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Abnormal Brain Connectivity Spectrum Disorders Following Thimerosal Administration: A Prospective Longitudinal Case–Control Assessment of Medical Records in the Vaccine Safety Datalink

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Abstract

Background: Autism spectrum disorder (ASD), tic disorder (TD), and hyperkinetic syndrome of childhood (attention deficit disorder [ADD]/attention deficit hyperactivity disorder [ADHD]) are disorders recently defined as abnormal connectivity spectrum disorders (ACSDs) because they show a similar pattern of abnormal brain connectivity. This study examines whether these disorders are associated with exposure to thimerosal, a mercury (Hg)-based preservative.

Methods: A hypothesis testing case-control study evaluated the Vaccine Safety Datalink for the potential dose-dependent odds ratios (ORs) for diagnoses of ASD, TD, and ADD/ADHD compared to controls, following exposure to Hg from thimerosal-containing *Haemophilus influenzae* type b vaccines administrated within the first 15 months of life. Febrile seizures, cerebral degeneration, and unspecified disorders of metabolism, which are not biologically plausibly linked to thimerosal, were examined as control outcomes.

Results: On a per 25 μg Hg basis, cases diagnosed with ASD (OR = 1.493), TD (OR = 1.428), or ADD/ADHD (OR = 1.503) were significantly ($P < .001$) more likely than controls to have received increased Hg exposure. Similar relationships were observed when separated by gender. Cases diagnosed with control outcomes were no more likely than controls to have received increased Hg exposure.

Conclusion: The results suggest that Hg exposure from thimerosal is significantly associated with the ACSDs of ASD, TD, and ADD/ADHD.

Keywords

Asperger, autism, ethylmercury, PDD-NOS, thimerosal, Tourette, ADD/ADHD, Mercury

Introduction

Autism spectrum disorder (ASD), tic disorder (TD), and hyperkinetic syndrome of childhood (also known as attention deficit disorder [ADD]/attention deficit hyperactivity disorder [ADHD]) are neurodevelopmental disorders.[1] Evidence suggests these children share similar neuropathology, symptomatology, and comorbid conditions. Moreover, these disorders present with a similar pattern of abnormal brain connectivity of long-range underconnectivity and short-range overconnectivity.[1] As a consequence, it was suggested that these disorders may be subsets in what could be termed an abnormal connectivity spectrum disorder (ACSD).

It has also been hypothesized that the etiological basis of ACSDs is plausibly related to neuronal insult (eg,

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neurotoxicity, neuroinflammation, excitotoxicity, sustained microglial activation, proinflammatory cytokines, toxic exposure, and oxidative stress), and the neuronal insult can ultimately cause loss of long-range connectivity and a resultant increase in short-range connectivity.[1] Certain neurotoxicants, including mercury (Hg), show neuropathological evidence of loss of long-range connections and a resultant increase in short-range connections.[1]

Mercury is a recognized ubiquitous environmental neurotoxicant, and there is mounting evidence linking it to ASD, TD, and ADD/ADHD.[2] A published meta-analysis of epidemiological evidence of the impact of prenatal and early infancy exposure to Hg and the long-term risk of a child being diagnosed with an ASD or ADD/ADHD revealed significant associations for both ASD (odds ratio [OR] = 1.66, 95% confidence interval [CI] = 1.14-2.17) and ADD/ADHD (OR = 1.60, 95% CI = 1.10-2.33).[3]

Of particular recent concern, the routine administration of thimerosal (49.55% Hg by weight)-containing childhood vaccines has become a significant source of organic Hg (thimerosal is 56.7% ethyl-Hg by weight) exposure from some vaccines.[4] Thimerosal is present in some influenza vaccines routinely recommended for administration to pregnant women, infants, and children in the United States, and it continues to remain in most multidose formulations of childhood vaccines recommended for routine administration in the developing world.[4] It is estimated that some children received more than 50% of their Hg exposure in early childhood from routine childhood vaccination, and that when combined with environmental Hg exposure, some children received instantaneous and total Hg doses manyfold in excess of the Hg safety limits established by the US Environmental Protection Agency (0.7 μg Hg/kg bodyweight/week), Health Canada (1.4 μg Hg/kg bodyweight/week), World Health Organization (1.6 μg Hg/kg bodyweight/week), the US Centers for Disease Control and Prevention (CDC)'s Agency for Toxic Substances and Disease Registry (2.1 μg Hg/kg bodyweight/week), and US Food and Drug Administration (2.8 μg Hg/kg bodyweight/week).[5]

It was previously revealed that thimerosal exposure is capable of inducing neuronal cell damage consistent with that observed in neuropathology studies of children diagnosed with neurodevelopmental disorders, including mitochondrial damage, reduced oxidative-reduction activity, cellular degeneration, and cell death.[6] Previous epidemiological studies of medical records showed that there were significant associations between exposures to increasing doses of Hg from thimerosal-containing hepatitis B vaccines (12.5 μg Hg/dose and a total of 37.5 μg Hg) administered within the first 6 months of life and the increasing long-term risk of a child being diagnosed with an ASD,[7] TD,[8] or ADD/ADHD.[9] Other epidemiological studies of medical records demonstrated that the relationship between increasing exposure to Hg from thimerosal-containing hepatitis B vaccines and the increased risk of diagnosed neurodevelopmental disorder outcomes was dose dependent.[10] Importantly, some of the aforementioned studies suggested that total cumulative exposure to Hg, timing of exposure to Hg, and gender may modulate the risks observed.

As a consequence of these previous studies, the present study undertook a secondary data analysis of existing medical records data using a case-control study methodology to further epidemiologically evaluate the potential relationship between exposure to Hg from thimerosal-containing vaccine administration in early childhood and the long-term risk of a child being diagnosed with the ACSDs of ASD, TD, and ADD/ADHD. The current study is differentiated from previous studies by examining a different type of vaccine, *Haemophilus influenzae* type b (Hib), administered to infants at different exposure windows (ie, at 2, 4, 6, and 15 months) and with different bolus and cumulative Hg doses (ie, 25 μg Hg/dose and a total of 100 μg Hg). In addition, the potential impacts of gender were examined by analyzing the data separated by gender. The specific new hypothesis tested in this study was that cases diagnosed with an ACSD in comparison to controls would, in a dose-dependent manner, be at greater odds of receiving increasing discrete doses of Hg exposure from thimerosal-containing Hib vaccines (50 μg Hg vs 25 μg Hg, 75 μg Hg vs 25 μg Hg, 100 μg Hg vs 25 μg Hg) administered within the first 15 months of life.

Methods

The study protocol was approved by the CDC, the institutional review board (IRB) of Kaiser Permanente Northwest (KPNW), and the IRB of Kaiser Permanente Northern California (KPNC). The data were analyzed between 2013 and 2014 at the secure Research Data Center of the National Center for Health Statistics in Hyattsville, Maryland. The views expressed in this study do not necessarily reflect those of the CDC or those of Kaiser Permanente.

Determining the Population at Risk

A cohort of over 1.95 million children in the Vaccine Safety Datalink (VSD) project (updated through the end of 2000) from KPNW, Kaiser Permanente Colorado, and KPNC were examined using SAS software (version 9.3). The VSD project was created in 1991 by the National Immunization Program of the CDC.[11-13] The project links medical event information, specific vaccine history, and selected demographic information from the computerized databases of several health maintenance organizations (HMOs). The cohort examined was comprised of children with nonmissing date of birth and nonmissing gender, who were continuously HMO-enrolled from their date of birth.

Determining Cases

The outcome files (inpatient and outpatient diagnoses) from the VSD population were reviewed to find the first instance of *International Classification of Disease, Ninth Revision* outcomes of ASD (299.xx), TD (307.2x), or ADD/ADHD (314.xx), which when referring to all 3 disorders will be

Table 1. A Summary of Various Types of Cases and Controls Examined in the Present Study.

Group Examined (*ICD-9* Code)	M	F	M/F Ratio	Birth Years	Mean Ages of Initial Diagnosis (SD)
ACSDs					
ASD cases (299.xx)	332	79	4.2	1991-2000	4.1 (1.6)
Controls	7529	7138	1.05	1991-1993	-
Tic disorder cases (307.2x)	181	60	3.02	1991-2000	5.1 (2.0)
Controls[a]	8259	7839	1.05	1991-1993	-
ADD/ADHD cases (314.xx)	820	221	3.71	1991-2000	5.7 (1.8)
Controls[a]	5039	4958	1.02	1991-1993	-
Non-thimerosal-related disorders[b]					
Febrile seizure cases (780.3x)	2839	2199	1.29	1991-2000	1.54 (1.29)
Controls	33 661	31 735	1.06	1991-1996	-
Unspecified disorders of metabolism cases (277.xx)	143	114	1.25	1991-2000	1.015 (1.47)
Controls	36 992	34 674	1.07	1991-1997	-
Cerebral degeneration cases (330.xx or 331.xx)	1104	875	1.26	1991-2000	0.63 (0.98)
Controls	55 057	52 547	1.05	1991-1998	-

Abbreviations: ACSD, abnormal connectivity spectrum disorder; ADD/ADHD, attention deficit disorder/attention deficit hyperactivity disorder; ASD, autism spectrum disorder; F, females; Hib, *Haemophilus influenzae* type b; *ICD-9*, *International Classification of Disease, Ninth Revision*; M, males; SD, standard deviation.
[a]These controls were followed to the mean age of initial diagnosis plus 1 SD.
[b]These outcomes were specifically chosen as not biologically plausibly linked to postnatal Hg exposure from thimerosal in Hib-containing vaccines.

referred to as an ACSD. Each specific diagnosis examined was analyzed independently, so children may have received 1 or more of the other diagnoses examined in this study. If there were multiple instances of the same diagnosis in a child, only the first instance was counted. All participants diagnosed with any of the ACSD diagnoses examined had to be continuously HMO-enrolled from birth until their initial ACSD diagnosis. In addition, only participants diagnosed with an ACSD following the administration of all vaccines under study were included in the present analyses as cases. Table 1 summarizes the demographics of the various ACSD diagnoses examined.

In addition, control outcomes were selected to be generally accepted as not biologically plausibly linked to Hg exposure. They were utilized to determine whether there was outcome specificity to the potential adverse effects of thimerosal exposure or whether any potential adverse effects of thimerosal exposure were simply the potential result of an unknown bias/confounder. The control outcomes of febrile seizure (780.3x), unspecified metabolic disturbances (277.xx), or cerebral degenerations (330.xx or 331.xx) were examined. These diagnoses were analyzed independently of the ACSD diagnoses examined and independently of each other, so a child diagnosed with any one of the control outcomes may have also been diagnosed with 1 or more other control outcomes and/or 1 or more of the ACSD diagnoses examined. The outcome files (inpatient and outpatient diagnoses) from the VSD population were reviewed to find the first instance of febrile seizures, unspecified metabolic disturbances, or cerebral degenerations. If there were multiple instances of the same diagnosis in a participant, only the first instance was counted. In addition, only participants diagnosed with febrile seizures, unspecified metabolic disturbances, or cerebral degenerations following the administration of all vaccines under study were included in the present analyses as cases. Table 1 summarizes the demographics of the various control outcomes examined in the present study.

Determining Controls

To identify controls who would have only a minimal chance of receiving one of the various ACSD diagnoses examined, controls had to have been continuously enrolled from birth until the mean age of initial diagnosis of the specific ACSD diagnosis examined plus twice the standard deviation of the mean age of initial diagnosis of that diagnosis. This length of follow-up was shortened to the mean age of initial diagnosis plus the standard deviation of the mean age of initial diagnosis for the outcomes of TD and ADHD. This was done because the mean ages of initial diagnosis for TD (5.1 years) and ADD/ADHD (5.7 years) were so long that, because of the time limitations on the years of records that were available to study, requiring continuous enrollment from birth until the mean age of initial diagnosis of the specific diagnosis being assessed plus more than 1 standard deviation of the mean age of initial diagnosis would have resulted in too few controls or no controls for comparison to those cases. For the outcomes of febrile seizure, unspecified metabolic disturbances, and cerebral degenerations, controls had to have been continuously enrolled from birth until the mean age of initial diagnosis of the specific diagnosis being assessed plus twice the standard deviation of the mean age of initial diagnosis of that specific diagnosis. Table 1 summarizes the demographics of the controls examined.

Haemophilus influenzae *type b Vaccine Exposure*

The vaccine file for cases and controls was then reviewed to determine the exact dates of Hib-containing vaccine administration. Data access restrictions by the CDC precluded

Table 2. A Summary of Exposure to Hg From Hib-Containing Vaccine Administration Within the First 15 Months of Life for the Cases Diagnosed With a Studied Abnormal Connectivity Spectrum Disorder and Controls.

Group Examined (*ICD-9* Code)	25 μg Hg (%; Reference Dose)	50 μg Hg (%)	75 μg Hg (%)	100 μg Hg (%)	Odds Ratio per 25 μg Hg (95% CI; *P* Value)
ASD cases (299.xx)	26 (6.5)	28 (7)	265 (66.1)	82 (20.4)	1.493 (1.359-1.627; <.001)
Controls[a]	2327 (15.8)	1960 (13.4)	8517 (58.1)	1863 (12.7)	
Tic disorder cases (307.2x)	14 (5.8)	20 (8.3)	162 (67.2)	45 (18.7)	1.428 (1.256-1.600; <.001)
Controls[b]	2371 (14.7)	2014 (12.5)	9639 (59.9)	2074 (12.9)	
ADD/ADHD cases (314.xx)	104 (10)	82 (7.9)	676 (64.9)	179 (17.2)	1.503 (1.425-1.582; <.001)
Controls[b]	2174 (21.7)	1748 (17.5)	4997 (50)	1078 (10.8)	

Abbreviations: ADD/ADHD, attention deficit disorder/attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CI, confidence interval; Hg, mercury; Hib, *Haemophilus influenzae* type b; *ICD-9*, *International Classification of Disease, Ninth Revision*.
[a]These controls were followed to the mean age of initial diagnosis plus twice the standard deviation.
[b]These controls were followed to the mean age of initial diagnosis plus 1 standard deviation.

examining more than 1 vaccine at a time, so Hib vaccine was selected as a vaccine that many children received during the 1990s, as described below. Overall, among the cases and controls, Hg exposure was assigned as follows—25 μg Hg per dose for whole-cell diphtheria-tetanus-pertussis-Hib vaccine; 25 μg Hg per dose for diphtheria-tetanus-acellular-pertussis (DTaP)-Hib vaccine; 25 μg Hg per dose for Hib vaccine manufactured by Lederle, Praxis Biologics, Wyeth-Ayerst, or Aventis Pasteur, Inc; 0 μg Hg per dose for Hib vaccine manufactured by Merck and Company, Inc or GlaxoSmithKline; 0 μg Hg per dose for Hib-hepatitis B vaccine; and 0 μg Hg per dose for those not receiving any type of Hib-containing vaccine. The thimerosal content for each of the aforementioned vaccines was determined from the official report by the Committee on Infectious Diseases and Committee on Environmental Health of the American Academy of Pediatrics.[14]

Statistical Analyses

The StatsDirect (version 3.0.150) software was utilized for statistical analyses, and a 2-sided *P* value <.05 was considered statistically significant. The logistic regression function was used for each of the case versus control comparisons examined in this study. Outcomes in the logistic regression function were based upon the presence of diagnosis (case) or absence of diagnosis (control). Exposure in the logistic regression function was based upon each additional 25 μg Hg unit of exposure from Hib-containing vaccines with thimerosal administered within the first 15 months of life. The reference group was selected as those receiving 25 μg Hg from Hib-containing vaccines rather than 0 μg Hg from Hib-containing vaccines administered within the first 15 months of life to potentially minimize the "healthy vaccine" effect associated with those receiving vaccines in comparison to those not receiving vaccines, as further described below. The ORs calculated from the logistic regression function were determined per additional 25 μg Hg from Hib-containing vaccines administered within the first 15 months of life. The data were then separated by gender and similar analyses were undertaken. Furthermore, in order to more fully evaluate the potential relationship between Hg exposure from Hib-containing vaccines administered within the first 15 months of life and the ACSD outcomes examined, Fisher exact statistical test was utilized to determine the discrete OR for exposure to 50 μg Hg, 75 μg Hg, or 100 μg Hg from Hib-containing vaccines in comparison to a reference group receiving 25 μg Hg from Hib-containing vaccines administered within the first 15 months of life. The overall null hypotheses for each comparison in the present study was that there would be no difference in the frequency of exposure to Hg from Hib-containing vaccines between cases and controls for each of the outcomes examined.

Results

Table 2 shows the increasing dose-dependent relationships between the various ACSD diagnoses examined and Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Cases diagnosed with ASD (OR = 1.493 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.359-1.627), TD (OR = 1.428 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.256-1.600), or ADD/ADHD (OR = 1.503 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.425-1.582) in comparison to controls were significantly more likely to receive increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Overall, cases diagnosed with ASD (OR = 2.478 per 75 μg Hg exposure, 95% CI = 2.075-2.881), TD (OR = 2.283 per 75 μg Hg exposure, 95% CI = 1.767-2.800), or ADD/ADHD (OR = 2.509 per 75 μg Hg exposure, 95% CI = 2.274–2.746) were significantly more likely than controls to receive increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life.

Table 3 reveals the dose-dependent relationship between the various ACSD diagnoses examined, for male cases in comparison to male controls and female cases in comparison to female controls, and Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Male cases diagnosed with ASD (OR = 1.479 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.329-1.629), TD (OR = 1.424 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.225-1.624), or ADD/ADHD (OR = 1.518 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.426-1.610) were significantly more likely than male controls to receive

Table 3. A Summary of Exposure to Hg From Hib-Containing Vaccine Administration Within the First 15 Months of Life When Separating Male and Female Abnormal Connectivity Spectrum Disorder Cases and Controls.

Group Examined (*ICD-9 Code*)	Gender	Odds Ratio per 25 μg Hg (95% CI; *P* Value)
ASD cases (299.xx)	Males	1.479 (1.329-1.629; <.001)
	Females	1.517 (1.217-1.821; <.001)
Tic disorder cases (307.2x)	Males	1.424 (1.225-1.624; <.001)
	Females	1.428 (1.085-1.772; <.05)
ADD/ADHD cases (314.xx)	Males	1.518 (1.426-1.610; <.001)
	Females	1.453 (1.290-1.616; <.001)

Abbreviations: ADD/ADHD, attention deficit disorder/attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CI, confidence interval; Hib, *Haemophilus influenzae* type b; Hg, mercury.

increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Overall, male cases diagnosed with ASD (OR = 2.437 per 75 μg Hg exposure, 95% CI = 1.987-2.888), TD (OR = 2.273 per 75 μg Hg exposure, 95% CI = 1.676-2.871), or ADD/ADHD (OR = 2.554 per 75 μg Hg exposure, 95% CI = 2.279-2.830) were significantly more likely than male controls to receive increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Female cases diagnosed with ASD (OR = 1.517 per 25 μg Hg exposure, $P < .001$, 95% CI = 1.217-1.821), TD (OR = 1.428 per 25 μg Hg exposure, $P < .05$, 95% CI = 1.085-1.772), or ADD/ADHD (OR = 1.453 per 25 μg Hg exposure, $P < .05$, 95% CI = 1.290-1.616) were significantly more likely than female controls to receive increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life. Overall, female cases diagnosed with ASD (OR = 2.557 per 75 μg Hg exposure, 95% CI = 1.649-3.466), TD (OR = 2.285 per 75 μg Hg exposure, 95% CI = 1.254-3.316), or ADD/ADHD (OR = 2.359 per 75 μg Hg exposure, 95% CI = 1.871-2.848) were significantly more likely than female controls to receive increased Hg exposure from Hib-containing vaccines administered within the first 15 months of life.

As summarized in Table 4 for the control outcomes not biologically plausibly linked to Hg exposure from Hib-containing vaccines, the cases diagnosed with febrile seizures (OR = 0.990 per 25 μg Hg exposure, $P = .607$, 95% CI = 0.950-1.029), cerebral degeneration (OR = 0.916 per 25 μg Hg exposure, $P < .001$, 95% CI = 0.859-0.974), or unspecified disorders of metabolism (OR = 0.687 per 25 μg Hg exposure, $P < .01$, 95% CI = 0.540-0.835) were no more likely than the controls to have received increased exposure to Hib-containing vaccines administered within the first 15 months of life.

Table 5 reports, for each of the various ACSD outcomes examined and controls, the discrete-point ORs (reference exposure = 25 μg Hg) and logistic regression estimates for an additional 25 μg Hg, 50 μg Hg, or 75 μg Hg exposure from Hib-containing vaccines administered within the first 15 months of life. It was observed from the discrete point ORs that cases diagnosed with ASD (OR = 2.785, 95% CI = 1.852-4.353), TD (OR = 2.846, 95% CI = 1.644-5.332), or ADD/ADHD (OR = 2.828, 95% CI = 2.282-3.532) were significantly more likely than controls to have received an additional 50 μg Hg exposure from Hib-containing vaccines. It was observed from the discrete-point ORs that cases diagnosed with ASD (OR = 3.939, 95% CI = 2.494-6.408), TD (3.674, 95% CI = 1.973-7.268), or ADD/ADHD (OR = 3.471, 95% CI = 2.697-4.468) were significantly ($P < .001$) more likely than controls to have received an additional 75 μg Hg exposure from Hib-containing vaccines. Overall, it was observed that the discrete-point ORs and logistic regression estimates of increasing Hg dose-dependent risks were similar.

Discussion

The present prospectively collected, longitudinal, hypothesis testing case–control epidemiological study was undertaken to evaluate medical records for the potential relationship between the ACSD disorder outcomes of ASD, TD, and ADD/ADHD and Hg exposure from thimerosal-containing childhood vaccines. It was observed that Hg exposure from thimerosal-containing childhood vaccines was associated with an overall and dose-dependent increased risk of these outcomes. In addition, the data were separated by gender to determine whether gender was a mediating factor for the overall associations observed. It was observed that the significant overall increased risks observed for the ACSD disorder outcomes examined remained significantly increased even when the data were separated by the gender status of the exposed individuals. By contrast, none of the 3 *a priori* selected outcomes that were hypothesized to be not biologically plausibly linked to Hg exposure from Hib-containing vaccines showed any increase in risk following additional doses of Hg from thimerosal-containing childhood vaccines.

The results observed in the present study are consistent with and build upon previously published epidemiological studies associating Hg exposure from thimerosal-containing childhood vaccines with adverse neurodevelopmental outcomes in children. For example, previous epidemiological studies have evaluated automated medical records in the VSD database using various epidemiological methods of study. It was revealed that increased Hg doses from thimerosal-containing childhood vaccines administered to infants at specific times were associated with overall increased risks for medically diagnosed ASDs and specific delays in development.[7,15] In addition, longitudinal case–control studies have revealed a significant dose-dependent increased risk for medically diagnosed ASDs, TD, ADD/ADHD, and specific delays in development following additional doses of Hg from thimerosal-containing childhood vaccines administered to infants at specific times during infancy,[10,16] and longitudinal cohort studies have revealed a significant dose-dependent increased risk for medically diagnosed ADD/ADHD and specific delays in development following the administration of various thimerosal-containing

Table 4. A Summary of Exposure to Hg From Hib-Containing Vaccine Administration Within the First 15 Months of Life Between the Cases Diagnosed With Medical Conditions A Priori not Biologically Plausibly Linked to Postnatal Hg Exposure From Thimerosal in Hib-Containing Vaccines and the Controls.

Group Examined (*ICD-9* Code)	25 μg Hg (%; Reference Dose)	50 μg Hg (%)	75 μg Hg (%)	100 μg Hg (%)	Odds Ratio per 25 μg Hg (95% CI; *P* Value)
Febrile seizure cases (780.3x)	329 (6.5)	463 (9.2)	3022 (60)	1224 (24.3)	0.990 (0.950-1.029; .607)
Controls[a]	3952 (6.0)	4297 (6.6)	42 746 (65.4)	14 402 (22.0)	
Unspecified disorders of metabolism cases (277.xx)	30 (11.7)	47 (18.3)	112 (43.6)	68 (26.4)	0.687 (0.540-0.835; <.001)
Controls[a]	4329 (6.0)	4742 (6.6)	46 578 (65)	16 018 (22.4)	
Cerebral degeneration cases (330.xx or 331.xx)	117 (5.9)	158 (8)	1283 (64.8)	421 (21.3)	0.916 (0.859-0.974; <.01)
Controls[a]	6633 (6.2)	7184 (6.7)	66 527 (61.8)	27 261 (25.3)	

Abbreviations: CI, confidence interval; Hg, mercury; Hib, *Haemophilus influenzae* type b; *ICD-9*, *International Classification of Disease, Ninth Revision*.
[a]These controls were followed to the mean age of initial diagnosis plus twice the standard deviation.

Table 5. A Summary of Exposure to Additional Hg from Hib-Containing Vaccines in Comparison to a 25 μg Hg Hib-Containing Vaccine Reference Dose Administered Within the First 15 Months of Life for the Cases Diagnosed With a Studied Abnormal Connectivity Spectrum Disorder and the Controls by Both Discrete-Point Odds Ratio Estimates and Logistic Regression Odds Ratio Estimates.

Group Examined (*ICD-9* Code)	Statistical Approach Used	25 μg Hg (95% CI)	50 μg Hg (95% CI)	75 μg Hg (95% CI)
ASD cases (299.xx)	Discrete-point odds ratio	1.279 (0.720-2.278)	2.785 (1.852-4.353)	3.939 (2.494-6.408)
	Logistic regression odds ratio estimate	1.493 (1.359-1.627)	1.986 (1.718-2.254)	2.478 (2.075-2.881)
Tic disorder cases (307.2x)	Discrete-point odds ratio	1.682 (0.805-3.609)	2.846 (1.644-5.332)	3.674 (1.973-7.268)
	Logistic regression odds ratio estimate	1.428 (1.256-1.600)	1.855 (1.511-2.200)	2.283 (1.767-2.800)
ADD/ADHD cases (314.xx)	Discrete-point odds ratio	0.981 (0.720-1.332)	2.828 (2.282-3.532)	3.471 (2.697-4.468)
	Logistic regression odds ratio estimate	1.503 (1.425-1.582)	2.006 (1.849-2.164)	2.509 (2.274-2.746)

Abbreviations: ADD/ADHD, attention deficit disorder/attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CI, confidence interval; Hib, *Haemophilus influenzae* type b; Hg, mercury.

childhood vaccines to infants at specific times.[17,18] Finally, a previous longitudinal ecological study revealed significant dose-dependent increased risks for medically diagnosed ASDs, TD, ADD/ADHD, emotional disorders, and specific delays in development following increasing doses of Hg from various thimerosal-containing childhood vaccines administered to infants at specific times.[19]

As another example, investigators have evaluated the relationship between increased Hg exposure from thimerosal-containing childhood vaccines administered during infancy and the subsequent risk of autism, speech disorder, mental retardation, and neurodevelopmental disorder adverse events being reported to the Vaccine Adverse Event Reporting System (VAERS) database. It was revealed using a cohort study design that a cohort of children who were administered thimerosal-containing diphtheria-tetanus-acellular-pertussis (DTaP) vaccine were significantly more likely to have autism reported as an adverse event to VAERS than a cohort of children administered thimerosal-free DTaP vaccine,[7] and it was also revealed using a case–control study design that adverse events specifying the outcomes of autism speech disorder, mental retardation, and neurodevelopmental disorders in general were more likely to be reported to the VAERS database following thimerosal-containing DTaP vaccine administration than its thimerosal-reduced formulation.[20]

Still, as a further example, investigators examined the relationship between thimerosal-containing hepatitis B vaccination and the risk of adverse neurodevelopmental outcomes in the CDC's National Health Interview Survey (NHIS). It was observed that males receiving thimerosal-containing hepatitis B vaccines as neonates were significantly more likely to be diagnosed with autism than boys never vaccinated with thimerosal-containing hepatitis B or vaccinated with thimerosal-containing hepatitis B vaccine after the first month of life.[21] It was also observed that males receiving 3 doses of thimerosal-containing hepatitis B vaccines as infants were significantly more likely than males receiving no doses of thimerosal-containing hepatitis B vaccines as infants to be diagnosed with developmental disabilities.[22]

Finally, investigators have evaluated the relationship between administration of thimerosal-containing childhood vaccines to infants and the subsequent neurodevelopmental status of children at specific points during childhood. It was observed in studies in Brazil[23-25] and Poland[26] that increasing doses of Hg exposure from thimerosal-containing childhood vaccines administered to infants were associated with negative impacts on the subsequent neurodevelopmental status of children at specific points during childhood.

The above studies and the results of the present study stand in contrast to 6 previous epidemiological studies that failed to

find a consistent significant relationship between Hg exposure from thimerosal-containing vaccines and adverse neurodevelopmental outcomes in children.[27-32] These studies have been criticized in detail for their methodological limitations and conflicts of interest.[33,34]

The results observed in this study are also supported by observations of biological plausibility made from a number of previous studies examining the adverse impacts of thimerosal exposure. Investigators published a critical review on the mechanisms by which limited thiol availability, abnormal sulfation chemistry, and decreased glutathione reserve capacity in children diagnosed with ACSD outcomes could make them more susceptible to the toxic effects of thimerosal routinely administered as part of childhood immunization schedules.[35] In addition, another critical review examined the adverse impacts of thimerosal exposure on human development.[36]

The mechanisms underlying the loss of neuronal connectivity from Hg exposure are multifold.[37] First, as described by Leong et al,[38] Hg binds to the tubulin/microtubule structure of the axon and causes the axon to degenerate. Second, neurons are damaged by Hg-induced oxidative stress.[37] Third, Hg induces neuronal excitotoxicity. For instance, Xu et al[39] found that Hg triggered an overactivation of *N*-methyl-D-aspartate receptors, which lead to cytoskeleton instability. Specifically, they found that Hg enhanced the amplitude and frequency of synaptic inward currents and increased spontaneous synaptic potentials followed by sustained membrane depolarization. They also found that Hg triggered a 2- to 5-fold sustained rise in intracellular calcium concentration. Others have found that Hg is linked to excitotoxicity by causing excessive accumulation of extracellular glutamate.[40] Fourth, Hg damages the neurons by inhibition of protein synthesis and methylation.[37] Fifth, Hg damages the neuronal mitochondria, which can result in neuronal degeneration.[6] These direct effects of Hg cause neuronal loss and subsequent loss of neuronal connectivity.

There are also indirect effects of Hg and these may be as damaging as the direct effects. Mercury can indirectly cause neuronal loss by triggering brain inflammation by causing the release of pieces of damaged neurons (debris) and by lowering brain glutathione levels. The resultant debris from the direct effects of Hg combined with lowered glutathione levels can result in sustained neuroinflammation. In situations of sustained inflammation over time, microglia can begin to engulf healthy tissue, particularly synapses. This results in further loss of connectivity. Acting like Pac Man, the activated microglia can cause loss of connectivity in their attempt to manage the direct damage to neurons from Hg.[41]

For many of the mechanisms just outlined, large, long-range neurons/axons are particularly vulnerable. As described by Wang and Michaelis,[42] a main factor in selective neuronal vulnerability, and particularly in oxidative stress, is the physical size of neurons. These authors stated that, for the most part, vulnerable neurons are large in size, with axons projecting over long distances to their targets. The reasons for the susceptibility of large, long-range neurons include the high demand for energy and mitochondrial activity, dependence on long-distance axonal transport, high content of neurofilaments, and a relatively large surface area for increased exposure to toxicants in the extracellular environment. Mattson and Magnus[43] found similar reasons for large, long-range neuronal vulnerability. They stated that large projection neurons have a high-energy requirement, reliance on axonal transport for sustained function, and a large cell surface area that increases exposure of the cells to toxicants. As described by Cavanagh,[44] neurons with long axons are unique among cells in having to maintain a very large area of membrane, and in conditions of energy deprivation or deprivation of antioxidant substances such as glutathione or when the transport of materials within the neuron is physically obstructed, the system may break down and the longest fibers will suffer first. Studies indicate that large caliber axons (which tend to be the long-range axons) are selectively vulnerable to Hg.[45]

The loss of these large, long-range neurons/axons is basically irreparable. A compensatory increase in short-range axons can result from long-range neurons/axons loss. In other words, short-range overconnectivity can result from long-range underconnectivity.[1]

A critical review has examined the underlying neuropathological basis by which Hg exposure may induce the ACSD outcomes of ASD, ADD/ADHD, and TD.[1] These investigators discussed in more detail how Hg targets long-range axons, and how long-range axons are selectively vulnerable to its toxic effects. They explain that after a critical (and relatively early) developmental period these long-range axons cannot be replaced. As a consequence of decreased long-range axon connectivity, an increase in short-range axon connectivity can occur and this is what is found in ASD, ADD/ADHD, and TD. Not only do ASD, ADD/ADHD, and TD share this neuropathological finding, but it was even shown that the greater the extent of long-range axon underconnectivity and short-range axon overconnectivity, the worse the symptoms of ASD, ADHD, and TD.[1]

Strengths/Limitations

An important strength of the present study stems from the data examined within the VSD database. Investigators from the CDC recently evaluated the data within the VSD database to determine whether the VSD population might be different than the general US population.[46] These investigators reported that the VSD population is representative of the general US population on several key demographic and socioeconomic variables, and further, the absolute number of the VSD population is large enough to ensure significant representation of a broad-spectrum of the general US population.

In addition, the VSD data analyzed were based upon retrospective assessment of prospectively collected medical records of children enrolled in various VSD-participating HMOs. As such, the VSD data examined were, in fact, collected independent of the study design and were generated as part of the routine health care that children received as part of their participation within their respective HMOs. As a consequence, at

Table 6. A Summary of the Effect of Inadequate Follow-Up of Controls on the Risk of Exposure to Hg From Hib-Containing Vaccine Administration Within the First 15 Months of Life for the Cases Diagnosed With a Studied Abnormal Connectivity Spectrum Disorder and the Reduced Follow-Up Controls.[a]

Group Examined (*ICD-9* Code)	25 μg Hg (%; Reference Dose)	50 μg Hg (%)	75 μg Hg (%)	100 μg Hg (%)	Odds Ratio per μg 25 Hg (95% CI; *P* Value)
ASD cases (299.xx)	26 (6.5)	28 (7)	265 (66.1)	82 (20.4)	0.950 (0.825-1.075; >.40)
Controls	4135 (6.1)	4505 (6.6)	44 577 (65.3)	15 020 (22)	
Tic cases (307.2x)	14 (5.8)	20 (8.3)	162 (67.2)	45 (18.7)	1.050 (0.875-1.225; >.50)
Controls	3573 (7.6)	3622 (7.7)	31 174 (65.9)	8911 (18.8)	
ADD/ADHD cases (314.xx)	104 (10)	82 (7.9)	676 (64.9)	179 (17.2)	0.975 (0.900-1.063; >.70)
Controls	3236 (9.2)	2968 (8.4)	23 126 (65.4)	6003 (17)	

Abbreviations: ADD/ADHD, attention deficit disorder/attention deficit hyperactivity disorder; ASD, autism spectrum disorder; Hib, *Haemophilus influenzae* type b; Hg, mercury.
[a]These controls were followed to the mean age of initial diagnosis.

the time the medical records were generated, the health-care providers were not thinking about the potential association between vaccine exposures and adverse health outcomes.

Another important strength of the study was the study design to evaluate the potential relationship between exposure and outcomes. Among the cases examined, they had to be continuously enrolled in their HMO from birth until a medical diagnosis was made for the specific outcome under study. Similarly, among the controls examined, they had to be continuously enrolled in their HMO from birth for a sufficient time period to ensure that their diagnosis status was appropriately ascertained. In order to be able to accurately ascertain the diagnosis status of controls, for ASD cases, they had to be at least enrolled from birth to the mean age of initial ASD diagnosis plus twice the standard deviation of initial ASD diagnosis, and for the outcomes of TD and ADD/ADHD, the controls had to be enrolled from birth to the mean age of the initial diagnosis examined plus the standard deviation of the initial diagnosis examined. For the cases diagnosed with the control outcomes of febrile seizures, unspecified disorders of metabolism, or cerebral degenerations, they had to be enrolled from birth to the mean age of initial diagnosis plus twice the standard deviation of initial diagnosis. As shown in Table 6 for the various ACSD diagnoses examined, a significant reduction in the length of follow-up to only the mean age of the initial ACSD diagnosis for the various outcomes examined resulted in cases having no differences in exposure to Hg from Hib-containing vaccines administered within the first 15 months of life in comparison to controls. This phenomena clearly establishes the importance of following controls in any study of ACSD outcomes for a sufficient period of time to ensure the risk of a control being diagnosed with a "case" outcome with additional follow-up be as small as possible, subject to the limitations of the records that are accessible for study.

The specific methods used to evaluate differences in cumulative doses of Hg received within the first 15 months of life from thimerosal in Hib-containing vaccines is a further strength of the present study. The differences in Hg doses that children received is related to the diverse thimerosal content of various licensed Hib vaccines and the timing of administration of Hib vaccines as recommended by the Advisory Committee on Immunization Practices.[14,47] As a result, the present study did not examine a few children with outlier exposures to thimerosal, but, instead, the differences in thimerosal dosing reflect genuine differences in the routine childhood vaccine schedule.

However, the results observed in the present study may have a number of potential limitations. For example, the results may have occurred from unknown biases or confounders present in the datasets examined. This seems unlikely because the control outcomes of febrile seizure, cerebral degenerations, and unspecified disorders of metabolism (ie, outcomes that are not biologically plausibly linked to postnatal organic Hg exposure from thimerosal-containing vaccines) were examined, using the same methodology used for the various ACSDs examined. As shown in Table 4, no increased dose-dependent risk was observed for cases diagnosed with these outcomes in comparison to controls.

Another potential limitation of the present study is that the results observed may be the result of statistical chance. However, such a possibility would be unlikely given the limited number of statistical tests performed, the highly significant results observed (most *P* values observed were <.01), and the consistency in the direction and magnitude of the results observed. In addition, similar ORs were observed for increasing Hg exposure among cases diagnosed with the various ACSDs in comparison to controls generated from the discrete-point estimates and logistic regression estimates, as shown in Table 5.

Still, other potential limitations of the present study include the possibilities that some of the individuals examined in the VSD may have had more subtle neurological dysfunction, which was not brought to the attention of their health-care providers; health-care providers may have misdiagnosed some individuals; there may be differences in the reliability and validity of diagnoses across different health providers; or some vaccine exposures may not have been appropriately classified. In addition, it is possible that some of the Hg exposures from the Hib-containing vaccines were different than the estimated amount. For example, ActHIB vaccine, manufactured by Aventis Pasteur, was always thimerosal-free, but it was usually

reconstituted with thimerosal-containing Tripedia; but it is possible that some doses were reconstituted with just plain saline. Similarly, some Hib vaccines by Merck and Company, Inc may have had 12.5 μg Hg per dose and not the assigned 0 μg Hg per dose. In addition, it is possible that some very small number of doses of HibTITER vaccine manufactured by Lederle may have come in single-dose vials with 0 μg Hg per dose and not the assigned 25 μg Hg per dose. These limitations, while possibly present in the data examined in the current study, should not have significantly impacted the results observed because it is unclear how differential application would have occurred to the study cohorts examined based upon the thimerosal doses that the children received. Moreover, misclassification occurring in the data examined would tend to bias any results observed toward the null hypothesis, since such effects would result in children being placed in the wrong exposure and/or outcome categories examined and result in decreased statistical power to determine true potential exposure–outcome relationships.

In addition, another potential limitation of the present study is that exposures to other vaccines or other sources of Hg were not evaluated. At this time, data analysis restrictions imposed by the CDC prevent us from studying more than 1 type of vaccine that participants received, despite the fact that it is very likely that the children examined incurred other Hg exposures from other thimerosal-containing childhood vaccines. It is also likely that children examined received Hg exposures from dental amalgams, fish, or other environmental sources. Although all these other sources of Hg may play a significant role in the pathogenesis of ACSDs, these unaccounted for Hg exposures would actually tend to bias the results observed toward the null hypothesis because they potentially would confound the specific exposure classifications of Hg examined. For example, children classified as having lower Hg exposure from thimerosal-containing vaccines may have actually received high doses of Hg from other sources, and children having higher Hg exposure from thimerosal-containing vaccines may have actually received low doses of Hg from other sources, with the net result tending to minimize the magnitude of the associations observed.

It is also possible that the findings observed may be the result of other components of the vaccines studied, which, in isolation or synergistically, interacted with the Hg exposures examined. These possibilities seem remote, since multiple different brands of Hib-containing vaccines were examined (with many different amounts/types of adjuvants and trace constituents), but significant overall and dose-dependent relationships were observed among cases diagnosed with the various ACSDs examined in comparison to controls for increasing Hg exposure. Moreover, any effects of other components of vaccines working in isolation or synergistically would tend to bias the results observed toward the null hypothesis because they were not considered in the statistical analyses undertaken.

Additionally, a further potential limitation of the present study is that there may be differences in the general health status among the children examined, independent of exposure to organic Hg from Hib-containing vaccine administration. As described previously by investigators from the CDC, confounding of this sort is a general problem for studies of adverse reactions to prophylactic interventions, as they may be withheld from some individuals precisely because they are already at high risk of the adverse event (this is the so-called healthy vaccine effect), and, as a consequence, studies that fail to adequately control for such confounding factors are likely to underestimate the risk of adverse events attributable to vaccination.[48] Subsequent studies by investigators from the CDC have further examined the impact of the healthy vaccine effect and have even attempted to develop methods to minimize its effects in certain types of studies.[49] These investigators have further described that for rare and more serious outcomes, the healthy vaccine effect may be nearly impossible to quantify.[49]

The current study utilized a variant of a technique previously developed by investigators from the CDC and further expanded by other investigators to attempt to minimize the healthy vaccine effect for rare and more serious outcomes.[50,51] This involved examining groups that were all vaccinated, but the groups differed with respect to the exact vaccines received. In the current study, a partially vaccinated reference group that received 25 μg Hg exposure from Hib-containing vaccines was utilized in all of our statistical analyses. Despite our attempt to minimize the healthy vaccine effect in our analyses using a partially vaccinated reference group, as shown in Table 4, we still did observe that there was an apparent protective association for increased organic Hg exposure from thimerosal in Hib-containing vaccines for our control outcomes of cerebral degenerations and unspecified disorders of metabolism compared to controls. As a consequence, there was a healthy vaccine effect in our data. In addition, the data were further examined for the control outcomes of cerebral degenerations and unspecified disorders of metabolism where an apparent healthy vaccine effect was observed to identify whether this phenomenon was present equally for males and females. It was observed that the apparent healthy vaccine effect for the outcome of cerebral degenerations that males (OR = 0.888 per 25 μg Hg, $P < .01$, 95% CI = 0.812-0.964) were more significantly impacted than females (OR = 0.952 per 25 μg Hg, $P = .284$, 95% CI = 0.865-1.040) and the outcome of unspecified disorders of metabolism that males (OR = 0.625 per 25 μg Hg, $P < .001$, 95% CI = 0.433-0.818) were more significantly impacted than females (OR = 0.770 per 25 μg Hg, $P = .051$, 95% CI = 0.542-1.001). Since this phenomenon is apparent in the data examined, the significantly increased positive dose-dependent associations observed for the various ACSDs examined probably underestimate the true extent of the relationship between Hg exposure from thimerosal in Hib-containing vaccines and the subsequent various ACSD outcomes studied.

Finally, the current study has the potential limitation that analyses were not conducted to further explore the precise timing and cumulative doses of organic Hg from all thimerosal-containing childhood vaccines associated with maximum adverse consequences. In future studies, it would be worthwhile to further explore these precise-timing and

cumulative-doses phenomena. In addition, it would be valuable to evaluate other neurodevelopmental outcomes, as well as other covariates such as race, birth weight, etc, that may further affect the magnitude of the adverse effects found. Despite the limitation of not examining the aforementioned covariates in the present study, it was observed that when the data were separated by gender, there were still significant dose-dependent relationships between increasing Hg exposure from thimerosal in Hib-containing vaccines and the various ACSDs outcomes examined.

Conclusion

The present prospective, longitudinal case–control study reveals consistent and significantly increased dose-dependent associations between exposure to Hg from thimerosal in Hib-containing vaccines administered within the first 15 months of life among children (both male and female) subsequently diagnosed with the ACSD outcomes of ASD, TD, or ADD/ADHD, in comparison to controls. The present study is both consistent with and extends previous epidemiological studies in the VSD, VAERS, and NHIS databases and provides compelling additional epidemiological evidence supporting a significant relationship between increasing Hg exposure from thimerosal-containing childhood vaccines and the subsequent risk of an ACSD diagnosis. Routine childhood vaccination is an important public health tool to reduce the morbidity and mortality associated with infectious diseases.[52] However, it is also a public health imperative to end the unnecessary addition of Hg to vaccines in the form of thimerosal, based on data showing an association between its administration and the increasing risk of the adverse ACSD outcomes studied.

Authors' Note

David Geier conceptualized and designed the study, carried out the initial analyses, drafted the initial manuscript. Janet Kern and Kristin Homme critically reviewed and revised the manuscript and approved the final manuscript as submitted. Mark Geier conceptualized and designed the study, drafted the initial manuscript. All authors approved the final manuscript as submitted.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: There are no competing financial interests. Three of the four authors have been involved in vaccine/biologic litigation. Dr. Mark Geier, Mr. David Geier, and Dr. Janet Kern have been involved as consultants and expert witnesses for petitioners in the No-Fault National Vaccine Injury Compensation Program (NVICP) and have also been consultants and expert witness for plaintiffs in civil litigation. The cases that Dr. Janet Kern, Mr. David Geier, and Dr. Mark Geier participated in involved the outcome of autism, and none of the other neurodevelopmental outcomes examined in this study. Dr. Mark Geier and Mr. David Geier are currently involved in no cases, have not been involved in any cases for several years, and have no plans to be involved in any future cases. Dr. Janet Kern is not involved in any current cases and has no plans to be involved in any future cases.

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