

# A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorders

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Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal-containing vaccines/Rho(D)-immune globulin preparations; and (f) had alternate causes for their regressive ASDs ruled out. There was a significant dose-response relationship between the severity of the regressive ASDs observed and the total mercury dose children received from Thimerosal-containing vaccines/Rho (D)-immune globulin preparations. Based upon differential diagnoses, 8 of 9 patients examined were exposed to significant mercury from Thimerosal-containing biologic/vaccine preparations during their fetal/infant developmental periods, and subsequently, between 12 and 24 mo of age, these previously normally developing children suffered mercury toxic encephalopathies that manifested with clinical symptoms consistent with regressive ASDs. Evidence for

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mercury intoxication should be considered in the differential diagnosis as contributing to some regressive ASDs.

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Autism is a neurodevelopmental syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns (California Department of Developmental Services, 2003). While genetic factors are recognized as being important in the pathogenesis of autistic disorders, the role for environmental factors has received considerable attention. Researchers have previously reported that exposure to mercury can produce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autistic disorders, and these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Bernard et al., 2001, 2002; Blaxill et al., 2004; Redwood et al., 2001). Furthermore, recent research observing children's communicative, social, affective and repetitive behaviors and toy play coded from videotapes of the toddlers' first and second birthday parties revealed there are children with regressive autistic disorders that manifest between the ages of 12 and 24 mo (Werner & Dawson, 2005), a temporal period concurrent with exposure of these children to mercury from Thimerosal-containing biologics/vaccines in the U.S. standard immunization schedule.

## MATERIALS AND METHODS

### Participants

The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. This study examines the cases of nine pediatric patients with neurodevelopmental disorders who presented to the Genetic Centers of America from June 2005 through February 2006 for outpatient genetic/developmental evaluations.

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David Geier has been a consultant in vaccine/biologic cases before the no-fault National Vaccine Injury Compensation Program (NVICP) and in civil litigation. Dr. Mark Geier has been an expert witness and a consultant in vaccine/biologic cases before the no-fault NVICP and in civil litigation.

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## Procedures

A detailed history was taken for each child, and the patient's past medical records were reviewed. All of the patients' mothers reported eating fish less than once per week, the mothers had a median of six dental amalgams during their pregnancies (no mother had dental work during her pregnancy), and none of the mothers were employed in occupational settings that would have exposed their children to environmental sources of mercury. Patients had testing for hair heavy metal levels (Doctors Data, Inc. [St. Charles, IL, CLIA ID: 14D0646470]; or Genova Diagnostics [Asheville, NC, CLIA ID: 34D0655571]), urine heavy metal levels (Doctors Data, Inc., or Genova Diagnostics), fecal heavy metal levels (Doctors Data, Inc.), transsulfuration metabolites (including urinary pyroglutamic acid—Great Plains Laboratory [Lenexa, KS, CLIA ID: 17D091496]; blood reduced glutathione, plasma cysteine, and plasma sulfate—Genova Diagnostics; or serum homocysteine and plasma cystathione—LabCorp [Burlington, NC, CLIA ID: 34D065520]), and androgen metabolites (including serum testosterone and serum dehydroepiandrosterone (DHEA)—LabCorp). Each patient also had testing performed to rule-out brain structural abnormalities (computed tomography [CT] or magnetic resonance imaging [MRI] head scans) and vision and hearing abnormalities. Additionally, laboratory testing was negative for fragile X syndrome, chromosomal abnormalities (structural and numeric), subtelomere chromosome rearrangements, thyroid function abnormalities, Prader Willi syndrome/Angelman, urine organic acid abnormalities, polychlorinated biphenyl (PCB) exposure, plasma amino acid abnormalities, and Rett's syndrome (except for one child) (LabCorp). A diagnostic psychiatric interview was conducted to evaluate each child's present clinical symptoms using the criteria specified in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. (DSM-IV). Additionally, an interview was conducted to evaluate the severity of each child's autistic symptoms using the Autism Treatment Evaluation Checklist (ATEC) (Autism Research Institute, San Diego, CA). The ATEC quantitatively evaluates (using a numeric scoring system) skills in a number of areas, including speech language/communication, sociability, sensory/cognitive awareness, and health/physical/behavior.

Each of the children in the present study was determined to have autism (*International Classification of Disease*, 9th rev. 299.00) or pervasive developmental delay—not otherwise specified (299.80). It was determined that eight of nine children presented in this study had regressive autistic spectrum disorders (ASDs). The ninth child was found to have Rett's syndrome. Based upon differential diagnoses of the eight children with regressive ASDs, these regressive ASDs were the apparent result of a toxic encephalopathy (349.82) induced by Thimerosal exposure from fetal exposure to mercury from the therapeutic use of Rho(D)-immune globulin (E934.6) and/or infant exposure to mercury from the therapeutic use of pertussis vaccine, including combinations with a pertussis component

(E948.6) and/or infant exposure to mercury from therapeutic use of other unspecified vaccines and biological substances (E949.9), and/or exposure to mercury from therapeutic use of influenza vaccine (E949.6). Based on their evaluations, each of these patients has a heightened sensitivity to mercury toxicity from disturbances of sulfur-bearing amino acid metabolism (270.4) and testicular hyperfunction (257.0).

## Statistical Analyses

The statistical package in StatsDirect (Version 2.4.2) was employed to evaluate the patients with toxic encephalopathies in the present study. The unweighted least squares test statistic (assuming an exponential distribution) was employed to evaluate the autism severity percentile (as derived from the ATEC form completed for each patient) in comparison to the total mercury exposure each child received from prenatal and/or postnatal Thimerosal-containing biologics/vaccines. Additionally, the data were examined using the unweighted least squares test statistic (assuming an exponential distribution) to evaluate the age of onset of regression in comparison to (1) the autism severity percentile (as derived from the ATEC form completed for each patient) and (2) total mercury exposure each child received from prenatal and/or postnatal Thimerosal-containing biologics/vaccines. The null hypothesis for each statistical test performed was that the slope of the line would be equal to zero. A  $p$  value of  $\leq .05$  was considered statistically significant.

## RESULTS

### Case Reports

#### Case 1

A 6-yr-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a full-term spontaneous vaginal delivery, with Activity, Pulse, Grimace (reflex irritability), Appearance, Respiration (APGAR) scores of 9 at both 1 and 5 min. The child went home with his mother from the hospital following birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression, so that by 14 mo his mother reported that her child had lost his language skills.

An analysis of the child's exposure to mercury indicated that he received 237.5  $\mu\text{g}$  mercury from Thimerosal-containing childhood vaccines (the first exposure being 50  $\mu\text{g}$  mercury, from Thimerosal-containing *Haemophilus influenzae* Type b [Hib] and diphtheria-tetanus-acellular-pertussis [DTaP] vaccines administered when the child was 2 mo old) by age 24 mo. Additionally, since the mother was Rh-negative, she was administered at 28 wk gestation a Thimerosal-containing Rho(D)-immune globulin preparation that contained 10.5  $\mu\text{g}$  mercury.

The child's first hair sample, collected when he was 2 yr old, was analyzed for heavy metal elements. The reported results found undetectable levels of mercury (<0.25 ppm, reference range = nondetectable–1 ppm), whereas other metals, such as lead, were shown to be elevated outside of the reference range (0.70 ppm, reference range = nondetectable–0.5 ppm), and still other metals, such as arsenic (0.089 ppm, reference range = nondetectable–0.1 ppm) and cadmium (0.028 ppm, reference range = nondetectable–0.15 ppm), were detectable and within their respective reference ranges. At 5 yr of age, the child was given a provocation dose of oral 2,3-dimercapto-1-propanesulfonic acid (DMPS), and a urine sample was collected. The test results showed a significantly elevated urinary concentration of mercury (31 µg mercury/g creatinine, unchelated reference range <5; creatinine = 29 mg/dl, reference range = 25–180 mg/dl) and an elevated urinary concentration of lead (22 µg lead/g creatinine, unchelated reference range <15), whereas other metals such as arsenic (24 µg arsenic/g creatinine, unchelated reference range <130) and cadmium (1.4 µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

At the same time, the results from a fecal sample also showed a significant concentration of mercury (0.081 mg mercury/kg, unchelated reference range <0.05; percent water content = 87.6%, expected range = 60–85%) and an elevated fecal concentration of lead (0.6 mg lead/kg, unchelated reference range <0.5), whereas other metals such as arsenic (0.19 mg arsenic/kg, unchelated reference range <0.3) and cadmium (0.47 mg cadmium/kg, unchelated reference range <0.5) were within their respective unchelated reference ranges. Moreover, the results from a concomitant blood analysis showed that the patient had significantly reduced levels of blood reduced glutathione (19 mg/dl, reference range ≥32 mg/dl), plasma sulfate (3.7 mg/dl, reference range = 4.8–5.3 mg/dl), and plasma cysteine (2.86 mg/dl, reference range = 3.1–3.9 mg/dl).

A series of biochemical and genomic tests was ordered on the patient. The patient was found to have an elevated serum level of testosterone (17 ng/dl, reference range <10 ng/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

When the child's autistic severity was assessed using an ATEC form, the patient had severe autistic symptoms (80–89th percentile of severity), with the child being most profoundly affected in the areas of sociability, sensory/cognitive awareness, and health/physical/behavior (80–89th percentile of severity).

#### Case 2

A 6-yr-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a full-term spontaneous vaginal delivery, with good APGAR scores. The child went home

with his mother from the hospital following his birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression starting at approximately 12 mo, so that by 18 mo his mother reported that her child had lost his language skills.

An analysis of the child's exposure to mercury indicated that he received 237.5 µg mercury from Thimerosal-containing childhood vaccines (the first exposure being 12.5 µg mercury, from a Thimerosal-containing hepatitis B vaccine administered on the day of birth) by 24 mo.

The child's first hair sample, collected when he was 3 yr old, was analyzed for heavy metals. The test results indicated undetectable levels of mercury (<0.25 ppm, reference range = nondetectable–1 ppm), whereas other metals, such as lead (0.18 ppm, reference range = nondetectable–0.5 ppm), arsenic (0.039 ppm, reference range = nondetectable–0.1 ppm), and cadmium (<0.025 ppm, reference range = nondetectable–0.15 ppm), were within their respective reference ranges.

At 5 yr of age, the child was administered a provocation dose of suppository *meso*-2,3-dimercaptosuccinic acid (DMSA). The test results from a subsequent urine sample indicated a significantly elevated urinary concentration of mercury (15 µg mercury/g creatinine, unchelated reference range <5; creatinine = 12 mg/dl, reference range = 40–142 mg/dl) and a significantly elevated urinary concentration of lead (91 µg lead/g creatinine, unchelated reference range <18), whereas other metals such as arsenic (68 µg arsenic/g creatinine, unchelated reference range <130) and cadmium (less than detectable level of cadmium/g creatinine, unchelated reference range <2) were within their respective unchelated reference ranges.

Subsequently, also at 5 yr, the child had been administered a provocation dose of oral DMPS. The results from the testing of a urine sample collected subsequently indicated an elevated urinary concentration of mercury (6.8 µg mercury/g creatinine, unchelated reference range <5 µg mercury/g creatinine; creatinine = 43 mg/dl, reference range = 40 mg/dl–142 mg/dl) and lead (29 µg lead/g creatinine, unchelated reference range <5), whereas other metals such as arsenic (110 µg arsenic/g creatinine, unchelated reference range <130) and cadmium (0.7 µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

A series of biochemical and genomic tests were ordered on the patient. The patient was found to have an elevated serum testosterone level (23 ng/dl, reference range <20 ng/dl). The patient had significantly reduced level of plasma cystathionine (nondetectable µmol/dl, reference range = nondetectable–0.4 µmol/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

When the child's autistic severity was assessed using an ATEC form, the patient had severe autistic symptoms (80–89th percentile of severity), with the child being most profoundly affected in the areas of sociability, sensory/cognitive awareness, and health/physical/behavior (80–89th percentile of severity).

### Case 3

A 9-yr-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a full-term spontaneous vaginal delivery, with APGAR scores of 8 at 1 min and 9 at 5 min, respectively. The child went home with his mother from the hospital following his birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression starting at approximately 15 mo, so that his mother reported that her child had lost his language skills by 18 mo, and, by 21 mo, her son had become unpredictable and volatile (on one occasion the child had bitten his father).

By age 24 mo, the child received 162.5 µg mercury from Thimerosal-containing childhood vaccines (the first dose of mercury being 12.5 µg mercury from a Thimerosal-containing hepatitis B vaccine administered when the child was 2 wk old). Additionally, since the mother was Rh-negative, she was administered at 28 wk of gestation a Thimerosal-containing Rho(D)-immune globulin preparation that contained 42 µg mercury.

The child's first hair sample, collected when he was 4 yr old, was analyzed for toxic elements. The test results found low levels of mercury (0.01 ppm, reference range = nondetectable–1 ppm), whereas other metals such as lead (0.59 ppm, reference range = nondetectable–0.5 ppm) and cadmium (0.156 ppm, reference range = nondetectable–0.15 ppm) were shown to be elevated outside of the reference range, and still other metals such as arsenic (0.087 ppm, reference range = nondetectable–0.1 ppm) were detectable and within their respective reference ranges.

Following collection of the hair sample, the patient, still age 4, was administered an oral DMSA challenge and a postchallenge urine sample was collected. The urine sample test results showed significantly elevated urinary concentrations of mercury (15 µg mercury/g creatinine, unchelated reference range <3; creatinine = 43.4 mg/dl, reference range = 21–76 mg/dl), whereas arsenic (98 µg arsenic/g creatinine, unchelated reference range <100), lead (12 µg lead/g creatinine, unchelated reference range <15), and cadmium (1.4 µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

Similarly, when the child was 5 yr of age and still on DMSA therapy, another urine sample was collected. Its test results showed mercury persisting at significantly elevated urinary concentrations (5.4 µg mercury/g creatinine), whereas arsenic (37 µg arsenic/g creatinine), lead (8.6 µg lead/g creatinine), and cadmium (0.5 µg cadmium/g creatinine) still remained within their respective unchelated reference ranges.

At 8 yr of age, the patient had an elevated serum testosterone level (25 ng/dl, reference range <25 ng/dl).

When the now 8-yr-old child's autistic severity was assessed using an ATEC form, the test scores indicated that the patient

had severe autistic symptoms (90–99th percentile of severity), with the most profoundly affected areas being those of sociability and sensory/cognitive awareness (90–99th percentile of severity).

A series of biochemical and genomic tests was ordered on the patient. The patient was found to have significantly reduced levels of blood reduced glutathione (20 mg/dl, reference range ≥32 mg/dl), plasma cysteine (2.72 mg/dl, reference range = 3.1–3.9 mg/dl), plasma sulfate (2.9 mg/dl, reference range = 4.8–5.3 mg/dl), and serum homocysteine (5 µmol/L, reference range = 5.1–13.9 µmol/L). The genomic testing found that the patient had none of the recognized genomic abnormalities.

### Case 4

A 6-yr-old Hispanic male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a spontaneous vaginal delivery (3 wk early) with an APGAR score of 9 at 5 min. The child went home with his mother from the hospital following birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression beginning at 14–15 mo.

The child's exposure to mercury indicated that he had been administered 200 µg mercury by age 24 mo of age from Thimerosal-containing childhood vaccines (the first dose of mercury being 12.5 µg mercury from a Thimerosal-containing hepatitis B vaccine administered when the child was 1 d old).

At 3 yr old the child underwent an oral provocation with DMSA and a urine sample was collected. That sample's test results indicated significantly elevated urinary concentrations of mercury (8 µg mercury/g creatinine, unchelated reference range <3; creatinine = 25 mg/dl, reference range = 21–76 mg/dl), with arsenic (34 µg arsenic/g creatinine, unchelated reference range <100), lead (0 µg lead/g creatinine, unchelated reference range <15), and cadmium (0 µg cadmium/g creatinine, unchelated reference range <2) remaining within their respective unchelated reference ranges.

Similarly, at 5 yr of age the child, while on transdermal DMPS therapy, had a fecal sample collected. The test results showed significant concentrations of mercury (0.05 mg mercury/kg, unchelated reference range <0.05; water content = 77.6%, expected range = 60–85%).

Previous blood analysis had shown that the patient had significantly reduced levels of blood reduced glutathione (28 mg/dl, reference range ≥32 mg/dl), plasma cysteine (2.79 mg/dl, reference range = 3.1–3.9 mg/dl), and plasma sulfate (3.8 mg/dl, reference range = 4.8–5.3 mg/dl).

A series of biochemical and genomic tests was ordered on the patient. The patient was found to have an elevated serum testosterone level (23 ng/dl, reference range <10 ng/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

Using an ATEC form, the child's autistic severity scores indicated that the patient had moderate autistic symptoms (50–59th percentile of severity), with the child being most profoundly affected in the areas of sensory/cognitive awareness (80–89th percentile of severity) and sociability (60–69th percentile of severity).

#### Case 5

A 10-yr-old Caucasian male presented for outpatient care with a developmental disorder and a family history remarkable for an older brother that had been previously diagnosed with Asperger's disorder. The child's past medical history indicated that he was the product of a spontaneous vaginal delivery (3 wk early) with APGAR scores of 9 at both 1 and 5 min. The child went home with his mother from the hospital following birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression starting at 17 mo in which the child rapidly lost the ability to speak all of the words he had previously spoken.

The child's exposure to mercury indicated that he received 200 µg mercury by age 24 mo from Thimerosal-containing childhood vaccines (the first exposure being 12.5 µg mercury, from a Thimerosal-containing hepatitis B vaccine administered when the child was 1 d old).

The child's first hair sample, collected when he was 5 yr of age, was analyzed for toxic elements. Test results revealed low levels of mercury (0.23 ppm, reference range <0.4 ppm), whereas other metals, such as lead (1.5 ppm, reference range <1 ppm), arsenic (0.14 ppm, reference range <0.08 ppm), and cadmium (0.22 ppm, reference range <0.15 ppm), were shown to be elevated outside of the reference range.

Subsequently, also at age 5, the child had been administered a provocation dose of oral DMSA, and a urine sample was collected. Its test results showed a significantly elevated urinary concentration of mercury (47 µg mercury/g creatinine, unchelated reference range <3; creatinine = 21 mg/dl, reference range = 40–142 mg/dl), whereas other metals such as arsenic (21 µg arsenic/g creatinine, unchelated reference range <100), lead (8.9 µg lead/g creatinine, unchelated reference range <15), and cadmium (0.2 µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

At 6 yr of age, the child had been administered another provocation dose of oral DMSA and a postprovocation urine sample was collected. Its test results showed a significantly elevated urinary concentration of mercury (12 µg mercury/g creatinine, unchelated reference range <3; creatinine = 20 mg/dl, reference range = 40–142 mg/dl), whereas other metals such as arsenic (19 µg arsenic/g creatinine, unchelated reference range <100), lead (8.4 µg lead/g creatinine, unchelated reference range <15), and cadmium (1.1 µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

At 10 yr of age, the results from blood tests indicated the patient had significantly reduced levels of plasma cysteine (173.71 µmol/L, reference range >200 µmol/L) and plasma reduced glutathione (0.933 µmol/L, reference range >2 µmol/L).

A series of biochemical and genomic tests was ordered on the patient. The test results indicated the patient had a significantly elevated serum level of testosterone (53 ng/dl, reference range <25 ng/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

When the child's autistic severity was assessed using an ATEC form, the patient had moderate autistic symptoms (50–59th percentile of severity), with the child being most profoundly affected in the area of health/physical/behavior (70–79th percentile of severity).

#### Case 6

An 8-yr-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a full-term cesarean section (breach presentation) delivery, with APGAR scores of 8 and 9 at 1 and 5 min, respectively. The child went home with his mother from the hospital following birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression beginning at approximately 14 mo.

The child's exposure to mercury indicated that he received 137.5 µg mercury by age 24 m from Thimerosal-containing childhood vaccines (the first exposure being 12.5 µg mercury, from Thimerosal-containing hepatitis B vaccine on the day of birth).

The child's first hair sample, collected when he was 3, was analyzed for toxic elements. Test results revealed undetectable levels of mercury (<0.03 ppm, reference range <0.4 ppm), whereas other metals, such as arsenic, were shown to be elevated outside of the reference range (0.25 ppm, reference range <0.08 ppm), and still other metals, such as lead (0.22 ppm, reference range <1 ppm) and cadmium (0.11 ppm, reference range <0.15 ppm), were detectable and within their respective reference ranges.

At age 6, the child had been administered a provocation dose of oral DMSA, and a postprovocation urine sample was collected. Its test results showed a significantly elevated urinary concentration of mercury (8.7 µg mercury/g creatinine, unchelated reference range <3; creatinine = 9.7 mg/dl, reference range = 40–142 mg/dl), whereas the urinary concentration of other metals such as lead (1.2 µg lead/g creatinine, unchelated reference range <15), arsenic (20 µg arsenic/g creatinine, unchelated reference range <100), and cadmium (less than detectable µg cadmium/g creatinine, unchelated reference range <2) remained within their respective unchelated reference ranges.

Based on the results from blood analysis, the patient was also found at this time to have significantly reduced levels of

blood reduced glutathione (27 mg/dl, reference range  $\geq 32$  mg/dl), plasma sulfate (3.6 mg/dl, reference range = 4.8–5.3 mg/dl), and plasma cysteine (2.38 mg/dl, reference range = 3.1–3.9 mg/dl).

A series of biochemical and genomic tests was ordered on the patient. Based on the results from the blood testing ordered, the patient had an elevated serum level of DHEA (181 ng/dl, reference range = 53–135 ng/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

When the child's autistic severity was assessed using an ATEC form, the patient had moderate autistic symptoms (60–69th percentile of severity), with the child being most profoundly affected in the areas of sociability and health/physical/behavior (70–79th percentile of severity).

#### Case 7

A 14-y-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a full-term spontaneous vaginal delivery, with good APGAR scores. The child went home with his mother from the hospital following his birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression starting at approximately 20 mo.

The child received 125  $\mu$ g mercury by age 24 mo from Thimerosal-containing childhood vaccines (the first dose of mercury being 25  $\mu$ g mercury from a Thimerosal-containing whole-cell diphtheria–tetanus–pertussis–*Haemophilus influenzae* type b [DTaP] vaccine administered when the child was 2 mo old).

The patient was administered an oral DMPS challenge at age 14 y and a postchallenge urine sample was collected. Test results showed significantly elevated urinary concentrations of mercury (15  $\mu$ g mercury/g creatinine, unchelated reference range  $< 4$ ; creatinine = 200 mg/dl, reference range = 45–225 mg/dl), whereas other metals such as lead showed an elevation (4.6  $\mu$ g lead/g creatinine, unchelated reference range  $< 4$ ), and still other metals such as arsenic (23  $\mu$ g arsenic/g creatinine, unchelated reference range  $< 130$ ) and cadmium (0.4  $\mu$ g cadmium/g creatinine, unchelated reference range  $< 2$ ) remained within their respective unchelated reference ranges.

Subsequently, also while the child was still 14 yr old, a post-DMPS-challenge hair sample was analyzed for heavy metals. Test results revealed a significant elevation in mercury (0.87 ppm, reference range  $< 0.4$  ppm), whereas other metals such as arsenic (0.025 ppm, reference range  $< 0.08$  ppm), cadmium (0.095 ppm, reference range  $< 0.15$  ppm) and lead (0.12 ppm, reference range  $< 1$  ppm) remained within their respective reference ranges. By contrast, a previously analyzed hair sample taken prior to DMPS challenge had shown a significantly lower level of mercury (0.46 ppm, reference range  $< 0.4$  ppm).

A series of biochemical and genomic tests was ordered on the patient. The results from the blood testing ordered indicated

the patient had a borderline elevated serum testosterone level (497 ng/dl, reference range = 15–500 ng/dl). The patient had previously been shown at age 9 to have significantly reduced hair sulfur levels (45,300 ppm, reference range = 45,500–53,000 ppm) and at 13 yr old to have significantly reduced urinary pyroglutamic levels (19.25 nmol/g creatinine, reference range = 20–115 nmol/g creatinine). The genomic testing found that the patient had none of the recognized genomic abnormalities.

Based on the results from an evaluation using an ATEC form, the child's autistic severity scores showed that patient's autistic symptoms were mild (10–19th percentile of severity), with the child being most profoundly affected in the area of health/physical/behavior (40–49th percentile of severity).

#### Case 8

A 4-yr-old Caucasian male presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that he was the product of a spontaneous vaginal delivery (3 wk early) with good APGAR scores. The child went home with his mother from the hospital following birth, and made the normal developmental milestones during the first year of life. Subsequently, this child underwent a developmental regression starting at 18 mo. Mother reported that her son had regressed so far that he was left with only 3 words by 24 mo (previously, by 18 mo, he had used about 25 words).

The child's exposure to mercury indicated that he received 100  $\mu$ g mercury by age 24 mo from Thimerosal-containing childhood vaccines (the first dose/exposure of mercury being 25  $\mu$ g mercury from a Thimerosal-containing DTaP vaccine administered when the child was 2 mo old).

The child's first hair sample was collected when he was 2 yr old. The hair sample was analyzed for heavy metals. The test results revealed that the mercury level was low (0.03 ppm, reference range  $< 0.4$  ppm), while arsenic (0.036 ppm, reference range  $< 0.08$  ppm), cadmium (0.037 ppm, reference range  $< 0.15$  ppm), and lead (0.18 ppm, reference range  $< 1$  ppm) were all within their respective reference ranges.

Subsequently, starting at 3 yr old, the child was administered a treatment course of oral DMSA followed by a treatment course of transdermal DMPS. A sample of the child's hair was collected at 4 yr old and analyzed for toxic elements. The test results for this sample showed significantly elevated mercury levels (0.76 ppm, reference range  $< 0.4$  ppm), whereas all the other metals, such as arsenic (0.025 ppm, reference range  $< 0.08$  ppm), cadmium (0.037 ppm, reference range  $< 0.15$  ppm), and lead (0.19, reference range  $< 1$  ppm), remained within their respective reference ranges.

Thus, as chelation therapy was conducted on the patient, it was observed that there was a significant  $> 25$ -fold increase in hair mercury levels from a "baseline" measured at 2 to the post-chelation-initiation level measured at 4 yr of age. It was also previously observed that the patient had significantly

reduced levels of serum homocysteine (4  $\mu\text{mol/L}$ , reference range = 6.3–15  $\mu\text{mol/L}$ ), blood reduced glutathione (18 mg/dl, reference range >32 mg/dl), plasma cysteine (2.47 mg/dl, reference range = 3.1–3.9 mg/dl), and plasma sulfate (4.5 mg/dl, reference range = 4.8–5.3 mg/dl).

A series of biochemical and genomic tests was ordered on the patient. The results of the blood testing ordered found the patient had an elevated serum level of testosterone (13 ng/dl, reference range <10 ng/dl). The genomic testing found that the patient had none of the recognized genomic abnormalities.

When the child's autistic severity was assessed using an ATEC form, it was determined that patient had mild autistic symptoms (10–19th percentile of severity), with the child being most profoundly affected in the area of sensory/cognitive awareness (40–49th percentile of severity).

#### Case 9

A 9-yr-old Caucasian female presented for outpatient care with a developmental disorder and a negative family history of developmental disorders. The child's past medical history indicated that she was the product of a full-term vaginal delivery (induced for failure to progress) with APGAR scores of 7 at 1 min and 8 at 5 min. The child went home with her mother from the hospital following birth, and the parent observed that the child was different than a normal child. The child had delayed developmental milestones during the first year of life. The child did not walk until she was 2 and had only developed the ability to speak about 4 words by 18 mo of age. The child's speech never reached the "speaking in phrases" stage.

A series of biochemical and genomic tests were ordered on the patient. The results of the blood testing ordered found the patient had an elevated serum level of testosterone (62 ng/dl, reference range <30 ng/dl) and a significantly reduced level of serum homocysteine (4.5  $\mu\text{mol/L}$ , reference range = 5.1–13.9  $\mu\text{mol/L}$ ). The child tested positive for Rett's syndrome (*MECP2* mutation R294X).

Using an ATEC form to evaluate the patient, the patient was found to have severe autistic symptoms (80–89th percentile of severity), with the child being most profoundly affected in the areas of sensory/cognitive awareness and speech/language/communication (80–89th percentile of severity).

#### Statistical Analyses

Table 1 summarizes the medical evaluations undertaken for each patient examined in the present study (excluding the child diagnosed with Rett's syndrome). Figure 1 summarizes the mercury exposure (prenatal, postnatal, and total mercury) each child received, and the severity of each child's autistic disorder (excluding the child diagnosed with Rett's syndrome). Figure 1 shows a significant direct dose-response relationship between autism severity in the mercury-affected children and total mercury doses from Thimerosal-containing biologics/vaccines. Additionally, a significant inverse relationship was found

between the age of the reported onset of the child's regression (in months) and the child's autism severity score (autism severity =  $19.9e^{-0.0044(\text{reported onset age in months})}$ ,  $R^2 = 0.68$ ,  $p < .02$ ). A similar inverse relationship was found between the reported age of regression onset in months and the total mercury dose from Thimerosal-containing biologics/vaccines (reported age of regression onset in months =  $691e^{-0.089(\text{total mercury dose})}$ ,  $R^2 = .49$ ,  $p = .05$ ).

#### DISCUSSION

In considering the present case series of patients, it is evident that eight of nine children examined in this study had apparently normal births and seemed to develop normally during the first year of life. Subsequently, these children had a developmental regression that was noticed by their parents during the second year of life. The observed second-year autistic regressions are consistent in timing with that recently reported by Werner and Dawson (2005). Werner and Dawson (2005) by reviewing videotapes of children were able to discern there are children that apparently develop normally during their first year of life, and subsequently regress into an autistic state. The importance of a normal developmental period followed by regression is that it suggests autistic disorders can be acquired, and one is not necessarily born with the disorder. It was previously described that there can be a significant latency period between exposure to mercury and when one subsequently manifests with mercury toxicity. For example, Harada (1978) described in intrauterine methylmercury poisoning in Japan that no conspicuous abnormalities were seen in newborns following birth, and only beginning at mo 6 after birth did patients begin to become symptomatic of mercury toxicity.

In evaluating the patients examined in the present study (excluding the child diagnosed with Rett's syndrome), it was observed that, except for Thimerosal-containing biologics/vaccines administered during the prenatal/infant period, the children apparently had minimal exposure to mercury because none of the mothers reported eating fish more than once per week, the mothers had a median of six dental amalgams during their pregnancies (no mother had dental work during their pregnancies), and none of the mothers were employed in occupational settings that would have exposed their children to environmental sources of mercury. Following exposure to mercury during these periods, these children subsequently developed toxic encephalopathies that manifested with clinical symptoms consistent with autistic disorders. In the course of evaluating these patients, differential diagnosis generally revealed no apparent factors contributing to/causing the children's autistic disorders other than their mercury exposure. Furthermore, subsequent workup revealed that the children examined in this study had significant biochemical susceptibility to mercury exposure. Following chelation therapy to remove heavy metals from the body, each of these children presented with significantly elevated concentrations of

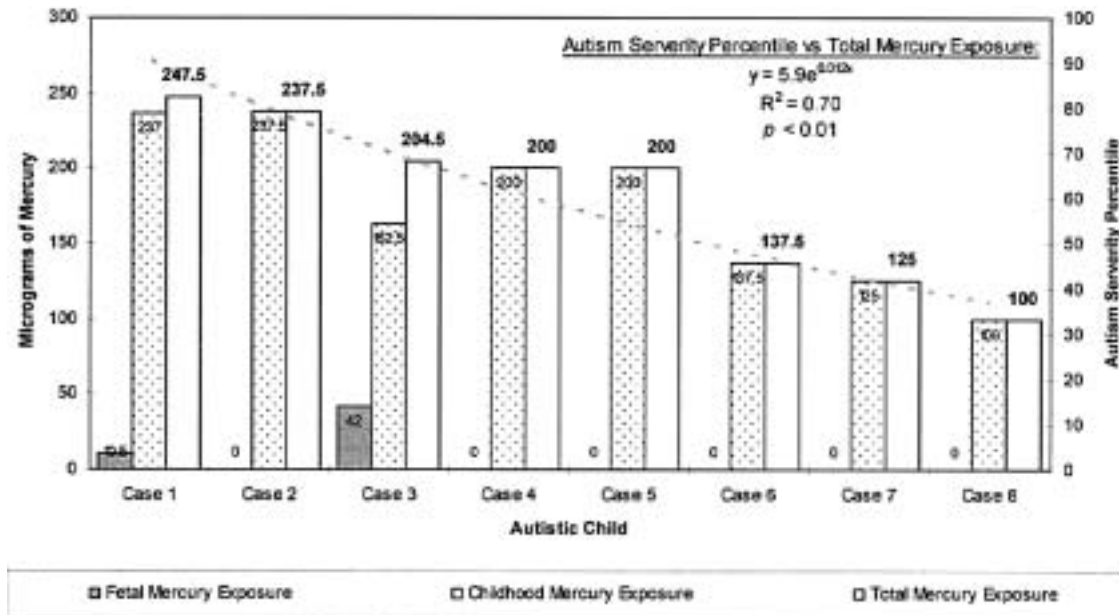
**TABLE 1**  
Summary of the Patient History for Each Case Examined in This Study

Autistic child <sup>a</sup>	Approximate age at start of regression (mo)	First childhood haircut (toxic metals)	Evidence of mercury toxicity (postchelation treatment)	Biochemical mercury susceptibility
Case 1	14	Low mercury	Prenatal exp. = 10.5 µg Postnatal exp. = 237 µg <i>Total exp. = 247.5 µg</i> <i>Significant urinary mercury</i> <i>Significant fecal mercury</i>	↑ Androgen metabolites
Case 2	12	<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic Low mercury	Prenatal exp. = 0 µg Postnatal exp. = 237.5 µg <i>Total exp. = 237.5 µg</i> <i>Significant urinary mercury</i>	↓ Glutathione metabolites ↑ Androgen metabolites
Case 3	15	<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic Low mercury	Prenatal exp. = 42 µg Postnatal exp. = 162.5 µg <i>Total exp. = 204.5 µg</i> <i>Significant urinary mercury</i>	↓ Glutathione metabolites ↑ Androgen metabolites
Case 4	14.5	<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic Not available	Prenatal exp. = 0 µg Postnatal exp. = 200 µg <i>Total exp. = 200 µg</i> <i>Significant urinary mercury</i> <i>Significant fecal mercury</i>	↓ Glutathione metabolites ↑ Androgen metabolites ↓ Glutathione metabolites



Case 5	17	Low mercury	Prenatal exp. = 0 µg Postnatal exp. = 200 µg Total exp. = 200 µg Significant urinary mercury	↑Androgen metabolites
Case 6	14	<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic Low mercury	Prenatal exp. = 0 µg Postnatal exp. = 137.5 µg Total exp. = 137.5 µg Significant urinary mercury	↓Glutathione metabolites ↑Androgen metabolites
Case 7	20	<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic Not available	Prenatal exp. = 0 µg Postnatal exp. = 125 µg Total exp. = 125 µg Significant urinary mercury Significant hair mercury	↓Glutathione metabolites ↑Androgen metabolites
Case 8	18	Low mercury	Prenatal exp. = 0 µg Postnatal exp. = 100 µg Total exp. = 100 µg Significant hair mercury	↓Glutathione metabolites ↑Androgen metabolites
		<i>In reference range or quantitative elevation</i> for cadmium, lead, and arsenic		↓Glutathione metabolites

<sup>a</sup>All the children examined in this case series had normal development during the first year of life (the child diagnosed with Rett's syndrome was excluded from the table).



**FIG. 1.** A summary of mercury exposure patterns and the severity of autism among the children examined (excluding the child diagnosed with Rett's syndrome). All the mothers reported eating fish less than once per week, the mothers had a median of six dental amalgams during their pregnancies (no mother had dental work during her pregnancy), and none of the mothers was employed in an occupational settings that would have exposed her child to environmental sources of mercury.

mercury in their urine, fecal, and/or hair samples. For the non-genetically damaged children, the facts contained in the case histories of these children appear to support a dose-response correlation between the severity of their developmental symptoms and the amount of mercury to which they were exposed from Thimerosal-containing biologics/vaccines. In the present cases, the important factors in determining/predicting how severely affected a child would be seem to be (1) how early in life the child began to be exposed to mercury and (2) how much total mercury the child received. Additionally, a significant inverse relationship was observed between the total amount of mercury a child was exposed to from Thimerosal-containing biologics/vaccines and how early the child regressed.

In considering the possibility that mercury from Thimerosal exposure induced autistic disorders in children, it is important to evaluate its biological effects. For example, it has been previously reported that "mercury can alter cell number and cell division; these impacts have been postulated as modes of action for the observed effects in neuronal development, and as a result the potential implications of such observations are evident when evaluated in context with research showing that altered cell proliferation and focal neuropathologic effects have been linked to specific behavioral deficits (e.g. autism)" (Faustman et al., 2000, p. 20).

Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) that dissociates in saline-based

formulations into ethylmercuric chloride and thiosalicylic acid (Reader & Lines, 1983). Thimerosal has historically been added to several vaccines/biologics at the preservative level (0.003% to 0.01%). In a series of molecular studies with neurons it was shown that nanomolar to micromolar concentrations of Thimerosal are capable of inducing neuronal death, degeneration, membrane damage, and DNA damage within hours of exposure (Baskin et al., 2003; Brown & Yel, 2003; Brunner et al., 1991; Humphrey et al., 2005; James et al., 2005; Parry, 1993; Wallin & Hartely-Asp, 1993; Yel et al., 2005). Additionally, it was reported that nanomolar to micromolar concentrations of Thimerosal were capable of disrupting critical signaling pathways/biochemical events necessary for neurons to undergo normal neuronal development (Mutkus et al., 2005; Parran et al., 2005; Waly et al., 2004).

The mercury kinetics of prenatal/postnatal Thimerosal administration showed that administration of Thimerosal-containing childhood vaccines resulted in blood levels of mercury in some children in excess of the U.S. Environmental Protection Agency (EPA) blood mercury limit (Stajich et al., 2000), and that the ethylmercury from Thimerosal is capable of crossing the placental and blood-brain barriers and results in an appreciable persistent bound inorganic mercury content in tissues including the brain (Blair et al., 1975; Burbacher et al., 2005; Fagan et al., 1977; Gasset et al., 1975; Matheson et al., 1980; Royhans et al., 1984; Slikker, 2000). Furthermore, it was reported in prenatal animal studies that ethylmercury compounds

readily pass through the placental barrier (to a greater extent than the corresponding methylmercury compound) (Leonard et al., 1983). Moreover, it was shown that exposure to ethylmercury results in a greater mercury concentration in fetal tissues than in the mother, especially in the fetal central nervous system (Ukita et al., 1967).

Burbacher et al. (2005) evaluated infant monkeys following injection of doses of Thimerosal comparable to the dosing schedule (weight- and age-adjusted) that U.S. children received during the 1990s. They determined that the maximum ethylmercury content in the brains of the Thimerosal-treated infant monkeys averaged approximately 40–50 ppb, and that its overall half-life in the brain of the infant monkeys examined was approximately 24 d. In addition, post-dosing-schedule testing found that the concentration of inorganic mercury (formed from the ethylmercury entering the brain) averaged 16 ppb in the brains of the Thimerosal-treated infant monkeys. Moreover, the half-life of this inorganic mercury in the monkeys' brains was too long to estimate a value from the available data (no significant measurable decline was detectable by 120 d). Additionally, it was previously reported as result of the significant inorganic fraction of mercury observed in the brain following injection of Thimerosal that a longer biological half-time for mercury in the brain was observed than for methylmercury (Suzuki et al., 1973).

The overall importance of persistent inorganic mercury in the brain stems from the fact that a number of recent studies showed that dealkylation of mercury in the brain is not a detoxification process (Charleston et al., 1994, 1995, 1996; Vahter et al., 1994, 1995). It was shown in monkeys following dosing with organic mercury that the half-life of inorganic mercury in the brain was estimated to vary significantly across different regions of the brain, from 227 d to 540 d. In other regions, the concentrations of inorganic mercury remained the same (thalamus) or doubled (pituitary) 6 mo after mercury dosing had ended (Vahter et al., 1994, 1995). Stereologic and autometallo-graphic studies on the brains of these monkeys indicated that the persistence of inorganic mercury in the brain was associated with a significant increase in the number of microglia in the brain, whereas the number of astrocytes declined. Notably, these effects were observed 6 mo after mercury dosing had ended, when inorganic mercury concentrations were at their highest levels, or in animals solely exposed to inorganic mercury (Charleston et al., 1994, 1995, 1996). It is important to note that "an active neuroinflammatory process" has been demonstrated in brains of autistic patients, including a marked activation of microglia (Vargas et al., 2005). Furthermore, one of the most consistent neurological abnormalities found in autistics is marked Purkinje cell loss in the cerebellum (as determined by histopathological postmortem examination) and atrophy of the cerebellar folia (as determined by *in vivo* neuroimaging (Baily et al., 1998; Courchesne, 1991; Courchesne et al., 1994; Kemper & Bauman, 1993; Ritvo et al., 1986). Several animal studies showed that Purkinje cells are vulnerable to

mercury (Sakamoto et al., 2002; Sorensen et al., 2000; Warfvinge, 2000).

Thimerosal has been recognized by the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, as a developmental toxin. This implies that Thimerosal may produce birth defects, low birth weight, biological dysfunctions, or psychological or behavior deficits that become manifest as the child grows, and maternal exposure during pregnancy may disrupt the development or even cause the death of the fetus.

It was found that human prenatal exposure to Thimerosal significantly increases the risk for birth defects (Heinonen et al., 1977). In animal models, it was shown that doses of Thimerosal resulting in a tissue concentration of mercury less than 1 ppm were able to induce significant lethality and teratogenicity in animal model systems (Digar et al., 1987; Gasset et al., 1975; Itoi et al., 1972). Additionally, exposure to low-dose ethylmercury (less than 760 µg mercury/kg body weight/d) in young animal models was found to produce severe neurodevelopmental toxicity (Tryphonas & Nielsen, 1973).

Hornig et al. (2004) administered Thimerosal postnatally to mice, mimicking the U.S. routine childhood immunization schedule of the 1990s (weight- and age-adjusted), and observed in a susceptible mouse strain autistic symptoms that included growth delay, reduced locomotion, exaggerated response to novelty, increased brain size, decreased numbers of Purkinje cells, significant abnormalities in brain architecture affecting areas subserving emotion and cognition, and densely packed hyperchromic hippocampal neurons with altered glutamate receptors and transporters.

Historically, similar types of significant regression displaying symptoms consistent with autistic disorders such as those observed in the present case series of children have been reported following infant/childhood mercury (including ethylmercury and Thimerosal) exposure (Chrysochoou et al., 2003; Derban, 1974; Engleson & Herner, 1952; Fagan et al., 1977; Pelclova et al., 2002; Rohyans et al., 1984, Zhang, 1984).

Amin-Saki et al. (1981) reported on a 5-yr longitudinal study in Iraq, of infants whose primary route of exposure to mercury (approximately 60% as organic mercury) was via breast milk. These researchers reported that abnormal neurological signs in these infants became more obvious with time, and that the earlier the exposure to mercury was, the more severe were the abnormal neurological signs observed. Additionally, it was reported that the mothers of the children examined in the study noticed the affliction of their children, and described their infants by saying, "They are like needles blunted by the poison." The researchers concluded the frequency of children with impaired intelligence was 1 out of 6, a frequency high enough to be noteworthy, and the combination of delayed motor development, retarded language development, and delayed toilet training, among several other symptoms, was considered evidence of damage to the central nervous system.

Counter et al. (2002) conducted an epidemiological study of mercury exposure on the prevalence of neuro-otological symptoms among 114 primarily school children from gold-mining areas of Ecuador. The sources of mercury exposure in the study areas were from inhalation of mercury vapors during the burning of mercury used to separate gold particulates from alluvial sediment and rock soil from within the mountain mines and rivers, and possibly from consumption of methylmercury-contaminated fish from local rivers, and of domestic chickens and pigs that eat from mercury-contaminated ground soil. These researchers observed prevalent learning disabilities, attention deficits, and autism among the school children examined.

Additionally, several recent epidemiological studies have evaluated the effects of mercury exposure on autism in the United States. It was observed in the state of California utilizing a case-control design, and in the state of Texas utilizing an ecological study design, that there was a significant association between mercury exposure and autism (Palmer et al., 2006; Windham et al., 2006).

In considering the present reported case series, the increased body burdens of mercury and biochemical mercury susceptibilities observed in the children are consistent with previous controlled observations of children with autistic disorders (Chauhan & Chauhan, 2006; Environmental Working Group, 2004; McGinnis, 2004; Mutter et al., 2005).

Bradstreet et al. (2003) showed that following DMSA chelation there were approximately three times significantly greater urinary mercury concentrations among autistics matched to neurotypical children, whereas autistics and matched neurotypical children had similar urinary cadmium and lead concentrations. Likewise, Holmes et al. (2003) examined first baby haircuts as a measure of mercury excretion, and determined that autistics had significantly lower levels of mercury in their first baby haircuts in comparison to nonautistic matched controls. Their research also demonstrated that the ability to excrete mercury in first baby haircuts was inversely proportional to the severity of symptoms observed in autistics. Furthermore, Nataf et al. (2006) have evaluated the potential environmental contribution to autism, by conducting a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 106 children with autistic disorder. It was observed that there was a significant approximately 2.5-fold elevation in urinary precoproporphyrin, a finding known to be associated with an increased body burden of mercury, among autistic children in comparison to matched controls.

James et al. (2005) reported that the neurotoxicity of Thimerosal is associated with depletion of glutathione. The ethylmercury in Thimerosal binds to cysteine thiol (-SH) groups on intracellular proteins and inactivates their function. The cysteine-SH group of glutathione binds mercury and protects essential proteins from functional inactivation. Glutathione is the major mechanism of mercury excretion, and individuals with genetic deficiencies in glutathione synthesis will be less able to excrete mercury and will be more sensitive to its

adverse effects (Environmental Working Group, 2004). James et al. (2004) actually evaluated such biochemical susceptibilities to mercury toxicity in autistic children in comparison to age- and gender-matched control children by evaluating the methionine cycle and transsulfuration metabolites. It was determined that there were significant decreases in the plasma concentrations of cysteine (19% reduction) and glutathione (46% reduction) in autistic children in comparison to control children, whereas there was a significant increase in the plasma concentration of oxidized glutathione (42% increase) in autistic children in comparison to control children. As a result, it was determined that overall biochemical susceptibility to mercury in autistic children resulted in a significant three fold decrease in the glutathione/oxidized glutathione ratio in comparison to control children.

In addition to biochemical susceptibilities in autistic disorders to mercury toxicity, several recent studies assessed genomic susceptibilities to mercury toxicity in autistic disorders. It was observed that there were significant correlations between genomic changes associated with reduced functioning mercury detoxification enzymes and autistic disorders, including glutathione *S*-transferase M1 (GSTM1) deletions (Buyske et al., 2006), 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms (Boris et al., 2004), metal-regulatory transcription factor 1 (MTF1) gene polymorphisms (Serajee et al., 2004), and divalent metal ion transporter SLC11A3 gene polymorphisms (Serajee et al., 2004).

Also, as was observed in the present study, autistics who present with no evidence of abnormality of early morphogenesis, manifested by either significant dysmorphology or microcephaly, have a considerable gender discrepancy with more than six males for every female (Miles et al., 2005). In considering this phenomenon, it was recently reported that testosterone and mercury work synergistically to produce increased toxicity, whereas estrogen significantly reduces mercury toxicity (M. R. Geier & D. A. Geier, 2005; Haley, 2005; Mutter et al., 2005). Sager et al. (1984) previously reported on the neurotoxic effects of alkyl mercury exposure on fetuses/infants of different genders. The researchers determined that with high-dose mercury exposure, 2-d-old male and female mice had neurons that were similarly adversely affected. With low-dose mercury exposure, however, the neurons of 2-d-old female mice were much less severely/adversely affected when compared with the neurons of male 2-d-old mice. The authors concluded males are considerably more sensitive than females to the neurotoxic effects of mercury, and that in some human fetal/infant population exposures to low-dose alkylmercury, it has been observed that males were more sensitive than females to psychomotor retardation (Clarkson et al., 1985; Grandjean et al., 1998).

The ability of Thimerosal exposure to induce toxic encephalopathies that clinically manifested as autistic disorders in the present case series of patients is further supported by epidemiological studies. In a series of previous epidemiological studies,

various databases, including the Vaccine Adverse Event Reporting System (VAERS), the U.S. Department of Education, the Vaccine Safety Datalink (VSD), and the California Department of Developmental Services (CDDS) (2003); were examined and a two- to eightfold significantly increased risk for neurodevelopmental disorders, depending upon the symptoms or outcomes examined, was observed following administration of Thimerosal-containing childhood vaccines (D.A. Geier & M.R. Geier 2003, 2004a, 2004b, 2005, 2006a, 2006b, 2006c, and 2006d. M. R. Geier & D. A. Geier, 2003a, 2003b). One other epidemiological study conducted in the United States that examined the relationship between Thimerosal-containing vaccines and neurodevelopmental disorders, by Verstraeten et al. (2003) from the U.S. Centers for Disease Control and Prevention, initially found a significant relationship between Thimerosal-containing childhood vaccines and some types of neurodevelopmental disorders, but upon further examination of a different data set, it did not find a consistent effect. The lead author concluded that this study was neutral (i.e., could neither accept nor reject a causal relationship) regarding the relationship between Thimerosal and neurodevelopmental disorders (Verstraeten, 2004).

## CONCLUSION

Eight of nine children examined in this study (a) had regressive autistic disorders, (b) had elevated levels of androgens, (c) excreted significant amounts of mercury post chelation challenge, (d) had biochemical evidence of decreased function in their glutathione pathways, (e) had no known significant mercury exposure except from Thimerosal in their vaccines/Rho(D)-immune globulin preparations, and (f) had extensive alternate causes for their regressive ASDs ruled out.

It is clear from these data, and other emerging data that have been recently published, that additional autistic disorder research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing vaccines. Additionally, studies should also be undertaken to evaluate other databases/registries of patients to assess the compatibility of the present results with clinical observations for other children with autistic disorders. In light of the results of this present case series examining mercury exposure and its consistency with previous controlled studies of autistic children, the mercury factor should be considered in the differential diagnosis factors of regressive autistic disorders in children.

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