

## HEPATITIS B VACCINATION OF MALE NEONATES AND AUTISM DIAGNOSIS, NHIS 1997–2002

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**Universal hepatitis B vaccination was recommended for U.S. newborns in 1991; however, safety findings are mixed. The association between hepatitis B vaccination of male neonates and parental report of autism diagnosis was determined. This cross-sectional study used weighted probability samples obtained from National Health Interview Survey 1997–2002 data sets. Vaccination status was determined from the vaccination record. Logistic regression was used to estimate the odds for autism diagnosis associated with neonatal hepatitis B vaccination among boys age 3–17 years, born before 1999, adjusted for race, maternal education, and two-parent household. Boys vaccinated as neonates had threefold greater odds for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life. Non-Hispanic white boys were 64% less likely to have autism diagnosis relative to nonwhite boys. Findings suggest that U.S. male neonates vaccinated with the hepatitis B vaccine prior to 1999 (from vaccination record) had a threefold higher risk for parental report of autism diagnosis compared to boys not vaccinated as neonates during that same time period. Nonwhite boys bore a greater risk.**

Universal newborn immunization with the hepatitis B vaccination was recommended in 1991 (CDC, 1991). A recent narrative review concluded that hepatitis B vaccines available since 1982 are safe and effective (Demirjian & Levy, 2009); however, safety findings from individual studies are mixed. In Vaccine Safety Datalink studies, Lewis et al. (2001) reported no evidence of a significant association between vaccination at birth and fever or neurological adverse events, Naleway et al. (2009) found an elevated, although not statistically significant, risk of immune hemolytic anemia in children vaccinated with hepatitis B vaccine, and Price et al. (2010)

reported no association between autism and vaccination with the hepatitis B vaccination during the first month of life. Additionally, Marques et al. (2007) found no association between time of hepatitis B vaccination, i.e., within 24 hrs versus 2–4 days postnatally, and neurodevelopment delays at 6 months of age. In contrast, increased risk for central nervous system inflammatory demyelination in childhood were associated with hepatitis B vaccination (Mikaeloff et al., 2009). Further, hepatitis B vaccination has been associated with acute ear infection and pharyngitis, chronic arthritis (Fisher et al., 2001), and liver problems, such as jaundice (Fisher & Eklund, 1999), as well as

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elevated risk for receipt of early intervention or special education services (EIS) (Gallagher & Goodman, 2008), in population-representative samples of U.S. children. Children with autism spectrum disorder (ASD) comprise a growing caseload for EIS in the United States (Rice et al., 2004; Cavnano, 2009). A study of incident ASD cases in California from 1990 through 2006 identified an increase from 6.2 to 42.5 per 10,000 births: a rise only partially explained by decreasing age at diagnosis and inclusion of milder cases (Hertz-Picciotto & Delwiche, 2009).

Recurrent ear infections and pharyngitis were reported by parents of children with developmental regression following either viral infection or immunization; the latter showed abnormal proinflammatory responses (Jyonouchi et al., 2001). Data suggested that aberrant innate immune responses of autistic children might enhance susceptibility to adverse reactions to vaccines (Jyonouchi et al., 2001). During the first 6 mo of life, the immature innate immune response develops a balance between T helper (Th) 1 and Th2 cytokines (Marodi, 2002). Th1–Th2 imbalances were also reported in autistic children (Gupta et al., 1998; Ashwood et al., 2006). Skewed cytokine production may result in or exacerbate autoimmune disease (Hemdan et al., 2007). Studies reported circulating anti-brain autoantibodies in 30 to 70% of autism subjects (Ashwood & Van de Water, 2004). Singh (2009) cited evidence of brain myelin basic protein autoantibodies in children with autism and suggested autoimmune autistic disorder (AAD) as a major subset of autism. Li et al. (2009) reported significantly increased proinflammatory cytokines with elevated Th1/Th2 ratio in the brain tissue of patients with autism (Li et al., 2009). In vitro findings showed increased proinflammatory cytokine responses in children with autism compared to controls; this dysfunctional innate immune response might alter long-term neuroimmune development and contribute to autism pathophysiology (Enstrom et al., 2009).

Boys have more than a fourfold risk for autism compared to girls (Fombonne, 2003).

In previous studies, black race has been shown to be associated with increased risk for autism (Bhasin & Schendel, 2007; Croen et al., 2002; Hillman et al., 2000). In contrast, a recent national study found that nonwhite children showed decreased risk for autism diagnosis (Kogan et al., 2009). Black race has also been associated with later autism diagnosis (Mandell et al., 2009); thus, autism prevalence may have been underreported in younger black children. Higher maternal education level has been associated with greater likelihood of autism diagnosis (Croen et al., 2002). Absence of one parent was shown to be a risk factor for delay in vaccination (Dombkowski et al., 2004), as well as decreased risk for autism diagnosis (Kogan et al., 2009).

The relationship between hepatitis B vaccination during the first month of life and autism, typically diagnosed at 3 years of age (Filipek et al., 2000), has not been evaluated using a probability sample of U.S. children. The objective of the current study was (1) to evaluate the association between hepatitis B vaccination of male neonates and autism diagnosis among boys age 3 to 17 years of age born before 1999, and (2) to adjust for potentially confounding influences of race/ethnicity, maternal education, and number of parents in household. The rationale for birth year restriction is to control for variations in exposures to vaccine mercury (Hg) content. In July 1999, the American Academy of Pediatrics (AAP) and the U.S. Public Health Service (USPHS) called for vaccine manufacturers to eliminate or reduce the Hg content of vaccines (AAP & USPHS, 1999). This action was, in large part, a response to the Food and Drug Administration (FDA) assessment that U.S. infants fully vaccinated during the first 6 mo of life may have been exposed to cumulative Hg at levels exceeding U.S. EPA safety recommendations (Ball et al., 2001). The U.S. EPA recommended limit for methyl-Hg dietary intake is 0.1  $\mu\text{g}/\text{kg}$  body weight/d (Ball et al., 2001). Applying this formula to a newborn with a normal birth weight of 3.0 kg, the safe Hg limit would be computed as  $0.1 \mu\text{g}/\text{kg}/\text{d} \times 3.0 \text{ kg} \times 1 \text{ d} = 0.3 \mu\text{g}$ . The FDA calculated 12.5  $\mu\text{g}$  ethyl-Hg per 0.5-ml dose of

thimerosal-containing hepatitis B vaccine (Ball et al., 2001). For a 1-day-old baby weighing 3.0 kg, this represents more than 40 times the safe limit for dietary Hg intake. In the current study, it was postulated that among boys born before 1999, there is a difference in the risk for autism diagnosis among boys vaccinated with the hepatitis B vaccination during the first month of life compared to later- or never-vaccinated boys.

## METHODS

The National Health Interview Survey (NHIS) uses a complex sample design that entails stratification, clustering, and multistage sampling methods and sampling weights to generate a probability sample that is representative of the U.S. population (NCHS, 2002a). Data were obtained from NHIS electronic immunization and sample child files for 1997 through 2002. Observations were limited to children included in the immunization file whose responsible household members were interviewed in their homes by trained NHIS survey interviewers. The parent sample contained 15,402 children in 1997; 14,775 in 1998; 13,881 in 1999; 14,618 in 2000; 14,709 in 2001; and 13,611 in 2002.

In order to conduct statistical analysis on the relevant subsamples in accordance with the complex survey design, SAS version 9.2 software was used to create domains stratified by gender, age group, and availability of the vaccination record. The resulting domain, or subsample, of primary interest included boys ages 3 through 17 years with a vaccination record available; this domain was further restricted to birth prior to 1999. Vaccination with the hepatitis B vaccine during the first month of life was determined by subtracting birth month and year from the first hepatitis B vaccination month and year. Birth month and year was equal to vaccination month and year for observations identified as having been vaccinated as neonates. A dual blank for month and year of vaccination was coded as no vaccination if the NHIS interviewer counted a total of zero hepatitis B vaccinations for that

individual. Refusals to answer or responses of "don't know," values not ascertained, or only month or year but not both indicated were coded as missing data. Either trained NHIS interviewers transcribed the vaccination dates directly from the vaccination record or the child's responsible household member read from the record in a face-to-face interview with NHIS staff (NCHS, 2002b). Subjects vaccinated during the first month of life were compared to subjects never vaccinated with the hepatitis B vaccine or vaccinated after the first month of life.

The outcome variable was a dichotomous (yes/no) variable created in response to the following survey question and presentation of a card with a choice of diagnoses: "Looking at this list, has a doctor or other professional ever told you that [sample child's name] had any of these conditions . . . ( i.e., autism)?" Refusals to answer, responses of "don't know," and missing values were counted as missing data. Non-Hispanic white race was defined by survey respondent reports of white race and non-Hispanic ethnicity; all others were categorized as nonwhite. Two-parent household was also a dichotomous variable representing survey respondent report of both parents in the household; answers refused, unknown, or not ascertained were coded as missing; all other responses were coded as non-two-parent household. Maternal education was a dichotomous variable representing respondent report that the child's mother's educational attainment was at least a high school diploma. Answers not known, refused, or not ascertained were coded as missing; all other responses were coded as maternal education less than high school completion.

SAS version 9.2 was used to conduct multivariable logistic regression analysis using NHIS immunization sample weights and Taylor series linearization methods, as recommended by the National Center for Health Statistics for hypothesis testing of complex sample designs (NCHS, 2002a). Analysis by domains yielded results stratified by gender. Values of  $p$  were calculated by SAS for the Wald chi-square statistic. The criterion for significance was set at  $p \leq .05$ .

## RESULTS

### Unadjusted Findings

There were in total 193 children with autism diagnosis and 79,690 without autism diagnosis included in the parent sample, or a rate of 2.42 per 1000 children (Table 1). The ratio of autistic boys to autistic girls was 5.43:1. In the total sample, 97% of children with an autism diagnosis were age 3 years and older. Sixty-one percent of children with an autism diagnosis were non-Hispanic white, compared to 60% of children without an autism diagnosis; this difference in unweighted proportions was not statistically significant. Sixty-one percent of children with an autism diagnosis and 68% of children without an autism diagnosis lived in a two-parent household; this difference in proportions was statistically significant. Among children with an autism diagnosis, 85% of mothers were high school educated or higher, compared to 79% of children without an autism diagnosis; this was also a statistically significant difference.

In the subsample limited to boys 3 to 17 years of age with a vaccination record, and whose first vaccinations were received before 1999, autism prevalence was greater, 4.32 per 1000 boys (Table 2a). There were no statistically significant differences between the unweighted proportions of boys with and without autism diagnosis, by race, two-parent household, or maternal education. Boys

vaccinated with the hepatitis B vaccine during the first month of life comprised 29% of children with autism diagnosis (9 of 31), and 17% of children without autism diagnosis (1258 of 7368). Non-Hispanic white children comprised 48% of children with autism diagnosis and 59% of children without autism diagnosis. Of the nine children with autism diagnosis who were vaccinated during the first month of life, four were white, three were black, one was American Indian, and one was of other/multiple ethnic origin; none were Asian (Table 2b). Fifty-eight percent of children with autism diagnosis lived in a two-parent household, compared to 71% of children without autism. The proportions of children of mothers with at least a high school education were similar between subgroups with (75%) and without (78%) autism diagnosis.

Univariate logistic regression using sample weights showed that boys who received the first hepatitis B dose during the first month of life had 2.82-fold greater odds for autism diagnosis (Table 2a). Non-Hispanic white boys were at 59% lower odds for autism diagnosis compared to nonwhite boys. Boys from two-parent households were at 66% lower odds for autism diagnosis relative to boys from households without two parents. Having a mother with at least a high school education was not statistically associated with odds for autism diagnosis.

**TABLE 1.** Sample Characteristics, All Children on Immunization File, Both With and Without Shot Record, NHIS 1997–2002

Characteristic	Children with autism, <i>n</i> = 193		Children without autism, <i>n</i> = 79,690	
Has vaccination record	45	23%	21,359	27%
Age 0–2 years	5	3%	14,519	18%
Age 3–17 years	188	97%	65,171	82%
Male	163	84%	40,966	51%
Non-Hispanic white	117	61%	47,692	60%
Two parent household	117 <sup>a</sup>	61%	54,486 <sup>b</sup>	68%
Maternal education, high school or higher	157 <sup>c</sup>	85%	58,717 <sup>d</sup>	79%

Note. For totals superscripted a–d, observations with missing values for indicated variables were excluded.

<sup>a</sup>*n* = 192.

<sup>b</sup>*n* = 79,667.

<sup>c</sup>*n* = 184.

<sup>d</sup>*n* = 74,480.



**TABLE 2A.** Subsample Characteristics and Univariate Logistic Regression Results for Odds for Autism, Boys Aged 3–17 Years, Born Prior to 1999, With Shot Record, Only, NHIS 1997–2002

Characteristic	Children with autism ( <i>n</i> = 33), number (%)	Children without autism ( <i>n</i> = 7640), number (%)	Unadjusted, weighted odds ratio	<i>p</i> Value
Received first dose of hepatitis B vaccine during first month of life <sup>a</sup>	9 <sup>b</sup> (29%)	1258 <sup>c</sup> (17%)	2.816	.038
Non-Hispanic white	16 (48%)	4495 (59%)	0.410	.027
Two-parent household	19 (58%)	5457 <sup>d</sup> (71%)	0.343	.008
Maternal education, high school or higher	24 <sup>e</sup> (75%)	5658 <sup>f</sup> (78%)	1.546	.353

Note. For totals superscripted *b–f*, observations with missing values for indicated variables were excluded.

<sup>a</sup>Birth month and year = hepatitis B vaccination month and year, compared to later- or never-vaccinated with hepatitis B vaccine. If both month and year of vaccination were blank and total number of hepatitis B vaccinations = 0 per NHIS interviewer count, counted as no vaccination. Counted as missing if either month or year of vaccination not reported, or date of birth missing, or respondent answered “don’t know,” refused, or answer was otherwise not ascertained. Source: Vaccination record.

<sup>b</sup>*n* = 31. Vaccination year (number vaccinated): 1993 (3); 1994 (2); 1995 (1); 1996 (3).

<sup>c</sup>*n* = 7368.

<sup>d</sup>*n* = 7637.

<sup>e</sup>*n* = 32.

<sup>f</sup>*n* = 7301.

**TABLE 2B.** Autism by Ethnic Origin Subgroup and Hepatitis B Neonatal Vaccination Status, Boys Aged 3–17 Years, Born Prior to 1999, With Shot Record Only, NHIS 1997–2002

Ethnic origin subgroups	Children with autism		Children without autism	
	First hepatitis B vaccination during first month of life, number (% of <i>n</i> )	No hepatitis B vaccination during first month of life, number (% of <i>n</i> )	First hepatitis B vaccination during first month of life, number (% of <i>n</i> )	No hepatitis B vaccination during first month of life, number (% of <i>n</i> )
Total	( <i>n</i> = 9) (100%)	( <i>n</i> = 22) (100%)	( <i>n</i> = 1258) (100%)	( <i>n</i> = 6092) (100%)
White	4 (44%)	9 (41%)	906 (72%)	4546 (75%)
Black	3 (33%)	3 (14%)	142 (11%)	785 (13%)
American Indian	1 (11%)	1 (5%)	6 (<1%)	55 (1%)
Chinese	0 (0%)	1 (5%)	12 (1%)	26 (<1%)
Other Asian	0 (0%)	1 (5%)	27 (2%)	87 (1%)
Filipino	0 (0%)	0 (0%)	8 (1%)	22 (<1%)
Asian Indian	0 (0%)	0 (0%)	6 (<1%)	26 (<1%)
Other/multiple ethnic origin	1 (11%)	7 (32%)	151 (12%)	545 (9%)

Note. Eighteen observations missing data on ethnic origin subgroups. Due to rounding error, percentages may not total to 100.

**Adjusted Findings**

Boys age 3 to 17 years (born before 1999 with a vaccination record) who received the first dose of hepatitis B vaccine during the first month of life had 3-fold greater odds for autism diagnosis (*n* = 30 with autism diagnosis and 7044 without autism diagnosis; OR = 3.002; 95% CI = 1.109, 8.126), relative to boys either vaccinated later or not at all, adjusted for race, family structure, and maternal education (Table 3). Non-Hispanic

white boys were 64% less likely to have autism diagnosis (OR = 0.357; 95% CI = 0.145, 0.880) and boys from two-parent households were 70% less likely to have autism diagnosis (OR = 0.304; 95% CI = 0.123, 0.749). Boys of mothers with at least a high school education were twofold more likely to have autism diagnosis; however, these odds were insignificant (OR = 2.320; 95% CI = 0.854, 6.303). Exclusion of the maternal education covariate from the model showed similar

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**TABLE 3.** Multivariate Logistic Regression Results for Odds For Autism Diagnosis,<sup>a</sup> Boys Aged 3–17 Years, Born Prior to 1999, With Shot Record, Only, NHIS 1997–2002

Parameter	Adjusted, weighted odds ratio	<i>p</i> Value	95% CI
Received first dose of hepatitis B vaccine during first month of life <sup>b</sup>	3.002	.031	1.109, 8.126
Non-Hispanic white	0.357	.025	0.145, 0.880
Two-parent household	0.304	.010	0.123, 0.749
Maternal education, high school or higher	2.320	.099	0.854, 6.303

<sup>a</sup>*n* = 30 with and 7044 without autism diagnosis; observations with missing values were excluded.

<sup>b</sup>Source: Vaccination record. Counted as missing if either month or year of vaccination was not reported, or date of birth missing, or respondent answered “don’t know,” refused, or answer was otherwise not ascertained; if both month and year of vaccination were blank and total number of hepatitis B vaccinations = 0 per NHIS interviewer count, counted as no vaccination.

effects estimates and *p* values. The convergence criterion was satisfied for all models, thus providing no indication to question the validity of model fit.

## DISCUSSION

Results support the hypothesis that, among boys born before 1999, there is a difference in the OR for autism diagnosis in boys vaccinated with the hepatitis B vaccination during the first month of life compared to later- or never-vaccinated boys. Our finding of increased risk for autism diagnosis among male neonates vaccinated with the hepatitis B vaccine is novel. It was previously shown, however, that vaccination with the triple series hepatitis B vaccine was associated with elevated risk of developmental disability, using receipt of early intervention or special education services (EIS) as a surrogate measure (Gallagher & Goodman, 2008). Of note, both of these U.S. probability sample-based studies demonstrated a paradoxically protective effect among girls. In the current study, however, there were only nine observations with autism diagnosis who were female—a likely contributing factor to the insignificant results, and results were borderline significant. In light of this paradox and small sample size, larger epidemiological studies stratified by gender are merited.

In response to previously reported findings of an association between hepatitis B vaccination and liver problems using the NHIS

database (Fisher & Eklund 1999), Evans and London (1999) suggested testing for specificity of exposure–disease association. In other words, might hepatitis-B vaccinated children also show greater OR for health problems other than autism? Similarly, van Damme et al. (2000) suggested that selection bias might play a role because parents of children diagnosed with a medical condition would be more likely to seek medical advice and preventive care for their child. First, to test for disease specificity of the outcome affected by the exposure, parental report of any one or more of a group of outcomes with no known relation to autism, autoimmunity, or vaccines—i.e., Down’s syndrome, cystic fibrosis, cerebral palsy, congenital or other heart problems—was substituted for autism diagnosis in the multivariate logistic regression model; null effects were found. Specificity of the exposure was also tested by separately substituting the varicella and measles–mumps–rubella vaccinations for the neonatal hepatitis B vaccination in the model for autism; again, significant associations were absent. Further, absence of a two-parent household was reported as a risk factor for delayed vaccination (Dombkowski et al. 2004). Therefore, to address potential confounding bias attributable to the possibility that children in two-parent households would be more likely to seek and receive both early vaccination and developmental evaluation, multivariable analysis adjusted for this factor. In the current study, however, a two-parent household

was protective against autism diagnosis. These findings provide evidence against selection bias associated with medical attention-seeking behavior.

Because the current study's sample represents infants born prior to the manufacture of thimerosal-free vaccines, questions are raised regarding the possible adverse effects of the vaccine preservative thimerosal. Of note, the relationship between neonatal hepatitis B vaccination and autism diagnosis among boys was also examined without restricting birth year to before 1999, and it was found that the association became marginally significant, and attenuated. There was one observation with autism diagnosis born in the later period, and this single observation was unvaccinated during the neonatal period. Thus, there is insufficient sample size to evaluate vaccination exposure before and after the availability of thimerosal-free vaccines.

There are contradictory findings reported in the literature regarding associations between Hg exposure and autism. Although an autism case-control study examined Hg-induced autoimmunity, as indicated by antibodies to metallothionein, a metal-detoxifying protein, and found no significant difference (Singh & Hanson 2006), a prospective study of U.S. children aged 2 to 16 years found associations between autism and biomarkers of Hg toxicity, i.e., urinary porphyrins (Geier & Geier, 2007). Young et al. (2008) found associations between neurodevelopmental disorders and increased exposures to thimerosal-containing vaccines (TCV), yet this study was limited by its ecological design. On the other hand, a recent Vaccine Safety Datalink (VSD) study reported no association between TCV, including the hepatitis birth dose, and autism (Price et al., 2010), supporting the findings of an earlier VSD study (Verstraeten et al., 2003). The Price et al. (2010) study assumed clerical error for records with the same vaccinations given within 15 days of the first hepatitis B dose received during the 1st month of life and within 30 days of Hib, DTP, DTaP vaccine receipt, and so, excluded duplicate exposures (Price et al., 2009). Mell et al. (2005), however,

found that 11.6% of VSD children had an extra immunization and, in a study using the 1997 NHIS, Feikema et al. (2000) reported an extraimmunization rate of 21% of U.S. children, with a rate of 4.9% for extraimmunization with the hepatitis B vaccine. Additionally, the authors of the earlier VSD study acknowledged that neonatal hepatitis B vaccination dose might have been incompletely ascertained (Verstraeten et al., 2003). Therefore, it is possible, although uncertain, that exposure misclassification might have biased results of both VSD studies toward the null. The Institute of Medicine (IOM, 2004) concluded that there is no causal association between TCV and autism based upon reviews of the Verstraeten et al. (2003) study, as well as cohort studies from the United Kingdom, Denmark, and Sweden, countries without recommendations for universal newborn hepatitis B vaccination (EUVAC.net, 2010). Each of the previous studies has a unique set of advantages and limitations. A major limitation of the current study is that, due to study design, it was not possible to test specific hypotheses regarding the risk attributable to specific vaccine component exposures (e.g., thimerosal, aluminum adjuvant, yeast protein, or the vaccine antigen itself). Our finding suggests an association; however, a large-scale, case-control study of two U.S. birth cohorts, i.e., one of children born before 1999, and the other of children born during or after 2003, when the last lot of TCV expired (CDC 2009), would be necessary to compare birth cohorts with and without thimerosal-containing hepatitis B vaccine exposure.

Another notable finding is the decreased OR for autism diagnosis among non-Hispanic white children compared to nonwhite children. Although consistent with findings in California (Croen et al., 2002), Atlanta, GA (Bhasin & Schendel 2007), and Missouri (Hillman et al., 2000), this racial disparity was not found in our previous study (Gallagher & Goodman, 2008), likely due to issues of access to EIS among some nonwhite populations (Bailey et al., 2004). Further, Kogan et al. (2009) showed decreased OR for autism diagnosis

among nonwhite children; however, results were not limited to boys. African-American male neonates are at increased risk for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and hyperbilirubinemia (Kaplan et al., 2004), and hyperbilirubinemia has been associated with an almost fourfold risk of infantile autism (Maimburg et al. 2008); however, the relation between G-6-PD deficiency and autism is uncertain and merits further research. Because greater hepatitis B surface antigen prevalence was found among pregnant Asian women in the United States compared to other race/ethnic groups (Euler et al. 2003), it is plausible that Asian neonates are more likely to have been vaccinated at birth; however, in this U.S. probability sample, none of the male children of Asian ethnicity vaccinated as neonates reported an autism diagnosis (Table 2b).

A limitation is that data were not available on emerging autism risk factors, e.g., familial history of an autoimmune disorder (Sweeten et al., 2003; Molloy et al., 2006); aberrant metabolic function, e.g., impaired methylation (James et al., 2004), porphyrin biomarkers of metal inhibition of the heme synthesis pathway (Austin & Shandley, 2008; Nataf et al., 2006; Geier & Geier, 2007), early antibiotic use (Adams et al., 2007); genetic variants among subjects of European ancestry (Wang et al., 2009); gamma-aminobutyric acid (GABA) receptor downregulation (Fatemi et al., 2009); and jaundice (May-Benson et al., 2009; Maimburg et al., 2008). Epidemiological associations may reflect common susceptibilities rather than causation; however, research that investigates the relationships between these risk factors, hepatitis B vaccination, and autism may increase our understanding of the etiology of this otherwise idiopathic disorder. Further, a review of the evidence suggests that interactions among multiple factors may result in postnatal neuronal damage and autism in susceptible children (Kern & Jones, 2006), rather than any one single factor.

In the current study, analysis was limited to children with a vaccination record in order

to minimize parent recall bias, as the vaccination record is more reliable than parent recall for individual vaccines (Bolton et al., 1998). On the other hand, parent report of autism diagnosis may be subject to case ascertainment bias, as diagnosis was not medically confirmed. Consequently, no data was available to differentiate specific autism spectrum diagnoses such as Asperger syndrome from low-functioning autism, or phenotypes such as autoimmune autistic disorder (Singh 2009), delays-plus-regression autism (Ozonoff et al. 2005), and autism-epilepsy (Tuchman et al. 2009). The autism diagnosis prevalence rate of 2.42 per 1000 children in the NHIS immunization sample is less than the *Morbidity Mortality Weekly Report (MMWR)* surveillance summary reported average rate for ASD among 8-year-olds in 2000: 6.7 per 1000 per children, with a range of 4.5 to 9.9, for 6 sites in the United States; sites included metropolitan areas (Rice, 2007) where children may have had greater access to autism diagnostic evaluation services. Differences between parental report of autism diagnosis versus records abstraction, sample age ranges, and use of a national probability versus a multisite sample likely contribute to this discrepancy. Further, the *MMWR* cited recent "best estimates" of between 2.0 and 6.0 autism cases per 1000 children (Rice, 2007), and the NHIS immunization file rate is within this range, as is the autism diagnosis rate among this study's subsample of boys 3 to 17 years of age (4.32 per 1000 boys). In the current study, a greater proportion of children without autism diagnosis had vaccination records. Because observations without a vaccination record were excluded, autism diagnosis prevalence may be underestimated. The percentage of mothers with at least a high school diploma dropped from 85% in the autism subgroup of the parent NHIS immunization sample to 75% in the subsample. Lower maternal education level has been associated with greater likelihood of having a vaccination record at home (Suarez et al., 1997); thus, the effect of maternal education may have been underestimated. Incomplete and missing data may introduce



bias in the analysis and is a notable limitation of the current study. Further, there may be unmeasured confounders associated with missing vaccination data or possible cohort effects, such as birth prior to/after universal hepatitis B recommendation; small sample size, however, precluded further stratified assessments.

The ratio of ASD boys to ASD girls in the NHIS sample was 5.43:1, quantitatively higher than a previously reported estimate of 4.3:1 (Fombonne, 2003). Most children with a diagnosis of ASD were older than 3 years of age—a finding consistent with the literature (Filipek et al., 2000). Overall, our descriptive findings show consistencies with previous reports.

As with all cross-sectional secondary data analyses, causality cannot be determined, and this study is subject to bias from unmeasured or uncontrolled confounding factors. Despite these limitations, the results of the study indicate that U.S. male neonates vaccinated with the hepatitis B vaccine prior to 1999 incurred a threefold greater risk for autism diagnosis. Nonwhite boys bore a disproportionate burden. In light of the dearth of large-scale studies that evaluate the long-term risks of neonatal hepatitis B vaccination, and recent findings of uncertain long-term protection against the hepatitis B virus among children vaccinated at birth (Giambi et al., 2008; Bialek et al., 2008), risk-benefit analysis may shed additional insights. Our findings do not suggest that the risks of autism outweigh the benefits of vaccination; however, future research into hepatitis B vaccination scheduling is warranted.

## REFERENCES

- American Academy of Pediatrics and U.S. Public Health Service. 1999. Joint Statement of the American Academy of Pediatrics (AAP) and the United States Public Health Services (USPHS). *Pediatrics* 104:568–569.
- Adams, J. B., Romdalvik, J., Ramanujam, V. M. S., and Legator, M. S. 2007. Mercury, lead, and zinc in baby teeth of children with autism versus controls. *J. Toxicol. Environ. Health A* 70:1046–1051.
- Ashwood, P., and Van de Water, J. 2004. Is autism an autoimmune disease? *Autoimmun. Rev.* 3:557–562.
- Ashwood, P., Wills, S., and Van de Water, J. 2006. The immune response in autism: a new frontier for autism research. *J. Leukocyte Biol.* 80:1–15.
- Austin, D. W., and Shandley, K. 2008. An investigation of porphyrinuria in Australian children with autism. *J. Toxicol. Environ. Health A* 71:1349–1351.
- Bailey, D. B., Hebbeler, D., Scarborough, A., Spike, D., and Mallik, S. 2004. First experiences with early interventions: A national perspective. *Pediatrics* 113:887–896.
- Ball, L. K., Ball, R., and Pratt, R. D. 2001. An assessment of thimerosal use in childhood vaccines. *Pediatrics* 107:1147–1154.
- Bhasin, T. K., and Schendel, D. 2007. Socio-demographic risk factors for autism in a U.S. metropolitan area. *J. Autism Dev. Disorders* 37:667–677.
- Bialek, S. R., Bower, W. A., Novak, R., Helgenberger, L., Auerbach, S. B., Williams, I. T., and Bell, B. P. 2008. Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. *Pediatr. Infect. Dis. J.* 27:881–885.
- Bolton, P., Holt, E., Ross, A., Hughart, N., and Guyer, B. 1998. Estimate vaccination coverage using parental recall, vaccination cards and medical records. *Public Health Rep.* 113:521–526.
- Cavagnaro, A. T. 2009. *California Department of Developmental Services. Autistic spectrum disorders, Changes in the California caseload, An update: June 1987–June 2007.* Updated 2009 July 21; accessed 2009 October 31. <http://www.dd.ca.gov/autism/docs/Autism/home.cfr>
- Centers for Disease Control. 1991. Hepatitis B virus: A comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm. Rep.* 40(RR-13):1–25.

- Centers for Disease Control. 2008. *Timeline: Thimerosal in vaccines (1999–2008)*. Accessed 2009 May 10. <http://www.cispimmunize.org>
- Croen, L. A., Grether, J. K., and Selvin, S. 2002. Descriptive epidemiology of autism in a California population: Who is at risk? *J. Autism Dev. Disorders* 32: 217–224.
- Demirjian, A., and Levy, O. 2009. Safety and efficacy of neonatal vaccination. *Eur. J. Immunol.* 39:36–46.
- Dombkowski, K. J., Lantz, P. M., and Freed, G. L. 2004. Risk factors for delay in age-appropriate vaccination. *Public Health Rep.* 119:144–155.
- Enstrom, A. M., Onore, C. E., Van de Water, J. A., and Ashwood, P. 2009. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain Behav. Immun.* doi: 10.1016/j.bbi.2009.08.001.
- Euler, G. L., Wooten, K. G., Baughman, A. L., and Williams, W. W. 2003. Hepatitis B surface antigen prevalence among pregnant women in urban areas: Implications for testing, reporting, and preventing perinatal transmission. *Pediatrics* 111:1192–1197.
- EUVAC.NET. 2010. *EUVAC.NET, A surveillance community network for vaccine preventable infectious diseases*. <http://www.euvac.net/graphics/euvac/background.htm>. Accessed 7 January 2010.
- Evans, A. A., and London, W. T. 1999. Letters: Hepatitis B vaccine and liver problems in U.S. children less than 6 years old. *Epidemiology* 10:790–791.
- Fatemi, S. H., Reutiman, T. H., Folsom, T. D., and Turas, P. D. 2009. GABA(A) receptor downregulation in brains of subjects with autism. *J. Autism Dev. Disorders* 39:223–230.
- Feikema, S. M., Klevens, R. M., Washington, M. L., Barker, L. 2000. Extraimmunization among US children. *JAMA* 283:1311–1317.
- Filipek, P. A., Accardo, P. J., Ashwal, S., Baranek, G. T., Cook, E. H., Dawson, G., Gordon, B., Gravel, J. S., Johnson, C. P., Kallen, R. J., Levy, S. E., Minshew, N. J., Ozonoff, S., Prizant, B. M., Rapin, I., Rogers, S. J., Stony, W. L., Teplin, S. W., Tuchman, R. F., and Volkmar, F. R. 2000. Practice parameter: Screening and diagnosis of autism: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Child Neurology Society. *Neurology* 55:468–479.
- Fisher, M. A., and Eklund, S. A. 1999. Hepatitis B vaccine and liver problems in U.S. children less than 6 years old, 1993 and 1994. *Epidemiology* 10:337–339.
- Fisher, M. A., Eklund, S. A., James, S. A., and Lin, X. 2001. Adverse events associated with hepatitis B vaccine in US children less than six years of age, 1993 and 1994. *Ann. Epidemiol.* 11:13–21.
- Fombonne, E. 2003. Epidemiological surveys of autism and other pervasive developmental disorders: An update. *J. Autism Dev. Disorders* 33:365–382.
- Gallagher, C., and Goodman, M. 2008. Hepatitis B triple series vaccine and developmental disability in US children aged 1–9 years. *Toxicol. Environ. Chem.* 90:997–1008.
- Giambi, C., Bella, A., Barale, A., Montu, D., Marchisio, M., Oddone, M., Zito, S., Rapicetta, M., Chionne, P., Madonna, E., and degli Atti, M. L. 2008. A cohort study to evaluate persistence of hepatitis B immunogenicity after administration of hexavalent vaccines. *BMC Infect. Dis.* 8:100. Accessed 22 May 2009, <http://www.biomedcentral.com/1471-2334/8/100>
- Geier, D. A., and Geier, M. R. 2007. A prospective study of mercury toxicity biomarkers in autistic spectrum disorders. *J. Toxicol. Environ. Health A.* 70:1723–1730.
- Gupta, S., Aggarwal, S., Roshanravan, B., and Lee, T. 1998. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J. Neuroimmunol.* 85:106–109.
- Hemdan, N. Y. A., Emmrich, F., Faber, S., Lehmann, J., and Sack, U. 2007. Alterations of Th1/Th2 reactivity by heavy metals. *Ann. NY Acad. Sci.* 1109:129–137.
- Hertz-Picciotto, I., and Delwiche, L. 2009. The rise in autism and the role of age at diagnosis. *Epidemiology* 20:84–90.

- Hillman, R. E., Kanafani, N., Takahashi, T. N., and Miles, J. H. 2000. Prevalence of autism in Missouri. *MO Med.* 97:159–163.
- Institute of Medicine. 2004. *IOM immunization safety review: Vaccines and autism, 2004*. Washington, DC: National Academy of Sciences, The National Academy Press. Accessed May 10, 2009. <http://www.iom.edu/?id=20155&redirect=0>
- James, S. J., Cutler, P., Melnyk, S., Jernigan, S., Janak, L., Gaylor, D. W., and Neubrandner, J. A. 2004. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am. J. Clin. Nutr.* 80:1611–11617.
- Jyonouchi, H., Sun, S., and Le, H. 2001. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J. Neuroimmunol.* 120:170–179.
- Kaplan, M., Herschel, M., Hammerman, C., Hoyer, J. D., and Stevenson, D. K. 2004. Hyperbilirubinemia among African American, glucose-6-phosphate dehydrogenase-deficient neonates. *Pediatrics* 114:e213–e219.
- Kern, J. K., and Jones, A. M. 2006. Evidence of toxicity, oxidative stress, and neuronal insult in autism. *J. Toxicol. Environ. Health B* 9:485–499.
- Kogan, M. D., Blumberg, S. J., Schieve, L. A., Boyle, C. A., Perrin, J. M., Ghandour, R. M., Singh, G. K., Strickland, B. B., Trevathan, E., and van Dyck, P. C. 2009. Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. *Pediatrics* 124:1395–1403.
- Lewis, E., Shinefield, H. R., Woodruff, B. A., Black, S. B., Destefano, F., Chen, R. T., Ensor, R., and Vaccine Safety Datalink Workgroup. 2001. Safety of neonatal hepatitis B vaccine administration. *Pediatr. Infect. Dis. J.* 20:1049–1054.
- Li, X., Chauhan, A., Sheikh, A. M., Patil, S., Chauhan, V., Li, X. M., Ji, L., Brown, T., and Malik, M. 2009. Elevated immune response in the brain of autistic patients. *J. Neuroimmunol.* 207:111–116.
- Mandell, D. S., Wiggins, L. D., Carpenter, L. A., Daniels, J., DiGiuseppi, C., Durkin, M. S., Giarelli, E., Morrier, M. J., Nicholas, J. S., Pinto-Martin, J. A., Shattuck, P. T., Tomas, K. C., Yeargin-Allsopp, M., and Kirby, R. S. 2009. Racial/ethnic disparities in the identification of children with autism spectrum disorders. *Am. J. Public Health* 99:493–498.
- Maimburg, R. D., Schendel, V. M., Bech, B. H., Olsen, J., and Thorsen, P. 2008. Neonatal jaundice: A risk factor for infantile autism? *Paediatr. Perinat. Epidemiol.* 22:562–568.
- Marodi, L. 2002. Down-regulation of Th1 responses in human neonates. *Clin. Exp. Immunol.* 128:1–2.
- Marques, R. C., Dorea, J. G., Manzatto, A. G., Bastos, W. R., Bernardi, J. V. E., and Malm, O. 2007. Time of perinatal immunization, thimerosal exposure and neurodevelopment at 6 months in breastfed infants. *Acta Paediatr.* 96:864–868.
- May-Benson, T. A., Koomar, J. A., and Teasdale, A. 2009. Incidence of pre-, peri-, and post-natal birth and developmental problems of children with sensory processing disorder and children with autism spectrum disorder. *Front. Integr. Neurosci.* 3:1–12.
- Mell, L. K., Ogren, D. S., Davis, R. L., Mullooly, J. P., Black, S. B., Shinefield, H. R., Zangwill, K. M., Ward, J. I., Marcy, S. M., Chen, R. T.: Centers for Disease Control and Prevention Vaccine Safety Datalink Project. 2005. Compliance with national immunization guidelines for children younger than 2 years, 1996–1999. *Pediatrics* 115:461–467.
- Mikaeloff, Y., Caridade, G., Suissa, S., and Tardieu, M. 2009. Hepatitis B vaccine and the risk of CNS inflammatory demyelination in childhood. *Neurology* 72:873–880.
- Molloy, C. A., Morrow, A. L., Meinzen-Derr, J., Dawson, G., Bernier, R., Dunn, M., Hyman, S. L., McMahon, W. M., Goudie-Nice, J., Hepburn, S., Minshew, N., Rogers, S., Sigman, M., Spence, M. A., Tager-Flusberg, H., Volkmar, F. R., and Lord, C. 2006. Familial autoimmune thyroid disease as a risk factor for regression in children with autism spectrum disorder: A CPEA study. *J. Autism Dev. Disorders* 36:317–324.

- Nataf, R., Skorupka, C., Amet, L., Lam, A., Springbett, A., and Lathe, R. 2006. Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity. *Toxicol. Appl. Pharmacol.* 214:99–108.
- Naleway, A. L., Belongia, E. A., Donahue, J. G., Kieke, B. A., Glanz, J. M., for the Vaccine Safety Datalink 2009. Risk of immune hemolytic anemia in children following immunization. *Vaccine* 27:7394–7397.
- National Center for Health Statistics. 2002a. *Data file documentation, National Health Interview Survey, 1997–2002*. National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD. Updated October 5, 2009; accessed October 31, 2009. [http://www.cdc.gov/nchs/nhis/quest\\_data\\_related\\_1997\\_forward.htm](http://www.cdc.gov/nchs/nhis/quest_data_related_1997_forward.htm)
- National Center for Health Statistics. 2002b. *Immunization core questions, Basic module, Version 00.3. National Health Interview Survey*. National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD. Updated October 5, 2009; accessed October 31, 2009. [http://www.cdc.gov/nchs/nhis/quest\\_data\\_related\\_1997\\_forward.htm](http://www.cdc.gov/nchs/nhis/quest_data_related_1997_forward.htm)
- Ozonoff, S., Williams, B. J., and Landa, R. 2005. Parental report of early development of children with regressive autism: The delays-plus-regression phenotype. *Autism* 5:461–486.
- Price, C., Robertson, A., Goodson, B. 2009. Thimerosal and autism. Technical report. Vol I. Bethesda, MD: Abt Associates Inc.
- Price, C. S., Thompson, W. W., Goodson, B., Weintraub, E. S., Croen, L. A., Hinrichsen, V. L., Marcy, M., Robertson, A., Eriksen, E., Lewis, E., Bernal, P., Shay, D., Davis, R. L., DeStefano F. 2010. Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism. *Pediatrics* [published online Sep 13, 2010; DOI: 10.1542/peds.2010-0309.
- Rice, C. 2007. Prevalence of autism spectrum disorders—Autism and developmental disabilities monitoring network, Six sites, United States, 2000. *MMWR Surveill. Summ.* 56:1–11.
- Rice, C., Schendel, D., Cunniff, C., and Doernberg, N. 2004. Public health monitoring of developmental disabilities with a focus on the autism spectrum disorders. *Am. J. Med. Genet.* 125C:22–27.
- Singh, V. K. 2009. Phenotypic expression of autoimmune autistic disorder (AAD): A major subset of autism. *Ann. Clin. Psychiatry* 21:148–161.
- Singh, V. K., and Hanson, J. 2006. Assessment of metallothionein and antibodies to metallothionein in normal and autistic children having exposure to vaccine-derived thimerosal. *Pediatr. Allergy Immunol.* 17:291–296.
- Suarez, L., Simpson, D. M., and Smith, D. R. 1997. Errors and correlates in parental recall of child immunizations: Effects on vaccination coverage estimates. *Pediatrics* 99:E3.
- Sweeten, T. L., Bowyer, S. L., Posey, D. J., Halberstadt, G. M., and McDougle, C. J. 2003. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics* 112:e420–424.
- Tuchman, R., Moshe, S. L., and Rapin, I. 2009. Convulsing toward the pathophysiology of autism. *Brain Dev.* 31:95–103.
- Van Damme, P., van Herck, K., and Meus, A. 2000. Letters: Hepatitis B vaccine is not associated with liver problems in U.S. children less than 6 years old. *Epidemiology* 11:364.
- Verstraeten, T., Davis, R. L., DeStefano, F., Lieu, T. A., Rhodes, P. H., Black, S. B., Shinefield, H., Chen, R. T., and Vaccine Safety Datalink Team. 2003. Safety of thimerosal-containing vaccines: A two-phased study of computerized health maintenance organization databases. *Pediatrics* 112:1039–1048.
- Wang, K., Zhang, H., Ma, D., Bucan, M., Glessner, J. T., Abrahams, B. S., Salyakina, D., Imielinski, M., Bradfield, J. P., Sleiman, P. M., Kim, C. E., Hou, C., Frackelton, E., Chiavacci, R., Takahashi, N., Sakurai, T., Rappaport, E., Lajonchere, C. M., Munson, J., Estes, A., Korvatska, O., Piven, J., Sonnenblick, L. I., Alvarez Retuerto, A. I., Herman, E. I., Dong, H., Hutman, T.,



Sigman, M., Ozonoff, S., Klin, A., Owley, T., Sweeney, J. A., Brune, C. W., Cantor, R. M., Bernier, R., Gilbert, J. R., Cuccaro, M. L., McMahon, W. M., Miller, J., State, M. W., Wassink, T. H., Coon, H., Levy, S. E., Schultz, R. T., Nurnberger, J. I., Haines, J. L., Sutcliffe, J. S., Cook, E. H., Minshew, N. J., Buxbaum, J. D., Dawson, G., Grant, S. F., Geschwind, D. H., Pericak-Vance, M. A., Schellenberg, G. D., and Hakonarson, H.

2009. Common genetic variants on 5p14.1 associated with autism spectrum disorders. *Nature*. doi: 10.1038/nature07999 [10 May 2009].

Young, H. A., Geier, D. A., and Geier, M. R. 2008. Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink. *J. Neurol. Sci.* 271:110–118.