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

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IMPACTO DE LOS FACTORES AMBIENTALES EN LA PREVALENCIA DEL TRASTORNO AUTISTA DESDE 1979

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ABSTRACT

El objetivo de este estudio fue investigar un factor ambiental previamente pasado por alto a pesar de tratarse de un factor universalmente introducido, que son los contaminantes fetales y retrovirales pocedentes de vacunas infantiles, ausentes antes de los puntos de cambio (PCs) en la prevalencia del trastorno autista (TA), con una conscuente evidencia en la relación dosis-efecto y con conocidos mecanismos de acción patológicos. Un estudio de cohorte basado en la población mundial se utilizó para el diseño de este estudio. Se utilizaron las configuraciones de Estados Unidos, Australia Occidental, Reino Unido y Dinamarca. Fueron incluidos todos los bebés nacidos vivos después del 1 de enero de 1970 que posteriormente desarrollaron un trastorno autista, cuyos informes sobre el diagnóstico de vacunación y trastorno autista están disponibles públicamente en bases de datos mantenidas por Gobierno Federal de los Estados Unidos, Australia Occidental, Reino Unido y Dinamarca. Los nacimientos vivos, agrupados según la edad parental, procedian de Estados Unidos y Australia. En niños vacunados con MMRII (triple vírica), Varicela y Hepatitis A, la edad de vacunación varió entre los 19 y 35 meses en el momento de la misma.

En el trastorno autista según el año de nacimiento, los puntos de cambio se identificaron como 1980.9, 1988.4 y 1996 para los EE. UU., 1987 para el Reino Unido, 1990.4 para Australia Occidental, y 1987.5 para Dinamarca. Los puntos de cambio en estos países correspondieron a la introducción o al aumento de las dosis de vacunas producidas en líneas de células fetales humanas, mientras que no se encontró relación entre la edad parental o las variaciones de criterio realizadas en el Manual de Diagnóstico Estadístico (DSM) y el diagnóstico de trastorno autista. Además, una regresión lineal (¿estudio retrospectivo?) reveló que la cobertura vacunal contra la varicela y la hepatitis A estaba significativamente correlacionada con los casos de trastorno autista. Se utilizó el software R para calcular los puntos de cambio. Los puntos de cambio del trastorno autista años coinciden con la introducción de vacunas fabricadas con líneas celulares fetales humanas, que contienen contaminantes fetales y retrovirales, en programas de vacunación infantil. Este patrón se repitió en los Estados Unidos, Reino Unido, Australia Occidental y Dinamarca. Por lo tanto, la prevalencia del aumento del trastorno autista está directamente relacionada con las vacunas fabricadas utilizando células fetales humanas. El incremento en la edad parentl y las revisiones del DSM no estaban relacionadas con el aumento de la prevalencia del trastorno autista.

Key words: trastorno de autismo, punto de cambio, vacuna, edad parental.

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INTRODUCCIÓN

El trastorno autista (TA) es un subconjunto de los Trastornos del Espectro Autista (TEAs), un grupo de discapacidades del desarrollo que ha alcanzado niveles epidémicos. En todo el mundo, 1988 ha sido identificado por la Agencia de Protección Ambiental (EPA) como un año de incidentes críticos para el TA (McDonald 2010). Los CDC publicaron un estudio en 2013 que estima la prevalencia de TEA en USA en 1 de cada 50 niños de 6 a 17 años en 2011 para 2012. Además de TEA, también hay niveles aparentemente epidémicos de otros neurodesarrollos de inicio temprano (ND), síndromes como la esquizofrenia de inicio en la infancia (0.4% de la población afectada) (Okkels et al., 2012) y trastorno bipolar (Leibenluft 2008). Características compartidas entre las epidemias infantiles de ND incluyen asociaciones con el género masculino, aptitud reproductiva reducida, aumento de la edad paterna y la excesiva presencia de tasas de mutaciones genómicas de novo. Actualmente, la edad paterna es la explicación más favorecida para la epidemia mundial de autismo. Sin embargo, conceptos en evolución sobre el espectro del autismo y otras enfermedades ND sugieren que estas enfermedades sean consecuencia "impactos múltiples", con componentes genéticos, genómicos y ambientales. ///

Hay una acumulación de evidencia del exoma familiar puntos de secuencia a la importancia de cientos de raros, diversas mutaciones de novo (DNMs) en la infancia ND enfermedades (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).
Las mutaciones de novo en estas enfermedades son encontrado constantemente en exones o regiones críticas de codificación de genes que conducirían a un stop or non-funcional prematuro proteínas (Awadalla et al., 2010; O'Roak et al., 2011; De Ligt et al., 2012; Luo et al., 2012). Además de aumento de los DNM en niños con enfermedad de ND, de novo las inserciones y deleciones genómicas son significativamente aumento de la discapacidad intelectual (De Ligt et al., 2012), trastorno autista (O'Roak et al., 2011) y infancia esquizofrenia de inicio (Xu et al., 2012). Diverso, raro Los DNM exigen que los factores ambientales sean conocidos por causar la inestabilidad genómica se evaluará por surelación con estas enfermedades. La consideración de posibles desencadenantes ambientales requiere evaluación estadística para identificar el cambio del año de nacimiento puntos (PC) asociados con una tasa creciente en la incidencia del autismo Requisitos para un factor ambiental como el desencadenante de la enfermedad incluye: (1) niveles ausentes o inferiores antes de un punto de cambio, (2) aumento continuo después de un se demuestra el punto de cambio (dosis-efecto), (3) biológico mecanismo consistente con la patología, y (4) en casos de enfermedades no geográficamente limitadas como autismo, esquizofrenia y discapacidad intelectual, debería tener una exposición casi universal (McDonald 2010). Este estudio investiga un pasado por alto, universalmente factor ambiental introducido, contaminantes fetales y retrovirales en vacunas infantiles, ausentes antes del cambio puntos en la prevalencia del trastorno autista con posterior evidencia de dosis-efecto y mecanismos patológicos conocidos de acción. Las vacunas han hecho un gran bien en el mundo; Sin embargo, una mayor investigación de la fabricación fetal contaminantes de la vacuna como un medio ambiente contribuyente a la actual epidemia de trastorno autista es pedido.

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 stopornon-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.

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 C D C ' 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 t h e 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 changepoints 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 during1973 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

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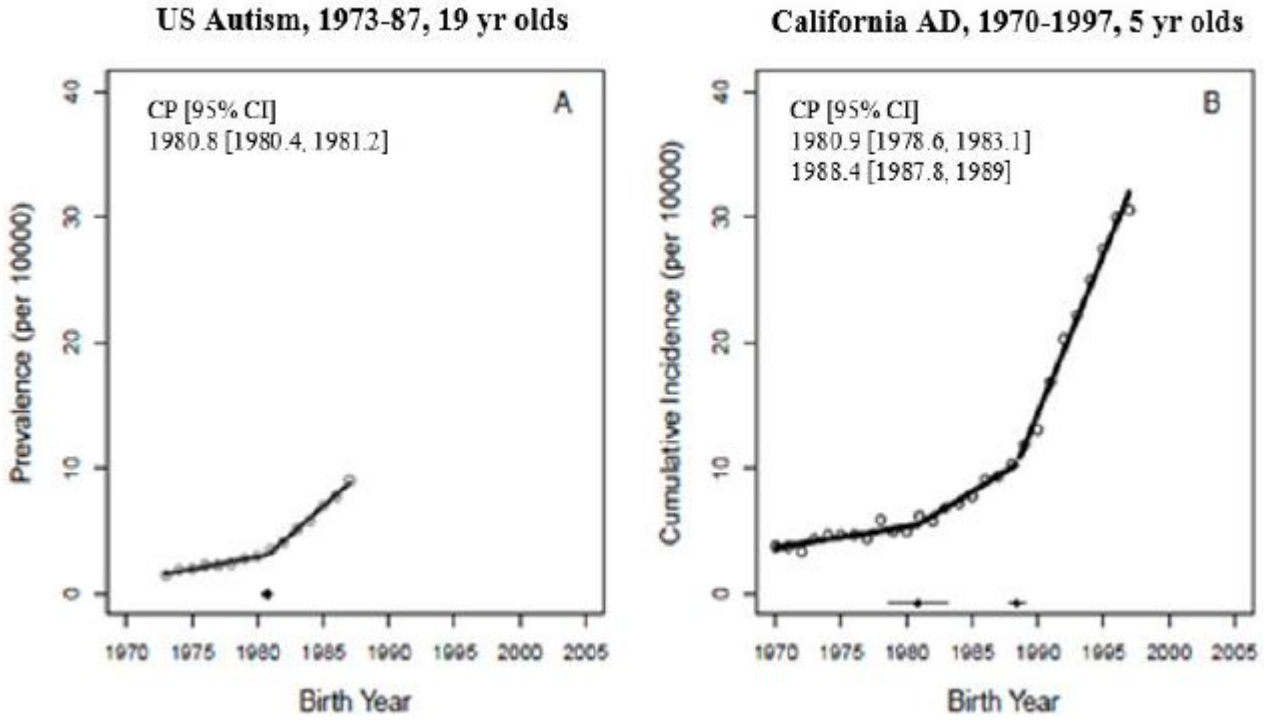
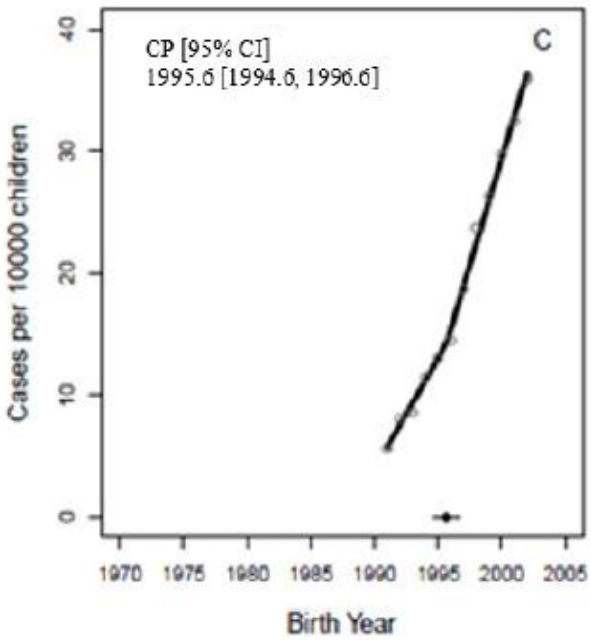
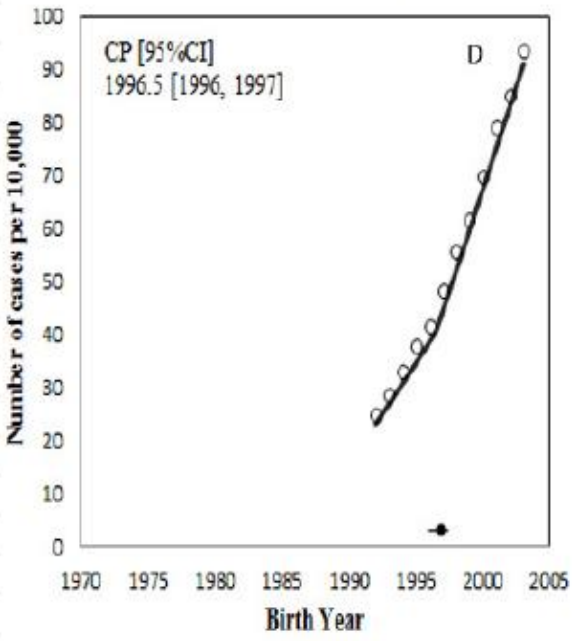


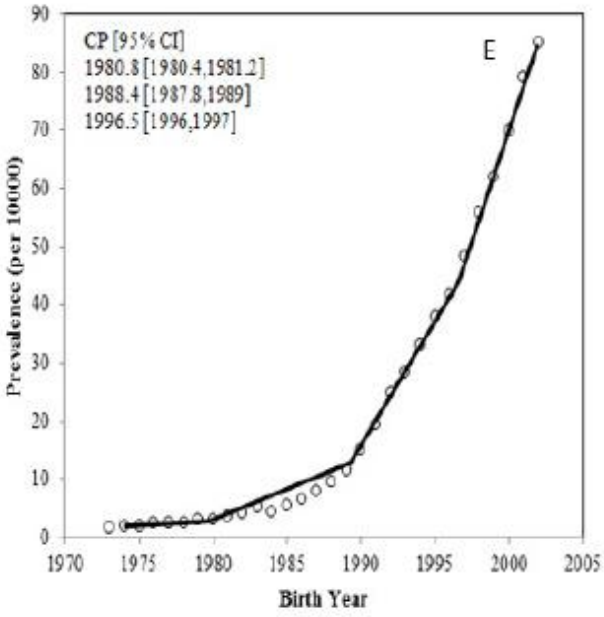
California AD, 1991-2002, 4 yr olds



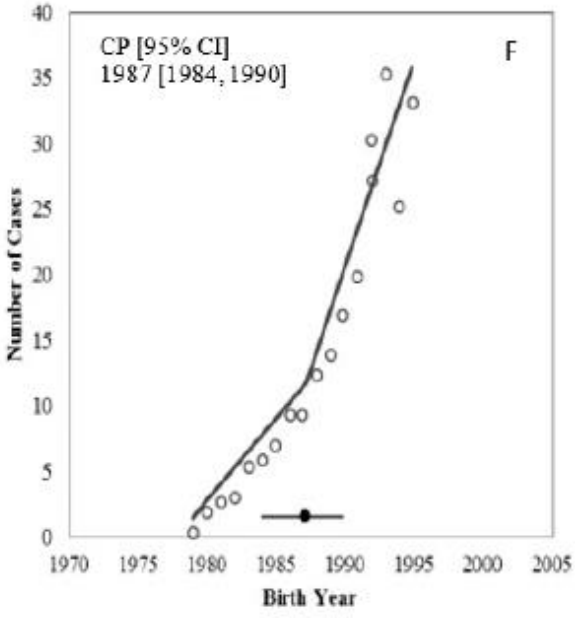
US, 1992-2003, 8 yr olds



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



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



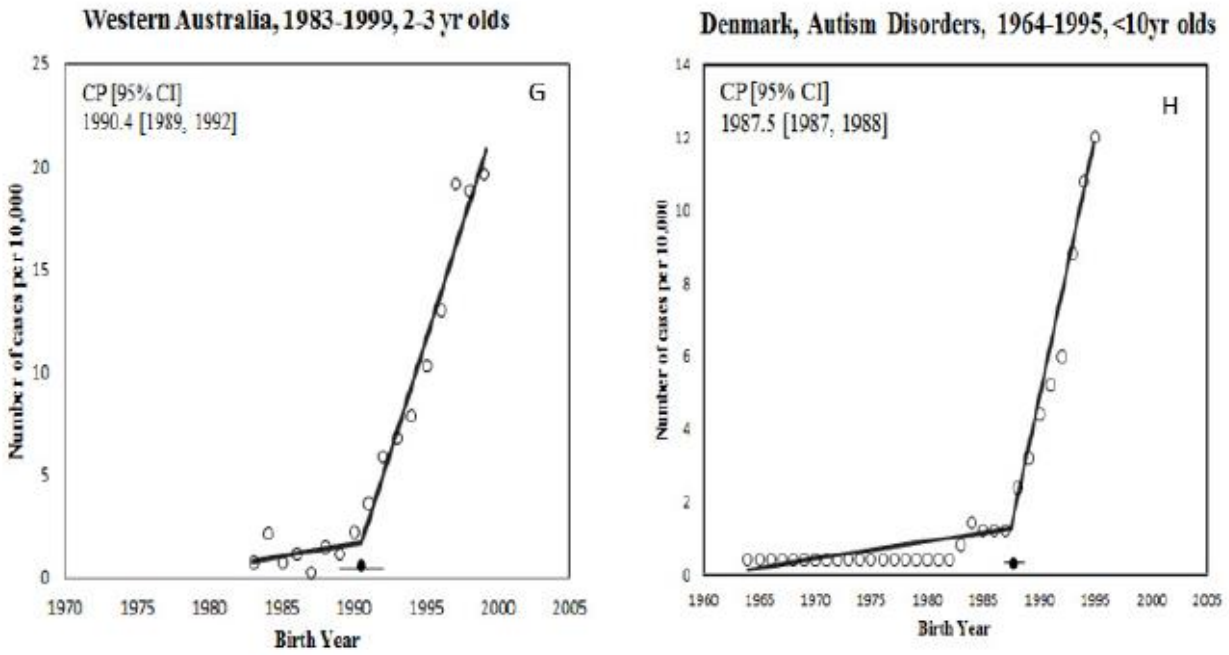


Figura 1. Análisis del punto de inflexión en TA, solidez y resultados.
La Figura 1A muestra los resultados del punto de cambio de TA para USA, California, Reino Unido, Australia Occidental y Dinamarca. La Figura 1B muestra una comparación de los ajustes de "hockey" y "segmentados" para los datos de California TA 1970-1997. Ambos análisis producen puntos de cambio con intervalos de confianza superpuestos cercanos 1988. Sin embargo, el análisis "segmentado" revela un segundo punto de cambio cerca de 1981.

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.

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DISCUSIÓN

El trastorno autista comenzó a aumentar en los Estados Unidos en los nacidos después de 1978 (Newschaffer y Gurney 2005). De acuerdo con las recomendaciones de la EPA, los puntos de inflexión según el año de nacimiento para la prevalencia del trastorno autista deben generar la consideración de desencadenantes ambientales, como para cualquier enfermedad (McDonald 2010). En este estudio, informamos tres autistas estadounidenses calculados trastorno puntos de inflexión del año de nacimiento para los años de nacimiento 1970 hasta 2002.

Algoritmos de ajuste iterativo identificados 1980.8 (1980.4 a 1981.2), 1988.4 (1987.8 a 1989) y 1996.5 (1994.6 a 1996.6) como años de "punto de inflexión" para autistas estadounidenses prevalencia del trastorno. Si bien ningún sistema de informes es perfecto, hemos tratado de minimizar cualquier efecto de error diagnóstico o codificación eligiendo el trastorno autista más ajustado o autismo infantil. Independientemente de la(s) causa(s), los diagnósticos de trastorno autista han aumentado dramáticamente, agregando una carga significativa de salud pública y por lo tanto se hace necesario exigir una evaluación crítica de los desencadenantes ambientales que puedan ser responsables de esta aparente epidemia. Los desencadenantes ambientales candidatos deben tener los siguientes atributos: exposición desde la concepción al menos a los 3 años de edad alrededor de cada punto de inflexión, ausente o sustancialmente menor antes del primer cambio identificado punto, un efecto dosis asociado con el cambio calculado puntos y mecanismos toxicológicos compatibles con interrupción en el desarrollo neuronal temprano, es decir, biológico plausibilidad.

Por lo tanto, preguntamos si la información sobre los criterios diagnósticos predeciría el trastorno autista cambiar puntos consistentes con nuestro trastorno autista calculado Cambiar puntos. Aunque los cambios en el diagnóstico criterios han ocurrido claramente, examen de DSM revisiones sugiere que el trastorno autista (no el más amplio ASD) diagnóstico no ha sido relajado. DSM IV introducido un requisito para excluir el trastorno de Rett, lo que implica que DSM-IV puede ser más restrictivo que DSM-III o IIIR. Curiosamente, el manual de DSM no suele aparecer en la lista entre las herramientas de diagnóstico utilizadas por cualquiera de los profesionales al hacer su diagnóstico inicial de cualquier trastorno autista o TEA de todos modos (Wiggins et al., 2006). Más Es importante destacar que solo analizamos los datos del trastorno autista; excluyendo conjuntos de datos que contenían diagnósticos de TEA, consistentes con la declaración de los CDC de que un niño con trastorno autista "puede ser menos complicado de diagnosticar que otro con trastornos del espectro ”(Victoria et al., 2010). Independientemente de si el trastorno autista es un diagnóstico de relajación ha ocurrido o no, e independientemente de si DSM se utiliza como herramienta para el trastorno autista inicial diagnóstico o no, trastorno autista predicho año de nacimiento cambiar puntos basados en cronogramas de impresión de revisión DSM no se correlaciona con el cambio del trastorno autista calculado puntos y no puede ser el principal medio ambiente o disparador sociológico responsable del autismo actual prevalencia del trastorno.

Múltiples publicaciones en los últimos años señalan a la importancia potencial de la proteína que interrumpe de novo mutaciones puntuales en la etiología del espectro autista trastornos y otras enfermedades ND de inicio en la infancia. En el EE. UU., El avance de la edad paterna tiene una asociación aparente con estos trastornos, si solo se miran las fechas de 1980 adelante. Sin embargo, como se muestra aquí, la consideración de vivir nacimientos de padres mayores desde 1960 disputa la importancia de la edad paterna como desencadenante primario para el aumento de la prevalencia del trastorno autista. Autista el diagnóstico de trastorno fue bajo y estable desde el año de nacimiento

1960 a 1978. Además, en su publicación sobre edad paterna avanzada y mutaciones de novo por Kong et al., 2012 señalan que nacen vivos de padres mayores en hielo la tierra fue sustancialmente más alta desde 1650 hasta 1940 de lo que son hoy (Kong et al., 2012), períodos de tiempo cuando el trastorno autista era extremadamente raro. Adicionalmente, no se han realizado estudios para determinar si el de novo mutaciones en niños con enfermedad de ND están ocurriendo en los padres del espermo folder o en las células somáticas de la niños. Sin embargo, se ha encontrado que la edad paterna es una factor de riesgo para el diagnóstico del trastorno del espectro autista (Kong et al., 2012). Nuestros datos, tomados junto con la evidencia que la edad avanzada está asociada con esperma susceptible a la formación de ruptura de doble cadena e inestabilidad genómica (McDonald 2010), puede explicar la asociación entre edad paterna e infancia ND.

En 1979, coincidente con el primer trastorno de autismo. c h a n g e p o i n t, fabricación de vacunas c h a n g e s introdujo fragmentos de ADN fetal humano y retrovirales contaminantes en vacunas infantiles (Victoria et al., 2010). Si bien no conocemos el mecanismo causal detrás de estas nuevas vacunas contaminantes y autistas trastorno, los fragmentos de ADN fetal humano son inductores de reacciones autoinmunes, mientras que ambos fragmentos de ADN y se sabe que los retrovirus potencian las inserciones genómicas y mutaciones (Yolken et al., 2000; Kurth 1998; U S Food y Drug Administration 2011). Los infantes y niños están casi universalmente expuesto a estas vacunas adicionales componentes / contaminantes, y estos eventos convergentes están asociados con el aumento del trastorno autista en una dosis dependiente moda debido al creciente número de humanos vacunas fetales fabricadas que se han agregado a Las pautas de vacunación de EE. UU., incluyendo Pentacel®, que desde 2008 contiene poliovirus inactivados crecido en la línea celular fetal humana MRC-5. Pentacel® es recomendado para niños de 2, 4 y 6 meses de edad, y puede explicar la idea reciente de que los científicos tienen volverse más experto en el diagnóstico de autismo en los más jóvenes años. El diagnóstico a una edad más temprana puede ser más probable resultado de la introducción de la vacuna de células fetales humanas contamina a los niños más pequeños. En 1979, coincidente con el primer trastorno de autismo c h a n g e p o i n t, fabricación de vacunas c h a n g e s introdujo fragmentos de ADN fetal humano y retrovirales contaminantes en vacunas infantiles (Victoria et al., 2010). Si bien no conocemos el mecanismo causal detrás de estas nuevas vacunas contaminantes y autistas trastorno, los fragmentos de ADN fetal humano son inductores de reacciones autoinmunes, mientras que ambos fragmentos de ADN y se sabe que los retrovirus potencian las inserciones genómicas y mutaciones (Yolken et al., 2000; Kurth 1998; U S Food & Drug Administration 2011). Los infantes y niños son casi universalmente expuesto a estas vacunas adicionales componentes / contaminantes, y estos eventos convergentes están asociados con el aumento del trastorno autista en una dosis dependiente moda debido al creciente número de humanos vacunas fetales fabricadas que se han agregado a Las pautas de vacunación de EE. UU., incluyendo Pentacel®, que desde 2008 contiene poliovirus inactivados crecido en la línea celular fetal humana

MRC-5. Pentacel® es recomendado para niños de 2, 4 y 6 meses de edad, y puede explicar la idea reciente de que los científicos tienen volverse más experto en el diagnóstico de autismo en los más jóvenes años. El diagnóstico a una edad más temprana puede ser más probable resultado de la introducción de la vacuna de células fetales humanas contamina a los niños más pequeños.

Vacunas cultivadas o fabricadas utilizando la línea celular fetal WI-38 como MeruvaxII®, MMRII®, Varivax®, Havrix® y Pentacel® son además contaminado con fragmentos de endógeno humano retrovirus HERVK (Victoria et al., 2010). Evidencia reciente ha demostrado que las transcripciones retrovirales endógenas humanas están elevados en el cerebro de pacientes con esquizofrenia o trastorno bipolar (Frank et al., 2005), en sangre periférica leucocitos mononucleares de pacientes con espectro autista (Freimanis et al., 2010), así como asociados con varias enfermedades autoinmunes (Tai et al., 2008). Los fuerte asociación ecológica entre células fetales humanas vacunas fabricadas en línea y cambio de trastorno autista puntos llama a una mayor investigación de estos niños contaminantes de vacunas y para preservar cobertura crítica de vacunación, incluso un retorno a animales fabricación. Fabricación de vacunas infantiles en células fetales humanas. líneas, con su ADN retroviral y humano asociado fragmentos de contaminantes, cumple con todos los requisitos requisitos como desencadenante primario de la enfermedad de ND, trastorno autista Los contaminantes no estaban presentes antes hasta el primer punto de cambio de trastorno autista de EE. UU. continuó aumentando el medio ambiente con aprobaciones adicionales y dosis de vacunas fetales humanas, y están clínicamente documentado adverso inmunológico y efectos secundarios mutagénicos Con la aprobación estadounidense de 2008 de Pentacel® para niños de 2, 4 y 6 meses de edad, nosotros puede estar viendo la edad de inicio del autismo regresivo disminuir dramáticamente. Este estudio es el primer estudio de laboratorio y ecológico. realizado hasta la fecha que ha examinado la cuestión de un relación entre la línea celular fetal humana fabricada, vacunas y autismo. El diagnóstico de trastorno autista tiene típicamente no se hace hasta la edad de 5 años, y se confirma el diagnóstico a menudo no se realiza hasta la edad de 8 años (Lord et al.,2006; Luyster et al., 2009). Por lo tanto, no pudimos para investigar correlaciones directas entre el trastorno autista prevalencia y cobertura de vacunas de otro feto humano vacunas fabricadas con células aprobadas después de la hepatitis A, como la vacuna Pentacel®. Sin embargo, entre el año de nacimiento 1992 hasta el año de nacimiento 1998, hay un número suficiente de niños vacunados o no vacunados con Varivax® (varicela), cuyos datos son mantenidos en el enlace de datos de seguridad de vacunas (VSD), que podrían usarse para determinar el riesgo relativo de un autista diagnóstico de trastorno para aquellos que recibieron o no recibieron esta vacuna fabricada con células fetales muy contaminadas (Yolken et al., 2000). Este potencial desencadenante pasado por alto para la epidemia mundial de trastornos del autismo exige estudios adicionales para garantizar la fabricación segura de las vacunas infantiles de rutina recomendadas, particularmente desde volver a los métodos de fabricación basados en animales Está fácilmente disponible.

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 IIIR. 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 relaxaion 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 ice land 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 spermofolder 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 c h a n g e p o i n t , vaccine manufacturing c h a n g e s

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 animalbased 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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