

Full Length Research Paper

Impact of environmental factors on the prevalence of autistic disorder after 1979

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The aim of this study was to investigate a previously overlooked, universally introduced environmental factor, fetal and retroviral contaminants in childhood vaccines, absent prior to change points (CPs) in autistic disorder (AD) prevalence with subsequent dose-effect evidence and known pathologic mechanisms of action. Worldwide population based cohort study was used for the design of this study. The United States, Western Australia, United Kingdom and Denmark settings were used. All live born infants who later developed autistic disorder delivered after 1 January 1970, whose redacted vaccination and autistic disorder diagnosis information is publicly available in databases maintained by the US Federal Government, Western Australia, UK, and Denmark. The live births, grouped by father's age, were from the US and Australia. The children vaccinated with MMRII, Varicella and Hepatitis A vaccines varied from 19 to 35 months of age at the time of vaccination. Autistic disorder birth year change points were identified as 1980.9, 1988.4 and 1996 for the US, 1987 for UK, 1990.4 for Western Australia, and 1987.5 for Denmark. Change points in these countries corresponded to introduction of or increased doses of human fetal cell line-manufactured vaccines, while no relationship was found between paternal age or Diagnostic and Statistical Manual (DSM) revisions and autistic disorder diagnosis. Further, linear regression revealed that Varicella and Hepatitis A immunization coverage was significantly correlated to autistic disorder cases. R software was used to calculate change points. Autistic disorder change points years are coincident with introduction of vaccines manufactured using human fetal cell lines, containing fetal and retroviral contaminants, into childhood vaccine regimens. This pattern was repeated in the US, UK, Western Australia and Denmark. Thus, rising autistic disorder prevalence is directly related to vaccines manufactured utilizing human fetal cells. Increased paternal age and DSM revisions were not related to rising autistic disorder prevalence.

Key words: Autism disorder, change point, vaccine, paternal age.

INTRODUCTION

Autistic disorder (AD) is a subset of the Autism Spectrum Disorders (ASDs), a group of developmental disabilities that have reached epidemic levels. Worldwide, 1988 has

been identified by the Environmental Protection Agency (EPA) as a critical incident year for AD (McDonald 2010). The CDC released a study in 2013 estimating US ASD

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prevalence at 1 in 50 children aged 6 to 17 in 2011 to 2012. In addition to ASD, there are also apparent epidemic levels of other early onset neuro-developmental (ND) syndromes such as childhood onset schizophrenia (0.4% of population affected) (Okkels et al., 2012) and bipolar disorder (Leibenluft 2008). Shared characteristics among childhood ND epidemics include associations with male gender, reduced reproductive fitness, increased paternal age and the presence of excess de novo genomic mutation rates. Paternal age is currently a favored explanation for the worldwide autism epidemic. However, evolving concepts about autism spectrum and other ND diseases suggest these diseases to be “multi-hit” with genetic, genomic and environmental contributors. Accumulating evidence from family-based exome sequencing points to the importance of hundreds of rare, diverse, de novo mutations (DNMs) in childhood ND diseases (Van Den Bossche et al., 2012; Robinson 2010; Awadalla et al., 2010; O’Roak et al., 2011; De Ligt et al., 2012; Girard et al., 2011; Xu et al., 2012).

The de novo mutations in these diseases are consistently found in exons or critical coding regions of genes that would lead to premature stop/non-functional proteins (Awadalla et al., 2010; O’Roak et al., 2011; De Ligt et al., 2012; Luo et al., 2012). In addition to the increase in DNMs in children with ND disease, de novo genomic insertions and deletions are significantly increased in intellectual disability (De Ligt et al., 2012), autistic disorder (O’Roak et al., 2011), and childhood onset schizophrenia (Xu et al., 2012). Diverse, rare DNMs mandate that environmental factors known to cause genomic instability be evaluated for their relationship to these diseases.

Consideration of potential environmental triggers requires statistical assessment to identify birth year change points (CPs) associated with a rising rate in the incidence of autism. Requirements for an environmental factor as a trigger for disease include: (1) absent or lower levels before a change point, (2) continued increase after a change point is demonstrated (dose-effect), (3) biological mechanism consistent with pathology, and (4) in instances of non-geographically limited disease such as autism, schizophrenia and intellectual disability, it should have almost universal exposure (McDonald 2010). This study investigates a previously overlooked, universally introduced environmental factor, fetal and retroviral contaminants in childhood vaccines, absent prior to change points in autistic disorder prevalence with subsequent dose-effect evidence and known pathologic mechanisms of action. Vaccinations have done tremendous good in the world; however, further investigation of fetal manufactured-vaccine contaminants as an environmental contributor to the current autistic disorder epidemic is called for.

METHODOLOGY

Previously published autistic disorder data obtained from large

populations and having a time span adequate for change point analyses were used. For the Diagnostic and Statistical Manual (DSM) revisions, change points are predicted based on the year of FDA approval of the vaccine and the month or year of publication of the DSM revision, respectively, and compared to the statistically calculated autistic disorder change points.

Data sources

Broadening changes in diagnostic criteria for ASD complicate interpretation of the current epidemic. Therefore, we focused on autistic disorder (previously called infantile autism), the most severe form of ASD, which has relatively constant diagnostic criteria over the past 5 decades, despite nomenclature changes from childhood schizophrenia to infantile autism to autistic disorder (McDonald 2010). To objectively assess suspected diagnostic relaxation for autistic disorder, printing dates were obtained for the DSM editions, found on the copyright page. Printing dates indicate the rapidity with which changes in diagnostic criterion were disseminated to the professional community. To determine whether DSM revisions were related to autistic disorder, we predicted a range of expected autistic disorder change point birth years based on the printing dates for the various DSM revisions. If DSM revisions cause an autistic disorder change point, children born prior to the new edition would be affected. Expected change point ranges are predicted to be 8 years prior to the earliest printing date and 3 years prior to the latest printing date for each revision based on first diagnosis of AD occurring after 3 years of age and firm diagnosis by 8 years of age (Lord et al., 2006; Luyster et al., 2009).

For the US, autistic disorder data were obtained from the California Department of Developmental Services (DDS) (McDonald 2010; Cavagnaro 2003; Schechter and Grether 2008) and from the Individuals with Disabilities Education Act (IDEA) program website of the Department of Education (IDEA 2012). Live birth data were extracted from the CDC’s “Annual reports of Vital Statistics of the United States”, (Centers of Disease Control and Prevention 2012a; Centers of Disease Control and Prevention 2012b) and birth year autistic disorder prevalence per 10,000 was then calculated. Male population data were obtained from the U.S. Census Bureau website, (US Census Bureau 2012a) for data prior to 2000 and from the “fact finder” web site for data after 2000 (US Census Bureau 2012b). Birth rates by age of father were obtained from the National Vital Statistics Reports: “Birth Final Data” (Centers of Disease Control and Prevention 2012). Varicella and Hepatitis A immunization coverage for children 19 to 35 months of age was obtained from the CDC National Immunization Survey (NIS) (Centers of Disease Control and Prevention 2012).

For Western Australia, autistic disorder prevalence for children aged 2 to 3, 4 to 5 and 6 to 8 years was obtained from (Nassar et al. 2009). Live births, live births by paternal age cohort, and male population data were obtained from the Australian Bureau of Statistics (Australian Bureau of Statistics 2013). Childhood autistic disorder data for North East London and Denmark were from (Lingam et al., 2002; Lauritsen et al. 2004), respectively.

Linear regression and change point analysis

Linear regression and R^2 analyses were used to assess correlations between autistic disorder prevalence and vaccine coverage or births by paternal age; associations with $P < 0.05$ were considered significant.

For change point determination, both the hockey-stick (Qian 2010) and segmented line fitting (Muggeo 2008) methods were employed. The robustness of our algorithm was tested by repeating the algorithm using deliberately chosen poor initial inputs. Our fit results were robust across a wide variation of input parameters (data

not shown).

The Akaike Information Criterion (AIC) (Sakamoto et al., 1986) and the Bayesian Information Criterion (BIC) (Tiwari et al., 2005) determined the optimal segmented line fits and associated change points. The R statistical software was used to run the 'segmented' and AIC algorithms. For the data presented, all possible pairs of input change point years were tested. All other input parameters were set to default values. Not all pairs of input years led to convergence; what are presented here are results from fits that converged and had the lowest AIC and BICs cores.

Cell substrate residuals in selected childhood vaccines

Residual human DNA (single and double stranded) levels from the human fetal cell lines used to manufacture Meruvax® (Rubella, Merck & Co. Inc.), the rubella component of MMRII®, and HAVRIX® (Hepatitis A, GSK Biologicals) were measured using commercially available ELISA kits (Pico Green (dsDNA) and OliGreen (ssDNA)) (Life Technologies). DNA fragment sizes were determined using SYBR gold staining after 4% agarose gel electrophoresis. Notably, the viruses in the Meruvax®, MMRII® and HAVRIX® vaccines are mRNA viruses, not DNA viruses, and since the mRNA was degraded by heat treatment prior to oligonucleotide measurements, the DNA results are indeed specific for human DNA, the only DNA in the mRNA virus vaccines (Oker-Blim et al., 1984; Wikipedia 2014a; b; c).

RESULTS

Autistic disorder change point analysis

Segmented line fitting analysis identified three change points from the US IDEA and CA DDS AD data for birth years 1970 to 2002; 1980.8 (Figure 1A; panels A and B), 1988.4 (panel B), and 1995.6; 1996.5 (panels C and D). Since hockey-stick analysis of IDEAAD data for 19-year-olds born during 1973 to 1987 identified an autistic disorder change point at birth year 1980.8 (Figure 1A; panel A) which had not been published by the EPA (McDonald 2010), hockey-stick was compared to segmented line fit for California DDS data which had been used in the EPA publication for birth years 1970 to 1997 (Figure 1B). Based on the AIC and BIC, the segmented algorithm with 2 change points (1980.9 and 1988.4) resulted in a better fit of the data than the hockey-stick method used by the EPA, which identified a single change point at birth year 1987.5. When directly compared, our software program analysis to the EPAs, use of the hockey-stick method yielded a change point for the CA DDS data for birth years 1970 to 1997 equivalent to the EPA's published change point to the nearest tenth (Figure 1B).

The graph in Panel E depicts change points when all autistic disorder data from US IDEA and CA DDS for children born between 1973 and 2002 used in panels A through D are combined and submitted to segmented line fitting algorithms. Using the combined data, three change points are calculated (1980.8, 1988.4 and 1996.5) demonstrating the robustness of segmented line fitting for change point analysis. Panel F shows segmented line fit

results for North East London (UK) for birth years 1979 to 1995 (core AD, CP: 1987). Panels G and H show results for Western Australia for birth years 1983 to 1999 (CP: 1990.4) and Denmark for birth years 1964 to 1995 (CP: 1987.5).

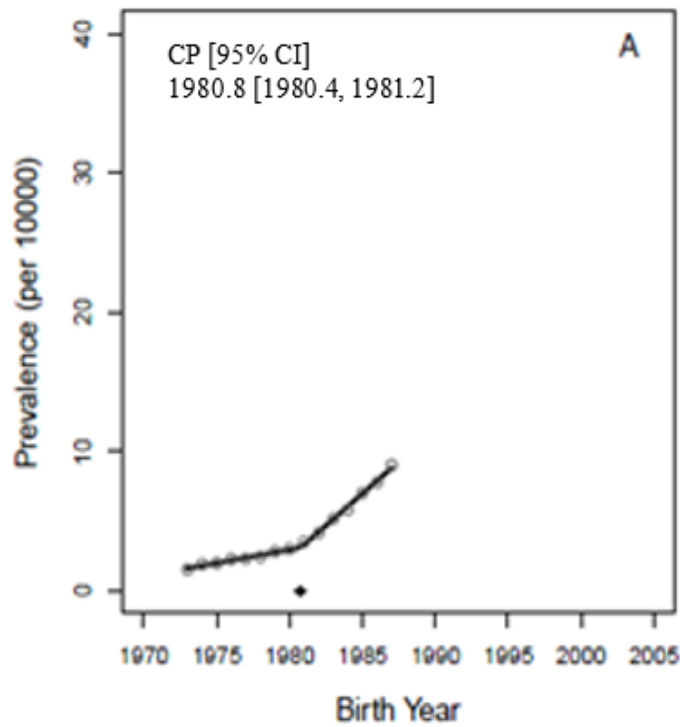
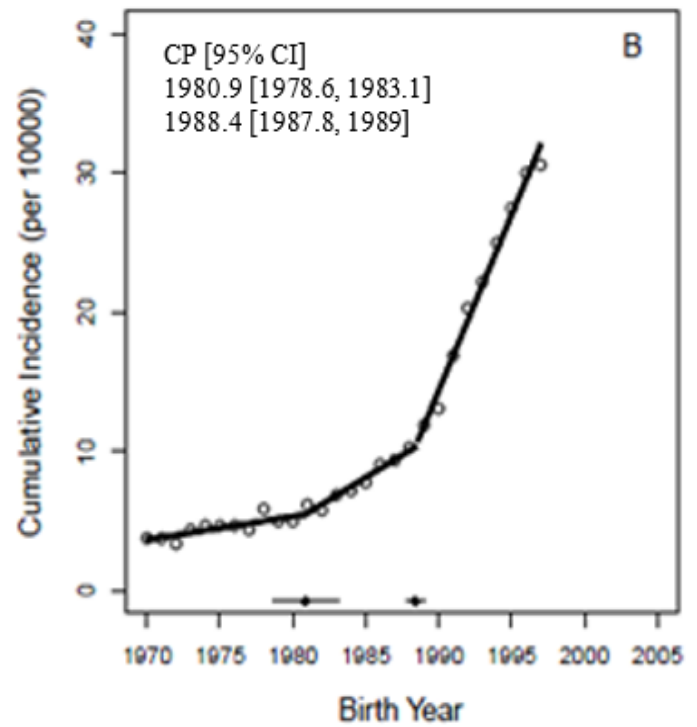
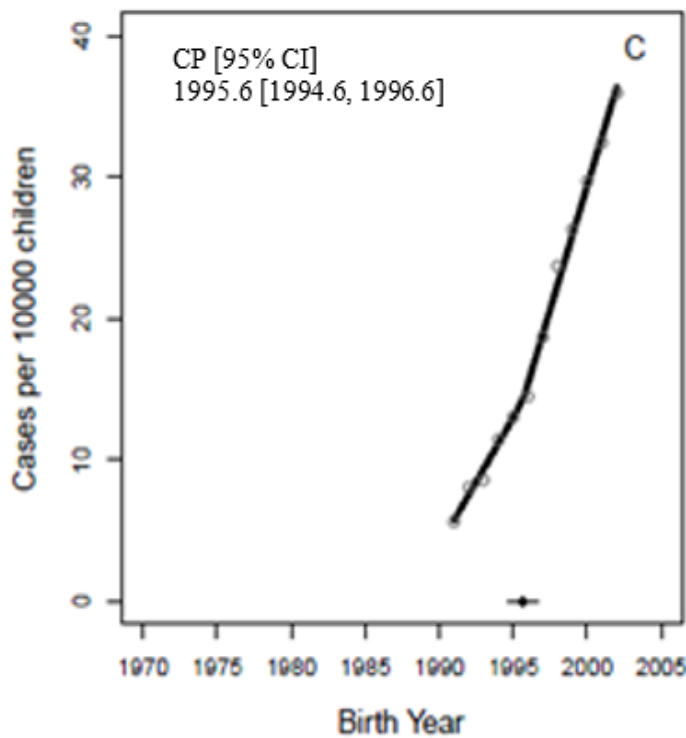
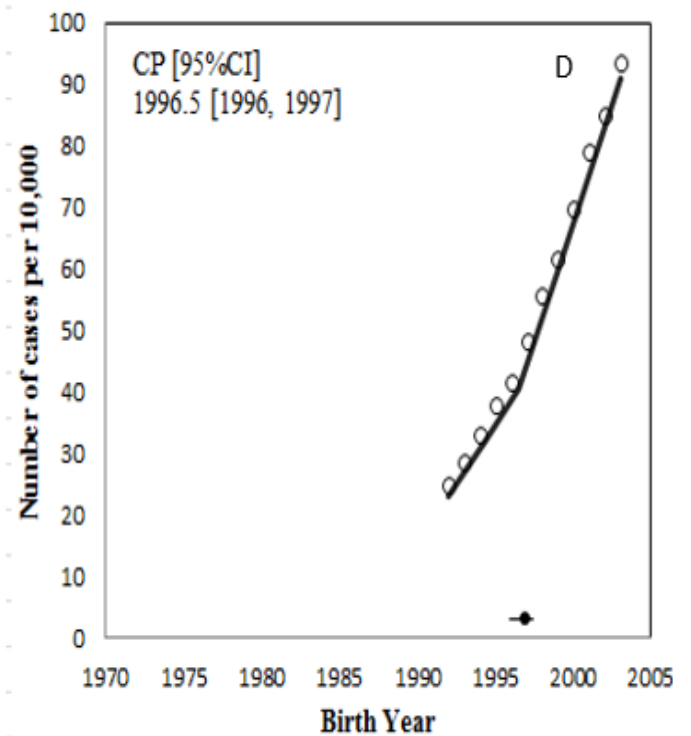
Diagnostic and statistical manual (DSM)

The first DSM of Mental Disorders, *DSM I*, was published by the American Psychiatric Association in 1952. Since then there have been five major revisions: *DSM II* (1968); *DSM III* (1980); *DSM III – R* (1987); *DSM IV* (1994) and *DSM IV – TR* (2000). The impact of DSM revisions on the diagnosis of autism depends on the significance of changes to diagnostic criteria and on the rapidity with which the DSM revisions are disseminated and applied. Table 1 compares diagnostic criteria for autistic disorder, but not the broader autism spectrum disorder, across DSM revisions. As the table demonstrates, DSM revisions differ primarily in that more examples of behaviors typical of autistic disorder were listed with each revision. However, the required number of behaviors for an autism diagnosis remains the same or actually increases with the revisions, rather than becoming less stringent as has been commonly suggested. Furthermore, if relaxed diagnosis were to lead to an increase in autistic disorder prevalence then one would expect a decrease in the number of symptom categories required for diagnosis, however, these symptom categories are consistent across DSM revisions.

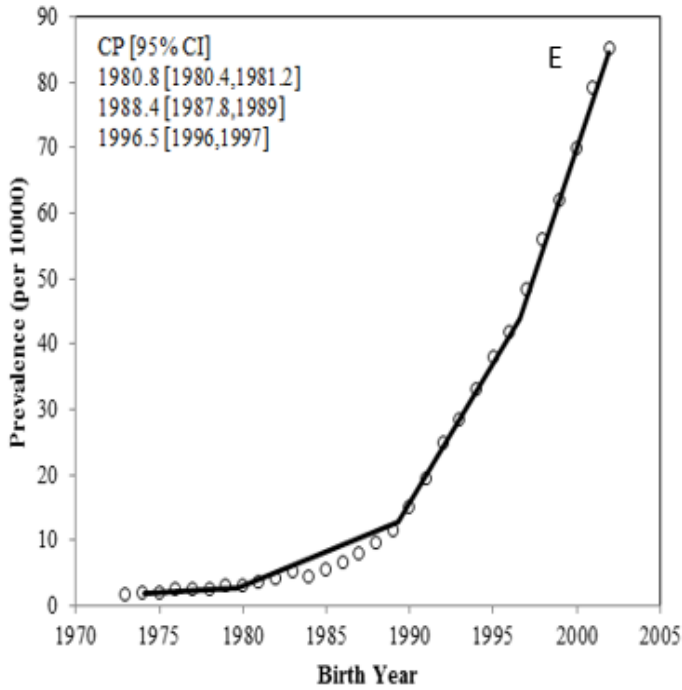
The DSM printing record (Table 2) suggests that the dissemination and application of the DSM revisions is quite rapid after the date of DSM publication, and therefore, the printing dates for DSM were used to predict expected birth year change points to determine whether DSM revisions affect autistic disorder diagnosis rates. Predicted expected birth year change point ranges are found in Table 2. Change point ranges are predicted to be 8 years prior to the earliest printing date and 3 years prior to the latest printing date for each revision based on first diagnosis of autistic disorder occurring after age 3 and firm diagnosis by age 8 (Lord et al., 2006; Luyster et al., 2009). Assuming that the DSMs are strictly followed, the latest predicted birth year change points as a result of DSM changes are 1978, 1984, and 1992 for DSM-III, IIIR, and IV, respectively. There is no corresponding calculated autistic disorder change points associated with those years (Table 2), therefore DSM revisions are unlikely to be the primary trigger for increased autistic disorder prevalence.

Association between paternal age and autistic disorder

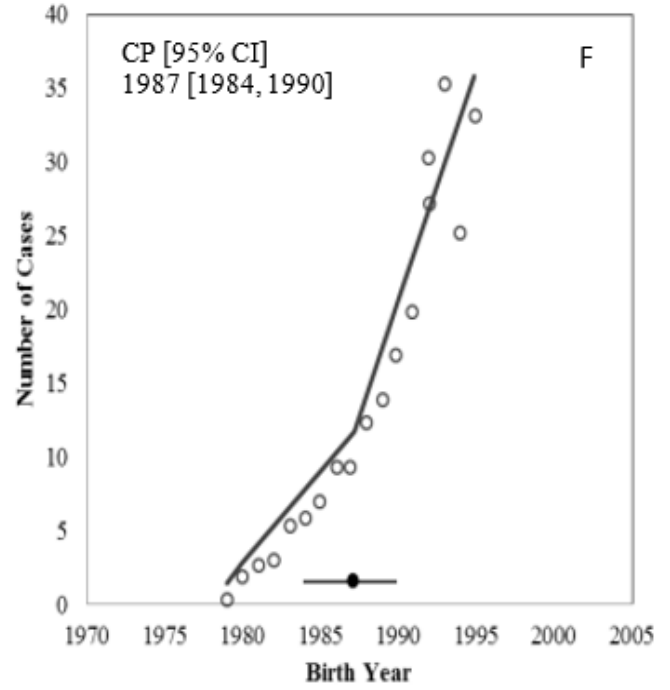
Figure 2A shows that US live births declined during the 1960s and 1970s in almost all paternal age groups, and then rebounded after 1978 in all paternal age groups above

US Autism, 1973-87, 19 yr olds**California AD, 1970-1997, 5 yr olds****California AD, 1991-2002, 4 yr olds****US, 1992-2003, 8 yr olds**

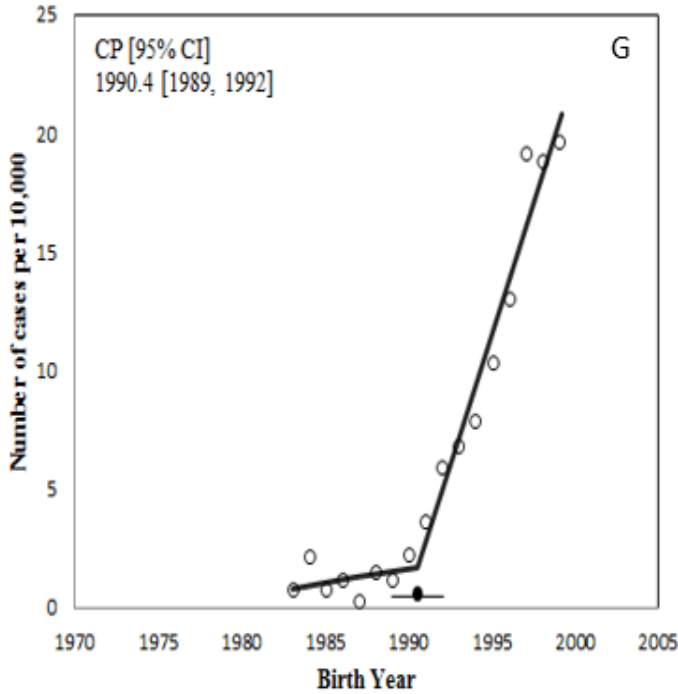
US Autism, 1973-2002, 8 and 19 yr olds



UK, Childhood Autism, 1979-1995, <=10 yr olds



Western Australia, 1983-1999, 2-3 yr olds



Denmark, Autism Disorders, 1964-1995, <10yr olds

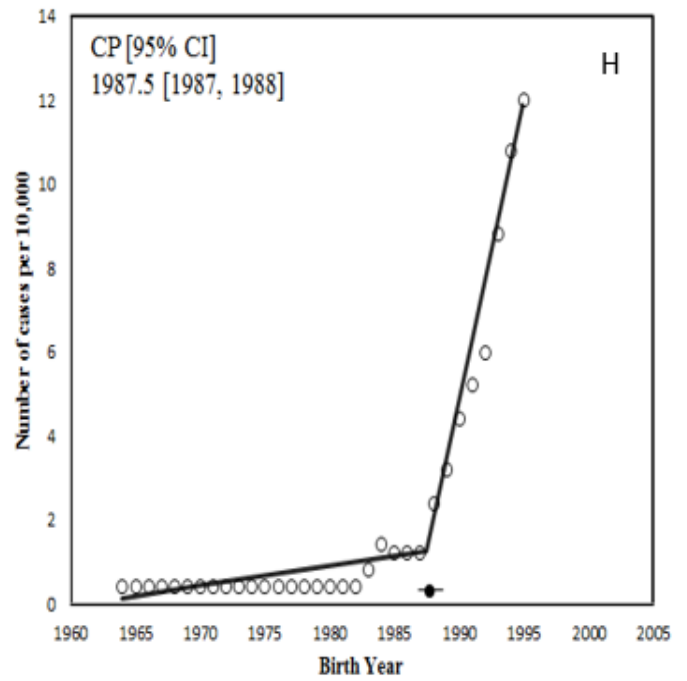


Figure 1. AD changepoint analysis robustness and results.

Figure 1A shows AD changepoint results for the U.S., California, UK, Western Australia, and Denmark. Figure 1B shows a comparison of 'hockey' and 'segmented' fits for California AD 1970-1997 data. Both analyses yield changepoints with overlapping confidence intervals near 1988. However, 'segmented' analysis reveals a second changepoint near 1981.

Table 1. Comparison of diagnostic criteria for autistic disorder (AD) across DSM revisions.

Autistic disorder symptom category	DSM II (1968) Schizophrenia, childhood type	DSM III (1980) Infantile autism	DSM III R (1987) Autistic disorder	DSM IV (1994) and DSM IV R (2000) Autistic disorder
Number of symptom examples listed/Number of examples required for diagnosis				
Impaired social interaction e.g. pervasive lack of responsiveness to other people	3 examples/3 requirement not listed	1 example/1 required	5 examples/2 required	4 examples/2 required
Impaired communication e.g. marked abnormalities in the production of speech, including volume, pitch, stress, rate, rhythm, and intonation; stereotyped and repetitive use of language or idiosyncratic language.	1 example/1 required	4 examples/4 requirement not listed	6 examples/1 required	4 examples/1 required
Atypical or withdrawn behavior e.g. stereotyped body movements (for example, hand flicking or twisting, spinning, head-banging, complex whole-body movements)	1 example/1 required	2 examples/2 requirement not listed	5 examples/1 required	4 examples/1 required
Age of onset	Before puberty	Before 30 months	Before 36 months unless specified	Before 36 months
Alternative diagnosis that must be excluded	Schizophrenia symptoms	None listed	None listed	Rett's disorder* or childhood disintegrative disorder

groups above the age of 30. Of note, fathers over the age of 40 had similar numbers of live births in 1963 (333,785) as they did in 2001 (342,030); therefore, if paternal age were a major trigger for autistic disorder, older fathers would have been fathering as many autistic children in 1963 as 2008. However, reported autistic disorder prevalence was 0.7 cases per 10,000 in 1963 (Treffert 1970) compared to 79 per 10,000 in 2001. In addition, linear regression analysis of paternal age versus autistic disorder for each specific birth year did not reveal a relationship (Figure 2B; $R^2 = 0.1027$).

In Western Australia, from 1975 to 2011, live births increased slightly for fathers over 40 years of age (Figure 2C). However, live births in 1999 to fathers over age 40 were less than 2-fold higher

than in 1989, while autistic disorder diagnosis had risen 10-fold between birth years 1989 and 1999. Linear regression analysis revealed no relationship between paternal age and autistic disorder diagnosis for Western Australia (Figure 2D; $R^2 < 1$).

Association between approval of human fetal cell line manufactured vaccines and autistic disorder change points

Published AD data for the UK (Taylor et al., 1999) and North East London (Lingam et al., 2002) suggested that autistic disorder rose conspicuously around 1988 to 1989, and our calculated change point for the North East London data is 1987. While MMR coverage was over 90% before this

time (Lingam et al., 2002), the autistic disorder change point followed a switch in the UK from animal cell line to human fetal cell line manufacture of MMR vaccine in October 1988 (Table 3). Similarly, our calculated change point result of 1987.5 for Denmark corresponds to the introduction of MMR vaccine in 1987. The relationship between autistic disorder prevalence and use of vaccines manufactured were therefore evaluated using human fetal cell lines elsewhere.

The US 1980 to 1981 autistic disorder change point followed the January 1979 approval of MeruvaxII® and MMRII®, which are manufactured in the human fetal cell line WI-38. An earlier human fetal cell vaccine, Diplovax, had been licensed in the US in 1972. However, it was withdrawn in 1976 because it never gained any US

Table 2. Printing schedules for DSM revisions/editions.

Parameter	Date of printing	Number printed	Predicted BYr CP range by DSM printings	Calculated BYr CP
DSM III	February-80	40,000	February 1972 - September 1978	1980.85*
	May-80	25,000		
	September-80	25,000		
	November-80	30,000		
	January-81	30,000		
	March-81	35,000		
	September-81	25,000		
DSM IIIR	May-87	75,000	May 1979 - November 1984	1988.4
	June-87	80,000		
	November-87	75,000		
DSM IV	May-94	Not given	May 1986 - January 1992	1995.6
	July-94	Not given		
	August-94	Not given		
	January-95	Not given		

market share, and therefore, the introduction of MeruvaxII® and MMRII® would be the first fetal cell vaccines to impact the US, and correspond to the 1980 to 1981 autistic disorder change point. The US 1988.4 change point corresponded to the addition of a second dose of MMRII® to a measles vaccination campaign that increased compliance from ≤ 50 to 82% between birth years 1987 and 1989 (Centers for Disease Control 1989; Kaye and Jick 2001) as well as to the introduction of Poliovax in 1987. The 1995.6 autistic disorder change point corresponded to the approval and introduction of the Varicella vaccine (Varivax®). The Western Australia 1990 autistic disorder change point came shortly after the 1989 addition of MMR vaccine to the vaccination schedule, supplied solely with MMRII® (Table 3).

Association between autistic disorder and fetal cell manufactured vaccination coverage

US autistic disorder prevalence began rising after birth year 1978 (Newschaffer and Gurney 2005), and has continued to rise through birth year 2008. Figure 3A illustrates the continuing rise in US autistic disorder for 8 year olds born between 1992 and 2003. IDEA data for 3 year olds (not shown) through birth year 2008 demonstrates a continuing rise in US autistic disorder after 2003. Figure 3B illustrates that varicella coverage increased steadily after its approval in 1995 for children whose birth years were 1993 through 1998 to 1999, leveling off after reaching just over 80% saturation.

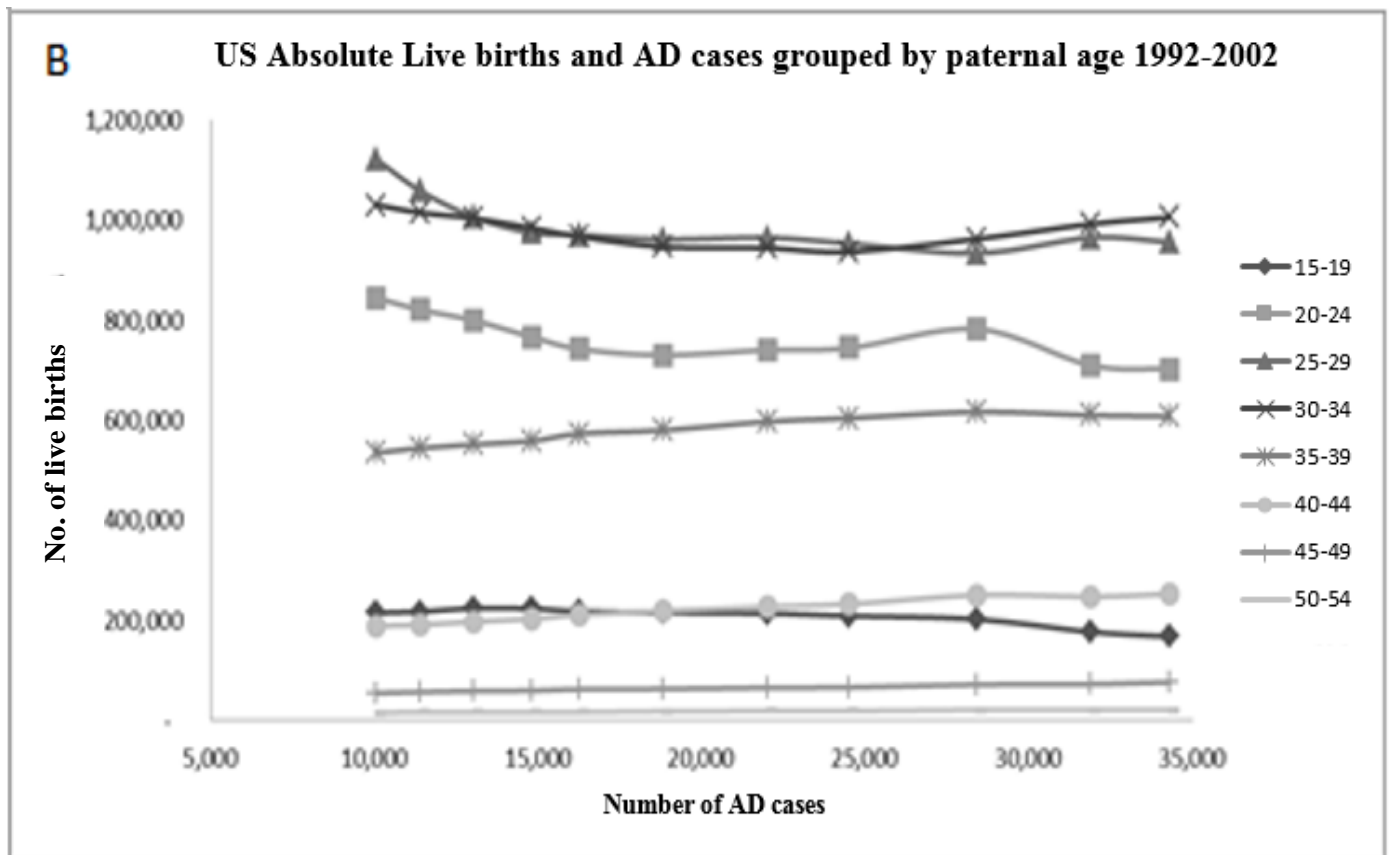
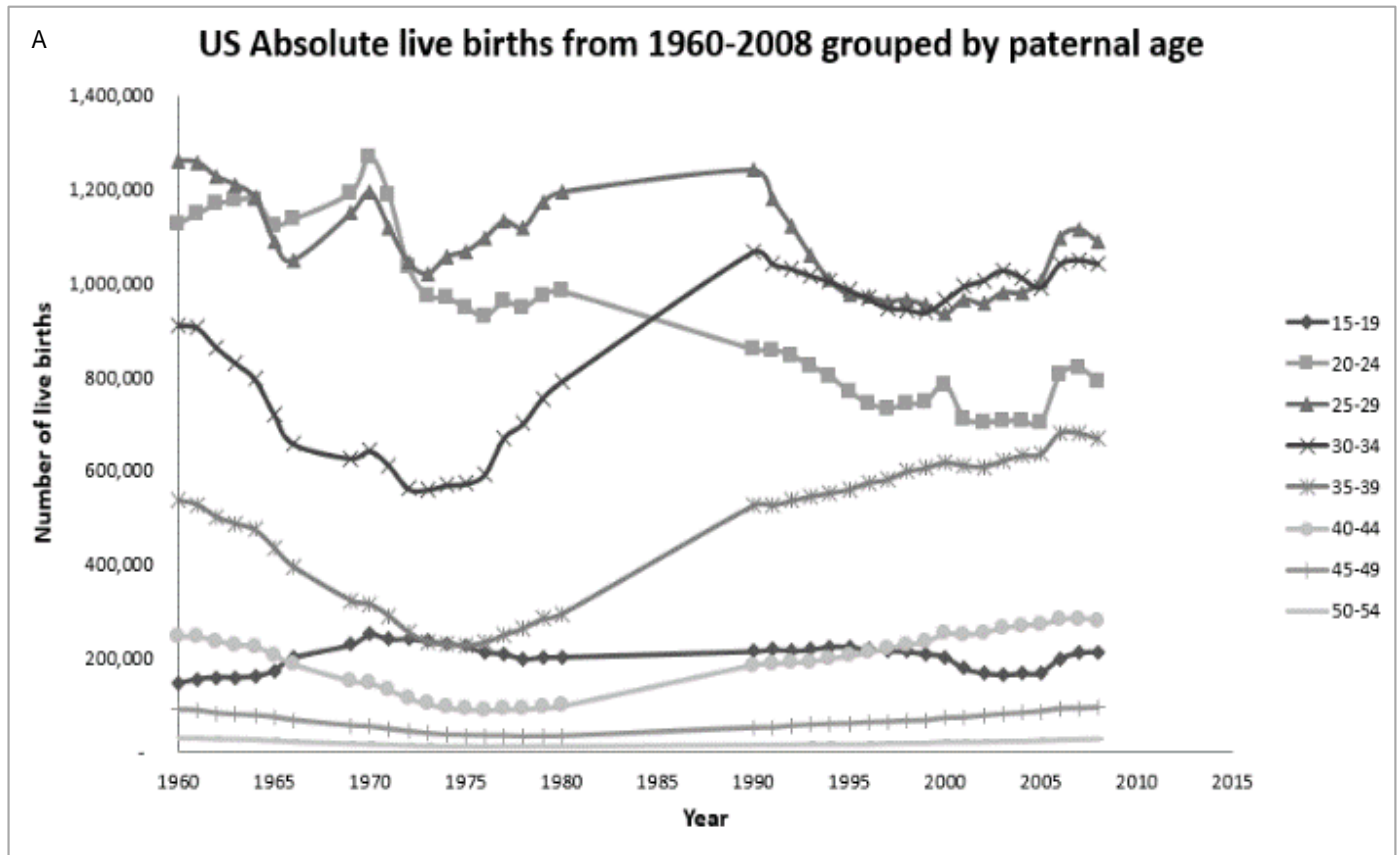
Hepatitis Avaccine (Havrix®) was approved for use in the US in 1995; however, it was neither part of the childhood immunization schedule nor recommended for use by any states. In 1999, 17 states began recommending/considering its use for children 24 months and older, and in 2005 it was included in the ACIP recommended

vaccination schedule for children 12 months and older (Table 3). Hepatitis A coverage (Figure 3D) shows a more complicated compliance due to the non-uniform state recommendations from 1999 through 2005. Based on approval dates and recommendation dates, Hepatitis A use could have affected autistic disorder rates for children born in 1997 or later, however, there is not public data tracking vaccination rates prior to 2006. Extrapolating from age of immunization to birth years, Hepatitis A immunization coverage has increased steadily for birth year 2003 through birth year 2008 (Figure 3D).

To compare absolute numbers of children diagnosed with autistic disorder to the absolute numbers of children vaccinated with Varivax®, we performed linear regression analysis for birth years 1992 to 1998, during which time Varivax® coverage increased linearly. Additionally, birth years 1992 to 1998 were chosen because state variation in use of Hepatitis A vaccine after 1999 confounds the use of Varivax® as a measure of exposure to vaccines manufactured in human fetal cell lines for birth years subsequent to 1998. Figure 3C illustrates the highly significant correlation between the absolute number of children vaccinated with Varivax® and the absolute number of children diagnosed with autistic disorder ($R^2=0.8774$; $P<0.001$). A similar strong correlation was also observed between the number of children vaccinated against Hepatitis A and the number of autistic disorder cases for birth years 2003 to 2008 ($R^2=0.6762$; $P<0.001$).

DNA residuals in human fetal cell line manufactured vaccines

In addition to the ingredients listed on the package



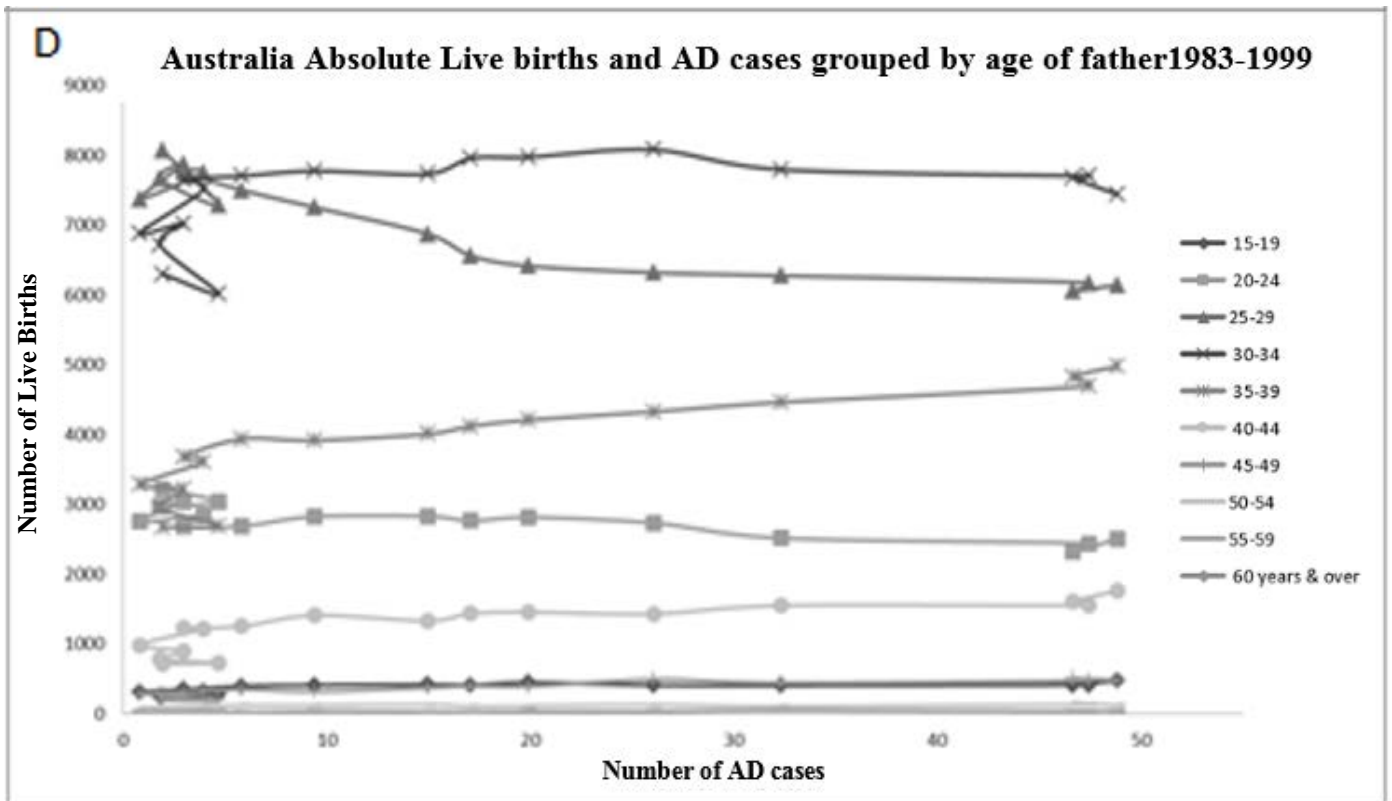
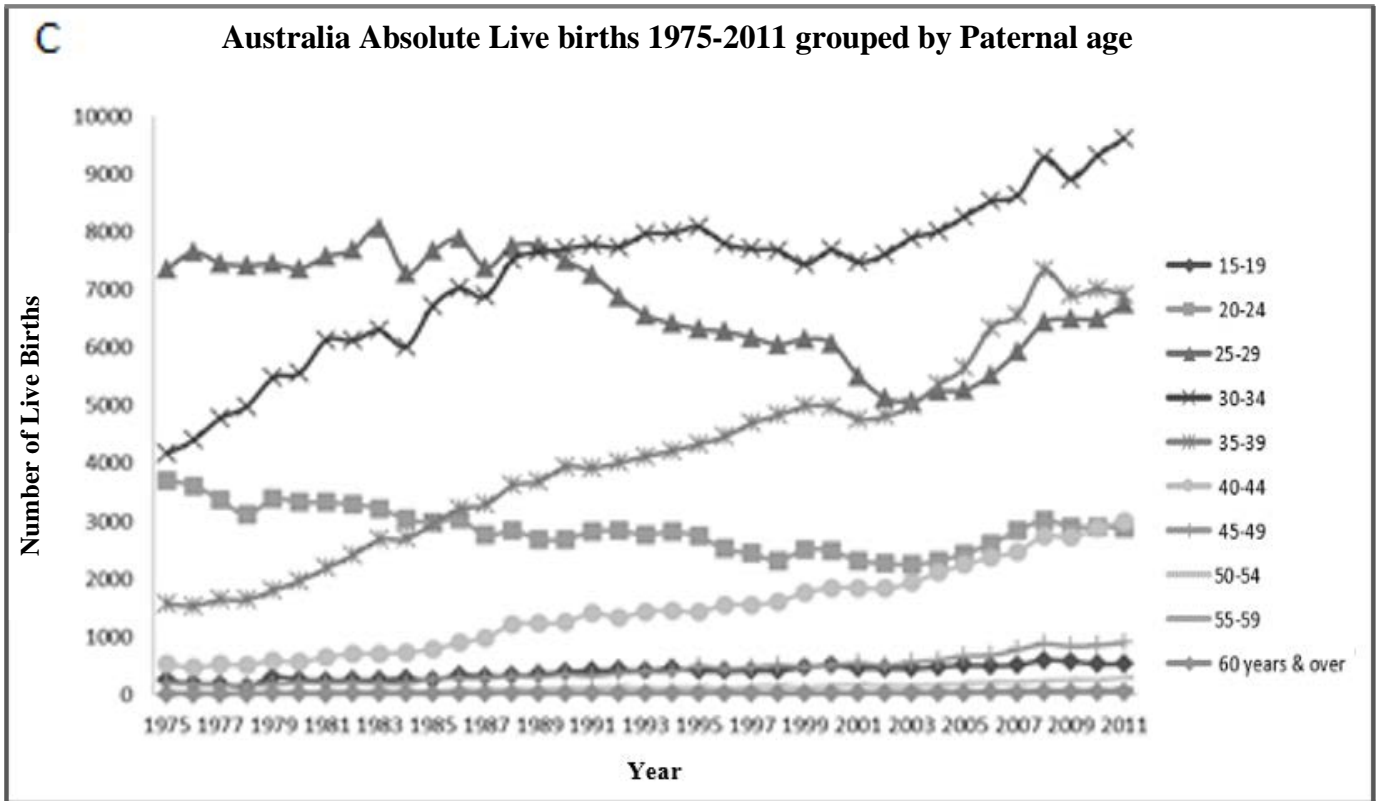


Figure 2. Number of live births grouped by paternal age and its correlation with AD cases over years of US and Western Australia. Panel A and C show number of live births at different father's age from 1960 to 2008 for the U.S. and from 1975 to 2011 for Western Australia. Panel B and D show the number of live births and number of AD cases by paternal age of the U.S. and Western Australia.

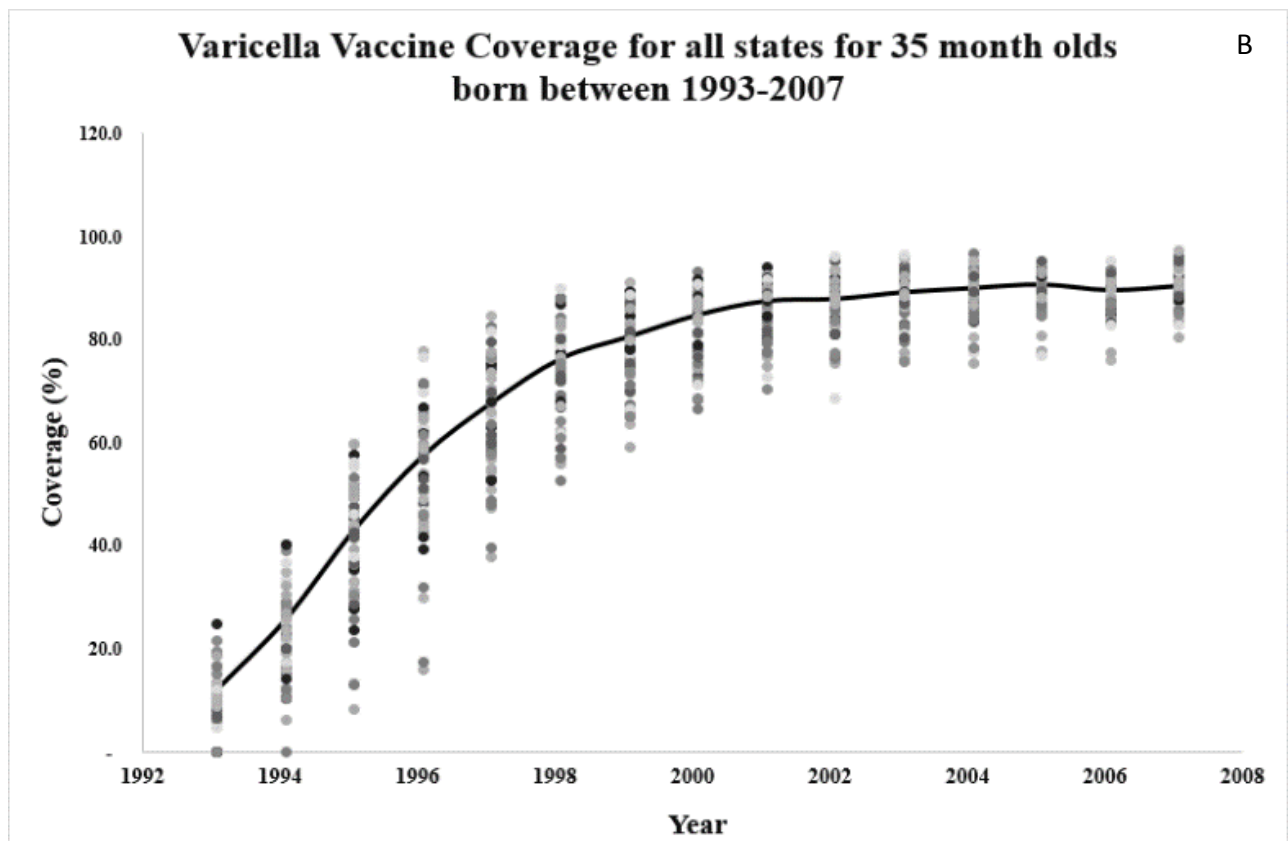
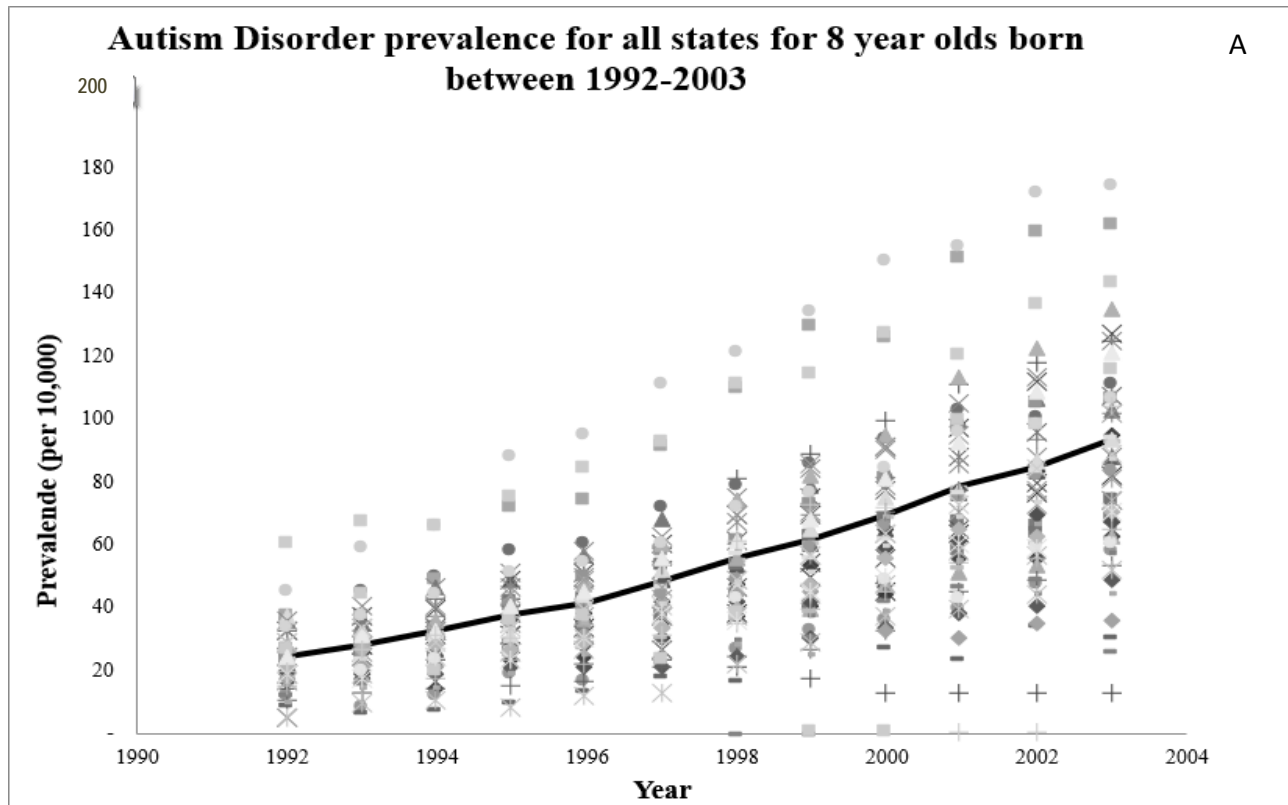
Table 3. History of MMR, varicella and hepatitis A vaccines approved for use in the US, UK and Western Australia manufactured using human fetal cell lines and contaminated with human fetal DNA and/or retroviral fragments.

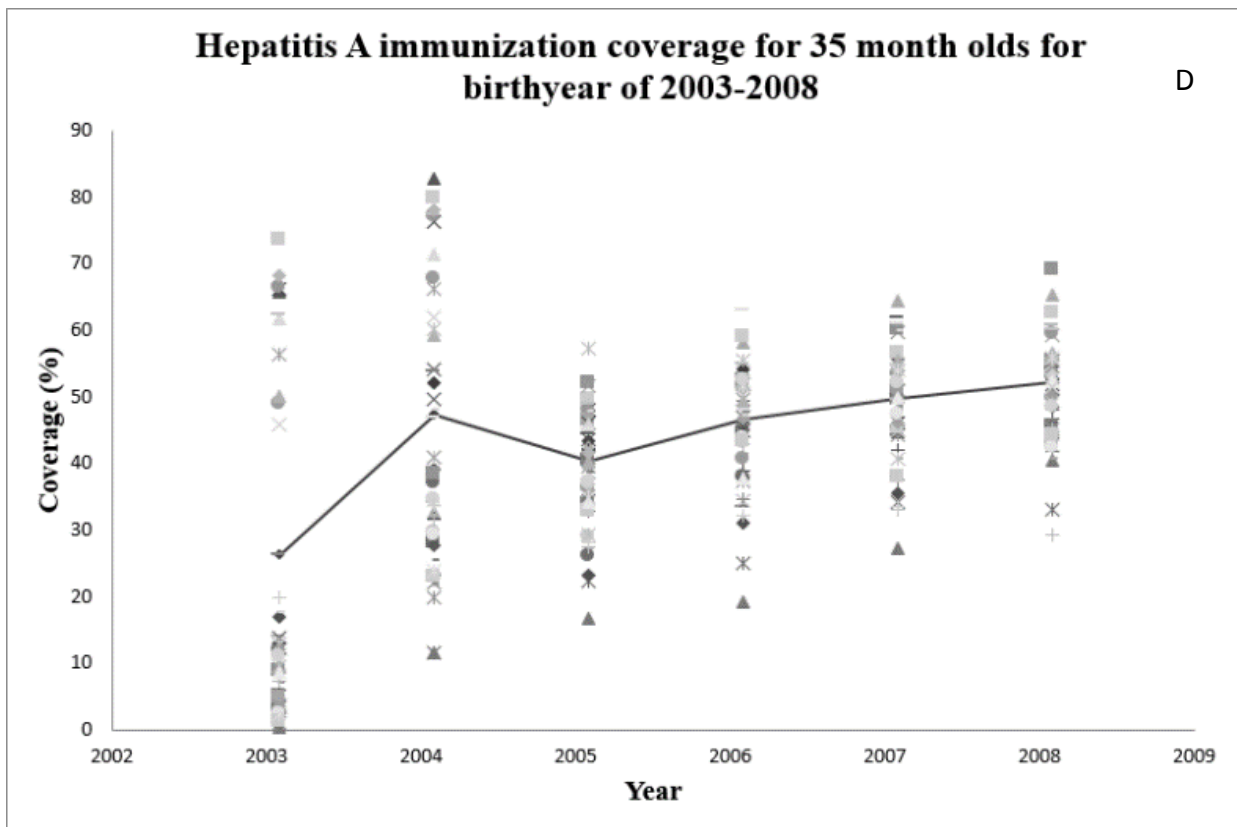
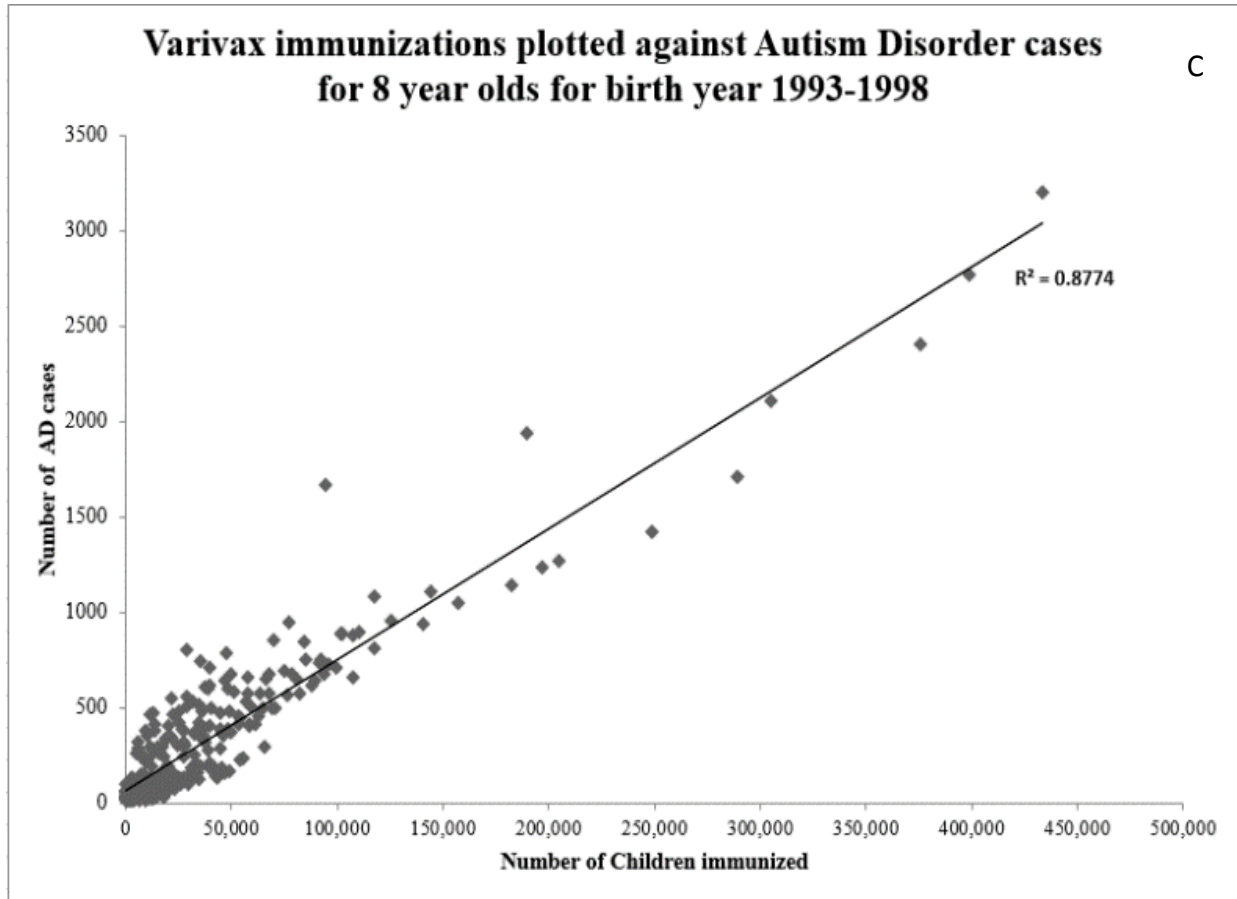
Date	Vaccine name	Type of vaccine	Manufacturer	Age of immunization	Events
History of vaccines approved for use in US					
01/1979	Meruvax II	A rubella vaccine with the RA 27/3 (human diploid fibroblast)	Merck	>=12 months	Licensed.
1979	MMR II	Combined measles, mumps and rubella with the RA 27/3 strain	Merck	>=12 months	Licensed
3/17/1995	Varivax	Varicella virus vaccine, live	Merck	>=12 months	Licensed
2/22/1995	Havrix	The first inactivated hepatitis A vaccine	SmithKline Beecham	>=24 months	Licensed
3/29/1996	Vaqta	A second inactivated hepatitis A vaccine	Merck	>=24 months	Licensed
1999	Vaqta				17 states considered for use
5/11/2001	Twinrix	A combined hepatitis A inactivated and hepatitis B (recombinant) vaccine	SmithKline Beecham	>=18 years	Licensed
8/11/2005	Vaqta	A second inactivated hepatitis A vaccine	Merck	>=12 months	FDA approved lowering the age limit to 12 months
2005	Vaqta				Included in ACIP recommendations
10/18/2005	Havrix	The inactivated hepatitis A vaccine	GSK	>=12 months	FDA approved lowering the age limit to 12 months
3/28/2007	Twinrix	A combined hepatitis A inactivated and hepatitis B (recombinant) vaccine	GSK	>=18 years	FDA approved an accelerated dosing schedule
History of vaccines approved for use in Australia					
1989	MMR II	Combined measles, mumps and rubella with the RA 27/3 strain	Merck, Sharp, Dohme - MSD	>=12 months	Licensed
1999	Varivax	Varicella virus vaccine, live	Merck, Sharp, Dohme - MSD	>=12 months	Licensed
1999	Varilrix	Varicella virus vaccine, live	GSK	>=12 months	Licensed
History of vaccines approved for use in UK					
10/1988	MMR II	Combined measles, mumps and rubella with the RA 27/3 strain	SmithKline Beecham, Merieux, Merck Sharpe	>=12 months	Licensed
History of vaccines approved for use in Denmark					
1987	MMRII	Combined measles, mumps and rubella with the RA 27/3 strain	Statens Serum Institut	15 months and 12 years of age	Licensed
1989	MMRII	Combined measles, mumps and rubella with the RA 27/3 strain	Statens Serum Institut	< 18 years	Extended

insert for Meruvax II® (rubella), we detected significant levels of human ssDNA (142 ± 8 ng/vial) as well as dsDNA (35 ± 10 ng/vial) fragmented to ~215 base pairs in length. The MMR II® package insert discloses the presence of human fetal residuals nor how much cell substrate dsDNA or ssDNA contaminates each dose. In each vial of

Havrix®, we detected ssDNA (301 ± 153 ng/vial) as well as dsDNA (44 ± 24 ng/vial) unfragmented residual DNA more than 48.5 K base pairs in length. The Havrix® package insert discloses the presence of human fetal cellular residuals from the MRC-5 cell line, but not the DNA contaminant levels specifically.

The Varivax® vaccine is manufactured using the human diploid cell line MRC5, and is contaminated with 2 micrograms of cell substrate double stranded DNA. Single stranded DNA levels are not reported in Merck's Varivax Summary Basis for Approval document nor are the length of the DNA fragments contaminating the vaccine (Merck 2011).





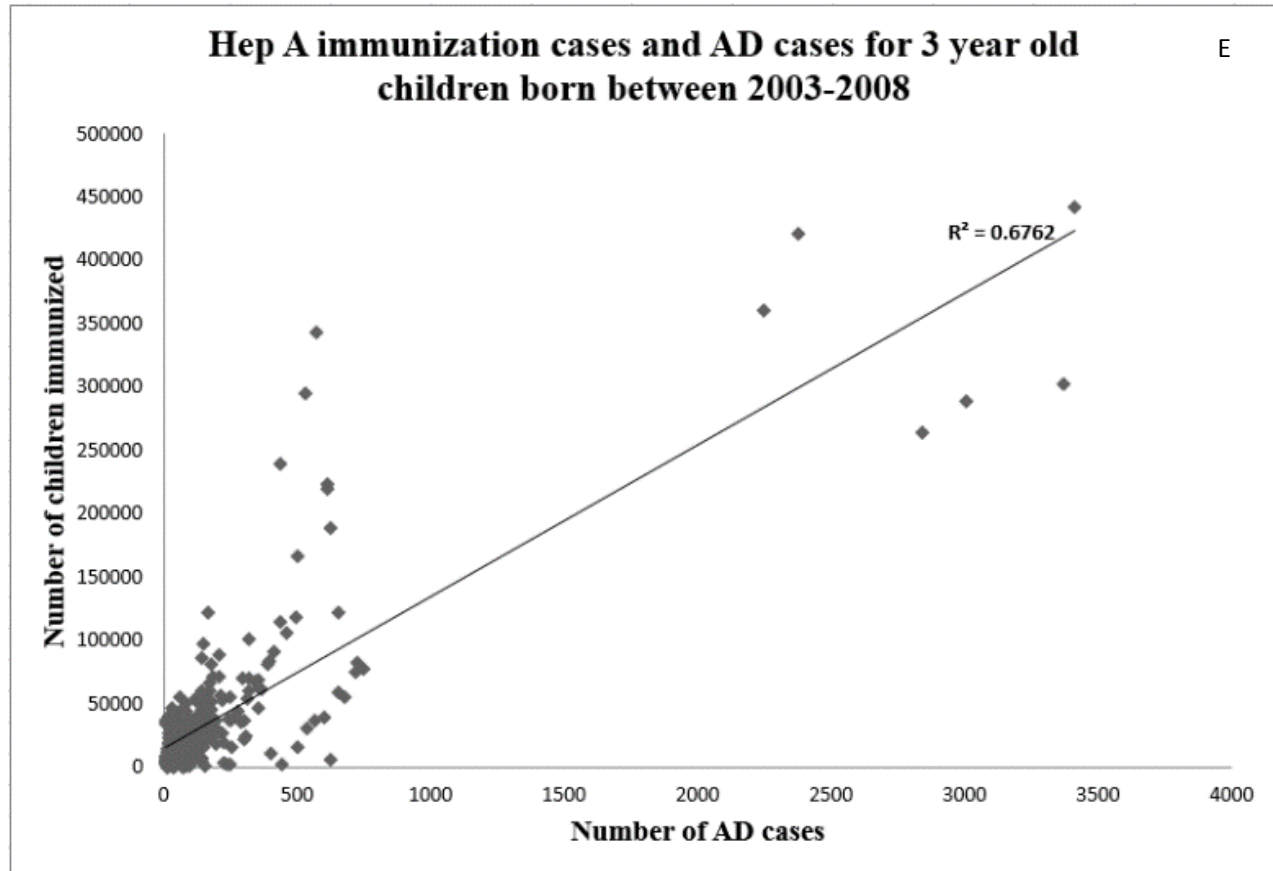


Figure 3. Varicella® and Hepatitis A vaccine coverage trend and their correlations with AD cases over years in the U.S. Panel A shows the increase in AD prevalence for children born between 1992 and 2003. Panel B and D show Varivax and Hepatitis A coverage for all states. Panel C and E show the high correlation between the numbers of children vaccinated with Varivax® and Hepatitis A with AD cases.

DISCUSSION

Autistic disorder began to rise in the US after birth year 1978 (Newschaffer and Gurney 2005). According to EPA recommendations, birth year change points for prevalence of autistic disorder should drive consideration of environmental triggers, as for any disease (McDonald 2010). In this study, we report three calculated US autistic disorder birth year change points for birth years 1970 through 2002. Iterative fitting algorithms identified 1980.8 (1980.4 to 1981.2), 1988.4 (1987.8 to 1989) and 1996.5 (1994.6 to 1996.6) as 'change point' years for US autistic disorder prevalence. While no reporting system is perfect, we have tried to minimize any effects of erroneous diagnosis or coding by choosing the narrower autistic disorder or infantile autism. Regardless of the cause(s) diagnoses of autistic disorder have risen dramatically, adding a significant public health burden and therefore demanding critical assessment of environmental triggers that may be responsible for this apparent epidemic. Candidate environmental triggers should have the following attributes: exposure from conception to at least

3 years of age around each change point, absent or substantially lower prior to the first identified change point, a dose-effect associated with calculated change points, and toxicological mechanisms compatible with disruption in early neural development, that is, biological plausibility.

Therefore, we asked the question whether information about diagnostic criteria would predict autistic disorder change points consistent with our calculated autistic disorder change points. Even though changes in diagnostic criteria have clearly occurred, examination of DSM revisions suggests that autistic disorder (not the broader ASD) diagnosis has not been relaxed. DSM IV introduced a requirement to exclude Rett's disorder, implying that DSM-IV may be more restrictive than DSM-III or III-R. Interestingly, the DSM manual is not typically listed among the diagnostic tools used by any of the practitioners when making their initial diagnosis of either autistic disorder or ASD anyway (Wiggins et al., 2006). More importantly, we analyzed only autistic disorder data; excluding datasets that contained ASD diagnoses, consistent with the CDC statement that a child with autistic

disorder “can be less complicated to diagnose than other spectrum disorders” (Victoria et al., 2010).

Regardless of whether autistic disorder diagnostic relaxation has or has not occurred, and regardless of whether

DSM is used as a tool for initial autistic disorder diagnosis or not, predicted autistic disorder birth year change points based on DSM revision printing schedules do not correlate with calculated autistic disorder change points and cannot be the primary environmental or sociological trigger responsible for current autistic disorder prevalence.

Multiple publications over the past several years point to the potential importance of protein disrupting *de novo* point mutations in the etiology of autism spectrum disorders and other childhood onset ND diseases. In the US, advancing paternal age has an apparent association with these disorders, if one looks only at dates from 1980 onward. However, as shown here, consideration of live births to older fathers back to 1960 disputes the importance of paternal age as a primary trigger for the increased prevalence of autistic disorder. Autistic disorder diagnosis was low and stable from birth year 1960 through 1978. Furthermore, in their publication on advanced paternal age and *de novo* mutations by Kong et al., 2012 point out that live births to older fathers in Iceland were substantially higher from 1650 through 1940 than they are today (Kong et al., 2012), time periods when autistic disorder was extremely rare. Additionally, no studies have been done to determine if the *de novo* mutations in children with ND disease are occurring in the sperm of fathers or in the somatic cells of the children. However, paternal age has been found to be a risk factor for autism spectrum disorder diagnosis (Kong et al., 2012). Our data, taken together with the evidence that advancing age is associated with sperm susceptible to double-strand break formation and genomic instability (McDonald 2010), may explain the association between paternal age and childhood ND.

In 1979, coincident with the first autism disorder change point, vaccine manufacturing changes introduced human fetal DNA fragments and retroviral contaminants into childhood vaccines (Victoria et al., 2010). While we do not know the causal mechanism behind these new vaccine contaminants and autistic disorder, human fetal DNA fragments are inducers of autoimmune reactions, while both DNA fragments and retroviruses are known to potentiate genomic insertions and mutations (Yolken et al., 2000; Kurth 1998; U S Food and Drug Administration 2011). Infants and children are almost universally exposed to these additional vaccine components/contaminants, and these converging events are associated with rising autistic disorder in a dose-dependent fashion due to the increasing numbers of human fetal manufactured vaccines which have been added to the US immunization guidelines, including Pentacel®, which since 2008, contains inactivated polioviruses grown on the MRC-5 human fetal cell line. Pentacel® is recommended for children at 2, 4 and 6 months of age,

and may account for the recent idea that scientists have become more adept at diagnosing autism at younger age. Diagnosis at younger age may more likely be the result of introducing human fetal cell vaccine contaminates to younger children.

Vaccines that have been cultured on or manufactured using the WI-38 fetal cell line such as MeruvaxII®, MMRII®, Varivax®, Havrix® and Pentacel® are additionally contaminated with fragments of human endogenous retrovirus HERVK (Victoria et al., 2010). Recent evidence has shown that human endogenous retroviral transcripts are elevated in the brains of patients with schizophrenia or bipolar disorder (Frank et al., 2005), in peripheral blood mononuclear leucocytes of patients with autism spectrum (Freimanis et al., 2010) as well as associated with several autoimmune diseases (Tai et al., 2008). The strong ecological association between human fetal cell line-manufactured vaccines and autistic disorder change points calls for further investigation of these childhood vaccine contaminants, and for the sake of preserving critical vaccination coverage, even a return to animal-based manufacturing.

Manufacture of childhood vaccines in human fetal cell lines, with its associated retroviral and human DNA fragment contaminants, fulfills all of the necessary requirements as a primary trigger for the ND disease, autistic disorder. The contaminants were not present prior to the first US autistic disorder change point, they have continued to increase the environment with additional human fetal vaccine approvals and doses, and they have clinically documented adverse immunologic and mutagenic side effects. With the 2008 US approval of Pentacel® for children at 2, 4, and 6 months of age, we may be seeing age of onset of regressive autism decrease dramatically.

This study is the first laboratory and ecological study conducted to date that has examined the question of a relationship between human fetal cell line manufactured vaccines and autism. Autistic disorder diagnosis has typically not been made until the age of 5, and confirmed diagnosis is often not made until the age of 8 (Lord et al., 2006; Luyster et al., 2009). Therefore, we were not able to investigate direct correlations between autistic disorder prevalence and vaccine coverage of other human fetal cell manufactured vaccines approved after Hepatitis A, such as the Pentacel® vaccine. Nevertheless, between birth year 1992 through birth year 1998, there are sufficient numbers of children vaccinated or not vaccinated with Varivax® (chickenpox), whose data is maintained in the Vaccine Safety Datalink (VSD), that could be used to determine the relative risk of an autistic disorder diagnosis for those who did or did not receive this heavily contaminated fetal cell manufactured vaccine (Yolken et al., 2000). This overlooked potential trigger for the worldwide autism disorder epidemic demands additional studies in order to assure the safe manufacture of routine recommended childhood vaccines, particularly since reverting to animal based manufacturing methods

is readily available.

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Ethics statement

All data used in this manuscript were from public data files and therefore is exempted from IRB approval according to guidelines from The National Human Subjects Protection Advisory Committee (NHRPAC) recommendations on Public Use Data Files approved at the January 28 to 29, 2002 Committee meeting. (<http://www.hhs.gov/ohrp/archive/nhrpac/documents/dataltr.pdf>): "Responsibility of Users of Public Use Data Files: Users of public use data files do not need to obtain IRB approval to use such files or seek a determination that the use of the public data files meets the criteria for being exempt from IRB review."

Conflict of Interests

The author(s) have not declared any conflict of interests.

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