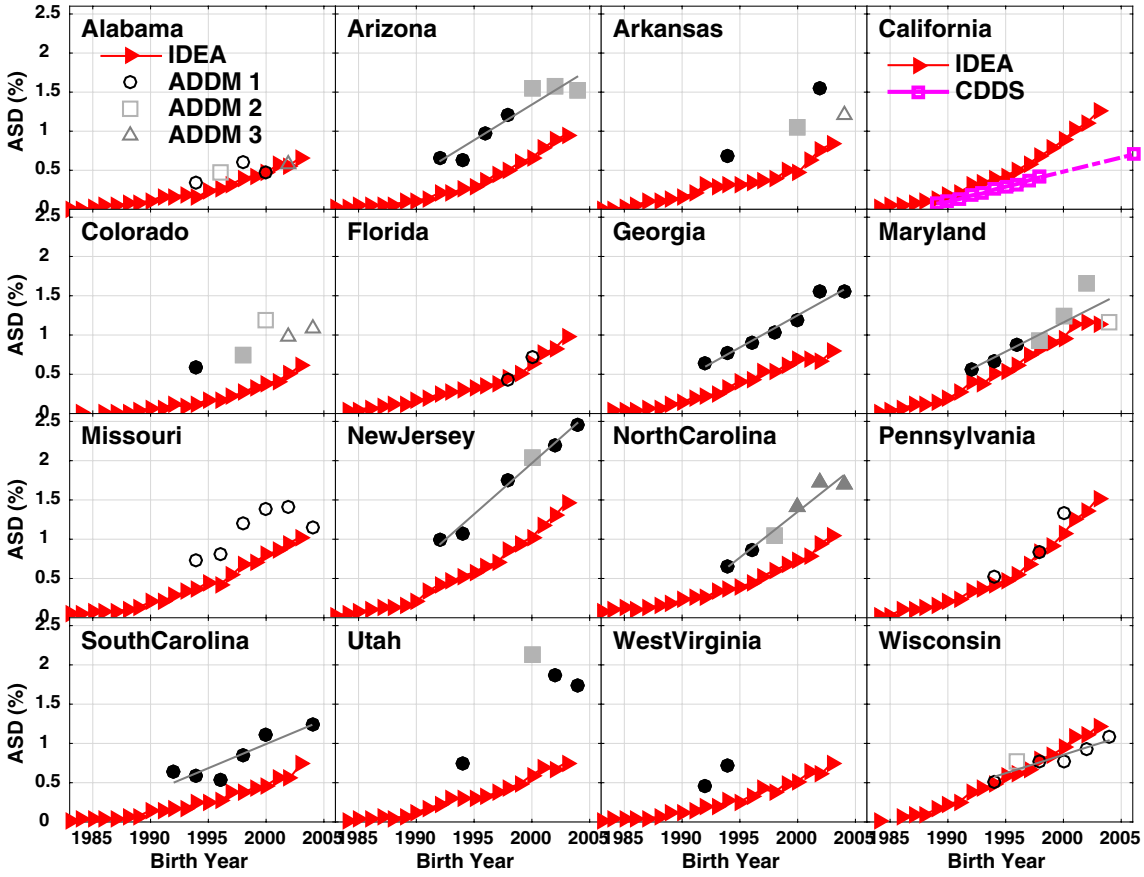

Fig. 5 Comparison of data tracking ASD prevalence among 8 year-olds from 3 different networks. IDEA data (red triangles) tracking 8 year-olds over report years 1991–2011 (corresponding to birth years 1983–2003) are available for all states. In California, the IDEA data are compared to CDDS 8 year-olds (magenta squares) tracked from report years 1997–2006 and 2014 (birth years 1989–1998 and 2006). For all other states, the IDEA data are compared to ADDM 8 year-olds tracked biannually from birth years 1992–2004. Up to 3 different black or grey symbols are used for the ADDM data to denote shifts and inconsistencies in the number of counties sampled within each state in successive reports. In addition, the ADDM data are plotted as solid symbols for prevalence derived based on both health and education records and as open symbols when only health records were available. (Color figure online)

around ~ 1980, ~ 1990, and ~ 2007, when the rate of growth accelerated.

The interpretation of the data is complicated by the redefinition of CDDS Code 1 from "autism, full syndrome" to AD in 2008, and by the further redefinition from AD to ASD at the end of 2014. The ~ 1980 and ~ 1990 change points noted above are relatively insensitive to these code changes, while the most recent birth cohorts are more likely to be affected. Although the entire 2017 snapshot in principle has been updated to the new DSM-5 definition of Code 1, due to the annual to triannual diagnosis update schedule, the point of entry into the CCDS system may be the single most important time for diagnosis; the CDDS regional centers in some cases may renew the date stamp of the Client Development Evaluation Report (CDER) without actively reviewing the diagnosis (Paul Choate, personal comm.). It is therefore possible that some of the new cases entering under Code 1 in the 2016 and 2017 snapshots would have been diagnosed with PDD-NOS or Asperger's under the earlier DSM-IV criteria and thus would not have been allowed into the CDDS caseload.

However, several considerations argue against changing diagnostic criteria as the only or primary cause of the new uptick in prevalence in recent years, which started around birth year 2007. First, the 2007 uptick is evident already in the 2014 snapshot, in which Code 1 was defined as AD based on DSM-IV. Second, the CDDS caseload historically has covered the more severe end of the ASD spectrum and likely continues to do so. The CDDS screening process is stringent in that it requires not only an autism diagnosis to qualify for services, but also demonstration of "significant functional disability" in at least 3 out of 7 life challenge areas. CDDS in fact raised the bar on this requirement in 2003 from a previously more lenient standard of 1 out of 7 (Autism Society San Francisco Bay Area 2015). This increased stringency makes the upward surge in prevalence around birth year

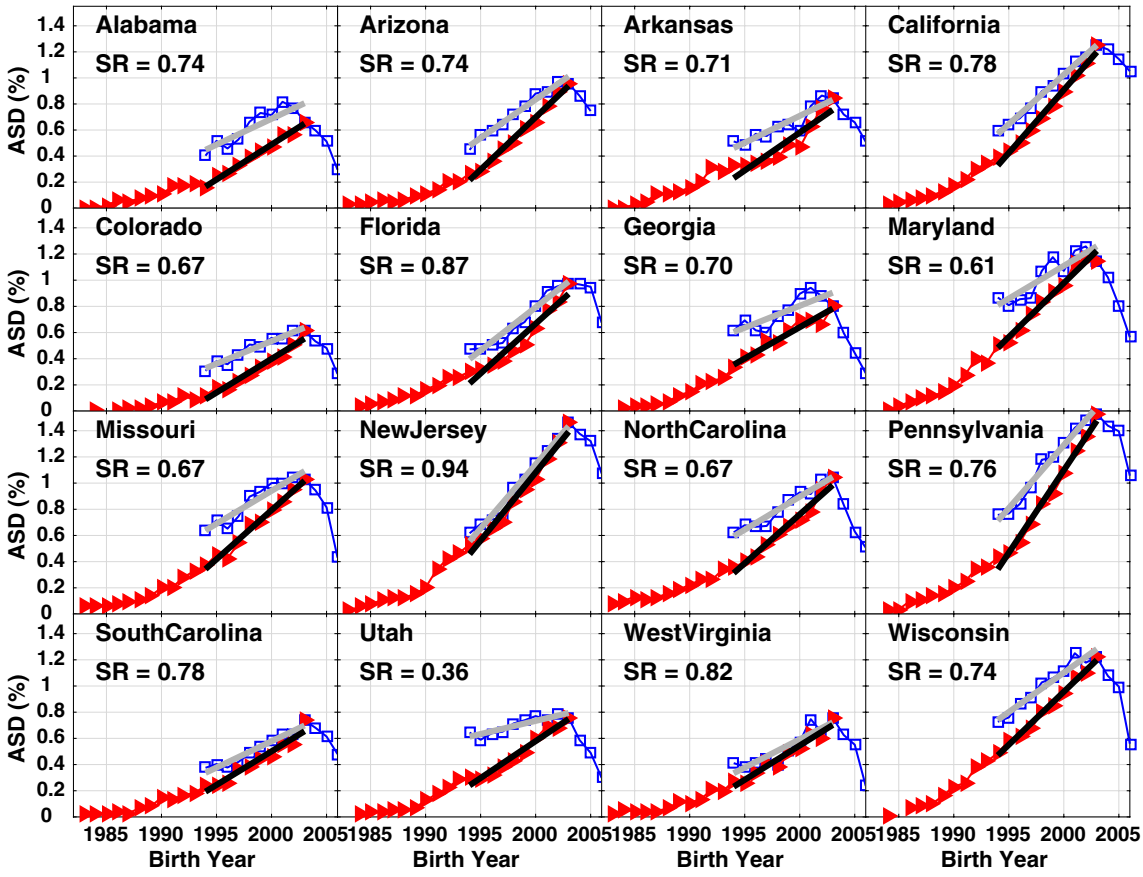

Fig. 6 IDEA data from 1991 to 2011 reports, comparing 8 year-old tracking data (red triangles) to 2011 IDEA age-resolved snapshot data (blue squares). The slopes of the ASD prevalence increase over birth year interval 1994–2003 are determined by least squares linear regression and plotted as gray and black lines for the 2011 snapshot and 8 year-old tracking, respectively. The b_{snap}:b_{track} slope ratio (*SR*) is shown in each panel, representing the ratio of the grey:black slopes. (Color figure online)

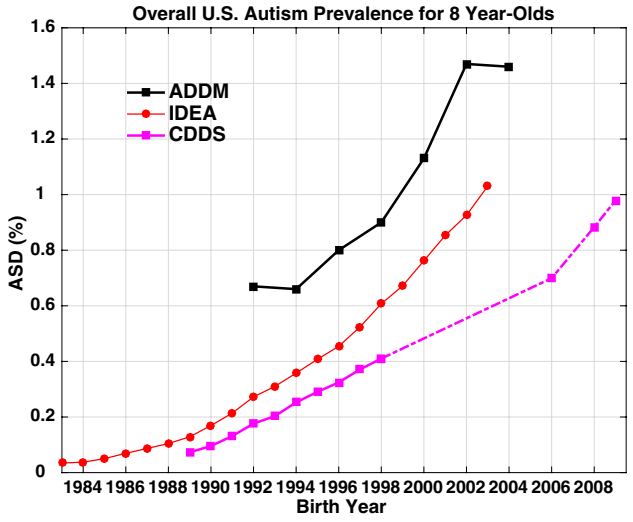

Fig. 7 Comparison of data tracking overall U.S. ASD prevalence among 8 year-olds from the IDEA and ADDM networks. Also shown are the CDDS 8 year-old data (covering CDER Code 1 autism cases in California only). (Color figure online)

2007 even more remarkable. Third, although some milder cases may be allowed in initially as very young children, the annual to triannual diagnosis update process screens them out relatively quickly. Of 34,711 new Code 1 cases entering CDDS services after November 2014, nearly 3000 have subsequently been discharged, due either to moving out of state or no longer meeting the qualifying requirements (Paul Choate, personal comm.). The latter is the more likely reason, since CDDS historically has had a substantially lower attrition rate. Of those new cases still qualifying for services, 68% had an ASD impact scale rating of moderate, 7.5% severe and 24% mild.

Snapshot Evolution and Snapshot Versus Tracking Analysis

The thirteen different age-resolved reports spanning 1997–2017 provide a means to examine how the diagnosed prevalence of CDDS autism has evolved over the years among the same birth cohorts. The passage of time across this 21-year span of reports has allowed

extensive opportunities for retroactive diagnosis, during an era of growing awareness, improved educational services and increasing availability of therapies and treatments. Accordingly, the birth cohorts from the late 1980s onward are characterized by modest but ongoing increases in diagnosed autism prevalence. These increases are evident in the evolution of the CDDS reports over time (Fig. 3), as well as the growth in prevalence among individual birth cohorts with age (Fig. 4).

Autism by definition is either present from birth or has been expressed by age 3. However, even using as a baseline the prevalence at age 8 [the tracking age used by ADDM (CDC 2016)], Fig. 4 shows an ongoing increase in prevalence into adulthood by factors ranging from 1.35 to 2.5 (corresponding to absolute rises in prevalence of 0.11–0.15%). Part of this increase may be due to net migration into California (Fountain and Bearman 2011; Paragon Real Estate Group 2017), which is not accounted for in our prevalence calculation, since we use static live birth data for the denominator. Still, the ongoing upward revision is curious and lends support to claims that better and expanded diagnosis is driving at least part of the reported increase in these later birth cohorts (assuming the increase is not explained by immigration). However, the increases of up to a factor of 2.5 over time from age 8 onward among individual birth cohorts (Fig. 4) are small compared to the much larger increases that have occurred with time across successive birth cohorts. Across birth cohorts in the 2017 snapshot, CDDS autism prevalence has increased by a factor of 25 from birth years 1970–2012 and by a factor of 1000 from birth years 1931–2012.

Focusing on the most recent birth years of 1989–2009, the age-resolved snapshot versus 8 year-old tracking analysis shows that the trend slopes computed from these two independent methods are largely consistent. Both methods indicate a steep increase over time in Code 1 autism prevalence, with a slope ratio of 0.87 (Fig. 2). Nevison (2014) suggested that the b_{snap}:b_{track} slope ratio of an autism prevalence versus time graph provides a rough estimate of the real fraction of the constant-age tracking trend. That interpretation, based on the slope ratio of 0.87, would suggest that 87% of the increase in CDDS autism tracked among 8 year-olds over the 1989–2009 birth year interval is due to a true rise in the condition. A caveat here is that the b_{snap}:b_{track} ratio is sensitive to tracking age. When we repeat the calculations in Fig. 2, but with 6 or 7 instead of 8 as the tracking age, we calculate b_{snap}:b_{track} ratios of 82 and 86%, respectively, over birth years 1991–2011 and 1990–2010. One reason for the sensitivity of the b_{snap}:b_{track} ratios to tracking age may be that the assumption of linearity, which is required for the calculation, starts to break down in recent years due to the new uptick in the prevalence data around birth year 2007. While the b_{snap}:b_{track} method involves substantial uncertainty, it nevertheless provides an empirical, quantitative estimate of the real fraction of the increase in autism across CDDS birth cohorts over time, suggesting that ~ 82–87% of the tracked increase since birth year ~ 1990 may be due to a true rise in the condition. By implication, the residual ~ 13–18% of the increase is likely not real and may be due instead to immigration or better and expanded diagnosis.

There are three main hypotheses for how better and expanded diagnosis might lead to an apparent but non-real increase in autism prevalence (Keyes et al. 2012): (1) diagnostic substitution, with intellectual disability typically named as the diagnostic substituent (Croen et al. 2002; Polyak et al. 2015), (2) diagnostic expansion, which often refers to the addition of Asperger's syndrome to the list of autism spectrum disorders, and (3) diagnostic oversight, which refers to the possibility that children who were overlooked in years past are now being identified, thanks to increased awareness among families and diagnosticians (Blaxill 2004).

Hypothesis 1 is unlikely, since the trend in intellectual disability in California has been more or less flat over the time frame of the steep rise in autism (Shattuck 2006; Nevison and Blaxill 2017). Hypothesis 2 may be a contributing factor, given the several revisions to CDER Code 1 over the years, but is unlikely to be the main driver of the increases shown in Figs. 1, 2, 3, for the reasons discussed earlier. The third hypothesis, diagnostic oversight, also may be a viable explanation for some of the upward revision over time among specific birth cohorts shown in Figs. 3 and 4. However, we cannot ascribe the upward revision definitively to any specific hypothesis based on the high-level statewide data presented in this study.

In contrast to the birth cohorts from the late 1980s onward, there is little or no upward revision in diagnosis among the birth cohorts of the 1930s, 1940s and 1950s, and only small upward revision for the birth cohorts of the 1960s, 1970s and early 1980s (Figs. 3, 4, Supplementary File S1). The CDDS datasets thus show no obvious evidence of a large, overlooked population of autistic adults in the 1931 through early 1980s birth cohorts. However, these cohorts were already in their teens or older by the time of the first available CDDS report in 1997. Thus, these cohorts, with the exception of the teenagers born in the early 1980s, in general were not covered by the Child Find mandate and it is unclear whether they would have the opportunity or incentive to be evaluated for ASD as adults if they were not already diagnosed as children.

Differences Among States and Data Networks

Previous studies based on assemblages of autism data from different places, reflecting different criteria, and grouped into irregular time or age bins have been unable to confirm a clear trend in the data. Further, the greater than threefold

difference among 8 year-olds in an (early) ADDM survey between New Jersey and Alabama has been cited as a reason why changing diagnostic criteria have played a major role in creating the apparent increase in autism (Fombonne 2009). Our study concurs that autism prevalence estimates differ substantially among CDDS, IDEA and ADDM datasets and among states within the IDEA and ADDM networks. These differences, combined with the likelihood of strong gradients in prevalence as a function of age, suggest the need for caution in combining widely disparate datasets when evaluating time trends in autism prevalence or, more generally, when citing a single number [e.g., 1 in 68 (CDC 2016)] as the overall rate of autism.

A comparison of New Jersey and Alabama is instructive for understanding some of the reasons behind the large apparent differences in ASD prevalence among states and data networks. Our analysis also finds a more than threefold difference between ADDM prevalence in New Jersey versus Alabama in the most recent available common birth year, 2002 (Fig. 5), but much of the difference can be attributed to several identifiable factors. First, 8 year-olds are still substantially under-ascertained in Alabama while in New Jersey they are not. For the 2001 birth year cohort, comparison of 8 year-old and 10 year-old IDEA data (Supplementary Dataset S3) shows that prevalence among Alabama children was revised upward by 40% from age 8 to 10, whereas in New Jersey 8 year-olds were more fully diagnosed, with only 4% upward revision from age 8 to 10.

A second factor involves differences in the regions sampled within each state. Taking birth year 2002 as an example, prevalence among 8 year-olds differs by a factor of 3.8 for ADDM between New Jersey and Alabama, but IDEA prevalence among 8 year-olds differs by only a factor of 2.3 between the two states. IDEA data cover the whole of both states. In contrast, ADDM typically surveys 4 urban counties in New Jersey, but samples Alabama broadly across 32 counties in the northern half of the state, comprising a mix of urban and rural areas. ASD prevalence in general tends to be higher in urban than rural areas, for reasons that are not clear but may involve enhanced exposure to beneficial environmental microbes in rural areas and/or higher levels of toxins in urban areas (Becker 2010; Dickerson et al. 2016). As a result, the different sampling strategies across the two states will tend to exaggerate the difference in ASD prevalence between New Jersey and Alabama.

If one combines these two factors (i.e., comparing at age 10 instead of age 8, and comparing IDEA prevalence across the whole of both states instead of ADDM prevalence in selected counties), one can account for much of the difference in ASD prevalence between New Jersey and Alabama. Indeed, the prevalence ratio is reduced from 3.8 to 1.5. This remaining ratio of 1.5 may or may not reflect a true regional difference between the two states. Regardless, each state individually shows a statistically significant increasing trend in ASD, and that trend furthermore is relatively consistent between the age-resolved snapshot and constant age tracking methods, when applied to IDEA data (Fig. 6; Table 2).

Another important consideration is that, within the ADDM network, New Jersey has more detailed and extensive records available and has access to information from both education and health sources in its ascertainment, whereas Alabama's case finding is limited to healthcare sources (CDC 2014). New Jersey's unusual alignment of snapshot and constant-age tracking slopes (cf. b_{snap}:b_{track} ratio of 0.94 shown in Fig. 6) may indicate that New Jersey's educational records are more comprehensive than those in other states, leading to more complete case finding. Here it is interesting and somewhat paradoxical to note that the IDEA and ADDM prevalence values tend to agree best in states in which the ADDM prevalence is estimated only based on health records (Alabama, Missouri, Wisconsin and Pennsylvania) (CDC 2014), while ADDM prevalence tends to exceed IDEA prevalence in states where ADDM has access to both health and educational records (Fig. 5). A detailed analysis in Utah found that ASD prevalence was higher when estimated based on both health and education data, with a greater proportion of cases ascertained from health records (Pinborough-Zimmerman et al. 2012). That analysis may explain part of the large discrepancy between ADDM and IDEA prevalence in Utah shown in Fig. 5.

Another important factor in these considerations is the extent to which milder forms of ASD are included in the definition of autism. Among the three data networks examined in this paper, autism prevalence increases in the following order: CDDS < IDEA ≤ ADDM (Figs. 5, 7). This ordering is broadly consistent with the inclusion or exclusion of milder ASD in these datasets. CDDS historically has been entirely AD and continues to focus on the more severely affected population under *DSM-5*, IDEA includes some milder forms of ASD in some states but not in others (MacFarlane and Kanaya 2009), while ADDM attempts to include all ASD subtypes. Here, it is notable that Asperger's cases have accounted consistently for only ~ 10% of the total ADDM cases over the reports (birth year 2000–2004) that provide this information (CDC 2012, 2014, 2016). However, given that the median age of Asperger's diagnosis is about 8 (Lingam et al. 2003), ADDM, which surveys 8 year-olds, likely misses the true number of Asperger's cases.

Inconsistencies in the ADDM Network and in Nationwide Prevalence

While ADDM data are commonly cited as the definitive metric of United States ASD prevalence, only 7 out of 15 ADDM states, as shown in Fig. 5, have an 8 year-old tracking slope b_{track} that is statistically different from 0 at the

$p < 0.01$ confidence level. This was surprising because we had expected to be able to reject the null hypothesis for all b_{track} slopes simply due to better and expanded ASD diagnosis over time. However, inconsistencies in the ADDM Network, as well as the small number of data points and missing years of data, may have contributed to the surprising ADDM b_{track} results. Among the 15 states surveyed over the history of the ADDM network, only 1 (Georgia) has consistently monitored ASD in the same subset of counties with complete coverage, including access to health and education data, over all seven available ADDM reports from birth year 1992–2004.

Another curious feature of ADDM data is that the network appears to underestimate the upward trend in nationwide ASD prevalence between birth years 1992–1994 and again between birth years 2002–2004 compared to IDEA and CDDS (Fig. 7). The ADDM numbers remain stable over both of these 2-year intervals, even as CDDS and IDEA prevalence continues to increase. One reason for the flat ADDM trend from 2002 to 2004 may have been that 2 states (Maryland and Arkansas), which had traditionally had access to health and education records in earlier ADDM reports, lost access to most of their education records in 2004 (CDC 2014, 2016). Accordingly, both these states reported a decrease in ASD prevalence from 2002 to 2004.

The flat ADDM trend from 1992 to 1994 trend may reflect the sensitivity of the nationwide ADDM mean to the changing set of counties and states surveyed. The overall mean is computed as the sum of all the ASD cases in the participating states divided by the sum of the total population surveyed. In both 1992 and 1994 the ADDM overall mean was about 0.66%, or 1/150. In 1992, six states were sampled, while in 1994, 14 states were sampled, including all original 6. If just the original 6 states had been sampled for birth year 1994, instead of remaining flat, the overall mean would have increased by 10% from 1992 to 0.74%. Each of the subsequent ADDM reports has brought a new shift in which and how many states are included. Each also has been accompanied by changes in the number of counties sampled within many of the participating states (Supplementary File S4). Given these uncertainties, it is unfortunate that restrictions on the availability of cohort-referenced IDEA data beyond the 2011 report will hinder systematic tracking of IDEA trends into more recent birth years, making it difficult to compare future IDEA and ADDM nationwide trends.

Conclusion

CDDS autism prevalence has risen dramatically over the last 35 years, increasing from ~0.05% in birth year 1970 to nearly 1.2% in birth year 2012. The available data extending back to 1931 show a prevalence of only 0.001% in that birth cohort. Prevalence slowly increased from ~1940 to 1980, at which time the first of several change points occurred, in ~1980, ~1990, and ~2007, each associated with a new uptick in the rate of growth. The CDDS dataset suggests that prevalence has increased by a factor of 25 from birth year 1970–2012 and by as much as a factor of 1000 from birth year 1931–2012.

CDDS continues to exclude most milder cases of autism, despite two different changes to its diagnostic criteria in the last decade. As a result, IDEA autism prevalence in California is substantially higher than CDDS prevalence. ADDM ASD prevalence in turn is substantially higher than IDEA prevalence in 11 out of 15 overlapping states, likely due to a combination of factors, including inclusion of all forms of ASD, access to health and education-based records, and disproportionate sampling of urban over rural areas in some states. While about half of ADDM states have non-significant 8 year-old tracking trend slopes, this is attributable in part to discontinuous or inconsistent data records and differences in completeness, suggesting the need for more consistent sampling strategies when evaluating time trends in overall ASD prevalence. The ADDM network states with the most consistent access to information from multiple (health and education) sources show the most strongly increasing ASD trends. Metropolitan New Jersey, for example, has been the leading indicator of autism prevalence in the ADDM network across the decade, with the most recent prevalence estimate showing ASD prevalence as high as 2.5% among 8 year-olds of the 2004 birth cohort (Zahorodny et al. 2014; CDC 2016).

Acknowledgments The authors thank the California Department of Developmental Services (CDDS) for providing much of the data used in this study. We are particularly grateful to CDDS data analyst Paul Choate for his detailed explanations of CDDS codes and practices. We also thank two anonymous reviewers for their helpful comments, which much improved the manuscript.

Author Contributions CN conceived of the study, compiled the IDEA, ADDM and most recent CDDS data, and drafted most of the manuscript. MB compiled the 1997–2006 CDDS data and wrote parts of the Discussion. WZ provided detailed information about the ADDM data and wrote parts of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest CN, MB and WZ declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Approval The ASD counts used in this study involved datasets in which all relevant personal information had been de-identified prior to our activities and in which the data were aggregated by age at the state level. This project therefore did not require institutional review and approval.

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EXHIBIT 61

Summary of Autism Spectrum Disorder (ASD) Prevalence Studies

Author	Year published	Country	Time period studied	Age range studied	Number of children in population	Criteria used	Methodology used	ASD prevalence (CI)	IQ<70 (%)
Lotter	1966	England	1964	8 to 10	78,000	Kanner	Case enumeration and direct exam	0.45 (0.31-0.62)	84
Brask	1970	Denmark	1962	2 to 14	46,500	Kanner	Case enumeration	0.43 (0.26-0.66)	NR
Treffert	1970	USA	1962-1967	3 to 12	899,750	Kanner	Case enumeration	0.07-0.31 (0.0-1.0)	NR
Wing & Gould	1979	England	1970	0 to 14	35,000	Kanner	Case enumeration and direct exam	0.49 (0.29-0.78)	70
Hoshino et al. (1)	1982	Japan	1977	0 to 17	234,039	Kanner	Case enumeration and direct exam	0.23 (0.19-0.27)	NR
Ishii & Takahashi	1983	Japan	1981	6 to 12	35,000	Rutter	Case enumeration and direct exam	1.6 (1.2-2.8)	NR
Bohman et al.	1983	Sweden	1979	0 to 20	69,000	Rutter	Case enumeration and direct exam	0.3 (0.2-0.5)	NR
McCarthy et al.	1984	Ireland	1978	8 to 10	65,000	Kanner	Case enumeration and direct exam	0.43 (0.29-0.59)	NR
Gillberg	1984	Sweden	1980	4 to 18	128,584	DSM-III	Case enumeration and direct exam	0.20 (0.13-0.30)	80, 77
Steinhausen et al.	1986	Germany	1982	0 to 14	279,616	Rutter	Case enumeration and direct exam	0.19 (0.14-0.24)	44

Author	Year published	Country	Time period studied	Age range studied	Number of children in population	Criteria used	Methodology used	ASD prevalence (CI)	IQ<70 (%)
Steffenberg & Gillberg	1986	Sweden	1984	<10	78,413	DSM-III	Case enumeration and direct exam	0.45 (0.31-0.62)	NR
Matsuishi et al.	1987	Japan	1983	4 to 12	32,834	DSM-III	Case enumeration and direct exam	1.55 (1.16-1.64)	NR
Burd et al.	1987	USA	1985	2 to 18	180,986	DSM-III	Case enumeration and direct exam	0.12 (0.00-0.20)	NR
Bryson et al.	1988	Canada	1985	6 to 14	20,800	DSM-III	Case enumeration and direct exam	1.01 (0.62-1.54)	76
Tanoue et al.	1988	Japan	1977-1985	3 to 7	95,394	DSM-III	Case enumeration	1.38 (1.16-1.64)	NR
Ciadella & Mamelle	1989	France	1986	3 to 9	135,180	DSM-III	Case enumeration	0.51 (0.39-0.63)	NR
Sugiyama & Abe	1989	Japan	1979-1984	2 to 5	12,263	DSM-III	Population screen and direct exam	1.3 (0.7-2.1)	38
Ritvo et al.	1989	USA	1984-1988	8 to 12	184,822	DSM-III	Case enumeration and direct exam	0.40 (0.31-0.50)	NR
Gillberg et al.	1991	Sweden	1988	4 to 13	78,106	DSM-III-R	Case enumeration and direct exam	0.95 (0.74-1.95)	82, 80
Fombonne & Mazaubrun (1)	1992	France	1985	9 to 13	274,816	ICD-10	Case enumeration and direct exam	0.49 (0.47-0.65)	87
Honda et al.	1996	Japan	1994	1.5 to 6	8,537	ICD-10	Population screen and direct exam	2.11 (1.25-3.33)	50

Author	Year published	Country	Time period studied	Age range studied	Number of children in population	Criteria used	Methodology used	ASD prevalence (CI)	IQ<70 (%)
Fombonne et al.	1997	France	1992-1993	6 to 16	325,347	ICD-10	Case enumeration and direct exam	0.54 (0.46-0.62)	88
Arivdsson et al.	1997	Sweden	1994	3 to 16	1,941	ICD-10	Population screen and direct exam	3.10 (1.14-6.72)	100
Webb et al.	1997	Wales	1992	3 to 15	73,300	DSM-III-R	Case enumeration and direct exam	0.72 (0.54-0.95)	NR
Sponheim & Skjeldae	1998	Norway	1992	3 to 14	65,688	ICD-10	Case enumeration and direct exam	0.38 (0.25-0.56)	64
Kadesjo et al.	1999	Sweden	1992	6.7 to 7.7	826	ICD-10	Case enumeration and direct exam	6.0 (1.97-14.1)	60
Baird et al.	2000	England	1998	1.5 to 8	16,235	ICD-10	Population screen and direct exam	3.1 (2.29-4.06)	40
Powell et al.	2000	England	1995	1 to 4	29,200	DSM-III-R or DSM-IV	Case enumeration	0.96 (0.64-1.39)	NR
Kielinen et al.	2000	Finland	1996	5 to 18	152,732	DSM-IV	Case enumeration	1.22 (1.06-1.41)	50
Magnusson & Saemundsen	2000	Iceland	1997	5 to 14	43,153	ICD-10	Population screen and direct exam	0.86 (0.60-1.18)	49
Chakrabarti & Fombonne	2001	England	1998	2.5 to 6.5	15,500	DSM-IV	Population screen and direct exam	1.68 (1.1-2.46)	24
Fombonne et al. (2)	2001	UK	1999	5 to 15	12,529	DSM-IV	Population screen and direct exam	2.61 (1.81-3.70)	44.4

Author	Year published	Country	Time period studied	Age range studied	Number of children in population	Criteria used	Methodology used	ASD prevalence (CI)	IQ<70 (%)
Bertrand et al.	2001	USA	1998	3 to 10	8,996	DSM-IV	Case enumeration and direct exam	4.0 (2.8-5.5)	49
Croen et al.	2001	USA	1987-1999	0 to 21	4,600,000	DSM-III-R or DSM-IV	Case enumeration	1.1 (1.06-1.14)	NR
Yeargin-Allsopp et al. (2)	2003	USA	1996	3 to 10	290,000	DSM-IV	Case enumeration	3.4 (3.2-3.6)	62
Gurney et al. (2)	2003	USA	1981-1982, 2001-2002	6 to 17		DSM-IV	Case enumeration	4.4 (4.3-4.5)	NR
Lingam et al.	2003	UK	2000	5 to 14	186,206	ICD-10	Case enumeration	1.5 (1.3-1.7)	NR
Icasiano et al.	2004	Australia	2002	2 to 17	45,153	DSM-IV	Case enumeration	3.9 (3.3-4.5)	47
Lauritsen et al.	2004	Denmark	2001	0 to 9	682,397	ICD-10	Case enumeration	1.2 (1.1-1.3)	NR
Fombonne et al.	2006	Canada	1987-1998	5 to 21	27,749	DSM-IV	Case enumeration	2.16 (1.65-2.78)	NR
Baird et al.	2006	UK	1990-1991	9 to 10	56,946	ICD-10	Case enumeration, screen, and direct exam	3.89 (3.39-4.43)	56
CDC ADDM Network (1)	2007	USA	2000	8	187,761	DSM-IV	Case enumeration and record review	6.7 (6.3-7.0)	36-61
CDC ADDM Network (1)	2007	USA	2002	8	444,050	DSM-IV	Case enumeration and record review	6.6 (6.3-6.8)	45

| Author | Year published | Country | Time period studied | Age range studied | Number of children in population | Criteria used | Methodology used | ASD prevalence (CI) | IQ<70 (%) |

Author	Year published	Country	Time period studied	Age range studied	Number of children in population	Criteria used	Methodology used	ASD prevalence (CI)	IQ<70 (%)
Oullette-Kuntz et al.	2007	Canada	1996-2004	4 to 9	2,240,537	Special education classification	Case enumeration from special education classification	1.2 (1996), 4.3 (2004)	NR
Wong et al. (1)	2008	Hong Kong	1986-2005	0 to 14	4,247,206	DSM-IV	Case enumeration	1.6	NR
Williams et al.	2008	Australia	2003-2004	6 to 12	5,459	DSM-IV	Questionnaires	1.0 (0.8-1.0) to 4.1 (3.8-4.4)	NR
Montiel-Nava et al.	2008	Venezuela	2005-2006	3 to 9	254,905	DSM-IV	Case enumeration	1.7 (1.3-2.0)	NR
Baron-Cohen et al.	2009	UK	2003-2004	5 to 9	5,484	Special Education Needs register	Case enumeration from survey and direct exam	15.7 (9.9-24.6)	NR
CDC ADDM Network (1)	2009	USA	2004	8	172,335	DSM-IV	Case enumeration and record review	8.0 (7.6-8.4)	44
CDC ADDM Network (1)	2009	USA	2006	8	308,038	DSM-IV	Case enumeration and record review	9.0 (8.6-9.3)	41
Al-Farsi et al.	2010	Oman	2009	0 to 14	798,913	DSM-IV	Case enumeration	0.1 (0.1-0.2)	NR
Parner et al.	2011	Denmark	1994-1999		404,816	DSM-IV	Case enumeration	6.9 (6.5-7.2)	NR
Parner et al.	2011	Western Australia	1994-1999		152,060	DSM-IV	Case enumeration	5.1 (4.7-5.5)	NR
Chien et al.	2011	Taiwan	1996-2005	0 to 18	372,642	ICD-9	Case enumeration	2.9	NR

Windham et al.	2011	USA	1994, 1996	0 to 8	82,153 (1994), 80,249 (1996)	DSM-IV	Case enumeration	4.7 (4.2-5.1) (1994); 4.7 (4.2-5.2) (1996)	NR
Kim et al.	2011	South Korea	2005-2009	7 to 12	55,266	DSM-IV	Case enumeration from survey and direct exam	26.4 (19.1-33.7)	59
Zimmerman et al.	2012	USA	2002, 2006, 2008	8	26,213 (2002); 29,494 (2006); 33,757 (2008)	ICD-9 and special education classification	Case enumeration	6.5 (2002), 10.2 (2006), 13.0 (2008)	NR
Kocovska et al.	2012	Faroe Islands	2002, 2009	7 to 16 (2002), 15 to 24 (2009)	7122 (2002), 7128 (2009)	DSM-IV, ICD-10	Screening and direct exam	5.6 (2002), 9.4 (2009)	NR
CDC ADDM Network (1)	2012	USA	2008	8	337,093	DSM-IV	Case enumeration and record review	11.3 (11.0-11.7)	38
Blumberg et al.	2013	USA	2011-2012	6 to 17	95,677	Parent report	Telephone survey	2%	NR
Zablotsky et al.	2015	USA	2011 -2014	3 to 17	43,283	Parent report	Household survey	2.4%	NR
Christensen et al. (1)	2016	USA	2012	8	346,978	DSM-IV	Case enumeration and record review	14.6 (8.2 – 24.6)	32

(1) The prevalence reported represents the average.
(2) The prevalence study provided overall rate only

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Psychiatry Research 287 (2020) 112922

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Review article

Prevalence of comorbid psychiatric disorders among people with autism spectrum disorder: An umbrella review of systematic reviews and meta-analyses

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ARTICLE INFO

Keywords:
Neurodevelopmental disorders
Mental disorders
Comorbidity
Multimorbidity
Epidemiology

ABSTRACT

With ever-increasing prevalence of various mental disorders worldwide, a comprehensive evaluation of the prevalence of co-occurring psychiatric disorders among individuals with autism spectrum disorder (ASD) is needed to strengthen the knowledge base. This umbrella review aims to summarize the current evidence on the prevalence of comorbid psychiatric disorders among people with ASD. A systematic search of 12 major databases and additional sources was conducted. Any systematically conducted narrative, qualitative, or meta-analytic review reporting the prevalence of psychiatric disorders among people with ASD with no age or geographical restriction were included. From a total of 2755 records, 26 articles representing 14 systematic reviews and 12 meta-analyses met the criteria of this review. The synthesized findings reveal a high burden of comorbid psychiatric disorders among people with ASD, including anxiety disorders, depressive disorders, bipolar and mood disorders, schizophrenia spectrum, suicidal behavior disorders, attention-deficit/hyperactivity disorder, disruptive, impulse-control and conduct disorders amongst diverse age groups, with a majority in younger participants. Most studies were conducted in developed nations, with limited evidence from low and middle-income countries. These synthesized findings provide high-quality evidence for clinical and policy-level decision-making from a global overview of the status of comorbid psychiatric disorders among people with ASD.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder of early-onset characterized by social-communication difficulties and repetitive or stereotypical behaviors (Masi et al., 2017). Nearly 1% of global prevalence makes ASD one of the widely discussed neuropsychiatric problems that critically affect the lives of the affected individuals, caregivers, and communities (Lyall et al., 2017). The long-term health outcomes associated with ASD continue to impose a high burden of care, which necessitates a scientific evaluation of how different comorbid conditions affect people with ASD (Hoetman et al., 2014; Masi et al., 2017). Among diverse co-occurring health conditions, the issue of psychiatric comorbidity in ASD has become more relevant in recent years as the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) no longer excludes additional diagnoses among individuals with ASD (Romero et al., 2016). This inclusion is consistent with past research that ASD can co-occur with other psychiatric disorders (Brereton et al., 2006; Yerys et al., 2011). It is estimated that nearly 70% of people with ASD experience at least one comorbid psychiatric disorder, whereas nearly 40% individuals may have two or more psychiatric disorders (DeFilippis, 2018). Individuals with ASD are likely to experience a higher prevalence of common mental disorders compared to the typically developed individuals (DeFilippis, 2018; Romero et al., 2016). Moreover, children with ASD have been found to have a higher burden of psychiatric comorbidity than children with intellectual disability (Romero et al., 2016). Previous studies have shown that psychiatric comorbidity in ASD significantly increases the difficulties in adaptive responses and affects the daily activities,

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https://doi.org/10.1016/j.psychres.2020.112922
Received 3 January 2020; Accepted 15 March 2020
Available online 18 March 2020
0165-1781/

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decreases the quality of living, and accentuates problems like restlessness, passivity, social isolation, aggressiveness, irritability, or self-injury (Fitzpatrick et al., 2016). These problems are difficult to identify and address, which they coexist with a complex condition like ASD. It is well documented that diagnosing ASD is a difficult task due to the unique clinical and subclinical manifestations among individuals (Huerta and Lord, 2012); therefore, diagnosing co-occurring psychiatric disorders in ASD may be challenging for the mental healthcare providers. In addition, contexts with fewer human resources for mental health are likely to have an under-recognized burden of psychiatric comorbidities among people with ASD (Tekola et al., 2016). These challenges should be acknowledged to improve the diagnostic capacities for ASD and comorbid psychiatric disorders for early diagnosis and management. In this discourse, it is essential to understand the epidemiological burden of psychiatric comorbidity in ASD, which may enable effective policymaking and capacity building across health systems to improve mental healthcare for people with ASD (Baxter et al., 2015).

To strengthen the knowledge base on how different psychiatric disorders are distributed in the ASD population, an extensive evaluation of the prevalence of mental disorders among individuals with ASD is needed. While observational studies conducted in different places evaluating one or more psychiatric disorders may provide a partial picture of the population-level psychiatric burden in ASD, such findings can be synthesized in evidence-based reviews minimizing the methodological issues in individual studies and providing a collective overview of the problems. Therefore, systematically conducted reviews of empirical studies offer high-quality evidence for clinical and policy-level decision-making (Garg et al., 2008). Given that more than 11 systematic reviews are published in a day (Bastian et al., 2010), there is a growing interest in synthesizing knowledge from published reviews, which is known as umbrella review, or systematic review of the reviews (Aromataris et al., 2015). This umbrella review systematically evaluated the current evidence on the prevalence of comorbid psychiatric disorders among people with ASD from systematic reviews and meta-analyses to inform future research, policymaking, and practice.

2. Materials and methods

2.1. Search strategy of the review

In this umbrella review, we adopted the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009), and the Joanna Briggs Institute (JBI) methodology for umbrella reviews (Aromataris et al., 2015). We searched MEDLINE, Academic Search Ultimate, PsycINFO, CINAHL Complete, Health Policy Reference Center, ERIC, Health Source (Nursing/Academic Edition), Child Development & Adolescent Studies, PsycARTICLES, EMBASE, PubMed, and the Cochrane Library using specific keywords (see Table 1). The titles, abstracts, subject headings, and general keywords were searched with no language restrictions. In addition, we searched the references of selected articles and citing articles from Google Scholar using the "cited by" function. All databases and additional sources were searched from their inception to November 10, 2019, and the entire search process was repeated on December 25, 2019, for the last time. All the retrieved citations were uploaded to Rayyan cloud-based systematic review management system for further assessment (Ouzzani et al., 2016).

2.2. Inclusion and exclusion criteria

In this umbrella review, we included the systematically conducted narrative, qualitative, or meta-analytic reviews reporting the prevalence of psychiatric disorders among people with ASD. Also, any quantitative measures of disease burden, including odds ratio or relative risk expressing the epidemiological burden of psychiatric comorbidity in ASD, were also considered in the absence of a prevalence estimation. To further specify the psychiatric disorders as well as ASD, mental health conditions were included if they were defined within the classifications of psychiatric disorders in the International Classification of Diseases (ICD) tenth revision (World Health Organization (WHO), 2016), or the Diagnostic and Statistical Manual (DSM) fifth edition (American Psychiatric Association, 2013). Upon considering the historical evolution of the definitions and conceptual constructs for different psychiatric conditions, any disorder reported in the earlier versions of ICD or DSM were considered if they had an equivalent diagnosis classified under the current versions of these guidelines. Further, the reviews were recruited if they fulfilled all the inclusion criteria and excluded if they had conflicts with at least one of the exclusion criteria, as listed in Table 2. Two authors independently evaluated all the retrieved citations. After the preliminary screening, potential conflicts on eligibility were addressed through discussion and consultation with a third author.

2.3. Data extraction and synthesis

We developed a data extraction tool using the JBI data extraction tool for systematic reviews and research synthesis (Munn et al., 2014). Two authors independently extracted data on the following domains: the objectives and types of the review articles, year of publication, names of databases, the timeframe of searching the literature, sample size and characteristics, study location, recruitment strategy, and key findings on the prevalence of psychiatric disorders among individuals with ASD. Considering the high heterogeneity in terms of methodological approaches, instruments, and psychiatric outcomes, a narrative synthesis of the research findings was performed reporting the prevalence rates (percentage, proportion, odds ratio [OR], relative risk [RR], or other quantitative measures) with specific estimation or 95%

Table 1
Search strategy and keywords for this umbrella review.

Search query	Keywords (searched within titles, abstracts, subject headings like MeSH, and general keywords)
1	"prevalence" OR "incidence" OR "epidemiology" OR "frequency" OR "cases" OR "rate"
2	"autism" OR "autism spectrum disorder" OR ASD OR "autistic" OR "autistic disorder" OR "Asperger" OR "pervasive developmental" OR "PDD" OR "PDD-NOS"
3	"mental disorders" OR "mental illness" OR "common mental disorders" OR "mental health" OR "mental health services" OR "psychological" OR "psychiatric" OR "psychosocial" OR "psychosomatic" OR "emotional" OR "psychiatric disorders" OR "psychiatric illness" OR "obsessive compulsive" OR "dementia" OR "depression" OR "depressive disorders" OR "suicide*" OR "self-harm" OR "schizophrenia" OR "bipolar disorder" OR "mood disorder" OR "affective disorders" OR "anxiety" OR "substance abuse" OR "substance use" OR "alcohol" OR "addiction" OR "addictive disorders" OR "panic" OR "posttraumatic" OR "post-traumatic" OR "PTSD" OR "dissociative disorders" OR "personality disorders" OR "Neurodevelopmental disorders" OR "intellectual disabilities" OR "communication disorders" OR "attention deficit hyperactivity disorder" OR "psychotic disorders" OR "Motor disorders" OR "Catatonia" OR "somatic symptom" OR "somatic disorders" OR "eating disorders" OR "sleep-wake disorders" OR "parasomnia" OR "sexual dysfunction" OR "gender dysphoria" OR "conduct disorders" OR "neurocognitive disorders" OR "paraphilic disorders" OR "unspecified mental disorders" OR "Alzheimer's"
4	"systematic review" OR "meta-analysis" OR "meta-regression" OR "pooled effect" OR "pooled estimate" OR "pooled prevalence"
Final search query	1 AND 2 AND 3 AND 4

Table 2
Eligibility criteria for this review.

Inclusion criteria	Exclusion criteria
1. Articles published as systematically conducted narrative, qualitative, or quantitative (meta-analytic) literature reviews	1. Articles which were not systematically conducted reviews (for example, narrative and unstructured reviews, primary studies, opinions, commentaries, letter, or editorials were excluded)
2. Articles reported prevalence (or other quantitative measures like incidence, cases with denominators, odds, or relative risks etc.) of psychiatric disorders	2. Articles which did not report prevalence (or other quantitative measures) of psychiatric disorders only were excluded; articles reporting psychiatric disorders within general health outcomes were also excluded
3. Articles reported primary studies where the overall prevalence was provided for people with ASD	3. Articles reported primary studies conducted in general population or mixed population groups without a focus on people with ASD (for example, psychiatric disorders among participants with diverse health conditions including ASD was evaluated, and those were excluded if they did not separately report the prevalence among ASD population)
4. Articles published in peer-reviewed journals	4. Articles not published in peer-reviewed journals were excluded (for example, dissertations, theses, policy papers, or institutional reports were excluded)
5. Articles with full-texts available in the English language	5. Articles were excluded if the full-texts were not available in English

confidence interval [CI] as reported in the respective reviews.

2.4. Evaluation of the methodological quality

In this umbrella review, we used the JBI critical appraisal checklist for systematic reviews and research synthesis checklist to assess the methodological quality of the included studies (Aromataris et al., 2015). Two authors independently evaluated each of the included articles, which was finalized at the end of the primary evaluation through discussion with a third author. The checklist consists of ten items; each item can receive one point, and the overall quality score of a study can range from zero to ten. In this review, studies receiving zero to four, five to seven, and eight to ten were categorized as the low, medium, and high-quality studies, respectively.

3. Results

We found 1963 citations from database searching and 792 citations from additional sources, totaling 2755 citations (see Fig. 1). After removing 960 duplicate citations, we screened the titles and abstracts of 1795 and excluded 1709 citations, which did not meet the criteria for this review. Further, we evaluated the full-texts of 86 articles and excluded 60 articles that did not meet all the criteria of this review. Finally, 26 articles meeting all the criteria were included in this umbrella review (see Table 3), including 14 systematic reviews (Arnevik and Helverschou, 2016; Hannon and Taylor, 2013; Hedley and Uljarević, 2018; Kalyva et al., 2016; Menezes et al., 2018; Nickel et al., 2019; Padgett et al., 2010; Richa et al., 2014; Segers and Rawana, 2014; Skokauskas and Gallagher, 2009; Stewart et al., 2006; Vannucchi et al., 2014; Wigham et al., 2017; Zahid and Upthegrove, 2017) and 12 meta-analyses (De Giorgi et al., 2019; Díaz-Román et al., 2018; Elrod and Hood, 2015; Hollocks et al., 2019; Hudson et al., 2019; Lai et al., 2019; Lugo-Marín et al., 2019; Lugo-Marín et al., 2018; Morgan et al., 2020; van Steensel et al., 2011; van Steensel and Heeman, 2017; Zheng et al., 2018).

3.1. Characteristics of the recruited reviews

The earliest review included in this umbrella review was published in 2006 (Stewart et al., 2006). Most (n = 17) reviews are published after 2015, whereas only nine reviews were published till 2015 (Elrod and Hood, 2015; Hannon and Taylor, 2013; Padgett et al., 2010; Richa et al., 2014; Segers and Rawana, 2014; Skokauskas and Gallagher, 2009; Stewart et al., 2006; van Steensel et al., 2011; Vannucchi et al., 2014). The number of the searched databases in the included reviews ranged from one to eight, with a median of four. Moreover, the number of primary studies in the included reviews ranged from four to 100 (Hannon and Taylor, 2013; Lai et al., 2019).

Among 26 reviews included in this review, 15 reviews reported the origin of the primary study populations. Most reviews included primary studies from the US, UK, Canada, Netherlands, Sweden, Italy, Norway, Germany, Australia, and other countries from North America, Europe, and Oceania whereas nine reviews included at least one country from Asia (Díaz-Román et al., 2018; Elrod and Hood, 2015; Hedley and Uljarević, 2018; Lai et al., 2019; Lugo-Marín et al., 2019; Lugo-Marín et al., 2018; Segers and Rawana, 2014; Wigham et al., 2017; Zahid and Upthegrove, 2017). No primary studies were found from Africa, Central America, or Latin America.

The quality evaluation found fourteen reviews with high quality (De Giorgi et al., 2019; Díaz-Román et al., 2018; Elrod and Hood, 2015; Hollocks et al., 2019; Hudson et al., 2019; Lai et al., 2019; Lugo-Marín et al., 2019; Lugo-Marín et al., 2018; Menezes et al., 2018; Morgan et al., 2020; van Steensel et al., 2011; van Steensel and Heeman, 2017; Wigham et al., 2017; Zheng et al., 2018), twelve reviews with medium quality (Arnevik and Helverschou, 2016; Hannon and Taylor, 2013; Hedley and Uljarević, 2018; Kalyva et al., 2016; Nickel et al., 2019; Padgett et al., 2010; Richa et al., 2014; Segers and Rawana, 2014; Skokauskas and Gallagher, 2009; Stewart et al., 2006; Vannucchi et al., 2014; Zahid and Upthegrove, 2017), and no studies had low quality (see the Supplementary material).

3.2. Sample size and characteristics of the study participants

Most reviews evaluated primary studies with ASD populations only among which the sample size ranged from three to 210,249 (Kalyva et al., 2016; Lai et al., 2019). A few studies compared psychiatric comorbidity among ASD population with those of typically-developed control population with sample size ranging from 12 to 1842,575 (Zheng et al., 2018). Most of the reviews reported multiple sites and strategies of recruiting study participants across primary studies. For example, Padgett and colleagues reported 19 primary studies that recruited study participants from clinical settings, registries, communities, and autism support groups (Padgett et al., 2010). Most of the reviews found participants from diverse age groups, including children, adolescents, and adults, with a majority of young participants. Five reviews evaluated psychiatric comorbidity only among the children and adolescents with ASD (Díaz-Román et al., 2018; Elrod and Hood, 2015; Menezes et al., 2018; van Steensel et al., 2011; van Steensel and Heeman, 2017). Moreover, four reviews focused only on the adult population with ASD and assessed the prevalence of psychiatric disorders among those participants (Hollocks et al., 2019; Lugo-Marín et al., 2019; Lugo-Marín et al., 2018; Morgan et al., 2020).

3.3. Prevalence of comorbid psychiatric disorders among people with ASD

The prevalence of comorbid disorders among people with ASD varied across reviews. For example, Lugo-Marin and colleagues

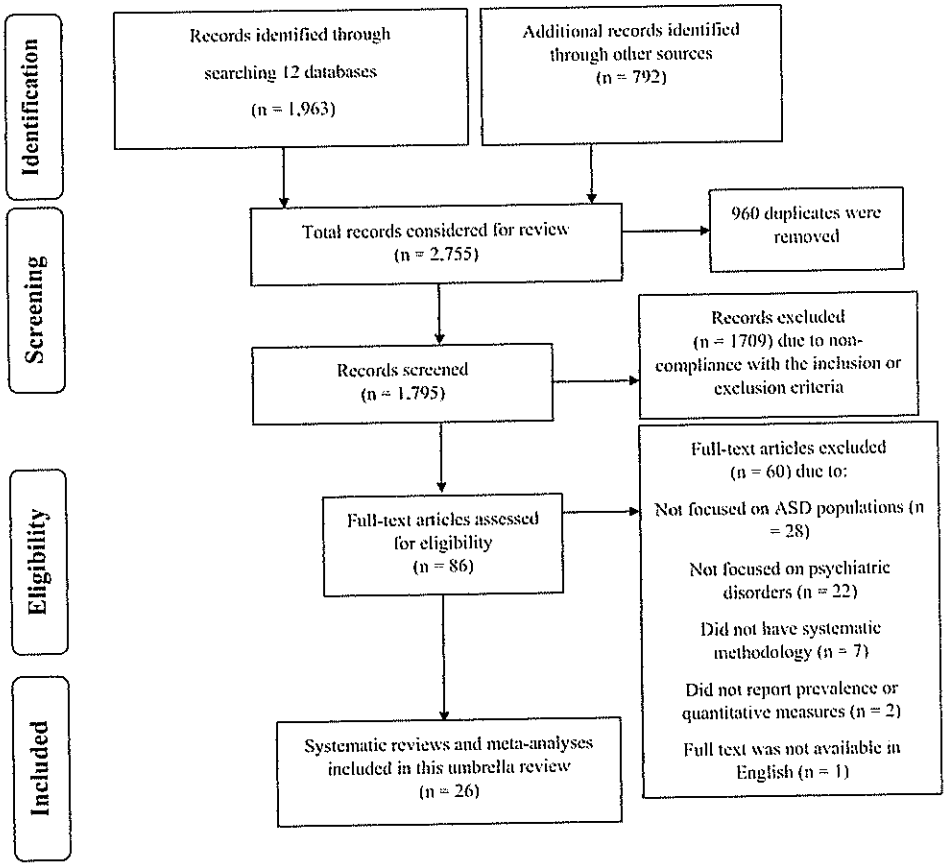

Fig. 1. Flow diagram of the umbrella review.

conducted a meta-analytic review of 47 studies and found the prevalence of at least one psychiatric disorder was 54.8% (95% CI: 46.6 – 62.7) among the study participants. Another systematic review by Richa and colleagues found up to 94% of the participants with ASD had psychiatric comorbidity (Richa et al., 2014). The prevalence of psychiatric disorders was different among population subgroups as well as for different methodologies and instruments used to assess those disorders. For example, Giorgi and colleagues found that participants aged above 18 years had a higher prevalence of psychiatric comorbidity compared to younger participants (De Giorgi et al., 2019). Moreover, studies with smaller sample sizes and instruments like DSM-III or later and ICD-10 had higher prevalence compared to studies with larger samples and older instruments (De Giorgi et al., 2019). The prevalence rates were different for specific psychiatric disorders, as reported in the synthesized findings of the respective reviews.

3.3.1. Anxiety disorders

Eight reviews reported the prevalence of anxiety disorders among people with ASD, which ranged between 1.47% and 54% across studies (Hollocks et al., 2019; Lai et al., 2019; Lugo-Marín et al., 2019; Richa et al., 2014; Skokauskas and Gallagher, 2009; van Steensel et al., 2011; van Steensel and Heeman, 2017; Zahid and Upthegrove, 2017). For example, van Steensel and colleagues evaluated anxiety disorders among 2121 children and adolescents with ASD in a meta-analytic review, which revealed the pooled prevalence as 39.6% and 34.8% in fixed and random effects models, respectively (van Steensel et al., 2011). Another review by Hollock and colleagues reported the pooled prevalence of anxiety disorders as 42% (95% CI: 35 – 50) among 26,070 adults with ASD (Hollocks et al., 2019).

3.3.2. Depressive disorders

The prevalence of depressive disorders among individuals with ASD was reported in six reviews, which ranged from 2.5% to 47.1% (Hollocks et al., 2019; Hudson et al., 2019; Lai et al., 2019; Menezes et al., 2018; Stewart et al., 2006; Wigham et al., 2017). For example, Lai and colleagues found that the prevalence of depressive disorders was 11% (95% CI: 9 – 13) among 162,671 samples comprising of children and adults (Lai et al., 2019). Another review by Hudson and colleagues evaluated depressive disorders in 66 studies and reported the current and lifetime prevalence as 12.3% (95% CI: 9.7 – 15.5) and 14.4% (95% CI: 10.3 – 19.8), respectively (Hudson et al., 2019).

3.3.3. Bipolar and mood disorders

Two reviews assessed the co-occurrence of bipolar disorders in ASD (Lai et al., 2019; Vannucchi et al., 2014). Vannucchi and colleagues found the prevalence of bipolar disorders ranged from 6% to 21.4% across studies (Vannucchi et al., 2014), whereas Lai and colleagues reported the prevalence as 5% (95% CI: 3 – 6) among 153,192 people with ASD (Lai et al., 2019). Moreover, five reviews reported the prevalence of mood disorders, which ranged from 4.4% to 37% across ASD samples (Lugo-Marín et al., 2019; Richa et al., 2014; Skokauskas and Gallagher, 2009; Stewart et al., 2006; Zahid and Upthegrove, 2017). For example, Lugo Marín and colleagues found the prevalence of mood disorders was 18.8% (95% CI: 10.6 – 31.1) among 21,797 participants with ASD (Lugo-Marín et al., 2019). Another review by Stewart and colleagues found 33% of the study participants with ASD diagnoses had mood disorders (Stewart et al., 2006).

Table 3
Characteristics and the key findings of the included systematic reviews and meta-analyses.

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
(Stewart et al., 2006)	MEDLINE, PsycINFO, Web of Science, and additional sources; studies published till 2003	27; Systematic review	Sample size ranged from 8 to 85	Participants were children, adolescents and adults with Asperger syndrome, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) and other diagnoses of ASD; mostly recruited from clinical settings; locations of the studies were not specified	The prevalence of depression or depressive symptoms ranged from 4% to 18%
(Mouridsen et al., 2007)	PubMed, PsycINFO, Embase, Science Direct, Ovid online, and additional sources; studies published till 2008	27; Systematic review	Sample size ranged from 10 to 200	Both children and adults with Asperger and other forms of ASD were included; recruitment strategy and locations of the studies were not specified	The prevalence rates of schizophrenia (ranged from 0% to 50%), mood disorders (ranged from 4.4% to 28.6%), and anxiety disorders (ranged from 1.47% to 84.1%) were reported across study samples
(Hudson et al., 2011)	MEDLINE, PsycINFO, and additional sources; from the inception of the databases to 2007	19; Systematic review	Sample size ranged from 18 to 322	Most samples had higher proportion of child participants with PDD and other ASD; samples were recruited from diverse settings including health facilities, clinic registries, autism organizations, and community settings; studies were conducted in the US, Denmark, and other non-specified locations	The rates of psychosis among individuals with pervasive developmental disorders (PDD) varied from 0% to 53% (median 0%, mean 11%); the incidence of co-morbid schizophrenia was up to 7.8% compared to 1% in general population
(van Steensel et al., 2011)	PsycINFO, PubMed, Web of Science, ERIC; timeframe not specified	31; Meta-analysis	The total sample was 2121 (ranged from 7 to 301)	Children and adolescent participants with different ASD subtypes were included; recruitment site and study locations were not specified	The prevalence of at least one comorbid DSM-IV anxiety disorder was 39.6% and 34.8% in fixed and random effects models, respectively; in random-effects analyses in subgroups, the prevalence rates were 17.4%, 16.6%, 29.8%, 15.4%, 9%, 1.8%, and 16.6% for obsessive compulsive disorders, social anxiety disorder, specific phobia, generalized anxiety disorder, separation anxiety disorder, panic disorder, and agoraphobia. Specific anxiety disorders were associated with different ASD subtype, age, IQ, and assessment methods
(Hossain et al., 2014)	PsycINFO, MEDLINE, Web of Science and the Cochrane Library; studies published up to 2012	4; Systematic review	Sample size ranged from 12 to 182	Three clinical and one community sample were recruited comprising of children, adolescents, and young adults with ASD; study locations were not specified	The lifetime prevalence of suicidal attempt was 7–8.5% among individuals with ASD and no history of abuse; the rates increased up to 31.6% and 40% for individuals who are abused physically or sexually, respectively; suicidal behavior was associated with the severity of ASD symptoms (p = .001)
(Hedley et al., 2018)	PsycINFO, Web of Science, MEDLINE, and additional sources; searched till 2013	10; Systematic review	Sample size ranged from 5 to 537	Most of the participants in the included studies were male with ASD; 40% studies had adult participants, 40% had child participants (3–10 years), 50% adolescent participants (11–17 years), and 20% conducted research with emerging adults; research settings included hospitals (40%), community clinics (30%), universities (10%), mail-based questionnaires (10%), and web-based interfaces (10%); most studies were from the US (n = 4), followed by European countries (n = 3; United Kingdom, Italy, Sweden), Japan (n = 2), and Turkey (n = 1).	Suicidality was prevalent in 10.9–50% of the ASD samples identified in the systematic review. Further, several large-scale studies found that individuals with ASD comprised 7.3–15% of suicidal populations. Individuals with peer victimization, behavioral problems, being Black or Hispanic, being male, lower socioeconomic status, and lower level of education had a higher prevalence of suicidal behavior disorder

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Table 3 (*continued*)

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
(Publication, 2017)	PubMed, PsycINFO, Embase, and MEDLINE; timeframe was not specified	14; Systematic review	Sample size ranged from 10 to 791	Children and adults with PDD and other ASD were included; recruitment was done in diverse settings including clinical and community settings, advertisements, referrals, and patient groups; studies from Sweden and Denmark were reported; location was not specified for most studies	The prevalence of suicidal ideation ranged from 10.9% to 50%, and suicidal attempt ranged from 7.2% to 15%; the prevalence of psychiatric co-morbidity among ASD samples was 94% for at least one comorbid condition including anxiety (54%), disruptive behavior disorder (48%), and mood disorder (37%)
(Author, Year)	PubMed, Scopus, PsycINFO; timeframe not specified	7; Systematic review	Sample size ranged from 44 to 4343	Samples comprised of children, adolescents, and adults with Asperger syndrome; participants were recruited from academic, clinical, and community settings; three studies from Sweden, France, and other developed countries were reported	The prevalence of bipolar disorder ranged from 6% to 21.4% among individuals with ASD
(Author, Year)	PubMed, Scopus, Google Scholar, and additional sources; studies published since 1994	10; Meta-analysis	The total sample was 564 (343 children with ASD, and 221 traditionally developed children)	Participants aged from 2 years to 19 years with ASD; recruited from clinical settings and registries; most studies were from the US (n = 5), followed by Italy (n = 4), and one study each from Israel and Sweden	Total sleep time (TST) for children with ASD was on average 32.8 min less per day (95% confidence interval [CI]: 16.6–49.0), the average sleep latency (SL) was 10.9 min longer (95% CI: 6.7–15.0), average sleep efficiency (SE) was 1.9% less (95% CI: 0.7–3.1) than their traditionally developed (TD) peers. Concurrent intellectual disability was reported as a moderator of TST; children with ASD and intellectual disability (ID) had a significantly decreased TST as compared with TD peers. Also, medication use, method of data collection, and age moderated the prevalence of sleep problems among children with ASD
(Author, Year)	PsycINFO, PsychARTICLES, ERIC, Psychology & Behavioral Sciences, Academic Search Primer, and MEDLINE; studies published till 2015	20; Systematic review	Sample size ranged from 3 to 447 in ASD populations	Both adults and children with ASD were recruited across studies from diverse settings including clinics and schools; study locations were not specified	The prevalence of Tourette syndrome or tic disorders ranged from 2.6% to 36% across different samples. The co-occurrence of those disorders and ASD ranged from 4% to 12% among the study populations
(Author, Year)	PubMed, PsycINFO, MEDLINE, and additional sources; studies published till January 2016	18; Systematic review	Sample size ranged from 14 to 414	Participants with ASD were recruited; most studies had a high male-to-female ratio; participants aged above 12 were included from clinical settings and registries; study locations were not reported	The prevalence of substance use disorder ranged from 0.7% to 36% among individuals with ASD
(Author, Year)	PsycINFO, PubMed, Web of Science and ERIC; studies published till 2016	83; Meta-analysis	Sample size ranged from 7 to 381	Children with ASD and typically developed children were recruited across studies; recruitment strategy was not specified; however, clinical samples were predominantly reported; study locations were not reported	Children with ASD had higher anxiety levels (effect sizes ranged between 0.11 to 0.91 in fixed-effect models and 0.12 to 1.21 in random-effects models) compared to typically developed or clinically referred youth without ASD; in contrast, children with ASD had lower anxiety levels (−0.12 and −0.45 in fixed and random-effects models, respectively) compared to youth with internalizing problems

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Table 3 (*continued*)

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
[illegible]	MEDLINE, Embase, CINAHL, ERIC, and PsycINFO; 1992–2015	19; Systematic review	Sample size ranged from 10 to 474	Children and adults with high functioning ASD were recruited clinical and educational settings, registries, autism support groups and web sources; most of the studies were conducted in the US (n = 6), followed by the UK (n = 4), Australia (n = 2), Sweden (n = 2), and one study each from Turkey, Finland, and the Netherlands; two studies recruited participants from more than one country	The self-reported prevalence of depressive disorder ranged from 4% to 47.1%. Moreover, the informant reported rates of depressive disorder ranged from 2.5% to 29%. The rates were different based on psychometric measures; for example, Autism Co-morbidity Interview found 15.8% of participants had depressive disorder whereas Child Symptom Inventory reported rates ranged between 0% to 6.2%
[illegible]	PsycINFO, MEDLINE, Embase, Web of Science and additional sources; studies published till 2014	12; Systematic review	Samples with ASD ranged from 10 to 791	Children, adolescents, and adults with ASD from diverse settings were recruited; most studies were from the US (n = 5), followed by Japan (n = 3), and one study each from the UK, Canada, Turkey, and Italy	The prevalence of suicidal ideation or attempts ranged between 3.8% to 66%. Studies have also reported a high prevalence of psychiatric co-morbidity among ASD samples including anxiety (54%), schizophrenia (67%), disruptive behavior disorder (48%), and mood disorder (37%)
[illegible]	PsycINFO, PubMed, and Web of Science; 2000–2017	35; Meta-analysis	Sample sizes were 26,070 for studies reporting anxiety and 26,117 for studies reporting depression (range 13 to 22,253)	All were adult participants with ASD; majority of male and most samples were collected from clinical settings followed by communities and referrals or contacts; Most studies (n = 10) were from the US, followed by the UK (n = 6), Sweden (n = 5), Netherlands (n = 3), and one study each from Germany and Norway; one study recruited participants from both France and Sweden	The pooled prevalence rates for any anxiety (lifetime 42% [95% CI: 35–50]), current 27% [95% CI: 17–37]), social phobia (lifetime 20% [95% CI: 7–38], current 29% [95% CI: 18–40]), obsessive compulsive disorder (lifetime 22% [95% CI: 10–27], current 24% [95% CI: 15–33]), generalized anxiety disorders (lifetime 26% [95% CI: 15–28], current 18% [95% CI: 10–26]), panic disorders (lifetime 18% [95% CI: 10–27], current 15% [95% CI: 8–23]), specific phobia (lifetime 31% [95% CI: 10–66], current 6% [95% CI: 1–32]), posttraumatic stress disorder (lifetime 5% [95% CI: 1–10], current 1% [95% CI: 0–5]), separation anxiety (lifetime 21% [95% CI: 17–29], current 3% [95% CI: not reported]), and depression (lifetime 37% [95% CI: 27–47], current 23% [95% CI: 17–29]) were reported
[illegible]	PubMed, Web of Science, PsycINFO, CINAHL, and ProQuest Dissertations and Theses; studies published till 2016	66; Meta-analysis	Sample size ranged from 4 to 22	Both children and adult participants with ASD were recruited from community and outpatient settings; study locations were not specified	The pooled prevalence of lifetime and current depressive disorders were 14.4% (95% CI 10.3–19.8) and 12.3% (95% CI 9.7–15.5), respectively; the prevalence rates were higher among studies with standardized interview methods assessing depressive disorders (lifetime = 28.5%, 95% CI 20.1–38.8; current = 15.3%, 95% CI 11.0–20.9), self-reported depressive symptoms (lifetime = 48.6%, 95% CI 33.3–64.2; current = 25.9%, 95% CI 17.0–37.3), studies with participants with higher intelligence; also, the prevalence was higher in samples with older and White participants

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Table 3 (*continued*)

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
[illegible]	PsycINFO, MEDLINE, CINAHL, Embase, and additional sources; studies published till 2016	10; Meta-analysis	Sample size ranged from 15 to 129	Adult participants with ASD with average IQ were recruited; recruitment sites were not specified; most studies (n = 6) were from Sweden, followed by the US (n = 2), and one study each from Italy, Netherlands, Germany, and Canada	The pooled prevalence of schizophrenia spectrum disorders was 6.4% (95% CI: 4–10.1) among adults with ASD
[illegible]	MEDLINE and PsycINFO; 2012–2016	43; Systematic review	Sample size ranged from 10 to 791 (except a case study)	Study participants were children and adolescents with ASD; recruited from both community and clinical samples; study locations were not specified	The prevalence of depression ranged from 7% to 47.1% across samples of individuals with ASD
[illegible]	PubMed (MEDLINE), PsycINFO, Embase + Embase classic, Ovid MEDLINE, Web of Science, biological abstracts, biosis, food science and technology abstracts; studies published till 2018	48; Meta-analysis	Sample size ranged from 75 to 5430	Children and adolescents with ASD were included as study participants; recruitment strategy was not specified; most studies were from the US (n = 9), Australia (n = 7), China (n = 5), Italy (n = 5), Japan (n = 3), two studies from Turkey and Israel, one study each from India, Oman, Sweden, Russia, Iran, Netherlands, Finland, France, and Korea	In subjective measures, the study participants with ASD, showed significantly higher bedtime resistance (SMD = 1.00, 95% CI 0.67–1.33), sleep onset delay (0.98, 95% CI: 0.66–1.29), sleep anxiety (0.96, 95% CI: 0.61–1.32), night awakenings (0.72, 95% CI: 0.44–1.01), parasomnias (0.88, 95% CI: 0.60–1.15), sleep-disordered breathing (0.48, 95% CI: 0.28–0.67), daytime sleepiness (0.34, 95% CI: 0.16–0.52), sleep onset latency (in min) (0.81, 95% CI: 0.59–1.02), restorative value of sleep (0.13, 95% CI: − 0.96–1.02), general sleep problems (0.93, 95% CI: 0.67–1.20) and lower sleep duration (− 0.88, 95% CI: − 1.18 to − 0.57). In objective measures, youth with ASD had lower total sleep time (− 0.90, 95% CI: − 1.51 to − 0.30), longer sleep onset latency (0.53, 95% CI: 0.21 to 0.86), higher time spent in stage 1 sleep (0.48, 95% CI: 0.06–0.90), lower time of REM sleep (− 0.88, 95% CI: − 1.56 to − 0.21), lower sleep efficiency (− 1.20, 95% CI: − 1.98 to − 0.41) and higher time awake after sleep onset (0.49, 95% CI: 0.11–0.87). However, no significant differences were observed between children with ASD and control individuals in stage 2 sleep, slow-wave sleep and REM latency
[illegible]	PubMed, PsycINFO, Web of Science and additional sources; 2012–17	13; Systematic review	The total sample size was 30,663 (ranging from 37 to 27,122)	Children and adults with ASD were recruited from general population and clinical samples; Most studies were from the US (n = 5), Japan (n = 3), and one each from Australia, Canada, Sweden, Turkey, and the UK	The prevalence of suicide ideation ranged from 11% to 66% and suicidal attempts from 1% to 35%; mortality due to suicide was 0.31% among people with ASD compared to 0.04% among traditionally developed individuals; the prevalence of comorbid attention-deficit hyperactivity syndrome was reported as high as 65% among study samples

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Table 3 (*continued*)

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
(Zhang et al., 2019)	PubMed, Embase, Cochrane Library, and additional sources; studies published till 2017	17; Meta-analysis	Sample size ranged from 12 to 9062 for people with ASD and 12 to 1842,575 among controls	Participants with ASD of all age group were included; the recruitment strategy was not specified; studies were conducted in Denmark (n = 4), the US (n = 4), Sweden (n = 3), and once study each in Norway, Canada, Australia, Finland, and the Netherlands	The pooled prevalence of schizophrenia was significantly higher among individuals with ASD compared to the controls (OR = 3.55, 95% CI: 2.08–6.05, P < .001); also, the pooled prevalence of ASD in individuals with schizophrenia ranged from 3.4 to 52%; ASD participants in the case-control studies had higher odds (8.2, 95% CI: 3.25–20.66) compare to cross-sectional studies (2.47, 95% CI: 1.31–4.66); samples from European countries had higher odds (4.21, 95% CI: 1.8–9.85) compared to the US samples (2.61, 95% CI: 1.07–6.39)
(Ke Hyperlink et al., 2019)	PubMed/MEDLINE, Web of Science, CINAHL; studies published till 2019	14; Meta-analysis	The total sample size was 1708 (ranged from 26 to 414)	Samples comprised of both children and adults with ASD; recruited from mostly outpatient settings (12 out of 14 studies); studies were conducted in Sweden (n = 3), Denmark (n = 3), The US (n = 2), and one study each from the UK, Italy, France, Canada, Norway; one study had samples from both Sweden and France	The pooled prevalence of non-affective psychoses or schizophrenia spectrum disorder was 9.5% (95% CI = 2.6 to 16.0) among participants with ASD; the subgroup analyses found higher prevalence (20.6% compared to 9.1%) for inpatient studies compared to outpatient studies, whereas participants older than 18 years had higher prevalence (10.1% compared to 8.47%) compared to younger participants; studies with lesser than 100 samples (19.6% compared to 6.36% in samples above 100) and recent diagnostic methods like DSM-III or later (11.7%) and ICD 10 (12.5%) had higher prevalence compared to earlier versions of diagnostic measures
(Jensen et al., 2014)	MEDLINE, Embase, PsycINFO, Scopus, Web of Science, and additional sources; 1993–2019	100; Meta-analysis	Sample size ranged from 23 to 48,762 across studies and 53,243 to 210,249 in pooled analyses	Both children and adult participants with ASD were recruited from population-based samples, registries, and clinical settings; studies were conducted in North America (n = 36), Europe (n = 38), Middle East (n = 6), Australia (n = 3), and Asia (n = 4)	The pooled prevalence estimates was 28% (95% CI: 25–32) for attention-deficit hyperactivity disorder; 20% (95% CI: 17–23) for anxiety disorders; 13% (95% CI: 9–17) for sleep-wake disorders; 12% (95% CI:10–15) for disruptive, impulse-control, and conduct disorders; 11% (95% CI: 9–13) for depressive disorders; 9% (95% CI: 7–10) for obsessive-compulsive disorder; 5% (95% CI: 3–6) for bipolar disorders; and 4% (95% CI: 3–5) for schizophrenia spectrum disorders
(Lai et al., 2019)	PsycINFO, PubMed, CINAHL, Web of Science, and additional sources; 2000–2016	47; Meta-analysis	The total sample size was 26,679	All participants were adults with ASD; mostly (74.35%) male; most of the studies (87%) were conducted in clinical settings; studies were conducted in Sweden (n = 13), UK (n = 8), USA (n = 6), Netherlands (n = 4), Denmark (n = 3), two studies each from Japan and Finland, one study each from Taiwan, Canada, Scotland, Australia, and Italy	The pooled prevalence rates of substance use disorder (8.3%, 95% CI: 4.1–16.1), schizophrenia spectrum disorders (11.8%, 95% CI: 7.7–17.6), mood disorder (18.8%, 95% CI: 10.6–31.1), anxiety disorders (17.8%, 95% CI: 12.3–25.2), eating disorders (3.6%, 95% CI: 2.1–6.1), personality disorders (12.6%, 95% CI: 4.8–29.3), attention deficit and hyperactivity disorder (25.7%, 95% CI: 18.6–34.3) and presence of any psychiatric disorder (54.8%, 95% CI: 46.6–62.7) were reported

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Table 3 (*continued*)

Author and year of publication	Name(s) and timeframe of searching Databases	Number of primary studies; type of review (meta-analysis or systematic review)	Sample sizes in the included primary studies (total or range as reported in respective reviews)	Characteristics of the study populations with Autism Spectrum Disorders or equivalent conditions (demographics, recruitment strategy, and location)	Prevalence rates (or other quantitative measures of epidemiological burden as specified), and related key findings
(Zahid et al., 2019)	Ovid MEDLINE; studies published till July 2019	17; Systematic review	Sample size ranged from 10 to 12,606	Both children and adults with ASD were included; the recruitment strategy and study locations were not specified	Three studies reported a higher risk of developing eating disorders among individuals with ASD; 7.9% had a current or previous eating disorder (ED) including anorexia nervosa (AN) 6.7%, bulimia nervosa (BN) 2.7% and binge eating disorder (BED) 1.4%)
(Zheng et al., 2020)	PubMed (MEDLINE), OVID databases (PsycINFO, Embase, MEDLINE, Embase classic, OVID MEDLINE) and Web of Knowledge (all databases); studies published till February 2019	8; Meta-analysis	Sample size ranged from 10 to 41	Adult participants with ASD; the mean age was 22 to 40.3 years; the recruitment strategy and study locations were not specified	Study participants with ASD had more impaired (six out of 11) subjective sleep parameters, including lower sleep efficiency (SE) (SMD = 0.87, 95% CI: −1.14 to −0.60), and objective outcomes (10 out of 17), including longer sleep onset latency (PSG) (SMD = 0.86, 95% CI: 0.29–1.07) and wake after sleep onset (WASO, actigraphy) (SMD = 0.57, 95% CI: 0.28–0.87)

3.3.4. Schizophrenia spectrum and other psychotic disorders

The prevalence of schizophrenia spectrum and other psychotic disorders was reported in eight reviews, which ranged from 4% to 67% (De Giorgi et al., 2019; Lai et al., 2019; Lugo-Marín et al., 2019; Lugo Marín et al., 2018; Padgett et al., 2010; Skokauskas and Gallagher, 2009; Zahid and Upthegrove, 2017; Zheng et al., 2018). For example, a meta-analytic review by Zheng and colleagues found that individuals with ASD had an odds ratio of 3.55 (95% CI: 2.08 – 6.05, $p < .001$) to develop schizophrenia spectrum disorders compared to the typically-developed controls (Zheng et al., 2018). Moreover, a random-effects meta-analysis by Lugo Marín found 11.8% (95% CI: 7.7 – 17.6) of the 22,176 participants with ASD had schizophrenia spectrum disorders (Lugo-Marín et al., 2019).

3.3.5. Suicidal behavior disorder

Five reviews reported the prevalence of suicidal ideation and attempts, which ranged from 10.9% to 66% and 1% to 35%, respectively (Hannon and Taylor, 2013; Hedley and Uljarević, 2018; Richa et al., 2014; Segers and Rawana, 2014; Zahid and Upthegrove, 2017). For example, Segers and Rawana found 10.9% to 50% of the ASD populations had suicidality (Segers and Rawana, 2014), whereas Richa and colleagues found the prevalence of suicidal attempts ranged from 7.2% to 15% among participants with ASD (Richa et al., 2014). In another review, Hannon and Taylor reported a high burden of suicidal behavior co-occurring in ASD, where 31.6% and 40% prevalence rates of suicidal attempt were found among participants with a history of physical and sexual abuse, respectively (Hannon and Taylor, 2013).

3.3.6. Sleep disorders

Five reviews reported a varying prevalence of sleep disorders among individuals with ASD (Díaz-Román et al., 2018; Elrod and Hood, 2015; Lai et al., 2019; Morgan et al., 2020). For example, a meta-analytic review by Diaz-Roman and colleagues reported that individuals with ASD had impaired objective indices of sleep disorders including reduced total sleep time (−0.90, 95% CI: −1.51 to −0.30), longer sleep onset latency (0.53, 95% CI: 0.21 to 0.86), higher time spent in stage 1 sleep (0.48, 95% CI: 0.06–0.90), lower time of REM sleep (−0.88, 95% CI: −1.56 to −0.21), lower sleep efficiency (−1.20, 95% CI: −1.98 to −0.41) and higher time awake after sleep onset (0.49, 95% CI: 0.11–0.87) (Díaz-Román et al., 2018). Another review by Lai and colleagues found that the prevalence of sleep-wake disorder was 13% (95% CI: 9 – 17) among 190,963 participants with ASD (Lai et al., 2019).

3.3.7. Obsessive-compulsive and related disorders

Three reviews assessed the prevalence of obsessive-compulsive disorders (OCD) co-occurring in ASD, which ranged from 9% to 22% (Hollocks et al., 2019; Lai et al., 2019; van Steensel et al., 2011). For example, Hollocks and colleagues found the lifetime prevalence of OCD was 22% (95% CI: 10 – 27) among people with ASD (Hollocks et al., 2019). In another review, van Steensel and colleagues reported 17.4% of participants with ASD had comorbid diagnoses of OCD (van Steensel et al., 2011).

3.3.8. Disruptive, impulse-control, and conduct disorders

Three reviews reported the prevalence of disruptive, impulse-control, and conduct disorders, which ranged from 12% to 48% (Lai et al., 2019; Richa et al., 2014; Zahid and Upthegrove, 2017). A meta-analytic review found the prevalence of comorbid disruptive, impulse-control, and conduct disorders were 12% (95% CI: 10 – 15), whereas Richa and colleagues reported the prevalence was 48% among participants with ASD (Lai et al., 2019; Richa et al., 2014).

3.3.9. Attention-deficit/hyperactivity disorder

Three reviews reported the prevalence of co-occurring Attention-deficit/hyperactivity disorder (ADHD) among people with autism,

which ranged from 25.7% to 65% (Hedley and Uljarević, 2018; Lai et al., 2019; Lugo-Marín et al., 2019). For example, Lugo-Marín and colleagues found the pooled prevalence of ADHD was 25.7% (95% CI: 18.6 – 34.3) among 24,511 individuals (Lugo-Marín et al., 2019). Another review by Lai and colleagues reported the pooled prevalence as 28% (95% CI: 25 – 32) among 210,249 participants with ASD (Lai et al., 2019).

3.3.10. Other comorbid psychiatric disorders

Several comorbid psychiatric disorders are reported across reviews with varying prevalence rates. Two reviews reported the prevalence of eating disorders among individuals with ASD, which ranged from 1.4% to 7.9% across studies (Lugo-Marín et al., 2019; Nickel et al., 2019). For example, Nickel and colleagues found 7.9% of individuals with ASD had a current or previous eating disorder (ED), including anorexia nervosa (6.7%), bulimia nervosa (2.7%), and binge eating disorder (1.4%) (Nickel et al., 2019). Moreover, two reviews reported the prevalence of substance use disorder in ASD, ranging from 0.7% to 36% (Arnevik and Helverschou, 2016; Lugo-Marín et al., 2019). For instance, Lugo-Marín and colleagues found the prevalence of substance use disorder was 8.3% (95% CI: 4.1 – 16.1) among 21,661 participants with ASD (Lugo-Marín et al., 2019). This review also reported the pooled prevalence of personality disorder as 12.6% (95% CI: 4.8 – 29.3) among 21,715 participants. Another review by Kalyva and colleagues reported the prevalence of Tourette syndrome or tic disorders ranged between 2.6% and 36% among study participants with ASD (Kalyva et al., 2016). Furthermore, Hollocks and colleagues reported the prevalence of posttraumatic stress disorder was 1% (95% CI: 0 – 5) and 5% (95% CI: 1 – 10) for recent time and lifetime, respectively (Hollocks et al., 2019).

4. Discussion

4.1. Overview of current evidence and recommendations for future research

In this umbrella review, we synthesized the current evidence on the prevalence of comorbid psychiatric disorders among people with ASD. The findings of this review suggest high epidemiological burden of various psychiatric disorders including anxiety disorders, depressive disorders, bipolar and mood disorders, schizophrenia spectrum and other psychotic disorders, suicidal behavior, eating disorders, substance use disorders, obsessive-compulsive and related disorders, Attention-deficit/hyperactivity disorder, disruptive, impulse-control, and conduct disorders in ASD population. In many reviews, the psychiatric burden reported among people with ASD was enormously higher compared to the typically developed individuals (Padgett et al., 2010; van Steensel and Heeman, 2017). Such mental health disparities resulting from the coexistence of these psychiatric morbidities may critically affect the mental health status, daily life, and overall quality of living among people who are already suffering from a complex neuropsychiatric condition like ASD. However, the prevalence rates for similar psychiatric disorders have shown marked heterogeneity across reviews. Such variability in the evidence can be attributable to several factors, which should be discussed to comprehend the findings of this umbrella review adequately.

First, the operational definitions and conceptual constructs associated with many psychiatric disorders have undergone many changes in the past years, particularly with the evolution of diagnostic criteria like DSM or ICD (De Giorgi et al., 2019). In addition, the instruments used to assess a psychiatric disorder were not consistent across studies. These issues may have substantially contributed to the changing prevalence of psychiatric morbidity in ASD. Future research and evidence synthesis should evaluate the effect of those changes on the overall epidemiological burden among the ASD population.

Second, another methodological challenge identified in this review is the sampling strategies in the primary studies, as reported in the respective reviews. A major proportion of the reviews did not report the sampling techniques, which may affect the generalizability of the findings. For example, studies conducted in clinical settings have shown higher prevalence rates for different psychiatric disorders compared to those conducted in community settings (De Giorgi et al., 2019; Hudson et al., 2019; Zheng et al., 2018). Therefore, further evaluation would be essential to understand the true burden of psychiatric comorbidity in ASD. Population-based observational research matched with clinical profiles may provide a better understanding in this regard, which is recommended for future research in this domain.

Third, this review synthesized the prevalence of several psychiatric disorders co-occurring in ASD; however, there are many disorders that are not reported in the existing reviews. For example, several psychiatric conditions like dementia or gender dysphoria are classified under DSM-V, which can co-occur among people with ASD, as reported in primary studies (Heylens et al., 2018; Rhodus et al., 2019). The epidemiological burden of such disorders is not yet synthesized in the existing reviews. This necessitates more primary research and efforts to synthesize such findings to better inform the prevalence of diverse psychiatric conditions in the future.

Fourth, this review aimed to synthesize the prevalence of comorbid psychiatric disorders in ASD. Therefore, evaluating the moderating effects of demographic and psychosocial variables was not within the scope of this review. However, varying prevalence rates among participants from different age groups, locations, and socioeconomic conditions inform that the epigenetic and psychosocial factors associated with the epidemiological burden of psychiatric morbidity can be different across ASD samples (De Giorgi et al., 2019; Hudson et al., 2019; Segers and Rawana, 2014), which should be evaluated using different observational approaches. Such research may explain the variability in the prevalence rates and inform which subgroups within the broader ASD population are more likely to experience psychiatric morbidity.

Fifth, mental health research is largely neglected around the world, particularly in resource-constrained contexts (Hossain and Purohit, 2019). This is also evident in the findings of this umbrella review as most of the primary studies were reported from North America, Europe, and Oceania in the included reviews, which highlights a lack of published research from Africa, Latin or Central America. Those countries have a high prevalence of ASD, but little evidence on psychiatric comorbidity is found from those countries. This gap necessitates future neuropsychiatric research to inform how co-occurring psychiatric disorders are distributed in those nations.

4.2. Implications of psychiatric practice and health policymaking

The findings of this review may have several implications for psychiatric practice. The co-occurrence of psychiatric disorders among individuals with ASD may create difficulties in reaching accurate diagnoses, provide specific treatments, and evaluate the prognoses. In such a scenario, mental health practitioners should evaluate the complex psychopathological dynamics that interplay between ASD and other psychiatric conditions. Moreover, a high prevalence of multiple psychiatric disorders within the same ASD population can impose a higher burden of diseases and compounding effects as psychosocial challenges, which may necessitate extensive evaluation and monitoring of mental health status among the affected individuals. Furthermore, many studies found a higher prevalence of psychiatric disorders among adults compared to younger participants. One implication of this phenomenon is the continued burden of psychosocial stressors across lifetime, or the expression of psychiatric diseases may increase over time, which would necessitate preventive measures for children and adolescents with ASD. Another reason for this may be the unmet need for preventive and therapeutic psychiatric services for children and adolescents, which continue to widen the gaps between mental health problems and efforts to address the same. From both of these perspectives, it is essential to strengthening psychiatric care, using both

pharmacological and psychosocial approaches, at the early stages to prevent potential psychiatric morbidity among people with ASD. Furthermore, a neurodevelopmental disorder like ASD requires continued care and support from informal caregivers within community settings. Therefore, it is essential to inform the family caregivers about how different psychiatric comorbidity can affect individuals with ASD, how to prevent such problems, and how to promote positive mental health among those individuals. Also, long term prevention and community-based care approaches for mental disorders are generally non-pharmacological in nature. To address the burden of psychiatric comorbidity in ASD, it is essential to explore how different innovative approaches can be adopted in non-clinical settings to better address mental health problems with minimal involvement of mental health professionals. Two studies have shown promising impacts of such interventions in improving health outcomes among children with ASD (Benvenuto et al., 2016; Narzisi et al., 2014). Mental healthcare providers and community health workers should consider such interventions to improve mental health among younger individuals with ASD.

There are several policy implications that can be discussed in the context of the synthesized findings of this review. A high burden of coexisting psychiatric morbidity warrants a broader perspective on how the healthcare community as well as health systems can rethink the existing models of care and improve the same to address psychiatric multimorbidity in this population. For example, the provisions of care and health systems financing can be very different for neurodevelopmental disorders and other psychiatric disorders. If an individual with ASD has access to the earlier one but may not pay for the later, persistent gaps in healthcare services will exist among such individuals, which may need policy approaches to resolve such health systems challenges. Global nations should revisit their health policies and programs to ensure the institutional measures adequately meet the psychiatric demands of the ASD populations. Another policy implication is the capacities of healthcare providers, institutions, and communities to acknowledge the high burden of psychiatric comorbidity among people with ASD. It would not be feasible to protect this population from psychiatric disorders unless formal and informal healthcare providers are aware of those problems and achieve essential capacities to fulfill their responsibilities to prevent, identify, and address the psychiatric epidemic that may occur across the continuum of life among ASD populations (Pellicano et al., 2014). It is essential to empower the health workforce so that they can contribute within their professional scopes and collectively provide holistic care to individuals with ASD. In this process, strengthening primary care and community-based health services to recognize ASD and psychiatric comorbidity among the affected individuals may enhance the overall health of people with ASD (Nicolaidis et al., 2014; Saqr et al., 2018). Last but not least, policymakers across health services organizations and multilateral institutions should foster collaboration to strengthen clinical, non-clinical, and social care through integrated and coordinated services for people with ASD (Aylott, 2010; Kałużna-Czaplińska et al., 2018). Given the high burden of ASD itself and the added challenges due to psychiatric morbidity, mobilizing collective resources may help in addressing those problems in a better way.

4.3. Strengths and limitations of this review

To the best of our knowledge, this is the first umbrella review that informs the prevalence of comorbid psychiatric disorders in ASD from evidence-based reviews. As we could not find another review of the reviews on this topic, it may not be appropriate to compare this review with another published article. Rather, this review critically appraised the global literature, identified systematic reviews and meta-analyses, and synthesized the findings on the prevalence of psychiatric comorbidity in ASD. Therefore, due to the methodological approaches, this review offers strong evidence on psychiatric comorbidity among people with ASD, which may facilitate future psychiatric research, practice, and policymaking around the world.

Despite those strengths, there are several limitations of this review, which should be acknowledged and addressed. First, we searched major databases and additional sources to retrieve relevant articles. However, this search was limited within the peer-reviewed journal articles. Therefore, we could not include articles that are not published within journals indexed in those databases, institutional reports, dissertations, or unpublished articles that had small effect sizes. This potential exclusion of studies which could meet our criteria highlights a publication bias within the existing body of knowledge. Secondly, our choice of databases or keywords may lead to a selection bias within the process of conducting this review, which may also affect the generalizability of this review. Third, we did not collect raw data from each primary study and conduct a patient-level quantitative analysis for each psychiatric disorder as the moderators for the same, which could have minimized the between-study variability of the synthesized findings. However, this umbrella review followed the methodological approaches as provided in the available guidelines for evidence-based reviews involving more than two reviewers as each step of the review, thus minimizing biases and methodological challenges. Future evidence syntheses adopting rigorous methods, and addressing the limitations of this review may improve the knowledge base on the prevalence of comorbid psychiatric disorders among people with ASD.

4.4. Conclusion

In this umbrella review, we synthesized the current evidence on the prevalence of comorbid psychiatric disorders among people with ASD from evidence-based systematic reviews and meta-analyses. The findings of this review suggest a high burden of different psychiatric disorders co-occurring among people with ASD, which is a major global mental health concern. It is essential to conduct more research to understand the burden of those disorders in low- and middle- income countries and different population groups experiencing varying levels of psychosocial distress around the world. Moreover, the limitations of the existing evidence should be addressed through multi-level research and strengthen the evidence base for better decision-making. Nonetheless, the findings of this review warrant multifaceted and multilevel interventions involving the mental healthcare providers, community health workers, informal caregivers, researchers, healthcare organizations, multilateral institutions, and health systems to ensure adequate measures for the optimal prevention, early diagnosis, and better pharmacological and psychosocial care of comorbid psychiatric disorders among people with ASD.

Role of the funding source

No funding was received at any stage of conducting this umbrella review or preparing this manuscript.

CRediT authorship contribution statement

Md Mahbub Hossain: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Nusrat Khan:** Data curation, Formal analysis, Writing - original draft. **Abida Sultana:** Data curation, Formal analysis. **Ping Ma:** Methodology, Writing - review & editing. **E. Lisako J. McKyer:** Methodology, Writing - review & editing. **Helal Uddin Ahmed:** Writing - review & editing. **Neetu Purohit:** Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

None.

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EXHIBIT 63

A range of physical and mental-health conditions frequently accompany autism. They include, but are not limited to, the following:

- Gastrointestinal (GI) problems
- Epilepsy
- Feeding issues
- Disrupted sleep
- Attention-deficit/hyperactivity disorder (ADHD)
- Anxiety
- Depression
- Obsessive compulsive disorder (OCD)
- Schizophrenia
- Bipolar Disorder

Autism and gastrointestinal (GI) disorders

GI disorders are nearly eight times more common among children with autism than other children.

They commonly include:

- Chronic constipation
- Abdominal pain
- Gastroesophageal reflux
- Bowel inflammation

The Autism Speaks Autism Treatment Network (ATN) has developed medical guidelines to help doctors recognize and manage these issues.

Also see: ATN/AIR-P Guide for Managing Constipation in Children

Autism and epilepsy

Epilepsy (seizure disorder) affects up to a third of people with autism. By contrast, it affects only 1 to 2 percent of the general population.

Red flags include:

- Unexplained staring spells
- Involuntary movements
- Unexplained confusion
- Severe headaches

Less-specific signs can include:

- Sleepiness
- Disrupted sleep
- Unexplained changes in abilities or emotions

Treatment of epilepsy is crucial to prevent brain damage.

If you suspect that you or your child may have epilepsy, seek evaluation from a neurologist. Evaluation typically involves an electroencephalogram (EEG) to check for seizure-related brain activity.

Also see these ATN/AIR-P Guidebooks:

- Having an Electroencephalogram (EEG): A Guide for Parents
- Having an Electroencephalogram (EEG): A Guide for Providers (caring for people with autism)

Epilepsy Resources

Below are some resources and websites that may be helpful to individuals with both diagnoses and their families:

Explaining Seizures to Children with Epilepsy and Their Peers

Sometimes it can be difficult for children to understand what is happening when they are having a seizure. In addition, it can be very scary for their peers or friends who witness them. Autism Speaks has put together Visual Stories to explain to children how people with epilepsy are just like everyone else!

Visual Story for Children with Epilepsy
Visual Story for Peers of Children with Epilepsy

If a family member suffers from seizures, you may want to consider a medical alert bracelet that can inform first responders of the seizure disorder and any medications that the individual may take. There are a variety of options available on the internet.

Autism and feeding/eating issues

Feeding and eating problems affect around 7 out of 10 children with autism.

These issues can include **extremely restricted food habits** and aversions to certain tastes and textures. Many adults with autism likewise describe food aversions and restricted eating patterns.

These challenges often stem from autism-related hypersensitivities and/or a strong need for sameness.

Chronic overeating leading to obesity is another challenge. It can stem from an inability to sense when "full" and/or eating as a soothing sensory behavior.

Pica – the eating of non-food items – is a particularly dangerous tendency often associated with autism. It appears to be most common among those severely affected by autism. See ATN/AIR-P's Pica: A Guide for Parents.

Many autism clinics – such as those in the Autism Speaks ATN – have specialized feeding programs staffed by behavioral therapists and nutritionists. Outside such programs, some speech, behavioral and occupational therapists can help.

You can find helpful strategies in Exploring Feeding Behavior in Autism

Autism and disrupted sleep

Over half of children with autism – and possibly as many as four in five – have one or more chronic sleep problems.

Many adults on the spectrum likewise have difficulty falling asleep and staying asleep through the night. These sleep issues tend to worsen behavioral challenges, interfere with learning and decrease overall quality of life.

Researchers with the Autism Speaks ATN have developed and tested autism-specific strategies for improving sleep. These can be found in three ATN/AIR-P guidebooks:

- Strategies to Improve Sleep in Children with Autism Spectrum Disorder
- Sleep Strategies for Teens with Autism
- Melatonin and Sleep Problems in ASD: An ATN/AIR-P Guide for Parents

Autism and attention deficit and hyperactivity disorder (ADHD)

ADHD affects an estimated 30 to 60 percent of people with autism, versus 6 to 7 percent of the general population.

ADHD involves a persistent pattern of inattention, difficulty remembering things, trouble with managing time, organizational tasks, hyperactivity and/or impulsivity that interferes with learn and daily life.

Symptoms of ADHD can overlap with those of autism. As a result, ADHD can be difficult to distinguish in someone on the spectrum.

If you suspect that you or your child has autism and ADHD, we recommend evaluation by a specialist familiar with both conditions. If the evaluation confirms ADHD, ask your healthcare provider to help you tailor a treatment plan appropriate to you or your child's needs.

Treatment may include behavioral strategies and in some cases medication for ADHD.

Autism and anxiety

Anxiety disorders affect up to 42 percent of people with autism. By contrast, they affect an estimated 3 percent of children and 15 percent of adults in the general population.

Because people with autism may have trouble assessing and expressing how they fell, behavior often provides the best clues in those experiencing anxiety. Anxiety can trigger racing heart, muscle tightness and stomaches, some people may event feel frozen in place.

Social anxiety – or extreme fear of new people, crowds and social situations – is especially common among people with autism. In addition, many people with autism have difficulty controlling anxiety once something triggers it.

Anxiety can be triggered at different points in time and by different activities – including some that were previously enjoyable.

Anxiety can be diagnosed by a medical professional.

Treatments include behavioral interventions including cognitive behavioral therapy programs adapted for people with autism. In some cases anti-anxiety medication may also be helpful.

Also see:

- Managing anxiety in children with autism
- Easing anxiety in children with autism and limited verbal skills

Autism and depression

Depression affects an estimated 7 percent of children and 26 percent of adults with autism. By contrast, it affects around 2 percent of children and 7 percent of adults in the general population.

Depression rates for people with autism rise with age and intellectual abiltiy.Autism-related communication challenges can mask depression. Telltale signs can include loss of interest in once-favorite activities, a noticeable worsening in hygiene,chronic feelings of sadness, hopelessness, worthlessness and irritability. At its most serious, depression can include frequent thoughts about death and/or suicide.

If you suspect that you or your child with autism is depressed, we urge you to seek evaluation and treatment.

Treatments may include cognitive behavioral therapy and in some cases anti-depressional medications.

Also see: What's the connection between autism and depression?

Obsessive Compulsive Disorder (OCD)

Research suggests that OCD is more common among teens and adults with autism than it is in the general population.

However, it can be difficult to distinguish OCD symptoms from the repetitive behaviors and restricted interests that are a hallmark of autism.

If you suspect that you or your child has developed OCD in addition to autism, we encourage you to seek evaluation by a mental health provider who has experience with both conditions.

Also see: A parent wonders: Are new repetitive behaviors OCD or 'just autism'

Autism and Schizophrenia

Autism and schizophrenia both involve challenges with **processing language** and **understanding other people's thoughts and feelings**. Clear differences include schizophrenia's psychosis which often involves **hallucinations**. In addition, autism's core symptoms typically emerge between ages 1 -3 years; schizophrenia emerges in early adulthood.

Treatments: Anti-psychotic medications

Autism and Bipolar Disorder

People with bipolar disorder tend to **alternate between** a frenzied state known as **mania** and episodes of **depression**.

It is important to understand the symptoms of true bipolar disorder from those of autism by looking at when the symptoms appeared and how long they lasted. For example, a child with autism may be consistently high-energy and socially intrusive through childhood. As such, her tendency to talk to strangers and make inappropriate comments are likely part of her autism, and not a symptom of a manic mood swing.

Treatments: Some of the medications used to treat bipolar disorder can be problematic for some with autism who has difficulty recognizing and expressing feelings. A psychiatrist can provide additional medications that may be safer.

Some of these conditions are described more extensively in *Autism and Health: A special report by Autism Speaks*.

autism speaks

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EXHIBIT 64

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Autism Spectrum Disorder (ASD)

What is Autism Spectrum Disorder?

Autism spectrum disorder (ASD) is a developmental disability that can cause significant social, communication and behavioral challenges. There is often nothing about how people with ASD look that sets them apart from other people, but people with ASD may communicate, interact, behave, and learn in ways that are different from most other people. The learning, thinking, and problem-solving abilities of people with ASD can range from gifted to severely challenged. Some people with ASD need a lot of help in their daily lives; others need less.

A diagnosis of ASD now includes several conditions that used to be diagnosed separately: autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome. These conditions are now all called autism spectrum disorder.

Signs and Symptoms

People with ASD often have problems with social, emotional, and communication skills. They might repeat certain behaviors and might not want change in their daily activities. Many people with ASD also have different ways of learning, paying attention, or reacting to things. Signs of ASD begin during early childhood and typically last throughout a person's life.

Children or adults with ASD might:

- not point at objects to show interest (for example, not point at an airplane flying over)
- not look at objects when another person points at them
- have trouble relating to others or not have an interest in other people at all
- avoid eye contact and want to be alone
- have trouble understanding other people's feelings or talking about their own feelings
- prefer not to be held or cuddled, or might cuddle only when they want to
- appear to be unaware when people talk to them, but respond to other sounds
- be very interested in people, but not know how to talk, play, or relate to them
- repeat or echo words or phrases said to them, or repeat words or phrases in place of normal language
- have trouble expressing their needs using typical words or motions
- not play "pretend" games (for example, not pretend to "feed" a doll)

- repeat actions over and over again
- have trouble adapting when a routine changes
- have unusual reactions to the way things smell, taste, look, feel, or sound
- lose skills they once had (for example, stop saying words they were using)

Learn more about symptoms »

Learn about developmental milestones that young children should reach »

Diagnosis

Diagnosing ASD can be difficult since there is no medical test, like a blood test, to diagnose the disorders. Doctors look at the child's behavior and development to make a diagnosis.

ASD can sometimes be detected at 18 months or younger. By age 2, a diagnosis by an experienced professional can be considered very reliable.[1] However, many children do not receive a final diagnosis until much older. This delay means that children with ASD might not get the early help they need.

Learn more about diagnosis »

Treatment

There is currently no cure for ASD. However, research shows that early intervention treatment services can improve a child's development.[2, 3] Early intervention services help children from birth to 3 years old (36 months) learn important skills. Services can include therapy to help the child talk, walk, and interact with others. Therefore, it is important to talk to your child's doctor as soon as possible if you think your child has ASD or other developmental problem.

Even if your child has not been diagnosed with an ASD, he or she may be eligible for early intervention treatment services. The Individuals with Disabilities Education Act (IDEA) says that children under the age of 3 years (36 months) who are at risk of having developmental delays may be eligible for services. These services are provided through an early intervention system in your state. Through this system, you can ask for an evaluation.

In addition, treatment for particular symptoms, such as speech therapy for language delays, often does not need to wait for a formal ASD diagnosis.

Learn about types of treatments »

Causes and Risk Factors

We do not know all of the causes of ASD. However, we have learned that there are likely many causes for multiple types of ASD. There may be many different factors that make a child more likely to have an ASD, including environmental, biologic and genetic factors.

- Most scientists agree that genes are one of the risk factors that can make a person more likely to develop ASD.[4, 19]
- Children who have a sibling with ASD are at a higher risk of also having ASD. [5-10]
- Individuals with certain genetic or chromosomal conditions, such as fragile X syndrome or tuberous sclerosis, can have a greater chance of having ASD. [11-14, 20]
- When taken during pregnancy, the prescription drugs valproic acid and thalidomide have been linked with a higher risk of ASD.[15-16]
- There is some evidence that the critical period for developing ASD occurs before, during, and immediately after birth. [17]
- Children born to older parents are at greater risk for having ASD. [18]

ASD continues to be an important public health concern. Like the many families living with ASD, CDC wants to find out what causes the disorder. Understanding the factors that make a person more likely to develop ASD will help us learn more about the causes. We are currently working on one of the largest U.S. studies to date, called Study to Explore Early Development (SEED). SEED is looking at many possible risk factors for ASD, including genetic, environmental, pregnancy, and behavioral factors.

Learn more about CDC's research on possible causes and risk factors for ASD »

Who is Affected

ASD occurs in all racial, ethnic, and socioeconomic groups, but is about 4 times more common among boys than among girls.

For over a decade, CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network has been estimating the number of children with ASD in the United States. We have learned a lot about how many U. S. children have ASD. It will be important to use the same methods to track how the number of children with ASD is changing over time in order to learn more about the disorder.

Learn more about CDC's tracking of the number of children with ASD »

If You're Concerned

If you think your child might have ASD or you think there could be a problem with the way your child plays, learns, speaks, or acts, **contact your child's doctor, and share your concerns**.

If you or the doctor is still concerned, **ask the doctor for a referral to a specialist** who can do a more in-depth evaluation of your child. Specialists who can do a more in-depth evaluation and make a diagnosis include:

- Developmental Pediatricians (doctors who have special training in child development and children with special needs)
- Child Neurologists (doctors who work on the brain, spine, and nerves)
- Child Psychologists or Psychiatrists (doctors who know about the human mind)

At the same time, call your state's public early childhood system to request a free evaluation to find out if your child qualifies for intervention services. This is sometimes called a Child Find evaluation. You do not need to wait for a doctor's referral or a medical diagnosis to make this call.

Where to call for a free evaluation from the state depends on your child's age:

- If your child is not yet 3 years old, contact your local early intervention system.
  - You can find the right contact information for your state by calling the Early Childhood Technical Assistance Center (ECTA) at 919-962-2001.
  - Or visit the ECTA website .
- If your child is 3 years old or older, contact your local public school system.
  - Even if your child is not yet old enough for kindergarten or enrolled in a public school, call your local elementary school or board of education and ask to speak with someone who can help you have your child evaluated.
  - If you're not sure who to contact, call the Early Childhood Technical Assistance Center (ECTA) at 919-962-2001.
  - Or visit the ECTA website .

Research shows that early intervention services can greatly improve a child's development.[2, 3] In order to make sure your child reaches his or her full potential, it is very important to get help for an ASD as soon as possible.

Economic Costs

- The total costs per year for children with ASD in the United States were estimated to be between $11.5 billion – $60.9 billion (2011 US dollars). This significant economic burden represents a variety of direct and in-direct costs, from medical care to special education to lost parental productivity. [Read article] [Read article]
- Children and adolescents with ASD had average medical expenditures that exceeded those without ASD by $4,110–$6,200 per year. On average, medical expenditures for children and adolescents with ASD were 4.1–6.2 times greater than for those without ASD. Differences in median expenditures ranged from $2,240 to $3,360 per year with median expenditures 8.4–9.5 times greater. [Read article]
- In 2005, the average annual medical costs for Medicaid-enrolled children with ASD were $10,709 per child, which was about six times higher than costs for children without ASD ($1,812). [Read summary]
- In addition to medical costs, intensive behavioral interventions for children with ASD cost $40,000 to $60,000 per child per year.[21]

Vaccine Safety

Some people have had concerns that ASD might be linked to the vaccines children receive, but studies have shown that there is no link between receiving vaccines and developing ASD. For more information about vaccines and ASD, click here

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Page last reviewed: March 25, 2020

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EXHIBIT 65

Neuropsychopharmacology REVIEWS (2017) 42, 284–298

The Role of the Immune System in Autism Spectrum Disorder

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Autism is a neurodevelopmental disorder characterized by deficits in communication and social skills as well as repetitive and stereotypical behaviors. While much effort has focused on the identification of genes associated with autism, research emerging within the past two decades suggests that immune dysfunction is a viable risk factor contributing to the neurodevelopmental deficits observed in autism spectrum disorders (ASD). Further, it is the heterogeneity within this disorder that has brought to light much of the current thinking regarding the subphenotypes within ASD and how the immune system is associated with these distinctions. This review will focus on the two main axes of immune involvement in ASD, namely dysfunction in the prenatal and postnatal periods. During gestation, prenatal insults including maternal infection and subsequent immunological activation may increase the risk of autism in the child. Similarly, the presence of maternally derived anti-brain autoantibodies found in ~20% of mothers whose children are at risk for developing autism has defined an additional subphenotype of ASD. The postnatal environment, on the other hand, is characterized by related but distinct profiles of immune dysregulation, inflammation, and endogenous autoantibodies that all persist within the affected individual. Further definition of the role of immune dysregulation in ASD thus necessitates a deeper understanding of the interaction between both maternal and child immune systems, and the role they have in diagnosis and treatment.

Neuropsychopharmacology Reviews (2017) **42,** 284–298; doi:10.1038/npp.2016.158; published online 21 September 2016

INTRODUCTION

First described by Kanner, 1943, autism is a neurodevelopmental disorder characterized by repetitive, stereotypical behaviors and impaired expressive communication, which has since been folded into the broader classification of autism spectrum disorders (ASD) (American Psychiatric Association, 2013). The most recent (2012) estimates in the United States indicate a rate of 14.6 per 1000 children (one in 68) aged eight (Christensen *et al*, 2016). With increased prevalence in the population has come increased level of attention to the causes of ASD, which remain elusive yet legion. It is known that autism is strongly influenced by the genome—in contrast to earlier speculation, ranges of heritability estimates generally center ~50 or 55% (Badcock, 2011; Colvert *et al*, 2015; Hallmayer *et al*, 2011)—but genes implicated by large genome-wide studies have been shown to account for only a fraction of ASD diagnoses (Abrahams and Geschwind, 2008; Badcock, 2011; Happe *et al*, 2006).

Nearly 50 years ago, an association of autism with congenital rubella infection was noted, and in the intervening years numerous other infections have been connected to the incidence of ASD. Since that time, mounting evidence for the ability of the immune system and abnormal immune function, including inflammation, cytokine dysregulation, and anti-brain autoantibodies, to act as a significant influence on ASD has prompted researchers to look more closely at the potential role of immune dysregulation and autoimmunity in ASD. This field of research has gained particular traction given that other neurodevelopmental disorders, such as schizophrenia, also present alongside a spectrum of changes in immune function. Here, we review the potential of the immune system to serve as a collective, complex etiology for autism and ASD in at least a subset of cases. A graphic overview of the various neuronal and immune system components involved in the topics under discussion are depicted in Figure 1, indicating the many cells and molecules that may lead from an altered immune system to altered neurodevelopment. We divided this work into two sections, separated into prenatal and postnatal exposures. It begins in the gestational period with an examination of

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Received 9 June 2016; revised 2 August 2016; accepted 5 August 2016; accepted article preview online 18 August 2016

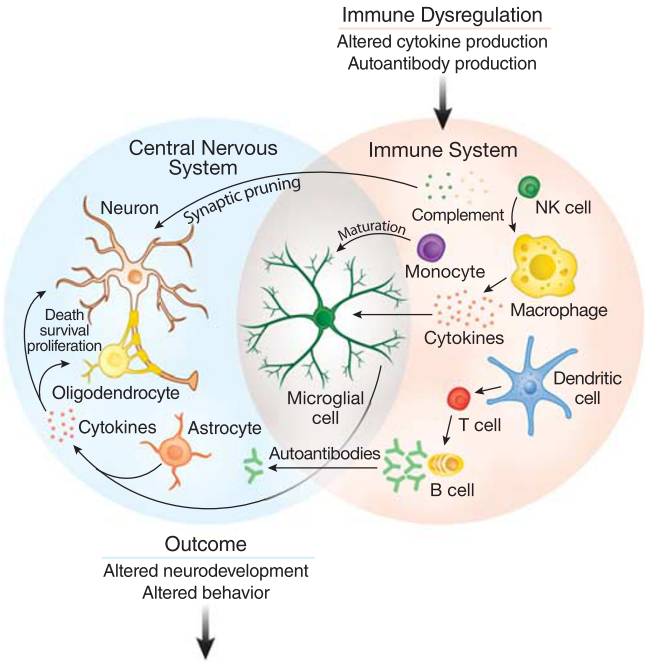

Figure 1. Overview of the immune system as a mediator of behavior. Understanding how immune dysfunction in ASD can lead to changes in behavior requires understanding a complex network of interactions between several cell types from both the innate and adaptive arms of the immune system. Several immune factors mediate effects of CNS function. T-cell and NK-cell subpopulations may have altered activity and an impaired reaction to stimulation. Some cytokines can inhibit neurogenesis and promote neuron death, while others can promote the growth and proliferation of neurons and oligodendrocytes. Complement proteins and microglia can participate in synaptic scaling and pruning, while brain-reactive autoantibodies can change the development or function of neurons. When the many components of the immune system are dysregulated, these networks can lead to changes in neurodevelopment and behavior.

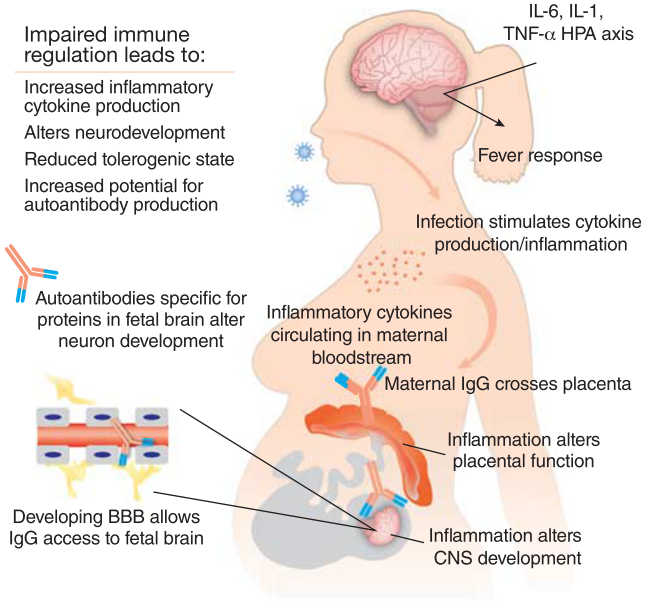

Figure 2. Maternal immune dysregulation during gestation is a risk factor for autism and numerous interrelated factors may lead to dysregulation of the maternal immune system. Infection during the pregnancy, such as by rubella or influenza virus, can create an inflammatory immune environment and spur the production of maternal cytokines, which can not only directly affect the placenta but also to a limited degree may cross the placenta and enter the fetal compartment to have lasting effects on the development of the fetus. These effects can also be achieved in the absence of active infection, whether though a generalized inflammatory response or loss of immune regulation. Animal models of MIA have been particularly significant in underscoring the importance of maternal immune regulation. In addition, a subset of women may produce anti-brain autoantibodies that can likewise gain access to the developing fetal brain and bind to fetal proteins, thereby altering the course of neurodevelopment. Activation of the maternal immune system during fetal development is thus an important factor in the etiology of ASD, and may lead to changes in neurodevelopment.

maternal infection, immune activation, and autoantibodies present during pregnancy, followed by the ongoing, postnatal role of dysregulation in the immune system in a child with ASD. We hope that through this comprehensive review, we will provide evidence supporting the notion that immune dysregulation in autism is a viable pathway towards the identification and subsequent treatment of this ASD subpopulation in the future.

THE PRENATAL/GESTATIONAL ENVIRONMENT

There are a number of ways in which the gestational environment can affect neurodevelopmental outcome in the child. This includes active infection as well as the immune response of the mother to infection as key areas of research when looking for prenatal risk factors. Indeed, it has now been demonstrated through animal models that merely an activated immune response in the mother, absent any infection, is sufficient to lead to changes in the offspring (Shi *et al*, 2003). Further, autoantibodies to proteins that are a key to healthy neurodevelopment are also thought to be a viable pathogenic mechanism in a subset of cases. Figure 2 summarizes the various prenatal maternal immune factors that can have a role in the development of ASD that will be addressed in the following sections.

Congenital Infection

In 1964, an epidemic of rubella spread throughout the United States, leading to between 20 000 and 30 000 infants with congenital defects. Following the outbreak, Dr Chess examined 243 preschool children with congenital rubella syndrome (CRS) and found a marked increase in the incidence of autism (Chess, 1971). The study reported 18 children with some degree of autism, implying an incidence of 741 cases per 10 000 among the population of CRS children, considered high even today and an especially high rate for the time. Chess followed-up as the children aged and found even higher rates of autism (905 per 10 000) (Chess, 1977, 1978), thus establishing a link between CRS and autism that has been supported by others (Deykin and MacMahon, 1979). A 2016 reappraisal of rubella-related ASD suggested

that the connection persists and offered several reasons for why the rubella–autism connection might be difficult to work out (eg, widespread vaccination, rubella may evade the fetal immune system, children with CRS often lack antibodies to rubella, etc) as well as some ideas for future research, such as using cord blood or prescreening newborns before rubella vaccination (Hutton, 2016).

Rubella is not the only virus, or indeed infection, to show a connection to autism when acquired congenitally (Atladottir *et al*, 2010; Wilkerson *et al*, 2009). Varying reports have also implicated measles and mumps (Deykin and MacMahon, 1979), cytomegalovirus (Libbey *et al*, 2005), polyomaviruses (Lintas *et al*, 2010), and influenza (Atladottir *et al*, 2012; Deykin and MacMahon, 1979; Shi *et al*, 2003; Zhang *et al*, 2010) in the incidence of ASD. Some studies suggest fever as a correlate (Atladottir *et al*, 2012), with this risk potentially tempered by anti-fever medications, such as Advil, Tylenol, or Nyquil (Zerbo *et al*, 2013). A more expansive study by Zerbo *et al* (2015) found that maternal infection diagnosed at a hospital admission, especially bacterial ones, correlated with increased risk of ASD. A yet-larger 2015 study of over 24 000 Swedish ASD cases observed increased ASD rates among offspring of mothers hospitalized the year before pregnancy; risk for ASD increased under any inpatient diagnosis, including both viral and bacterial (Lee *et al*, 2015). The authors found no connection with the gestational timing of infection, however, in contrast to previous suggestions of viral or bacterial infection (Atladottir *et al*, 2010). As research into the role of direct or retrospective infection continues, the evidence presented thus far strongly suggests a connection between maternal infection and altered neurodevelopment. The implication is that the response to the infection, not just a few select pathogens, can lead to ASD in the offspring. This changes how we view the risk of a child developing ASD following a gestational exposure to an infectious agent: we should be looking at the maternal response to the exposure rather than focusing on the specific infectious agent.

Maternal Immune Activation

Some of the strongest support for maternal infection as a contributor to autism has come from the maternal immune activation (MIA) model, which has repeatedly shown consistent results, helping to establish a subset of autism that is supported by observations of autism-relevant atypical behaviors. The MIA model in its most common design involves challenging pregnant rodents via direct infection (eg, influenza, *Escherichia coli*) or the dsRNA mimic poly (I:C); significant immunological, neurodevelopmental, and behavioral changes are then observed in the offspring (Gilmore *et al*, 2005; Meyer *et al*, 2005; Urakubo *et al*, 2001; Zuckerman and Weiner, 2005). These changes have been linked and employed to model several neurodevelopmental disorders, including schizophrenia (Meyer and Feldon, 2009; Meyer *et al*, 2005; Zuckerman and Weiner, 2005), cerebral palsy (Boksa, 2010), and autism (Brown *et al*, 2015).

Shi *et al* (2003) infected two strains of pregnant mice with human influenza virus and observed abnormal behavior, exploration, and social interaction in their offspring. In support of the congenital infection model, the authors observed similar aberrant phenotypes in the same mice when using only poly(I:C) treatment rather than infection, suggesting that it was the maternal immune response rather than the infection itself that was responsible for the effects of gestational exposure to an infectious agent. The authors followed-up by challenging pregnant dams with either a single injected dose of influenza on embryonic day E9.5 or poly(I:C) on day E12.5; both groups had offspring with altered cerebellar development, akin to that seen in autism (Shi *et al*, 2009). A more expansive study injected pregnant mice three times with poly(I:C), each with one-fourth the dose of Shi, *et al*, starting on E10.5 and spaced with 2 days between injections (Malkova *et al*, 2012). The authors looked at ultrasonic vocalization (USV) responses among male offspring and found lower rates and total length of vocalizations. Sociobehavioral changes were observed using an isolation test and the three-chamber test, a system that presents a test animal with the choice to either explore or avoid novel animals or objects (Kaidanovich-Beilin *et al*, 2011). The male MIA offspring also showed several autism-relevant changes, such as reduced sociability in the three-chamber apparatus, spending more time in the nonsocial chamber and less time with the social object, as well as significantly higher rates of repetitive behaviors such as self-grooming and marble burying.

A 2014 study by Bauman *et al* (2014), broadened the poly (I:C) maternal activation model to rhesus macaques, showing many abnormal behaviors, including in vocal communication and social interaction, as well as repetitive behaviors in the offspring of treated females. Pregnant monkeys were injected with poly(I:C) three times in either the late first or late second trimester, and their offspring were subsequently monitored for 2 years. Although there were some differences between the offspring of the two treatment groups, each set of young primates nonetheless presented aberrancies and atypical phenotypes relevant to both ASD and schizophrenia.

Weber-Stadlbauer expanded greatly on this in 2016, finding that offspring of pregnant mice given a single poly (I:C) injection at day E9 showed reduced interaction (via the three-chamber apparatus) and increased fear expression in a cued Pavlovian conditioning test (Weber-Stadlbauer *et al*, 2016). Significantly, they showed that several autism-relevant traits only persisted through the paternal line, without any further intervention, through the F2 and even the F3 generations, resulting in significant differences in gene expression. A 2015 epigenetic study also using a single poly (I:C) injection found significantly increased rates of 5-methylcytosine and 5-hydroxymethylcytosine on the glutamic acid decarboxylase (GAD1) promoter, which synthesizes the neurotransmitter GABA (Labouesse *et al*, 2015). These changes correlated with social interaction and

working memory impairments and are similar to GABA and GAD1 changes observed in related disorders such as schizophrenia.

Cytokines

Although the alterations in behavior and neurodevelopment of the MIA model have been fairly well established (Gilmore *et al*, 2005; Meyer *et al*, 2005; Urakubo *et al*, 2001; Zuckerman and Weiner, 2005), less well understood is how these changes are mediated through to the offspring. Given the diversity of infections that show a connection to the spectrum of autism disorders (Boksa, 2010; Libbey *et al*, 2005), as well as evidence from MIA models that reaction to infection rather than infection itself may lead to autism-related symptoms (Shi *et al*, 2003), a possible common etiology may be the influence of the immune system (Gottfried *et al*, 2015; Lintas *et al*, 2010; Zhang *et al*, 2010). Cytokines, small cell-signaling proteins that act as immunomodulatory and endocrine messengers, have been implicated throughout the process of CNS development (Boulanger, 2009; Jones and Thomsen, 2013). The immune cells that produce cytokines following activation are separated into two populations of cells: innate and adaptive. Both cell populations have the ability to produce cytokines that are linked to inflammation, including monocytes/macrophages and T cells. T cells are divided into subsets based on their function and their cytokine profile. Th or T-helper cells are classified into Th1 cells that produce pro-inflammatory cytokines, Th2 cells that are responsible for driving antibody production and downregulation of the inflammatory response, Th17 cells that also drive inflammation, and Th3 regulatory cells. As we investigate the relationship between the immune system and neurological disorders such as ASD, we look for skewing of the immune response towards an inflammatory or regulatory cytokine profile, whether it is during gestation or in the child.

There is strong evidence that disruption of normal cytokine levels has a significant role as a risk factor for several neurodevelopmental defects, including schizophrenia and autism (Boulanger and Shatz, 2004; Brown *et al*, 2004b; Deverman and Patterson, 2009; Hsiao *et al*, 2013; Ponzio *et al*, 2007). Smith *et al* (2007) showed in 2007 that a single injection of the pro-inflammatory cytokine IL-6 to pregnant mice at embryonic day 12.5 lead to offspring deficits in prepulse inhibition (PPI) and latent inhibition (LI), both autism-relevant behaviors; these results were not seen following injection of the pro-inflammatory cytokines IL-1α, TNF-α, or IFN-γ. The authors also used a poly(I:C) MIA model, observing basic behavioral changes in agreement with those previously described. When the authors co-administered a neutralizing anti-IL-6 antibody to pregnant dams, they observed an apparent rescue of the aberrant behaviors and reversal of transcriptional changes; these rescue effects were not seen in the context of co-administered anti-IFN-γ or anti-IL-1β antibody, suggesting a specific role for IL-6 in the etiology of ASD.

In further support of IL-6-mediated MIA autism, attempting the poly(I:C) MIA model in IL-6 knockout mice showed no statistically significant differences in PPI or social behavior via the three-chamber test from untreated IL-6 knockouts. Another team also used a poly(I:C) MIA model to show increased IL-6 levels, and found that treatment with *Bacteroides fragilis*, which can repair tight junction integrity in the gut, corrected and restored IL-6 levels, aberrant behavior, stereotypies, and anxiety-like symptoms (Hsiao *et al*, 2013).

Although previous work had shown that IL-6 may readily cross the placenta (Zaretsky *et al*, 2004), Choi *et al* (2016) extended this pathway further downstream and established that poly(I:C) injections in pregnant dams increased post-induction maternal serum levels of IL-6 3 h later and IL-17 2 days later. These increases did not occur in IL-6 knockout mice; however, increased IL-17 levels were found following injection of IL-6 to wild-type, untreated mothers, indicating that increased IL-6 leads to IL-17 production. MIA pups showed several aberrancies in USVs, sociality, and marble burying, but maternal pretreatment with an anti-IL-17 antibody restored these behaviors to typical measures. Fetal MIA offspring showed increased brain mRNA levels of the IL-17 receptor subunit, effects that were also rescued by maternal anti-IL-17 pretreatment. Injection of IL-17 to E14.5 fetal brain was enough to induce structural changes similar to those seen in MIA offspring. Finally, treatment of the anti-IL-17 antibody 2 days after MIA induction saw rescue of some phenotypes, such as USV calls and marble burying, but not others, including interaction deficits. Put together, this suggests that the Th17 cell/IL-17 pathway may be critical in MIA-induced autism, caused by upregulated IL-17 acting downstream of increased IL-6 levels. Choi *et al* (2016) also showed that their MIA model induced the production of several other cytokines, finding increased levels of TNF-α, IFN-β, and IL-1β 3 h following MIA induction, but not the anti-inflammatory IL-10. A poly(I:C) MIA model by Meyer *et al* (2008) suggested that maternal IL-10 may be able to attenuate behavioral and pharmacological dysfunctions.

More recent human studies found increased levels of IFN-γ, IL-4, and IL-5 in sera from mid-gestational mothers with a child that would later be diagnosed (Goines *et al*, 2011b) and elevated MCP-1 (Abdallah *et al*, 2012a), IL-4, TNF-α, and TNF-β levels in amniotic fluid (Abdallah *et al*, 2013). Most recently, studies have demonstrated that elevated mid-gestational levels of inflammatory cytokines and chemokines are more highly associated with the ASD subphentoype that presents with intellectual disability (ID), compared with ASD without ID, developmental delay (DD) without ASD, and typically developing controls (Jones *et al*, 2016). These included higher levels of GM-CSF, TNF-α, IFN-γ, IL-1α, IL-1β, IL-4, and IL-6. A 2016 study found maternal blood C-reactive protein levels to be lower in weeks 15–19 of pregnancies of a child with ASD than the general population, and although the results appear not to be genetic, the authors lacked reports of disease status and could not say whether infection during pregnancy was a factor (Zerbo *et al*, 2016).

This study was in contrast to an earlier investigation of Finnish children that showed an association between autism and elevated early gestational maternal C-reactive protein (Brown *et al*, 2014). One proposal suggested three pathways through which a prenatal inflammatory cytokine response may leads to ASD: maternal, in which cytokines from the mother cross the placenta; placental, in which MIA leads to inflammation and cytokine production in the placenta; or fetal, where MIA leads immune and gene dysregulation in the fetus itself (Abdallah *et al*, 2013). However, it should be considered that there might be a combination of these three pathways. Maternal inflammation history and the pro-inflammatory/anti-inflammatory cytokine balance are thus particularly important avenues for further study, especially at the interface between mother and fetus (Meyer *et al*, 2008; Jones and Thomsen, 2013; Young *et al*, 2016).

Transplacental Maternal Autoantibodies to the Fetal Brain

The notion that maternal autoantibodies to elements in the fetal compartment could elicit changes in neurodevelopment began over 25 years ago, in a study that examined serum from 11 mothers of young children and found six with circulating antibodies that targeted lymphocyte antigens of the children (Warren *et al*, 1990). A decade later, researchers described a mother with serum antibodies that tested positive for reactivity to rat neuronal tissue (Dalton *et al*, 2003). A subsequent study used samples taken from 11 mothers with autistic children to strengthen these results, finding serum reactivity in those mothers to prenatal, postnatal, and adult rat brain proteins while finding none in control mothers (Zimmerman *et al*, 2007). Of particular note is that the children in this study ranged in age from 2 to 18 years old, indicating that the presence of autoantibodies in maternal serum is not a transient phenomenon and may persist for many years. Although generally consistent in their findings, the previous studies lacked the large sample sizes that provide convincing evidence.

Braunschweig *et al* (2008) looked at plasma from 61 mothers of autistic children and 102 controls (62 TD and 40 with other, non-ASD DD) for reactivity to fetal brain proteins. The authors identified two bands, at 37 and 73 kDa, that were significantly more common among mothers with autistic children, in particular children with regression rather than early onset. More importantly, the authors found that 12% of samples from mothers of children with ASD showed both 37 and 73 kDa bands, while none of the mothers of TD or DD children did. Another study published that year looked at serum from 100 mothers of children with autism, again finding higher rates of anti-fetal brain antibodies as compared with 100 control mothers (Singer *et al*, 2008). These antibodies were positive to adult and fetal human and rat brain proteins, including targets at 73 kDa and 36 kDa, similar to those shown by Braunschweig *et al* (2008). These authors used specific brain samples rather than a medley, identifying numerous other targets specific to certain brain regions (Singer *et al*, 2008). In a large study using the Simons Simplex collection, Brimberg *et al* (2013) examined plasma from 2431 mothers of ASD children and compared it with plasma from 654 GP women, finding that mothers of children with ASD were four times as likely to have circulating anti-brain antibodies. The sample screening process for this study differed from previous research in that the serum samples were tested against mouse tissue sections using immunofluorescence rather than detection of denatured proteins by western blot. This underscores the importance of multiple investigators using different approaches to tackle this critical area of autism research.

Analysis of samples before the birth of the child is a critical next step from the associative studies noted above. In a study utilizing banked mid-pregnancy (prospective) blood samples, the authors noted that maternal autoantibodies reactive to proteins near 37 and 73 kDa were only found in women whose children later received a diagnosis of ASD (Croen and Braunschweig, 2008a). Although the specific maternal anti-fetal brain antibodies were detected more often in mothers of children with ASD, there was still minimal evidence supporting the idea that maternal autoantibodies could alter behavior. Moreover, additional studies are needed determine the predictive ability of the maternal autoantibodies for autism risk; such studies are currently underway utilizing multiple sample populations.

To further explore the relationship between the maternal autoantibodies and behavioral outcome, an expanded study was conducted with a larger cohort of patients to provide additional support for the role of maternal autoantibodies in the pathogenesis of ASD. The authors reported an association between the presence of anti-fetal brain antibodies in the mother and ASD-related deficits in the child, finding that paired brain reactivity (ie, to the 37 and 73 kDa bands) correlated with lower expressive language (Braunschweig *et al*, 2012a). Furthermore, reactivity to a protein band near 39 kDa was discovered, and paired reactivity to proteins at 39 and 73 kDa correlated with broader diagnosis of ASD as well as increased irritability on the Aberrant Behavior Checklist (ABC) scale, results that have since been supported by subsequent studies (Piras *et al*, 2014). In addition, it was noted in a follow-up report that children born to mothers with this pattern of autoantibody binding also exhibited higher total cerebral volume when compared both to children with ASD born to autoantibody-negative mothers as well as typically developing control children (Nordahl *et al*, 2013).

To understand how these anti-brain antibodies could potentially lead to changes in neurodevelopment, it was first necessary to determine the identity of the proteins corresponding to the 37, 39, and 73 kDa bands. Studies by Braunschweig *et al* (2013) utilized two-dimensional gel electrophoresis followed by tandem mass spectrometry peptide sequencing to identify seven developmentally regulated proteins in the fetal brain that are recognized by

maternal autoantibodies. These are lactate dehydrogenase A and B (LDH-A and LDH-B), stress-induced phosphoprotein 1 (STIP1), collapsin response mediator proteins 1 and 2 (CRMP1 and CRMP2), cypin, and Y-box binding protein 1 (YBX1). Confirmatory analysis of the identified targets confirmed that 23% of mothers whose children had ASD had reactivity to the highly specific antigen patterns compared with 1% of TD mothers. This preliminary analysis suggests a specificity of 98% with a sensitivity of 23%. Further, when behavioral outcomes were characterized in the children of the 7% of mothers with a combined reactivity to LDH, STIP1, and CRMP1 (corresponding to the original 37/73 kDa band pattern) this pattern of reactivity correlated with an increase in stereotypic behaviors in the child (Braunschweig *et al*, 2013). In specific combinations, autoantibodies to these proteins were predictive for autism risk, which, although still at the level of laboratory research, suggests the presence of a distinct, maternal autoantibody-driven subset of ASD. Future studies will build upon this work to determine the clinical utility of the maternal autoantibodies as a marker for autism risk.

Animal Models Strongly Support the Maternal Autoantibody Model

As maternal autoantibodies have become increasingly implicated in the behavioral and cognitive deficits that characterize ASD, it has been difficult to determine the pathogenic mechanism by which these antibodies lead to the behaviors observed in children with ASD by relying solely on retrospective clinical studies conducted with human subjects or samples. Toward this goal, animal models have been repeatedly utilized to bolster the findings of maternal anti-brain autoantibodies, suggesting a directly causative effect. As previously mentioned, investigators injected maternal serum containing anti-brain antibodies into pregnant mice daily between E10 and E17, and observed reduced exploration and motor control in their offspring as compared with the offspring of untreated dams (Dalton *et al*, 2003).

Singer *et al* (2009) exposed pregnant mice to IgG from mothers of children with autism daily between E13 and E18, observing alterations in sociability and anxiety. Braunschweig *et al* (2012b) performed a similar study to demonstrate atypical anxiety and sociality alongside impaired motor and sensory abilities following a single intravenous injection. A 2014 study injected pregnant mice only once, intraventricularly, on E14, with IgG from either mothers with the 37 and 73 kDa autoantibodies or control mothers; the authors observed atypical behaviors in the murine offspring, including stereotypical self-grooming and increased marble burying (Camacho *et al*, 2014). A follow on study using the same technique showed structural changes in the offspring of pregnant mice injected with autoreactive IgG, including higher proliferation of radial glial cells and increased brain and neuron size (Martinez-Cerdeno *et al*, 2016).

A related study collected sera from both mothers of autistic and TD children, and exposed pregnant macaques to IgG either reactive or nonreactive to monkey brain protein, verified via western blot. The authors found higher levels of motor activity and stereotypies among patient-treated macaque offspring, again supporting a causative agent present in maternal circulation (Martin *et al*, 2008). Of particular note is that the exogenous IgG was present for only 25% of the pregnancy, given in only three doses at the end of the first trimester, meaning that developing fetuses were likely under-dosed compared with what would be the case during human ASD development.

In a more targeted approach designed to increase gestational exposure, Bauman, *et al*, purified IgG from mothers who tested positive for antibodies that recognized the 37/73 kDa proteins (LDH, STIP1, and CRMP1) and had children with autism. The authors then treated eight pregnant macaques with that IgG six times over the course of the second and third trimesters, and assessed macaque offspring for 2 years using a battery of developmental and social tests. They observed a number of deviations from typical behavior as compared with animals treated with IgG from mothers of TD children as well as untreated controls, including rebuffed and unreciprocated friendship approaches and inappropriate vocalization (Bauman *et al*, 2013). These animals also had increased total cerebral volume, further supporting previous work in mice showing that these specific maternal autoantibodies appear to increase brain growth and total cerebral volume (Bauman *et al*, 2013; Martinez-Cerdeno *et al*, 2016).

The ideal study would make use of endogenously produced maternal anti-brain auto-antibodies to more readily mimic the theorized pathway. It is also worth keeping in mind that one can have an excellent biomarker of a disease without any direct evidence of pathological significance for that marker. However, while work in this area is currently underway from several lines of investigation and in multiple laboratories, it seems evident that maternal autoantibodies appear to influence, if not define, a specific subset of ASD. Given the existence of autoantibodies associated with other neurological disorders such as schizophrenia (Henneberg *et al*, 1994) and multiple sclerosis (Ryberg, 1982), there is clearly more to be addressed as to both the genesis and function of these antibodies in neurodevelopmental disorders. It is also critical that this work is validated and replicated by more than one investigator. However, the ultimate payoff for these studies has the potential to be of great benefit to families.

POSTNATAL/ONGOING EFFECTS

Alterations in immune function have also been noted as factors in children with autism, although this line of research has been somewhat hampered by the heterogeneity that is inherent in ASD. However, as we now recognize that autism would be more accurately labeled as 'autisms', the field has begun to look more closely at the relationship between

immune dysfunction and the behavioral endophenotypes. To begin with, children may have their own, endogenous anti-brain autoantibodies that are connected with atypical development, distinct from the previously described maternal autoantibodies. Immunogenetic factors are also connected to ASD, including *MET* and the HLA family of genes. Broad pathways of genetic involvement in immune dysregulation have also been identified, in particular natural killer cells and cytokine regulation. The immune system of children with ASD can also be significantly altered, with a skew toward a pro-inflammatory and elevated Th1 response. Figure 3 is a graphic representation of the various factors found among the pediatric ASD population that will be discussed in more detail in this section.

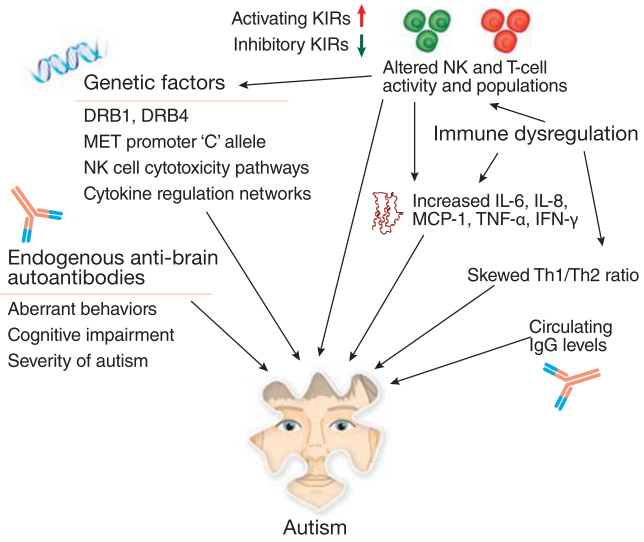

Figure 3. Ongoing immune dysregulation persists in autism. After birth and at least throughout childhood, an individual with ASD may have endogenous anti-brain autoantibodies, separate from any maternal IgG, which correlate with aberrant behaviors and impaired development. There also exists a broad picture of ASD-related immune dysregulation, including an increased inflammatory cytokine milieu (eg, IL-6, IL-8, and MCP-1), thus leading to an increased, pro-inflammatory Th1/Th2 ratio. T-cell and NK-cell populations may also be skewed, displaying a shift in cell subpopulations. NK cells in particular show an increased baseline activity but a decreased response to activation, rendering the cells unable to properly respond to stimuli. NK cells interact with activating and inhibitory KIRs, many of which are genetically linked to ASD. Other genetic factors include the oncogene *MET* and members of the diverse family of HLA genes. The broader background of immunogenetic factors of ASD includes multiple networks of the immune system, such as pathways that regulate cytokines and NK cells, which together constitute a broad, endogenous environment of atypical immune regulation and response.

Anti-brain Antibodies in Children with ASD

In addition to the prenatal, maternal autoantibodies previously discussed, numerous studies have reported the presence of circulating anti-brain immunoglobulins in patients with ASD, as well as related conditions such as multiple sclerosis (Ryberg, 1982), schizophrenia (Henneberg *et al*, 1994), and systemic lupus erythematosus (SLE) (Libbey and Fujinami, 2010; Mackay *et al*, 2015; Suurmond and Diamond, 2015). Singer *et al* (2006) found that children with autism were more likely than their non-autistic siblings or controls to have serum that tested positive via western blot to fresh human brain samples (caudate, putamen, and prefrontal cortex). A similar result found that children with autism showed positive serum anti-brain reactivity against prenatal rat proteins by western blot, with a different set of banding patterns relative to their non-autistic siblings and other controls (Zimmerman *et al*, 2007). A 2009 study of plasma from 63 young children with ASD found that 21% of them had antibodies positive against both human and macaque cerebellum samples via western blot, while only one out of the 63 typical developing (TD) controls (2%) tested positive (Wills *et al*, 2009). When a subset of subject samples was examined via immunohistochemistry (IHC), 21% of those from children with ASD had 'intense immunoreactivity' while none of the TD controls exhibited such reactivity. The western blot and IHC results were strongly correlative, reinforcing the findings of an ASD subset with persistent circulating anti-brain antibodies.

A larger 2011 study expanded on these findings, testing plasma from a cohort of 277 children with ASD and 189 TD controls for reactivity to human and macaque cerebellar protein medleys, with ASD children more likely to test positive for anti-brain autoantibodies via western blotting (Goines *et al*, 2011a). The authors also found strong correlations between the presence of those antibodies and aberrant behaviors, as well as decreased cognitive and adaptive functioning.

These results were reemphasized by Mostafa and Al-Ayadhi (2012), who found a higher rate of anti-neuronal antibodies in a cohort of 80 Saudi children with ASD *vs* 80 -GP controls, as tested via immunofluorescence against monkey cerebellum (62.5 *vs* 5%); the presence of such antibodies was higher in severe diagnoses (87.5%) than mild/moderate diagnoses (25%) as well as in females (90%) compared with males (53.3%). A later study found autoimmunoreactivity to neuronal progenitor cells (NPCs) to be far more common in children with autism than controls, and among positive samples, those with ASD immunostained more strongly than controls (Mazur-Kolecka *et al*, 2014).

An examination of anti-brain autoantibodies in a cohort of 355 Italian children with autism and their mothers and unaffected siblings found that autoantibodies in autistic individuals correlated with increased severity, stereotypies, and cognitive impairment (Piras *et al*, 2014). The authors showed that 45 and 62 kDa antibodies in the children correlated with severity of autism, IQ, and impaired motor function and social interaction, as scored on the Vineland Adaptive Behavior Scales. Of particular interest, the authors also examined the 37 and 73 kDa maternal anti-brain autoantibodies mentioned previously, showing that while correlations between the two discrete autoantibody populations do exist, maternal- and child-borne autoantibodies each retain their own distinct and defining characteristics.

Immunogenetic Factors

Numerous studies have shown a connection between the members of the expansive family of HLA alleles and incidence of autism (Torres *et al*, 2012), in particular the class II DRB1. The hypervariable region 3 of DRB1*0401 has been associated with an increased relative risk for autism in children from Utah (Warren *et al*, 1996). DRB1 was also associated with autism in Han Chinese (Chien *et al*, 2012), Egyptian (Mostafa *et al*, 2013), and Saudi populations (Al-Hakbany *et al*, 2014). The DRB1*11 (Mostafa *et al*, 2013) and DRB1*1104 (Al-Hakbany *et al*, 2014) alleles have specifically been noted to occur at a higher rate in individuals with ASD. Multiple studies have suggested HLA-DR4 as another risk factor, appearing at increased rates in children with autism as well as their mothers (Johnson *et al*, 2009; Lee *et al*, 2006). Other positively associated alleles are the class I A*01, A*02, and B*07, while DRB1*03 and some DQB1 alleles have been negatively associated with rates of autism (Al-Hakbany *et al*, 2014; Mostafa *et al*, 2013; Torres *et al*, 2006).

Interestingly, Guerini *et al* (2015) discovered a 14-basepair insertion in HLA-G that was found more often in individuals with ASD and their mothers. HLA-G, along with HLA-E, -F, and perhaps others, is a non-canonical class I MHC gene that behaves very differently from the classical perception of MHC. These genes, in contrast to the exceptionally polymorphic HLA-A, -B, and -C genes, possess few alleles (Carosella *et al*, 2003). HLA-G interacts with natural killer cells as part of the innate immune system and is primarily expressed in placental tissues as part of immune tolerance during pregnancy (Carosella *et al*, 2008). The full meaning of the insertion/deletion is not yet known, but an altered efficacy and faulty tolerance toward the fetus by maternal NK cells during pregnancy, thus compromising the privileged status of the fetus, could provide a mechanism for atypical development.

The *MET* oncogene, which encodes a receptor tyrosine kinase known as C-Met or hepatocyte growth factor receptor (HGFR), is typically associated with cancer metastasis. However, this pleiotropic receptor is also critical for neuronal migration during development of the cerebellum and cerebral cortex, and functions as a negative immune regulator on antigen presenting cells. Linkage studies by Campbell *et al* (2006), have found an association between ASD and a single SNP (rs1858830) in the promoter of *MET*. Recent estimates at this site suggest that the CC and CG alleles represent a 1.76 and 1.59 relative risk, respectively, over the GG allele (Campbell *et al*, 2008), with an odds ratio of 1.64 for the C variant (Jackson *et al*, 2009). This *MET* variant also appears connected to both an increase in 37/73 kDa maternal autoantibodies and a decrease in maternal IL-10, suggesting another mechanism through which *MET* may associate with ASD as a susceptibility factor (Heuer *et al*, 2011).

More recently, researchers have utilized the advanced techniques of next-generation sequencing and genome-wide association studies (GWAS) in an attempt to divine the genetic causes of autism, generally finding a complex and varied genomic landscape (Abrahams and Geschwind, 2008; Gaugler *et al*, 2014; Michel *et al*, 2012). Voineagu, *et al*, examined gene expression in ASD and control brains, and as expected found hundreds of genes that were differentially expressed (Voineagu *et al*, 2011). In the control brains, dozens of genes exhibited different expression levels between the temporal and frontal lobes of the brain; these differences were not mirrored in the ASD samples. The genes significantly upregulated in ASD were heavily enriched for immune and inflammatory response functions, immunoglobulin domains, and other immune-regulatory ontologies.

The authors also defined a module of genes upregulated in ASD that was enriched for astrocytes and activated microglia. They describe a model whereby a significantly altered neuroimmune milieu persists in constant dysregulation. As a complex network of altered expression, the authors suggest that these differences are unlikely to be directly pathological in their own right; rather, the evidence suggests broad immunomodulation as symptomatic of a larger cellular dysregulation (discussed later in more detail).

Several other studies searching for genetic factors of autism and ASD have also found strong connections to the immune system. A 2008 study explored transcriptomic changes in the temporal cortex of children and adults with autism, finding 152 transcripts with varied expression, 130 of which were upregulated (Garbett *et al*, 2008). When grouped by ontology, the authors found 31 sets of genes that were differentially expressed between autistic and control samples; of these, 19 were connected to the immune system. These immune-relevant gene sets fell into six classes: antigen-specific immune response, inflammation, cell death, autoimmune diseases, migration, and the natural killer T cell pathway. Many of these genes showed increased variability in the autism cohort, which suggests a general dysregulation of the autistic immune system and overall heterogeneous activation response. Another 2008 study identified 11 genes that were differentially expressed in children with autism, as well as within the subsets of early onset and developmental regression, as compared with the general population (overexpression in autism samples were confirmed for seven of 11 via qPCR) (Gregg *et al*, 2008). These genes are heavily overrepresented in the ontological KEGG natural killer cytotoxicity pathway, and are highly expressed in NK and CD8 T cells.

Ziats and Rennert (2011) performed an *in silico* analysis using genes from the annotated AutDB portal (Basu *et al*, 2009) in 2011, and constructed a gene interactome network from highly expressed ASD genes that heavily suggests the immune system has a central role in ASD, specifically via cytokine signaling, as well as in schizophrenia. The authors suggest that immune system genes may function as a fundamental convergence for autistic disorders. A 2014 RNA-seq study built upon this concept with a gene ontology analysis showing that the autism transcriptome was heavily enriched for immune response genes (Gupta *et al*, 2014).

When Lintas *et al* (2012) reviewed several genome-wide expression studies in 2012 (including the two just mentioned), they found a strong connection to the immune system; of the 464 genes identified, six out of the top 10 KEGG ontology pathways were functions of the immune systems. Immune dysregulation was a common trend in genomic studies, particularly in the NK cell and cytokine pathways. A 2014 study by Guerini *et al* (2014) expanded on this trend by looking at the killer-cell immunoglobulin-like receptors (KIRs), the family of receptors responsible for mediating the innate NK cell response. The authors demonstrated that among a cohort of 90 continental Italian children with ASD, rates of activating KIR/HLA complexes (aKIR2DS2) were increased, while inhibitory KIR/HLA complexes (KIR 3DL1/HLABw4-80I) were decreased in the genome relative to controls. Similarly significant differences were seen following genotyping of the mothers, suggesting a broad influence of the genetic background of NK activity, especially given that KIR/HLA complexes are expressed and active in the placenta (Guerini *et al*, 2014; Parham, 2005).

Lintas *et al* (2012) proposed a model whereby the dysregulated immune system leads to increased metabolism and reactive oxygen species (ROS) in both the pre- and postnatal environment. They suggest that such alterations would result in prenatal changes in proliferation, migration, and neuronal wiring, while postnatal mitochondrial dysfunction and ROS stress would lead to abnormal synaptic formation.

Taken together, the genetic forces noted in both children and adults with autism are myriad, but insufficiently explanatory. While there appears to be a strong immunogenetic component to many examples of ASD, there is no one single thread. Most studies to date have focused on specific targets, whether they are HLA alleles, inflammation, or NK pathways, but the larger picture is one of a cascading network of genetic interactions that leads to ongoing, persistent immune imbalance. A deeper comprehension of these networks is critical to our understanding of the significant genetic impacts on a life with ASD.

Immune Dysregulation

Skewed and dysfunctioning cell populations. A significant focus of the postnatal immune imbalance has been on specific immune cell subsets, including helper $CD4^+$ and cytotoxic $CD8^+$ T cells (Garbett *et al*, 2008; Gregg *et al*, 2008), which may behave differently in individuals with autism (Gupta *et al*, 1998). A 1986 study found a reduced number of T cells in individuals with autism, as well as an altered helper to suppressor T-cell ratio (Warren *et al*, 1986), while a supporting 1990 study suggested $CD4^+$ cell levels were decreased in autism (Yonk *et al*, 1990). A more recent report noted a decrease in $CD4^+CD25^{high}$ regulatory T cells in children with autism (Mostafa *et al*, 2010). Further, Enstrom *et al* (2010) showed that cultured monocytes from ASD patients responded differently to TLR stimulation, while Ashwood *et al* (2011b) demonstrated a differential response by PHA-stimulated peripheral blood mononuclear cells (PBMCs), including reduced numbers of CD134- and CD25-activated $CD3^+$, $CD4^+$, and $CD8^+$ T cells. A previously mentioned mouse model also implicated T cell skewing in ASD-relevant behavioral deficits (Ponzio *et al*, 2007), as did an examination of neuroinflammation in brains from individuals with autism (Vargas *et al*, 2005).

Natural killer cells have been frequently implicated in autism from a genetic/genomic standpoint (Lintas *et al*, 2012), and several studies have now examined the broader landscape of NK cell immune dysregulation in ASD. A reduction in NK cell activity was shown in teenage and adult autism patients, with 12 out of 31 patients showing lower activity in PBMCs via K562 chromium-release assays (Warren *et al*, 1987). Autistic patients showed no decrease in overall NK count, a similar result to schizophrenia (DeLisi *et al*, 1983), suggesting that it is the cells themselves that are dysfunctional.

A more recent study of NK cells recapitulates the genetic changes previously alluded to, showing that NK cells in children with autism had higher expression of NK cytotoxicity genes (Enstrom *et al*, 2009b). Among all upregulated genes found in NK cells, annotations were highly enriched for NK activity and immune response and defense, while protein biosynthesis and metabolism were downregulated. The authors also found considerable levels of immune dysregulation, observing significantly more $CD56^+$ NK cells in children with ASD compared with controls. The authors then showed that unstimulated NK cells—specifically the $CD56^{dim}$ subset—stained positive for perforin, granzyme B, and IFN-γ at higher frequencies in ASD patients as compared with general population (GP) controls. Interestingly, upon stimulation, NK cells showed much lower staining to these same targets as compared with GP. In essence, NK cells from individuals with ASD show higher resting but reduced stimulated cytolytic activity as compared with TD controls. NK cells in ASD individuals appear to have a baseline level of activity that is already at peak function, rendering them ill equipped for normal circulation and unable to respond further to stimulation.

A more recent study examined a different subset of natural killer cells, those that are $CD57^+CD3^-$ (Siniscalco *et al*, 2016). These NK cells are functionally different than traditional, $CD56^+$ NK cells—they are terminally differentiated and have higher cytolytic capabilities, and generally act as innate immune regulators despite being less responsive to cytokines and connected to autoimmunity and immunodeficiency (Lopez-Verges *et al*, 2010; Nielsen *et al*, 2013). Siniscalco *et al* (2016) examined 104 children with ASD, and found significantly lower, atypical levels of $CD57^+$ NK cells as compared with both age-matched and adult controls. Just 30% of children with ASD had normal $CD57^+$ NK cell levels, despite typical levels of $CD56^+$ NK cells, suggesting that it is the $CD57^+$ cells that are implicated in autism.

Immunoglobulins. Looking at the humoral immune system, a 2008 study of over 250 children found a reduction of plasma IgG and IgM levels circulating in young children with autism, in particular IgG, the levels of which correlated negatively with scores on the ABC, regardless of ASD status (Heuer *et al*, 2008). A later study with children from the same cohort found increased plasma levels of the weakly active IgG4 subclass among ASD patients (Enstrom *et al*, 2009a), as did an earlier study comparing ASD children to their siblings (Trajkovski *et al*, 2004). These findings are further supported by a recent report of lower IgG1 levels in the serum of boys with ASD, along with a similarly negative correlation to communication as scored by the ADI-R (Zaman *et al*, 2016).

Although there appears to be some discrepancy between studies that show increased (Croonenberghs *et al*, 2002b; Trajkovski *et al*, 2004) and decreased (Enstrom *et al*, 2009a) IgG levels in children with autism, age of the children is most likely a contributing factor, with IgG levels changing significantly and rapidly through the first decade of life (Heuer *et al*, 2008). A 2012 follow-up study ruled out abnormal B-cell function as the source of aberrant IgG levels (Heuer *et al*, 2012). Although the contribution of total IgG levels needs to be more thoroughly examined, the apparent shift in subclass to IgG4 means that children with autism are likely to have a higher percentage of circulating IgG with an inherently lower affinity for the antibody receptors on leukocytes (Enstrom *et al*, 2009a; Zaman *et al*, 2016).

Cytokines, chemokines, and inflammation. Numerous studies have demonstrated a correlation between cytokine levels and ASD status, and while there is a consensus that cytokines and chemokines are part of the altered immune environment noted in autism (Depino, 2013; Enstrom *et al*, 2010), there has been some disagreement on the manifestation of such alterations, specifically whether the pro-inflammatory Th1 or anti-inflammatory Th2 cellular response dominates. A 2006 study showed increases in the anti-inflammatory Th2 cytokines IL-4, IL-5, and IL-13 from PHA-stimulated PBMCs (Molloy *et al*, 2006), which was supported by a later study that indicated a Th2-like response was present at birth and connected increased IL-4 to more severe autism (Krakowiak *et al*, 2015). On the other hand, a 2002 study demonstrated an increase in the blood cytokines IL-1RA and IFN-γ, suggesting a more Th1-like, pro-inflammatory response (Croonenberghs *et al*, 2002a), while a mouse model in 2007 showed Th1-skewed T-cell development in amniotic fluid alongside stereotypies in the offspring following treatment of pregnant dams with IL-2 (Ponzio *et al*, 2007).

The notion of a more pro-inflammatory environment was supported by Li *et al* (2009) who not only found elevated levels of the chemokine IL-8, pro-inflammatory cytokines IL-6, GM-CSF, and TNF-α, and the Th1 cytokine IFN-γ in autistic brain tissue, but also saw no increase in IL-4 or IL-5; as such, the authors claimed an increase in the Th1/Th2 ratio, suggesting that the Th1 arm is activated in autism. In the context of dietary proteins and food hypersensitivity, PBMCs from children with ASD produce higher levels of TNF-α and IFN-γ but not IL-5, further lending credence to a Th1-dominated response (Jyonouchi *et al*, 2005; Jyonouchi *et al*, 2002). Vargas *et al* (2005) likewise showed the presence of an active neuroinflammatory environment, finding higher levels of MCP-1, TGF-β, and IL-6 in the brains of autistic patients. Of particular note is that many of the above studies found no increase in the Th2 cytokine IL-10 (Croonenberghs *et al*, 2002a; Krakowiak *et al*, 2015; Li *et al*, 2009; Molloy *et al*, 2006).

A large 2011 study of children with ASD found an increase in plasma levels of a broad range of cytokines, including the Th1-like IL-12p40 and pro-inflammatory cytokines IL-1β, IL-6, IL-8, and GM-CSF (Ashwood *et al*, 2011a). Examining children from the same cohort, the authors also published on increased levels of the chemokines MCP-1, RANTES, and eotaxin (Ashwood *et al*, 2011c). In both studies, increased cytokine or chemokine levels were correlated with increased aberrant behavior and impaired development (Ashwood *et al*, 2011a, 2011c). A related study by the same team supported these findings using PHA-stimulated PBMCs, and correlated a Th1-like response with greater impairments and behavior aberrancies and a Th2-like response to better cognitive and adaptive function (Ashwood *et al*, 2011b). In contrast to most of the above studies, a 2012 report using dried blood samples suggested that children with ASD had lower levels of both Th1-like and Th2-like cytokines (Abdallah *et al*, 2012b).

Less clear is the role of pro-inflammatory cytokines IL-17 and IL-23. Enstrom *et al* (2008) found decreased plasma intracellular IL-23 in children with ASD, but no such change in IL-17, while Al-Ayadhi and Mostafa (2012) and Akintunde *et al* (2015) found evidence of elevated IL-17 levels among such children in serum and PHA-stimulated PBMCs, respectively. Given that IL-23 induces IL-17 and the importance of IL-17 for inducing the MIA model of autism (Choi *et al*, 2016), it is clear that these cytokines and the role of Th17 cells in autism warrants further study. This is especially so given the outsized role IL-17 has in other autoimmune conditions such as SLE and rheumatoid arthritis (Konya *et al*, 2015).

Although an increased pro-inflammatory environment seems well supported (Haroon *et al*, 2012), there remains a wealth of data to scrutinize and reach agreement on. Further studies should attempt to elucidate the network of interactions leading to systemic immune dysregulation in individuals with autism, in particular the role of IL-17 and the Th1/Th2 balance, with guidance from observations from the MIA model. Given the ability of the immune environment to affect behavior (Filiano *et al*, 2016), parsing out the different pathways, especially over the rapidly changing immune environment in early childhood as well as the heterogeneity that is inherent in ASD, remains both a challenge and an opportunity for the field.

OVERLAP

Anti-brain Antibodies

The presence of both maternal and patient circulating autoantibodies that target the brain raises the specter of a common provenance. The evidence strongly supports distinct roles for both maternal (Braunschweig *et al*, 2008; Croen *et al*, 2008b; Singer *et al*, 2008; Zimmerman *et al*, 2007) and patient (Henneberg *et al*, 1994; Libbey and Fujinami, 2010; Ryberg, 1982) autoantibodies in ASD, including an ability to affect structural brain development (Nordahl *et al*, 2013; Rossi *et al*, 2011). Offending antibodies of either origin appear to persist systemically for extended periods of time (Mostafa and Al-Ayadhi, 2012; Piras *et al*, 2014; Singer *et al*, 2006; Zimmerman *et al*, 2007), and although there exists a correlation between the two there does appear to be at least some differences in effect (Piras *et al*, 2014). Although reports indicate the presence of these antibodies may correlate with adverse conditions in the patients (Braunschweig *et al*, 2012a; Goines *et al*, 2011a; Mostafa and Al-Ayadhi, 2012; Piras *et al*, 2014), the nature of those connections needs to be further investigated, especially as regards different subpopulations of ASD. Doing so could elucidate not only the action but also the cause of these functional autoantibodies, and would allow more accurate models of autoantibody-related autism pathology to be developed.

ASD as an Autoimmune Disorder

The research community has long noticed a correspondence between ASD and autoimmune diseases, with some suggesting autism should itself be considered an autoimmune disorder (Ashwood and Van de Water, 2004). Singh (2009) proposed a theoretical autoimmune mechanism for autism, suggesting that an environmental trigger (eg, a virus) could provoke faulty immune regulation that results in autoimmunity to the brain, leading to the observed neuropathology. Autoantibodies, such as those described above, have been found in patients of other autoimmune disorders, including multiple sclerosis (Ryberg, 1982), schizophrenia (Henneberg *et al*, 1994), and SLE (Libbey and Fujinami, 2010). Evidence for the association has mounted, including a 2015 study that found a more than double risk of ASD in children of mothers with SLE than those of controls (Vinet *et al*, 2015). This is especially significant since the authors note that SLE patients present with SLE-related autoantibodies.

Inflammation and immune signaling dysregulation can strongly influence neuropsychiatric behavior beyond just ASD (Haroon *et al*, 2012; Meyer and Feldon, 2009), with evidence pointing toward roles in bipolar disorder and PTSD (Jones and Thomsen, 2013), and perhaps most emphatically in schizophrenia (Brown *et al*, 2004b; Meyer and Feldon, 2009; Michel *et al*, 2012; Patterson, 2009). Schizophrenia and autism share many immune similarities, including a subset of patients with an etiology based in maternal infection and immune activation (Bauman *et al*, 2014; Boksa, 2010; Brown *et al*, 2004a; Meyer *et al*, 2005, 2007; Patterson, 2009; Urakubo *et al*, 2001). Further lending credence to this association are numerous studies showing that a significant risk factor for autism is a family history of autoimmune disease (Brimberg *et al*, 2013; Gesundheit *et al*, 2013; Gottfried *et al*, 2015; Wu *et al*, 2015), alongside direct genetic evidence that links ASD and schizophrenia (Cantor and Geschwind, 2008; Ellis *et al*, 2016; Zhou *et al*, 2016). Despite the differences between these two disorders, the similarities between them are such that results from animal models like the MIA model may be overlapping and confounded (Bauman *et al*, 2014; Young *et al*, 2016).

FUTURE STEPS

The field investigating the immunobiology of autism has grown rapidly in the past decade and is rife with opportunity. In the prenatal environment, a strong line of evidence is beginning to develop in support of maternal immune dysregulation as it affects neurodevelopment based on studies on maternal infection, immune activation, and maternal autoantibodies directed toward the developing fetal brain. Pro-inflammatory cytokines such as IL-6 and IL-17 have been implicated in this process, but the mechanisms remain under investigation. The successful and increasingly prolific MIA model provides an important tool in understanding the pathways involved in maternal immune system-influenced neurodevelopmental disorders.

The postnatal environment echoes those lines of development, with anti-brain antibodies and systemic inflammation providing evidence of anomalies in immune regulation in a subpopulation of children with ASD. Data from various studies regarding immune dysregulation in ASD are beginning to coalesce, but given the heterogeneity of ASD phenotypes, it remains difficult to explain different behavioral outcomes based solely on immune phenotype, and this may contribute to spurious conclusions. However, as more studies in this research area look at both the biological and behavioral phenotype as well as medical co-morbidities such as gastrointestinal symptoms in the same individual, the relationship between immune dysregulation and ASD will likely become clearer. Also exciting and potentially relevant is the recent groundbreaking work by Louveau *et al* (2015) showing active lymphatic vessels connecting the CNS to the lymphatic system that may possibly act as a conduit for neuroimmunological dysfunction. Further work should be undertaken to understand how this newly revealed lymphatic system in the CNS could function as a pipeline for immunological molecules relevant for the development of ASD.

The application of massive GWAS panels has also greatly informed our understanding of the genetic composition of ASD, and in doing so hundreds of potential avenues of research have opened up, further diversifying patient populations. Regulators of the immune system have long been featured prominently among the genetic components of

autism and are likely to continue to do so as we learn more about the myriad interactions taking place between the immune system and the brain. The intersection of genetic underpinnings and neuroimmune dysregulation may provide a door through which both clinical testing and directed therapy may emerge.

FUNDING AND DISCLOSURE

This study was funded by the NIEHS Center for Children's Environmental Health and Environmental Protection Agency (EPA) grants (2P01ES011269-11, 83543201 respectively), the NIEHS-funded CHARGE study (R01ES015359), and the NICHD funded IDDRC 054 (U54HD079125). There are three patents associated with the work at UCD noted in this review: A provisional 'Maternal Diagnostic Marker for Autism Risk and Therapeutic Intervention' UC Case No. 2014-467-1. There are 2 issued patents: The US patent Methods of Diagnosing and Treating Autism, US Patent No. 8,383,360 B2 or Pat. 8,383,360 was filed on 12 August 2010 and issued on 26 February 2013. The client reference number for this patent is 2009-737-2US. This patent is currently in the process of National filing. The first issued patent is Diagnostic Methods for Detection of Autism Spectrum Disorder, US Patent No. US 7,452,681 B2. This patent was filed on 5 May 2006, and was issued 18 November 2008. This patent was filed in the US only. Dr Van de Water received consulting fees from Pediatric Bioscience from 1 Jan 2015 until October 2015. Amory Meltzer reports no biomedical financial interests or potential conflicts of interest.

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