

**222 Research Papers Supporting Vaccine/Autism Causation**  
**Ginger Taylor, MS**  
**6/26/2023**

Mainstream research has found that vaccines and their ingredients can cause the underlying medical conditions that committed physicians and researchers are commonly finding in children who have been given an an autism diagnosis. These conditions include gastrointestinal damage, immune system impairment, chronic infections, mitochondrial disorders, autoimmune conditions, neurological regression, glial cell activation, interleukin-6 secretion dysregulation, brain inflammation, loss of integrity of the blood–brain barrier, seizures, synaptic dysfunction, dendritic cell dysfunction, mercury poisoning, aluminum toxicity, gene activation and alteration, glutathione depletion, impaired methylation, oxidative stress, impaired thioredoxin regulation, mineral deficiencies, impairment of the opioid system, endocrine dysfunction, cellular apoptosis, and other disorders.

These papers, sorted by category, can be found at [HowDoVaccinesCauseAutism.org](http://HowDoVaccinesCauseAutism.org)

1. [Increased risk of developmental neurologic impairment after high exposure to thimerosal-containing vaccine in first month of life.](#)

Division of Epidemiology and Surveillance, Vaccine Safety and Development Branch, National Immunization Program, Centers for Disease Control and Prevention. 1999.

Thomas M. Verstraeten, Robert Davis, David Gu, Frank DeStefano

Background: Concern has risen on the presence of the ethylmercury containing preservative thimerosal in vaccines. We assessed the risk for neurologic and renal impairment associated with past exposure to thimerosal-containing vaccine using automated data from the Vaccine Safety Data link (VSD). VSD is a large linked database from four health maintenance organizations in Washington, Oregon and California, containing immunization, medical visit and demographic data on over 400,000 infants born between '91 and '97.

Methods: We categorized the cumulative ethylmercury exposure from Thimerosal containing vaccines after one month of life and assessed the subsequent risk of degenerative and developmental neurologic disorders and renal disorders before the age of six. We applied proportional hazard models adjusting for HMO, year of birth, and gender, excluding premature babies.

Results: We identified 286 children with degenerative and 3702 with developmental neurologic disorders, and 310 with renal disorders. The relative risk (RR) of developing a neurologic development disorder was 1.8 ( 95% confidence intervals [CI] =1.1-2.8) when comparing the highest exposure group at 1 month of age (cumulative dose> 25 ug) to the unexposed group. **Within this group we also found an elevated risk for the following disorders: autism (RR 7.6, 95% CI = 1.8-31.5), non organic sleep disorders (RR 5.0, 95% CI = 1.6-15.9), and speech disorders (RR 2.1, 95% (1=1.1-4.0).** For the neurologic degenerative and renal disorders group we found no significantly increased risk

or a decreased risk.

Conclusion: **This analysis suggests that high exposure to ethyl mercury from thimerosal-containing vaccines in the first month of life increases the risk of subsequent development of neurologic development impairment,** but not of neurologic degenerative or renal impairment. Further confirmatory studies are needed.

2. [Pilot comparative study on the health of vaccinated and unvaccinated 6- to 12-year old U.S. children](#)

J Transl Sci 3: DOI: 10.15761/JTS.1000186, April 24, 2017

Anthony R Mawson, Azad R Bhuiyan, Brian D Ray, Binu Jacob  
Department of Epidemiology and Biostatistics, School of Public Health, Jackson State University, Jackson, MS 39213, USA  
National Home Education Research Institute, PO Box 13939, Salem, OR 97309, USA

Abstract

Vaccinations have prevented millions of infectious illnesses, hospitalizations and deaths among U.S. children, yet the long-term health outcomes of the vaccination schedule remain uncertain. Studies have been recommended by the U.S. Institute of Medicine to address this question. This study aimed 1) to compare vaccinated and unvaccinated children on a broad range of health outcomes, and 2) to determine whether an association found between vaccination and neurodevelopmental disorders (NDD), if any, remained significant after adjustment for other measured factors. A cross-sectional study of mothers of children educated at home was carried out in collaboration with homeschool organizations in four U.S. states: Florida, Louisiana, Mississippi and Oregon. Mothers were asked to complete an anonymous online questionnaire on their 6- to 12-year-old biological children with respect to pregnancy-related factors, birth history, vaccinations, physician-diagnosed illnesses, medications used, and health services. NDD, a derived diagnostic measure, was defined as having one or more of the following three closely-related diagnoses: a learning disability, Attention Deficient Hyperactivity Disorder, and Autism Spectrum Disorder. A convenience sample of 666 children was obtained, of which 261 (39%) were unvaccinated. The vaccinated were less likely than the unvaccinated to have been diagnosed with chickenpox and pertussis, but more likely to have been diagnosed with pneumonia, otitis media, allergies and NDD. After adjustment, vaccination, male gender, and preterm birth remained significantly associated with NDD. However, in a final adjusted model with interaction, vaccination but not preterm birth remained associated with NDD, while the interaction of preterm birth and vaccination was associated with a 6.6-fold increased odds of NDD (95% CI: 2.8, 15.5). **In conclusion, vaccinated homeschool children were found to have a higher rate of allergies and NDD than unvaccinated homeschool children.** While vaccination remained significantly associated with NDD after controlling for other factors, preterm birth coupled with vaccination was associated with an apparent synergistic increase in

the odds of NDD. Further research involving larger, independent samples and stronger research designs is needed to verify and understand these unexpected findings in order to optimize the impact of vaccines on children's health.

Exerpts:

**"NDD, a derived diagnostic measure, was defined as having one or more of the following three closely-related diagnoses: a learning disability, Attention Deficient Hyperactivity Disorder, and Autism Spectrum Disorder."**

"Chronic illness

**Vaccinated children were significantly more likely than the unvaccinated to have been diagnosed with the following:** allergic rhinitis (10.4% vs. 0.4%,  $p < 0.001$ ; OR 30.1, 95% CI: 4.1, 219.3), other allergies (22.2% vs. 6.9%,  $p < 0.001$ ; OR 3.9, 95% CI: 2.3, 6.6), eczema/atopic dermatitis (9.5% vs. 3.6%,  $p = 0.035$ ; OR 2.9, 95% CI: 1.4, 6.1), a learning disability (5.7% vs. 1.2%,  $p = 0.003$ ; OR 5.2, 95% CI: 1.6, 17.4), ADHD (4.7% vs. 1.0%,  $p = 0.013$ ; OR 4.2, 95% CI: 1.2, 14.5), **ASD (4.7% vs. 1.0%,  $p = 0.013$ ; OR 4.2, 95% CI: 1.2, 14.5)**, any neurodevelopmental disorder (i.e., learning disability, ADHD or ASD) (10.5% vs. 3.1%,  $p < 0.001$ ; OR 3.7, 95% CI: 1.7, 7.9) and any chronic illness (44.0% vs. 25.0%,  $p < 0.001$ ; OR 2.4, 95% CI: 1.7, 3.3)."

3. [Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997-2002.](#)

J Toxicol Environ Health A. 2010;73(24):1665-77. doi: 10.1080/15287394.2010.519317.

Gallagher CM, Goodman MS.

Abstract

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.

4. [Associations of prenatal and early childhood mercury exposure with autistic behaviors at 5 years of age: The Mothers and Children's Environmental Health \(MOCEH\) study](#)

Science of The Total Environment  
Volumes 605–606, 15 December 2017, Pages 251-257

JiaRyua. , Eun-HeeHaa, Boong-NyunKimb, MinaHac, YanghoKimd,  
HyesookParke, Yun-ChulHongf, Kyoung-NamKim

Department of Occupational and Environmental Medicine, School of Medicine,  
Ewha Womans University, Seoul, Republic of Korea

Division of Child & Adolescent Psychiatry, Department of Psychiatry and Institute  
of Human Behavioral Medicine, College of Medicine, Seoul National University,  
Seoul, Republic of Korea

Department of Preventive Medicine, College of Medicine, Dankook University,  
Cheonan, Republic of Korea

Department of Occupational and Environmental Medicine, Ulsan University  
Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea

Department of Preventive Medicine, School of Medicine, Ewha Womans  
University, Seoul, Republic of Korea

Department of Preventive Medicine, Seoul National University College of  
Medicine, Seoul, Republic of Korea

Institute of Public Health and Medical Service, Seoul National University Hospital,  
Seoul, Republic of Korea

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#### Abstract

##### Background

Although mercury is an established neurotoxin, only few longitudinal studies have investigated the association between prenatal and early childhood mercury exposure and autistic behaviors.

##### Methods

We conducted a longitudinal cohort study using an ongoing prospective birth cohort initiated in 2006, wherein blood mercury levels were measured at early and late pregnancy; in cord blood; and at 2 and 3 years of age. We analyzed 458 mother-child pairs. Autistic behaviors were assessed using the Social Responsiveness Scale (SRS) at 5 years of age. Both continuous SRS T-scores and T-scores dichotomized by a score of  $\geq 60$  or  $< 60$  were used as outcomes.

## Results

The geometric mean of mercury concentrations in cord blood was 5.52 µg/L. In adjusted models, a doubling of blood mercury levels at late pregnancy ( $\beta = 1.84$ , 95% confidence interval [CI]: 0.39, 3.29), in cord blood ( $\beta = 2.24$ , 95% CI: 0.22, 4.27), and at 2 years ( $\beta = 2.12$ , 95% CI: 0.54, 3.70) and 3 years ( $\beta = 2.80$ , 95% CI: 0.89, 4.72) of age was positively associated with the SRS T-scores. When the SRS T-scores were dichotomized, we observed positive associations with mercury levels at late pregnancy (relative risk [RR] = 1.31, 95% CI: 1.08, 1.60) and in cord blood (RR = 1.28, 95% CI: 1.01, 1.63).

## Conclusion

**We found that blood mercury levels at late pregnancy and early childhood were associated with more autistic behaviors in children at 5 years of age.** Further study on the long-term effects of mercury exposure is recommended.

5. [The association between mercury levels and autism spectrum disorders: A systematic review and meta-analysis](#)

Journal of Trace Elements in Medicine and Biology  
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Tina Jafari, Noushin Rostampour, Aziz A.Fallah, Afshin Hesamia

Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Sharhekord, Iran  
Department of Biochemistry and Nutrition, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran  
Department of Internal Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran  
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord 34141, Iran

## Abstract

### Background & aims

The relationship between mercury and autism spectrum disorders (ASD) has always been a topic of controversy among researchers. This study aimed to assess the relationship between ASD and mercury levels in hair, urine, blood, red blood cells (RBC), and brain through a meta-analysis.

### Methods

A systematic search was performed in several databases including PubMed, ISI Web of Science, Cochrane register of controlled trials, Google Scholar, Scopus, and MagIran until June 2017. Case-control studies evaluating concentration of total mercury in different tissues of ASD patients and comparing them to the healthy subjects (control group) were identified. Necessary data were extracted and random effects model was used to calculate overall effect and its 95% corresponding confidence interval (CI) from the effect sizes.

## Results

A total of 44 studies were identified that met the necessary criteria for meta-analysis. The mercury level in whole blood (Hedges = 0.43, 95% CI: 0.12, 0.74, P = 0.007), RBC (Hedges = 1.61, 95% CI: 0.83, 2.38, P < 0.001), and brain (0.61 ng/g, 95% CI, 0.02, 1.19, P = 0.043) was significantly higher in ASD patients than healthy subjects, whereas mercury level in hair (-0.14 mg/g, 95% CI: -0.28, -0.01, P = 0.039) was significantly lower in ASD patients than healthy subjects. The mercury level in urine was not significantly different between ASD patients and healthy subjects (0.51 mg/g creatinine, 95% CI: -0.14, 1.16, P = 0.121).

## Conclusions

**Results of the current meta-analysis revealed that mercury is an important causal factor in the etiology of ASD. It seems that the detoxification and excretory mechanisms are impaired in ASD patients which lead to accumulation of mercury in the body.** Future additional studies on mercury levels in different tissues of ASD patients should be undertaken.

### 6. [The Relationship Between the Level of Copper, Lead, Mercury and Autism Disorders: A Meta-Analysis](#)

Authors Jafari Mohammadabadi H, Rahmatian A, Sayehmiri F, Rafiei M

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1School of Medicine, Shiraz University of Medical Sciences, Shiraz, Fars, Iran;

2Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran;

3Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran;

4Department of Biostatistics and Epidemiology, Arak University of Medical Sciences, Arak, Iran

**Background and Objectives:** There is a likelihood of a possible relationship between the concentrations of copper, lead, and mercury and autism. The present review was carried out to determine the relationship between the concentrations of these elements and autism by meta-analysis.

**Methods:** In this study, searching Scopus, PubMed, and Science Direct databases, 18 articles conducted in different countries from 1982 to 2019 were collected. Studies' heterogeneity was investigated using the I<sup>2</sup> index. The data were analyzed using R and STATA software.

**Results:** In these 18 studies, 1797 patients (981 cases and 816 controls) aged 2 to 16 years were examined. Concentration of the samples (blood, hair, and nails) for both case and control groups was evaluated. There was no significant relationship between copper concentration and autism (SMD (95% CI): 0.02 (-1.16, 1.20); I<sup>2</sup>=97.7%; P=0.972); there was a significant relationship between mercury concentration and autism (SMD (95% CI): 1.96 (0.56, 3.35); I<sup>2</sup>=98.6%;

P=0.006); there was also a significant relationship between lead concentration and autism (SMD (95% CI): 2.81 (1.64,3.98); I<sup>2</sup>=97.8%; P=0.000).

**Conclusion: There is, nevertheless, a significant relationship between mercury concentration and autism. Thus, the concentration of mercury can be listed as a pathogenic cause (disease-causing) for autism.**

7. [The Putative Role of Environmental Mercury in the Pathogenesis and Pathophysiology of Autism Spectrum Disorders and Subtypes](#)

Molecular Neurobiology, First Online: 22 July 2017

G. Morris, K. Puri, R. E. Frye, M. Maes

1. Tir Na Nog Llanelli UK

2. Department of Medicine, Hammersmith Hospital Imperial College London, London UK

3. Division of Child and Adolescent Neurology and Children's Learning Institute, Department of Pediatrics University of Texas, Austin USA

4. Department of Psychiatry Chulalongkorn University Bangkok, Thailand

Abstract

Exposure to organic forms of mercury has the theoretical capacity to generate a range of immune abnormalities coupled with chronic nitro-oxidative stress seen in children with autism spectrum disorder (ASD). The paper discusses possible mechanisms explaining the neurotoxic effects of mercury and possible associations between mercury exposure and ASD subtypes. **Environmental mercury is neurotoxic at doses well below the current reference levels considered to be safe, with evidence of neurotoxicity in children exposed to environmental sources including fish consumption and ethylmercury-containing vaccines. Possible neurotoxic mechanisms of mercury include direct effects on sulfhydryl groups, pericytes and cerebral endothelial cells, accumulation within astrocytes, microglial activation, induction of chronic oxidative stress, activation of immune-inflammatory pathways and impairment of mitochondrial functioning. (Epi-)genetic factors which may increase susceptibility to the toxic effects of mercury in ASD include the following: a greater propensity of males to the long-term neurotoxic effects of postnatal exposure and genetic polymorphisms in glutathione transferases and other glutathione-related genes and in selenoproteins. Furthermore, immune and inflammatory responses to immunisations with mercury-containing adjuvants are strongly influenced by polymorphisms in the human leukocyte antigen (HLA) region and by genes encoding effector proteins such as cytokines and pattern recognition receptors. Some epidemiological studies investigating a possible relationship between high environmental exposure to methylmercury and impaired neurodevelopment have reported a positive dose-dependent effect. Retrospective studies, on the other hand, reported no relationship between a range of ethylmercury-containing vaccines and chronic neuropathology or ASD. On the basis of these results, we would argue that more clinically relevant research is required to examine whether**

environmental mercury is associated with ASD or subtypes. Specific recommendations for future research are discussed.

8. [A case series of children with apparent mercury toxic encephalopathies manifesting with clinical symptoms of regressive autistic disorders](#)

J Toxicol Environ Health A. 2007 May 15;70(10):837-51. doi: 10.1080/15287390701212141.

David A Geier<sup>1</sup>, Mark R Geier

PMID: 17454560 DOI: 10.1080/15287390701212141

#### Abstract

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

9. [Association of autism with toxic metals: A systematic review of case-control studies](#)

Pharmacol Biochem Behav. 2022 Jan;212:173313.



doi: 10.1016/j.pbb.2021.173313. Epub 2021 Dec 9.

Cecilia N Amadi <sup>1</sup> , Chinna N Orish <sup>2</sup> , Chiara Frazzoli <sup>3</sup> , Orish E Orisakwe <sup>4</sup>

<sup>1</sup> Department of Experimental Pharmacology & Toxicology, Faculty of Pharmacy, University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

<sup>2</sup> Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

<sup>3</sup> Department for Cardiovascular, Dysmetabolic and Aging Diseases, Istituto Superiore di Sanità, Rome, Italy.

<sup>4</sup> Department of Experimental Pharmacology & Toxicology, Faculty of Pharmacy, University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria; African Centre of Excellence for Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

#### Abstract

Environmental factors have been associated with the etiology of autism spectrum disorder ASD in recent times. The involvement of toxic metals in the generation of reactive oxygen species and their epigenetics effects have been implicated in ASD. This systemic review examines the association of toxic metals with autism in children. A systematic literature search was performed in scientific databases such as PubMed, Google scholar, and Scopus. Case-control studies evaluating toxic metal levels in different tissues of ASD children and comparing them to healthy children (control group) were identified. The Newcastle-Ottawa Scale was used to evaluate the risk of bias of the included studies. Six case-control studies with 425 study subjects met our inclusion criteria. A total of four studies indicated higher levels of As, Pb, Hg, Cd, Al, Sn, Sb, Ba, Tl, W, and Zr in whole blood, RBC, in whole blood, RBC, and hair samples of children with autism compared with control suggestive of a greater toxic metal exposure (immediate and long-term). Three studies identified significantly higher concentrations of Cd, Pb and Hg in urine and hair samples of autistic children compared to control suggesting decreased excretion and possible high body burden of these metals. The findings from this review demonstrate that high levels of toxic metals are associated with ASD, therefore, critical care is necessary to reduce body burden of these metals in children with ASD as a major therapeutic strategy.

#### 10. [Analysis of Whole Blood and Urine Trace Elements in Children with Autism Spectrum Disorders and Autistic Behaviors](#)

Biol Trace Elem Res. 2023 Feb;201(2):627-635.

doi: 10.1007/s12011-022-03197-4. Epub 2022 Mar 19.

Gang Zhao <sup>1 2</sup> , Si-Jin Liu <sup>3</sup> , Xin-Yu Gan <sup>4 5</sup> , Jun-Ru Li <sup>3</sup> , Xiao-Xue Wu <sup>3</sup> , Si-Yan Liu <sup>3</sup> , Yi-Si Jin <sup>6</sup> , Ke-Rang Zhang <sup>7</sup> , Hong-Mei Wu <sup>8</sup>

- 1 Department of Psychiatry, First Hospital of Shanxi Medical University, Taiyuan, 030001, China.
- 2 Department of Child Health Care, Maternity and Child Healthcare Hospital of Nanshan District, 1 Wanxia Road, Nanshan District, Shenzhen, 518067, China.
- 3 Department of Nursing, Harbin Medical University in Daqing, Daqing, 163319, China.
- 4 Department of Rehabilitation of the Heilongjiang Province Land Reclamation Headquarters General Hospital, Harbin, 150081, China.
- 5 Harbin Medical University in Daqing, Daqing, 163319, Heilongjiang, China.
- 6 Department of Rehabilitation, The Fifth Affiliated Hospital of Harbin Medical University, Daqing, 163000, China.
- 7 Department of Psychiatry, First Hospital of Shanxi Medical University, Taiyuan, 030001, China. [krangzhang\\_sxmu@vip.163.com](mailto:krangzhang_sxmu@vip.163.com).
- 8 Department of Nursing, Harbin Medical University in Daqing, Daqing, 163319, China.

## Abstract

The relationship between trace elements and neurological development is an emerging research focus. We performed a case-control study to explore (1) the differences of 13 trace elements chromium (Cr), manganese (Mn), cobalt (Co), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), stannum (Sn), stibium (Sb), mercury (Hg), titanium (Ti), and plumbum (Pb) concentration in whole blood and urine between autism spectrum disorder (ASD) children and their typical development peers, and (2) the association between the 13 trace elements and core behaviors of ASD. Thirty ASD subjects (cases) and 30 age-sex-matched healthy subjects from Baise City, Guangxi Zhuang Autonomous Region, China, were recruited. Element analysis was carried out by inductively coupled plasma-optical emission spectrometry. Autistic behaviors were assessed using Autism Behavior Checklist (ABC), Childhood Autism Rating Scale (CARS), and Children Neuropsychological and Behavior Scale (CNBS). **The whole blood concentrations of Mo ( $p = 0.004$ ), Cd ( $0.007$ ), Sn ( $p = 0.003$ ), and Pb ( $p = 0.037$ ) were significantly higher in the ASD cases than in the controls. Moreover, Se ( $0.393$ ), Hg ( $0.408$ ), and Mn ( $- 0.373$ ) concentrations were significantly correlated between whole blood and urine levels in ASD case subjects. There were significant correlations between whole blood Sb ( $0.406$ ), Ti ( $0.365$ ), Mo ( $- 0.4237$ ), Mn ( $- 0.389$ ), Zn ( $0.476$ ), and Se ( $0.375$ ) levels and core behaviors of ASD. Although the mechanism of trace element imbalance in ASD is unclear, these data demonstrate that core behaviors of ASD may be affected by certain trace elements. Further studies are recommended for exploring the mechanism of element imbalance and providing corresponding clinical treatment measures.**

11. [Blood Mercury, Arsenic, Cadmium, and Lead in Children with Autism Spectrum Disorder.](#)

Li H, Li H, Li Y, Liu Y, Zhao Z

Children's Hospital of Zhejiang University School of Medicine, Hangzhou, People's Republic of China.

Laboratory of Neuroinflammation, StVincent's Centre for Applied Medical Research and University of New South Wales, Sydney, NSW, Australia.

Children's Hospital of Zhejiang University School of Medicine, Hangzhou, People's Republic of China. zhaozy@zju.edu.cn.

Department of Pediatric Health Care, Children's Hospital of Zhejiang University School of Medicine, 57 Zhuganxiang Road, Hangzhou, People's Republic of China

#### Abstract

Environmental factors have been implicated in the etiology of autism spectrum disorder (ASD); however, the role of heavy metals has not been fully defined. This study investigated whether blood levels of mercury, arsenic, cadmium, and lead of children with ASD significantly differ from those of age- and sex-matched controls. One hundred eighty unrelated children with ASD and 184 healthy controls were recruited. **Data showed that the children with ASD had significantly ( $p < 0.001$ ) higher levels of mercury and arsenic and a lower level of cadmium.** The levels of lead did not differ significantly between the groups. **The results of this study are consistent with numerous previous studies, supporting an important role for heavy metal exposure, particularly mercury, in the etiology of ASD.** It is desirable to continue future research into the relationship between ASD and heavy metal exposure.

#### 12. [The role of environmental trace element toxicants on autism: A medical biogeochemistry perspective](#)

Ecotoxicol Environ Saf. 2023 Feb;251:114561.  
doi: 10.1016/j.ecoenv.2023.114561. Epub 2023 Jan 23.

Salih Muhammad Awadh <sup>1</sup>, Zaher Mundher Yaseen <sup>2</sup>, Mohammad Saleh Al-Suwaiyan <sup>3</sup>

<sup>1</sup> Department of Geology, College of Science, University of Baghdad, Baghdad, Iraq. Electronic address: salih.awadh@sc.uobaghdad.edu.iq.

<sup>2</sup> Civil and Environmental Engineering Department, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia; Interdisciplinary Research Center for Membranes and Water Security, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia. Electronic address: z.yaseen@kfupm.edu.sa.

<sup>3</sup> Civil and Environmental Engineering Department, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia; Interdisciplinary Research Center for Construction and Building Materials, King Fahd University of

Petroleum and Minerals, Dhahran 31261, Saudi Arabia. Electronic address: msaleh@kfupm.edu.sa.

#### Abstract

Since genetic factors alone cannot explain most cases of Autism, the environmental factors are worth investigating as they play an essential role in the development of some cases of Autism. This research is a review paper that aims to clarify the role of the macro elements (MEs), Trace elements (TEs) and ultra-trace elements (UTEs) on human health if they are greater or less than the normal range. Aluminium (Al), cadmium (Cd), lead (Pb), chromium (Cr), zinc (Zn), copper (Cu), nickel (Ni), arsenic (As), mercury (Hg), manganese (Mn), and iron (Fe) have been reviewed. Exposure to toxicants has a chemical effect that may ultimately lead to autism spectrum disorder (ASD). **The Cr, As and Al are found in high concentrations in the blood of an autistic child when compared to normal child reference values. The toxic metals, particularly aluminium, are primarily responsible for difficulties in socialization and language skills disabilities. Zinc and copper are important elements in regulating the gene expression of metallothioneins (MTs), and zinc deficiency may be a risk factor for ASD pathogenesis. Autistics frequently have zinc deficiency combined with copper excess; as part of the treatment protocol, it is critical to monitor zinc and copper levels in autistic people, particularly those with zinc deficiency. Zinc deficiency is linked to epileptic seizures, which are common in autistic patients. Higher serum manganese and copper significantly characterize people who have ASD. Autistic children have significantly decreased lead and cadmium in urine, whereas they have significantly higher urine Cr. A higher level of As and Hg was found in the ASD individual's blood.**

#### 13. [Hair Heavy Metal And Essential Trace Element Concentration In Children With Autism Spectrum Disorder](#)

T Tabatadze<sup>1</sup>, L Zhorzholiani<sup>1</sup>, M Kherkheulidze<sup>1</sup>, E Kandelaki<sup>1</sup>, T Ivanashvili<sup>1</sup>

Georgian Med News. 2015 Nov;(248):77-82.

<sup>1</sup> M. Iashvili Children's Central Hospital, Child Development Center; Iv. Javakhishvili Tbilisi State University, Georgia.

#### Abstract

Our study aims evaluation of level of essential trace elements and heavy metals in the hair samples of children with autistic spectrum disorder (ASD) and identification of changes that are associated with autistic spectrum disorders. Case-control study was conducted at Child Development Center of Iashvili Children's Central Hospital (LD). We studied 60 children aged from 4 to 5 years old. The concentrations of 28 elements among (Ca, Zn, K, Fe, Cu, Se, Mn, Cr, S,

Br, Cl, Co, Ag, V, Ni, Rb, Mo, Sr, Ti, Ba, Pb, As, Hg, Cd, Sb, Zr, Sn, Bi) them trace elements and toxic metals) were determined in scalp hair samples of children (n=30) with autistic spectrum disorder (ASD) and from control group of healthy children (n=30) with matched sex and age. Micro-elemental status was detected in the hair, with roentgen-fluorescence spectrometer method (Method MBI 081/12-4502-000, Apparatus ALVAX- CIP, USA - UKRAIN) .To achieve the similarity of study and control groups, pre and postnatal as well as family and social history were assessed and similar groups were selected. Children with genetic problems, malnourished children, children from families with social problems were excluded from the study. The diagnosis of ASD were performed by pediatrician and psychologist (using M-CHAT and ADOS) according to DSM IV (Diagnostic and Statistical Manual of Mental Disorders from the American Psychiatric association) criteria. The study was statistically analyzed using computer program SPSS 19. Deficiencies of essential trace microelements revealed in both group, but there was significant difference between control and studied groups. **The most deficient element was zinc (92% in target and 20% in control), then - manganese (55% and 8%) and selenium (38% and 4%). In case of cooper study revealed excess concentration of this element only in target group in 50% of cases. The contaminations to heavy metals were detected in case of lead (78% and 16), mercury (43% and 10%) and cadmium (38% and 8%). The study statistical results indicated, that deficient concentrations of trace elements such as zinc, manganese, molybdenum and selenium in hair significantly linked with ASD (Kramer's V was 0,740; 0,537; 0,333; 0,417 accordingly). In case of cooper we got excess levels of this element and this data was highly linked with autism spectrum disorder. We got high associations and significant values between of lead, mercury and cadmium concentrations and ASD. Study results indicate that there are significant differences of hair essential trace elements concentrations in children with autism spectrum disorder comparing with healthy children group. The result obtained also showed high contamination to heavy metals such as lead, mercury and cadmium in ASD children compared to healthy ones. So, our study demonstrated alteration in levels of toxic heavy metals and essential trace elements in children with autistic spectrum disorders as compared to healthy children. This suggests a possible pathophysiological role of heavy metals and trace elements in the genesis of symptoms of autism spectrum disorders.**

14. [The levels of blood mercury and inflammatory-related neuropeptides in the serum are correlated in children with autism spectrum disorder](#)

Metab Brain Dis. 2016 Jun;31(3):593-9.  
doi: 10.1007/s11011-015-9784-8. Epub 2016 Jan 6.

Gehan Ahmed Mostafa <sup>1 2</sup> , Geir Bjørklund <sup>3</sup> , Mauricio A Urbina <sup>4 5</sup> , Laila Yousef Al-Ayadhi <sup>6</sup>

<sup>1</sup> Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt. gehan.mostafa2000@yahoo.com.

2 Autism Research and Treatment Center, AL-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. [gehan.mostafa2000@yahoo.com](mailto:gehan.mostafa2000@yahoo.com).

3 Council for Nutritional and Environmental Medicine, Toften 24, 8610, Mo i Rana, Norway.

4 Department of Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK.

5 Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.

6 Autism Research and Treatment Center, AL-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia.

## Abstract

Tachykinins (substance P, neurokinin A, and neurokinin B) are pro-inflammatory neuropeptides that may play an important role in some autoimmune neuroinflammatory diseases, including autism spectrum disorder (ASD). Mercury (Hg) is a neurotoxicant, and potentially one of the main environmental triggers for ASD as it induces neuroinflammation with a subsequent release of neuropeptides. This is the first study to explore the potentially causal relationship between levels of serum neurokinin A and blood mercury (BHg) in children with ASD. Levels of serum neurokinin A and BHg were measured in 84 children with ASD, aged between 3 and 10 years, and 84 healthy-matched children. **There was a positive linear relationship between the Childhood Autism Rating Scale (CARS) and both serum neurokinin A and BHg. ASD children had significantly higher levels of serum neurokinin A than healthy controls ( $P < 0.001$ ). Increased levels of serum neurokinin A and BHg were respectively found in 54.8 % and 42.9 % of the two groups. There was significant and positive linear relationship between levels of serum neurokinin A and BHg in children with moderate and severe ASD, but not in healthy control children. It was found that 78.3 % of the ASD patients with increased serum levels of neurokinin A had elevated BHg levels ( $P < 0.001$ ). Neuroinflammation, with increased levels of neurokinin A, is seen in some children with ASD, and may be caused by elevated BHg levels. Further research is recommended to determine the pathogenic role of increased levels of serum neurokinin A and BHg in ASD. The therapeutic role of tachykinin receptor antagonists, a potential new class of anti-inflammatory medications, and Hg chelators, should also be studied in ASD.**

15. [Protective role of alpha-lipoic acid in impairments of social and stereotyped behaviors induced by early postnatal administration of thimerosal in male rat.](#)

Neurotoxicol Teratol. 2018 Feb 23. pii: S0892-0362(17)30086-7. doi: 10.1016/j.ntt.2018.02.002.

Namvarpour Z, Nasehi M, Amini A, Zarrindast MR.

Institute for Cognitive Science Studies (ICSS), Tehran, Iran.

Institute for Cognitive Science Studies (ICSS), Tehran, Iran; Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.

Department of Biology and Anatomy, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Institute for Cognitive Science Studies (ICSS), Tehran, Iran; Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

#### Abstract

Aim Thimerosal, a mercury-containing preservative has been widely used in a number of biological and drug products, including many vaccines, and has been studied as a possible etiological factor for some neurodevelopmental disabilities. Here, the protective effects of Alpha Lipoic Acid (ALA), an organosulfur compound derived from Octanoic Acid, on Thimerosal-induced behavioral abnormalities in rat were examined.

#### METHODS:

108 male Wistar rats were divided into three cohorts and treated as follows: 1) Thimerosal at different doses (30, 300, or 3000  $\mu\text{g Hg/kg}$ ) in four i.m. injections on 7, 9, 11, 15 postnatal days. 2) ALA (at doses of 5, 10 and 20 mg/kg), following the same order; 3) single dose of Thimerosal (3000  $\mu\text{g Hg/kg}$ ) plus ALA at different doses (5, 10 or 20 mg/kg), by the previously described method. A saline treated control group and a ALA vehicle control (0.1% NaOH) were also included. At 5 and 8 weeks after birth, rats were evaluated with behavioral tests, to assess locomotor activity, social interactions and stereotyped behaviors, respectively.

#### RESULTS:

The data showed that Thimerosal at all doses (30, 300 and 3000  $\mu\text{g Hg/kg}$ ) significantly impacted locomotor activity. Thimerosal at doses of 300 and 3000 but not 30  $\mu\text{g Hg/kg}$  impaired social and stereotyped behaviors. In contrast, ALA (at doses of 5, 10 and 20 mg/kg) did not alter behaviors by itself, at doses of 20 mg/kg, it reduced social interaction deficits induced by the highest dose of Thimerosal (3000  $\mu\text{g Hg/kg}$ ). Moreover, ALA, at all doses prevented the adverse effects of Thimerosal on stereotyped behaviors.

#### CONCLUSIONS:

the results of this preclinical study, consistent with previous studies on mice and rats, reveals that **neonatal dose-dependent exposure to Thimerosal mimicking the childhood vaccine schedule can induce abnormal social interactions and stereotyped behaviors similar to those observed in neurodevelopmental disorders such as autism**, and, for the first time, revealed that these abnormalities may be ameliorated by ALA. This indicates that ALA may protect against mercurial-induced abnormal behaviors.

J Trace Elem Med Biol. 2020 Jan;57:126409.  
doi: 10.1016/j.jtemb.2019.126409. Epub 2019 Sep 25.

Maria Fiore <sup>1</sup>, Rita Barone <sup>2</sup>, Chiara Copat <sup>3</sup>, Alfina Grasso <sup>3</sup>, Antonio Cristaldi <sup>3</sup>, Renata Rizzo <sup>2</sup>, Margherita Ferrante <sup>3</sup>

<sup>1</sup> Environmental and Food Hygiene Laboratory (LIAA), Department "G.F. Ingrassia", University of Catania, Catania, Italy. Electronic address: mfiore@unict.it.

<sup>2</sup> Child Neurology and Psychiatry Unit, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy.

<sup>3</sup> Environmental and Food Hygiene Laboratory (LIAA), Department "G.F. Ingrassia", University of Catania, Catania, Italy.

#### Abstract

**Background:** Autism Spectrum Disorder (ASD) is a complex disorder with heterogeneous etiology and wide clinical severity which supports the needs of recognizing biological and clinical features in patient subsets. The present study aimed to understand possible associations between the hair levels of metals and essential elements and some specific features of ASD measured by the Autism Diagnostic Observation Schedule (ADOS) that represents the gold-standard instrument to objectively confirm ASD diagnosis.

**Methods:** A cross-sectional study was performed in the province of Catania (Sicily, South Italy). Forty-eight subjects with ASD (70.8% male), aged from 2 to 17 years were studied. Metals (Li, Be, Al, Ni, As, Mo, Cd, Hg, U, Pb) and essential trace elements (Cr, Co, Mn, Zn, Cu, Se) were quantified in hair by inductively coupled plasma mass spectrometry analysis. Participants were characterized by measuring the severity of autism symptoms and cognitive levels.

**Results:** A significant and positive correlation was found between hair metal burden (lead, aluminum, arsenic and cadmium levels) and severity of ASD symptoms (social communication deficits and repetitive, restrictive behaviors). Hair zinc level were inversely related with age while there was a negative, significant association between hair zinc level and severity of autistic symptoms (defective functional play and creativity and increase of stereotyped behavior). Lead, molybdenum and manganese hair levels were inversely correlated with cognitive level (full intelligence quotient) in ASD individuals.

**Conclusions:** The present study suggests the importance to combine metallomics analysis with pertinent disease features in ASD to identify potential environmental risk factors on an individual level possibly in the early developmental period.



Exp Toxicol Pathol. 2009 Mar;61(2):133-6. doi: 10.1016/j.etp.2008.07.002. Epub 2008 Sep 3.

Departments of Medicine and Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada. don.branch@utoronto.ca

#### Abstract

A recent report shows a correlation of the historical use of thimerosal in therapeutic immunizations with the subsequent development of autism; however, this association remains controversial. Autism occurs approximately four times more frequently in males compared to females; thus, studies of thimerosal toxicity should take into consideration gender-selective effects. The present study was originally undertaken to determine the maximum tolerated dose (MTD) of thimerosal in male and female CD1 mice. However, during the limited MTD studies, it became apparent that thimerosal has a differential MTD that depends on whether the mouse is male or female. **At doses of 38.4-76.8mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal.** Although the thimerosal levels used were very high, as we were originally only trying to determine MTD, it was completely unexpected to observe a difference of the MTD between male and female mice. Thus, our studies, although not directly addressing the controversy surrounding thimerosal and autism, and still preliminary due to small numbers of mice examined, provide, nevertheless, the first report of gender-selective toxicity of thimerosal and indicate that any future studies of thimerosal toxicity should take into consideration gender-specific differences.

#### 18. [Mercury toxicokinetics--dependency on strain and gender.](#)

Toxicol Appl Pharmacol. 2010 Mar 15;243(3):283-91. doi: 10.1016/j.taap.2009.08.026. Epub 2009 Sep 2.

Ekstrand J1, Nielsen JB, Havarinasab S, Zalups RK, Söderkvist P, Hultman P. Molecular and Immunological Pathology, Department of Clinical and Experimental Medicine, Linköping University, Sweden.

#### Abstract

Mercury (Hg) exposure from dental amalgam fillings and thimerosal in vaccines is not a major health hazard, but adverse health effects cannot be ruled out in a small and more susceptible part of the exposed population. Individual differences in toxicokinetics may explain susceptibility to mercury. Inbred, H-2-congenic A.SW and B10.S mice and their F1- and F2-hybrids were given HgCl<sub>2</sub> with 2.0 mg Hg/L drinking water and traces of (203)Hg. Whole-body retention (WBR) was monitored until steady state after 5 weeks, when the organ Hg content was assessed. Despite similar Hg intake, **A.SW males attained a 20-30% significantly higher WBR and 2- to 5-fold higher total renal Hg retention/concentration than A.SW females and B10.S mice. A selective renal Hg accumulation but of lower magnitude was seen also in B10.S males compared with females.** Differences in WBR and organ Hg accumulation

are therefore regulated by non-H-2 genes and gender. Lymph nodes lacked the strain- and gender-dependent Hg accumulation profile of kidney, liver and spleen. After 15 days without Hg A.SW mice showed a 4-fold higher WBR and liver Hg concentration, but 11-fold higher renal Hg concentration, showing the key role for the kidneys in explaining the slower Hg elimination in A.SW mice. The trait causing higher mercury accumulation was not dominantly inherited in the F1 hybrids. F2 mice showed a large inter-individual variation in Hg accumulation, showing that multiple genetic factors influence the Hg toxicokinetics in the mouse. The genetically heterogeneous human population may therefore show a large variation in mercury toxicokinetics.

19. [A Review of the Differences in Developmental, Psychiatric, and Medical Endophenotypes Between Males and Females with Autism Spectrum Disorder](#)

J Dev Phys Disabil. 2015 Feb; 27(1): 119–139.

Eric Rubenstein, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Lisa D. Wiggins, and Li-Ching Lee

Abstract

**Autism spectrum disorder (ASD) is over four times more prevalent in males compared to females.** Increased understanding of sex differences in ASD endophenotypes could add insight into possible etiologies and the assessment and management of the disorder. Consequently, the purpose of this review is to describe current literature regarding sex differences in the developmental, psychiatric, and medical endophenotypes of ASD in order to illustrate current knowledge and areas in need of further research. Our review found that repetitive behaviors and restricted interests are more common in males than females with ASD. Intellectual disability is more common in females than males with ASD. Attention to detail may be more common in males than females with ASD and epilepsy may be more common in females than males with ASD, although limited research in these areas prevent definitive conclusions from being drawn. There does not appear to be a sex difference in other developmental, psychiatric, and medical symptoms associated with ASD, or the research was contradictory or too sparse to establish a sex difference. Our review is unique in that it offers detailed discussion of sex differences in three major endophenotypes of ASD. Further research is needed to better understand why sex differences exist in certain ASD traits and to evaluate whether phenotypic sex differences are related to different pathways of development, assessment, and treatment of the disorder.

20. [Mercury toxicity: Genetic susceptibility and synergistic effects](#)

Medical Veritas 2 (2005) 535–542

Boyd E. Haley, PhD. Professor and Chair, Department of Chemistry, University of Kentucky

## Abstract

Mercury toxicity and intoxication (poisoning) are realities that every American needs to face. Both the Environmental Protection Agency and National Academy of Science state that between 8 to 10% of American women have mercury levels that would render any child they gave birth to neurological disorders. One of six children in the USA have a neurodevelopmental disorder according to the Centers for Disease Control and Prevention. Yet our dentistry and medicine continue to expose all patients to mercury. This article discusses the obvious sources of mercury exposures that can be easily prevented. It also points out that genetic susceptibility and exposures to other materials that synergistically enhance mercury and ethylmercury toxicity need to be evaluated, and that by their existence prevent the actual determination of a "safe level" of mercury exposure for all. The mercury sources we consider are from dentistry and from drugs, mainly vaccines, that, in today's world are not only unnecessary sources, but also sources that are being increasingly recognized as being significantly deleterious to the health of many.

## Excerpt

"4. Hormonal effects: Testosterone and Estrogen

Testosterone and estrogen-like compounds give vastly different results. Using female hormones we found them not toxic to the neurons alone and to be consistently protective against thimerosal toxicity. In fact, at high levels they could afford total protection for 24 hours against neuronal death in this test system (data not plotted). **However, testosterone which appeared protective at very low levels (0.01 to 0.1 micromolar), dramatically increased neuron death at higher levels (0.5 to 1.0 micromolar). In fact, 1.0 micromolar levels of testosterone that by itself did not significantly increase neuron death (red flattened oval), within 3 hours when added with 50 nanomolar thimerosal (solid circles) caused 100% neuron death.** Fifty nanomolar thimerosal at this time point did not significantly cause any cell death.

**These testosterone results, while not conclusive because of the in vitro neuron culture type of testing, clearly demonstrated that male versus female hormones may play a major role in autism risk and may explain the high ratio of boys to girls in autism (4 to 1) and autism related disorders."**

## 21. [Autism: a form of lead and mercury toxicity](#)

Environ Toxicol Pharmacol. 2014 Nov;38(3):1016-24. doi: 10.1016/j.etap.2014.10.005. Epub 2014 Nov 6.

Yassa HA

## Abstract

AIM: Autism is a developmental disability characterized by severe deficits in social interaction and communication. The definite cause of autism is still unknown. The aim of this study is to find out the relation between exposure to

Lead and/or mercury as heavy metals and autistic symptoms, dealing with the heavy metals with chelating agents can improve the autistic symptoms.

**METHOD:** Blood and hair samples were obtained from 45 children from Upper Egypt with autism between the ages of 2 and 10 years and 45 children served as controls in the same age range, after taken an informed consent and fill a questionnaire to assess the risk factors. The samples were analyzed blindly for lead and mercury by using atomic absorption and ICP-MS. Data from the two groups were compared, then follow up of the autistic children after treatment with chelating agents were done.

**RESULTS:** The results obtained showed significant difference among the two groups, there was high level of mercury and lead among those kids with autism. Significant decline in the blood level of lead and mercury with the use of DMSA as a chelating agent. In addition, there was decline in the autistic symptoms with the decrease in the lead and mercury level in blood.

**CONCLUSION:** **Lead and mercury considered as one of the main causes of autism. Environmental exposure as well as defect in heavy metal metabolism is responsible for the high level of heavy metals. Detoxification by chelating agents had great role in improvement of those kids.**

## 22. Do aluminum vaccine adjuvants contribute to the rising prevalence of autism?

J Inorg Biochem. 2011 Nov;105(11):1489-99. Epub 2011 Aug 23.  
Tomljenovic L, Shaw CA.

Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, 828 W. 10th Ave, Vancouver, BC, Canada V5Z 1L8.

### Abstract

Autism spectrum disorders (ASD) are serious multisystem developmental disorders and an urgent global public health concern. Dysfunctional immunity and impaired brain function are core deficits in ASD. Aluminum (Al), the most commonly used vaccine adjuvant, is a demonstrated neurotoxin and a strong immune stimulator. Hence, adjuvant Al has the potential to induce neuroimmune disorders. When assessing adjuvant toxicity in children, two key points ought to be considered: (i) children should not be viewed as "small adults" as their unique physiology makes them much more vulnerable to toxic insults; and (ii) if exposure to Al from only few vaccines can lead to cognitive impairment and autoimmunity in adults, is it unreasonable to question whether the current pediatric schedules, often containing 18 Al adjuvanted vaccines, are safe for children? By applying Hill's criteria for establishing causality between exposure and outcome we investigated whether exposure to Al from vaccines could be contributing to the rise in ASD prevalence in the Western world. Our results show that: (i) children from countries with the highest ASD prevalence appear to have the highest exposure to Al from vaccines; (ii) the increase in exposure to Al adjuvants

significantly correlates with the increase in ASD prevalence in the United States observed over the last two decades (Pearson  $r=0.92$ ,  $p<0.0001$ ); and (iii) a significant correlation exists between the amounts of AI administered to preschool children and the current prevalence of ASD in seven Western countries, particularly at 3-4 months of age (Pearson  $r=0.89-0.94$ ,  $p=0.0018-0.0248$ ). **The application of the Hill's criteria to these data indicates that the correlation between AI in vaccines and ASD may be causal.** Because children represent a fraction of the population most at risk for complications following exposure to AI, a more rigorous evaluation of AI adjuvant safety seems warranted.

23. [The putative role of environmental aluminium in the development of chronic neuropathology in adults and children. How strong is the evidence and what could be the mechanisms involved?](#)

Metabolic Brain Disease, October 2017, Volume 32, Issue 5, pp 1335–1355

Gerwyn Morris, Basant K. Puri, Richard E. Frye  
Tir Na Nog, Llanelli, UK, Department of Medicine/Imperial College London, Hammersmith Hospital, London UK, College of Medicine, Department of Pediatrics/University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock

#### Abstract

**The conceptualisation of autistic spectrum disorder** and Alzheimer's disease has undergone something of a paradigm shift in recent years and rather than being viewed as single illnesses with a unitary pathogenesis and pathophysiology they are increasingly considered to be heterogeneous syndromes with a complex multifactorial aetiopathogenesis, involving a highly complex and diverse combination of genetic, epigenetic and environmental factors. **One such environmental factor implicated as a potential cause in both syndromes is aluminium**, as an element or as part of a salt, received, for example, in oral form or as an adjuvant. Such administration has the potential to induce pathology via several routes such as provoking dysfunction and/or activation of glial cells which play an indispensable role in the regulation of central nervous system homeostasis and neurodevelopment. Other routes include the generation of oxidative stress, depletion of reduced glutathione, direct and indirect reductions in mitochondrial performance and integrity, and increasing the production of proinflammatory cytokines in both the brain and peripherally. The mechanisms whereby environmental aluminium could contribute to the development of the highly specific pattern of neuropathology seen in transsulfuration in Alzheimer's disease are described. Also detailed are several mechanisms whereby significant quantities of aluminium introduced via immunisation could produce chronic neuropathology in genetically susceptible children. **Accordingly, it is recommended that the use of aluminium salts in immunisations should be discontinued and that adults should take steps to minimise their exposure to environmental aluminium.**

24. [Administration of aluminium to neonatal mice in vaccine-relevant amounts is associated with adverse long term neurological outcomes.](#)

J Inorg Biochem. 2013 Nov;128:237-44. doi: 10.1016/j.jinorgbio.2013.07.022. Epub 2013 Jul 19.

Shaw CA, Li Y, Tomljenovic L.

Dept. of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, British Columbia, Canada; Program in Experimental Medicine, University of British Columbia, Vancouver, British Columbia, Canada; Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada. Electronic address: cashawlab@gmail.com.

#### Abstract

Our previous ecological studies of autism spectrum disorder (ASD) has demonstrated a correlation between increasing ASD rates and aluminium (Al) adjuvants in common use in paediatric vaccines in several Western countries. The correlation between ASD rate and Al adjuvant amounts appears to be dose-dependent and satisfies 8 of 9 Hill criteria for causality. We have now sought to provide an animal model to explore potential behavioural phenotypes and central nervous system (CNS) alterations using s.c. injections of Al hydroxide in early postnatal CD-1 mice of both sexes. Injections of a "high" and "low" Al adjuvant levels were designed to correlate to either the U.S. or Scandinavian paediatric vaccine schedules vs. control saline-injected mice. Both male and female mice in the "high Al" group showed significant weight gains following treatment up to sacrifice at 6 months of age. **Male mice in the "high Al" group showed significant changes in light-dark box tests and in various measures of behaviour in an open field. Female mice showed significant changes in the light-dark box at both doses, but no significant changes in open field behaviours. These current data implicate Al injected in early postnatal life in some CNS alterations that may be relevant for a better understanding of the aetiology of ASD.**

Repetitive administration of aluminium to neonatal mice in amounts comparable to those to children receive via routine vaccinations significantly increases anxiety and reduces exploratory behaviour and locomotor activities. The neurodisruptive effects of aluminium are long-lasting and persist for 6 months following injection.

25. [Aluminum-Induced Entropy in Biological Systems: Implications for Neurological Disease](#)

Journal of Toxicology, Volume 2014 (2014), Article ID 491316, 27 pages

Christopher A. Shaw,<sup>1,2,3</sup> Stephanie Seneff,<sup>4</sup> Stephen D. Kette,<sup>5</sup> Lucija Tomljenovic,<sup>1</sup> John W. Oller Jr.,<sup>6</sup> and Robert M. Davidson<sup>7</sup>

<sup>1</sup>Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, 828 W. 10th Avenue, Vancouver, British Columbia, Canada V5Z 1L8

<sup>2</sup>Program Experimental Medicine, University of British Columbia, Vancouver, Canada V5Z 1L8

<sup>3</sup>Program in Neurosciences, University of British Columbia, Vancouver, Canada V5Z 1L8

<sup>4</sup>MIT Computer Science and Artificial Intelligence Laboratory, 32 Vassar Street, Cambridge, MA 02139, USA

<sup>5</sup>Hudson, FL 34667, USA

<sup>6</sup>Department of Communicative Disorders, University of Louisiana, Lafayette, LA 70504-3170, USA

<sup>7</sup>Internal Medicine Group Practice, PhyNet Inc., 4002 Technology Center, Longview, TX 75605, USA

Over the last 200 years, mining, smelting, and refining of aluminum (Al) in various forms have increasingly exposed living species to this naturally abundant metal. Because of its prevalence in the earth's crust, prior to its recent uses it was regarded as inert and therefore harmless. However, Al is invariably toxic to living systems and has no known beneficial role in any biological systems. Humans are increasingly exposed to Al from food, water, medicinals, vaccines, and cosmetics, as well as from industrial occupational exposure. Al disrupts biological self-ordering, energy transduction, and signaling systems, thus increasing biosemiotic entropy. Beginning with the biophysics of water, disruption progresses through the macromolecules that are crucial to living processes (DNAs, RNAs, proteoglycans, and proteins). It injures cells, circuits, and subsystems and can cause catastrophic failures ending in death. Al forms toxic complexes with other elements, such as fluorine, and interacts negatively with mercury, lead, and glyphosate. Al negatively impacts the central nervous system in all species that have been studied, including humans. Because of the global impacts of Al on water dynamics and biosemiotic systems, CNS disorders in humans are sensitive indicators of the Al toxicants to which we are being exposed.

**Exerpts: "Animal models of neurological disease plainly suggest that the ubiquitous presence of Al in human beings implicates Al toxicants as causally involved in Lou Gehrig's disease (ALS), Alzheimer's disease and autism spectrum disorders."**

**"All these findings plausibly implicate Al adjuvants in pediatric vaccines as causal factors contributing to increased rates of autism spectrum disorders in countries where multiple doses are almost universally administered."**

26. [Clinical clues for autoimmunity and neuroinflammation in patients with autistic regression.](#)

Dev Med Child Neurol. 2017 Apr 6. doi: 10.1111/dmcn.13432.

Scott O, Shi D, Andriashek D, Clark B, Goetz HR.

Department of Paediatrics, The Hospital for Sick Children, Toronto, ON, Canada.  
Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.  
Department of Pediatrics, Glenrose Rehabilitation Hospital, Edmonton, AB,  
Canada.  
Division of Pediatric Neurology, University of Alberta, Edmonton, AB, Canada

#### Abstract

##### AIM:

Autistic regression is a unique variant within the autism spectrum disorders (ASDs), with recent reports raising the possibility of immune aetiology. This study explores clinical clues for an association between autistic regression and autoimmunity.

##### METHOD:

Single-centre charts of children diagnosed with ASD in 2014 were reviewed. We compared the rates of: (1) familial autoimmunity in first-degree and second-degree relatives; (2) febrile illness preceding initial parental concern, as a potential precipitant of immune activation; and (3) possible non-immune precipitants such as pregnancy and postnatal complications.

##### RESULTS:

The charts of 206 children with ASD and 33 diagnosed with autistic regression variant were reviewed. The incidence of febrile illness in the 6 months prior to initial parental concern was significantly higher in the children with autistic regression compared with those with ASD (30% vs 0%;  $p < 0.001$ ). The overall prevalence of familial autoimmunity was also higher in children with autistic regression compared with those with ASD (33% vs 12%;  $p < 0.001$ ). Type 1 diabetes and autoimmune thyroiditis were both more common in families with children with autistic regression. Other non-immune risk factors did not differ between the two groups.

##### INTERPRETATION:

**Our findings suggest that predisposition to autoimmunity, and immune/inflammatory activation, may be associated with autistic regression.**

#### 27. [Biological plausibility of the gut-brain axis in autism.](#)

Ann N Y Acad Sci. 2017 Nov;1408(1):5-6. doi: 10.1111/nyas.13516. Epub 2017 Nov 1.

Vasquez A

#### Abstract

Organic abnormalities with neuroinflammatory and psychiatric consequences involving abnormal kynurenine and purine metabolism, neurotransmitter and



cytokine imbalances, and altered levels of nutrients and metabolites are noted in autism, and many of these abnormalities—specifically including increased intestinal permeability, microbial metabolites, and heightened serum levels of endotoxin—originate from the gut.

28. [A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors](#)

Environ Health. 2014; 13: 73.

Cynthia D Nevison

Institute for Arctic and Alpine Research, University of Colorado, Boulder, Boulder, CO 80309-0450 USA

The prevalence of diagnosed autism has increased rapidly over the last several decades among U.S. children. Environmental factors are thought to be driving this increase and a list of the top ten suspected environmental toxins was published recently.

#### Methods

Temporal trends in autism for birth years 1970–2005 were derived from a combination of data from the California Department of Developmental Services (CDDS) and the United States Individuals with Disabilities Education Act (IDEA). Temporal trends in suspected toxins were derived from data compiled during an extensive literature survey. Toxin and autism trends were compared by visual inspection and computed correlation coefficients. Using IDEA data, autism prevalence vs. birth year trends were calculated independently from snapshots of data from the most recent annual report, and by tracking prevalence at a constant age over many years of reports. The ratio of the snapshot:tracking trend slopes was used to estimate the "real" fraction of the increase in autism.

#### Results

The CDDS and IDEA data sets are qualitatively consistent in suggesting a strong increase in autism prevalence over recent decades. The quantitative comparison of IDEA snapshot and constant-age tracking trend slopes suggests that ~75-80% of the tracked increase in autism since 1988 is due to an actual increase in the disorder rather than to changing diagnostic criteria. Most of the suspected environmental toxins examined have flat or decreasing temporal trends that correlate poorly to the rise in autism. Some, including lead, organochlorine pesticides and vehicular emissions, have strongly decreasing trends. **Among the suspected toxins surveyed, polybrominated diphenyl ethers, aluminum adjuvants, and the herbicide glyphosate have increasing trends that correlate positively to the rise in autism.**

#### Conclusions

Diagnosed autism prevalence has risen dramatically in the U.S over the last several decades and continued to trend upward as of birth year 2005. The increase is mainly real and has occurred mostly since the late 1980s. In contrast, children's exposure to most of the top ten toxic compounds has remained flat or decreased over this same time frame. Environmental factors with increasing temporal trends can help suggest hypotheses for drivers of autism that merit further investigation.

29. [Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism](#)

Maedica (Buchar). 2012 Jan; 7(1): 38–48.

Eleonor BLAUROCK-BUSCH,<sup>a</sup> Omnia R. AMIN,<sup>b</sup> Hani H. DESSOKI,<sup>c</sup> and Thanaa RABAH <sup>d</sup>

<sup>a</sup>Lecturer and Advisor, International Board of Clinical Metal Toxicology & German Medical Association of Clinical Metal Toxicology, Hersbruck, Germany

<sup>b</sup>Associate Professor of Psychiatry, Cairo University, Egypt

<sup>c</sup>Associate Professor of Psychiatry, Beni-Suef University, Egypt - Beni-Suef University

<sup>d</sup>Researcher of Public Health and Biostatistics, National Research Center, Egypt

Address for correspondence: Eleonor Blaurock-Busch, Laboratory for Clinical and Environmental Analyses. Robenstr 20, D-912217, Hersbruck, Germania.

Phone: +0049 91514332 ; Email: ed.ecartorcim@bbew

#### ABSTRACT

**Objective:** The objective of this study was to assess the levels of ten toxic metals and essential elements in hair samples of children with autism, and to correlate the level of these elements with the severity of autism.

**Method:** The participants were 44 children, age 3 to 9 years, with Autistic Spectrum Disorder (ASD) according to Diagnostic and Statistical Manual of Mental Disorders 4th Edition, (DSM-IV). The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS). Hair analysis was performed to evaluate the long term metal exposure and mineral level.

**Results:** By comparing hair concentration of autistic vs nonautistic children, elevated hair concentrations were noted for aluminum, arsenic, cadmium, mercury, antimony, nickel, lead, and vanadium. Hair levels of calcium, iron, iodine, magnesium, manganese, molybdenum, zinc, and selenium were considered deficient. There was a significant positive correlation between lead & verbal communication ( $p = 0.020$ ) and general impression ( $p = 0.008$ ). In addition, there was a significant negative correlation between zinc & fear and nervousness ( $p = 0.022$ ).

Conclusion: **Our data supports the historic evidence that heavy metals play a role in the development of ASD. In combination with an inadequate nutritional status the toxic effect of metals increase along with the severity of symptoms.**

30. [Autism is an Acquired Cellular Detoxification Deficiency Syndrome with Heterogeneous Genetic Predisposition](#)

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James Lyons-Weiler\*

Institute for Pure and Applied Knowledge, USA

Abstract

Neurodevelopmental disorders, including autism spectrum disorders, have a complex biological and medical basis involving diverse genetic risk and myriad environmental exposures. Teasing apart the role of specific stressors is made challenging due to the large number of apparently contributing associations, gene x environment interactions and phenomimicry. Historically, these conditions have been rare, making causality assessment at the population level infeasible. Only a few vaccines have been tested for association with autism, and it has been shown that improved diagnosis only explains a percentage of the increase in diagnosis. Now the rates are so high in some countries that public school programs cannot handle to large numbers of special needs students, and professionals are quitting their jobs due to security concerns. Here, I present a mechanistic biomedical process model (theory) of the pathophysiology of autism that reconciles the apparent paradox between the high degree of causal heterogeneity in environmental toxins, the absence of common "autism genes" and the high degree of genetic concordance (heritability) of ASD and ASD-like traits. In brief, the environmental toxin sampling liability for ASD varies among families involving different local exposures following injury to normal cellular endoplasmic detoxification and mitochondrial processes from toxic metals. The literature strongly supports that autism is most accurately seen as an acquired cellular detoxification deficiency syndrome with heterogeneous genetic predisposition that manifests pathophysiologic consequences of accumulated, run-away cellular toxicity. At a more general level, it is a form of a toxicant-induced loss of tolerance of toxins, and of chronic and sustained ER overload ("ER hyperstress"), contributing to neuronal and glial apoptosis via the unfolded protein response (UPR). Inherited risk of impaired cellular detoxification and circulating metal re-toxification in neurons and glial cells accompanied by chronic UPR is key. This model explains the aberrant protein disorder observed in ASD; the great diversity of genes that are found to have low, but real contributions to ASD risk and the sensitivity of individuals with ASD to environmental toxins. The hindrance of detoxification and loss of cellular energetics leads to apoptosis,

release of cytokines and chronic neuroinflammation and microglial activation, all observed hallmarks of ASD. Interference with the development of normal complex (redundant) synapses leads to a pathological variation in neuronal differentiation, axon and dendrite outgrowth, and synaptic protein expression. The most general outcomes are overall simplification of gross synaptic anatomy and, neurofunctionally, a loss of inhibitory feedback and aberrations in long-term connections between distant regions of the brain. Failed resolution of the ER stress response leads to re-distribution of neurotoxic metals, and the impaired neurocellular processes lead to subsequent accumulation of a variety of additional types of toxins with secondary, sometime life-threatening comorbidities such as seizures, with overlapping (not mutually exclusive) causality. Reduction of exposure to toxins known to cause mitopathy (mercury) and endoplasmic reticulum dysfunction (mercury and aluminum) during pregnancy and during the early years of development will reduce the risk of ER overload and ER hyperstress, and of ASD diagnosis. This knowledge has immediate clinical translational relevance: Post-vaccination symptoms should be heeded as a sign of susceptibility to toxin; Vitamin D can be increased to drive the healthy early phases of the unfolded protein response (UPR), and mutations in ASD genes encoding proteins with high intrinsic disorder may contraindicate the use of aluminum and mercury for carriers of risk alleles. Clinicians should be alert to a patient's Vitamin D receptor (VDR) mutational status prior to recommending increased doses. Approaches to improving overall brain health in autistics must be de-stigmatized and given high priority. Reduction of lifetime exposures of industrial and agricultural toxins will improve brain health for the entire human population. Purely genetic studies of ASD, and studies that do not include vaccination as an environmental exposure with potential liability and interactions with genes, are unethical. To qualify as science, studies must test plausible hypotheses, and the absence of association from poorly designed, unethically executed, and underpowered and unsound whole-population association studies have been harmful distractions in the quest for understanding. Skilled pediatricians and ob/gyns will seek evidence of genetic predisposition to environmental susceptibility in the form of non-synonymous substitutions in brain proteins that require ER-folding, and they will provide informed cautions on exposures (from all sources) to environmental toxins to patients and parents of patients with signs of metal and chemical sensitivity. To aid in this, a list of ASD environmental susceptibility protein-encoded genes is presented. A clinical exome sequence test, followed by loss-of-function prediction analysis, would point to individuals most susceptible to vaccine metal-induced ER hyper stress.

31. [Assessment of infantile mineral imbalances in autism spectrum disorders \(ASDs\).](#)

Int J Environ Res Public Health. 2013 Nov 11;10(11):6027-43. doi: 10.3390/ijerph10116027.

Yasuda H1, Tsutsui T.

La Belle Vie Research Laboratory, 8-4 Nihonbashi-Tomizawacho, Chuo-ku, Tokyo 103-0006, Japan. yasuda@lbv.co.jp

#### Abstract

The interactions between genes and the environment are now regarded as the most probable explanation for autism. In this review, we summarize the results of a metallomics study in which scalp hair concentrations of 26 trace elements were examined for 1,967 autistic children (1,553 males and 414 females aged 0-15 years-old), and discuss recent advances in our understanding of epigenetic roles of infantile mineral imbalances in the pathogenesis of autism. In the 1,967 subjects, 584 (29.7%) and 347 (17.6%) were found deficient in zinc and magnesium, respectively, and the incidence rate of zinc deficiency was estimated at 43.5% in male and 52.5% in female infantile subjects aged 0-3 years-old. In contrast, 339 (17.2%), 168 (8.5%) and 94 (4.8%) individuals were found to suffer from high burdens of aluminum, cadmium and lead, respectively, and 2.8% or less from mercury and arsenic. High toxic metal burdens were more frequently observed in the infants aged 0-3 years-old, whose incidence rates were 20.6%, 12.1%, 7.5%, 3.2% and 2.3% for aluminum, cadmium, lead, arsenic and mercury, respectively. **These findings suggest that infantile zinc- and magnesium-deficiency and/or toxic metal burdens may be critical and induce epigenetic alterations in the genes and genetic regulation mechanisms of neurodevelopment in the autistic children, and demonstrate that a time factor "infantile window" is also critical for neurodevelopment and probably for therapy. Thus, early metallomics analysis may lead to early screening/estimation and treatment/prevention for the autistic neurodevelopment disorders.**

32. [Intracellular Aluminium in Inflammatory and Glial Cells in Cerebral Amyloid Angiopathy: A Case Report](#)

Int J Environ Res Public Health. 2019 Apr; 16(8): 1459. Published online 2019 Apr 24. doi: 10.3390/ijerph16081459

Matthew Mold,<sup>1</sup> Jason Cottle,<sup>2</sup> Andrew King,<sup>3</sup> and Christopher Exley<sup>1,\*</sup>

<sup>1</sup>The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire ST5 5BG, UK;

<sup>2</sup>School of Medicine, David Weatherly Building, Keele University, Staffordshire ST5 5BG, UK;

<sup>3</sup>Department of Clinical Neuropathology, Kings College Hospital, London SE5 9RS, UK;

#### Abstract

(1) Introduction: In 2006, we reported on very high levels of aluminium in brain tissue in an unusual case of cerebral amyloid angiopathy (CAA). The individual concerned had been exposed to extremely high levels of aluminium in their potable water due to a notorious pollution incident in Camelford, Cornwall, in the

United Kingdom. The recent development of aluminium-specific fluorescence microscopy has now allowed for the location of aluminium in this brain to be identified. (2) Case Summary: We used aluminium-specific fluorescence microscopy in parallel with Congo red staining and polarised light to identify the location of aluminium and amyloid in brain tissue from an individual who had died from a rare and unusual case of CAA. Aluminium was almost exclusively intracellular and predominantly in inflammatory and glial cells including microglia, astrocytes, lymphocytes and cells lining the choroid plexus. Complementary staining with Congo red demonstrated that aluminium and amyloid were not co-located in these tissues. (3) Discussion: The observation of predominantly intracellular aluminium in these tissues was novel and something similar has only previously been observed in cases of autism. The results suggest a strong inflammatory component in this case and support a role for aluminium in this rare and unusual case of CAA.

33. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism.

J Biomed Sci. 2002 Jul-Aug;9(4):359-64.

Singh VK, Lin SX, Newell E, Nelson C., Department of Biology and Biotechnology Center, Utah State University, Logan, Utah 84322, USA. [singhvk@cc.usu.edu](mailto:singhvk@cc.usu.edu)

**Abstract**

Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73-75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that **an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism.**

34. [Acute Encephalopathy Followed by Permanent Brain Injury or Death Associated With Further Attenuated Measles Vaccines: A Review of Claims Submitted to the National Vaccine Injury Compensation Program](#)

Pediatrics (1998) 101 (3): 383–387. <https://doi.org/10.1542/peds.101.3.383>

Robert E. Weibel, MD; Vito Caserta, MD; David E. Benor, JD; Geoffrey Evans, MD

Reprint requests to (R.E.W.) National Vaccine Injury Compensation Program, Health Resources and Services Administration

**Objective.** To determine if there is evidence for a causal relationship between acute encephalopathy followed by permanent brain injury or death associated with the administration of further attenuated measles vaccines (Attenuvax or Lirugen, Hoechst Marion Roussel, Kansas City, MO), mumps vaccine (Mumpsvax, Merck and Co, Inc, West Point, PA), or rubella vaccines (Meruvax or Meruvax II, Merck and Co, Inc, West Point, PA), combined measles and rubella vaccine (M-R-Vax or M-R-Vax II, Merck and Co, Inc, West Point, PA), or combined measles, mumps, and rubella vaccine (M-M-R or M-M-R II, Merck and Co, Inc, West Point, PA), the lead author reviewed claims submitted to the National Vaccine Injury Compensation Program.

**Methods.** The medical records of children who met the inclusion criteria of receiving the first dose of these vaccines between 1970 and 1993 and who developed such an encephalopathy with no determined cause within 15 days were identified and analyzed.

**Results.** A total of 48 children, ages 10 to 49 months, met the inclusion criteria after receiving measles vaccine, alone or in combination. Eight children died, and the remainder had mental regression and retardation, chronic seizures, motor and sensory deficits, and movement disorders. The onset of neurologic signs or symptoms occurred with a nonrandom, statistically significant distribution of cases on days 8 and 9. No cases were identified after the administration of monovalent mumps or rubella vaccine.

**Conclusions.** **This clustering suggests that a causal relationship between measles vaccine and encephalopathy may exist as a rare complication of measles immunization.**

35. [Acetaminophen \(paracetamol\) use, measles-mumps-rubella vaccination, and autistic disorder: the results of a parent survey](#)

Autism. 2008 May;12(3):293-307. doi: 10.1177/1362361307089518.

Stephen T Schultz<sup>1</sup>, Hillary S Klonoff-Cohen, Deborah L Wingard, Natacha A Akshoomoff, Caroline A Macera, Ming Ji

<sup>1</sup> University of California San Diego, USA. [Stephen.schultz@med.navy.mil](mailto:Stephen.schultz@med.navy.mil)

Abstract

The present study was performed to determine whether acetaminophen (paracetamol) use after the measles-mumps-rubella vaccination could be associated with autistic disorder. This case-control study used the results of an online parental survey conducted from 16 July 2005 to 30 January 2006, consisting of 83 children with autistic disorder and 80 control children. Acetaminophen use after measles-mumps-rubella vaccination was significantly associated with autistic disorder when considering children 5 years of age or less (OR 6.11, 95% CI 1.42-26.3), after limiting cases to children with regression in development (OR 3.97, 95% CI 1.11-14.3), and when considering only children who had post-vaccination sequelae (OR 8.23, 95% CI 1.56-43.3), adjusting for age, gender, mother's ethnicity, and the presence of illness concurrent with measles-mumps-rubella vaccination. Ibuprofen use after measles-mumps-rubella vaccination was not associated with autistic disorder. **This preliminary study found that acetaminophen use after measles-mumps-rubella vaccination was associated with autistic disorder.**

36. [Infection, vaccines and other environmental triggers of autoimmunity.](#)

Autoimmunity. 2005 May;38(3):235-45.

Molina V, Shoenfeld Y., Department of Medicine B and The Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel.

Abstract

The etiology of autoimmune diseases is still not clear but genetic, immunological, hormonal and environmental factors are considered to be important triggers. Most often autoimmunity is not followed by clinical symptoms unless an additional event such as an environmental factor favors an overt expression. Many environmental factors are known to affect the immune system and may play a role as triggers of the autoimmune mosaic. Infections: bacterial, viral and parasitic infections are known to induce and exacerbate autoimmune diseases, mainly by the mechanism of molecular mimicry. This was studied for some syndromes as for the association between SLE and EBV infection, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection and more. **Vaccines, in several reports were found to be temporally followed by a new onset of autoimmune diseases. The same mechanisms that act in infectious invasion of the host, apply equally to the host response to vaccination.** It has been accepted for diphtheria and tetanus toxoid, polio and measles vaccines and GBS. Also this theory has been accepted for MMR vaccination and development of autoimmune thrombocytopenia, MS has been associated with HBV vaccination. Occupational and other chemical exposures are considered as triggers for autoimmunity. A debate still exists about the role of silicone implants in induction of scleroderma like disease. Not only foreign chemicals and agents have been associated with induction of autoimmunity, but also an intrinsic hormonal exposure, such as estrogens. This might explain the



sexual dimorphism in autoimmunity. Better understanding of these environmental risk factors will likely lead to explanation of the mechanisms of onset and progression of autoimmune diseases and may lead to effective preventive involvement in specific high-risk groups. So by diagnosing a new patient with autoimmune disease a wide anamnesis work should be done.

37. [Impact of environmental factors on the prevalence of autistic disorder after 1979](#)

Journal of Public Health and Epidemiology, Vol.6(9), pp. 271-284, September 2014

Theresa A. Deisher, Ngoc V. Doan, Angelica Omaiye, Kumiko Koyama, Sarah Bwabye

Abstract

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

38. [An assessment of the impact of thimerosal on childhood neurodevelopmental disorders](#)

Pediatr Rehabil. 2003 Apr-Jun;6(2):97-102. doi: 10.1080/1363849031000139315.

David A Geier<sup>1</sup>, Mark R Geier

The Genetic Centers of America, 14 Redgate Court, Silver Spring, MD 20905, USA

Abstract

The prevalence of autism in the US has risen from 1 in approximately 2500 in the mid-1980s to 1 in approximately 300 children in the mid-1990s. The purpose of this study was to evaluate whether mercury from thimerosal in childhood vaccines contributed to neurodevelopmental disorders. Neurodevelopmental disorder dose-response curves for increasing mercury doses of thimerosal in childhood vaccines were determined based upon examination of the Vaccine Adverse Events Reporting System (VAERS) database and the 2001 US' Department of Education Report. The instantaneous dosage of mercury children received in comparison to the Food and Drug Administration (FDA)'s maximum permissible dose for the oral ingestion of methylmercury was also determined. The dose-response curves showed increases in odds ratios of neurodevelopmental disorders from both the VAERS and US Department of Education data closely linearly correlated with increasing doses of mercury from thimerosal-containing childhood vaccines and that for overall odds ratios statistical significance was achieved. Similar slopes and linear regression coefficients for autism odds ratios in VAERS and the US Department of Education data help to mutually validate each other. Controls employed in the VAERS and US Department of Education data showed minimal biases. **The evidence presented here shows that the occurrence of neurodevelopmental disorders following thimerosal-containing childhood vaccines does not appear to be coincidental.**

39. A Positive Association found between Autism Prevalence and Childhood Vaccination uptake across the U.S. Population

Journal of Toxicology and Environmental Health, Part A: Current Issues  
Volume 74, Issue 14, 2011, Pages 903 – 916

Author: Gayle DeLonga

Abstract

The reason for the rapid rise of autism in the United States that began in the 1990s is a mystery. Although individuals probably have a genetic predisposition to develop autism, researchers suspect that one or more environmental triggers are also needed. One of those triggers might be the battery of vaccinations that young children receive. Using regression analysis and controlling for family

income and ethnicity, the relationship between the proportion of children who received the recommended vaccines by age 2 years and the prevalence of autism (AUT) or speech or language impairment (SLI) in each U.S. state from 2001 and 2007 was determined. A positive and statistically significant relationship was found: The higher the proportion of children receiving recommended vaccinations, the higher was the prevalence of AUT or SLI. A 1% increase in vaccination was associated with an additional 680 children having AUT or SLI. Neither parental behavior nor access to care affected the results, since vaccination proportions were not significantly related (statistically) to any other disability or to the number of pediatricians in a U.S. state. **The results suggest that although mercury has been removed from many vaccines, other culprits may link vaccines to autism.** Further study into the relationship between vaccines and autism is warranted.

40. Neonatal administration of a vaccine preservative, thimerosal, produces lasting impairment of nociception and apparent activation of opioid system in rats.

Brain Res. 2009 Dec 8;1301:143-51. Epub 2009 Sep 9.

Olczak M, Duszczyk M, Mierzejewski P, Majewska MD. Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland.

#### Abstract

Thimerosal (THIM), an organomercury preservative added to many child vaccines is a suspected factor in pathogenesis of neurodevelopmental disorders. We examined the pharmacokinetics of Hg in the brain, liver and kidneys after i.m. THIM injection in suckling rats and we tested THIM effect on nociception. THIM solutions were injected to Wistar and Lewis rats in a vaccination-like mode on PN days 7, 9, 11 and 15 in four equal doses. For Wistar rats these were: 12, 48, 240, 720, 1440, 2160, 3000 microg Hg/kg and for Lewis: 54, 216, 540 and 1080 microg Hg/kg. Pharmacokinetic analysis revealed that Hg from THIM injections accumulates in the rat brain in significant amounts and remains there longer than 30 days after the injection. At the 6th week of age animals were examined for pain sensitivity using the hot plate test. THIM treated rats of both strains and sexes manifested statistically significantly elevated pain threshold (latency for paw licking, jumping) on a hot plate (56 degrees C). Wistar rats were more sensitive to this effect than Lewis rats. Prolonged THIM-induced hypoalgesia was reversed by naloxone (5 mg/kg, i.p.) injected before the hot plate test, indicative of involvement of endogenous opioids. This was confirmed by augmented catalepsy after morphine (2.5 mg/kg, s.c.) injection. Acute THIM injection to 6-week-old rats also produced hypoalgesia, but this effect was transient and was gone within 14 days. **Present findings show that THIM administration to suckling or adult rats impairs sensitivity to pain, apparently due to activation the endogenous opioid system.**

41. [Effect of thimerosal on the neurodevelopment of premature rats.](#)

World J Pediatr. 2013 Nov;9(4):356-60. doi: 10.1007/s12519-013-0443-z. Epub 2013 Nov 14.

Chen YN1, Wang J, Zhang J, Li SJ, He L, Shao DD, Du HY.

The Key Laboratory of Biomedical Information Engineering of Ministry of Education, and Institute of Biomedical Engineering, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, 710049, China.

Abstract

BACKGROUND:

This study was undertaken to determine the effect of thimerosal on the neurodevelopment of premature rats.

METHODS:

Thimerosal was injected into premature SD rats at a dose of 32.8, 65.6, 98.4 or 131.2 µg/kg on postnatal day 1. Expression of dopamine D4 receptor (DRD4) and serotonin 2A receptor (5-HT2AR), apoptosis in the prefrontal cortex on post-injection day 49, and learning and memory function were studied and compared with those in a control group injected with saline.

RESULTS:

Expression of DRD4 and 5-HT2AR and learning function decreased, and apoptosis increased significantly in the 131.2 µg/kg group ( $P < 0.001$ ). Memory function was significantly impaired by 65.6 ( $P < 0.05$ ), 98.4 and 131.2 µg/kg ( $P < 0.001$ ).

CONCLUSIONS:

**The negative adverse consequences on neurodevelopment observed in the present study are consistent with previous studies; this study raised serious concerns about adverse neurodevelopmental disorder such as autism in humans following the ongoing worldwide routine administration of thimerosal containing vaccines to infants.**

42. [Transcriptomic analyses of neurotoxic effects in mouse brain after intermittent neonatal administration of thimerosal.](#)

Toxicol Sci. 2014 Jun;139(2):452-65. doi: 10.1093/toxsci/kfu049. Epub 2014 Mar 27.

State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. Li X1, Qu F, Xie W, Wang F, Liu H, Song S, Chen T, Zhang Y, Zhu S, Wang Y, Guo C, Tang TS.

## Abstract

Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopmental disorders including autism. The association between infant vaccine thimerosal exposure and autism remains an open question. Although thimerosal has been removed from mandatory childhood vaccines in the United States, thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries. Notably, thimerosal-containing vaccines are being given to the newborns within the first 12-24 h after birth in some countries. To examine the possible neurotoxic effects of early neonatal exposure to a higher level of thimerosal, FVB mice were subcutaneously injected with thimerosal-mercury at a dose which is 20× higher than that used for regular Chinese infant immunization during the first 4 months of life. Thimerosal-treated mice exhibited neural development delay, social interaction deficiency, and inclination of depression. Apparent neuropathological changes were also observed in adult mice neonatally treated with thimerosal. High-throughput RNA sequencing of autistic-behaved mice brains revealed the alternation of a number of canonical pathways involving neuronal development, neuronal synaptic function, and the dysregulation of endocrine system. Intriguingly, the elevation of anterior pituitary secreting hormones occurred exclusively in male but not in female thimerosal-treated mice, demonstrating for the first time the gender bias of thimerosal-mercury toxicity with regard to endocrine system. **Our results indicate that higher dose of neonatal thimerosal-mercury (20× higher than that used in human) is capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be the causal involvements of autistic-like behavior in mice.**

43. Lasting neuropathological changes in rat brain after intermittent neonatal administration of thimerosal.

Folia Neuropathol. 2010;48(4):258-69. Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T, Majewska MD.

Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, ul. Sobieskiego 9, Warsaw, Poland.

## Abstract

Thimerosal, an organomercurial added as a preservative to some vaccines, is a suspected iatrogenic factor, possibly contributing to paediatric neurodevelopmental disorders including autism. We examined the effects of early postnatal administration of thimerosal (four i.m. injections, 12 or 240 µg THIM-Hg/kg, on postnatal days 7, 9, 11 and 15) on brain pathology in Wistar rats. Numerous neuropathological changes were observed in young adult rats which were treated postnatally with thimerosal. They included: ischaemic degeneration of neurons and "dark" neurons in the prefrontal and temporal cortex, the hippocampus and the cerebellum, pathological changes of the blood vessels in the temporal cortex, diminished synaptophysin reaction in the hippocampus,

atrophy of astroglia in the hippocampus and cerebellum, and positive caspase-3 reaction in Bergmann astroglia. **These findings document neurotoxic effects of thimerosal, at doses equivalent to those used in infant vaccines or higher, in developing rat brain, suggesting likely involvement of this mercurial in neurodevelopmental disorders.**

44. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats.

Behav Brain Res. 2011 Sep 30;223(1):107-18. doi: 10.1016/j.bbr.2011.04.026. Epub 2011 Apr 28.

Olczak M, Duszczyk M, Mierzejewski P, Meyza K, Majewska MD. Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, 02-957 Warsaw, Poland.

#### **Abstract**

The neurotoxic organomercurial thimerosal (THIM), used for decades as vaccine preservative, is a suspected factor in the pathogenesis of some neurodevelopmental disorders. Previously we showed that neonatal administration of THIM at doses equivalent to those used in infant vaccines or higher, causes lasting alterations in the brain opioid system in rats. Here we investigated neonatal treatment with THIM (at doses 12, 240, 1440 and 3000 µg Hg/kg) on behaviors, which are characteristically altered in autism, such as locomotor activity, anxiety, social interactions, spatial learning, and on the brain dopaminergic system in Wistar rats of both sexes. Adult male and female rats, which were exposed to the entire range of THIM doses during the early postnatal life, manifested impairments of locomotor activity and increased anxiety/neophobia in the open field test. In animals of both sexes treated with the highest THIM dose, the frequency of prosocial interactions was reduced, while the frequency of asocial/antisocial interactions was increased in males, but decreased in females. Neonatal THIM treatment did not significantly affect spatial learning and memory. THIM-exposed rats also manifested reduced haloperidol-induced catalepsy, accompanied by a marked decline in the density of striatal D<sub>2</sub> receptors, measured by immunohistochemical staining, suggesting alterations to the brain dopaminergic system. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of THIM. **These data document that early postnatal THIM administration causes lasting neurobehavioral impairments and neurochemical alterations in the brain, dependent on dose and sex. If similar changes occur in THIM/mercurial-exposed children, they could contribute do neurodevelopmental disorders.**

45. [B-Lymphocytes from a Population of Children with Autism Spectrum Disorder and Their Unaffected Siblings Exhibit Hypersensitivity to Thimerosal](#)

J Toxicol. 2013;2013:801517. Epub 2013 Jun 9.

Sharpe MA, Gist TL, Baskin DS.

Department of Neurosurgery, The Methodist Neurological Institute, Houston, TX.

#### Abstract

The role of thimerosal containing vaccines in the development of autism spectrum disorder (ASD) has been an area of intense debate, as has the presence of mercury dental amalgams and fish ingestion by pregnant mothers. We studied the effects of thimerosal on cell proliferation and mitochondrial function from B-lymphocytes taken from individuals with autism, their nonautistic twins, and their nontwin siblings. Eleven families were examined and compared to matched controls. B-cells were grown with increasing levels of thimerosal, and various assays (LDH, XTT, DCFH, etc.) were performed to examine the effects on cellular proliferation and mitochondrial function. A subpopulation of eight individuals (4 ASD, 2 twins, and 2 siblings) from four of the families showed thimerosal hypersensitivity, whereas none of the control individuals displayed this response. The thimerosal concentration required to inhibit cell proliferation in these individuals was only 40% of controls. Cells hypersensitive to thimerosal also had higher levels of oxidative stress markers, protein carbonyls, and oxidant generation. **This suggests certain individuals with a mild mitochondrial defect may be highly susceptible to mitochondrial specific toxins like the vaccine preservative thimerosal.**

46. [Thimerosal-Derived Ethylmercury Is a Mitochondrial Toxin in Human Astrocytes: Possible Role of Fenton Chemistry in the Oxidation and Breakage of mtDNA](#)

J Toxicol. 2012; 2012: 373678. Published online Jun 28, 2012. doi: 10.1155/2012/373678

Martyn A. Sharpe, \* Andrew D. Livingston, and David S. Baskin

#### Abstract

Thimerosal generates ethylmercury in aqueous solution and is widely used as preservative. We have investigated the toxicology of Thimerosal in normal human astrocytes, paying particular attention to mitochondrial function and the generation of specific oxidants. **We find that ethylmercury not only inhibits mitochondrial respiration leading to a drop in the steady state membrane potential, but also concurrent with these phenomena increases the formation of superoxide, hydrogen peroxide, and Fenton/Haber-Weiss generated hydroxyl radical. These oxidants increase the levels of cellular aldehyde/ketones. Additionally, we find a five-fold increase in the levels of oxidant damaged mitochondrial DNA bases and increases in the levels of mtDNA nicks and blunt-ended breaks. Highly damaged mitochondria are characterized by having very low membrane potentials, increased superoxide/hydrogen peroxide production, and extensively damaged mtDNA and proteins. These mitochondria appear to have undergone a permeability transition, an observation supported by the five-fold increase**

**in Caspase-3 activity observed after Thimerosal treatment.**

47. [Thioredoxin: A novel, independent diagnosis marker in children with autism.](#)

Int J Dev Neurosci. 2014 Nov 26. pii: S0736-5748(14)00191-9. doi: 10.1016/j.ijdevneu.2014.11.007.

Zhang QB1, Gao SJ1, Zhao HX2.

Abstract

**BACKGROUND:**

Oxidative stress increases serum thioredoxin (TRX), a redox-regulating protein with antioxidant activity recognized as an oxidative-stress marker. The aim of this study was to assess the clinical significance of serum TRX levels in Autism spectrum disorders (ASD).

**METHODS:**

Eighty patients diagnosed with ASD and 100 sex and age matched typically developing children were assessed for serum TRX content at admission. TRX were assayed with solid-phase sandwich ELISA, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score.

**RESULTS:**

The results indicated that the median serum TRX levels were significantly ( $P < 0.0001$ ) higher in children with ASD as compared to typically developing children [17.9(IQR: 10.7-25.8)ng/ml and 5.5(3.6-9.2)ng/ml, respectively]. Levels of TRX increased with increasing severity of ASD as defined by the CARS score. After adjusting for all other possible covariates, TRX still was an independent diagnosis marker of ASD with an adjusted OR of 1.454 (95% CI, 1.232-1.892;  $P < 0.0001$ ). Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of serum TRX levels as an indicator for auxiliary diagnosis of autism was projected to be 10.6ng/ml. Further, we found that an increased diagnosis of ASD was associated with TRX levels  $\geq 10.6$ ng/ml (adjusted OR 15.31, 95% CI: 7.36-31.85) after adjusting for possible confounders.

**CONCLUSIONS:**

**Our study demonstrated that serum TRX levels were associated with ASD, and elevated levels could be considered as a novel, independent diagnosis indicator of ASD.**

48. Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity.

J Biol Chem. 2008 May 2;283(18):11913-23. doi: 10.1074/jbc.M710133200. Epub 2008 Mar 4.

Carvalho CM1, Chew EH, Hashemy SI, Lu J, Holmgren A.

Abstract



Mercury toxicity mediated by different forms of mercury is a major health problem; however, the molecular mechanisms underlying toxicity remain elusive. We analyzed the effects of mercuric chloride (HgCl<sub>2</sub>) and monomethylmercury (MeHg) on the proteins of the mammalian thioredoxin system, thioredoxin reductase (TrxR) and thioredoxin (Trx), and of the glutaredoxin system, glutathione reductase (GR) and glutaredoxin (Grx). HgCl<sub>2</sub> and MeHg inhibited recombinant rat TrxR with IC<sub>50</sub> values of 7.2 and 19.7 nM, respectively. Fully reduced human Trx1 bound mercury and lost all five free thiols and activity after incubation with HgCl<sub>2</sub> or MeHg, but only HgCl<sub>2</sub> generated dimers. Mass spectra analysis demonstrated binding of 2.5 mol of Hg(2+) and 5 mol of MeHg(+)/mol of Trx1 with the very strong Hg(2+) complexes involving active site and structural disulfides. Inhibition of both TrxR and Trx activity was observed in HeLa and HEK 293 cells treated with HgCl<sub>2</sub> or MeHg. GR was inhibited by HgCl<sub>2</sub> and MeHg in vitro, but no decrease in GR activity was detected in cell extracts treated with mercurials. Human Grx1 showed similar reactivity as Trx1 with both mercurial compounds, with the loss of all free thiols and Grx dimerization in the presence of HgCl<sub>2</sub>, but no inhibition of Grx activity was observed in lysates of HeLa cells exposed to mercury. Overall, mercury inhibition was selective toward the thioredoxin system. In particular, the remarkable potency of the mercury compounds to bind to the selenol-thiol in the active site of TrxR should be a major molecular mechanism of mercury toxicity.

49. Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning.

FASEB J. 2011 Jan;25(1):370-81. doi: 10.1096/fj.10-157594. Epub 2010 Sep 1.

Carvalho CM1, Lu J, Zhang X, Arnér ES, Holmgren A.

Abstract

Mercury toxicity is a highly interesting topic in biomedicine due to the severe endpoints and treatment limitations. Selenite serves as an antagonist of mercury toxicity, but the molecular mechanism of detoxification is not clear. Inhibition of the selenoenzyme thioredoxin reductase (TrxR) is a suggested mechanism of toxicity. Here, we demonstrated enhanced inhibition of activity by inorganic and organic mercury compounds in NADPH-reduced TrxR, consistent with binding of mercury also to the active site selenolthiol. On treatment with 5 μM selenite and NADPH, TrxR inactivated by HgCl<sub>2</sub> displayed almost full recovery of activity. Structural analysis indicated that mercury was complexed with TrxR, but enzyme-generated selenide removed mercury as mercury selenide, regenerating the active site selenocysteine and cysteine residues required for activity. The antagonistic effects on TrxR inhibition were extended to endogenous antioxidants, such as GSH, and clinically used exogenous chelating agents BAL, DMPS, DMSA, and α-lipoic acid. Consistent with the in vitro results, recovery of TrxR activity and cell viability by selenite was observed in HgCl<sub>2</sub>-treated HEK 293 cells. These results stress the role of TrxR as a target of mercurials and provide the mechanism of selenite as a detoxification agent for mercury poisoning.

50. Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism.

Clin Immunol Immunopathol. 1998 Oct;89(1):105-8.

Singh VK, Lin SX, Yang VC. College of Pharmacy, University of Michigan, Ann Arbor, Michigan, 48109-1065, USA.

**Abstract**

Considering an autoimmunity and autism connection, brain autoantibodies to myelin basic protein (anti-MBP) and neuron-axon filament protein (anti-NAFP) have been found in autistic children. In this current study, we examined associations between virus serology and autoantibody by simultaneous analysis of measles virus antibody (measles-IgG), human herpesvirus-6 antibody (HHV-6-IgG), anti-MBP, and anti-NAFP. We found that measles-IgG and HHV-6-IgG titers were moderately higher in autistic children but they did not significantly differ from normal controls. Moreover, we found that a vast majority of virus serology-positive autistic sera was also positive for brain autoantibody: (i) 90% of measles-IgG-positive autistic sera was also positive for anti-MBP; (ii) 73% of measles-IgG-positive autistic sera was also positive for anti-NAFP; (iii) 84% of HHV-6-IgG-positive autistic sera was also positive for anti-MBP; and (iv) 72% of HHV-6-IgG-positive autistic sera was also positive for anti-NAFP. **This study is the first to report an association between virus serology and brain autoantibody in autism; it supports the hypothesis that a virus-induced autoimmune response may play a causal role in autism.**

51. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism

American Journal of Clinical Nutrition, Vol. 80, No. 6, 1611-1617, December 2004

Department of Pediatrics, University of Arkansas for Medical Sciences, and the Arkansas Children's Hospital Research Institute

**Abstract**

Background: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism.

Objective: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism.

Design: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children.

Results: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children.

Conclusions: **An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism.**

52. [Classification and adaptive behavior prediction of children with autism spectrum disorder based upon multivariate data analysis of markers of oxidative stress and DNA methylation](#)

Daniel P. Howsmon, Uwe Kruger, Stepan Melnyk, S. Jill James, Juergen Hahn

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Daniel P. Howsmon

Affiliations Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, United States of America

ORCID logo <http://orcid.org/0000-0002-7177-1342>

Uwe Kruger

Affiliation Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America

ORCID logo <http://orcid.org/0000-0001-5664-9499>

Stepan Melnyk

Affiliation Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States of America

S. Jill James

Affiliation Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States of America

Juergen Hahn

E-mail: hahnj@rpi.edu

Affiliations Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, United States of America, Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America

#### Abstract

The number of diagnosed cases of Autism Spectrum Disorders (ASD) has increased dramatically over the last four decades; however, there is still considerable debate regarding the underlying pathophysiology of ASD. This lack of biological knowledge restricts diagnoses to be made based on behavioral observations and psychometric tools. However, physiological measurements should support these behavioral diagnoses in the future in order to enable earlier and more accurate diagnoses. **Stepping towards this goal of incorporating biochemical data into ASD diagnosis, this paper analyzes measurements of metabolite concentrations of the folate-dependent one-carbon metabolism and transulfuration pathways taken from blood samples of 83 participants with ASD and 76 age-matched neurotypical peers.** Fisher Discriminant Analysis enables multivariate classification of the participants as on the spectrum or neurotypical which **results in 96.1% of all neurotypical participants being correctly identified as such while still correctly identifying 97.6% of the ASD cohort.** Furthermore, kernel partial least squares is used to predict adaptive behavior, as measured by the Vineland Adaptive Behavior Composite score, where measurement of five metabolites of the pathways was sufficient to predict the Vineland score with an R2 of 0.45 after cross-validation. This level of accuracy for classification as well as severity prediction far exceeds any other approach in this field and is a strong indicator that the metabolites under consideration are strongly correlated with an ASD diagnosis but also that the statistical analysis used here offers tremendous potential for extracting important information from complex biochemical data sets.

#### 53. [Newborn screening for autism: in search of candidate biomarkers.](#)

Biomark Med. 2013 Apr;7(2):247-60. doi: 10.2217/bmm.12.108.

Mizejewski GJ1, Lindau-Shepard B, Pass KA.

Division of Translational Medicine, Wadsworth Center, NYS Department of Health, PO Box 509, Albany, NY 12201 0509, USA.

#### Abstract

**BACKGROUND:**

Autism spectrum disorder (ASD) represents a wide range of neurodevelopmental disorders characterized by impairments in social interaction, language, communication and range of interests. Autism is usually diagnosed in children 3-5 years of age using behavioral characteristics; thus, diagnosis shortly after birth would be beneficial for early initiation of treatment.

**AIM:**

This retrospective study sought to identify newborns at risk for ASD utilizing bloodspot specimens in an immunoassay.

**MATERIALS & METHODS:**

The present study utilized stored frozen specimens from ASD children already diagnosed at 15-36 months of age. The newborn specimens and controls were analyzed by immunoassay in a multiplex system that included 90 serum biomarkers and subjected to statistical analysis.

**RESULTS:**

Three sets of five biomarkers associated with ASD were found that differed from control groups. The 15 candidate biomarkers were then discussed regarding their association with ASD.

**CONCLUSION:**

This study determined that a statistically selected panel of 15 biomarkers successfully discriminated presumptive newborns at risk for ASD from those of nonaffected controls.

**Exerpt:**

**"GST [Glutathione S-transferase] is a metabolic biomarker directly associated with ASD. The human gene product for GST constitutes a candidate susceptibility protein due to its tissue distribution and role in oxidative stress and methionine metabolism, which results in neuronal injury and death."**

**"Results of a recent study further demonstrated that glutathione, total glutathione and activity levels of GST were significantly lower in autistic patients as compared with control subjects; however, homocysteine, thioredoxin reductase and peroxidoxin levels were remarkably higher."**

**"Autistic children with metabolic disturbances are known to display reduced metabolic activities of GST, cysteine, glutathione and methionine, which are associated with methionine transmethyltion and trans-sulfation."**

54. [Altered urinary porphyrins and mercury exposure as biomarkers for autism severity in Egyptian children with autism spectrum disorder](#)

*Metab Brain Dis* **31**, 1419–1426 (2016). <https://doi.org/10.1007/s11011-016-9870-6>

Eman M. Khaled, Nagwa A. Meguid, Geir Bjørklund, Amr Gouda, Mohamed H. Bahary, Adel Hashish, Nermin M. Sallam, Salvatore Chirumbolo & Mona A. El-Bana

#### Abstract

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects social, communication, and behavioral development. Recent evidence supported but also questioned the hypothetical role of compounds containing mercury (Hg) as contributors to the development of ASD. Specific alterations in the urinary excretion of porphyrin-containing ring catabolites have been associated with exposure to Hg in ASD patients. In the present study, the level of urinary porphyrins, as biomarkers of Hg toxicity in children with ASD, was evaluated, and its correlation with severity of the autistic behavior further explored. A total of 100 children was enrolled in the present study. They were classified into three groups: children with ASD (40), healthy controls (40), and healthy siblings of the ASD children (20). Children with ASD were diagnosed using DSM-IV-TR, ADI-R, and CARS tests. Urinary porphyrins were evaluated within the three groups using high-performance liquid chromatography (HPLC), after plasma evaluation of mercury (Hg) and lead (Pb) in the same groups.

**Results showed that children with ASD had significantly higher levels of Hg, Pb, and the porphyrins pentacarboxyporphyrin, coproporphyrin, precoproporphyrin, uroporphyrins, and hexacarboxyporphyrin compared to healthy controls and healthy siblings of the ASD children.** However, there was no significant statistical difference in the level of heptacarboxyporphyrin among the three groups, while a significant positive correlation between the levels of coproporphyrin and precoproporphyrin and autism severity was observed. **Mothers of ASD children showed a higher percentage of dental amalgam restorations compared to the mothers of healthy controls suggesting that high Hg levels in children with ASD may relate to the increased exposure to Hg from maternal dental amalgam during pregnancy and lactation.** The results showed that the ASD children in the present study had increased blood Hg and Pb levels compared with healthy control children indicating that disordered porphyrin metabolism might interfere with the pathology associated with the autistic neurologic phenotype. **The present study indicates that coproporphyrin and precoproporphyrin may be utilized as possible biomarkers for heavy metal exposure and autism severity in children with ASD.**

55. Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity

*Toxicology and Applied Pharmacology* 214 (2006) 99 – 108, Received 13 February 2006; revised 23 March 2006; accepted 5 April 2006

Robert Natafa, Corinne Skorupkab, Lorene Ametb, Alain Lama, Anthea Springbettc and Richard Lathed, aLaboratoire Philippe Auguste, Paris, France,

Association ARIANE, Clichy, France, Department of Statistics, Roslin Institute, Roslin, UK, Pieta Research,

This new study from France utilizes a new and sophisticated measurement for environmental toxicity by assessing porphyrin levels in autistic children. It provides clear and unequivocal evidence that children with autism spectrum disorders are more toxic than their neurotypical peers.

Excerpt: "Coproporphyrin levels were elevated in children with autistic disorder relative to control groups...the elevation was significant. These data implicate environmental toxicity in childhood autistic disorder."

#### Abstract

To address a possible environmental contribution to autism, we carried out a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 269 children with neurodevelopmental and related disorders referred to a Paris clinic (2002–2004), including 106 with autistic disorder. Urinary porphyrin levels determined by high-performance liquid chromatography were compared between diagnostic groups including internal and external control groups. Coproporphyrin levels were elevated in children with autistic disorder relative to control groups. Elevation was maintained on normalization for age or to a control heme pathway metabolite (uroporphyrin) in the same samples. The elevation was significant ( $P < 0.001$ ). Porphyrin levels were unchanged in Asperger's disorder, distinguishing it from autistic disorder. The atypical molecule precoproporphyrin, a specific indicator of heavy metal toxicity, was also elevated in autistic disorder ( $P < 0.001$ ) but not significantly in Asperger's. A subgroup with autistic disorder was treated with oral dimercaptosuccinic acid (DMSA) with a view to heavy metal removal. Following DMSA there was a significant ( $P = 0.002$ ) drop in urinary porphyrin excretion. **These data implicate environmental toxicity in childhood autistic disorder.**

#### 56. [An investigation of porphyrinuria in Australian children with autism.](#)

Journal of Toxicology and Environmental Health, Part A, 71: 1403–1405, 2008  
Copyright © Taylor & Francis Group, LLC ISSN: 1528-7394 print / 1087-2620  
online DOI: 10.1080/15287390802271723

Austin DW, Shandley K.

Swinburne Autism Bio-Research Initiative (SABRI), Faculty of Life and Social Sciences, Swinburne University of Technology, Melbourne, Australia.  
daustin@swin.edu.au

#### Abstract

Two recent studies, from France (Nataf et al., 2006) and the United States (Geier & Geier, 2007), identified atypical urinary porphyrin profiles in children with an autism spectrum disorder (ASD). These profiles serve as an indirect measure of

environmental toxicity generally, and mercury (Hg) toxicity specifically, with the latter being a variable proposed as a causal mechanism of ASD (Bernard et al., 2001; Mutter et al., 2005). To examine whether this phenomenon occurred in a sample of Australian children with ASD, an analysis of urinary porphyrin profiles was conducted. A consistent trend in abnormal porphyrin levels was evidenced when data was compared with those previously reported in the literature. The results are suggestive of environmental toxic exposure impairing heme synthesis. **Three independent studies from three continents have now demonstrated that porphyrinuria is concomitant with ASD, and that Hg may be a likely xenobiotic to produce porphyrin profiles of this nature.**

57. [Porphyrinuria in Korean children with autism: correlation with oxidative stress.](#)

J Toxicol Environ Health A. 2010;73(10):701-10. doi: 10.1080/15287391003614000.

Youn SI1, Jin SH, Kim SH, Lim S.

Department of Basic Eastern Medical Science, Graduate School, KyungHee University, Seoul, Republic of Korea.

Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder believed to be associated with heavy metal exposure, especially mercury (Hg), and is characterized by disturbances in metal elimination. Various studies correlated elevated heavy metal body burden with ASD diagnoses as evidenced by increased urinary porphyrin levels in patients. Urinary porphyrins were also determined in Korean patients diagnosed with ASD (n = 65) who visited AK Eastern Medicinal Clinic in Kangnam-gu, Seoul, from June 2007 to September 2008, compared to controls (n = 9) residing in the same area, by means of Metamatrix (CLIA-approved) laboratory testing. Further, urinary organic acids as indicators of hepatic detoxification/oxidative stress were also analyzed among patients diagnosed with ASD. Significant increases were found in patients diagnosed with ASD for proporphyrins, pentacarboxyporphyrin, precoproporphyrin, coproporphyrins, and total porphyrins. Significant correlations were observed between hepatic detoxification/oxidative stress markers and urinary porphyrins. **In agreement with published data, the present results demonstrated that measurement of porphyrins serves as a reliable tool for diagnosis of heavy metal involvement in ASD.**

58. [Uncoupling of ATP-mediated Calcium Signaling and Dysregulated IL-6 Secretion in Dendritic Cells by Nanomolar Thimerosal](#)

Environ Health Perspect. 2006 Jul; 114(7): 1083–1091. Published online 2006 Mar 21. doi: 10.1289/ehp.8881



Samuel R. Goth, Ruth A. Chu Jeffrey P. Gregg

1 National Institute of Environmental Health Sciences Center for Children's Environmental Health

2 Department of Veterinary Molecular Biosciences and

3 Department of Medical Pathology, University of California–Davis, Davis, California, USA

4 MIND (Medical Investigation of Neurodevelopmental Disorders) Institute, University of California–Davis, Sacramento, California, USA

#### Abstract

Dendritic cells (DCs), a rare cell type widely distributed in the soma, are potent antigen-presenting cells that initiate primary immune responses. DCs rely on intracellular redox state and calcium ( $\text{Ca}^{2+}$ ) signals for proper development and function, but the relationship between these two signaling systems is unclear. Thimerosal (THI) is a mercurial used to preserve vaccines and consumer products, and is used experimentally to induce  $\text{Ca}^{2+}$  release from microsomal stores. We tested adenosine triphosphate (ATP)-mediated  $\text{Ca}^{2+}$  responses of DCs transiently exposed to nanomolar THI. Transcriptional and immunocytochemical analyses show that murine myeloid immature DCs (IDCs) and mature DCs (MDCs) express inositol 1,4,5-trisphosphate receptor (IP3R) and ryanodine receptor (RyR)  $\text{Ca}^{2+}$  channels, known targets of THI. IDCs express the RyR1 isoform in a punctate distribution that is densest near plasma membranes and within dendritic processes, whereas IP3Rs are more generally distributed. RyR1 positively and negatively regulates purinergic signaling because ryanodine (Ry) blockade a) recruited 80% more ATP responders, b) shortened ATP-mediated  $\text{Ca}^{2+}$  transients > 2-fold, and c) produced a delayed and persistent rise ( $\geq 2$ -fold) in baseline  $\text{Ca}^{2+}$ . THI (100 nM, 5 min) recruited more ATP responders, shortened the ATP-mediated  $\text{Ca}^{2+}$  transient ( $\geq 1.4$ -fold), and produced a delayed rise ( $\geq 3$ -fold) in the  $\text{Ca}^{2+}$  baseline, mimicking Ry. THI and Ry, in combination, produced additive effects leading to uncoupling of IP3R and RyR1 signals. THI altered ATP-mediated interleukin-6 secretion, initially enhancing the rate of cytokine secretion but suppressing cytokine secretion overall in Dcs. **Dendritic cells are exquisitely sensitive to Thimerosal, with one mechanism involving the uncoupling of positive and negative regulation of  $\text{Ca}^{2+}$  signals contributed by RyR1.**

Excerpt: "Our findings that DCs primarily express the RyR1 channel complex and that this complex is uncoupled by very low levels of THI with dysregulated IL-6 secretion raise intriguing questions about a molecular basis for immune dysregulation and the possible role of the RyR1 complex in genetic susceptibility of the immune system to mercury."

59. [Myeloid dendritic cells frequencies are increased in children with autism spectrum disorder and associated with amygdala volume and repetitive behaviors](#)

Brain Behav Immun. 2013 July ; 31: 69–75. doi:10.1016/j.bbi.2012.10.006.

Elizabeth Breecea, b, Brian Paciottib, Christine Wu Nordahlb, c, Sally Ozonoffb, c, Judy A. Van de Waterb, d, Sally J. Rogersb, c, David Amaralb, c, Paul Ashwood

a Department of Medical Microbiology and Immunology, University of California, Davis, USA

b The M.I.N.D. Institute, University of California, Davis, USA

c Department of Psychiatry and Behavioral Sciences, University of California, Davis, USA

d Division of Rheumatology, Allergy and Clinical Immunology, University of California, Davis, USA

#### Abstract

The pathophysiology of autism spectrum disorder (ASD) is not yet known; however, studies suggest that dysfunction of the immune system affects many children with ASD. Increasing evidence points to dysfunction of the innate immune system including activation of microglia and perivascular macrophages, increases in inflammatory cytokines/chemokines in brain tissue and CSF, and abnormal peripheral monocyte cell function. Dendritic cells are major players in innate immunity and have important functions in the phagocytosis of pathogens or debris, antigen presentation, activation of naïve T cells, induction of tolerance and cytokine/chemokine production. In this study, we assessed circulating frequencies of myeloid dendritic cells (defined as Lin-1-BDCA1+CD11c+ and Lin-1-BDCA3+CD123-) and plasmacytoid dendritic cells (Lin-1-BDCA2+CD123+ or Lin-1-BDCA4+ CD11c-) in 57 children with ASD, and 29 typically developing controls of the same age, all of who were enrolled as part of the Autism Phenome Project (APP). The frequencies of dendritic cells and associations with behavioral assessment and MRI measurements of amygdala volume were compared in the same participants. The frequencies of myeloid dendritic cells were significantly increased in children with ASD compared to typically developing controls ( $p < 0.03$ ). Elevated frequencies of myeloid dendritic cells were positively associated with abnormal right and left amygdala enlargement, severity of gastrointestinal symptoms and increased repetitive behaviors. The frequencies of plasmacytoid dendritic cells were also associated with amygdala volumes as well as developmental regression in children with ASD. **Dendritic cells play key roles in modulating immune responses and differences in frequencies or functions of these cells may result in immune dysfunction in children with ASD. These data further implicate innate immune cells in the complex pathophysiology of ASD.**

60. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosal

Environmental Health Perspectives, Aug 2005.

Thomas Burbacher, PhD [University of Washington].

This study demonstrates clearly and unequivocally that ethyl mercury, the kind of mercury found in vaccines, not only ends up in the brain, but leaves double the amount of inorganic mercury as methyl mercury, the kind of mercury found in fish. Methyl mercury (organic mercury) has a half-life in the brain measured in days (Rice), while thimerosal (organic mercury) once in the brain converts to inorganic mercury at much higher rates, and inorganic mercury has a half-life in the brain measured in years and decades (Rooney). This work is groundbreaking because little is known about ethyl mercury, and many health authorities have asserted that the mercury found in vaccines is the "safe kind." This study also delivers a strong rebuke of the Institute of Medicine's recommendation in 2004 to no longer pursue the mercury-autism connection.

Excerpt: "A recently published IOM review (IOM 2004) appears to have abandoned the earlier recommendation [of studying mercury and autism] as well as back away from the American Academy of Pediatrics goal [of removing mercury from vaccines]. This approach is difficult to understand, given our current limited knowledge of the toxicokinetics and developmental neurotoxicity of thimerosal, a compound that has been (and will continue to be) injected in millions of newborns and infants."

Excerpt: " The average brain-to-blood partitioning ratio of total Hg in the thimerosal group was slightly higher than that in the MeHg group ( $3.5 \pm 0.5$  vs.  $2.5 \pm 0.3$ , t-test,  $p = 0.11$ ). **Thus, the brain to-blood Hg concentration ratio established for MeHg will underestimate the amount of Hg in the brain after exposure to thimerosal. "**

#### Abstract

Thimerosal is a preservative that has been used in manufacturing vaccines since the 1930s. Reports have indicated that infants can receive ethylmercury (in the form of thimerosal) at or above the U.S. Environmental Protection Agency guidelines for methylmercury exposure, depending on the exact vaccinations, schedule, and size of the infant. In this study we compared the systemic disposition and brain distribution of total and inorganic mercury in infant monkeys after thimerosal exposure with those exposed to MeHg. Monkeys were exposed to MeHg (via oral gavage) or vaccines containing thimerosal (via intramuscular injection) at birth and 1, 2, and 3 weeks of age. Total blood Hg levels were determined 2, 4, and 7 days after each exposure. Total and inorganic brain Hg levels were assessed 2, 4, 7, or 28 days after the last exposure. The initial and terminal half-life of Hg in blood after thimerosal exposure was 2.1 and 8.6 days, respectively, which are significantly shorter than the elimination half-life of Hg after MeHg exposure at 21.5 days. Brain concentrations of total Hg were significantly lower by approximately 3-fold for the thimerosal-exposed monkeys when compared with the MeHg infants, whereas the average brain-to-blood concentration ratio was slightly higher for the thimerosal-exposed monkeys ( $3.5 \pm 0.5$  vs.  $2.5 \pm 0.3$ ). **A higher percentage of the total Hg in the brain was in the form of inorganic Hg for the thimerosal-exposed monkeys (34% vs. 7%).** The results indicate that MeHg is not a suitable reference for risk assessment from exposure to thimerosal-derived Hg. Knowledge of the toxicokinetics and developmental toxicity of thimerosal is needed to afford a meaningful

assessment of the developmental effects of thimerosal-containing vaccines. Key words: brain and blood distribution, elimination half-life, ethylmercury, infant nonhuman primates, methylmercury, thimerosal.

61. [Mercury and autism: accelerating evidence?](#)

Neuro Endocrinol Lett. 2005 Oct;26(5):439-46.

Joachim Mutter<sup>1</sup>, Johannes Naumann, Rainer Schneider, Harald Walach, Boyd Haley

<sup>1</sup> Institute for Environmental Medicine and Hospital Epidemiology, University Hospital Freiburg, Germany.

PMID: 16264412

Abstract

The causes of autism and neurodevelopmental disorders are unknown. Genetic and environmental risk factors seem to be involved. Because of an observed increase in autism in the last decades, which parallels cumulative mercury exposure, it was proposed that autism may be in part caused by mercury. We review the evidence for this proposal. Several epidemiological studies failed to find a correlation between mercury exposure through thimerosal, a preservative used in vaccines, and the risk of autism. Recently, it was found that autistic children had a higher mercury exposure during pregnancy due to maternal dental amalgam and thimerosal-containing immunoglobulin shots. It was hypothesized that children with autism have a decreased detoxification capacity due to genetic polymorphism. In vitro, mercury and thimerosal in levels found several days after vaccination inhibit methionine synthetase (MS) by 50%. **Normal function of MS is crucial in biochemical steps necessary for brain development, attention and production of glutathione, an important antioxidative and detoxifying agent. Repetitive doses of thimerosal leads to neurobehavioral deteriorations in autoimmune susceptible mice, increased oxidative stress and decreased intracellular levels of glutathione in vitro. Subsequently, autistic children have significantly decreased level of reduced glutathione. Promising treatments of autism involve detoxification of mercury, and supplementation of deficient metabolites.**

62. [The retention time of inorganic mercury in the brain--a systematic review of the evidence.](#)

Toxicol Appl Pharmacol. 2014 Feb 1;274(3):425-35. doi: 10.1016/j.taap.2013.12.011. Epub 2013 Dec 22.

Rooney JP.

Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College, 152-160 Pearse Street, Dublin 2, Ireland. Electronic address: jrooney@rcsi.ie.

#### Abstract

Reports from human case studies indicate a half-life for inorganic mercury in the brain in the order of years-contradicting older radioisotope studies that estimated half-lives in the order of weeks to months in duration. This study systematically reviews available evidence on the retention time of inorganic mercury in humans and primates to better understand this conflicting evidence. A broad search strategy was used to capture 16,539 abstracts on the Pubmed database. Abstracts were screened to include only study types containing relevant information. 131 studies of interest were identified. Only 1 primate study made a numeric estimate for the half-life of inorganic mercury (227-540 days). Eighteen human mercury poisoning cases were followed up long term including autopsy. Brain inorganic mercury concentrations at death were consistent with a half-life of several years or longer. 5 radionucleotide studies were found, one of which estimated head half-life (21 days). This estimate has sometimes been misinterpreted to be equivalent to brain half-life-which ignores several confounding factors including limited radioactive half-life and radioactive decay from surrounding tissues including circulating blood. No autopsy cohort study estimated a half-life for inorganic mercury, although some noted bioaccumulation of brain mercury with age. Modelling studies provided some extreme estimates (69 days vs 22 years). Estimates from modelling studies appear sensitive to model assumptions, however predications based on a long half-life (27.4 years) are consistent with autopsy findings. In summary, shorter estimates of half-life are not supported by evidence from animal studies, human case studies, or modelling studies based on appropriate assumptions. **Evidence from such studies point to a half-life of inorganic mercury in human brains of several years to several decades.** This finding carries important implications for pharmacokinetic modelling of mercury and potentially for the regulatory toxicology of mercury.

#### 63. [Alkyl Mercury-Induced Toxicity: Multiple Mechanisms of Action.](#)

In: de Voogt, P. (eds) Reviews of Environmental Contamination and Toxicology, vol 240. Springer, Cham. [https://doi.org/10.1007/398\\_2016\\_1](https://doi.org/10.1007/398_2016_1)

Risher JF, Tucker P.

Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road (MS F-58), Atlanta, GA, 30333, USA.

#### Abstract

There are a number of mechanisms by which alkylmercury compounds cause toxic action in the body. Collectively, published studies reveal that there are some similarities between the mechanisms of the toxic action of the mono-alkyl

mercury compounds methylmercury (MeHg) and ethylmercury (EtHg). This paper represents a summary of some of the studies regarding these mechanisms of action in order to facilitate the understanding of the many varied effects of alkylmercurials in the human body. The similarities in mechanisms of toxicity for MeHg and EtHg are presented and compared. **The difference in manifested toxicity of MeHg and EtHg are likely the result of the differences in exposure, metabolism, and elimination from the body, rather than differences in mechanisms of action between the two.**

Exerpts:

### Summary and Conclusions

**There are many commonalities/similarities in the mechanisms of toxic action of methylmercury and ethylmercury (from thimerosal)... Evidence for the similarity of the various mechanisms of toxicity include the following:**

- **Both MeHg and EtHg bind to the amino acid cysteine (Clarkson 1995; Wu et al. 2008)...**
- **Both decrease glutathione activity, thus providing less protection from the oxidative stress caused by MeHg and EtHg (Carocci et al. 2014; Ndountse and Chan (2008); Choi et al. 1996; Franco et al. 2006; Mori et al. 2007; Muller et al. 2001; Ndountse and Chan 2008; Wu et al. 2008)...**
- **Both disrupt glutamate homeostasis (Farina et al. 2003a, b; Manfroi et al. 2004; Mutkus et al. 2005; Yin et al. 2007).**
- **Both cause oxidative stress/creation of ROS (Dreiem and Seegal 2007; Garg and Chang 2006; Myhre et al. 2003; Sharpe et al. 2012; Yin et al. 2007)...**
- **Both cause effects on receptor binding/neurotransmitter release involving one or more transmitters (Basu et al. 2008; Coccini et al. 2000; Cooper et al. 2003; Fonfria et al. 2001; Ida-Eto et al. 2011; Ndountse and Chan 2008; Yuan and Atchison 2003).**
- **Both cause DNA damage or impair DNA synthesis (Burke et al. 2006; Sharpe et al. 2012; Wu et al. 2008).**

64. [Autism and clostridium tetani](#)

Medical Hypotheses, Volume 51, Issue 2, August 1998, Pages 133-144,  
[https://doi.org/10.1016/S0306-9877\(98\)90107-4](https://doi.org/10.1016/S0306-9877(98)90107-4)

Received 10 October 1996, Accepted 13 May 1997, Available online 23 June 2004.

Abstract

Autism is a severe developmental disability believed to have multiple etiologies. This paper outlines the possibility of a subacute, chronic tetanus infection of the intestinal tract as the underlying cause for symptoms of autism observed in some individuals.

A significant percentage of individuals with autism have a history of extensive antibiotic use. Oral antibiotics significantly disrupt protective intestinal microbiota, creating a favorable environment for colonization by opportunistic pathogens. *Clostridium tetani* is an ubiquitous anaerobic bacillus that produces a potent neurotoxin. Intestinal colonization by *C. tetani*, and subsequent neurotoxin release, have been demonstrated in laboratory animals which were fed vegetative cells. The vagus nerve is capable of transporting tetanus neurotoxin (TeNT) and provides a route of ascent from the intestinal tract to the CNS. This route bypasses TeNT's normal preferential binding sites in the spinal cord, and therefore the symptoms of a typical tetanus infection are not evident. Once in the brain, TeNT disrupts the release of neurotransmitters by the proteolytic cleavage of synaptobrevin, a synaptic vesicle membrane protein. This inhibition of neurotransmitter release would explain a wide variety of behavioral deficits apparent in autism. Lab animals injected in the brain with TeNT have exhibited many of these behaviors. Some children with autism have also shown a significant reduction in stereotyped behaviors when treated with antimicrobials effective against intestinal clostridia.

When viewed as sequelae to a subacute, chronic tetanus infection, many of the puzzling abnormalities of autism have a logical basis. A review of atypical tetanus cases, and strategies to test the validity of this paper's hypothesis, are included.

65. [Purkinje cell vulnerability and autism: a possible etiological connection](#)

Brain Dev. 2003 Sep;25(6):377-82. doi: 10.1016/s0387-7604(03)00056-1.

Janet Kinnear Kern 1

1 Department of Psychiatry, University of Texas Southwestern Medical Center at Dallas, St. Paul Professional Building I, 5959 Harry Hines Boulevard, Suite 520, Dallas, TX 75390-9101, USA.

Abstract

Autism is a neurological disorder of unknown etiology. The onset of the abnormal growth and development within the brain is also not known. Current thought by experts in autism is that the time of onset is prenatal, occurring prior to 30 weeks gestation. **However, autism comprises a heterogeneous population in that parents report either that their child was abnormal from birth, or that their child was developmentally normal until sometime after birth, at which time the child began to regress or deteriorate. Anecdotal reports suggest that some children with autism have significant illness or clinical events prior to the development of autistic symptoms. Conceivably, these children may become autistic from neuronal cell death or brain damage sometime after**

birth as result of insult. To support this theory is that marked Purkinje cell loss, the most consistent finding in the autistic disorder, can result from insult. Evidence suggests that the Purkinje cell is selectively vulnerable. This article discusses a theory that the selective vulnerability of the Purkinje cell may play a role in the etiology of autism, and suggests that a future direction in autism research may be to investigate the possibility of neuronal cell loss from insult as a cause of autism. Results of a small pilot survey are also discussed.

66. [The Neurobiology of Autism](#)

Brain Pathology Volume 17, Issue 4, Oct 4 2007, Pagesv-vi, 347-480

Carlos A. Pardo<sup>1,2</sup>; Charles G. Eberhart<sup>2</sup>

Departments of <sup>1</sup>Neurology and <sup>2</sup>Pathology,  
Johns Hopkins University School of Medicine, Baltimore, Md.

Abstract

Improving clinical tests are allowing us to more precisely classify autism spectrum disorders and diagnose them at earlier ages. This raises the possibility of earlier and potentially more effective therapeutic interventions. To fully capitalize on this opportunity, however, will require better understanding of the neurobiological changes underlying this devastating group of developmental disorders. It is becoming clear that the normal trajectory of neurodevelopment is altered in autism, with aberrations in brain growth, neuronal patterning and cortical connectivity. Changes to the structure and function of synapses and dendrites have also been strongly implicated in the pathology of autism by morphological, genetic and animal modeling studies. Finally, environmental factors are likely to interact with the underlying genetic profile, and foster the clinical heterogeneity seen in autism spectrum disorders. In this review we attempt to link the molecular pathways altered in autism to the neurodevelopmental and clinical changes that characterize the disease. We focus on signaling molecules such as neurotrophin, Reelin, PTEN and hepatocyte growth factor, neurotransmitters such as serotonin and glutamate, and synaptic proteins such as neurexin, SHANK and neuroligin. **We also discuss evidence implicating oxidative stress, neuroglial activation and neuroimmunity in autism.**

Excerpt:

**“Oxidative stress is another possible cause of Purkinje cell loss and other neuroanatomical changes described in autistic brains (reviewed in (37, 113)). Oxidative stress occurs when the levels of reactive oxygen species exceed the antioxidant capacities of a cell, often leading to cell death. Because of its very high oxygen demands and limited anti-oxidant capacity, the brain is thought to be relatively vulnerable to oxidative stress (111). Several studies have shown decreased levels of antioxidants such as superoxide dismutase, transferrin and ceruloplasmin in the blood or serum**



of patients with ASD (38, 108, 222). Significant elevations in biomarker profiles indicating increased oxidative stress, such as increased lipid peroxidation, have also been documented in autism (38, 107, 229). Interestingly, in one report the alterations in antioxidant proteins were linked specifically to regressive autism, suggesting a postnatal environmental effect (38). Polymorphisms in metabolic pathway genes may contribute to the increased oxidative stress in autism (108). Advanced glycation end products have also been reported to be elevated in both the brain tissue and serum of autistic patients, a change which can also lead to increased oxidative damage (23,110).”

67. [Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism.](#)

Am J Med Genet B Neuropsychiatr Genet. 2006 Dec 5;141B(8):947-56.

James SJ1, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K, Boris M, Bradstreet JJ, Baker SM, Gaylor DW.

Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas

Abstract

Autism is a behaviorally defined neurodevelopmental disorder usually diagnosed in early childhood that is characterized by impairment in reciprocal communication and speech, repetitive behaviors, and social withdrawal. Although both genetic and environmental factors are thought to be involved, none have been reproducibly identified. The metabolic phenotype of an individual reflects the influence of endogenous and exogenous factors on genotype. As such, it provides a window through which the interactive impact of genes and environment may be viewed and relevant susceptibility factors identified. Although abnormal methionine metabolism has been associated with other neurologic disorders, these pathways and related polymorphisms have not been evaluated in autistic children. Plasma levels of metabolites in methionine transmethylation and transsulfuration pathways were measured in 80 autistic and 73 control children. In addition, common polymorphic variants known to modulate these metabolic pathways were evaluated in 360 autistic children and 205 controls. **The metabolic results indicated that plasma methionine and the ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), an indicator of methylation capacity, were significantly decreased in the autistic children relative to age-matched controls. In addition, plasma levels of cysteine, glutathione, and the ratio of reduced to oxidized glutathione, an indication of antioxidant capacity and redox homeostasis, were significantly decreased. Differences in allele frequency and/or significant gene-gene interactions were found for relevant genes encoding the reduced folate carrier (RFC 80G > A), transcobalamin II (TCN2 776G > C), catechol-O-methyltransferase (COMT 472G > A), methylenetetrahydrofolate reductase (MTHFR 677C > T and 1298A > C), and glutathione-S-transferase (GST M1). We propose that an increased vulnerability to oxidative stress (endogenous or environmental) may**

**contribute to the development and clinical manifestations of autism.**

68. [Brain and tissue levels of mercury after chronic methylmercury exposure in the monkey.](#)

J Toxicol Environ Health. 1989;27(2):189-98.

Rice DC

Toxicology Research Division, Health Protection Branch, Health and Welfare,  
Ottawa, Ontario, Canada.

Abstract

Estimated half-lives of mercury following methylmercury exposure in humans are 52-93 d for whole body and 49-164 d for blood. In its most recent 1980 review, the World Health Organization concluded that there was no evidence to suggest that brain half-life differed from whole-body half-life. In the present study, female monkeys (*Macaca fascicularis*) were dosed for at least 1.7 yr with 10, 25, or 50 micrograms/kg.d of mercury as methylmercuric chloride. Dosing was discontinued, and blood half-life was determined to be about 14 d. Approximately 230 d after cessation of dosing, monkeys were sacrificed and organ and regional brain total mercury levels determined. One monkey that died while still being dosed had brain mercury levels three times higher than levels in blood.

Theoretical calculations were performed assuming steady-state brain: blood ratios of 3, 5, or 10. Brain mercury levels were at least three orders of magnitude higher than those predicted by assuming the half-life in brain to be the same as that in blood. Estimated half-lives in brain were between 56 (brain: blood ratio of 3) and 38 (brain: blood ratio of 10) days. In addition, there was a dose-dependent difference in half-lives for some brain regions. **These data clearly indicate that brain half-life is considerably longer than blood half-life in the monkey under conditions of chronic dosing.**

69. [Neurodevelopmental disorders after thimerosal-containing vaccines: a brief communication](#)

Exp Biol Med (Maywood). 2003 Jun;228(6):660-4. doi:  
10.1177/153537020322800603.

Mark R Geier 1 , David A Geier

The Genetic Centers of America, Silver Spring, Maryland 20905, USA.

Abstract

We were initially highly skeptical that differences in the concentrations of thimerosal in vaccines would have any effect on the incidence rate of neurodevelopmental disorders after childhood immunization. This study presents the first epidemiologic evidence, based upon tens of millions of doses of vaccine administered in the United States, that associates increasing thimerosal from vaccines with neurodevelopmental disorders. Specifically, an analysis of the **Vaccine Adverse Events Reporting System (VAERS) database showed statistical increases in the incidence rate of autism (relative risk [RR] = 6.0), mental retardation (RR = 6.1), and speech disorders (RR = 2.2) after thimerosal-containing diphtheria, tetanus, and acellular pertussis (DTaP) vaccines in comparison with thimerosal-free DTaP vaccines.** The male/female ratio indicated that autism (17) and speech disorders (2.3) were reported more in males than females after thimerosal-containing DTaP vaccines, whereas mental retardation (1.2) was more evenly reported among male and female vaccine recipients. Controls were employed to determine if biases were present in the data, but none were found. It was determined that overall adverse reactions were reported in similar-aged populations after thimerosal-containing DTaP (2.4 +/- 3.2 years old) and thimerosal-free DTaP (2.1 +/- 2.8 years old) vaccinations. Acute control adverse reactions such as deaths (RR = 1.0), vasculitis (RR = 1.2), seizures (RR = 1.6), ED visits (RR = 1.4), total adverse reactions (RR = 1.4), and gastroenteritis (RR = 1.1) were reported similarly after thimerosal-containing and thimerosal-free DTaP vaccines. **An association between neurodevelopmental disorders and thimerosal-containing DTaP vaccines was found,** but additional studies should be conducted to confirm and extend this study.

70. [Impact of environmental factors on the prevalence of autistic disorder after 1979](#)

Journal of Public Health and Epidemiology. Vol. 6(9), pp. 271-286, September 2014, Received 13 May, 2014; Accepted 9 July, 2014 DOI: 10.5897/JPHE2014.0649

Theresa A. Deisher\*, Ngoc V. Doan, Angelica Omaiye, Kumiko Koyama and Sarah Bwabye

Sound Choice Pharmaceutical Institute, 1749 Dexter Ave N, Seattle, WA 98109, USA.

Abstract

The aim of this study was to investigate a previously overlooked, universally introduced environmental factor, fetal and retroviral contaminants in childhood vaccines, absent prior to change points (CPs) in autistic disorder (AD) prevalence with subsequent dose-effect evidence and known pathologic mechanisms of action. Worldwide population based cohort study was used for the design of this study. The United States, Western Australia, United Kingdom and Denmark settings were used. All live born infants who later developed autistic disorder delivered after 1 January 1970, whose redacted vaccination and autistic disorder diagnosis information is publicly available in databases maintained by the US Federal Government, Western Australia, UK, and

Denmark. The live births, grouped by father's age, were from the US and Australia. The children vaccinated with MMRII, Varicella and Hepatitis A vaccines varied from 19 to 35 months of age at the time of vaccination. Autistic disorder birth year change points were identified as 1980.9, 1988.4 and 1996 for the US, 1987 for UK, 1990.4 for Western Australia, and 1987.5 for Denmark. Change points in these countries corresponded to introduction of or increased doses of human fetal cell line-manufactured vaccines, while no relationship was found between paternal age or Diagnostic and Statistical Manual (DSM) revisions and autistic disorder diagnosis. Further, linear regression revealed that Varicella and Hepatitis A immunization coverage was significantly correlated to autistic disorder cases. R software was used to calculate change points. Autistic disorder change points years are coincident with introduction of vaccines manufactured using human fetal cell lines, containing fetal and retroviral contaminants, into childhood vaccine regimens. This pattern was repeated in the US, UK, Western Australia and Denmark. Thus, rising autistic disorder prevalence is directly related to vaccines manufactured utilizing human fetal cells. Increased paternal age and DSM revisions were not related to rising autistic disorder prevalence.

71. [Interplay of glia activation and oxidative stress formation in fluoride and aluminium exposure.](#)

Pathophysiology. 2015 Mar;22(1):39-48. doi: 10.1016/j.pathophys.2014.12.001. Epub 2014 Dec 13.

Akinrinade ID1, Memudu AE2, Ogundele OM3, Ajetunmobi OI4.

**BACKGROUND:**

Oxidative stress formation is pivotal in the action of environmental agents which trigger the activation of glial cells and neuroinflammation to stimulate compensatory mechanisms aimed at restoring homeostasis.

**AIM:**

This study sets to demonstrate the interplay of fluoride (F) and aluminium (Al) in brain metabolism. Specifically, it reveals how oxidative stress impacts the activation of astrocytes (GFAP), mediates proinflammatory responses (microglia and B-cells: CD68 and CD 20 respectively) and shows the pattern of lipid peroxidation in the brain following fluoride and (or) aluminium treatment in vivo.

**METHOD:**

Male adult Wistar rats were treated with low and high doses of fluoride, aluminium or combination of fluoride-aluminium for 30 days. The control group received distilled water for the duration of the treatment. Blood and brain tissue homogenates were prepared for colorimetric assay of stress biomarkers [malonaldehyde (MDA) and superoxide dismutase (SOD)]. Subsequent analysis involved immunodetection of astrocytes (anti-GFAP), microglial (anti-CD68) and B-cells (anti-CD20) in coronal sections of the prefrontal cortex using antigen retrieval immunohistochemistry.

**RESULT AND CONCLUSION:**

Aluminium, fluoride and a combination of aluminium-fluoride treatments caused an increase in brain lipid peroxidation products and reactive oxygen species (ROS) formation. Similarly, an increase in glial activation and inflammatory

response were seen in these groups versus the control. Oxidative stress induced glial activation (GFAP) and increased the expression of B cells (CD20). This also corresponded to the extent of tissue damage and lipid peroxidation observed.

**Taken together, the results suggest a close link between oxidative stress neuroinflammation and degeneration in aluminium-fluoride toxicity.**

72. Increases in the Number of Reactive Glia in the Visual Cortex of *Macaca fascicularis* Following Subclinical Long-Term Methyl Mercury Exposure

Toxicol Appl Pharmacol, 1994 Dec;129(2):196-206. doi: 10.1006/taap.1994.1244.

Charleston JS, Bolender RP, Mottet NK, Body RL, Vahter ME, Burbacher TM., Department of Pathology, School of Medicine, University of Washington

Abstract

The number of neurons, astrocytes, reactive glia, oligodendrocytes, endothelia, and pericytes in the cortex of the calcarine sulcus of adult female *Macaca fascicularis* following long-term subclinical exposure to methyl mercury (MeHg) and mercuric chloride (inorganic mercury; IHg) has been estimated by use of the optical volume fractionator stereology technique. Four groups of monkeys were exposed to MeHg (50 micrograms Hg/kg body wt/day) by mouth for 6, 12, 18, and 12 months followed by 6 months without exposure (clearance group). A fifth group of monkeys was administered IHg (as HgCl<sub>2</sub>; 200 micrograms Hg/kg body wt/day) by constant rate intravenous infusion via an indwelling catheter for 3 months. Reactive glia showed a significant increase in number for every treatment group, increasing 72% in the 6-month, 152% in the 12-month, and 120% in the 18-month MeHg exposed groups, and the number of reactive glia in the clearance group remained elevated (89%). The IHg exposed group showed a 165% increase in the number of reactive glia. The IHg exposed group and the clearance group had low levels of MeHg present within the tissue; however, the level of IHg was elevated in both groups. **These results suggest that the IHg may be responsible for the increase in reactive glia.** All other cell types, including the neurons, showed no significant change in number at the prescribed exposure level and durations. The identities of the reactive glial cells and the implications for the long-term function and survivability of the neurons due to changes in the glial population following subclinical long-term exposure to mercury are discussed.

73. [Elevated levels of measles antibodies in children with autism](#)

Pediatr Neurol. 2003 Apr;28(4):292-4. doi: 10.1016/s0887-8994(02)00627-6.

Vijendra K Singh<sup>1</sup>, Ryan L Jensen

Department of Biology and Biotechnology Center, Utah State University, Logan, Utah, USA.

#### Abstract

Virus-induced autoimmunity may play a causal role in autism. To examine the etiologic link of viruses in this brain disorder, we conducted a serologic study of measles virus, mumps virus, and rubella virus. Viral antibodies were measured by enzyme-linked immunosorbent assay in the serum of autistic children, normal children, and siblings of autistic children. The level of measles antibody, but not mumps or rubella antibodies, was significantly higher in autistic children as compared with normal children ( $P = 0.003$ ) or siblings of autistic children ( $P < 0.0001$ ). Furthermore, immunoblotting of measles vaccine virus revealed that the antibody was directed against a protein of approximately 74 kd molecular weight. The antibody to this antigen was found in 83% of autistic children but not in normal children or siblings of autistic children. **Thus autistic children have a hyperimmune response to measles virus, which in the absence of a wild type of measles infection might be a sign of an abnormal immune reaction to the vaccine strain or virus reactivation.**

74. [Phenotypic expression of autoimmune autistic disorder \(AAD\): a major subset of autism](#)

Ann Clin Psychiatry . 2009 Jul-Sep;21(3):148-61.

Vijendra K Singh 1

Brain State International Research Center, Scottsdale, AZ 85260, USA.  
vj1000s@yahoo.com

#### Abstract

**Background:** Autism causes incapacitating neurologic problems in children that last a lifetime. The author of this article previously hypothesized that autism may be caused by autoimmunity to the brain, possibly triggered by a viral infection. This article is a summary of laboratory findings to date plus new data in support of an autoimmune pathogenesis for autism.

**Methods:** Autoimmune markers were analyzed in the sera of autistic and normal children, but the cerebrospinal fluid (CSF) of some autistic children was also analyzed. Laboratory procedures included enzyme-linked immunosorbent assay and protein immunoblotting assay.

**Results:** Autoimmunity was demonstrated by the presence of brain autoantibodies, abnormal viral serology, brain and viral antibodies in CSF, a positive correlation between brain autoantibodies and viral serology, elevated levels of proinflammatory cytokines and acute-phase reactants, and a positive response to immunotherapy. Many autistic children harbored brain myelin basic

protein autoantibodies and elevated levels of antibodies to measles virus and measles-mumps-rubella (MMR) vaccine. Measles might be etiologically linked to autism because measles and MMR antibodies (a viral marker) correlated positively to brain autoantibodies (an autoimmune marker)--salient features that characterize autoimmune pathology in autism. Autistic children also showed elevated levels of acute-phase reactants--a marker of systemic inflammation.

Conclusions: **The scientific evidence is quite credible for our autoimmune hypothesis, leading to the identification of autoimmune autistic disorder (AAD) as a major subset of autism.** AAD can be identified by immune tests to determine immune problems before administering immunotherapy. The author has advanced a speculative neuroautoimmune (NAI) model for autism, in which virus-induced autoimmunity is a key player. The latter should be targeted by immunotherapy to help children with autism.

75. [Modeling the interplay between neurons and astrocytes in autism using human induced pluripotent stem cells](#)

Biological Psychiatry, Available online 3 October 2017

Fabiele Baldino Russo, Beatriz Camille Freitas, Graciela Conceição Pignatari, Isabella Rodrigues Fernandes, Jonathan Sebat, Alysso Renato Muotri, Patricia Cristina Baleeiro Beltrão-Braga

Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil

Department of Surgery, School of Veterinary Medicine, University of São Paulo, São Paulo, SP, Brazil

Department of Pediatrics/Rady Children's Hospital San Diego, Department of Cellular & Molecular Medicine, Stem Cell Program, University of California San Diego School of Medicine, Sanford Consortium for Regenerative Medicine, La Jolla, CA, USA

Department of Psychiatry, Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA

Department of Obstetrics, School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, SP, Brazil

Received 12 September 2016, Revised 14 August 2017, Accepted 17 September 2017,

Abstract  
Background

Autism Spectrum Disorders (ASD) are neurodevelopmental disorders with unclear etiology and imprecise genetic causes. The main goal of this work was to investigate neuronal connectivity and the interplay between neurons and

astrocytes from non-syndromic ASD individuals using induced Pluripotent Stem Cells (iPSCs).

## Methods

Our iPSCs were derived from a clinically well-characterized cohort of three non-syndromic ASD individuals, sharing common behaviors, and three controls, two clones each. We generated mixed neural cultures analyzing synaptogenesis and neuronal activity using a multi-electrode array (MEA) platform. Furthermore, using an enriched astrocytes population we investigated their role in neuronal maintenance.

## Results

Our results revealed that ASD-derived neurons had a significant decrease in synaptic gene expression and protein levels, glutamate neurotransmitter release and, consequently, reduced spontaneous firing rate. Based on co-culture experiments, we observed that ASD-derived astrocytes interfered with proper neuronal development. In contrast, control-derived astrocytes rescued the morphological neuronal phenotype and synaptogenesis defects from ASD neuronal co-cultures. Furthermore, after identifying IL-6 secretion from astrocytes in our ASD individuals as a possible culprit for neural defects, we were able to increase synaptogenesis by blocking IL-6 levels.

## Conclusions

**Our findings reveal astrocytes contribution to neuronal phenotype and confirm previous studies linking IL-6 and autism, suggesting potential novel therapeutic pathways for a subtype of ASD individuals.** This is the first report demonstrating that glial dysfunctions could contribute to non-syndromic autism pathophysiology using iPSCs modeling disease technology.

## 76. Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

Ann Neurol 2005 Jan;57(1):67-81. doi: 10.1002/ana.20315.

Diana L. Vargas, MD, Johns Hopkins University.

### Abstract

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained



at autopsy from 11 patients with autism were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

Excerpt: "**Because this neuroinflammatory process appears to be associated with an ongoing and chronic mechanism of CNS dysfunction, potential therapeutic interventions should focus on the control of its detrimental effects and thereby eventually modify the clinical course of autism.**"

#### 77. [Aluminium in brain tissue in autism](#)

Journal of Trace Elements in Medicine and Biology, Volume 46, March 2018, Pages 76-82

Matthew Mold, Dorcas Umar, Andrew King, Christopher Exley,

The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, United Kingdom

Life Sciences, Keele University, Staffordshire, ST5 5BG, United Kingdom

Department of Clinical Neuropathology, Kings College Hospital, London, SE5 9RS, United Kingdom

26 November 2017

#### Abstract

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17)  $\mu\text{g/g}$  dry wt. for the occipital, frontal, temporal and parietal lobes respectively. **These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content**

**of the occipital lobe of a 15 year old boy would be 8.74 (11.59) µg/g dry wt.?** Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. **While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.**

78. [Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism.](#)

Biol Psychiatry. 2010 Aug 15;68(4):368-76. doi: 10.1016/j.biopsych.2010.05.024.

Morgan JT1, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP.  
Department of Neuroscience, School of Medicine, University of California, San Diego

**BACKGROUND:**

In the neurodevelopmental disorder autism, several neuroimmune abnormalities have been reported. However, it is unknown whether microglial somal volume or density are altered in the cortex and whether any alteration is associated with age or other potential covariates.

**METHODS:**

Microglia in sections from the dorsolateral prefrontal cortex of nonmacrencephalic male cases with autism (n = 13) and control cases (n = 9) were visualized via ionized calcium binding adapter molecule 1 immunohistochemistry. In addition to a neuropathological assessment, microglial cell density was stereologically estimated via optical fractionator and average somal volume was quantified via isotropic nucleator.

**RESULTS:**

Microglia appeared markedly activated in 5 of 13 cases with autism, including 2 of 3 under age 6, and marginally activated in an additional 4 of 13 cases. Morphological alterations included somal enlargement, process retraction and thickening, and extension of filopodia from processes. Average microglial somal volume was significantly increased in white matter (p = .013), with a trend in gray matter (p = .098). Microglial cell density was increased in gray matter (p = .002). Seizure history did not influence any activation measure.

**CONCLUSIONS:**

The activation profile described represents a neuropathological alteration in a sizeable fraction of cases with autism. Given its early presence, microglial activation may play a central role in the pathogenesis of autism in a substantial proportion of patients. Alternatively, activation may represent a response of the innate neuroimmune system to synaptic, neuronal, or neuronal network disturbances, or reflect genetic and/or environmental abnormalities impacting multiple cellular populations.

79. [Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism](#)

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Department of Medicine, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA  
Simone Gupta, Shannon E. Ellis, Foram N. Ashar, Anna Moes, Joel S. Bader  
Dan E. Arking  
Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA  
Joel S. Bader & Jianan Zhan  
Department of Neurology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA  
Andrew B. West

#### Abstract

Recent studies of genomic variation associated with autism have suggested the existence of extreme heterogeneity. Large-scale transcriptomics should complement these results to identify core molecular pathways underlying autism. Here we report results from a large-scale RNA sequencing effort, utilizing region-matched autism and control brains to identify neuronal and microglial genes robustly dysregulated in autism cortical brain. **Remarkably, we note that a gene expression module corresponding to M2-activation states in microglia is negatively correlated with a differentially expressed neuronal module, implicating dysregulated microglial responses in concert with altered neuronal activity-dependent genes in autism brains.** These observations provide pathways and candidate genes that highlight the interplay between innate immunity and neuronal activity in the aetiology of autism.

80. [Nanomolar aluminum induces pro-inflammatory and pro-apoptotic gene expression in human brain cells in primary culture.](#)

J Inorg Biochem. 2005 Sep;99(9):1895-8.

Lukiw WJ1, Percy ME, Kruck TP.

Neuroscience Center of Excellence and Department of Ophthalmology, Louisiana State University Health Sciences Center, 2020 Gravier Street, Suite 8B8, New Orleans, LA 70112-2272, USA. wlukiw@lsuhsc.edu

#### Abstract

Aluminum, the most abundant neurotoxic metal in our biosphere, has been implicated in the etiology of several neurodegenerative disorders including Alzheimer's disease (AD). To further understand aluminum's influence on gene expression, we examined total messenger RNA levels in untransformed human neural cells exposed to 100 nanomolar aluminum sulfate using high density DNA microarrays that interrogate the expression of every human gene. Preliminary data indicate that of the most altered gene expression levels, 17/24 (70.8%) of aluminum-affected genes, and 7/8 (87.5%) of aluminum-induced genes exhibit expression patterns similar to those observed in AD. The seven genes found to be significantly up-regulated by aluminum encode pro-inflammatory or pro-apoptotic signaling elements, including NF-kappaB subunits, interleukin-1beta precursor, cytosolic phospholipase A2, cyclooxygenase-2, beta-amyloid precursor protein and DAXX, a regulatory protein known to induce apoptosis and repress transcription. **The promoters of genes up-regulated by aluminum are enriched in binding sites for the stress-inducible transcription factors HIF-1 and NF-kappaB, suggesting a role for aluminum, HIF-1 and NF-kappaB in driving atypical, pro-inflammatory and pro-apoptotic gene expression.** The effect of aluminum on specific stress-related gene expression patterns in human brain cells clearly warrant further investigation.

81. [Hypothesis: conjugate vaccines may predispose children to autism spectrum disorders](#)

Med Hypotheses. 2011 Dec;77(6):940-7. doi: 10.1016/j.mehy.2011.08.019. Epub 2011 Oct 10.

Brian J Richmand

PMID: 21993250 DOI: 10.1016/j.mehy.2011.08.019

Abstract

The first conjugate vaccine was approved for use in the US in 1988 to protect infants and young children against the capsular bacteria *Haemophilus influenzae* type b (Hib). Since its introduction in the US, this vaccine has been approved in most developed countries, including Denmark and Israel where the vaccine was added to their national vaccine programs in 1993 and 1994, respectively. There have been marked increases in the reported prevalence of autism spectrum disorders (ASDs) among children in the US beginning with birth cohorts in the late 1980s and in Denmark and Israel starting approximately 4-5 years later. Although these increases may partly reflect ascertainment biases, an exogenous trigger could explain a significant portion of the reported increases in ASDs. It is hypothesized here that the introduction of the Hib conjugate vaccine in the US in 1988 and its subsequent introduction in Denmark and Israel could explain a substantial portion of the initial increases in ASDs in those countries. The continuation of the trend toward increased rates of ASDs could be further explained by increased usage of the vaccine, a change in 1990 in the recommended age of vaccination in the US from 15 to 2 months, increased

immunogenicity of the vaccine through changes in its carrier protein, and the subsequent introduction of the conjugate vaccine for *Streptococcus pneumoniae*. Although conjugate vaccines have been highly effective in protecting infants and young children from the significant morbidity and mortality caused by Hib and *S. pneumoniae*, the potential effects of conjugate vaccines on neural development merit close examination. Conjugate vaccines fundamentally change the manner in which the immune systems of infants and young children function by deviating their immune responses to the targeted carbohydrate antigens from a state of hypo-responsiveness to a robust B2 B cell mediated response. This period of hypo-responsiveness to carbohydrate antigens coincides with the intense myelination process in infants and young children, and conjugate vaccines may have disrupted evolutionary forces that favored early brain development over the need to protect infants and young children from capsular bacteria.

82. [Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation.](#)

Front Psychiatry. 2011 May 13;2:27. doi: 10.3389/fpsy.2011.00027. eCollection 2011.

Young AM1, Campbell E, Lynch S, Suckling J, Powis SJ.

Bute Medical School, University of St. Andrews Fife, Scotland, UK.

Abstract

Autism spectrum condition (ASC) is recognized as having an inflammatory component. Post-mortem brain samples from patients with ASC display neuroglial activation and inflammatory markers in cerebrospinal fluid, although little is known about the underlying molecular mechanisms. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) is a protein found in almost all cell types and mediates regulation of immune response by inducing the expression of inflammatory cytokines and chemokines, establishing a feedback mechanism that can produce chronic or excessive inflammation. This article describes immunodetection and immunofluorescence measurements of NF- $\kappa$ B in human post-mortem samples of orbitofrontal cortex tissue donated to two independent centers: London Brain Bank, Kings College London, UK (ASC: n = 3, controls: n = 4) and Autism Tissue Program, Harvard Brain Bank, USA (ASC: n = 6, controls: n = 5). The hypothesis was that concentrations of NF- $\kappa$ B would be elevated, especially in activated microglia in ASC, and pH would be concomitantly reduced (i.e., acidification). Neurons, astrocytes, and microglia all demonstrated increased extranuclear and nuclear translocated NF- $\kappa$ B p65 expression in brain tissue from ASC donors relative to samples from matched controls. These between-groups differences were increased in astrocytes and microglia relative to neurons, but particularly pronounced for highly mature microglia. Measurement of pH in homogenized samples demonstrated a 0.98-unit difference in means and a strong ( $F = 98.3$ ;  $p = 0.00018$ ) linear relationship to the expression of nuclear translocated NF- $\kappa$ B in mature microglia. Acridine orange staining localized pH reductions to lysosomal compartments. **In summary, NF- $\kappa$ B is aberrantly expressed in orbitofrontal cortex in patients**

**with ASC, as part of a putative molecular cascade leading to inflammation, especially of resident immune cells in brain regions associated with the behavioral and clinical symptoms of ASC.**

83. [A Study of Nuclear Transcription Factor-Kappa B in Childhood Autism](#)

PLoS One. 2011; 6(5): e19488.

Usha S. Naik,<sup>1</sup> Charitha Gangadharan,<sup>2</sup> Kanakalatha Abbagani,<sup>1</sup> Balakrishna Nagalla,<sup>3</sup> Niranjana Dasari,<sup>1</sup> and Sunil K. Manna<sup>2,\*</sup>  
Monica Uddin, Editor

Department of Psychiatry, Osmania Medical College, Hyderabad, India  
Laboratory of Immunology, Centre for DNA Fingerprinting and Diagnostics,  
Nampally, Hyderabad, India  
National Institute of Nutrition, Hyderabad, India  
University of Michigan, United States of America

**Abstract**

**Background**

Several children with autism show regression in language and social development while maintaining normal motor milestones. A clear period of normal development followed by regression and subsequent improvement with treatment, suggests a multifactorial etiology. The role of inflammation in autism is now a major area of study. Viral and bacterial infections, hypoxia, or medication could affect both foetus and infant. These stressors could upregulate transcription factors like nuclear factor kappa B (NF- $\kappa$ B), a master switch for many genes including some implicated in autism like tumor necrosis factor (TNF). On this hypothesis, it was proposed to determine NF- $\kappa$ B in children with autism.

**Methods**

Peripheral blood samples of 67 children with autism and 29 control children were evaluated for NF- $\kappa$ B using electrophoretic mobility shift assay (EMSA). A phosphor imaging technique was used to quantify values. The fold increase over the control sample was calculated and statistical analysis was carried out using SPSS 15.

**Results**

We have noted significant increase in NF- $\kappa$ B DNA binding activity in peripheral blood samples of children with autism. When the fold increase of NF- $\kappa$ B in cases (n=67) was compared with that of controls (n=29), there was a significant difference (3.14 vs. 1.40, respectively; p<0.02).

**Conclusion**

**This finding has immense value in understanding many of the known biochemical changes reported in autism. As NF- $\kappa$ B is a response to stressors of several kinds and a master switch for many genes, autism may**

**then arise at least in part from an NF- $\kappa$ B pathway gone awry.**

84. Autism: A Brain Disorder, or A Disorder That Affects the Brain?

Clinical Neuropsychiatry, 2005

Martha R. Herbert M.D., Ph.D., Harvard University

Autism is defined behaviorally, as a syndrome of abnormalities involving language, social reciprocity and hyperfocus or reduced behavioral flexibility. It is clearly heterogeneous, and it can be accompanied by unusual talents as well as by impairments, but its underlying biological and genetic basis is unknown. Autism has been modeled as a brain-based, strongly genetic disorder, but emerging findings and hypotheses support a broader model of the condition as a genetically influenced and systemic. These include imaging, neuropathology and psychological evidence of pervasive (and not just specific) brain and phenotypic features; postnatal evolution and chronic persistence of brain, behavior and tissue changes (e.g. inflammation) and physical illness symptomatology (e.g. gastrointestinal, immune, recurrent infection); overlap with other disorders; and reports of rate increases and improvement or recovery that support a role for modulation of the condition by environmental factors (e.g. exacerbation or triggering by toxins, infectious agents, or other stressors, or improvement by treatment). Modeling autism more broadly encompasses previous work, but also encourages the expansion of research and treatment to include intermediary domains of molecular and cellular mechanisms, as well as chronic tissue, metabolic and somatic changes previously addressed only to a limited degree. The heterogeneous biologies underlying autism may conceivably converge onto the autism profile via multiple mechanisms on the one hand and processing and connectivity abnormalities on the other may illuminate relevant final common pathways and contribute to focusing on the search for treatment targets in this biologically and etiologically heterogeneous behavioral syndrome.

85. [Multivariate techniques enable a biochemical classification of children with autism spectrum disorder versus typically-developing peers: A comparison and validation study](#)

Daniel P. Howsmon Troy Vargason Robert A. Rubin Leanna Delhey Marie Tippet Shannon Rose Sirish C. Bennuri John C. Slattery Stepan Melnyk S. Jill James Richard E. Frye Juergen Hahn

Bioengineering & Translational Medicine, 14 May 2018

<https://doi.org/10.1002/btm2.10095>

Funding information National Institutes of Health, Grant/Award Number: 1R01AI110642

## Abstract

Autism spectrum disorder (ASD) is a developmental disorder which is currently only diagnosed through behavioral testing. Impaired folate-dependent one carbon metabolism (FOCM) and transsulfuration (TS) pathways have been implicated in ASD, and recently a study involving multivariate analysis based upon Fisher Discriminant Analysis returned very promising results for predicting an ASD diagnosis. This article takes another step toward the goal of developing a biochemical diagnostic for ASD by comparing five classification algorithms on existing data of FOCM/TS metabolites, and also validating the classification results with new data from an ASD cohort. The comparison results indicate a high sensitivity and specificity for the original data set and up to a 88% correct classification of the ASD cohort at an expected 5% misclassification rate for typically-developing controls. These results form the foundation for the development of a biochemical test for ASD which promises to aid diagnosis of ASD and provide biochemical understanding of the disease, applicable to at least a subset of the ASD population.

## 86. Activation of Methionine Synthase by Insulin-like Growth Factor-1 and Dopamine: a Target for Neurodevelopmental Toxins and Thimerosal

Mol Psychiatry. 2004 Apr;9(4):358-70.

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Charnitsky VA, Deth RC. Department of Pharmaceutical Sciences, Northeastern University, Boston, MA

## Abstract

Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu(2+) promoted enzyme activity and methylation, while Cu(+), Pb(2+), Hg(2+) and Al(3+) were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. **The potent inhibition of this pathway by**



**ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.**

87. [Thimerosal Induces DNA Breaks, Caspase-3 Activation, Membrane Damage, and Cell Death in Cultured Human Neurons and Fibroblasts](#)

David S. Baskin, Hop Ngo, Vladimir V. Didenko

Toxicological Sciences, Volume 74, Issue 2, August 2003, Pages 361–368,  
<https://doi.org/10.1093/toxsci/kfg126>  
Published: 01 August 2003

Abstract

Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250- $\mu$ M concentrations of thimerosal for 45 min to 24 h. A 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify nonviable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2  $\mu$ M based on the manual detection of the fluorescent attached cells and at a 1- $\mu$ M level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250  $\mu$ M thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. **We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3-dependent apoptosis in human neurons and fibroblasts.** We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.

88. Validation of the Phenomenon of Autistic Regression Using Home Videotapes

Archives of General Psychiatry, 2005

Emily Werner, PhD; Geraldine Dawson, PhD, University of Washington

## Abstract

**Objective** To validate parental report of autistic regression using behavioral data coded from home videotapes of children with autism spectrum disorder (ASD) vs typical development taken at 12 and 24 months of age.

**Design** Home videotapes of 56 children's first and second birthday parties were collected from parents of young children with ASD with and without a reported history of regression and typically developing children. Child behaviors were coded by raters blind to child diagnosis and regression history. A parent interview that elicited information about parents' recall of early symptoms from birth was also administered.

**Setting** Participants were recruited from a multidisciplinary study of autism conducted at a major university.

**Participants** Fifteen children with ASD with a history of regression, 21 children with ASD with early-onset autism, and 20 typically developing children and their parents participated.

**Main Outcome Measures** Observations of children's communicative, social, affective, repetitive behaviors, and toy play coded from videotapes of the toddlers' first and second birthday parties.

**Results** Analyses revealed that infants with ASD with regression show similar use of joint attention and more frequent use of words and babble compared with typical infants at 12 months of age. In contrast, infants with ASD with early onset of symptoms and no regression displayed fewer joint attention and communicative behaviors at 12 months of age. By 24 months of age, both groups of toddlers with ASD displayed fewer instances of word use, vocalizations, declarative pointing, social gaze, and orienting to name as compared with typically developing 24-month-olds.

Parent interview data suggested that some children with regression displayed difficulties in regulatory behavior before the regression occurred.

**Conclusion** **This study validates the existence of early autistic regression.**

89. [Searching for the 'Trigger': An ethnographic analysis of parental beliefs regarding autism causation and vaccination in Puerto Rico](#)

Vaccine. 2023 Jan 9;41(2):540-546. doi: 10.1016/j.vaccine.2022.11.064. Epub 2022 Dec 7.

Melissa Anderson-Chavarria 1 , Jane Turner 2

1 DO-PhD Program, Michigan State University College of Osteopathic Medicine and the Department of Anthropology, 909 Wilson Road, West Fee Hall, Rm 317,

East Lansing, MI 48824, United States.  
2 Department of Pediatrics and Human Development, Michigan State University  
College of Human Medicine, Life Sciences Bldg, 1355 Bogue St., B240, East  
Lansing, MI 48824, United States.

#### Abstract

This study examines the personal beliefs held by parents of autistic children in Puerto Rico regarding the cause of their child's autism and how these beliefs may influence parental vaccination decision-making. This study seeks to contribute towards diversifying the autism literature by focusing on an autism community living in a relatively lower income, resource-deficit context. These findings expand our understandings of how parents of autistic children may perceive vaccines and how these perceptions are informed by various sources of knowledge. This ethnographic research study was conducted between May 2017 and August 2019. Methods included 350+ hours of participant-observation and semi-structured interviewing of 35 Puerto Rican parents of autistic children. 32 of these 35 parents interviewed believed autism to be the result of genetic risks that are 'triggered' by an unknown environmental factor. Suggested 'triggers' included various environmental contaminants and vaccinations. The subject of vaccination came up in every interview; 18 interviewed parents did not believe vaccines 'triggered' autism, 3 parents attributed their child's autism entirely to vaccines, while 14 considered vaccines to be one of several possible 'triggers'. It is important to note that no parents interviewed perceived vaccinations to be inherently or universally harmful. Rather, **they perceived vaccinations to be one of many possible 'triggers' for a child predisposed to develop autism. In some cases, this perception prompted parents to oppose mandatory vaccination policies on the island. Parents shared nuanced, complex understandings of autism causation that may carry implications for COVID-19 vaccine uptake within the Puerto Rican autistic community.**

#### 90. Blood Levels of Mercury Are Related to Diagnosis of Autism: A Reanalysis of an Important Data Set

Journal of Child Neurology, Vol. 22, No. 11, 1308-1311 (2007)

M. Catherine DeSoto, PhD, Robert T. Hitlan, PhD -Department of Psychology,  
University of Northern Iowa, Cedar Falls, Iowa

#### Abstract

The question of what is leading to the apparent increase in autism is of great importance. Like the link between aspirin and heart attack, even a small effect can have major health implications. If there is any link between autism and mercury, it is absolutely crucial that the first reports of the question are not falsely stating that no link occurs. **We have reanalyzed the data set originally reported by Ip et al. in 2004 and have found that the original p value was in error and that a significant relation does exist between the blood levels of**

**mercury and diagnosis of an autism spectrum disorder. Moreover, the hair sample analysis results offer some support for the idea that persons with autism may be less efficient and more variable at eliminating mercury from the blood.**

91. Empirical Data Confirm Autism Symptoms Related to Aluminum and Acetaminophen Exposure

Entropy, November 7, 2012

Stephanie Seneff, Robert M. Davidson and Jingjing Liu

Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, Internal Medicine Group Practice, PhyNet, Inc., Longview, TX 75604, USA

Abstract

Autism is a condition characterized by impaired cognitive and social skills, associated with compromised immune function. The incidence is alarmingly on the rise, and environmental factors are increasingly suspected to play a role. This paper investigates word frequency patterns in the U.S. CDC Vaccine Adverse Events Reporting System (VAERS) database. Our results provide strong evidence supporting a link between autism and the aluminum in vaccines. A literature review showing toxicity of aluminum in human physiology offers further support. Mentions of autism in VAERS increased steadily at the end of the last century, during a period when mercury was being phased out, while aluminum adjuvant burden was being increased. Using standard log-likelihood ratio techniques, we identify several signs and symptoms that are significantly more prevalent in vaccine reports after 2000, including cellulitis, seizure, depression, fatigue, pain and death, which are also significantly associated with aluminum-containing vaccines. **We propose that children with the autism diagnosis are especially vulnerable to toxic metals such as aluminum and mercury due to insufficient serum sulfate and glutathione. A strong correlation between autism and the MMR (Measles, Mumps, Rubella) vaccine is also observed, which may be partially explained via an increased sensitivity to acetaminophen administered to control fever.**

92. [Mitochondrial dysfunction in autism spectrum disorders: a population-based study](#)

Dev Med Child Neurol. 2005 Mar;47(3):185-9. doi: 10.1017/s0012162205000332.

G Oliveira 1 , L Diogo, M Grazina, P Garcia, A Ataíde, C Marques, T Miguel, L Borges, A M Vicente, C R Oliveira

1 Outpatient Clinic of Autism, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra, 3000-076 Coimbra, Portugal.

#### Abstract

A minority of cases of autism has been associated with several different organic conditions, including bioenergetic metabolism deficiency. In a population-based study, we screened associated medical conditions in a group of 120 children with autism (current age range 11y 5mo to 14y 4mo, mean age 12y 11mo [SD 9.6mo], male:female ratio 2.9:1). Children were diagnosed using Diagnostic and Statistical Manual of Mental Disorders criteria, the Autism Diagnostic Interview--Revised, and the Childhood Autism Rating Scale; 76% were diagnosed with typical autism and 24% with atypical autism. Cognitive functional level was assessed with the Griffiths scale and the Wechsler Intelligence Scale for Children and was in the normal range in 17%. Epilepsy was present in 19 patients. Plasma lactate levels were measured in 69 patients, and in 14 we found hyperlactacidemia. **Five of 11 patients studied were classified with definite mitochondrial respiratory chain disorder, suggesting that this might be one of the most common disorders associated with autism (5 of 69; 7.2%) and warranting further investigation.**

#### 93. [Autistic disorder in 2 children with mitochondrial disorders](#)

J Child Neurol. 2007 Sep;22(9):1121-3. doi: 10.1177/0883073807306266.

Chang-Yong Tsao<sup>1</sup>, Jerry R Mendell

<sup>1</sup> Department of Pediatrics, The Ohio State University, Columbus, Ohio, USA. tsaoc@pediatrics.ohio-state.edu

#### Abstract

Autistic disorder is a heterogeneous disorder. The majority of the cases are idiopathic, and only a small number of the autistic children have associated secondary diagnosis. **This article reports 2 children with mitochondrial disorders associated with autistic disorder** fulfilling the diagnostic criteria of the American Psychiatric Association Manual of Psychiatric Diseases, 4th edition, and briefly reviews the literature on autistic disorder associated with mitochondrial disorders.

#### 94. [Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism](#)

FASEB J 2009 Aug;23(8):2374-83. doi: 10.1096/fj.08-128926. Epub 2009 Mar 23.

S Jill James<sup>1</sup>, Shannon Rose, Stepan Melnyk, Stefanie Jernigan, Sarah Blossom, Oleksandra Pavliv, David W Gaylor

Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, 1120 Marshall St., Little Rock, AR 72202, USA.

#### Abstract

Research into the metabolic phenotype of autism has been relatively unexplored despite the fact that metabolic abnormalities have been implicated in the pathophysiology of several other neurobehavioral disorders. Plasma biomarkers of oxidative stress have been reported in autistic children; however, intracellular redox status has not yet been evaluated. Lymphoblastoid cells (LCLs) derived from autistic children and unaffected controls were used to assess relative concentrations of reduced glutathione (GSH) and oxidized disulfide glutathione (GSSG) in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. **Exposure to oxidative stress via the sulfhydryl reagent thimerosal resulted in a greater decrease in the GSH/GSSG ratio and increase in free radical generation in autism compared to control cells. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the autism LCLs, although GSH/GSSG and ATP concentrations were similarly decreased in both cell lines. These results suggest that the autism LCLs exhibit a reduced glutathione reserve capacity in both cytosol and mitochondria that may compromise antioxidant defense and detoxification capacity under prooxidant conditions.**

95. [Homozygous gene deletions of the glutathione S-transferases M1 and T1 are associated with thimerosal sensitization](#)

Int Arch Occup Environ Health. 2000 Aug;73(6):384-8. doi: 10.1007/s004200000159.

G A Westphal<sup>1</sup>, A Schnuch, T G Schulz, K Reich, W Aberer, J Brasch, P Koch, R Wessbecher, C Szliska, A Bauer, E Hallier

<sup>1</sup> Abteilung für Arbeits- und Sozialmedizin, Georg-August-Universität Göttingen, Germany.

#### Abstract

Objective: Thimerosal is an important preservative in vaccines and ophthalmologic preparations. The substance is known to be a type IV sensitizing

agent. High sensitization rates were observed in contact-allergic patients and in health care workers who had been exposed to thimerosal-preserved vaccines. There is evidence for the involvement of the glutathione system in the metabolism of thimerosal or its decomposition products (organomercury alkyl compounds). Thus detoxification by polymorphically expressed glutathione S-transferases such as GSTT1 and GSTM1 might have a protective effect against sensitization by these substances.

Methods: To address this question, a case control study was conducted, including 91 Central European individuals with a positive patch-test reaction to thimerosal. This population was compared with 169 healthy controls and additionally with 114 individuals affected by an allergy against para-substituted aryl compounds. The latter population was included in order to test whether possible associations were due to substance-specific effects, or were a general feature connected with type IV immunological diseases. Homozygous deletions of GSTT1 and GSTM1 were determined by polymerase chain reaction.

Results: Glutathione S-transferase M1 deficiency was significantly more frequent among patients sensitized to thimerosal (65.9%,  $P = 0.013$ ) compared with the healthy control group (49.1%) and the "para-compound" group (48%,  $P = 0.034$ ). Glutathione S-transferase T1 deficiency in the thimerosal/mercury group (19.8%) was barely elevated versus healthy controls (16.0%) and the "para-compound" group (14.0%). The combined deletion (GSTT1-/GSTM1-) was markedly more frequent among thimerosal-sensitized patients than in healthy controls (17.6% vs. 6.5%,  $P = 0.0093$ ) and in the "para-compound" group (17.6% vs. 6.1%,  $P = 0.014$ ), revealing a synergistic effect of these enzyme deficiencies (healthy controls vs. thimerosal GSTM1 negative individuals,  $OR = 2.0$  [CI = 1.2-3.4], GSTT1-,  $OR = 1.2$  [CI = 0.70-2.1], GSTM1/T1-,  $OR = 3.1$  [CI = 1.4-6.5]).

Conclusions: **Since the glutathione-dependent system was repeatedly shown to be involved in the metabolism of thimerosal decomposition products, the observed association may be of functional relevance.**

96. [Neuroinflammation and autism: toward mechanisms and treatments](#)

Neuropsychopharmacology. 2013 Jan;38(1):241-2. doi: 10.1038/npp.2012.174.

Christopher J McDougle <sup>1</sup>, William A Carlezon Jr <sup>2</sup>

<sup>1</sup> Department of Psychiatry, Massachusetts General Hospital and Mass General Hospital for Children, Boston, MA, USA.

<sup>2</sup> Harvard Medical School, Boston, MA, USA; <sup>3</sup> Department of Psychiatry, McLean Hospital, Belmont, MA, USA

Autism spectrum disorders (ASDs) were originally described by Kanner (1943). The relatively consistent clinical phenotype will likely be shown to comprise numerous etiologic subtypes. Approximately 10% of ASD cases are linked to disorders of genetic etiology, such as Fragile X syndrome, tuberous sclerosis, and Rett disorder. The majority of cases, however, remain idiopathic.

A role for immunological involvement in ASDs has long been hypothesized. Kanner did not comment on this in his initial descriptions, but a detailed review of the original 11 cases reveals important observations. One patient was 'kept in bed often because of colds, bronchitis, chickenpox, streptococcus infection, impetigo and rheumatic fever'. Another was 'given anterior pituitary and thyroid preparations and her father, aged 36 years, was one of those chronically thin persons, nervous energy readily expended', suggesting hyperthyroidism.

In our clinical work and review of the literature, we have been impressed by the possible role of autoimmune disorders as influencing the pathophysiology of a distinct, objectively defined etiologic subtype of ASDs. Money et al (1971) published what is widely accepted as the first connection between autism and autoimmune disorders. They described a family in which the youngest child had multiple diagnoses, including autism, Addison's disease, moniliasis, and diabetes mellitus. The next older brother had hypoparathyroidism, Addison's disease, moniliasis, and alopecia totalis. The oldest son was symptom-free. The mother had ulcerative colitis, the father had 'chronic athlete's foot', and a paternal uncle had diabetes mellitus. Consistent with these observations, we showed that first- and second-degree relatives of children with an ASD have a higher number of autoimmune disorders than family members of healthy children (Sweeten et al, 2003). In a recent post-mortem study of 13 males with autism and 9 control cases, microglia appeared markedly activated in 5 of 13 cases with autism, including 2 of 3 under the age of 6 years, and marginally activated in an additional 4 of 13 cases (Morgan et al, 2010), suggesting ongoing inflammatory processes in brain.

Observations in humans are supported by experiments in laboratory animals. As one example, Martin et al (2008) exposed pregnant rhesus monkeys to human IgG collected from mothers of children diagnosed with ASDs, while controls received IgG collected from mothers of normally developing children. Those offspring that were gestationally exposed to IgG class antibodies from mothers of children with ASDs consistently demonstrated increases in stereotypies and hyperactivity. These findings suggest that some ASD-like behaviors can be triggered by environmental (non-genetic) manipulations.

The notion that environmental factors contribute to ASD prevalence continues to evolve. Once-influential theories suggesting links among exposure to vaccines containing attenuated virus or toxins, conditions such as inflammatory bowel disease, and ASDs have fallen from favor since the retraction of a key study (Wakefield et al, 1998). It is important to emphasize, however, that the major reason for retraction was poor scientific method rather than theoretical flaws. Although ASDs are currently within the realm of psychiatrists and neurologists, it is becoming clear that at least some subtypes represent whole-body disorders, offering exciting new possibilities for therapy.

97. [Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children](#)



Original Investigation | Pediatrics, JAMA Network Open. 2018;1(2):e180279.  
doi:10.1001/jamanetworkopen.2018.0279

Guifeng Xu, MD; Linda G. Snetselaar, PhD; Jin Jing, MD, PhD; Buyun Liu, MD, PhD; Lane Strathearn, MBBS, FRACP, PhD; Wei Bao, MD, PhD

## Abstract

**IMPORTANCE** The prevalence of autism spectrum disorder (ASD) in US children has increased during the past decades. Immunologic dysfunction has recently emerged as a factor associated with ASD. Although children with ASD are more likely to have gastrointestinal disorders, little is known about the association between food allergy and ASD.

**OBJECTIVE** To examine the association of food allergy and other allergic conditions with ASD in US children.

**DESIGN, SETTING, AND PARTICIPANTS** This population-based, cross-sectional study used data from the National Health Interview Survey collected between 1997 and 2016. The data analysis was performed in 2018. All eligible children aged 3 to 17 years were included. Food allergy, respiratory allergy, and skin allergy were defined based on an affirmative response in the questionnaire by a parent or guardian.

**MAIN OUTCOMES AND MEASURES** Reported ASD diagnosed by a physician or other health professional.

**RESULTS** This analysis included 199 520 children (unweighted mean [SD] age, 10.21 [4.41] years; 102 690 boys [51.47%]; 55 476 Hispanic [27.80%], 97 200 non-Hispanic white [48.72%], 30 760 non-Hispanic black [15.42%], and 16 084 non-Hispanic other race [8.06%]). Among them, 8734 (weighted prevalence, 4.31%) had food allergy, 24 555 (12.15%) had respiratory allergy, and 19 399 (9.91%) had skin allergy. A diagnosis of ASD was reported in 1868 children (0.95%). The weighted prevalence of reported food, respiratory, and skin allergies was higher in children with ASD (11.25%, 18.73%, and 16.81%, respectively) compared with children without ASD (4.25%, 12.08%, and 9.84%, respectively). In analyses adjusting for age, sex, race/ethnicity, family highest education level, family income level, geographical region, and mutual adjustment for other allergic conditions, the associations between allergic conditions and ASD remained significant. The odds ratio (OR) of ASD increased in association with food allergy (OR, 2.29; 95% CI, 1.87-2.81), respiratory allergy (OR, 1.28; 95% CI, 1.10-1.50), and skin allergy (OR, 1.50; 95% CI, 1.28-1.77) when comparing children with these conditions and those without.

**CONCLUSIONS AND RELEVANCE** In a nationally representative sample of US children, a significant and positive association of common allergic conditions, in particular food allergy, with ASD was found. Further investigation is warranted to elucidate the causality and underlying mechanisms.

98. [Another Step Toward Defining an Immune-Mediated Subtype of Autism Spectrum Disorder](#)

Invited Commentary | Pediatrics, June 8, 2018

Christopher J. McDougle, MD<sup>1,2</sup>

<sup>1</sup>Lurie Center for Autism, Massachusetts General Hospital, Lexington  
<sup>2</sup>Department of Psychiatry, Harvard Medical School, Boston, Massachusetts

JAMA Netw Open. 2018;1(2):e180280. doi:10.1001/jamanetworkopen.2018.0280

Original Investigation

Association of Allergies With Autism Spectrum Disorder

Guifeng Xu, MD; Linda G. Snetselaar, PhD; Jin Jing, MD, PhD; Buyun Liu, MD, PhD; Lane Strathearn, MBBS, FRACP, PhD; Wei Bao, MD, PhD

In their article “Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder,” Xu and colleagues<sup>1</sup> present new data that add to the growing body of literature supporting an immune-mediated subtype of autism spectrum disorder (ASD). The investigators analyzed data from the National Health Interview Survey, a continuous, ongoing, nationally representative annual health survey conducted in the United States since 1957. The National Health Interview Survey is the primary source of information on health conditions of the US population. Data from 1997 to 2016, collected via an in-person household interview, were used in this study. All children aged 3 to 17 years with available information about allergic conditions and ASD were included. Respondents were asked about the occurrence of a food or digestive allergy, any kind of respiratory allergy, or eczema or any kind of skin allergy during the past 12 months. Respondents were also asked whether the child received a diagnosis of ASD from a physician or other health professional. Among the 199 520 children in the analysis, 8734 had food allergy, 24 555 had respiratory allergy, and 19 399 had skin allergy. Health professional–diagnosed ASD was reported in 1868 children. Children with ASD were significantly more likely than those without ASD to have food allergy (11.25% vs 4.25%), respiratory allergy (18.73% vs 12.08%), and skin allergy (16.81% vs 9.84%). **The likelihood of the child having ASD more than doubled among children with food allergy compared with those without food allergy; children with respiratory and skin allergy were also significantly more likely to have ASD, but at a lesser magnitude. While no sex difference was found for food allergy, boys with ASD were significantly more likely than girls with ASD to have respiratory and skin allergy.**

Previous studies have identified a positive association of respiratory allergy and skin allergy with ASD, as detailed in the current article. To my knowledge, the results of Xu et al<sup>1</sup> are the first to document the association of food allergy with ASD with confidence, in part based on the large sample size they accessed. The authors wonder whether this association may be related to gut-brain-behavior axis abnormalities that have been hypothesized to exist in a subset of individuals with ASD. Such an association has been reported in both patients with ASD and animal models of ASD, particularly those using the maternal immune activation model of ASD.<sup>2</sup> From a clinical perspective, patients with ASD who are minimally

verbal to nonverbal may be unable to describe the pain and discomfort they experience secondary to food allergy and subsequent inflammation in the gastrointestinal (GI) tract. Instead, their physical distress may manifest as irritability, aggression, and/or self-injury. It is important to underscore the need for health care professionals to conduct a thorough history and physical examination to rule out identifiable medical causes of aberrant behavior, including food allergy and secondary GI inflammation, before proceeding with treatments designed to reduce behavior problems. In addition to GI pathology, other common comorbid medical disorders that occur with ASD include seizures and sleep disturbance. Interestingly, each of these comorbidities has also been associated with inflammatory processes.<sup>3,4</sup> **It may be that GI dysfunction, seizures, and sleep disorder, in addition to food, respiratory, and skin allergies, are medical comorbidities that characterize the immune-mediated subtype of ASD.**

Another interesting and potentially important finding in the current article is the lack of significant association of respiratory allergy and skin allergy with ASD in girls. These findings are in line with recent reports of a striking difference in vulnerability to early-life immune insult between male and female mice in animal models of ASD.<sup>5,6</sup>

**In the Discussion section of their article, Xu and colleagues<sup>1</sup> review other aspects of immune dysfunction reported in ASD, including abnormalities in peripheral immunoglobulins, imbalance of T-cell subsets, and increased levels of proinflammatory cytokines in postmortem brains of patients with ASD. Considering the significant association between food, respiratory, and skin allergy in children with ASD reported by Xu and colleagues, in conjunction with numerous studies documenting aspects of immune dysfunction in patients with ASD and specific animal models of ASD,<sup>7</sup> evidence continues to mount that an immune-mediated subtype of ASD should continue to be pursued and defined.**

99. [Glutathione-related factors and oxidative stress in autism, a review.](#)

Curr Med Chem. 2012;19(23):4000-5.

Ghanizadeh A1, Akhondzadeh S, Hormozi M, Makarem A, Abotorabi-Zarchi M, Firoozabadi A.

Research Center for Psychiatry and Behavioral Sciences, Shiraz University of Medical Sciences, School of Medicine, Shiraz, Iran. ghanizad@sina.tums.ac.ir

Abstract

Autism spectrum disorders are complex neuro-developmental disorders whose neurobiology is proposed to be associated with oxidative stress which is induced by reactive oxygen species. The process of oxidative stress can be a target for therapeutic interventions. In this study, we aimed to review the role of oxidative stress, plasma glutathione (GSH), and related factors as the potential sources of damage to the brain as well as the possible related factors which reduce the

oxidative stress. Methylation capacity, sulfates level, and the total glutathione level are decreased in autism. On the other hand, both oxidized glutathione and the ratio of oxidized to reduced glutathione are increased in autism. In addition, the activity of glutathione peroxidase, superoxide dismutase, and catalase, as a part of the antioxidative stress system are decreased. **The current literature suggests an imbalance of oxidative and anti-oxidative stress systems in autism. Glutathione is involved in neuro-protection against oxidative stress and neuro-inflammation in autism by improving the anti-oxidative stress system. Decreasing the oxidative stress might be a potential treatment for autism.**

100. Developmental Regression and Mitochondrial Dysfunction in a Child With Autism

J Child Neurol. 2006 Feb;21(2):170-2.

Jon S. Poling, MD, PhD, Department of Neurology and Neurosurgery  
Johns Hopkins Hospital

Jon S. Poling, Richard E. Frye, John Shoffner and Andrew W. Zimmerman

Abstract

Autistic spectrum disorders can be associated with mitochondrial dysfunction. We present a singleton case of developmental regression and oxidative phosphorylation disorder in a 19-month-old girl. Subtle abnormalities in the serum creatine kinase level, aspartate aminotransferase, and serum bicarbonate led us to perform a muscle biopsy, which showed type I myofiber atrophy, increased lipid content, and reduced cytochrome c oxidase activity. There were marked reductions in enzymatic activities for complex I and III. Complex IV (cytochrome c oxidase) activity was near the 5% confidence level. To determine the frequency of routine laboratory abnormalities in similar patients, we performed a retrospective study including 159 patients with autism (Diagnostic and Statistical Manual of Mental Disorders-IV and Childhood Autism Rating Scale) not previously diagnosed with metabolic disorders and 94 age-matched controls with other neurologic disorders. Aspartate aminotransferase was elevated in 38% of patients with autism compared with 15% of controls ( $P < .0001$ ). The serum creatine kinase level also was abnormally elevated in 22 (47%) of 47 patients with autism. These data suggest that further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent.

Excerpt: **"Children who have (mitochondrial-related) dysfunctional cellular energy metabolism might be more prone to undergo autistic regression between 18 and 30 months of age if they also have infections or immunizations at the same time."**

101. Oxidative Stress in Autism: Elevated Cerebellar 3-nitrotyrosine Levels

American Journal of Biochemistry and Biotechnology 4 (2): 73-84, 2008

Elizabeth M. Sajdel-Sulkowska, - Dept of Psychiatry, Harvard Medical School

Shows a potential link between mercury and the autopsied brains of young people with autism. A marker for oxidative stress was 68.9% higher in autistic brain tissue than controls (a statistically significant result), while mercury levels were 68.2% higher.

**Abstract**

It has been suggested that oxidative stress and/or mercury compounds play an important role in the pathophysiology of autism. This study compared for the first time the cerebellar levels of the oxidative stress marker 3-nitrotyrosine (3-NT), mercury (Hg) and the antioxidant selenium (Se) levels between control and autistic subjects. Tissue homogenates were prepared in the presence of protease inhibitors from the frozen cerebellar tissue of control (n=10; mean age, 15.5 years; mean PMI, 15.5 hours) and autistic (n=9; mean age 12.1 years; mean PMI, 19.3 hours) subjects. The concentration of cerebellar 3-NT, determined by ELISA, in controls ranged from 13.69 to 49.04 pmol g<sup>-1</sup> of tissue; the concentration of 3-NT in autistic cases ranged from 3.91 to 333.03 pmol g<sup>-1</sup> of tissue. Mean cerebellar 3-NT was elevated in autism by 68.9% and the increase was statistically significant (p=0.045). Cerebellar Hg, measured by atomic absorption spectrometry ranged from 0.9 to 35 pmol g<sup>-1</sup> tissue in controls (n=10) and from 3.2 to 80.7 pmol g<sup>-1</sup> tissue in autistic cases (n=9); the 68.2% increase in cerebellar Hg was not statistically significant. However, there was a positive correlation between cerebellar 3-NT and Hg levels (r=0.7961, p=0.0001). A small decrease in cerebellar Se levels in autism, measured by atomic absorption spectroscopy, was not statistically significant but was accompanied by a 42.9% reduction in the molar ratio of Se to Hg in the autistic cerebellum. While preliminary, the results of the present study add elevated oxidative stress markers in brain to the growing body of data reflecting greater oxidative stress in autism.

**Excerpt: The preliminary data suggest a need for more extensive studies of oxidative stress, its relationship to the environmental factors and its possible attenuation by antioxidants in autism."**

102. Large Brains in Autism: The Challenge of Pervasive Abnormality

Neuroscientist. 2005 Oct;11(5):417-40.

Herbert MR., Harvard University

Pediatric Neurology, Center for Morphometric Analysis, Massachusetts General Hospital, Charleston, MA

## Abstract

The most replicated finding in autism neuroanatomy—a tendency to unusually large brains—has seemed paradoxical in relation to the specificity of the abnormalities in three behavioral domains that define autism. We now know a range of things about this phenomenon, including that brains in autism have a growth spurt shortly after birth and then slow in growth a few short years afterward, that only younger but not older brains are larger in autism than in controls, that white matter contributes disproportionately to this volume increase and in a nonuniform pattern suggesting postnatal pathology, that functional connectivity among regions of autistic brains is diminished, and that neuroinflammation (including microgliosis and astrogliosis) appears to be present in autistic brain tissue from childhood through adulthood. Alongside these pervasive brain tissue and functional abnormalities, there have arisen theories of pervasive or widespread neural information processing or signal coordination abnormalities (such as weak central coherence, impaired complex processing, and underconnectivity), which are argued to underlie the specific observable behavioral features of autism. This convergence of findings and models suggests that a systems- and chronic disease-based reformulation of function and pathophysiology in autism needs to be considered, and it opens the possibility for new treatment targets..

**Excerpt: "Oxidative stress, brain inflammation, and microgliosis have been much documented in association with toxic exposures including various heavy metals...the awareness that the brain as well as medical conditions of children with autism may be conditioned by chronic biomedical abnormalities such as inflammation opens the possibility that meaningful biomedical interventions may be possible well past the window of maximal neuroplasticity in early childhood because the basis for assuming that all deficits can be attributed to fixed early developmental alterations in neural architecture has now been undermined."**

### 103. Evidence of Toxicity, Oxidative Stress, and Neuronal Insult in Autism

J Toxicol Environ Health B Crit Rev. 2006 Nov-Dec;9(6):485-99.

Kern JK, Jones AM.

Department of Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

## Abstract

According to the Autism Society of America, autism is now considered to be an epidemic. The increase in the rate of autism revealed by epidemiological studies and government reports implicates the importance of external or environmental factors that may be changing. This article discusses the evidence for the case that some children with autism may become autistic from neuronal cell death or brain damage sometime after birth as result of insult; and addresses the hypotheses that toxicity and oxidative stress may be a cause of neuronal insult in autism. The article first describes the Purkinje cell loss found in autism, Purkinje

cell physiology and vulnerability, and the evidence for postnatal cell loss. Second, the article describes the increased brain volume in autism and how it may be related to the Purkinje cell loss. Third, the evidence for toxicity and oxidative stress is covered and the possible involvement of glutathione is discussed. Finally, the article discusses what may be happening over the course of development and the multiple factors that may interplay and make these children more vulnerable to toxicity, oxidative stress, and neuronal insult.

#### 104. Oxidative Stress in Autism

Pathophysiology. 2006 Aug;13(3):171-81. Epub 2006 Jun 12.

Chauhan A, Chauhan V.

NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY

##### Abstract

Autism is a severe developmental disorder with poorly understood etiology. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipid peroxidation, detoxifying agents (such as glutathione), and antioxidants involved in the defense system against reactive oxygen species (ROS). Lipid peroxidation markers are elevated in autism, indicating that oxidative stress is increased in this disease. Levels of major antioxidant serum proteins, namely transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), are decreased in children with autism. There is a positive correlation between reduced levels of these proteins and loss of previously acquired language skills in children with autism. The alterations in ceruloplasmin and transferrin levels may lead to abnormal iron and copper metabolism in autism. The membrane phospholipids, the prime target of ROS, are also altered in autism. The levels of phosphatidylethanolamine (PE) are decreased, and phosphatidylserine (PS) levels are increased in the erythrocyte membrane of children with autism as compared to their unaffected siblings. Several studies have suggested alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in autism. Additionally, altered glutathione levels and homocysteine/methionine metabolism, increased inflammation, excitotoxicity, as well as mitochondrial and immune dysfunction have been suggested in autism. Furthermore, environmental and genetic factors may increase vulnerability to oxidative stress in autism. Taken together, these studies suggest increased oxidative stress in autism that may contribute to the development of this disease. A mechanism linking oxidative stress with membrane lipid abnormalities, inflammation, aberrant immune response, impaired energy metabolism and excitotoxicity, leading to clinical symptoms and pathogenesis of autism is proposed.

**Excerpt: "Upon completion of this article, participants should be able to: 1. Be aware of laboratory and clinical evidence of greater oxidative stress in autism. 2. Understand how gut, brain, nutritional, and toxic status in autism are consistent with greater oxidative stress. 3. Describe how anti-oxidant**

**nutrients are used in the contemporary treatment of autism."**

105. Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors

Neurotoxicology. 2005 Jan;26(1):1-8.

James SJ, Slikker W 3rd, Melnyk S, New E, Pogribna M, Jernigan S.

Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR

Abstract

Thimerosal is an antiseptic containing 49.5% ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Environmental methyl mercury has been shown to be highly neurotoxic, especially to the developing brain. Because mercury has a high affinity for thiol (sulfhydryl (-SH)) groups, the thiol-containing antioxidant, glutathione (GSH), provides the major intracellular defense against mercury-induced neurotoxicity. Cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosal toxicity compared to glioblastoma cells that have higher basal levels of intracellular GSH. **Thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH in both cell lines.** Pretreatment with 100 microM glutathione ethyl ester or N-acetylcysteine (NAC), but not methionine, resulted in a significant increase in intracellular GSH in both cell types. Further, pretreatment of the cells with glutathione ethyl ester or NAC prevented cytotoxicity with exposure to 15 microM Thimerosal. Although Thimerosal has been recently removed from most children's vaccines, it is still present in flu vaccines given to pregnant women, the elderly, and to children in developing countries. The potential protective effect of GSH or NAC against mercury toxicity warrants further research as possible adjunct therapy to individuals still receiving Thimerosal-containing vaccinations.

106. [Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage.](#)

Curr Top Med Chem. 2001 Dec;1(6):529-39.

Ercal N1, Gurer-Orhan H, Aykin-Burns N.

University of Missouri-Rolla, Department of Chemistry, 65409-0010, USA.  
nercal@umr.edu

Abstract



Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. Redox-active metals, such as iron, copper and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury and others deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes. Either redox-active or redox-inactive metals may cause an increase in production of reactive oxygen species (ROS) such as hydroxyl radical (HO.), superoxide radical (O<sub>2</sub><sup>-</sup>) or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Enhanced generation of ROS can overwhelm cells' intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. **Consequently, it is suggested that metal-induced oxidative stress in cells can be partially responsible for the toxic effects of heavy metals. Several studies are underway to determine the effect of antioxidant supplementation following heavy metal exposure. Data suggest that antioxidants may play an important role in abating some hazards of heavy metals.** In order to prove the importance of using antioxidants in heavy metal poisoning, pertinent biochemical mechanisms for metal-induced oxidative stress should be reviewed.

107. Aluminum adjuvant linked to gulf war illness induces motor neuron death in mice

Neuromolecular Med. 2007;9(1):83-100.

Petrik MS, Wong MC, Tabata RC, Garry RF, Shaw CA.

Department of Ophthalmology and Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada.

#### Abstract

Gulf War illness (GWI) affects a significant percentage of veterans of the 1991 conflict, but its origin remains unknown. Associated with some cases of GWI are increased incidences of amyotrophic lateral sclerosis and other neurological disorders. Whereas many environmental factors have been linked to GWI, the role of the anthrax vaccine has come under increasing scrutiny. Among the vaccine's potentially toxic components are the adjuvants aluminum hydroxide and squalene. To examine whether these compounds might contribute to neuronal deficits associated with GWI, an animal model for examining the potential neurological impact of aluminum hydroxide, squalene, or aluminum hydroxide combined with squalene was developed. Young, male colony CD-1 mice were injected with the adjuvants at doses equivalent to those given to US military service personnel. All mice were subjected to a battery of motor and cognitive-behavioral tests over a 6-mo period postinjections. Following sacrifice, central nervous system tissues were examined using immunohistochemistry for evidence of inflammation and cell death. Behavioral testing showed motor

deficits in the aluminum treatment group that expressed as a progressive decrease in strength measured by the wire-mesh hang test (final deficit at 24 wk; about 50%). Significant cognitive deficits in water-maze learning were observed in the combined aluminum and squalene group (4.3 errors per trial) compared with the controls (0.2 errors per trial) after 20 wk. Apoptotic neurons were identified in aluminum-injected animals that showed significantly increased activated caspase-3 labeling in lumbar spinal cord (255%) and primary motor cortex (192%) compared with the controls. Aluminum-treated groups also showed significant motor neuron loss (35%) and increased numbers of astrocytes (350%) in the lumbar spinal cord. The findings suggest a possible role for the aluminum adjuvant in some neurological features associated with GWI and possibly an additional role for the combination of adjuvants.

108. [Enrichment of Elevated Plasma F2t-Isoprostane Levels in Individuals with Autism Who Are Stratified by Presence of Gastrointestinal Dysfunction](#)

PLoS ONE 8(7): e68444.

Gorrindo P, Lane CJ, Lee EB, McLaughlin B, Levitt P (July 3, 2013)

Funding: This work was supported in part by National Institutes of Health awards National Institute of Child Health and Human Development R21HD065289 (PL), National Institute of General Medical Sciences T32GM07347 for the Vanderbilt Medical Scientist Training Program (PG), National Center for Research Resources TL1RR024978 (PG), and National Center for Advancing Translational Sciences UL1TR000445 for the Vanderbilt Institute for Clinical and Translational Research. Additional support was provided by the Marino Autism Research Institute, the Pediatric Clinical Research Center at Vanderbilt University, The Scott Family Foundation, and the Vanderbilt Autism Treatment Network Site, a program funded by Autism Speaks.

Abstract Etiology is unknown in the majority of individuals with autism spectrum disorder (ASD). One strategy to investigate pathogenesis is to stratify this heterogeneous disorder based on a prominent phenotypic feature that enriches for homogeneity within population strata. Co-occurring gastrointestinal dysfunction (GID) characterizes a subset of children with ASD. Our current objective was to investigate a potential pathophysiological measure to test the hypothesis that children with both ASD and GID have a more severe metabolic dysfunction than children with ASD-only, given that the highly metabolically active brain and gastrointestinal system may additively contribute measurable impairment. Plasma levels of F2t-Isoprostanes (F2-IsoPs), a gold standard biomarker of oxidative stress, were measured in 87 children in four groups: ASD-GID, ASD-only, GID-only and Unaffected. F2-IsoP levels were elevated in all 3 clinical groups compared to the Unaffected group, with the ASD-GID group significantly elevated above the ASD-only group (mean, SD in pg/mg: ASD-GID 53.6, 24.4; ASD-only 36.5, 13.3;  $p = 0.007$ ). Adjusting for age, sex, and triglyceride levels, F2-IsoP levels remained significantly different between study groups, with a moderate effect size of  $\eta^2 = 0.187$  ( $p = 0.001$ ). **Elevation in peripheral oxidative stress is consistent with, and may contribute to, the**

**more severe functional impairments in the ASD-GID group.** With unique medical, metabolic, and behavioral features in children with ASD-GID, the present findings serve as a compelling rationale for both individualized approaches to clinical care and integrated studies of biomarker enrichment in ASD subgroups that may better address the complex etiology of ASD.

109. [Inflammation and Autophagy: A Convergent Point between Autism Spectrum Disorder \(ASD\)-Related Genetic and Environmental Factors: Focus on Aluminum Adjuvants](#)

Toxics 2022 Aug 31;10(9):518. doi: 10.3390/toxics10090518.

Loïc Angrand <sup>1 2 3 4 5</sup>, Jean-Daniel Masson <sup>1 2</sup>, Alberto Rubio-Casillas <sup>6 7</sup>, Marika Nosten-Bertrand <sup>3 4 5</sup>, Guillemette Crépeaux <sup>1 2</sup>

Affiliations

- 1 Univ Paris Est Créteil, INSERM, IMRB, F-94010 Créteil, France.
- 2 Ecole Nationale Vétérinaire d'Alfort IMRB, F-94700 Maisons-Alfort, France.
- 3 INSERM UMR-S 1270, 75005 Paris, France.
- 4 Sorbonne Université, Campus Pierre et Marie Curie, 75005 Paris, France.
- 5 Institut du Fer à Moulin, 75005 Paris, France.
- 6 Biology Laboratory, Autlán Regional Preparatory School, University of Guadalajara, Autlán 48900, Jalisco, Mexico.
- 7 Autlán Regional Hospital, Health Secretariat, Autlán 48900, Jalisco, Mexico.

Abstract

Autism spectrum disorder (ASD), schizophrenia, and bipolar disorder are genetically complex and heterogeneous neurodevelopmental disorders (NDDs) resulting from genetic factors and gene-environment (GxE) interactions for which onset occurs in early brain development. Recent progress highlights the link between ASD and (i) immunogenetics, neurodevelopment, and inflammation, and (ii) impairments of autophagy, a crucial neurodevelopmental process involved in synaptic pruning. Among various environmental factors causing risk for ASD, aluminum (Al)-containing vaccines injected during critical periods have received special attention and triggered relevant scientific questions. The aim of this review is to discuss the current knowledge on the role of early inflammation, immune and autophagy dysfunction in ASD as well as preclinical studies which question Al adjuvant impacts on brain and immune maturation. We highlight the most recent breakthroughs and the lack of epidemiological, pharmacokinetic and pharmacodynamic data constituting a “scientific gap”. We propose additional research, such as genetic studies that could contribute to identify populations at genetic risk, improving diagnosis, and potentially the development of new therapeutic tools.

Excerpts:

**“As a result of these pieces of evidence (epidemiological, clinical and**

**preclinical data) pointing to a potential causal association between early ABA exposure and increased ASD risk, new hypotheses regarding neurological and immunological consequences of ABA-containing vaccines and novel clinical strategies (i.e., postponing of ABA-containing vaccines and replacement of ABAs with calcium phosphate are now being considered.“**

**“Our review presents the lack of fundamental scientific data demonstrating that Al adjuvants are safe and do not induce any long-term side effects. It also supports further investigation related to the effects of early Al adjuvant exposures occurring in combination with genetic susceptibility factors, including autophagy, immune and inflammation process genes. As accumulating evidence shows that modulating the levels of autophagy may increase the risk of NDDs, such studies will elucidate a new etiology for these complex disorders and contribute to develop potential new diagnostic and therapeutic tools.”**

110. [Reduced levels of mercury in first baby haircuts of autistic children.](#)

Int J Toxicol. 2003 Jul-Aug;22(4):277-85.

Holmes AS, Blaxill MF, Haley BE.

**Abstract**

Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair

analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

111. A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorder

J Toxicol Environ Health A. 2007 May 15;70(10):837-51.

Geier DA, Geier MR.

Institute of Chronic Illnesses, Inc., Silver Spring, Maryland, USA.

Abstract

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

112. [The Changing Prevalence of Autism In California](#)

Journal of Autism and Developmental Disorders, April 2003

Mark Blaxill, MBA

This study helps to refute the supposition made by some researchers that autism's epidemic may only be due to "diagnostic substitution".

Excerpt: **"They have suggested that 'diagnostic substitution' accounts for an apparent increase in the incidence of autism in California that is not real. This hypothesized substitution is not supported by proper and detailed analyses of the California data."**

113. [California Autism Prevalence Trends from 1931 to 2014 and Comparison to National ASD Data from IDEA and ADDM.](#)

J Autism Dev Disord. 2018 Jul 5.

Nevison C, Blaxill M, Zahorodny W.

Abstract

Time trends in U.S. autism prevalence from three ongoing datasets [Individuals with Disabilities Education Act, Autism and Developmental Disabilities Monitoring Network, and California Department of Developmental Services (CDDS)] are calculated using two different methods: (1) constant-age tracking of 8 year-olds and (2) age-resolved snapshots. The data are consistent across methods in showing a strong upward trend over time. The prevalence of autism in the CDDS dataset, the longest of the three data records, increased from 0.001% in the cohort born in 1931 to 1.2% among 5 year-olds born in 2012. **This increase began around ~ 1940 at a rate that has gradually accelerated over time, including notable change points around birth years 1980, 1990 and, most recently, 2007.**

114. [Cytokine dysregulation in autism spectrum disorders \(ASD\): Possible role of the environment](#)

Paula E. Goines<sup>1</sup> and Paul Ashwood<sup>2</sup>

Neurotoxicol Teratol. Author manuscript; available in PMC 2014 Mar 1.

University of California, Davis, School of Veterinary Medicine, Department of Molecular Biosciences, Sacramento, CA, USA<sup>b</sup>

University of California, Davis, School of Medicine, Department of Medical Microbiology and Immunology, Sacramento, CA, USA

Abstract

Autism spectrum disorders (ASD) are neurodevelopmental diseases that affect an alarming number of individuals. The etiological basis of ASD is unclear, and

evidence suggests it involves both genetic and environmental factors. There are many reports of cytokine imbalances in ASD. These imbalances could have a pathogenic role, or they may be markers of underlying genetic and environmental influences. Cytokines act primarily as mediators of immunological activity, but they also have significant interactions with the nervous system. They participate in normal neural development and function, and inappropriate activity can have a variety of neurological implications. It is therefore possible that cytokine dysregulation contributes directly to neural dysfunction in ASD. Further, cytokine profiles change dramatically in the face of infection, disease, and toxic exposures. Therefore, imbalances may represent an immune response to environmental contributors to ASD. The following review is presented in two main parts. First, we discuss select cytokines implicated in ASD, including IL-1B, IL-6, IL-4, IFN- $\gamma$ , and TGF-B, and focus on their role in the nervous system. Second, we explore several neurotoxic environmental factors that may be involved in the disorders, and focus on their immunological impacts. **This review represents an emerging model that recognizes the importance of both genetic and environmental factors in ASD etiology. We propose that the immune system provides critical clues regarding the nature of the gene by environment interactions that underlie ASD pathophysiology.**

115. [Diagnostic Substitution for Intellectual Disability: A Flawed Explanation for the Rise in Autism](#)

Journal of Autism and Developmental Disorders First Online: 06 June 2017, DOI: 10.1007/s10803-017-3187-0

Cynthia D. Nevison, Mark Blaxill

Abstract

Time trends in autism spectrum disorder (ASD) and intellectual disability (ID) prevalence from the United States Individuals with Disabilities Education Act data were computed from 2000 to 2011 for each state and each age from 6 to 17.

**These trends did not support the hypothesis that diagnostic substitution for ID can explain the ASD rise over recent decades**, although the hypothesis appeared more plausible when the data were aggregated across all states and ages. Nationwide ID prevalence declined steeply over the last two decades, but the decline was driven mainly by ~15 states accounting for only one-fourth of the U.S. school population. **More commonly, including in the most populous states, ID prevalence stayed relatively constant while ASD prevalence rose sharply.**

116. Mitochondrial Energy-Deficient Endophenotype in Autism

American Journal of Biochemistry and Biotechnology 4 (2): 198-207, 2008

J. Jay Gargus and Faiqa Imtiaz

Department of Physiology and Biophysics and Department of Pediatrics, Section of Human Genetics, School of Medicine, University of California, Irvine, Arabian Diagnostics Laboratory, King Faisal Specialist Hospital and Research Centre

Abstract: While evidence points to a multigenic etiology of most autism, the pathophysiology of the disorder has yet to be defined and the underlying genes and biochemical pathways they subserve remain unknown. Autism is considered to be influenced by a combination of various genetic, environmental and immunological factors; more recently, evidence has suggested that increased vulnerability to oxidative stress may be involved in the etiology of this multifactorial disorder.

Furthermore, recent studies have pointed to a subset of autism associated with the biochemical endophenotype of mitochondrial energy deficiency, identified as a subtle impairment in fat and carbohydrate oxidation. This phenotype is similar, but more subtle than those seen in classic mitochondrial defects. In some cases the beginnings of the genetic underpinnings of these mitochondrial defects are emerging, such as mild mitochondrial dysfunction and secondary carnitine deficiency observed in the subset of autistic patients with an inverted duplication of chromosome 15q11-q13. In addition, rare cases of familial autism associated with sudden infant death syndrome (SIDS) or associated with abnormalities in cellular calcium homeostasis, such as malignant hyperthermia or cardiac arrhythmia, are beginning to emerge. **Such special cases suggest that the pathophysiology of autism may comprise pathways that are directly or indirectly involved in mitochondrial energy production** and to further probe this connection three new avenues seem worthy of exploration: 1) metabolomic clinical studies provoking controlled aerobic exercise stress to expand the biochemical phenotype, 2) high-throughput expression arrays to directly survey activity of the genes underlying these biochemical pathways and 3) model systems, either based upon neuronal stem cells or model genetic organisms, to discover novel genetic and environmental inputs into these pathways.

117. Bridging from Cells to Cognition in Autism Pathophysiology: Biological Pathways to Defective Brain Function and Plasticity

American Journal of Biochemistry and Biotechnology 4 (2): 167-176, 2008

Matthew P. Anderson, Brian S. Hooker and Martha R. Herbert  
Departments of Neurology and Pathology, Harvard Medical School/Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, High Throughput Biology Team, Fundamental Science Directorate, Pacific Northwest National Laboratory, Pediatric Neurology/Center for Morphometric Analysis, Massachusetts General Hospital/Harvard Medical School, and Center for Child and Adolescent Development, Cambridge Health Alliance/Harvard Medical School

Abstract: We review evidence to support a model where the disease process



underlying autism may begin when an in utero or early postnatal environmental, infectious, seizure, or autoimmune insult triggers an immune response that increases reactive oxygen species (ROS) production in the brain that leads to DNA damage (nuclear and mitochondrial) and metabolic enzyme blockade and that these inflammatory and oxidative stressors persist beyond early development (with potential further exacerbations), producing ongoing functional consequences. In organs with a high metabolic demand such as the central nervous system, the continued use of mitochondria with damaged DNA and impaired metabolic enzyme function may generate additional ROS which will cause persistent activation of the innate immune system leading to more ROS production. Such a mechanism would self-sustain and possibly progressively worsen. The mitochondrial dysfunction and altered redox signal transduction pathways found in autism would conspire to activate both astroglia and microglia. These activated cells can then initiate a broad-spectrum proinflammatory gene response. Beyond the direct effects of ROS on neuronal function, receptors on neurons that bind the inflammatory mediators may serve to inhibit neuronal signaling to protect them from excitotoxic damage during various pathologic insults (e.g., infection). **In autism, over-zealous neuroinflammatory responses could not only influence neural developmental processes, but may more significantly impair neural signaling involved in cognition in an ongoing fashion.** This model makes specific predictions in patients and experimental animal models and suggests a number of targets sites of intervention. Our model of potentially reversible pathophysiological mechanisms in autism motivates our hope that effective therapies may soon appear on the horizon.

118. Heavy-Metal Toxicity—With Emphasis on Mercury

John Neustadt, ND, and Steve Pieczenik, MD, PhD

Research Review

Conclusion: Metals are ubiquitous in our environment, and exposure to them is inevitable. However, not all people accumulate toxic levels of metals or exhibit symptoms of metal toxicity, suggesting that genetics play a role in their potential to damage health. **Metal toxicity creates multisystem dysfunction, which appears to be mediated primarily through mitochondrial damage from glutathione depletion.**

Accurate screening can increase the likelihood that patients with potential metal toxicity are identified. The most accurate screening method for assessing chronic-metals exposure and metals load in the body is a provoked urine test.

119. Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment

American Journal of Biochemistry and Biotechnology 4 (2): 208-217, 2008

Daniel A. Rossignol, J. Jeffrey Bradstreet, International Child Development Resource Center,

#### Abstract

Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. **Exposure to environmental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions.**

Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.

120. [Evidence of Mitochondrial Dysfunction in Autism: Biochemical Links, Genetic-Based Associations, and Non-Energy-Related Mechanisms](#)

Oxid Med Cell Longev. 2017 May 29.

Keren K. Griffiths and Richard J. Levy

Department of Anesthesiology, Columbia University Medical Center, New York, NY, USA

#### Abstract

Autism spectrum disorder (ASD), the fastest growing developmental disability in the United States, represents a group of neurodevelopmental disorders characterized by impaired social interaction and communication as well as restricted and repetitive behavior. The underlying cause of autism is unknown and therapy is currently limited to targeting behavioral abnormalities. Emerging studies suggest a link between mitochondrial dysfunction and ASD. Here, we review the evidence demonstrating this potential connection. We focus specifically on biochemical links, genetic-based associations, non-energy related

mechanisms, and novel therapeutic strategies.

#### Conclusion

**The literature reviewed here suggests a link between abnormalities in mitochondrial homeostasis and ASD and provides biochemical and genetic evidence to support a role for mitochondrial dysfunction in the pathogenesis of the autism phenotype.** Mechanistically, the connection may involve defects in bioenergetic capacity as well as non-energy related pathways. However, it is not clear if mitochondrial impairments cause ASD or if they are merely associated with the disease process. Positive patient behavioral responses to conventional mitochondrial disease therapies are promising, however, further investigation is necessary. Future work should focus on determining how mitochondrial dysfunction causes the autistic phenotype as well as how defects in mitochondrial homeostasis predispose individuals to ASD via interaction with environmental toxins, dietary factors, and epigenetic modifications during critical periods of development. Establishing a causative relationship between mitochondrial dysfunction and ASD and elucidating the exact mechanisms will permit the development of more precisely targeted therapies in the future. Ultimately, with improved knowledge and innovation, we may one day be able to prevent or cure autism.

121. Proximity to point sources of environmental mercury release as a predictor of autism prevalence

Health & Place, 2008

Raymond F. Palmer, Stephen Blanchard, Robert Wood  
University of Texas Health Science Center, San Antonio Department of Family and Community Medicine, Our Lady of the Lake University, San Antonio Texas, Chair, Department of Sociology

This study should be viewed as hypothesis-generating - a first step in examining the potential role of environmental mercury and childhood developmental disorders. Nothing is known about specific exposure routes, dosage, timing, and individual susceptibility. **We suspect that persistent low-dose exposures to various environmental toxicants, including mercury, that occur during critical windows of neural development among genetically susceptible children (with a diminished capacity for metabolizing accumulated toxicants) may increase the risk for developmental disorders such as autism.** Successfully identifying the specific combination of environmental exposures and genetic susceptibilities can inform the development of targeted prevention intervention strategies.

122. Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions

Developmental Medicine & Child Neurology, 2007

Guiomar Oliveira MD PhD, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra; Assunção Ataíde BSc, Direcção Regional de Educação do Centro Coimbra;

Carla Marques MSc, Centro de Desenvolvimento da Criança, Hospital Pediátrico de Coimbra; Teresa S Miguel BSc, Direcção Regional de Educação do Centro, Coimbra;

Ana Margarida Coutinho BSc, Instituto Gulbenkian de Ciência, Oeiras; Luísa Mota-Vieira PhD, Unidade de Genética e Patologia moleculares, Hospital do Divino Espírito Santo, Ponta Delgada, Açores; Esmeralda Gonçalves PhD; Nazaré Mendes Lopes PhD, Faculdade de Ciências e Tecnologia, Universidade de Coimbra; Vítor Rodrigues MD PhD; Henrique Carmona da Mota MD PhD, Faculdade de Medicina, Universidade de Coimbra, Coimbra; Astrid Moura Vicente PhD, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

\*Correspondence to first author at Hospital Pediátrico de Coimbra, Av Bissaya Barreto, 3000-076 Coimbra, Portugal. E-mail: [guiomar@hpc.chc.min-saude.pt](mailto:guiomar@hpc.chc.min-saude.pt)

**Abstract:** The objective of this study was to estimate the prevalence of autistic spectrum disorder (ASD) and identify its clinical characterization, and medical conditions in a paediatric population in Portugal. A school survey was conducted in elementary schools, targeting 332 808 school-aged children in the mainland and 10 910 in the Azores islands. Referred children were directly assessed using the Diagnostic and Statistical Manual of Mental Disorders (4th edn), the Autism Diagnostic Interview–Revised, and the Childhood Autism Rating Scale. Clinical history and a laboratory investigation was performed. In parallel, a systematic multi-source search of children known to have autism was carried out in a restricted region. The global prevalence of ASD per 10 000 was 9.2 in mainland, and 15.6 in the Azores, with intriguing regional differences. **A diversity of associated medical conditions was documented in 20%, with an unexpectedly high rate of mitochondrial respiratory chain disorders.**

123. Thimerosal induces neuronal cell apoptosis by causing cytochrome c and apoptosis-inducing factor release from mitochondria.

International Journal of Molecular Medicine, 2006

Yel L, Brown LE, Su K, Gollapudi S, Gupta S. Department of Medicine, University of California, Irvine, CA 92697, USA. [lyel@uci.edu](mailto:lyel@uci.edu)

There is a worldwide increasing concern over the neurological risks of thimerosal (ethylmercury thiosalicylate) which is an organic mercury compound that is commonly used as an antimicrobial preservative. In this study, we show that thimerosal, at nanomolar concentrations, induces neuronal cell death through the

mitochondrial pathway. Thimerosal, in a concentration- and time-dependent manner, decreased cell viability as assessed by calcein-ethidium staining and caused apoptosis detected by Hoechst 33258 dye. Thimerosal-induced apoptosis was associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, and release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria to cytosol. Although thimerosal did not affect cellular expression of Bax at the protein level, we observed translocation of Bax from cytosol to mitochondria. Finally, caspase-9 and caspase-3 were activated in the absence of caspase-8 activation. Our data suggest that thimerosal causes apoptosis in neuroblastoma cells by changing the mitochondrial microenvironment.

124. Mitochondrial mediated thimerosal-induced apoptosis in a human neuroblastoma cell line (SK-N-SH).

Neurotoxicology. 2005

Humphrey ML, Cole MP, Pendergrass JC, Kinningham KK. Department of Pharmacology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25704-9388, USA.

Environmental exposure to mercurials continues to be a public health issue due to their deleterious effects on immune, renal and neurological function. Recently the safety of thimerosal, an ethyl mercury-containing preservative used in vaccines, has been questioned due to exposure of infants during immunization. Mercurials have been reported to cause apoptosis in cultured neurons; however, the signaling pathways resulting in cell death have not been well characterized. Therefore, the objective of this study was to identify the mode of cell death in an in vitro model of thimerosal-induced neurotoxicity, and more specifically, to elucidate signaling pathways which might serve as pharmacological targets. Within 2 h of thimerosal exposure (5 microM) to the human neuroblastoma cell line, SK-N-SH, morphological changes, including membrane alterations and cell shrinkage, were observed. Cell viability, assessed by measurement of lactate dehydrogenase (LDH) activity in the medium, as well as the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, showed a time- and concentration-dependent decrease in cell survival upon thimerosal exposure. In cells treated for 24 h with thimerosal, fluorescence microscopy indicated cells undergoing both apoptosis and oncosis/necrosis. To identify the apoptotic pathway associated with thimerosal-mediated cell death, we first evaluated the mitochondrial cascade, as both inorganic and organic mercurials have been reported to accumulate in the organelle. Cytochrome c was shown to leak from the mitochondria, followed by caspase 9 cleavage within 8 h of treatment. In addition, poly(ADP-ribose) polymerase (PARP) was cleaved to form a 85 kDa fragment following maximal caspase 3 activation at 24 h. Taken together these findings suggest deleterious effects on the cytoarchitecture by thimerosal and initiation of mitochondrial-mediated apoptosis.

125. Possible Immunological Disorders in Autism: Concomitant Autoimmunity and Immune Tolerance

The Egyptian Journal of Immunology, 2006

Maha I. Sh. Kawashti, Omnia R. Amin Nadia G. Rowehey

Microbiology Department, Faculty of Medicine (For Girls), Al Azhar University, Cairo, Egypt, Psychiatry Department, Faculty of Medicine, Cairo University, Cairo, Egypt and Serology Lab King Fahad General Hospital, Jeddah, K.S.A.

**Abstract:** Autism is a pervasive developmental disorder that affect children early in their life. Immunological disorders is one of several contributing factors that have been suggested to cause autism. Thirty autistic children aged 3-6 years and thirty non-autistic psychologically-free siblings were studied. Circulating IgA and IgG autoantibodies to casein and gluten dietary proteins were detected by enzyme-immunoassays (EIA). Circulating IgG antibodies to measles, mumps and rubella vaccine (M.M.R) and cytomeglovirus were investigated by EIA. Results revealed high seropositivity for autoantibodies to casein and gluten: 83.3% and 50% respectively in autistic children as compared to 10% and 6.7% positivity in the control group. Surprisingly, circulating anti-measles, anti-mumps and anti-rubella IgG were positive in only 50%, 73.3% and 53.3% respectively as compared to 100% positivity in the control group. Anti-CMV IgG was positive in 43.3% of the autistic children as compared to 7% in the control group. **It is concluded that, autoimmune response to dietary proteins and deficient immune response to measles, mumps and rubella vaccine antigens might be associated with autism, as a leading cause or a resulting event.** Further research is needed to confirm these findings.

126. Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink.

Young HA, Geier DA, Geier MR.

The George Washington University School of Public Health and Health Services, Department of Epidemiology and Biostatistics, United States.

**Abstract**

The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990-1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of

Disease, 9th revision (ICD-9) specific NDs and control outcomes were calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. **Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs.** By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and Nds.

127. [Is fever a predictive factor in the autism spectrum disorders?](#)

Med Hypotheses. 2013 Apr;80(4):391-8. doi: 10.1016/j.mehy.2013.01.007. Epub 2013 Feb 8.

Amalia S F Megremi 1

1 Ilion Socio-Medical Center, General University Hospital "Attikon", Athens, Greece.

Abstract

Autism spectrum disorders (ASD) display such a marked increase in recent decades that researchers speak of "epidemic outbreak" of the disease. Although the diagnostic framework has been expanded and thus more disorders now fall within the autistic spectrum, no one disputes the increased incidence of autism in modern societies, making it a major public health problem. On the other hand, heterogeneity is a major feature of the disorder, both in terms of the etiopathogenesis as well as to the phenotypic expression, natural history and evolution. Consequently, there is considerable research interest in determining factors which are etiopathogenetically, prognostically, preventively or/and therapeutically associated with the disorder. Literature data indicate that probably there are differences in susceptibility to various infections between normal and autistic children. In addition, some autistic children show improvement in the characteristics of their autistic behavior during febrile incident and repression of fever (through antipyretics) might be associated with the onset of autistic disorder. Since fever has been associated with mental illness since the time of Hippocrates already and the presence of fever is associated with a favorable outcome in various pathologic conditions, it is assumed that there are probably two subgroups of autistic children: those who have the possibility to develop acute febrile incidents and those who develop acute incidents without fever. If this is the case, it is important to know whether there are differences between the two subgroups in various biological markers (cytokines/chemokines, autoantibodies), neuroimaging findings, personal and family history of these

children (use of drugs, vaccinations, history of autoimmunity, etc.) and, if the first subgroup consists of autistic people of higher functionality and better outcome, or not. If such a classification is real, is there a possibility for the fever to be used as a predictor of the autistic disorder outcome and of whether that person will achieve an acceptable level of functionality in the future? If there are positive answers to these questions, are autistic children, who develop fever, at a very critical stage in evolutionary terms, where it is very important not to lose the defense mechanism of fever development and thus must use the fever repression methods (antipyretic drugs for example) with caution and chariness? **If it is confirmed that autistic children with high fevers are of higher functionality, it is possible for preventive intervention programs to be developed where children are exposed to the least possible chemical drugs intervention (antipyretics, antibiotics, etc.) or even selective vaccination.** Further experimental, epidemiological and clinical studies are necessary to investigate the above.

128. [Post-Vaccination Inflammatory Syndrome: a new syndrome](#)

Clin Case Rep Rev 5: DOI: 10.15761/CCRR.1000454

Giannotta G1 and Giannotta N2

1Pediatrician, Provincial Health Authority of Vibo Valentia, Vibo Valentia, Italy

2Università Magna Grecia, Medical and Surgery Sciences, Catanzaro, Italy

Abstract

**Background:** The relationship between vaccines and neuroinflammation have consistent molecular biology bases. In a recent paper we have already analyzed this kind of relationship.

**Hypothesis:** In this paper, we have gained additional evidence to support the link between vaccines and neuroinflammation. Furthermore, we found the molecular bases that support the link between HPV vaccines and certain adverse events (AEs). The peripheral proinflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ), expressed after the injection of the vaccines can reach the brain and can cause neuroinflammation after microglia activation. After vaccine injection significant systemic immune activation may occur with signs suggesting reactive brain inflammation, such as acute crying, fever, restlessness and failure to eat. It is a warning of danger to the brain in front of which we should reflect before causing irreversible damage. We also hypothesized the existence of a post-vaccination inflammatory syndrome caused by the proinflammatory cytokines strongly expressed after HPV vaccine injections. In addition, the molecular explanation of the chronic pain that has affected many girls in the world, including the complex regional pain syndrome (CRPS) in Japanese girls.

**Conclusion:** All vaccines can cause neuroinflammation. HPV vaccines can cause a post-vaccination inflammatory syndrome characterized by chronic pain and neuroinflammation. In this case, the phenomena of central sensitization is



responsible for all the symptoms associated with chronic pain. The strong expression of proinflammatory cytokines, secreted after HPV vaccinations, brings to process that can produce irreversible neurological results in HPV vaccinated girls.

129. Glutathione, oxidative stress and neurodegeneration

Schulz JB, Lindenau J, Seyfried J, Dichgans J.  
Neurodegeneration Laboratory, Department of Neurology, University of Tübingen, Germany.

Eur J Biochem. 2000 Aug;267(16):4904-11.

Abstract

**There is significant evidence that the pathogenesis of several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Friedreich's ataxia and amyotrophic lateral sclerosis, may involve the generation of reactive oxygen species and mitochondrial dysfunction.**

Here, we review the evidence for a disturbance of glutathione homeostasis that may either lead to or result from oxidative stress in neurodegenerative disorders. Glutathione is an important intracellular antioxidant that protects against a variety of different antioxidant species. An important role for glutathione was proposed for the pathogenesis of Parkinson's disease, because a decrease in total glutathione concentrations in the substantia nigra has been observed in preclinical stages, at a time at which other biochemical changes are not yet detectable. Because glutathione does not cross the blood-brain barrier other treatment options to increase brain concentrations of glutathione including glutathione analogs, mimetics or precursors are discussed.

130. Environ Sci Pollut Res Int. 2021; 28(33): 44587–44597.  
Published online 2021 Jul 1. doi: 10.1007/s11356-021-14700-0

[Aluminum environmental pollution: the silent killer](#)

Reema H. Alasfar and Rima J. Isaifan

Division of Sustainable Development (DSD), College of Science and Engineering (CSE), Hamad Bin Khalifa University (HBKU)/Qatar Foundation (QF), P.O. Box 5825, Doha, Qatar

Abstract

The concern about aluminum (Al) toxicity has been proven in various cases. Some cases are associated with the fact that Al is a neurotoxic substance that has been found in high levels in the brain tissues of Alzheimer's disease (AD), epilepsy, and autism patients. Other cases are related to infants, especially premature infants and ones with renal failure, who are at the risk of developing the central nervous system (CNS) and bone toxicity. This risk is a result of infants' exposure to Al from milk formulas, intravenous-feeding solutions, and possibly from aluminum-containing vaccinations. Furthermore, most antiperspirants contain aluminum compounds that raise human exposure to toxic Al. This review paper is intended to discuss in detail the above concerns associated with aluminum, and hence urges the need for more studies exploring the effects of overexposure to Al and recommending mitigation actions.

131. [Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years](#)

Carolyn Gallagher a; Melody Goodman, Graduate Program in Public Health, Stony Brook University Medical Center, Health Sciences Center, New York, USA

Journal Toxicological & Environmental Chemistry, Volume 90, Issue 5 September 2008 , pages 997 - 1008

Abstract

This study investigated the association between vaccination with the Hepatitis B triple series vaccine prior to 2000 and developmental disability in children aged 1–9 years (n = 1824), proxied by parental report that their child receives early intervention or special education services (EIS). National Health and Nutrition Examination Survey 1999–2000 data were analyzed and adjusted for survey design by Taylor Linearization using SAS version 9.1 software, with SAS callable SUDAAN version 9.0.1. The odds of receiving EIS were approximately nine times as great for vaccinated boys (n = 46) as for unvaccinated boys (n = 7), after adjustment for confounders. **This study found statistically significant evidence to suggest that boys in United States who were vaccinated with the triple series Hepatitis B vaccine, during the time period in which vaccines were manufactured with thimerosal, were more susceptible to developmental disability than were unvaccinated boys.**

132. [IL-4 mediates the delayed neurobehavioral impairments induced by neonatal hepatitis B vaccination that involves the down-regulation of the IL4 receptor in the hippocampus](#)

Cytokine

Xiao Wang, Junhua Yang, Zhiwei Xing, Hongyang Zhang, Yaru Wen, Fangfang Qi, Zejie Zuo, Jie Xu, Zhibin Yao

Department of Anatomy and Neurobiology, Zhongshan School of Medicine, Sun Yat-sen University, PR China  
Guangdong Province Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, PR China

#### ABSTRACT

We have previously verified that neonatal hepatitis B vaccination induced hippocampal neuroinflammation and behavior impairments in mice. However, the exact mechanism of these effects remain unclear. In this study, we observed that neonatal hepatitis B vaccination induced an anti-inflammatory cytokine response lasting for 4–5 weeks in both the serum and the hippocampus, primarily indicated by elevated IL-4 levels. Three weeks after the vaccination schedule, however, hepatitis B vaccine (HBV)-mice showed delayed hippocampal neuroinflammation. In periphery, IL-4 is the major cytokine induced by this vaccine. Correlation analyses showed a positive relationship in the IL-4 levels between serum and hippocampus in HBV-mice. Thus, we investigated whether neonatal over-exposure to systemic IL-4 influences brain and behavior. We observed that mice injected intraperitoneally with recombinant mouse IL-4 (mIL-4) during early life had similar neuroinflammation and cognition impairment similar to those induced by neonatal hepatitis B vaccination. Next, the mechanism underlying the effects of IL-4 on brain in mice was explored using a series of experiments. In brief, these experiments showed that IL-4 mediates the delayed neurobehavioral impairments induced by neonatal hepatitis B vaccination, which involves the permeability of neonatal blood–brain barrier and the down-regulation of IL-4 receptor. **This finding suggests that clinical events concerning neonatal IL-4 over-exposure, including neonatal hepatitis B vaccination and allergic asthma in human infants, may have adverse implications for brain development and cognition.**

133. [The risk of neurodevelopmental disorders at age 10 years associated with blood concentrations of interleukins 4 and 10 during the first postnatal month of children born extremely preterm.](#)

Cytokine. 2018 May 12;110:181-188. doi: 10.1016/j.cyto.2018.05.004.

Leviton A, Joseph RM, Allred EN, Fichorova RN, O'Shea TM, Kuban KKC, Dammann O7.

Boston Children's Hospital and Harvard Medical School, Boston, MA, USA.  
Electronic address: alan.leviton@childrens.harvard.edu.  
Boston University School of Medicine, Boston, MA, USA.  
Boston Children's Hospital and Harvard Medical School, Boston, MA, USA.  
Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.

University of North Carolina School of Medicine, Chapel Hill, NC, USA.  
Boston Medical Center and Boston University School of Medicine, Boston, MA, USA.

Tufts University School of Medicine, Boston, MA 02111, USA; Perinatal Neuroepidemiology Unit, Department of Gynecology and Obstetrics, Hannover Medical School, 30623 Hannover, Germany

#### Abstract

##### BACKGROUND:

Interleukin (IL)-4 and IL-10 are viewed mainly as anti-inflammatory cytokines. Yet, high concentrations have also been associated with inflammation-related diseases in newborns.

##### METHODS:

We measured the concentrations of IL-4 and IL-10, as well as IL-8 and ICAM-1 in blood specimens collected on postnatal day 21 (N = 555), day 28 (N = 521), and both days 21 and 28 (N = 449) from children born extremely preterm (EP) (<28 weeks gestation) who at age 10 years had a DAS-II IQ Z-score > -2 (which approximates a score of >70) and the following assessments, CCC-2, and CSI-4, DAS-II, NEPSY-II, OWLS-II, SCQ, and WIAT-III. Selected children also were assessed with the ADI-R and the ADOS-2. We modeled the risk of low scores or dysfunctions associated with top quartile concentrations of IL-4 and IL-10 on each day and on both days.

##### RESULTS:

The risks of low scores on the Animal Sorting and Arrows components of the NEPSY-II, both components of the OWLS-II, and the PseudoWord and Spelling components of the WIAT-III were heightened among children who had top quartile concentrations of IL-4 on postnatal days 21 and 28. Children who had high concentrations of IL-10 on days 21 and 28, individually and collectively, were at increased risk of low scores on the WIAT-III Spelling component. High concentrations of IL-4 on day 28 were associated with autism spectrum disorder (ASD). High concentrations of IL-10 on day 28 were also associated with a doubling of ASD risk, but this did not achieve statistical significance. Top quartile concentrations of IL-4 and IL10 on both days were not associated with increased risk of social, language, or behavioral dysfunctions.

##### CONCLUSION:

**Among children born EP, those who had top quartile concentrations of IL-4 and/or IL-10 on postnatal days 21 and/or 28 were more likely than their peers to have low scores on components of the NEPSY-II, OWLS-II, and WIAT-III assessments, as well as identification as having an ASD.**

134. Induction of metallothionein in mouse cerebellum and cerebrum with low-dose thimerosal injection.

Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S., Department of Life

Sciences, School of Science & Engineering, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka, 577-8502, Japan, minamita@life.kindai.ac.jp.

Cell Biology and Toxicology. 2009 Apr 9. [Epub ahead of print]

#### Abstract

Thimerosal, an ethyl mercury compound, is used worldwide as a vaccine preservative. We previously observed that the mercury concentration in mouse brains did not increase with the clinical dose of thimerosal injection, but the concentration increased in the brain after the injection of thimerosal with lipopolysaccharide, even if a low dose of thimerosal was administered. Thimerosal may penetrate the brain, but is undetectable when a clinical dose of thimerosal is injected; therefore, the induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection, as MT is an inducible protein. MT-1 mRNA was expressed at 6 and 9 h in both the cerebrum and cerebellum, but MT-1 mRNA expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12 microg/kg thimerosal. MT-2 mRNA was not expressed until 24 h in both organs. MT-3 mRNA was expressed in the cerebellum from 6 to 15 h after the injection, but not in the cerebrum until 24 h. MT-1 and MT-3 mRNAs were expressed in the cerebellum in a dose-dependent manner. Furthermore, MT-1 protein was detected from 6 to 72 h in the cerebellum after 12 microg/kg of thimerosal was injected and peaked at 10 h. MT-2 was detected in the cerebellum only at 10 h. In the cerebrum, little MT-1 protein was detected at 10 and 24 h, and there were no peaks of MT-2 protein in the cerebrum. In conclusion, MT-1 and MT-3 mRNAs but not MT-2 mRNA are easily expressed in the cerebellum rather than in the cerebrum by the injection of low-dose thimerosal. It is thought that the cerebellum is a sensitive organ against thimerosal. **As a result of the present findings, in combination with the brain pathology observed in patients diagnosed with autism, the present study helps to support the possible biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with autism.**

#### 135. Mercury induces inflammatory mediator release from human mast cells

Duraisamy Kempuraj, Shahrzad Asadi, Bodi Zhang, Akrivi Manola, Jennifer Hogan, Erika Peterson, Theoharis C Theoharides

Journal of Neuroinflammation 2010, 7:20 doi:10.1186/1742-2094-7-20

#### Abstract

Background: Mercury is known to be neurotoxic, but its effects on the immune system are less well known. Mast cells are involved in allergic reactions, but also in innate and acquired immunity, as well as in inflammation. Many patients with Autism Spectrum Disorders (ASD) have “allergic” symptoms; moreover, the prevalence of ASD in patients with mastocytosis, characterized by numerous hyperactive mast cells in most tissues, is 10-fold higher than the general

population suggesting mast cell involvement. We, therefore, investigated the effect of mercuric chloride (HgCl<sub>2</sub>) on human mast cell activation.

Methods: Human leukemic cultured LAD2 mast cells and normal human umbilical cord blood-derived cultured mast cells (hCBMCs) were stimulated by HgCl<sub>2</sub> (0.1-10 μM) for either 10 min for beta-hexosaminidase release or 24 hr for measuring vascular endothelial growth factor (VEGF) and IL-6 release by ELISA.

Results: HgCl<sub>2</sub> induced a 2-fold increase in β-hexosaminidase release, and also significant VEGF release at 0.1 and 1 μM (311±32 pg/10<sup>6</sup> cells and 443±143 pg/10<sup>6</sup> cells, respectively) from LAD2 mast cells compared to control cells (227±17 pg/10<sup>6</sup> cells, n=5, p<0.05). Addition of HgCl<sub>2</sub> (0.1 μM) to the proinflammatory neuropeptide substance P (SP, 0.1 μM) had synergistic action in inducing VEGF from LAD2 mast cells. HgCl<sub>2</sub> also stimulated significant VEGF release (360 ± 100 pg/10<sup>6</sup> cells at 1 μM, n=5, p<0.05) from hCBMCs compared to control cells (182 ±57 pg/10<sup>6</sup> cells), and IL-6 release (466±57 pg/10<sup>6</sup> cells at 0.1 μM) compared to untreated cells (13±25 pg/10<sup>6</sup> cells, n=5, p<0.05). Addition of HgCl<sub>2</sub> (0.1 μM) to SP (5 μM) further increased IL-6 release. Conclusions: HgCl<sub>2</sub> stimulates VEGF and IL-6 release from human mast cells. **This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation. As a result, the findings of the present study provide a biological mechanism for how low levels of mercury may contribute to ASD pathogenesis.**

136. [Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders](#)

Neuroscience

Medical Investigation of Neurodevelopmental Disorders (M.I.N.D.) Institute and Department of Psychiatry and Behavioral Sciences, UC Davis School of Medicine, University of California, Sacramento, CA 95817; Departments of Psychiatry and Biostatistics, Harvard University Schools of Medicine and Public Health, McLean Hospital, Belmont, MA 02478; and Department of Radiology, UC Davis School of Medicine, University of California, Sacramento, CA 95817

Proc Natl Acad Sci U S A. 2011 Dec 13; 108(50): 20195–20200.  
Published online 2011 Nov 28. doi: 10.1073/pnas.1107560108

Christine Wu Nordahl, Nicholas Lange, Deana D. Li, Lou Ann Barnett, Aaron Lee, Michael H. Buonocore, Tony J. Simon, Sally Rogers, Sally Ozonoff, and David G. Amarala,

ABSTRACT

Autism is a heterogeneous disorder with multiple behavioral and biological phenotypes. Accelerated brain growth during early childhood is a well-established biological feature of autism. Onset pattern, i.e., early onset or regressive, is an intensely studied behavioral phenotype of autism. There is currently little known, however, about whether, or how, onset status maps onto the abnormal brain growth. We examined the relationship between total brain volume and onset status in a large sample of 2- to 4-y-old boys and girls with autism spectrum disorder (ASD) [n = 53, no regression (nREG); n = 61, regression (REG)] and a comparison group of age-matched typically developing controls (n = 66). We also examined retrospective head circumference measurements from birth through 18 mo of age. **We found that abnormal brain enlargement was most commonly found in boys with regressive autism. Brain size in boys without regression did not differ from controls.** Retrospective head circumference measurements indicate that head circumference in boys with regressive autism is normal at birth but diverges from the other groups around 4–6 mo of age. There were no differences in brain size in girls with autism (n = 22, ASD; n = 24, controls). **These results suggest that there may be distinct neural phenotypes associated with different onsets of autism.** For boys with regressive autism, divergence in brain size occurs well before loss of skills is commonly reported. Thus, rapid head growth may be a risk factor for regressive autism.

137. [Abnormal intestinal permeability in children with autism](#)

Acta Paediatr. 1996 Sep;85(9):1076-9. doi: 10.1111/j.1651-2227.1996.tb14220.x.

P D'Eufemia 1, M Celli, R Finocchiaro, L Pacifico, L Viozzi, M Zaccagnini, E Cardì, O Giardini

Affiliation

Institute of Pediatrics, La Sapienza University of Rome, Italy.

Abstract

**We determined the occurrence of gut mucosal damage using the intestinal permeability test in 21 autistic children who had no clinical and laboratory findings consistent with known intestinal disorders.** An altered intestinal permeability was found in 9 of the 21 (43%) autistic patients, but in none of the 40 controls. Compared to the controls, these nine patients showed a similar mean mannitol recovery, but a significantly higher mean lactulose recovery (1.64% +/- 1.43 vs 0.38% +/- 0.14; P < 0.001). **We speculate that an altered intestinal permeability could represent a possible mechanism for the increased passage through the gut mucosa of peptides derived from foods with subsequent behavioural abnormalities.**

138. [Blood–brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders](#)

Molecular Autism Brain, Cognition and Behavior, Published: 29 November 2016

Maria Fiorentino, Anna Sapone, Stefania Senger, Stephanie S. Camhi, Sarah M. Kadzielski, Timothy M. Buie, Deanna L. Kelly, Nicola Cascella and Alessio Fasano

#### Abstract

##### Background

Autism spectrum disorders (ASD) are complex conditions whose pathogenesis may be attributed to gene–environment interactions. There are no definitive mechanisms explaining how environmental triggers can lead to ASD although the involvement of inflammation and immunity has been suggested. Inappropriate antigen trafficking through an impaired intestinal barrier, followed by passage of these antigens or immune-activated complexes through a permissive blood–brain barrier (BBB), can be part of the chain of events leading to these disorders. Our goal was to investigate whether an altered BBB and gut permeability is part of the pathophysiology of ASD.

##### Methods

Postmortem cerebral cortex and cerebellum tissues from ASD, schizophrenia (SCZ), and healthy subjects (HC) and duodenal biopsies from ASD and HC were analyzed for gene and protein expression profiles. Tight junctions and other key molecules associated with the neurovascular unit integrity and function and neuroinflammation were investigated.

##### Results

Claudin (CLDN)-5 and -12 were increased in the ASD cortex and cerebellum. CLDN-3, tricellulin, and MMP-9 were higher in the ASD cortex. IL-8, tPA, and IBA-1 were downregulated in SCZ cortex; IL-1b was increased in the SCZ cerebellum. Differences between SCZ and ASD were observed for most of the genes analyzed in both brain areas. CLDN-5 protein was increased in ASD cortex and cerebellum, while CLDN-12 appeared reduced in both ASD and SCZ cortexes. In the intestine, 75% of the ASD samples analyzed had reduced expression of barrier-forming TJ components (CLDN-1, OCLN, TRIC), whereas 66% had increased pore-forming CLDNs (CLDN-2, -10, -15) compared to controls.

##### Conclusions

**In the ASD brain, there is an altered expression of genes associated with BBB integrity coupled with increased neuroinflammation and possibly impaired gut barrier integrity.** While these findings seem to be specific for ASD, the possibility of more distinct SCZ subgroups should be explored with additional studies.



139. [A possible central mechanism in autism spectrum disorders, part 1](#)

Altern Ther Health Med. 2008 Nov-Dec;14(6):46-53.

Russell L Blaylock

Belhaven College, Jackson, Mississippi, USA.

PMID: 19043938

Abstract

The autism spectrum disorders (ASD) are a group of related neurodevelopmental disorders that have been increasing in incidence since the 1980s. Despite a considerable amount of data being collected from cases, a central mechanism has not been offered. A careful review of ASD cases discloses a number of events that adhere to an immunoexcitotoxic mechanism. This mechanism explains the link between excessive vaccination, use of aluminum and ethylmercury as vaccine adjuvants, food allergies, gut dysbiosis, and abnormal formation of the developing brain. It has now been shown that chronic microglial activation is present in autistic brains from age 5 years to age 44 years. A considerable amount of evidence, both experimental and clinical, indicates that repeated microglial activation can initiate priming of the microglia and that subsequent stimulation can produce an exaggerated microglial response that can be prolonged. It is also known that one phenotypic form of microglia activation can result in an outpouring of neurotoxic levels of the excitotoxins, glutamate and quinolinic acid. Studies have shown that careful control of brain glutamate levels is essential to brain pathway development and that excesses can result in arrest of neural migration, as well as dendritic and synaptic loss. It has also been shown that certain cytokines, such as TNF-alpha, can, via its receptor, interact with glutamate receptors to enhance the neurotoxic reaction. To describe this interaction I have coined the term immunoexcitotoxicity, which is described in this article.

140. [Glial fibrillary acidic protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders](#)

Biol Psychiatry. 1993 May 15;33(10):734-43. doi: 10.1016/0006-3223(93)90124-v.

G Ahlsén<sup>1</sup>, L Rosengren, M Belfrage, A Palm, K Haglid, A Hamberger, C Gillberg

<sup>1</sup> Department of Child Neuropsychiatry, University of Göteborg, Sweden

Abstract

The cerebrospinal fluid (CSF) of 47 children and adolescents with autism was analyzed for the contents of two astroglial proteins, the glial fibrillary acidic protein (GFA) and S 100. The results were contrasted with those obtained in similarly aged cases with other neuropsychiatric disorders (n = 25) and in normal children (n = 10). S-100 did not discriminate the groups from each other. However, **GFA in autism and autistic-like conditions was at a level almost three times that in the normal group. The results could implicate gliosis and unspecific brain damage in autism.** An alternative model would be increased synapse turnover regardless of underlying cause.

141. [A possible central mechanism in autism spectrum disorders, part 2: immunoexcitotoxicity](#)

Altern Ther Health Med. 2009 Jan-Feb;15(1):60-7.

Russell L Blaylock

Belhaven College, Jackson, Mississippi, USA.

PMID: 19161050

Abstract

In this section, I explore the effects of mercury and inflammation on transsulfuration reactions, which can lead to elevations in androgens, and how this might relate to the male preponderance of autism spectrum disorders (ASD). It is known that mercury interferes with these biochemical reactions and that chronically elevated androgen levels also enhance the neurodevelopmental effects of excitotoxins. Both androgens and glutamate alter neuronal and glial calcium oscillations, which are known to regulate cell migration, maturation, and final brain cytoarchitectural structure. Studies have also shown high levels of DHEA and low levels of DHEA-S in ASD, which can result from both mercury toxicity and chronic inflammation. Chronic microglial activation appears to be a hallmark of ASD. Peripheral immune stimulation, mercury, and elevated levels of androgens can all stimulate microglial activation. Linked to both transsulfuration problems and chronic mercury toxicity are elevations in homocysteine levels in ASD patients. Homocysteine and especially its metabolic products are powerful excitotoxins. Intimately linked to elevations in DHEA, excitotoxicity and mercury toxicity are abnormalities in mitochondrial function. A number of studies have shown that reduced energy production by mitochondria greatly enhances excitotoxicity. Finally, I discuss the effects of chronic inflammation and elevated mercury levels on glutathione and metallothionein.

142. [A neurochemical theory of autism](#)

Points of view| Volume 2, P174-177, 1979, DOI:[https://doi.org/10.1016/0166-2236\(79\)90071-7](https://doi.org/10.1016/0166-2236(79)90071-7)

Jaak Panksepp

J. Panksepp is Research Professor in the Department of Psychology, Bowling Green State University, Bowling Green, OH 43403, U.S.A.

#### Abstract

The appreciation of the existence of a brain disorder as encapsulated in early childhood autism has only come to the fore in recent years. Possibly for this reason, there is no apparent agreement as to which of the symptoms of the disorder, namely emotional disturbances or cognitive defects, are primary, and which are secondary. Nor is there yet either a generally accepted medical treatment or a coherent neurochemical theory of autism. In this article, Jaak Panksepp puts forward the idea that autism is an emotional disturbance arising from an upset in the opiate systems in the brain, and hence proposes, as a possibility, opiate antagonist therapy for the autistic syndrome.

143. Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study

Acta Neurobiol Exp 2010, 70: 147–164 Polish Neuroscience Society - PTBUN, Nencki Institute of Experimental Biology

Laura Hewitson<sup>1,2,\*</sup>, Brian J. Lopresti<sup>3</sup>, Carol Stott<sup>4</sup>, N. Scott Mason<sup>3</sup> and Jaime Tomko<sup>1</sup>

Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; Thoughtful House Center for Children, Austin, TX, USA; Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; <sup>4</sup>Independent Chartered Scientist, Cambridge, UK;

#### Abstract

This longitudinal, case-control pilot study examined amygdala growth in rhesus macaque infants receiving the complete US childhood vaccine schedule (1994-1999). Longitudinal structural and functional neuroimaging was undertaken to examine central effects of the vaccine regimen on the developing brain. Vaccine-exposed and saline-injected control infants underwent MRI and PET imaging at approximately 4 and 6 months of age, representing two specific timeframes within the vaccination schedule. Volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume that was observed in unexposed animals. After controlling for left amygdala volume, the binding of the opioid antagonist [<sup>11</sup>C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [<sup>11</sup>C]DPN binding occurred. **These results suggest that maturational changes in amygdala volume and the binding capacity of [<sup>11</sup>C]DPN in the amygdala was**

**significantly altered in infant macaques receiving the vaccine schedule. The macaque infant is a relevant animal model in which to investigate specific environmental exposures and structural/functional neuroimaging during neurodevelopment.**

144. Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to thimerosal challenge.

Neurotoxicology. 2006 Sep;27(5):685-92. Epub 2006 Jun 16.

Walker SJ, Segal J, Aschner M.

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27156, USA. swalker@wfubmc.edu

Abstract

Abstract

There are reports suggesting that some autistic children are unable to mount an adequate response following exposure to environmental toxins. This potential deficit, coupled with the similarity in clinical presentations of autism and some heavy metal toxicities, has led to the suggestion that heavy metal poisoning might play a role in the etiology of autism in uniquely susceptible individuals. Thimerosal, an anti-microbial preservative previously added routinely to childhood multi-dose vaccines, is composed of 49.6% ethyl mercury. Based on the levels of this toxin that children receive through routine immunization schedules in the first years of life, it has been postulated that thimerosal may be a potential triggering mechanism contributing to autism in susceptible individuals. One potential risk factor in these individuals may be an inability to adequately up-regulate metallothionein (MT) biosynthesis in response to presentation of a heavy metal challenge. To investigate this hypothesis, cultured lymphocytes (obtained from the Autism Genetic Resource Exchange, AGRE) from autistic children and non-autistic siblings were challenged with either 10 microM ethyl mercury, 150 microM zinc, or fresh media (control). Following the challenge, total RNA was extracted and used to query "whole genome" DNA microarrays. Cultured lymphocytes challenged with zinc responded with an impressive up-regulation of MT transcripts (at least nine different MTs were over-expressed) **while cells challenged with thimerosal responded by up-regulating numerous heat shock protein transcripts, but not MTs. Although there were no apparent differences between autistic and non-autistic sibling responses in this very small sampling group, the differences in expression profiles between those cells treated with zinc versus thimerosal were dramatic.** Determining cellular response, at the level of gene expression, has important implications for the understanding and treatment of conditions that result from exposure to neurotoxic compounds.

145.       Sorting out the spinning of autism: heavy metals and the question of incidence

Acta Neurobiol Exp 2010, 70: 165–176

Mary Catherine DeSoto\* and Robert T. Hitlan, Department of Psychology, University of Northern Iowa, Cedar Falls, Iowa, USA

The reasons for the rise in autism prevalence are a subject of heated professional debate. Featuring a critical appraisal of some research used to question whether there is a rise in cases and if rising levels of autism are related to environmental exposure to toxins (Soden et al. 2007, Thompson et al. 2007, Barbaresi et al. 2009) we aim to evaluate the actual state of scientific knowledge. In addition, we surveyed the empirical research on the topic of autism and heavy metal toxins. Overall, the various causes that have led to the increase in autism diagnosis are likely multi-faceted, and understanding the causes is one of the most important health topics today. We argue that scientific research does not support rejecting the link between the neurodevelopmental disorder of autism and toxic exposures.

146.       Urinary Porphyrin Excretion in Neurotypical and Autistic Children

Environ Health Perspect. 2010 Oct;118(10):1450-7. Epub 2010 Jun 24.

Woods JS, Armel SE, Fulton DI, Allen J, Wessels K, Simmonds PL, Granpeesheh D, Mumper E, Bradstreet JJ, Echeverria D, Heyer NJ, Rooney JP., Department of Environmental and Occupational Health Sciences, University of Washington

**Abstract**

**BACKGROUND:** Increased urinary concentrations of pentacarboxyl-, precopro- and copro-porphyrins have been associated with prolonged mercury (Hg) exposure in adults, and comparable increases have been attributed to Hg exposure in children with autism (AU).

**OBJECTIVES:** This study was designed to measure and compare urinary porphyrin concentrations in neurotypical (NT) children and same-age children with autism, and to examine the association between porphyrin levels and past or current Hg exposure in children with autism.

**METHODS:** This exploratory study enrolled 278 children 2-12 years of age. We evaluated three groups: AU, pervasive developmental disorder-not otherwise specified (PDD-NOS), and NT. Mothers/caregivers provided information at enrollment regarding medical, dental, and dietary exposures. Urine samples from all children were acquired for analyses of porphyrin, creatinine, and Hg.

Differences between groups for mean porphyrin and Hg levels were evaluated. Logistic regression analysis was conducted to determine whether porphyrin levels were associated with increased risk of autism.

**RESULTS:** Mean urinary porphyrin concentrations are naturally high in young children and decline by as much as 2.5-fold between 2 and 12 years of age. Elevated copro- ( $p < 0.009$ ), hexacarboxyl- ( $p < 0.01$ ) and pentacarboxyl- ( $p < 0.001$ ) porphyrin concentrations were significantly associated with AU but not with PDD-NOS. No differences were found between NT and AU in urinary Hg levels or in past Hg exposure as determined by fish consumption, number of dental amalgam fillings, or vaccines received. **CONCLUSIONS: These findings identify disordered porphyrin metabolism as a salient characteristic of autism.** Hg exposures were comparable between diagnostic groups, and a porphyrin pattern consistent with that seen in Hg-exposed adults was not apparent.

147. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

Molecular Psychiatry advance online publication 25 January 2011;doi: 10.1038/mp.2010.136

D A Rossignol and R E Frye

**Abstract**

A comprehensive literature search was performed to collate evidence of mitochondrial dysfunction in autism spectrum disorders (ASDs) with two primary objectives. First, features of mitochondrial dysfunction in the general population of children with ASD were identified. Second, characteristics of mitochondrial dysfunction in children with ASD and concomitant mitochondrial disease (MD) were compared with published literature of two general populations: ASD children without MD, and non-ASD children with MD. The prevalence of MD in the general population of ASD was 5.0% (95% confidence interval 3.2, 6.9%), much higher than found in the general population (~0.01%). The prevalence of abnormal biomarker values of mitochondrial dysfunction was high in ASD, much higher than the prevalence of MD. Variances and mean values of many mitochondrial biomarkers (lactate, pyruvate, carnitine and ubiquinone) were significantly different between ASD and controls. Some markers correlated with ASD severity. Neuroimaging, in vitro and post-mortem brain studies were consistent with an elevated prevalence of mitochondrial dysfunction in ASD. Taken together, these findings suggest children with ASD have a spectrum of mitochondrial dysfunction of differing severity. Eighteen publications representing a total of 112 children with ASD and MD (ASD/MD) were identified. The prevalence of developmental regression (52%), seizures (41%), motor delay (51%), gastrointestinal abnormalities (74%), female gender (39%), and elevated lactate (78%) and pyruvate (45%) was significantly higher in ASD/MD compared with the general ASD population. The prevalence of many of these abnormalities was similar to the general population of children with MD, suggesting that ASD/MD represents a

distinct subgroup of children with MD. Most ASD/MD cases (79%) were not associated with genetic abnormalities, raising the possibility of secondary mitochondrial dysfunction. Treatment studies for ASD/MD were limited, although improvements were noted in some studies with carnitine, co-enzyme Q10 and B-vitamins. Many studies suffered from limitations, including small sample sizes, referral or publication biases, and variability in protocols for selecting children for MD workup, collecting mitochondrial biomarkers and defining MD. **Overall, this evidence supports the notion that mitochondrial dysfunction is associated with ASD.** Additional studies are needed to further define the role of mitochondrial dysfunction in ASD.

148. Sensitization effect of thimerosal is mediated in vitro via reactive oxygen species and calcium signaling.

Toxicology. 2010 July - August;274(1-3):1-9. Epub 2010 May 10.

Migdal C, Foggia L, Tailhardat M, Courtellemont P, Haftek M, Serres M.

Thimerosal, a mercury derivative composed of ethyl mercury chloride (EtHgCl) and thiosalicylic acid (TSA), is widely used as a preservative in vaccines and cosmetic products and causes cutaneous reactions. Since dendritic cells (DCs) play an essential role in the immune response, the sensitization potency of chemicals was studied in vitro using U937, a human promyelomonocytic cell line that is used as a surrogate of monocytic differentiation and activation. Currently, this cell line is under ECVAM (European Center for the Validation of Alternative Methods) validation as an alternative method for discriminating chemicals. Thimerosal and mercury derivatives induced in U937 an overexpression of CD86 and interleukin (IL)-8 secretion similarly to 1-chloro-2,4-dinitrobenzene (DNCB), a sensitizer used as a positive control for DC activation. Non-sensitizers, dichloronitrobenzene (DCNB), TSA and sodium dodecyl sulfate (SDS), an irritant, had no effect. U937 activation was prevented by cell pretreatment with N-acetyl-L-cysteine (NAC) but not with thiol-independent antioxidants except vitamin E which affected CD86 expression by preventing lipid peroxidation of cell membranes. Thimerosal, EtHgCl and DNCB induced glutathione (GSH) depletion and reactive oxygen species (ROS) within 15min; another peak was detected after 2h for mercury compounds only. MitoSOX, a specific mitochondrial fluorescent probe, confirmed that ROS were essentially produced by mitochondria in correlation with its membrane depolarization. Changes in mitochondrial membrane permeability induced by mercury were reversed by NAC but not by thiol-independent antioxidants. Thimerosal and EtHgCl also induced a calcium (Ca<sup>2+</sup>) influx with a peak at 3h, suggesting that Ca<sup>2+</sup> influx is a secondary event following ROS induction as Ca<sup>2+</sup> influx was suppressed after pretreatment with NAC but not with thiol-independent antioxidants. Ca<sup>2+</sup> influx was also suppressed when culture medium was deprived of Ca<sup>2+</sup> confirming the specificity of the measure. **In conclusion, these data suggest that thimerosal induced U937 activation via oxidative stress from mitochondrial stores and mitochondrial membrane depolarization with a**

**primordial effect of thiol groups.** A cross-talk between ROS and Ca(2+) influx was demonstrated.

149. [What's going on? The question of time trends in autism.](#)

Public Health Rep. 2004 Nov-Dec;119(6):536-51.

Blaxill MF.

Abstract

Increases in the reported prevalence of autism and autistic spectrum disorders in recent years have fueled concern over possible environmental causes. The author reviews the available survey literature and finds evidence of large increases in prevalence in both the United States and the United Kingdom that cannot be explained by changes in diagnostic criteria or improvements in case ascertainment. Incomplete ascertainment of autism cases in young child populations is the largest source of predictable bias in prevalence surveys; however, this bias has, if anything, worked against the detection of an upward trend in recent surveys. Comparison of autism rates by year of birth for specific geographies provides the strongest basis for trend assessment. Such comparisons show large recent increases in rates of autism and autistic spectrum disorders in both the U.S. and the U.K. Reported rates of autism in the United States increased from < 3 per 10,000 children in the 1970s to > 30 per 10,000 children in the 1990s, a 10-fold increase. In the United Kingdom, autism rates rose from < 10 per 10,000 in the 1980s to roughly 30 per 10,000 in the 1990s. Reported rates for the full spectrum of autistic disorders rose from the 5 to 10 per 10,000 range to the 50 to 80 per 10,000 range in the two countries. A precautionary approach suggests that the rising incidence of autism should be a matter of urgent public concern.

150. [Retracted - Vaccines and Autism](#)

Laboratory medicine, September 2002, number 9, volume 33

Bernard Rimland, PhD, Woody McGinnis, MD

Autism Research Institute, San Diego, CA

Excerpt: "**Vaccinations may be one of the triggers for autism. Substantial data demonstrate immune abnormality in many autistic children consistent with impaired resistance to infection, activation of inflammatory response, and autoimmunity. Impaired resistance may predispose to vaccine injury in autism.**"



151. Theoretical aspects of autism: Causes—A review

Journal of Immunotoxicology, January-March 2011, Vol. 8, No. 1 , Pages 68-79

Helen V. Ratajczak, PhD

Autism, a member of the pervasive developmental disorders (PDDs), has been increasing dramatically since its description by Leo Kanner in 1943. First estimated to occur in 4 to 5 per 10,000 children, the incidence of autism is now 1 per 110 in the United States, and 1 per 64 in the United Kingdom, with similar incidences throughout the world. Searching information from 1943 to the present in PubMed and Ovid Medline databases, this review summarizes results that correlate the timing of changes in incidence with environmental changes. Autism could result from more than one cause, with different manifestations in different individuals that share common symptoms. Documented causes of autism include genetic mutations and/or deletions, viral infections, and encephalitis following vaccination. **Therefore, autism is the result of genetic defects and/or inflammation of the brain. The inflammation could be caused by a defective placenta, immature blood-brain barrier, the immune response of the mother to infection while pregnant, a premature birth, encephalitis in the child after birth, or a toxic environment.**

152. [Preterm birth, vaccination and neurodevelopmental disorders: a cross-sectional study of 6- to 12-year-old vaccinated and unvaccinated children](#)

Anthony R Mawson, Azad R Bhuiyan, Brian D Ray, Binu Jacob  
Department of Epidemiology and Biostatistics, School of Public Health, Jackson State University, Jackson, MS 39213, USA  
National Home Education Research Institute, PO Box 13939, Salem, OR 97309, USA

J Transl Sci 3: DOI: 10.15761/JTS.1000187, April 24, 2017

Abstract

From about 8% to 27% of extremely preterm infants develop symptoms of autism spectrum disorder, but the causes are not well understood. Preterm infants receive the same doses of the recommended vaccines and on the same schedule as term infants. The possible role of vaccination in neurodevelopmental disorders (NDD) among premature infants is unknown, in part because pre-licensure clinical trials of pediatric vaccines have excluded ex-preterm infants. This paper explores the association between preterm birth, vaccination and NDD, based on a secondary analysis of data from an anonymous survey of mothers, comparing the birth history and health outcomes of vaccinated and unvaccinated homeschool children 6 to 12 years of age. A convenience sample of 666 children was obtained, of which 261 (39%) were unvaccinated, 7.5% had an NDD (defined as a learning disability, Attention Deficit Hyperactivity Disorder and/or Autism Spectrum Disorder), and 7.7% were born preterm. **No association was**

**found between preterm birth and NDD in the absence of vaccination, but vaccination was significantly associated with NDD in children born at term (OR 2.7, 95% CI: 1.2, 6.0). However, vaccination coupled with preterm birth was associated with increasing odds of NDD, ranging from 5.4 (95% CI: 2.5, 11.9) compared to vaccinated but non-preterm children, to 14.5 (95% CI: 5.4, 38.7) compared to children who were neither preterm nor vaccinated.** The results of this pilot study suggest clues to the epidemiology and causation of NDD but question the safety of current vaccination practices for preterm infants. Further research is needed to validate and investigate these associations in order to optimize the impact of vaccines on children's health.

153. [iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants](#)

The Journal of Pediatrics, Volume 136, Issue 5, May 2000, Pages 679–681

Gregory V. Stajich, PharmD, Gaylord P. Lopez, PharmD, ABAT, Sokei W. Harry, MBBS, MPH, William R. Sexson, MD  
Mercer University, Southern School of Pharmacy, Atlanta, Georgia; Georgia Poison Center, Grady Health System, Atlanta; Georgia Poison Center, Georgia Health System, Atlanta and Emory University, School of Medicine, Atlanta, Georgia.

Thimerosal, a derivative of mercury, is used as a preservative in hepatitis B vaccines. We measured total mercury levels before and after the administration of this vaccine in 15 preterm and 5 term infants. Comparison of pre- and post-vaccination mercury levels showed a significant increase in both preterm and term infants after vaccination. **Additionally, post-vaccination mercury levels were significantly higher in preterm infants as compared with term infants.** Because mercury is known to be a potential neurotoxin to infants, further study of its pharmacodynamics is warranted.

154. [Hypothesis: conjugate vaccines may predispose children to autism spectrum disorders](#)

Med Hypotheses. 2011 Dec;77(6):940-7. doi: 10.1016/j.mehy.2011.08.019. Epub 2011 Oct 10.

Brian J Richmand

PMID: 21993250 DOI: 10.1016/j.mehy.2011.08.019

Abstract

The first conjugate vaccine was approved for use in the US in 1988 to protect infants and young children against the capsular bacteria *Haemophilus influenzae* type b (Hib). Since its introduction in the US, this vaccine has been approved in most developed countries, including Denmark and Israel where the vaccine was added to their national vaccine programs in 1993 and 1994, respectively. There have been marked increases in the reported prevalence of autism spectrum disorders (ASDs) among children in the US beginning with birth cohorts in the late 1980s and in Denmark and Israel starting approximately 4-5 years later. Although these increases may partly reflect ascertainment biases, an exogenous trigger could explain a significant portion of the reported increases in ASDs. It is hypothesized here that the introduction of the Hib conjugate vaccine in the US in 1988 and its subsequent introduction in Denmark and Israel could explain a substantial portion of the initial increases in ASDs in those countries. The continuation of the trend toward increased rates of ASDs could be further explained by increased usage of the vaccine, a change in 1990 in the recommended age of vaccination in the US from 15 to 2 months, increased immunogenicity of the vaccine through changes in its carrier protein, and the subsequent introduction of the conjugate vaccine for *Streptococcus pneumoniae*. Although conjugate vaccines have been highly effective in protecting infants and young children from the significant morbidity and mortality caused by Hib and *S. pneumoniae*, the potential effects of conjugate vaccines on neural development merit close examination. **Conjugate vaccines fundamentally change the manner in which the immune systems of infants and young children function by deviating their immune responses to the targeted carbohydrate antigens from a state of hypo-responsiveness to a robust B2 B cell mediated response. This period of hypo-responsiveness to carbohydrate antigens coincides with the intense myelination process in infants and young children, and conjugate vaccines may have disrupted evolutionary forces that favored early brain development over the need to protect infants and young children from capsular bacteria.**

155. [Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age.](#)

J Pediatr. 2015 Feb;166(2):269-75.e3. doi: 10.1016/j.jpeds.2014.10.053. Epub 2014 Dec 2.

Guy A1, Seaton SE2, Boyle EM2, Draper ES2, Field DJ2, Manktelow BN2, Marlow N3, Smith LK2, Johnson S4.

1Department of Health Sciences, University of Leicester, Leicester, United Kingdom; School of Psychology, University of Warwick, Coventry, United Kingdom.

2Department of Health Sciences, University of Leicester, Leicester, United Kingdom.

3Department of Academic Neonatology, Institute for Women's Health, University College London, London, United Kingdom.

4Department of Health Sciences, University of Leicester, Leicester, United Kingdom. Electronic address: [sjj19@le.ac.uk](mailto:sjj19@le.ac.uk).

#### Abstract

##### OBJECTIVES:

To assess the prevalence of positive screens using the Modified Checklist for Autism in Toddlers (M-CHAT) questionnaire and follow-up interview in late and moderately preterm (LMPT; 32-36 weeks) infants and term-born controls.

##### STUDY DESIGN:

Population-based prospective cohort study of 1130 LMPT and 1255 term-born infants. Parents completed the M-CHAT questionnaire at 2-years corrected age. Parents of infants with positive questionnaire screens were followed up with a telephone interview to clarify failed items. The M-CHAT questionnaire was re-scored, and infants were classified as true or false positives. Neurosensory, cognitive, and behavioral outcomes were assessed using parent report.

##### RESULTS:

Parents of 634 (57%) LMPT and 761 (62%) term-born infants completed the M-CHAT questionnaire. LMPT infants had significantly higher risk of a positive questionnaire screen compared with controls (14.5% vs 9.2%; relative risk [RR] 1.58; 95% CI 1.18, 2.11). After follow-up, significantly more LMPT infants than controls had a true positive screen (2.4% vs 0.5%; RR 4.52; 1.51, 13.56). This remained significant after excluding infants with neurosensory impairments (2.0% vs 0.5%; RR 3.67; 1.19, 11.3).

##### CONCLUSIONS:

**LMPT infants are at significantly increased risk for positive autistic screen.**

An M-CHAT follow-up interview is essential as screening for autism spectrum disorders is especially confounded in preterm populations. Infants with false positive screens are at risk for cognitive and behavioral problems.

156. [Preterm birth and mortality and morbidity: a population-based quasi-experimental study.](#)

JAMA Psychiatry. 2013 Nov;70(11):1231-40. doi: 10.1001/jamapsychiatry.2013.2107.

D'Onofrio BM1, Class QA, Rickert ME, Larsson H, Långström N, Lichtenstein P.

Department of Psychological and Brain Sciences, Indiana University-Bloomington.

#### Abstract

##### IMPORTANCE:

Preterm birth is associated with increased mortality and morbidity. However, previous studies have been unable to rigorously examine whether confounding factors cause these associations rather than the harmful effects of being born preterm.

##### OBJECTIVE:

To estimate the extent to which the associations between early gestational age and offspring mortality and morbidity are the result of confounding factors by using a quasi-experimental design, the sibling-comparison approach, and by controlling for statistical covariates that varied within families.

**DESIGN, SETTING, AND PARTICIPANTS:**

A population-based cohort study, combining Swedish registries to identify all individuals born in Sweden from 1973 to 2008 (3,300,708 offspring of 1,736,735 mothers) and link them with multiple outcomes.

**MAIN OUTCOMES AND MEASURES:**

Offspring mortality (during infancy and throughout young adulthood) and psychiatric (psychotic or bipolar disorder, autism, attention-deficit/hyperactivity disorder, suicide attempts, substance use, and criminality), academic (failing grades and educational attainment), and social (partnering, parenthood, low income, and social welfare benefits) outcomes through 2009.

**RESULTS:**

In the population, there was a dose-response relationship between early gestation and the outcome measures. **For example, extreme preterm birth (23-27 weeks of gestation) was associated with infant mortality (odds ratio, 288.1; 95% CI, 271.7-305.5), autism (hazard ratio [HR], 3.2; 95% CI, 2.6-4.0),** low educational attainment (HR, 1.7; 1.5-2.0), and social welfare benefits (HR, 1.3; 1.2-1.5) compared with offspring born at term. The associations between early gestation and mortality and psychiatric morbidity generally were robust when comparing differentially exposed siblings and controlling for statistical covariates, whereas the associations with academic and some social problems were greatly or completely attenuated in the fixed-effects models.

**CONCLUSIONS AND RELEVANCE:**

The mechanisms responsible for the associations between preterm birth and mortality and morbidity are outcome-specific. Associations between preterm birth and mortality and psychiatric morbidity are largely independent of shared familial confounds and measured covariates, consistent with a causal inference. However, some associations, particularly predicting suicide attempt, educational attainment, and social welfare benefits, are the result of confounding factors. The findings emphasize the importance of both reducing preterm birth and providing wraparound services to all siblings in families with an offspring born preterm.

157. [Reviewing the association between aluminum adjuvants in the vaccines and autism spectrum disorder](#)

J Trace Elem Med Biol. 2021 Jul;66:126764.  
doi: 10.1016/j.jtemb.2021.126764. Epub 2021 Apr 27.

Alberto Boretti 1

Affiliations

Prince Mohammad Bin Fahd University, P.O. Box 1664, Al Khobar, 31952, Saudi Arabia.

#### Abstract

The manuscript reviews the association between aluminum adjuvants (AIAd) in vaccines and autism spectrum disorder (ASD). Aluminum (Al) is neurotoxic. Infants who have received AIAd in vaccines show a higher rate of ASD. The behavior of mice changes with Al injection. Patients suffering from ASD have higher concentrations of Al in their brains. Thus, AIAd is an etiologic factor in ASD. Immune efficacy led to the use of the AIAd in vaccines; however, the safety of those who are vaccinated with such vaccines has not been considered. The mechanisms of action of AIAd and the pharmacodynamics of injected AIAd used in vaccines are not well-characterized. **The association between aluminum adjuvants in the vaccines and autism spectrum disorder is suggested by multiple lines of evidence.**

158. Ancestry of pink disease (infantile acrodynia) identified as a risk factor for autism spectrum disorders.

J Toxicol Environ Health A. 2011 Sep 15;74(18):1185-94.  
Shandley K, Austin DW.

Swinburne Autism Bio-Research Initiative (SABRI), Brain and Psychological Sciences Research Centre , Swinburne University of Technology , Hawthorn , Victoria , Australia.

#### Abstract

Pink disease (infantile acrodynia) was especially prevalent in the first half of the 20th century. Primarily attributed to exposure to mercury (Hg) commonly found in teething powders, the condition was developed by approximately 1 in 500 exposed children. The differential risk factor was identified as an idiosyncratic sensitivity to Hg. Autismspectrum disorders (ASD) have also been postulated to be produced by Hg. Analogous to the pink disease experience, Hg exposure is widespread yet only a fraction of exposed children develop an ASD, suggesting sensitivity to Hg may also be present in children with an ASD. The objective of this study was to test the hypothesis that individuals with a known hypersensitivity to Hg (pink disease survivors) may be more likely to have descendants with an ASD. Five hundred and twenty-two participants who had previously been diagnosed with pink disease completed a survey on the health outcomes of their descendants. The prevalence rates of ASD and a variety of other clinical conditions diagnosed in childhood (attention deficit hyperactivity disorder, epilepsy, Fragile X syndrome, and Down syndrome) were compared to well-established general population prevalence rates. The results showed the prevalence rate of ASD among the grandchildren of pink disease survivors (1 in

22) to be significantly higher than the comparable general population prevalence rate (1 in 160). **The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD.**

159. Risk Factors for Autistic Regression: Results of an Ambispective Cohort Study.

J Child Neurol. 2012 Jan 30. [Epub ahead of print]

Zhang Y, Xu Q, Liu J, Li SC, Xu X., Department of Child Health Care, Children's Hospital of Fudan University, Shanghai, China.

Abstract

A subgroup of children diagnosed with autism experience developmental regression featured by a loss of previously acquired abilities. The pathogeny of autistic regression is unknown, although many risk factors likely exist. To better characterize autistic regression and investigate the association between autistic regression and potential influencing factors in Chinese autistic children, we conducted an ambispective study with a cohort of 170 autistic subjects.

**Analyses by multiple logistic regression showed significant correlations between autistic regression and febrile seizures (OR = 3.53, 95% CI = 1.17-10.65, P = .025), as well as with a family history of neuropsychiatric disorders (OR = 3.62, 95% CI = 1.35-9.71, P = .011). This study suggests that febrile seizures and family history of neuropsychiatric disorders are correlated with autistic regression.**

160. [Early Seizures Prematurely Unsilence Auditory Synapses to Disrupt Thalamocortical Critical Period Plasticity.](#)

Cell Rep. 2018 May 29;23(9):2533-2540. doi: 10.1016/j.celrep.2018.04.108.

Sun H, Takesian AE, Wang TT, Lippman-Bell JJ, Hensch TK, Jensen FE.

Department of Neurology, Perelman School of Medicine, University of Pennsylvania

F.M. Kirby Neurobiology Center, Department of Neurology, Boston Children's Hospital, Harvard Medical School

Department of Neuroscience, Carleton University

Abstract

**Heightened neural excitability in infancy and childhood results in increased susceptibility to seizures. Such early-life seizures are associated with language deficits and autism that can result from aberrant development of**

**the auditory cortex.** Here, we show that early-life seizures disrupt a critical period (CP) for tonotopic map plasticity in primary auditory cortex (A1). We show that this CP is characterized by a prevalence of "silent," NMDA-receptor (NMDAR)-only, glutamate receptor synapses in auditory cortex that become "unsilenced" due to activity-dependent AMPA receptor (AMPA) insertion. Induction of seizures prior to this CP occludes tonotopic map plasticity by prematurely unsilencing NMDAR-only synapses. Further, brief treatment with the AMPAR antagonist NBQX following seizures, prior to the CP, prevents synapse unsilencing and permits subsequent A1 plasticity. These findings reveal that early-life seizures modify CP regulators and suggest that therapeutic targets for early post-seizure treatment can rescue CP plasticity.

161. [MMR vaccination and febrile seizures: evaluation of susceptible subgroups and long-term prognosis.](#)

Vestergaard M1, Hviid A, Madsen KM, Wohlfahrt J, Thorsen P, Schendel D, Melbye M, Olsen J.

JAMA. 2004 Jul 21;292(3):351-7.

Abstract

CONTEXT:

**The rate of febrile seizures increases following measles, mumps, and rubella (MMR) vaccination** but it is unknown whether the rate varies according to personal or family history of seizures, perinatal factors, or socioeconomic status. Furthermore, little is known about the long-term outcome of febrile seizures following vaccination.

OBJECTIVES:

To estimate incidence rate ratios (RRs) and risk differences of febrile seizures following MMR vaccination within subgroups of children and to evaluate the clinical outcome of febrile seizures following vaccination.

DESIGN, SETTING, AND PARTICIPANTS:

A population-based cohort study of all children born in Denmark between January 1, 1991, and December 31, 1998, who were alive at 3 months; 537,171 children were followed up until December 31, 1999, by using data from the Danish Civil Registration System and 4 other national registries.

MAIN OUTCOME MEASURES:

Incidence of first febrile seizure, recurrent febrile seizures, and subsequent epilepsy.

RESULTS:

A total of 439,251 children (82%) received MMR vaccination and 17,986 children developed febrile seizures at least once; 973 of these febrile seizures occurred within 2 weeks of MMR vaccination. The RR of febrile seizures increased during the 2 weeks following MMR vaccination (2.75; 95% confidence interval [CI], 2.55-2.97), and thereafter was close to the observed RR for nonvaccinated children. The RR did not vary significantly in the subgroups of children that had been



defined by their family history of seizures, perinatal factors, or socioeconomic status. At 15 to 17 months, the risk difference of febrile seizures within 2 weeks following MMR vaccination was 1.56 per 1000 children overall (95% CI, 1.44-1.68), 3.97 per 1000 (95% CI, 2.90-5.40) for siblings of children with a history of febrile seizures, and 19.47 per 1000 (95% CI, 16.05-23.55) for children with a personal history of febrile seizures. Children with febrile seizures following MMR vaccinations had a slightly increased rate of recurrent febrile seizures (RR, 1.19; 95% CI, 1.01-1.41) but no increased rate of epilepsy (RR, 0.70; 95% CI, 0.33-1.50) compared with children who were nonvaccinated at the time of their first febrile seizure.

CONCLUSIONS:

**MMR vaccination was associated with a transient increased rate of febrile seizures** but the risk difference was small even in high-risk children. The long-term rate of epilepsy was not increased in children who had febrile seizures following vaccination compared with children who had febrile seizures of a different etiology.

162. [Common variants associated with general and MMR vaccine-related febrile seizures](#)

Bjarke Feenstra, Björn Pasternak, Frank Geller, Lisbeth Carstensen, Tongfei Wang, Fen Huang, Jennifer L Eitson, Mads V Hollegaard, Henrik Svanström, Mogens Vestergaard, David M Hougaard, John W Schoggins, Lily Yeh Jan, Mads Melbye & Anders Hviid

Nature Genetics (2014) doi:10.1038/ng.3129

Received 20 May 2014 Accepted 03 October 2014 Published online 26 October 2014

Abstract

**Febrile seizures represent a serious adverse event following measles, mumps and rubella (MMR) vaccination.** We conducted a series of genome-wide association scans comparing children with MMR-related febrile seizures, children with febrile seizures unrelated to vaccination and controls with no history of febrile seizures. Two loci were distinctly associated with MMR-related febrile seizures, harboring the interferon-stimulated gene IFI44L (rs273259:  $P = 5.9 \times 10^{-12}$  versus controls,  $P = 1.2 \times 10^{-9}$  versus MMR-unrelated febrile seizures) and the measles virus receptor CD46 (rs1318653:  $P = 9.6 \times 10^{-11}$  versus controls,  $P = 1.6 \times 10^{-9}$  versus MMR-unrelated febrile seizures). Furthermore, four loci were associated with febrile seizures in general, implicating the sodium channel genes SCN1A (rs6432860:  $P = 2.2 \times 10^{-16}$ ) and SCN2A (rs3769955:  $P = 3.1 \times 10^{-10}$ ), a TMEM16 family gene (ANO3; rs114444506:  $P = 3.7 \times 10^{-20}$ ) and a region associated with magnesium levels (12q21.33; rs11105468:  $P = 3.4 \times 10^{-11}$ ). Finally, we show the functional relevance of ANO3 (TMEM16C) with electrophysiological experiments in wild-type and knockout rats.

163. [Adverse events following 12 and 18 month vaccinations: a population-based, self-controlled case series analysis.](#)

PLoS One. 2011;6(12):e27897. Epub 2011 Dec 12.

Wilson K, Hawken S, Kwong JC, Deeks S, Crowcroft NS, Van Walraven C, Potter BK, Chakraborty P, Keelan J, Pluscauskas M, Manuel D. Department of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Canada. kwilson@ohri.ca

#### Abstract

##### BACKGROUND:

Live vaccines have distinct safety profiles, potentially causing systemic reactions one to 2 weeks after administration. In the province of Ontario, Canada, live MMR vaccine is currently recommended at age 12 months and 18 months.

##### METHODS:

Using the self-controlled case series design we examined 271,495 12 month vaccinations and 184,312 18 month vaccinations to examine the relative incidence of the composite endpoint of emergency room visits or hospital admissions in consecutive one day intervals following vaccination. These were compared to a control period 20 to 28 days later. In a post-hoc analysis we examined the reasons for emergency room visits and the average acuity score at presentation for children during the at-risk period following the 12 month vaccine.

##### RESULTS:

Four to 12 days post 12 month vaccination, children had a 1.33 (1.29-1.38) increased relative incidence of the combined endpoint compared to the control period, or at least one event during the risk interval for every 168 children vaccinated. Ten to 12 days post 18 month vaccination, the relative incidence was 1.25 (95%, 1.17-1.33) which represented at least one excess event for every 730 children vaccinated. The primary reason for increased events was statistically significant elevations in emergency room visits following all vaccinations. There were non-significant increases in hospital admissions. **There were an additional 20 febrile seizures for every 100,000 vaccinated at 12 months.**

##### CONCLUSIONS:

There are significantly elevated risks of primarily emergency room visits approximately one to two weeks following 12 and 18 month vaccination. Future studies should examine whether these events could be predicted or prevented.

164. [Measles-Mumps-Rubella-Varicella Combination Vaccine and the Risk of Febrile Seizures](#)

Pediatrics (2010) 126 (1): e1–e8. <https://doi.org/10.1542/peds.2010-0665>

Nicola P. Klein, MD, PhD; Bruce Fireman, MS; W. Katherine Yih, MPH, PhD; Edwin Lewis, MPH; Martin Kulldorff, PhD; Paula Ray, MPH; Roger Baxter, MD; Simon Hambidge, MD, PhD; James Nordin, MD, MPH; Allison Naleway, PhD; Edward A. Belongia, MD; Tracy Lieu, MD, MPH; James Baggs, PhD; Eric Weintraub, MPH; for the Vaccine Safety Datalink

Kaiser Permanente Vaccine Study Center, 1 Kaiser Plaza, 16th Floor, Oakland, CA 94612.

## Abstract

### OBJECTIVE:

In February 2008, we alerted the Advisory Committee on Immunization Practices to preliminary evidence of a twofold increased risk of febrile seizures after the combination measles-mumps-rubella-varicella (MMRV) vaccine when compared with separate measles-mumps-rubella (MMR) and varicella vaccines. Now with data on twice as many vaccine recipients, our goal was to reexamine seizure risk after MMRV vaccine.

### METHODS:

Using 2000–2008 Vaccine Safety Datalink data, we assessed seizures and fever visits among children aged 12 to 23 months after MMRV and separate MMR + varicella vaccines. We compared seizure risk after MMRV vaccine to that after MMR + varicella vaccines by using Poisson regression as well as with supplementary regressions that incorporated chart-review results and self-controlled analyses.

### RESULTS:

MMRV vaccine recipients (83 107) were compared with recipients of MMR + varicella vaccines (376 354). Seizure and fever significantly clustered 7 to 10 days after vaccination with all measles-containing vaccines but not after varicella vaccination alone. Seizure risk during days 7 to 10 was higher after MMRV than after MMR + varicella vaccination (relative risk: 1.98 [95% confidence interval: 1.43–2.73]). Supplementary analyses yielded similar results. The excess risk for febrile seizures 7 to 10 days after MMRV compared with separate MMR + varicella vaccination was 4.3 per 10 000 doses (95% confidence interval: 2.6–5.6).

### CONCLUSIONS:

**Among 12- to 23-month-olds who received their first dose of measles-containing vaccine, fever and seizure were elevated 7 to 10 days after vaccination. Vaccination with MMRV results in 1 additional febrile seizure for every 2300 doses given instead of separate MMR + varicella vaccines. Providers who recommend MMRV should communicate to parents that it increases the risk of fever and seizure over that already associated with measles-containing vaccines.**

165. [Reduced GABAergic Action in the Autistic Brain.](#)

Curr Biol. 2015 Dec 16. pii: S0960-9822(15)01413-X. doi: 10.1016/j.cub.2015.11.019.

Robertson CE1, Ratai EM2, Kanwisher N3.

1Harvard Society of Fellows, Harvard University, Cambridge, MA 02138, USA; McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02138, USA. Electronic address: carolinerobertson@fas.harvard.edu.

2Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.

3McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02138, USA.

Abstract

An imbalance between excitatory/inhibitory neurotransmission has been posited as a central characteristic of the neurobiology of autism [1], inspired in part by the striking prevalence of seizures among individuals with the disorder [2]. Evidence supporting this hypothesis has specifically implicated the signaling pathway of the inhibitory neurotransmitter,  $\gamma$ -aminobutyric acid (GABA), in this putative imbalance: GABA receptor genes have been associated with autism in linkage and copy number variation studies [3-7], fewer GABA receptor subunits have been observed in the post-mortem tissue of autistic individuals [8, 9], and GABAergic signaling is disrupted across heterogeneous mouse models of autism [10]. Yet, empirical evidence supporting this hypothesis in humans is lacking, leaving a gulf between animal and human studies of the condition. Here, we present a direct link between GABA signaling and autistic perceptual symptomatology. We first demonstrate a robust, replicated autistic deficit in binocular rivalry [11], a basic visual function that is thought to rely on the balance of excitation/inhibition in visual cortex [12-15]. **Then, using magnetic resonance spectroscopy, we demonstrate a tight linkage between binocular rivalry dynamics in typical participants and both GABA and glutamate levels in the visual cortex. Finally, we show that the link between GABA and binocular rivalry dynamics is completely and specifically absent in autism.** These results suggest a disruption in inhibitory signaling in the autistic brain and forge a translational path between animal and human models of the condition.

166. [Administration of thimerosal to infant rats increases overflow of glutamate and aspartate in the prefrontal cortex: protective role of dehydroepiandrosterone sulfate.](#)

Neurochem Res. 2012 Feb;37(2):436-47. Epub 2011 Oct 21.

Duszczyk-Budhathoki M, Olczak M, Lehner M, Majewska MD. Marie Curie

Chairs Program, Department of Pharmacology and Physiology of Nervous System, Institute of Psychiatry and Neurology, 02-957, Warsaw, Poland.

#### Abstract

Thimerosal, a mercury-containing vaccine preservative, is a suspected factor in the etiology of neurodevelopmental disorders. We previously showed that its administration to infant rats causes behavioral, neurochemical and neuropathological abnormalities similar to those present in autism. Here we examined, using microdialysis, the effect of thimerosal on extracellular levels of neuroactive amino acids in the rat prefrontal cortex (PFC). Thimerosal administration (4 injections, i.m., 240 µg Hg/kg on postnatal days 7, 9, 11, 15) induced lasting changes in amino acid overflow: an increase of glutamate and aspartate accompanied by a decrease of glycine and alanine; measured 10-14 weeks after the injections. Four injections of thimerosal at a dose of 12.5 µg Hg/kg did not alter glutamate and aspartate concentrations at microdialysis time (but based on thimerosal pharmacokinetics, could have been effective soon after its injection). Application of thimerosal to the PFC in perfusion fluid evoked a rapid increase of glutamate overflow. Coadministration of the neurosteroid, dehydroepiandrosterone sulfate (DHEAS; 80 mg/kg; i.p.) prevented the thimerosal effect on glutamate and aspartate; the steroid alone had no influence on these amino acids. Coapplication of DHEAS with thimerosal in perfusion fluid also blocked the acute action of thimerosal on glutamate. In contrast, DHEAS alone reduced overflow of glycine and alanine, somewhat potentiating the thimerosal effect on these amino acids. **Since excessive accumulation of extracellular glutamate is linked with excitotoxicity, our data imply that neonatal exposure to thimerosal-containing vaccines might induce excitotoxic brain injuries, leading to neurodevelopmental disorders.** DHEAS may partially protect against mercurials-induced neurotoxicity.

#### 167. Neonatal Administration of Thimerosal Causes Persistent Changes in Mu Opioid Receptors in the Rat Brain

Neurochem Res. 2010 November; 35(11): 1840–1847.

Mieszko Olczak, Michalina Duszczyk, Pawel Mierzejewski, Teresa Bobrowicz, and Maria Dorota Majewska<sup>1</sup>, Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Sobieskiego 9 str., 02-957 Warsaw, Poland, Department of Forensic Medicine, Medical University of Warsaw, Oczki 1 str., 02-007 Warsaw, Poland, Department of Neuropathology, Institute of Psychiatry and Neurology, 02-957 Warsaw, Poland, Department of Biology and Environmental Science, University of Cardinal Stefan Wyszyński, Wóycickiego Str. 1/3, 01-815 Warsaw, Poland

#### Abstract

Thimerosal added to some pediatric vaccines is suspected in pathogenesis of several neurodevelopmental disorders. Our previous study showed that thimerosal administered to suckling rats causes persistent, endogenous opioid-mediated hypoalgesia. Here we examined, using immunohistochemical staining

technique, the density of  $\mu$ -opioid receptors (MORs) in the brains of rats, which in the second postnatal week received four i.m. injections of thimerosal at doses 12, 240, 1,440 or 3,000  $\mu\text{g Hg/kg}$ . The periaqueductal gray, caudate putamen and hippocampus were examined. Thimerosal administration caused dose-dependent statistically significant increase in MOR densities in the periaqueductal gray and caudate putamen, but decrease in the dentate gyrus, where it was accompanied by the presence of degenerating neurons and loss of synaptic vesicle marker (synaptophysin). **These data document that exposure to thimerosal during early postnatal life produces lasting alterations in the densities of brain opioid receptors along with other neuropathological changes, which may disturb brain development.**

168. [Adverse Effects of Pertussis and Rubella Vaccines: A Report of the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines](#)

National Academies of Sciences, Engineering, and Medicine. 1991. Washington, DC: The National Academies Press. <https://doi.org/10.17226/1815>.

A Report of the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines

Christopher P. Howson, Cynthia J. Howe,  
and Harvey V. Fineberg, Editors

Division of Health Promotion and Disease Prevention

Institute of Medicine

NATIONAL ACADEMY PRESS

Washington, D.C. 1991

National Academies of Sciences, Engineering, and Medicine. 1991. Adverse Effects of Pertussis and Rubella Vaccines. Washington, DC: The National Academies Press. <https://doi.org/10.17226/1815>.

Preface

Although vaccines have markedly reduced the toll of many childhood illnesses, the practice of vaccination is not always without risk. Minor side effects are common, and serious side effects, although less numerous, have been observed on rare occasions with certain vaccines. Whether there are increased risks of serious adverse events following whole-cell pertussis and rubella vaccines, however, is controversial. The fact that pertussis and rubella vaccination is mandatory in many states has heightened public awareness of controversy and concern about the safety of the two vaccines...

**In November 1989, IOM established the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines. The specific charge**

to the committee, as outlined in Section 312 of the National Childhood Vaccine Injury Act, was to: identify and review all available medical and scientific literature on the nature, circumstance, and extent of the relationship, if any, between vaccines containing pertussis (including whole cells, extracts, and specific antigens) and the following illnesses and conditions: hemolytic anemia, hypersarrhythmia, infantile spasms, Reye syndrome, peripheral mononeuropathy, deaths classified as sudden infant death syndrome (SIDS), aseptic meningitis, juvenile diabetes, autism, learning disabilities, hyperactivity, and other such illnesses as recommended by the committee or the Advisory Commission on Childhood Vaccines, and inquire into the possible association between pertussis vaccines and permanent neurologic damage...”

#### Summary

**No data** were identified that address the question of a relation between vaccination with DPT or its pertussis component and autism. There are **no experimental data** bearing on a possible biologic mechanism.

#### Conclusion

There is **no evidence** to indicate a causal relation between DPT vaccine or the pertussis component of DPT vaccine and autism.

#### 169. Unanswered Questions: A Review of Compensated Cases of Vaccine-Induced Brain Injury

Pace Environmental Law Review, vol. 28, no. 2, 2011

Mary Holland, Louis Conte, Robert Krakow and Lisa Colin

#### Executive Summary

In 1986, Congress created the Vaccine Injury Compensation Program (VICP) under the National Childhood Vaccine Injury Act (1986 Law). This Program has original jurisdiction for children’s claims of vaccine injury. Because almost all children receive multiple vaccinations for daycare and school, it is critically important that the Program provides fundamental fairness, due process and transparency.

**This empirical investigation, published in a peer-reviewed law journal, examines claims that the VICP compensated for vaccine-induced encephalopathy and seizure disorder. The VICP has compensated approximately 2,500 claims of vaccine injury since the inception of the program. This study found 83 cases of acknowledged vaccine-induced brain damage that include autism, a disorder that affects speech, social communication and behavior. In 21 published cases of the Court of Federal Claims, which administers the VICP, the Court stated that the petitioners had**

autism or described autism unambiguously. In 62 remaining cases, the authors identified settlement agreements where Health and Human Services (HHS) compensated children with vaccine-induced brain damage, who also have autism or an autism spectrum disorder.

Parents reported the existence of autism in telephone interviews and supplied supplemental materials including medical diagnoses, school records, and completed, standard autism screening questionnaires to verify their reports. In 39 of the 83 cases, or 47% of the cases of vaccine injury reviewed, there is confirmation of autism or autism spectrum disorder beyond parental report.

**This finding of autism in compensated cases of vaccine injury is significant. U.S. government spokespeople have been asserting no vaccine-autism link for more than a decade. This finding calls into question the decisions of the Court of Federal Claims in the Omnibus Autism Proceeding in 2009 and 2010 and the statement of Health and Human Services on its website that “HHS has never concluded in any case that autism was caused by vaccination.”**

Using publicly available information, the investigation shows that the VICP has been compensating cases of vaccine-induced brain damage associated with autism for more than twenty years. This investigation suggests that officials at HHS, the Department of Justice and the Court of Federal Claims may have been aware of this association but failed to publicly disclose it.

The study calls on Congress to thoroughly investigate the VICP, including a medical investigation of compensated claims of vaccine injury. This investigation calls on Congress to get answers to these critically important unanswered questions.

170. [Integrating experimental \(in vitro and in vivo\) neurotoxicity studies of low-dose thimerosal relevant to vaccines.](#)

Neurochem Res. 2011 Jun;36(6):927-38. doi: 10.1007/s11064-011-0427-0. Epub 2011 Feb 25.

Dórea JG, Faculty of Health Sciences, Universidade de Brasília, CP 04322, 70919-970, Brasília, DF, Brazil. dorea@rudah.com.br

### **Abstract**

There is a need to interpret neurotoxic studies to help deal with uncertainties surrounding pregnant mothers, newborns and young children who must receive repeated doses of Thimerosal-containing vaccines (TCVs). This review integrates information derived from emerging experimental studies (in vitro and in vivo) of low-dose Thimerosal (sodium ethyl mercury thiosalicylate). Major databases (PubMed and Web-of-science) were searched for in vitro and in vivo experimental studies that addressed the effects of low-dose Thimerosal (or ethylmercury) on neural tissues and animal behaviour. Information extracted from



studies indicates that: (a) activity of low doses of Thimerosal against isolated human and animal brain cells was found in all studies and is consistent with Hg neurotoxicity; (b) the neurotoxic effect of ethylmercury has not been studied with co-occurring adjuvant-AI in TCVs; (c) animal studies have shown that exposure to Thimerosal-Hg can lead to accumulation of inorganic Hg in brain, and that (d) doses relevant to TCV exposure possess the potential to affect human neuro-development. Thimerosal at concentrations relevant for infants' exposure (in vaccines) is toxic to cultured human-brain cells and to laboratory animals. The persisting use of TCV (in developing countries) is counterintuitive to global efforts to lower Hg exposure and to ban Hg in medical products; its continued use in TCV requires evaluation of a sufficiently nontoxic level of ethylmercury compatible with repeated exposure (co-occurring with adjuvant-AI) during early life.

171. [\[11C\]PBR28 MR–PET imaging reveals lower regional brain expression of translocator protein \(TSPO\) in young adult males with autism spectrum disorder](#)

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N. R. Zürcher 1,2 • M. L. Loggia 1,2 • J. E. Mullett 3 • C. Tseng 1,2 • A. Bhanot 1 • L. Richey 1 • B. G. Hightower 1 • C. Wu 1 •  
A. J. Parmar 1 • R. I. Butterfield 1 • J. M. Dubois 1,2 • D. B. Chonde 1,2 • D. Izquierdo-Garcia 1,2 • H. Y. Wey 1,2 • C. Catana 1,2 •  
N. Hadjikhani 1,2,4 • C. J. McDougle 2,3 • J. M. Hooker 1,2

N. R. Zürcher, [zurcher@nmr.mgh.harvard.edu](mailto:zurcher@nmr.mgh.harvard.edu)

J. M. Hooker [jhooker@mgh.harvard.edu](mailto:jhooker@mgh.harvard.edu)

1Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, USA

2Harvard Medical School, Boston, MA, USA

3Lurie Center for Autism, Massachusetts General Hospital, Lexington, MA, USA

4Gillberg Neuropsychiatry Center, University of Gothenburg, Sahlgrenska Academy, Gothenburg, Sweden

#### Abstract

**Mechanisms of neuroimmune and mitochondrial dysfunction have been repeatedly implicated in autism spectrum disorder (ASD).** To examine these mechanisms in ASD individuals, we measured the in vivo expression of the 18 kDa translocator protein (TSPO), an activated glial marker expressed on mitochondrial membranes. Participants underwent scanning on a simultaneous magnetic resonance–positron emission tomography (MR–PET) scanner with the second-generation TSPO radiotracer [11C]PBR28. By comparing TSPO in 15 young adult males with ASD with 18 age- and sex-matched controls, we showed that individuals with ASD exhibited lower regional TSPO expression in several brain regions, including the bilateral insular cortex, bilateral precuneus/posterior cingulate cortex, and bilateral temporal, angular, and supramarginal gyri, which

have previously been implicated in autism in functional MR imaging studies. No brain region exhibited higher regional TSPO expression in the ASD group compared with the control group. A subset of participants underwent a second MR–PET scan after a median interscan interval of 3.6 months, and we determined that TSPO expression over this period of time was stable and replicable. Furthermore, voxelwise analysis confirmed lower regional TSPO expression in ASD at this later time point. **Lower TSPO expression in ASD could reflect abnormalities in neuroimmune processes or mitochondrial dysfunction.**

**Press Release from Harvard Magazine:**

**“Inflammation link for autism**

**A neuroimaging study has shown that the brains of young men with autism spectrum disorder have low levels of translocator protein, a substance that appears to play a role in inflammation and metabolism.**

**This discovery by a team of HMS researchers at Massachusetts General Hospital provides an important insight into the possible origins of autism spectrum disorder.**

**This developmental disorder, which affects one in fifty-nine children in the United States, emerges in early childhood and is characterized by difficulty communicating and interacting with others. Although the cause is unknown, growing evidence has linked it to neuroinflammation.**

**One sign of neuroinflammation is elevated levels of translocator protein, which can be measured in the brain using positron-emission tomography and anatomic magnetic resonance imaging.**

**The research team used these imaging tools to scan the brains of fifteen young adult males with the disorder. The group included both high- and low-functioning participants with varying degrees of intellectual ability. As a control, the team scanned the brains of eighteen non-autistic young men of similar age.**

**The scans showed that the brains of the young men with the disorder had lower levels of the protein, compared with the brains of non-autistic participants. In fact, those participants with the most severe symptoms of the disorder tended to have the lowest expression of the protein.**

**The brain regions found to have low expression of the protein have previously been linked to autism spectrum disorder and are thought to govern social and cognitive capacities such as processing emotions, interpreting facial expressions, and empathy.**

**The researchers point out that the translocator protein has multiple complex roles, some of which promote brain health. Adequate levels of the protein are, for example, necessary for normal functioning of mitochondria.**

**Earlier research has linked malfunctioning mitochondria in brain cells to autism spectrum disorder.**

Zürcher NR et al., Molecular Psychiatry, February 2020

172. [Biomarkers in Autism](#)

Front Psychiatry. 2014; 5: 100. Published online 2014 Aug 12. doi: 10.3389/fpsy.2014.00100

Andre A. S. Goldani,<sup>1</sup>Susan R. Downs,<sup>2</sup>Felicia Widjaja,<sup>2</sup>Brittany Lawton,<sup>2</sup> and Robert L. Hendren<sup>2,\*</sup>

<sup>1</sup>Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

<sup>2</sup>Department of Psychiatry, University of California San Francisco, San Francisco, CA, USA

Edited by: Paul Croarkin, Mayo Clinic, USA

Abstract

Autism spectrum disorders (ASDs) are complex, heterogeneous disorders caused by an interaction between genetic vulnerability and environmental factors. In an effort to better target the underlying roots of ASD for diagnosis and treatment, efforts to identify reliable biomarkers in genetics, neuroimaging, gene expression, and measures of the body's metabolism are growing. For this article, we review the published studies of potential biomarkers in autism and conclude that while there is increasing promise of finding biomarkers that can help us target treatment, there are none with enough evidence to support routine clinical use unless medical illness is suspected. **Promising biomarkers include those for mitochondrial function, oxidative stress, and immune function.** Genetic clusters are also suggesting the potential for useful biomarkers.

Excerpt: **“A recent review assessed the research on physiological abnormalities associated with ASD (44). The authors identified four main mechanisms that have been increasingly studied during the past decade: immunologic/inflammation, oxidative stress, environmental toxicants, and mitochondrial abnormalities. In addition, there is accumulating research on the lipid, GI systems, microglial activation, and the microbiome, and how these can also contribute to generating biomarkers associated with ASD (45, 46).**

**Pathways are interconnected with a defect in one likely leading to dysfunction in others. Many metabolic disorders can lead to endpoints such as impaired methylation, sulfuration, and detoxification pathways and**

**nutritional deficiencies. Mitochondrial dysfunction, environmental risk factors, metabolic imbalances, and genetic susceptibility can all lead to oxidative stress (47), which in turn leads to inflammation, damaged cell membranes, autoimmunity (48), impaired methylation (49), cell death (48), and neurological deficits (50). The brain is highly vulnerable to oxidative stress (51), particularly in children (52) during the early part of development (47). As environmental events and metabolic imbalances affect oxidative stress and methylation, they also can affect the expression of genes.“**

173. [Gestational Exposure to Air Pollution Alters Cortical Volume, Microglial Morphology, and Microglia-Neuron Interactions in a Sex-Specific Manner](#)

Front Synaptic Neurosci. 2017 May 31;9:10. doi: 10.3389/fnsyn.2017.00010. eCollection 2017.

Jessica L Bolton <sup>1</sup>, Steven Marinero <sup>2</sup>, Tania Hassanzadeh <sup>1</sup>, Divya Natesan <sup>1</sup>, Dominic Le <sup>1</sup>, Christine Belliveau <sup>1</sup>, S N Mason <sup>3</sup>, Richard L Auten <sup>3</sup>, Staci D Bilbo <sup>1 2 4</sup>

<sup>1</sup> Department of Psychology and Neuroscience, Duke University, DurhamNC, United States.

<sup>2</sup> Department of Neurobiology, Duke University Medical Center, DurhamNC, United States.

<sup>3</sup> Department of Pediatrics, Division of Neonatal Medicine, Duke University Medical Center, DurhamNC, United States.

<sup>4</sup> Department of Pediatrics and Program in Neuroscience, Lurie Center for Autism, Harvard Medical School, Massachusetts General Hospital for Children, BostonMA, United States.

#### Abstract

Microglia are the resident immune cells of the brain, important for normal neural development in addition to host defense in response to inflammatory stimuli. Air pollution is one of the most pervasive and harmful environmental toxicants in the modern world, and **several large scale epidemiological studies have recently linked prenatal air pollution exposure with an increased risk of neurodevelopmental disorders such as autism spectrum disorder (ASD)**. Diesel exhaust particles (DEP) are a primary toxic component of air pollution, and markedly activate microglia in vitro and in vivo in adult rodents. **We have demonstrated that prenatal exposure to DEP in mice, i.e., to the pregnant dams throughout gestation, results in a persistent vulnerability to behavioral deficits in adult offspring, especially in males, which is intriguing given the greater incidence of ASD in males to females (~4:1)**. Moreover, there is a striking upregulation of toll-like receptor (TLR) 4 gene expression within the brains of the same mice, and this expression is primarily in microglia. Here we explored the impact of gestational exposure to DEP or vehicle on microglial morphology in the developing brains of male and female mice. **DEP**

**exposure increased inflammatory cytokine protein and altered the morphology of microglia, consistent with activation or a delay in maturation, only within the embryonic brains of male mice; and these effects were dependent on TLR4. DEP exposure also increased cortical volume at embryonic day (E)18, which switched to decreased volume by post-natal day (P)30 in males, suggesting an impact on the developing neural stem cell niche. Consistent with this hypothesis, we found increased microglial-neuronal interactions in male offspring that received DEP compared to all other groups. Taken together, these data suggest a mechanism by which prenatal exposure to environmental toxins may affect microglial development and long-term function, and thereby contribute to the risk of neurodevelopmental disorders.**

174. [The impact of neuroimmune alterations in autism spectrum disorder](#)

Front. Psychiatry, 09 September 2015 Sec. Systems Biology Archive  
Volume 6 - 2015 | <https://doi.org/10.3389/fpsy.2015.00121>

Carmem Gottfried<sup>1,2\*</sup>, Victorio Bambini-Junior<sup>1,2,3</sup>, Fiona Francis<sup>4,5,6</sup>, Rudimar Riesgo<sup>1,7</sup> and Wilson Savino<sup>3\*</sup>

<sup>1</sup>Translational Research Group in Autism Spectrum Disorder (GETTEA),  
Federal University of Rio Grande do Sul, Porto Alegre, Brazil

<sup>2</sup>Research Group in Neuroglial Plasticity, Department of Biochemistry, Federal  
University of Rio Grande do Sul, Porto Alegre, Brazil

<sup>3</sup>Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz  
Foundation, Rio de Janeiro, Brazil

<sup>4</sup>Sorbonne Université, Université Pierre et Marie Curie, Paris, France

<sup>5</sup>INSERM UMR-S 839, Paris, France

<sup>6</sup>Institut du Fer à Moulin, Paris, France

<sup>7</sup>Child Neurology Unit, Clinical Hospital of Porto Alegre, Federal University of  
Rio Grande do Sul, Porto Alegre, Brazil

Abstract

Autism spectrum disorder (ASD) involves a complex interplay of both genetic and environmental risk factors, with immune alterations and synaptic connection deficiency in early life. In the past decade, studies of ASD have substantially increased, in both humans and animal models. Immunological imbalance (including autoimmunity) has been proposed as a major etiological component in ASD, taking into account increased levels of pro-inflammatory cytokines observed in postmortem brain from patients, as well as autoantibody production. Also, epidemiological studies have established a correlation of ASD with family history of autoimmune diseases; associations with major histocompatibility complex haplotypes and abnormal levels of immunological markers in the blood. Moreover, the use of animal models to study ASD is providing increasing information on the relationship between the immune system and the

pathophysiology of ASD. **Herein, we will discuss the accumulating literature for ASD, giving special attention to the relevant aspects of factors that may be related to the neuroimmune interface in the development of ASD, including changes in neuroplasticity.**

A commentary has been posted on this article:

GENERAL COMMENTARY article  
Front. Psychiatry, 08 October 2015 Sec. Systems Biology Archive  
Volume 6 - 2015 | <https://doi.org/10.3389/fpsy.2015.00145>

[Commentary: The impact of neuroimmune alterations in autism spectrum disorder](#)

Dario Siniscalco 1,2,3\*

1Department of Experimental Medicine, Second University of Naples, Naples, Italy

2Centre for Autism – La Forza del Silenzio, Caserta, Italy

3Cancellautismo – No Profit Association for Autism Care, Florence, Italy

The dramatic increasing prevalence of autism spectrum disorders (ASDs) (1), together with the influence on the quality of life and the lifetime societal cost of caring, has called for newest research on both the development of these diseases and the therapeutic options. Nowadays, it is well recognized that multifactorial and polygenic features (complex combination of genetic, epigenetic, and environmental interactions) characterize ASDs (2). Prenatal immune alterations and early inflammatory processes could be the autism etiological events. The authors Gottfried et al. (3) in this hypothesis-and-theory article discuss the recent findings in autism discovery. Starting from a brief historical way on autism development, the main topic of the article is to focus on the state-of-the-art of the novel findings in autism studies. **The authors rightly highlight the newest challenging frontier of autism research: the neuroimmune axis alterations. These alterations are first evident in the cells early responsible for immune responses, as they are the precursors for macrophages, dendritic, and microglial cells: monocytes or peripheral blood mononuclear cells (PBMCs). These cells show strong dysfunctions in ASD children and are committed to a pro-inflammatory state, which in turn result in long-term immune alterations (4). In ASDs, altered PBMCs are responsible for elevated pro-inflammatory cytokine production. The up-regulation of inflammatory cytokines is also reflected in brain centers of autistic patients (5): the consequences are the induction of blood–brain barrier (the immunological interface between peripheral immune system and central nervous system) disruption. Changes in BBB permeability directly influence neural plasticity, connectivity and function, triggering impairments in social interaction, communication, and behavior (3). Immunological abnormalities also influence the gastrointestinal system and the microglial innate immune cells of the central nervous system (6).**

**The authors also discuss the role of autoimmunity in the pathogenesis of autism. Familial or virus/bacteria-infected autoimmunity could be a risk factor for autism. Even if the exact cellular and molecular pathways responsible for the induction of neuroimmune alterations are still to be further clarify, a complex interaction among epigenetic and environmental risk factors (7) could trigger the neuroimmune abnormalities, such as abnormal neuron and glia responses.**

Taken together, these autism-associated neuroimmune changes could help in identifying novel therapeutic target for a better future management of ASDs.

175. Hepatitis B vaccine induces apoptotic death in Hepa1-6 cells

Apoptosis. 2012 Jan 17. Hamza H, Cao J, Li X, Li C, Zhu M, Zhao S.

Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China, Heyam68\_hamza@yahoo.com.

Abstract

Vaccines can have adverse side-effects, and these are predominantly associated with the inclusion of chemical additives such as aluminum hydroxide adjuvant. The objective of this study was to establish an in vitro model system amenable to mechanistic investigations of cytotoxicity induced by hepatitis B vaccine, and to investigate the mechanisms of vaccine-induced cell death. The mouse liver hepatoma cell line Hepa1-6 was treated with two doses of adjuvanted (aluminium hydroxide) hepatitis B vaccine (0.5 and 1 µg protein per ml) and cell integrity was measured after 24, 48 and 72 h. Hepatitis B vaccine exposure increased cell apoptosis as detected by flow cytometry and TUNEL assay. Vaccine exposure was accompanied by significant increases in the levels of activated caspase 3, a key effector caspase in the apoptosis cascade. Early transcriptional events were detected by qRT-PCR. We report that hepatitis B vaccine exposure resulted in significant upregulation of the key genes encoding caspase 7, caspase 9, Inhibitor caspase-activated DNase (ICAD), Rho-associated coiled-coil containing protein kinase 1 (ROCK-1), and Apoptotic protease activating factor 1 (Apaf-1). Upregulation of cleaved caspase 3,7 were detected by western blot in addition to Apaf-1 and caspase 9 expressions argues that cell death takes place via the intrinsic apoptotic pathway in which release of cytochrome c from the mitochondria triggers the assembly of a caspase activation complex. **We conclude that exposure of Hepa1-6 cells to a low dose of adjuvanted hepatitis B vaccine leads to loss of mitochondrial integrity, apoptosis induction, and cell death, apoptosis effect was observed also in C2C12 mouse myoblast cell line after treated with low dose of vaccine (0.3, 0.1, 0.05 µg/ml).** In addition In vivo apoptotic effect of hepatitis B vaccine was observed in mouse liver.

176. [Profile of autism spectrum disorders in Morocco: cross-sectional retrospective study of parents of children with autism](#)

Med Pharm Rep. 2023 Jan;96(1):71-78. doi: 10.15386/mpr-2308. Epub 2023 Jan 25.

Mohamed Réda Sefrioui 1 , Imane Elidrissi 2 , Ibrahim Sbai El Othmani 1 , Sanae Derfoufi 1 , Amal Ait Haj Said 1 , Adnane Benmoussa 1 , Soufiane Derraji 2

1 Laboratory of Drug Science, Biomedical and Biotechnological Research, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, Casablanca, Morocco.

2 Laboratory of Pharmacology-Toxicology, Faculty of Medicine and Pharmacy, Mohamed V University in Rabat, Rabat, Morocco.

#### Abstract

**Context and objective:** In Morocco, autism is a frequent disorder and no epidemiological studies have been carried out. The aim of this work is to analyze the characteristics of the onset of autism in children.

**Settings and design:** We conducted a cross-sectional retrospective study, spread over a period of 10 months.

**Methods:** We included in our survey children who, according to the DSM 5 definition, had one of the autism spectrum disorders and had a well-defined diagnosis. For the statistical analysis we used Excel® software. We used the percentages for the description of the qualitative variables.

**Results:** Of the parents of children with ASD, 49% were biological mothers. Of these, 30% women had their child when they were between 30 and 35 years of age. Males accounted for 61% of children with a sex ratio of 2.6. The first autistic traits appeared at the age of 18 months for 22% of our population. Of mothers with children with ASD, 83% had regular medical follow-up throughout their pregnancy. All autistic children in our population were vaccinated according to the National Immunization Program. According to parent reports, 70% affirmed that the first autistic features appeared after vaccination with the measles-mumps-rubella (MMR) vaccine, knowing that this statement is not based on evidence.

**Conclusions:** The quality of care depends on the age at which the diagnosis of autism is established.



177. [Thimerosal Induces Apoptosis in a Neuroblastoma Model via the cJun N-Terminal Kinase Pathway.](#)

Toxicological Sciences 92 (1). 246-253

ML Herdman, A Marcelo, Y Huang, RM Niles, Dhar S & Kiningham KK. (2006).

Department of Pharmacology, Joan C. Edwards School of Medicine, 1542 Spring Valley Drive, Marshall University, Huntington, WV USA

EXCERPT: In recent years, controversy has surrounded the use of thimerosal in vaccines as mercury is a known neurotoxin and nephrotoxin. Since the controversy began in the late 1990's, much of the thimerosal has been removed from vaccines administered to children in the United States. However, it remains in some, such as the influenza vaccine, and is added to multidose vials used in countries around the world. Studies concentrating on thimerosal-induced neurotoxicity are limited, and exposure guidelines, such as those set by the Food and Drug Administration, are based on research with methylmercury. Interestingly, some in vitro and in vivo studies suggest that ethylmercury may react differently than methylmercury (Aschner and Aschner, 1990; Harry et al., 2004; Magos et al., 1985). Few studies with thimerosal have focused on determining specific signaling pathways involved in neurotoxicity. Establishing these pathways may be an important step in discovering methods of alleviating toxic outcomes in patients exposed to thimerosal....Our study is the first demonstration that thimerosal can induce the activation of JNK and AP-1 in the SK-N-SH neuroblastoma cell line. We showed that activation of cJun and AP-1 transcriptional activity following thimerosal treatment does not appear to be involved in the induction of apoptosis, as demonstrated with the studies using the cJun dominant negative. Furthermore, we were able to show that JNK is an essential upstream component of this pathway through the use of the JNK inhibitor SP600125. This compound was able to attenuate activation of downstream components of mitochondrial-mediated cell death and subsequently protect the cells from apoptosis. These results are significant because identifying specific signaling pathways activated in response to thimerosal exposure presents pharmacological targets for attenuating potential toxicity in patients exposed to thimerosal-containing products.

178. [JNK Signaling Regulates Cellular Mechanics of Cortical Interneuron Migration](#)

eNeuro. 2020 Aug 20;7(4):ENEURO.0132-20.2020. doi: 10.1523/ENEURO.0132-20.2020. Print 2020 Jul/Aug.

Skye E Smith 1 2 3 , Nicholas K Coker 1 , Eric S Tucker 4 3

Department of Neuroscience, West Virginia University School of Medicine, Morgantown, WV 26506.

2Biochemistry and Molecular Biology Graduate Program, West Virginia University School of Medicine, Morgantown, WV 26506.

3WVU Rockefeller Neuroscience Institute, West Virginia University School of Medicine, Morgantown, WV 26506.

4Department of Neuroscience, West Virginia University School of Medicine, Morgantown, WV 26506 [etucker@hsc.wvu.edu](mailto:etucker@hsc.wvu.edu).

Abstract

**Aberrant migration of inhibitory interneurons can alter the formation of cortical circuitry and lead to severe neurologic disorders including epilepsy, autism, and schizophrenia.** However, mechanisms involved in directing the migration of interneurons remain incompletely understood. Using a mouse model, we performed live-cell confocal microscopy to explore the mechanisms by which the c-Jun NH2-terminal kinase (JNK) pathway coordinates leading process branching and nucleokinesis, two cell biological processes that are essential for the guided migration of cortical interneurons. Pharmacological inhibition of JNK signaling disrupts the kinetics of leading process branching, rate and amplitude of nucleokinesis, and leads to the rearward mislocalization of the centrosome and primary cilium to the trailing process. Genetic loss of Jnk from interneurons also impairs leading process branching and nucleokinesis, suggesting that important mechanics of interneuron migration depend on the intrinsic activity of JNK. **These findings highlight key roles for JNK signaling in leading process branching, nucleokinesis, and the trafficking of centrosomes and cilia during interneuron migration, and further implicates JNK signaling as an important mediator of cortical development.**

179. Maternal thimerosal exposure results in aberrant cerebellar oxidative stress, thyroid hormone metabolism, and motor behavior in rat pups; sex- and strain-dependent effects.

Cerebellum. 2012 Jun;11(2):575-86. doi: 10.1007/s12311-011-0319-5.

Sulkowski ZL, Chen T, Midha S, Zavacki AM, Sajdel-Sulkowska EM, Department of Psychiatry, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA.

**Abstract**

Methylmercury (Met-Hg) and ethylmercury (Et-Hg) are powerful toxicants with a range of harmful neurological effects in humans and animals. While Met-Hg is a recognized trigger of oxidative stress and an endocrine disruptor impacting neurodevelopment, the developmental neurotoxicity of Et-Hg, a metabolite of thimerosal (TM), has not been explored. We hypothesized that TM exposure during the perinatal period impairs central nervous system development, and

specifically the cerebellum, by the mechanism involving oxidative stress. To test this, spontaneously hypertensive rats (SHR) or Sprague-Dawley (SD) rat dams were exposed to TM (200 µg/kg body weight) during pregnancy (G10-G15) and lactation (P5-P10). Male and female neonates were evaluated for auditory and motor function; cerebella were analyzed for oxidative stress and thyroid metabolism. TM exposure resulted in a delayed startle response in SD neonates and decreased motor learning in SHR male (22.6%), in SD male (29.8%), and in SD female (55.0%) neonates. TM exposure also resulted in a significant increase in cerebellar levels of the oxidative stress marker 3-nitrotyrosine in SHR female (35.1%) and SD male (14.0%) neonates. The activity of cerebellar type 2 deiodinase, responsible for local intra-brain conversion of thyroxine to the active hormone, 3',3,5-triiodothyronine (T3), was significantly decreased in TM-exposed SHR male (60.9%) pups. This coincided with an increased (47.0%) expression of a gene negatively regulated by T3, *Odf4* suggesting local intracerebellar T3 deficiency. **Our data thus demonstrate a negative neurodevelopmental impact of perinatal TM exposure which appears to be both strain- and sex-dependent.**

180. The rise in autism and the role of age at diagnosis.

Epidemiology. 2009 Jan;20(1):84-90. doi: 10.1097/EDE.0b013e3181902d15.

Hertz-Picciotto I, Delwiche L., Department of Public Health Sciences, University of California, Davis, California 95616, USA. [ihp@ucdavis.edu](mailto:ihp@ucdavis.edu)

**Abstract**

**BACKGROUND:**

Autism prevalence in California, based on individuals eligible for state-funded services, rose throughout the 1990s. The extent to which this trend is explained by changes in age at diagnosis or inclusion of milder cases has not been previously evaluated.

**METHODS:**

Autism cases were identified from 1990 through 2006 in databases of the California Department of Developmental Services, which coordinates services for individuals with specific developmental disorders. The main outcomes were population incident cases younger than age 10 years for each quarter, cumulative incidence by age and birth year, age-specific incidence rates stratified by birth year, and proportions of diagnoses by age across birth years.

**RESULTS:**

Autism incidence in children rose throughout the period. Cumulative incidence to 5 years of age per 10,000 births rose consistently from 6.2 for 1990 births to 42.5 for 2001 births. Age-specific incidence rates increased most steeply for 2- and 3-year olds. The proportion diagnosed by age 5 years increased only slightly, from 54% for 1990 births to 61% for 1996 births. Changing age at diagnosis can explain a 12% increase, and inclusion of milder cases, a 56% increase.

**CONCLUSIONS:**

Autism incidence in California shows no sign yet of plateauing. Younger ages at diagnosis, differential migration, changes in diagnostic criteria, and inclusion of

milder cases do not fully explain the observed increases. Other artifacts have yet to be quantified, and as a result, the extent to which the continued rise represents a true increase in the occurrence of autism remains unclear.

181. [Slow CCL2-dependent translocation of biopersistent particles from muscle to brain](#)

Zakir Khan<sup>1,2</sup>, Christophe Combadière<sup>3,4,5</sup>, François-Jérôme Authier<sup>1,2,6</sup>, Valérie Itier<sup>1,11,2</sup>, François Lux<sup>7,8</sup>, Christopher Exley<sup>9</sup>, Meriem Mahrouf-Yorgov<sup>1,11,2</sup>, Xavier Decrouy<sup>1,2</sup>, Philippe Moretto<sup>10</sup>, Olivier Tillement<sup>7,8</sup>, Romain K Gherardi<sup>1,12,2,6\*†</sup> and Josette Cadusseau<sup>1,11,12,2\*†</sup>

Author Affiliations

1 Inserm, U955, 8 rue du Général Sarrail, Créteil, 94010, France

2 Université Paris Est, Faculté de Médecine, 8 rue du Général Sarrail, Créteil, 94010, France

3 Inserm, UMR-S 945, 91 Boulevard de l'Hôpital, Paris, 75013, France

4 Université Pierre et Marie Curie, Faculté de Médecine, 11 Boulevard de l'Hôpital, Paris, 75013, France

5 AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service d'Immunologie, 11 Boulevard de l'Hôpital, Paris, 75013, France

6 AP-HP, Hôpital H. Mondor - A. Chenevier, Service d'Histologie, Centre de Référence Neuromusculaire GNMH, 51 Avenue du Maréchal de Lattre de Tassigny, Créteil, 94000, France

7 CNRS UMR 5620, Laboratoire de Physico-Chimie des Matériaux Luminescents, 2 rue Victor Grignard, Villeurbanne, 69622, France

8 Université Claude Bernard Lyon 1, 2 rue Victor Grignard, Villeurbanne, 69622, France

9 The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, UK

10 CNRS UMR 5797, Centre d'Etudes Nucléaires de Bordeaux Gradignan, Allée du haut Vignaud, Gradignan, 33175, France

11 Faculté des Sciences et Technologie, UPEC, 61 Avenue du Général de Gaulle, Créteil, France

12 IMRB Team 10, Faculté de Médecine, 8 rue du Général Sarrail, Créteil, F-

94010, France

BMC Medicine 2013, 11:99 doi:10.1186/1741-7015-11-99, 4 April 2013

## Abstract

### Background

Long-term biodistribution of nanomaterials used in medicine is largely unknown. This is the case for alum, the most widely used vaccine adjuvant, which is a nanocrystalline compound spontaneously forming micron/submicron-sized agglomerates. Although generally well tolerated, alum is occasionally detected within monocyte-lineage cells long after immunization in presumably susceptible individuals with systemic/neurologic manifestations or autoimmune (inflammatory) syndrome induced by adjuvants (ASIA).

### Methods:

On the grounds of preliminary investigations in 252 patients with alum-associated ASIA showing both a selective increase of circulating CCL2, the major monocyte chemoattractant, and a variation in the CCL2 gene, we designed mouse experiments to assess biodistribution of vaccine-derived aluminum and of alum-particle fluorescent surrogates injected in muscle. Aluminum was detected in tissues by Morin stain and particle induced X-ray emission (PIXE) Both 500 nm fluorescent latex beads and vaccine alum agglomerates-sized nanohybrids (Al-Rho) were used.

### Results:

**Intramuscular injection of alum-containing vaccine was associated with the appearance of aluminum deposits in distant organs, such as spleen and brain where they were still detected one year after injection.** Both fluorescent materials injected into muscle translocated to draining lymph nodes (DLNs) and thereafter were detected associated with phagocytes in blood and spleen. Particles linearly accumulated in the brain up to the six-month endpoint; they were first found in perivascular CD11b+ cells and then in microglia and other neural cells. DLN ablation dramatically reduced the biodistribution. Cerebral translocation was not observed after direct intravenous injection, but significantly increased in mice with chronically altered blood-brain-barrier. Loss/gain-of-function experiments consistently implicated CCL2 in systemic diffusion of Al-Rho particles captured by monocyte-lineage cells and in their subsequent neurodelivery. Stereotactic particle injection pointed out brain retention as a factor of progressive particle accumulation.

### Conclusion

Nanomaterials can be transported by monocyte-lineage cells to DLNs, blood and spleen, and, similarly to HIV, may use CCL2-dependent mechanisms to penetrate the brain. This occurs at a very low rate in normal conditions explaining good overall tolerance of alum despite its strong neurotoxic potential. **However, continuously escalating doses of this poorly biodegradable adjuvant in the population may become insidiously unsafe, especially in the case of overimmunization or immature/alterd blood brain barrier or high constitutive CCL-2 production.**

182. [Blood–brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders](#)

Maria Fiorentino, Anna Sapone, Stefania Senger, Stephanie S. Camhi, Sarah M. Kadzielski, Timothy M. Buie, Deanna L. Kelly, Nicola Cascella & Alessio Fasano

Molecular Autism volume 7, Article number: 49 (2016)

Abstract

Background

Autism spectrum disorders (ASD) are complex conditions whose pathogenesis may be attributed to gene–environment interactions. There are no definitive mechanisms explaining how environmental triggers can lead to ASD although the involvement of inflammation and immunity has been suggested. Inappropriate antigen trafficking through an impaired intestinal barrier, followed by passage of these antigens or immune-activated complexes through a permissive blood–brain barrier (BBB), can be part of the chain of events leading to these disorders. Our goal was to investigate whether an altered BBB and gut permeability is part of the pathophysiology of ASD.

Methods

Postmortem cerebral cortex and cerebellum tissues from ASD, schizophrenia (SCZ), and healthy subjects (HC) and duodenal biopsies from ASD and HC were analyzed for gene and protein expression profiles. Tight junctions and other key molecules associated with the neurovascular unit integrity and function and neuroinflammation were investigated.

Results

Claudin (CLDN)-5 and -12 were increased in the ASD cortex and cerebellum. CLDN-3, tricellulin, and MMP-9 were higher in the ASD cortex. IL-8, tPA, and IBA-1 were downregulated in SCZ cortex; IL-1b was increased in the SCZ cerebellum. Differences between SCZ and ASD were observed for most of the genes analyzed in both brain areas. CLDN-5 protein was increased in ASD cortex and cerebellum, while CLDN-12 appeared reduced in both ASD and SCZ cortexes. In the intestine, 75% of the ASD samples analyzed had reduced expression of barrier-forming TJ components (CLDN-1, OCLN, TRIC), whereas 66% had increased pore-forming CLDNs (CLDN-2, -10, -15) compared to controls.

Conclusions

**In the ASD brain, there is an altered expression of genes associated with BBB integrity coupled with increased neuroinflammation and possibly impaired gut barrier integrity.** While these findings seem to be specific for ASD, the possibility of more distinct SCZ subgroups should be explored with additional studies.

183. [Thimerosal and autism? A plausible hypothesis that should not be dismissed.](#)

Med Hypotheses. 2004;62(5):788-94.

Blaxill MF, Redwood L, Bernard S.

Abstract

The autism-mercury hypothesis first described by Bernard et al. has generated much interest and controversy. The Institute of Medicine (IOM) reviewed the connection between mercury-containing vaccines and neurodevelopmental disorders, including autism. They concluded that the hypothesis was biologically plausible but that there was insufficient evidence to accept or reject a causal connection and recommended a comprehensive research program. Without citing new experimental evidence, a number of observers have offered opinions on the subject, some of which reject the IOM's conclusions. In a recent review, Nelson and Bauman argue that a link between the preservative thimerosal, the source of the mercury in childhood vaccines, is improbable. In their defense of thimerosal, these authors take a narrow view of the original hypothesis, provide no new evidence, and rely on selective citations and flawed reasoning. We provide evidence here to refute the Nelson and Bauman critique and to defend the autism-mercury hypothesis.

184. Autism Spectrum Disorders in Relation to Distribution of Hazardous Air Pollutants in the SF Bay Area

Environmental Health Perspectives – Vol. 114 No. 9, September, 2006

Gayle Windham, Div. of Environmental and Occupational Disease Control, California Department of Health Services

284 ASD children & 657 controls, born in 1994 in Bay Area, were assigned exposure levels by birth tract for 19 chemicals. Risks for autism were elevated by 50% in tracts with the highest chlorinated solvents and heavy metals. The highest risk compounds were mercury, cadmium, nickel, trichloroethylene, and vinyl chloride, and the risk from heavy metals was almost twice as high as solvents.

Excerpt: "Our results suggest a potential association between autism and estimated metal concentrations, and possibly solvents, in ambient air around the birth residence."

185. Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas

Health Place. 2006 Jun;12(2):203-9.

Palmer RF, Blanchard S, Stein Z, Mandell D, Miller C.

University of Texas Health Science Center, San Antonio Department of Family and Community Medicine, 7703 Floyd Curl Drive, San Antonio, Texas

#### Abstract

The association between environmentally released mercury, special education and autism rates in Texas was investigated using data from the Texas Education Department and the United States Environmental Protection Agency. A Poisson regression analysis adjusted for school district population size, economic and demographic factors was used. There was a significant increase in the rates of special education students and autism rates associated with increases in environmentally released mercury. On average, for each 1,000 lb of environmentally released mercury, there was a 43% increase in the rate of special education services and a 61% increase in the rate of autism. The association between environmentally released mercury and special education rates were fully mediated by increased autism rates. This ecological study suggests the need for further research regarding the association between environmentally released mercury and developmental disorders such as autism. These results have implications for policy planning and cost analysis.

186. [Autism spectrum disorder prevalence and proximity to industrial facilities releasing arsenic, lead or mercury.](#)

Sci Total Environ. 2015 Dec 1;536:245-51. doi: 10.1016/j.scitotenv.2015.07.024. Epub 2015 Jul 25.

Dickerson AS1, Rahbar MH2, Han I3, Bakian AV4, Bilder DA5, Harrington RA6, Pettygrove S7, Durkin M8, Kirby RS9, Wingate MS10, Tian LH11, Zahorodny WM12, Pearson DA13, Moyé LA 3rd14, Baio J15.

1Biostatistics/Epidemiology/Research Design (BERD) Core, Center for Clinical and Translational Sciences (CCTS), University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address:

Aisha.S.Dickerson@uth.tmc.edu.

2Biostatistics/Epidemiology/Research Design (BERD) Core, Center for Clinical and Translational Sciences (CCTS), University of Texas Health Science Center at Houston, Houston, TX 77030, USA; Division of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address:

Mohammad.H.Rahbar@uth.tmc.edu.

3Division of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, University of Texas Health Science Center at Houston, Houston, TX 77030, USA. Electronic address: Inkyu.Han@uth.tmc.edu.



- 4Division of Child Psychiatry, Department of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. Electronic address: Amanda.Bakian@hsc.utah.edu.
- 5Division of Child Psychiatry, Department of Psychiatry, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. Electronic address: Deborah.Bilder@hsc.utah.edu.
- 6Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA. Electronic address: rharrin5@jhu.edu.
- 7Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ 85721, USA. Electronic address: sydney@u.arizona.edu.
- 8Waisman Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726, USA. Electronic address: mdurkin@wisc.edu.
- 9Department of Community and Family Health, College of Public Health, University of South Florida, Tampa, FL 33612, USA. Electronic address: rkirby@health.usf.edu.
- 10Department of Health Care Organization and Policy, School of Public Health, University of Alabama at Birmingham, Birmingham, AL 35205, USA.. Electronic address: mslay@uab.edu.
- 11National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. Electronic address: bsr4@cdc.gov.
- 12Department of Pediatrics, Rutgers New Jersey Medical School, Newark, NJ 07103, USA. Electronic address: zahorodn@njms.rutgers.edu.
- 13Department of Psychiatry and Behavioral Sciences, University of Texas Medical School, Houston, TX 77054, USA. Electronic address: Deborah.A.Pearson@uth.tmc.edu.
- 14Division of Biostatistics, University of Texas School of Public Health at Houston, Houston, TX 77030, USA. Electronic address: Lemuel.A.Moye@uth.tmc.edu.
- 15National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. Electronic address: xzb1@cdc.gov.

#### Abstract

Prenatal and perinatal exposures to air pollutants have been shown to adversely affect birth outcomes in offspring and may contribute to prevalence of autism spectrum disorder (ASD). **For this ecologic study, we evaluated the association between ASD prevalence, at the census tract level, and proximity of tract centroids to the closest industrial facilities releasing arsenic, lead or mercury** during the 1990s. We used 2000 to 2008 surveillance data from five sites of the Autism and Developmental Disabilities Monitoring (ADDM) network and 2000 census data to estimate prevalence. Multi-level negative binomial regression models were used to test associations between ASD prevalence and proximity to industrial facilities in existence from 1991 to 1999 according to the US Environmental Protection Agency Toxics Release Inventory (USEPA-TRI). Data for 2489 census tracts showed that after adjustment for demographic and socio-economic area-based characteristics, ASD prevalence was higher in census tracts located in the closest 10th percentile compared of distance to those in the furthest 50th percentile (adjusted RR=1.27, 95% CI: (1.00, 1.61), P=0.049). **The findings observed in this study**

are suggestive of the association between urban residential proximity to industrial facilities emitting air pollutants and higher ASD prevalence.

187. [Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children](#)

Pediatr Neurol. 2007 Jun;36(6):361-5. doi: 10.1016/j.pediatrneurol.2007.01.012.

Michael G Chez<sup>1</sup>, Tim Dowling, Pikul B Patel, Pavan Khanna, Matt Kominsky

<sup>1</sup> Department of Neurology, Rosalind Franklin University, and the Chicago Medical School, North Chicago, IL, USA.

Abstract

Recent reports implicating elevated cytokines in the central nervous system in a small number of patients studied with autism have reported clinical regression. These studies have not focused on tumor necrosis factor-alpha as a possible marker for inflammatory damage. A series of 10 children with autism had clinical evaluation of their serum and spinal fluid for inflammatory changes and possible metabolic disease as part of their neurological evaluation. **Elevation of cerebrospinal fluid levels of tumor necrosis factor-alpha was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in all of the patients studied. The ratio of the cerebrospinal fluid levels to serum levels averaged 53.7:1. This ratio is significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum tumor necrosis factor-alpha levels have been simultaneously measured.** This observation may offer a unique insight into central nervous system inflammatory mechanisms that may contribute to the onset of autism and may serve as a potential clinical marker. More controlled study of this potentially important observation may prove valuable.

188. [Inflammatory Responses to Trivalent Influenza Virus Vaccine Among Pregnant Women](#)

Vaccine. 2011 Nov 8;29(48):8982-7. doi: 10.1016/j.vaccine.2011.09.039. Epub 2011 Sep 22.

Christian LM, Iams JD, Porter K, Glaser R.

Department of Psychiatry, The Ohio State University Medical Center, Columbus, OH

Abstract

Objective

In the U.S., seasonal trivalent influenza vaccination (TIV) is currently universally

recommended for all pregnant women. However, data on the maternal inflammatory response to vaccination is lacking and would better delineate the safety and clinical utility of immunization. In addition, for research purposes, vaccination has been used as a mild immune trigger to examine in vivo inflammatory responses in nonpregnant adults. The utility of such a model in pregnancy is unknown. Given the clinical and empirical justifications, the current study examined the magnitude, time course, and variance in inflammatory responses following seasonal influenza virus vaccination among pregnant women.

#### Methods

Women were assessed prior to and at one day (n=15), two days (n=10), or approximately one week (n=21) following TIV. Serum interleukin (IL)-6, tumor necrosis factor (TNF)- $\alpha$ , C-reactive protein (CRP), and macrophage migration inhibitory factor (MIF) were determined by high sensitivity immunoassay.

#### Results

Significant increases in CRP were seen at one and two days post-vaccination (ps <.05). A similar effect was seen for TNF- $\alpha$ , for which an increase at two days post-vaccination approached statistical significance (p = .06). There was considerable variability in magnitude of response; coefficients of variation for change at two days post-vaccination ranged from 122% to 728%, with the greatest variability in IL-6 responses at this timepoint.

#### Conclusions

Trivalent influenza virus vaccination elicits a measurable inflammatory response among pregnant women. There is sufficient variability in response for testing associations with clinical outcomes. As adverse perinatal health outcomes including preeclampsia and preterm birth have an inflammatory component, a tendency toward greater inflammatory responding to immune triggers may predict risk of adverse outcomes, providing insight into biological mechanisms underlying risk. The inflammatory response elicited by vaccination is substantially milder and more transient than seen in infectious illness, arguing for the clinical value of vaccination. However, further research is needed to confirm that the mild inflammatory response elicited by vaccination is benign in pregnancy

### 189. [The Role of the Immune System in Autism Spectrum Disorder](#)

Neuropsychopharmacology. 2017 Jan; 42(1): 284–298. Published online 2016 Sep 21. Prepublished online 2016 Aug 18. doi: 10.1038/npp.2016.158

Amory Meltzer<sup>1</sup> and Judy Van de Water<sup>1,2,3,\*</sup>

<sup>1</sup>Division of Rheumatology/Allergy and Clinical Immunology, Department of Internal Medicine, University of California, Davis, CA, USA

<sup>2</sup>The M.I.N.D. Institute, University of California, Davis, CA, USA

<sup>3</sup>NIEHS Center for Children's Environmental Health, University of California, Davis, CA, USA

\*Division of Rheumatology/Allergy and Clinical Immunology; 451 E. Health Sciences Dr, Suite 6510; University of California Davis

## Abstract

Autism is a neurodevelopmental disorder characterized by deficits in communication and social skills as well as repetitive and stereotypical behaviors. While much effort has focused on the identification of genes associated with autism, **research emerging within the past two decades suggests that immune dysfunction is a viable risk factor contributing to the neurodevelopmental deficits observed in autism spectrum disorders (ASD).** Further, it is the heterogeneity within this disorder that has brought to light much of the current thinking regarding the subphenotypes within ASD and how the immune system is associated with these distinctions. This review will focus on the two main axes of immune involvement in ASD, namely dysfunction in the prenatal and postnatal periods. **During gestation, prenatal insults including maternal infection and subsequent immunological activation may increase the risk of autism in the child. Similarly, the presence of maternally derived anti-brain autoantibodies found in ~20% of mothers whose children are at risk for developing autism has defined an additional subphenotype of ASD.** The postnatal environment, on the other hand, is characterized by related but distinct profiles of immune dysregulation, inflammation, and endogenous autoantibodies that all persist within the affected individual. Further definition of the role of immune dysregulation in ASD thus necessitates a deeper understanding of the interaction between both maternal and child immune systems, and the role they have in diagnosis and treatment.

## 190. [Elevated maternal C-reactive protein and autism in a national birth cohort.](#)

Mol Psychiatry. 2013 Jan 22. doi: 10.1038/mp.2012.197.

Brown AS, Sourander A, Hinkka-Yli-Salomäki S, McKeague IW, Sundvall J, Surcel HM.

Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York State Psychiatric Institute, New York, NY, USA, Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY, USA.

### Abstract

Autism is a complex neuropsychiatric syndrome with a largely unknown etiology. Inflammation during pregnancy may represent a common pathway by which infections and other insults increase risk for the disorder. Hence, we investigated the association between early gestational C-reactive protein (CRP), an established inflammatory biomarker, prospectively assayed in maternal sera, and childhood autism in a large national birth cohort with an extensive serum biobank. Other strengths of the cohort included nearly complete ascertainment of pregnancies in Finland (N=1.2 million) over the study period and national psychiatric registries consisting of virtually all treated autism cases in the population. Increasing maternal CRP levels, classified as a continuous variable,

were significantly associated with autism in offspring. For maternal CRP levels in the highest quintile, compared with the lowest quintile, there was a significant, 43% elevated risk. This finding suggests that maternal inflammation may have a significant role in autism, with possible implications for identifying preventive strategies and pathogenic mechanisms in autism and other neurodevelopmental disorders. *Molecular Psychiatry* advance online publication, 22 January 2013; doi:10.1038/mp.2012.197.

191. [What is regressive autism and why does it occur? Is it the consequence of multi-systemic dysfunction affecting the elimination of heavy metals and the ability to regulate neural temperature?](#)

*N Am J Med Sci.* 2009 July; 1(2): 28–47.

Graham E. Ewing

Montague Healthcare, Nottingham United Kingdom

#### Abstract

There is a compelling argument that the occurrence of regressive autism is attributable to genetic and chromosomal abnormalities, arising from the overuse of vaccines, which subsequently affects the stability and function of the autonomic nervous system and physiological systems. That sense perception is linked to the autonomic nervous system and the function of the physiological systems enables us to examine the significance of autistic symptoms from a systemic perspective. Failure of the excretory system influences elimination of heavy metals and facilitates their accumulation and subsequent manifestation as neurotoxins: the long-term consequences of which would lead to neurodegeneration, cognitive and developmental problems. It may also influence regulation of neural hyperthermia. This article explores the issues and concludes that sensory dysfunction and systemic failure, manifested as autism, is the inevitable consequence arising from subtle DNA alteration and consequently from the overuse of vaccines.

192. [Neurologic adverse events following vaccination](#)

*Prog Health Sci* 2012, Vol 2 , No1

Sienkiewicz D.\*, Kułak W., Okurowska-Zawada B., Paszko-Patej G.

Department of Pediatric Rehabilitation of the Medical University of Białystok, Poland

#### Abstract

The present review summarizes data on neurological adverse events following vaccination in the relation to intensity, time of onset, taking into account the

immunological and non-immunological mechanisms. The authors described the physiological development of the immune system and the possible immune system responses following vaccination. Toxic property of thimerosal - a mercury-containing preservative used in some vaccines was presented. The neurological complications after vaccination were described. The role of vaccination in the natural course of infectious diseases and the current immunizations schedule in Poland was discussed.

Discussion by Sienkiewicz et. al.:

**"Among the "major" neurological complications, usually manifesting more than 48 hours after vaccination and which might be the cause of permanent damage to the central nervous system (CNS), the following are listed: seizures - especially if there is no increase in body temperature, hypotonic-hyporesponsive episodes, postvaccinal encephalitis, postvaccinal encephalopathy [6, 8-11] and autism [10, 12-14]."**

193. [Hypotonic-hyporesponsive episodes following pertussis vaccination: a cause for concern?](#)

Drug Saf. 2002;25(2):85-90. doi: 10.2165/00002018-200225020-00003.

Michael S Gold 1

1 University Department of Paediatrics, and Immunisation Coordination Unit, Adelaide, Australia.

Abstract

Vaccine safety has become a major community concern and of particular importance for parents, vaccine recipients and vaccine providers. **A hypotonic-hyporesponsive episode (HHE) is a sudden and unexpected episode of loss of tone, unresponsiveness and colour change which uncommonly affects infants and children after vaccination. Although any vaccine may be associated with this adverse event, HHE usually follows administration of a pertussis containing vaccine.** There has been renewed interest in this adverse event in the light of community concerns regarding vaccine safety. The focus of this interest has been to formulate an acceptable case definition, to document possible risk factors and to better define the outcome of HHE. In addition, studies have documented the outcome of revaccination of children who have had an HHE. Although much remains to be learnt about HHE it would appear that there are no long-term sequelae and that children who have had an HHE can be revaccinated. Parents should be provided with the available information such that they can make an assessment of the risks and benefits of pertussis vaccination. The benefits of pertussis vaccination still outweigh the risk and universal childhood pertussis vaccination should continue to be advocated.

194. [The Weak Link: Hypotonia in Infancy and Autism Early Identification](#)

Front. Neurol., 04 February 2021. Sec. Pediatric Neurology, Volume 12 - 2021 | <https://doi.org/10.3389/fneur.2021.612674>

Lidia V. Gabis<sup>1,2\*</sup>, Meirav Shaham<sup>3</sup>, Odelia Leon Attia<sup>1</sup>, Shahar Shefer<sup>1</sup>, Ruth Rosenan<sup>1</sup>, Tal Gabis<sup>4</sup> and Michal Daloya<sup>3</sup>

<sup>1</sup>Weinberg Child Development Center at Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel

<sup>2</sup>Sackler Faculty of Medicine at Tel Aviv University, Tel Aviv, Israel

<sup>3</sup>Department of Statistics, University of Haifa, Haifa, Israel

<sup>4</sup>Faculty of Medicine, Charles University, Prague, Czechia

**Background:** Presenting symptoms and age specific differential diagnosis of Autism Spectrum Disorder (ASD), determine the age of initial assessment and the age of a definite diagnosis. The AAP recommends screening all children for ASD at 18 and 24 months followed by a comprehensive evaluation for children with developmental concerns. More recently it has been recommended that the evaluation should be performed at a younger age, with a diagnosis being made as early as the beginning of the second year of life resulting in earlier intensive intervention.

**Objective:** To assess early developmental milestones in a cohort of children diagnosed with Autism Spectrum Disorder (ASD) in order to find an objective and reliable early marker. We suggest that low muscle tone- hypotonia, is a sign that meets the above criteria of consistency and reliability and may be related to early diagnosis.

**Methods:** We compared age distributions of ASD diagnosis in the presence of hypotonia in a dataset of 5,205 children diagnosed at Keshet Center. One thousand, one hundred eighty-two children (953 males) were diagnosed with ASD and compared to other developmental diagnoses. Within the ASD cohort we further analyzed for gender and pre-maturity differences.

**Results:** In the presence of hypotonia, the mean age for ASD diagnosis was significantly lower (by 1.5 years for males and females) and this effect increased in children born at term as compared to pre-maturity.

**Conclusions:** Hypotonia is a recognizable marker of ASD and may serve as a “red flag” to prompt earlier recognition and neurodevelopmental evaluation toward an autism diagnosis.

195. [Hypotonic-hyporesponsive episodes reported to the Vaccine Adverse Event Reporting System \(VAERS\), 1996-1998](#)

Pediatrics. 2000 Oct;106(4):E52. doi: 10.1542/peds.106.4.e52.

## Abstract

**Background:** A hypotonic-hyporesponsive episode (HHE) is the sudden onset of hypotonia, hyporesponsiveness, and pallor or cyanosis that occurs within 48 hours after childhood immunizations. This syndrome has been primarily associated with pertussis-containing vaccines administered to children <2 years of age, and has been estimated to occur once every 1750 diphtheria-tetanus-pertussis (DTwP) vaccinations. Previous studies of HHE were limited by small numbers of cases and, sometimes, by limited details of the event.

**Objectives:** To characterize a large number of HHE cases reported to the Vaccine Adverse Event Reporting System (VAERS), to assist clinicians in identifying HHE, and to assist researchers in investigating the risk factors, cause, and pathogenesis of this syndrome.

**Methods:** More than 40,000 VAERS reports received between 1996 and 1998 were screened for HHE by a computer algorithm and reviewed, and a telephone follow-up questionnaire was administered to the witness of HHE.

**Results:** There were 215 HHE cases, all nonfatal. The median age of onset of HHE was 4.0 months (range: 1.1-107 months). Over half of the reports (53%) concerned females. The median birth weight was 3.36 kg (range: 1.27-4.96 kg); 4.7% had a birth weight <2500 g. The median interval between vaccination and HHE was 210 minutes (range: 1 minute-2 days). Among children with HHE who were <24 months of age, the episode occurred within 5 minutes in only 8.5%, compared with 66.7% of children with HHE >24 months of age. There were no relevant findings regarding family medical history or the mothers' gestational history. Nearly all of the children (98.6%) returned to their prevaccination state according to the telephone questionnaire; median time to return was 6 hours (range: 1 minute- 4 months). The 3 children reported as not returning to their prevaccination state all had VAERS reports submitted after they developed conditions (autism, complex partial epilepsy, and developmental delays with infantile spasms) that are not known to be causally associated with immunization. The vast majority of children (93%) with HHE received a pertussis-containing vaccine, either diphtheria-tetanus-acellular pertussis (DTaP, 28%), DTwP (11%), or diphtheria-tetanus-pertussis-Haemophilus influenzae type b (DTwP-HIB, 61%). During the HHE episode, 90.1% of the children had pallor and 49% had cyanosis. Because of the HHE event, 6.8% of children had had all vaccines withheld as of the date of the interview. Of the remainder, 66.5% of children have had 1 or more subsequent vaccinations or vaccine components withheld, and 26.7% have not had any subsequent vaccinations withheld. Only 1 child was reported to have had a repeat episode of HHE, occurring after hepatitis B vaccination. From 1996 to 1998, the number of HHE reports decreased from 99 to 38, when the predominant pertussis vaccine administered to infants changed from whole-cell to acellular.

**Conclusion:** This study represents the largest published case series of children with HHE and supports the generally benign, self-limited, nonrecurrent nature of



this syndrome. Although HHE has been less frequently reported to VAERS after increased use of DTaP, HHE does occur after the administration of DTaP and other nonpertussis-containing vaccines. Although most parents and pediatricians withheld the pertussis component of subsequent vaccinations, many did not, with no reported adverse events occurring in the children after the subsequent immunizations. Restricting the definition of HHE to a more narrow age range (eg, <2 years of age) is also proposed because most of the older children probably experienced vasovagal syncope rather than HHE within 5 minutes of immunization.

196. [Neurological adverse events associated with vaccination](#)

Curr Opin Neurol. 2002 Jun;15(3):333-8. doi: 10.1097/00019052-200206000-00018.

Sucheep Piyasirisilp 1 , Thiravat Hemachudha

1 Division of Neurology, Department of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Abstract

Public tolerance to adverse reactions is minimal. Several reporting systems have been established to monitor adverse events following immunization. The present review summarizes data on neurologic complications following vaccination, and provides evidence that indicates whether they were directly associated with the vaccines. These complications include autism (measles vaccine), multiple sclerosis (hepatitis B vaccine), meningoencephalitis (Japanese encephalitis vaccine), Guillain-Barré syndrome and giant cell arteritis (influenza vaccine), and reactions after exposure to animal rabies vaccine. **Seizures and hypotonic/hyporesponsive episodes following pertussis vaccination** and potential risks associated with varicella vaccination, as well as vaccine-associated paralytic poliomyelitis following oral poliovirus vaccination, are also described. In addition, claims that complications are caused by adjuvants, preservatives and contaminants [i.e. macrophagic myofasciitis (aluminium), neurotoxicity (thimerosal), and new variant Creutzfeldt-Jakob disease (bovine-derived materials)] are discussed.

197. [Immunological and autoimmune considerations of Autism Spectrum Disorders.](#)

J Autoimmun. 2013 Jul 15. pii: S0896-8411(13)00073-5. doi: 10.1016/j.jaut.2013.05.005.

Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Procházka V, Melamed M, Kristt DA, Steinberg A, Shulman C, Hwang P, Koren G, Walfisch A, Passweg JR, Snowden JA, Tamouza R, Leboyer M, Farge-Bancel D, Ashwood P.

Jerusalem, Israel.

#### Abstract

Autism Spectrum Disorders (ASD) are a group of heterogeneous neurodevelopmental conditions presenting in early childhood with a prevalence ranging from 0.7% to 2.64%. Social interaction and communication skills are impaired and children often present with unusual repetitive behavior. The condition persists for life with major implications for the individual, the family and the entire health care system. While the etiology of ASD remains unknown, various clues suggest a possible association with altered immune responses and ASD. Inflammation in the brain and CNS has been reported by several groups with notable microglia activation and increased cytokine production in postmortem brain specimens of young and old individuals with ASD. Moreover several laboratories have isolated distinctive brain and CNS reactive antibodies from individuals with ASD. Large population based epidemiological studies have established a correlation between ASD and a family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. In addition, there is evidence that antibodies that are only present in some mothers of children with ASD bind to fetal brain proteins and may be a marker or risk factor for ASD. Studies involving the injection of these ASD specific maternal serum antibodies into pregnant mice during gestation, or gestational exposure of Rhesus monkeys to IgG subclass of these antibodies, have consistently elicited behavioral changes in offspring that have relevance to ASD. We will summarize the various types of studies associating ASD with the immune system, critically evaluate the quality of these studies, and attempt to integrate them in a way that clarifies the areas of immune and autoimmune phenomena in ASD research that will be important indicators for future research.

198. [Toward an immune-mediated subtype of autism spectrum disorder](#)

Brain Res. 2015 Aug 18;1617:72-92. doi: 10.1016/j.brainres.2014.09.048. Epub 2014 Nov 13.

Christopher J McDougle <sup>1</sup> , Samantha M Landino <sup>2</sup> , Arshya Vahabzadeh <sup>3</sup> , Julia O'Rourke <sup>4</sup> , Nicole R Zurcher <sup>5</sup> , Beate C Finger <sup>6</sup> , Michelle L Palumbo <sup>4</sup> , Jessica Helt <sup>7</sup> , Jennifer E Mullett <sup>7</sup> , Jacob M Hooker <sup>5</sup> , William A Carlezon Jr <sup>6</sup>

<sup>1</sup> Lurie Center for Autism, Lexington, MA, United States; Massachusetts General Hospital, Boston, MA, United States; Harvard Medical School, Boston, MA, United States. Electronic address: cmcdougle@partners.org.

<sup>2</sup> Behavioral Genetics Laboratory, Belmont, MA, United States; McLean Hospital, Belmont, MA, United States.

3 Massachusetts General Hospital, Boston, MA, United States; McLean Hospital, Belmont, MA, United States; Harvard Medical School, Boston, MA, United States.

4 Lurie Center for Autism, Lexington, MA, United States; Massachusetts General Hospital, Boston, MA, United States; Harvard Medical School, Boston, MA, United States.

5 Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA, United States; Massachusetts General Hospital, Boston, MA, United States; Harvard Medical School, Boston, MA, United States.

6 Behavioral Genetics Laboratory, Belmont, MA, United States; McLean Hospital, Belmont, MA, United States; Harvard Medical School, Boston, MA, United States.

7 Lurie Center for Autism, Lexington, MA, United States; Massachusetts General Hospital, Boston, MA, United States.

PMID: 25445995 DOI: 10.1016/j.brainres.2014.09.048

#### Abstract

A role for immunological involvement in autism spectrum disorder (ASD) has long been hypothesized. This review includes four sections describing (1) evidence for a relationship between familial autoimmune disorders and ASD; (2) results from post-mortem and neuroimaging studies that investigated aspects of neuroinflammation in ASD; (3) findings from animal model work in ASD involving inflammatory processes; and (4) outcomes from trials of anti-inflammatory/immune-modulating drugs in ASD that have appeared in the literature. Following each section, ideas are provided for future research, suggesting paths forward in the continuing effort to define the role of immune factors and inflammation in the pathophysiology of a subtype of ASD. This article is part of a Special Issue entitled SI: Neuroimmunology in Health And Disease.

#### 199. [Immune mediated conditions in autism spectrum disorders](#)

Brain Behav Immun. 2015 May;46:232-6. doi: 10.1016/j.bbi.2015.02.001. Epub 2015 Feb 11.

Ousseny Zerbo <sup>1</sup>, Albin Leong <sup>2</sup>, Lisa Barcellos <sup>3</sup>, Pilar Bernal <sup>4</sup>, Bruce Fireman <sup>5</sup>, Lisa A Croen <sup>5</sup>

Affiliations

PMID: 25681541 PMCID: PMC4414798 DOI: 10.1016/j.bbi.2015.02.001

#### Abstract

We conducted a case-control study among members of Kaiser Permanente Northern California (KPNC) born between 1980 and 2003 to determine the prevalence of immune-mediated conditions in individuals with autism, investigate whether these conditions occur more often than expected, and explore the timing

of onset relative to autism diagnosis. Cases were children and young adults with at least two autism diagnoses recorded in outpatient records (n=5565). Controls were children without autism randomly sampled at a ratio of 5 to 1, matched to cases on birth year, sex, and length of KPNC membership (n=27,825). The main outcomes - asthma, allergies, and autoimmune diseases - were identified from KPNC inpatient and outpatient databases. Chi-square tests were used to evaluate case-control differences. Allergies and autoimmune diseases were diagnosed significantly more often among children with autism than among controls (allergy: 20.6% vs. 17.7%, Crude odds ratio (OR)=1.22, 95% confidence interval (CI) 1.13-1.31; autoimmune disease: 1% vs. 0.76%, OR=1.36, 95% CI 1.01-1.83), and asthma was diagnosed significantly less often (13.7% vs. 15.9%; OR=0.83, 95% CI 0.76-0.90). Psoriasis occurred more than twice as often in cases than in controls (0.34% vs. 0.15%; OR=2.35, 95% CI 1.36-4.08). Our results support previous observations that children with autism have elevated prevalence of specific immune-related comorbidities.

200. [Examples of Outcome Reporting Bias in Vaccine Studies: Illustrating How Perpetuating Medical Consensus Can Impede Progress in Public Health](#)

Cureus. 2022 Sep 21;14(9):e29399. doi: 10.7759/cureus.29399. eCollection 2022 Sep.

Gary S Goldman

Research, Independent Computer Scientist, Bogue Chitto, USA.

Abstract

Introduction: Outcome reporting bias in vaccine studies is a widespread problem among all researchers who have a tendency to report selective results and conclusions that support their beliefs and values or those of sponsoring agencies. Especially during the COVID-19 pandemic, this bias surfaced through the unprecedented proliferation of conflicting vaccine studies. Many researchers strongly recommend and report on the safety and effectiveness of the COVID-19 vaccine. Those researchers who embrace the COVID-19 vaccine and vaccines, in general, are often dismissive of other researchers who present views that differ from medical orthodoxy and oppose medical consensus.

Methods: The aim of this analysis is to critically evaluate seven vaccine studies using qualitative and/or quantitative approaches to identify outcome reporting bias and assess its potential impact on the stated conclusions that align with medical consensus. Four studies claim to have found no association between autism and (a) blood levels of mercury, (b) measles, mumps, and rubella (MMR) vaccine, and (c) thimerosal-containing vaccines. Three other studies claim no association exists between infant mortality rate and the number of vaccine doses, universal varicella vaccination and herpes zoster, and pandemic influenza vaccines and fetal losses.

Results: The presence of outcome reporting bias and independent reanalysis demonstrated an impact on both the direction and magnitude of the observed effect - raising questions concerning the robustness of the original study design and conclusions and challenging the current medical consensus. Medical consensus has exonerated vaccines as having any causal relationship to autism spectrum disorders (ASDs), yet no other reasonable cause has been proposed. Medical consensus attributes significant ASD increases to better case ascertainment and broadened clinical diagnosis. According to 2018 data, an estimated 1 in 44 eight-year-olds has been identified with ASD. From 1990 to 2019, there have been an estimated two million new cases of ASD in the US, with lifetime social costs exceeding \$7 trillion (in 2019 dollars). Can perpetuating medical consensus impede the advancement of public health? Or has it already done so?

Conclusions: **Conflicts of interest (e.g., financial) that abound between health regulatory agencies and the pharmaceutical industry impact what is ultimately reckoned as medical consensus. Outcome reporting bias that is inherent to all researchers to some degree, obscures medical and scientific truth. Advancement of public health requires that researchers have integrity and an openness and willingness to collaborate to resolve contradictory findings. In fact, it is usually through meticulous, rigorous, scientific investigation of contradictory findings that medical science has advanced and contributed to improvements in public health - since medical consensus and orthodoxy can be incorrect.**

201. [Identification of Unique Gene Expression Profile in Children with Regressive Autism Spectrum Disorder \(ASD\) and Ileocolitis](#)

PLoS ONE 8(3): e58058. doi:10.1371/journal.pone.0058058

Walker SJ, Fortunato J, Gonzalez LG, Krigsman A

Abstract

Gastrointestinal symptoms are common in children with autism spectrum disorder (ASD) and are often associated with mucosal inflammatory infiltrates of the small and large intestine. Although distinct histologic and immunohistochemical properties of this inflammatory infiltrate have been previously described in this ASDGI group, molecular characterization of these lesions has not been reported. In this study we utilize transcriptome profiling of gastrointestinal mucosal biopsy tissue from ASDGI children and three non-ASD control groups (Crohn's disease, ulcerative colitis, and histologically normal) in an effort to determine if there is a gene expression profile unique to the ASDGI group. Comparison of differentially expressed transcripts between the groups demonstrated that non-pathologic (normal) tissue segregated almost completely from inflamed tissue in all cases. Gene expression profiles in intestinal biopsy tissue from patients with Crohn's disease, ulcerative colitis, and ASDGI, while having significant overlap with each other, also showed distinctive features for each group. **Taken together, these results demonstrate that ASDGI children**

**have a gastrointestinal mucosal molecular profile that overlaps significantly with known inflammatory bowel disease (IBD), yet has distinctive features that further supports the presence of an ASD-associated IBD variant, or, alternatively, a prodromal phase of typical inflammatory bowel disease.** Although we report qPCR confirmation of representative differentially expressed transcripts determined initially by microarray, these findings may be considered preliminary to the extent that they require further confirmation in a validation cohort.

202. [Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4+ T cells](#)

Immunity. 2022 Jan 11;55(1):145-158.e7. doi: 10.1016/j.immuni.2021.11.005. Epub 2021 Dec 7.

Eunha Kim <sup>1</sup>, Donggi Paik <sup>1</sup>, Ricardo N Ramirez <sup>1</sup>, Delaney G Biggs <sup>1</sup>, Youngjun Park <sup>1</sup>, Ho-Keun Kwon <sup>1</sup>, Gloria B Choi <sup>2</sup>, Jun R Huh <sup>3</sup>

<sup>1</sup> Department of Immunology, Blavatnik Institute, Harvard Medical School, Boston, MA 02115, USA.

<sup>2</sup> The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. Electronic address: gbchoi@mit.edu.

<sup>3</sup> Department of Immunology, Blavatnik Institute, Harvard Medical School, Boston, MA 02115, USA; Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA. Electronic address: jun\_huh@hms.harvard.edu.

PMID: 34879222 PMCID: PMC8755621 DOI: 10.1016/j.immuni.2021.11.005

Abstract

**Children with autism spectrum disorders often display dysregulated immune responses and related gastrointestinal symptoms.** However, the underlying mechanisms leading to the development of both phenotypes have not been elucidated. Here, we show that mouse offspring exhibiting autism-like phenotypes due to prenatal exposure to maternal inflammation were more susceptible to developing intestinal inflammation following challenges later in life. In contrast to its prenatal role in neurodevelopmental phenotypes, interleukin-17A (IL-17A) generated immune-primed phenotypes in offspring through changes in the maternal gut microbiota that led to postnatal alterations in the chromatin landscape of naive CD4+ T cells. The transfer of stool samples from pregnant mice with enhanced IL-17A responses into germ-free dams produced immune-primed phenotypes in offspring. **Our study provides mechanistic insights into why children exposed to heightened inflammation in the womb might have an increased risk of developing inflammatory diseases in addition to**

**neurodevelopmental disorders.**

203. [Early Disruption of the Microbiome Leading to Decreased Antioxidant Capacity and Epigenetic Changes: Implications for the Rise in Autism](#)

Front. Cell. Neurosci., 15 August 2018 | <https://doi.org/10.3389/fncel.2018.00256>

Rebecca S. Eshraghi, Richard C. Deth, Rahul Mittal, Mayank Aranke, Sae-In S. Kay<sup>4</sup>, Baharak Moshiree and Adrien A. Eshraghi

Currently, 1 out of every 59 children in the United States is diagnosed with autism. While initial research to find the possible causes for autism were mostly focused on the genome, more recent studies indicate a significant role for epigenetic regulation of gene expression and the microbiome. In this review article, **we examine the connections between early disruption of the developing microbiome and gastrointestinal tract function, with particular regard to susceptibility to autism. The biological mechanisms that accompany individuals with autism are reviewed in this manuscript including immune system dysregulation, inflammation, oxidative stress, metabolic and methylation abnormalities as well as gastrointestinal distress.** We propose that these autism-associated biological mechanisms may be caused and/or sustained by dysbiosis, an alteration to the composition of resident commensal communities relative to the community found in healthy individuals and its redox and epigenetic consequences, changes that in part can be due to early use and over-use of antibiotics across generations. Further studies are warranted to clarify the contribution of oxidative stress and gut microbiome in the pathophysiology of autism. A better understanding of the microbiome and gastrointestinal tract in relation to autism will provide promising new opportunities to develop novel treatment modalities.

204. [Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits](#)

Mol Psychiatry. 2014 Apr;19(4):495-503. doi: 10.1038/mp.2013.41. Epub 2013 Apr 23.

Wong CC<sup>1</sup>, Meaburn EL<sup>2</sup>, Ronald A<sup>2</sup>, Price TS<sup>3</sup>, Jeffries AR<sup>1</sup>, Schalkwyk LC<sup>1</sup>, Plomin R<sup>1</sup>, Mill J<sup>4</sup>.

<sup>1</sup> King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London, UK.

2 1] King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London, UK [2] Department of Psychological Sciences, Birkbeck, University of London, London, UK.

3 1] King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London, UK [2] Institute of Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, PA, USA.

4 1] King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London, UK [2] University of Exeter Medical School, Exeter University, St Luke's Campus, Exeter, UK.

#### Abstract

Autism spectrum disorder (ASD) defines a group of common, complex neurodevelopmental disorders. Although the aetiology of ASD has a strong genetic component, there is considerable monozygotic (MZ) twin discordance indicating a role for non-genetic factors. Because MZ twins share an identical DNA sequence, disease-discordant MZ twin pairs provide an ideal model for examining the contribution of environmentally driven epigenetic factors in disease. We performed a genome-wide analysis of DNA methylation in a sample of 50 MZ twin pairs (100 individuals) sampled from a representative population cohort that included twins discordant and concordant for ASD, ASD-associated traits and no autistic phenotype. Within-twin and between-group analyses identified numerous differentially methylated regions associated with ASD. **In addition, we report significant correlations between DNA methylation and quantitatively measured autistic trait scores across our sample cohort. This study represents the first systematic epigenomic analyses of MZ twins discordant for ASD and implicates a role for altered DNA methylation in autism.**

#### "Excerpt

**These findings concur with mounting data suggesting that environmentally mediated effects on the epigenome may be relatively common and important for disease."**

205. [Correlations between gene expression and mercury levels in blood of boys with and without autism.](#)

Neurotox Res. 2011 Jan;19(1):31-48. doi: 10.1007/s12640-009-9137-7. Epub 2009 Nov 24.

Stamova B1, Green PG, Tian Y, Hertz-Picciotto I, Pessah IN, Hansen R, Yang X, Teng J, Gregg JP, Ashwood P, Van de Water J, Sharp FR.

Department of Neurology, University of California at Davis Medical Center, Sacramento, CA 95817, USA. boryana.stamova@ucdmc.ucdavis.edu



#### Abstract

Gene expression in blood was correlated with mercury levels in blood of 2- to 5-year-old boys with autism (AU) compared to age-matched typically developing (TD) control boys. This was done to address the possibility that the two groups might metabolize toxicants, such as mercury, differently. RNA was isolated from blood and gene expression assessed on whole genome Affymetrix Human U133 expression microarrays. Mercury levels were measured using an inductively coupled plasma mass spectrometer. Analysis of covariance (ANCOVA) was performed and partial correlations between gene expression and mercury levels were calculated, after correcting for age and batch effects. To reduce false positives, only genes shared by the ANCOVA models were analyzed. Of the 26 genes that correlated with mercury levels in both AU and TD boys, 11 were significantly different between the groups ( $P(\text{Diagnosis} \times \text{Mercury}) \leq 0.05$ ). The expression of a large number of genes ( $n = 316$ ) correlated with mercury levels in TD but not in AU boys ( $P \leq 0.05$ ), the most represented biological functions being cell death and cell morphology. Expression of 189 genes correlated with mercury levels in AU but not in TD boys ( $P \leq 0.05$ ), the most represented biological functions being cell morphology, amino acid metabolism, and antigen presentation. These data and those in our companion study on correlation of gene expression and lead levels show that AU and TD children display different correlations between transcript levels and low levels of mercury and lead. These findings might suggest different genetic transcriptional programs associated with mercury in AU compared to TD children.

206. [Abnormal immune response to brain tissue antigen in the syndrome of autism.](#)

Am J Psychiatry. 1982 Nov;139(11):1462-5.

Weizman A, Weizman R, Szekely GA, Wijzenbeek H, Livni E.

#### Abstract

Cell-mediated immune response to human myelin basic protein was studied by the macrophage migration inhibition factor test in 17 autistic patients and a control group of 11 patients suffering from other mental diseases included in the differential diagnosis of the syndrome of autism. Of the 17 autistic patients, 13 demonstrated inhibition of macrophage migration, whereas none of the nonautistic patients showed such a response. **The results indicate the existence of a cell-mediated immune response to brain tissue in the syndrome of autism.**

207. [Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism.](#)

Dig Dis Sci. 2000 Apr;45(4):723-9.

Kawashima H, Mori T, Kashiwagi Y, Takekuma K, Hoshika A, Wakefield A.

Department of Paediatrics, Tokyo Medical University, Japan.

#### Abstract

It has been reported that measles virus may be present in the intestine of patients with Crohn's disease. Additionally, a new syndrome has been reported in children with autism who exhibited developmental regression and gastrointestinal symptoms (autistic enterocolitis), in some cases soon after MMR vaccine. It is not known whether the virus, if confirmed to be present in these patients, derives from either wild strains or vaccine strains. In order to characterize the strains that may be present, we have carried out the detection of measles genomic RNA in peripheral mononuclear cells (PBMC) in eight patients with Crohn's disease, three patients with ulcerative colitis, and nine children with autistic enterocolitis. As controls, we examined healthy children and patients with SSPE, SLE, HIV-1 (a total of eight cases). RNA was purified from PBMC by Ficoll-paque, followed by reverse transcription using AMV; cDNAs were subjected to nested PCR for detection of specific regions of the hemagglutinin (H) and fusion (F) gene regions. Positive samples were sequenced directly, in nucleotides 8393-8676 (H region) or 5325-5465 (from noncoding F to coding F region). One of eight patients with Crohn disease, one of three patients with ulcerative colitis, and three of nine children with autism, were positive. Controls were all negative. The sequences obtained from the patients with Crohn's disease shared the characteristics with wild-strain virus. **The sequences obtained from the patients with ulcerative colitis and children with autism were consistent with being vaccine strains.** The results were concordant with the exposure history of the patients. Persistence of measles virus was confirmed in PBMC in some patients with chronic intestinal inflammation.

208. [Mechanisms of aluminum adjuvant toxicity and autoimmunity in pediatric populations](#)

Lupus. 2012 Feb;21(2):223-30. doi: 10.1177/0961203311430221.

L Tomljenovic, CA Shaw

Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada

Departments of Ophthalmology and Visual Sciences and Experimental Medicine and the Graduate Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada

Lucija Tomljenovic, Post-doctoral fellow, Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia

## Abstract

Immune challenges during early development, including those vaccine-induced, can lead to permanent detrimental alterations of the brain and immune function. Experimental evidence also shows that simultaneous administration of as little as two to three immune adjuvants can overcome genetic resistance to autoimmunity. In some developed countries, by the time children are 4 to 6 years old, they will have received a total of 126 antigenic compounds along with high amounts of aluminum (Al) adjuvants through routine vaccinations. According to the US Food and Drug Administration, safety assessments for vaccines have often not included appropriate toxicity studies because vaccines have not been viewed as inherently toxic. Taken together, these observations raise plausible concerns about the overall safety of current childhood vaccination programs. When assessing adjuvant toxicity in children, several key points ought to be considered: (i) infants and children should not be viewed as “small adults” with regard to toxicological risk as their unique physiology makes them much more vulnerable to toxic insults; (ii) in adult humans Al vaccine adjuvants have been linked to a variety of serious autoimmune and inflammatory conditions (i.e., “ASIA”), yet children are regularly exposed to much higher amounts of Al from vaccines than adults; (iii) it is often assumed that peripheral immune responses do not affect brain function. However, **it is now clearly established that there is a bidirectional neuro-immune cross-talk that plays crucial roles in immunoregulation as well as brain function. In turn, perturbations of the neuro-immune axis have been demonstrated in many autoimmune diseases encompassed in “ASIA” and are thought to be driven by a hyperactive immune response; and (iv) the same components of the neuro-immune axis that play key roles in brain development and immune function are heavily targeted by Al adjuvants.** In summary, research evidence shows that increasing concerns about current vaccination practices may indeed be warranted. Because children may be most at risk of vaccine-induced complications, a rigorous evaluation of the vaccine-related adverse health impacts in the pediatric population is urgently needed.

209. [Autism pathogenesis: Piecing it all together, from end to beginning ...](#)

J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2787-2789

1 Vinu Arumugham, 2 Maxim V.Trushin

1 - Independent Researcher, San Jose, USA

2 - Kazan Federal University, Kazan, Russia

**Increased extra-axial cerebrospinal fluid (EA-CSF) have been observed in imaging studies of infant brains, who go on to develop autism. Folate deficiency can cause defects in neural development that can affect CSF production and drainage. Folate receptor alpha antibodies (FRAA) are observed in 75% of autism patients. Maternal FRAA have also been observed in the case of neural tube defects.**

**Folate deficiency can cause aluminum accumulation in the brain. Autistic brains have been shown to accumulate aluminum. FRAA in the child or mother can therefore explain all the observations.**

Further, autism patients have a higher genetic risk for cancer but have lower cancer rates. Many cancer cells express folate receptor alpha to transport folate required for rapid growth. Once again FRAA in autism can thus explain lower rates of cancer occurrence as FRAA block FRA expressed on cancer cells, affecting folate transport.

A majority of FRAA are of the IgG4 subclass and bind with higher affinity to the bovine folate receptor than the human folate receptor. The human and bovine FR have 90% protein sequence homology.

From allergies and parasite infections we know that IgG4 is the second stage of the immune response. The first stage is IgE against FRA. The US Institute of Medicine concluded that antigens in vaccines do cause IgE mediated sensitization. Many vaccines contain cow's milk proteins, one of which is the bovine folate receptor protein. Bovine casein and casamino acids used as growth media for vaccine manufacture are derived from cow's milk.

The solution for vaccine-induced IgE against FRA, is to immediately remove all non-target proteins from all vaccines by using processes such as affinity chromatography.

210. [Etiology of autism spectrum disorders: Genes, environment, or both?](#)

OA Autism 2014 Jun 10;2(2):11

C Shaw, S Sheth, D Li, L Tomljenovic  
University of British Columbia, Vancouver, British Columbia, Canada

Introduction

Thus far, most of the research on both neurodevelopmental and neurodegenerative disorders has been focused on finding the presumed underlying genetic causes, while much less emphasis has been put on potential environmental factors. While some forms of autism are clearly genetic, the fact remains that heritability factors cannot adequately explain all reported cases nor their drastic increase over the last few decades. In particular, studies on twins have now shown that common environmental factors account for 55% of their risk for developing autism while genetic susceptibility explains only 37% of cases. Because the prenatal environment and early postnatal environment are shared between twins and because overt symptoms of autism emerge around the end of the first year of life, it is likely that at least some of the environmental factors contributing to the risk of autism exert their deleterious neurodevelopmental effect during this early period of life. Indeed, evidence has now emerged showing

that autism may in part result from early-life immune insults induced by environmental xenobiotics. One of the most common xenobiotic with immunostimulating as well as neurotoxic properties to which infants under two years of age are routinely exposed worldwide is the aluminum (Al) vaccine adjuvant. In this review we discuss the mechanisms by which Al can induce adverse neurological and immunological effects and how these may provide important clues of Al's putative role in autism. Because of the tight connection between the development of the immune and the central nervous system, the possibility that immune-overstimulation in early infancy via vaccinations may play a role in neurobehavioural disorders needs to be carefully considered.

#### Conclusion

There is now sufficient evidence from both human and animal studies showing that cumulative exposure to aluminium adjuvants is not as benign as previously assumed. Given that vaccines are the only medical intervention that we attempt to deliver to every living human on earth and that by far the largest target population for vaccination are healthy children, a better appreciation and understanding of vaccine adjuvant risks appears warranted.

211. [Thiol-modulated mechanisms of the cytotoxicity of thimerosal and inhibition of DNA topoisomerase II alpha.](#)

Chem Res Toxicol. 2008 Feb;21(2):483-93.

Wu X, Liang H, O'Hara KA, Yalowich JC, Hasinoff BB.

Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada.

#### Abstract

Thimerosal is an organic mercury compound that is widely used as a preservative in vaccines and other solution formulations. The use of thimerosal has caused concern about its ability to cause neurological abnormalities due to mercury accumulation during a normal schedule of childhood vaccinations. While the chemistry and the biological effects of methylmercury have been well-studied, those of thimerosal have not. Thimerosal reacted rapidly with cysteine, GSH, human serum albumin, and single-stranded DNA to form ethylmercury adducts that were detectable by mass spectrometry. These results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. **Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase II alpha, likely through reaction with critical free cysteine thiol groups.** Thimerosal, however, did not act as a topoisomerase II poison and the lack of cross-resistance with a K562 cell line with a decreased level of topoisomerase II alpha (K/VP.5 cells) suggested that inhibition of topoisomerase II alpha was not a significant mechanism for the inhibition of cell growth. **Depletion of intracellular GSH with buthionine sulfoximine treatment greatly increased the K562 cell growth inhibitory**

**effects of thimerosal, which showed that intracellular glutathione had a major role in protecting cells from thimerosal.** Pretreatment of thimerosal with glutathione did not, however, change its K562 cell growth inhibitory effects, a result consistent with the rapid exchange of the ethylmercury adduct among various thiol-containing cellular reactants. Thimerosal-induced single and double strand breaks in K562 cells were consistent with a rapid induction of apoptosis. In conclusion, these studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase II alpha and protein and nonprotein thiols and with DNA.

212. [Topoisomerases facilitate transcription of long genes linked to autism](#)

Nature (2013) doi:10.1038/nature12504

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Ian F. King, Chandri N. Yandava, Angela M. Mabb, Jack S. Hsiao, Hsien-Sung Huang, Brandon L. Pearson, J. Mauro Calabrese, Joshua Starmer, Joel S. Parker, Terry Magnuson, Stormy J. Chamberlain, Benjamin D. Philpot & Mark J. Zylka

**Abstract**

Topoisomerases are expressed throughout the developing and adult brain and are mutated in some individuals with autism spectrum disorder (ASD). However, how topoisomerases are mechanistically connected to ASD is unknown. Here we find that topotecan, a topoisomerase 1 (TOP1) inhibitor, dose-dependently reduces the expression of extremely long genes in mouse and human neurons, including nearly all genes that are longer than 200 kilobases. Expression of long genes is also reduced after knockdown of Top1 or Top2b in neurons, highlighting that both enzymes are required for full expression of long genes. By mapping RNA polymerase II density genome-wide in neurons, we found that this length-dependent effect on gene expression was due to impaired transcription elongation. Interestingly, many high-confidence ASD candidate genes are exceptionally long and were reduced in expression after TOP1 inhibition. **Our findings suggest that chemicals and genetic mutations that impair topoisomerases could commonly contribute to ASD and other neurodevelopmental disorders.**

213. [Aluminum in the central nervous system \(CNS\): toxicity in humans and animals, vaccine adjuvants, and autoimmunity.](#)

Immunol Res. 2013 Jul;56(2-3):304-16.

Shaw CA, Tomljenovic L.

#### Abstract

We have examined the neurotoxicity of aluminum in humans and animals under various conditions, following different routes of administration, and provide an overview of the various associated disease states. The literature demonstrates clearly negative impacts of aluminum on the nervous system across the age span. In adults, aluminum exposure can lead to apparently age-related neurological deficits resembling Alzheimer's and has been linked to this disease and to the Guamanian variant, ALS-PDC. Similar outcomes have been found in animal models. In addition, injection of aluminum adjuvants in an attempt to model Gulf War syndrome and associated neurological deficits leads to an ALS phenotype in young male mice. In young children, a highly significant correlation exists between the number of pediatric aluminum-adjuvanted vaccines administered and the rate of autism spectrum disorders. Many of the features of aluminum-induced neurotoxicity may arise, in part, from autoimmune reactions, as part of the ASIA syndrome.

214. [Transcriptomic Analyses of Neurotoxic Effects in Mouse Brain After Intermittent Neonatal Administration of Thimerosal.](#)

Toxicol Sci. 2014 Apr 4.

Li X1, Qu F, Xie W, Wang F, Liu H, Song S, Chen T, Zhang Y, Zhu S, Wang Y, Guo C, Tang TS.

#### Abstract

Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopmental disorders including autism. The association between infant vaccine thimerosal exposure and autism remains an open question. Although thimerosal has been removed from mandatory childhood vaccines in the United States, thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries. Notably, thimerosal-containing vaccines are being given to the newborns within the first 12-24 h after birth in some countries. To examine the possible neurotoxic effects of early neonatal exposure to a higher level of thimerosal, FVB mice were subcutaneously injected with thimerosal-mercury at a dose which is 20× higher than that used for regular Chinese infant immunization during the first 4 months of life. Thimerosal-treated mice exhibited neural development delay, social interaction deficiency, and inclination of depression. Apparent neuropathological changes were also observed in adult mice neonatally treated with thimerosal. High-throughput RNA sequencing of autistic-behaved mice brains revealed the alternation of a number of canonical pathways involving neuronal development, neuronal synaptic function, and the dysregulation of endocrine system. Intriguingly, the elevation of anterior pituitary secreting hormones occurred exclusively in male but not in female thimerosal-treated mice, demonstrating for the first time the gender bias of thimerosal-mercury toxicity with regard to endocrine system. Our results indicate that higher dose of

neonatal thimerosal-mercury (20× higher than that used in human) is capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be the causal involvements of autistic-like behavior in mice.

215. [Increased Prevalence of Familial Autoimmunity in Probands With Pervasive Developmental Disorders](#)

Electronic Articles| November 01 2003, Pediatrics (2003) 112 (5): e420.  
<https://doi.org/10.1542/peds.112.5.e420>

Thayne L. Sweeten, PhD; Suzanne L. Bowyer, MD; David J. Posey, MD; Gary M. Halberstadt, DO; Christopher J. McDougle, MD

Department of Psychiatry, Indiana University School of Medicine

Abstract

**Objectives.** Increased prevalence of familial autoimmune disease is a common finding among probands with various autoimmune disorders. Autistic disorder (autism) is a highly genetic disorder with known immune and immunogenetic abnormalities. Previous research has found an increased frequency of autoimmune disorders in families with autistic probands. We further investigated this association by determining the frequency of autoimmune disorders in families that have probands with pervasive developmental disorders (PDDs), including autism, compared with 2 control groups.

**Methods.** Three well-defined study groups, including 1) families that have a child with a PDD, 2) families that have a child with an autoimmune disorder, and 3) families with a healthy control child, constituted the sample. A questionnaire inquiring about which first- and second-degree family members had received a diagnosis of having specific autoimmune disorders was completed by 101 families in each group.

**Results.** The frequency of autoimmune disorders was significantly higher in families of the PDD probands compared with families of both the autoimmune and healthy control probands. Autoimmunity was highest among the parents of PDD probands compared with parents of the healthy control subjects. Hypothyroidism/Hashimoto's thyroiditis and rheumatic fever were significantly more common in families with PDD probands than in the healthy control families.

**Conclusions.** Autoimmunity was increased significantly in families with PDD compared with those of healthy and autoimmune control subjects. These preliminary findings warrant additional investigation into immune and autoimmune mechanisms in autism.



216. [Self-organized criticality theory of autoimmunity.](#)

PLoS One. 2009 Dec 31;4(12):e8382. doi: 10.1371/journal.pone.0008382.

Tsumiyama K1, Miyazaki Y, Shiozawa S.

Department of Biophysics, Kobe University Graduate School of Health Science, Kobe, Japan.

Abstract

BACKGROUND:

The cause of autoimmunity, which is unknown, is investigated from a different angle, i.e., the defect in immune 'system', to explain the cause of autoimmunity.

METHODOLOGY/PRINCIPAL FINDINGS:

Repeated immunization with antigen causes systemic autoimmunity in mice otherwise not prone to spontaneous autoimmune diseases. Overstimulation of CD4(+) T cells led to the development of autoantibody-inducing CD4(+) T (aiCD4(+) T) cell which had undergone T cell receptor (TCR) revision and was capable of inducing autoantibodies. The aiCD4(+) T cell was induced by de novo TCR revision but not by cross-reaction, and subsequently overstimulated CD8(+) T cells, driving them to become antigen-specific cytotoxic T lymphocytes (CTL). These CTLs could be further matured by antigen cross-presentation, after which they caused autoimmune tissue injury akin to systemic lupus erythematosus (SLE).

CONCLUSIONS/SIGNIFICANCE:

**Systemic autoimmunity appears to be the inevitable consequence of overstimulating the host's immune 'system' by repeated immunization with antigen, to the levels that surpass system's self-organized criticality.**

217. [Can Awareness of Medical Pathophysiology in Autism Lead to Primary Care Autism Prevention Strategies?](#)

Elizabeth Mumper, MD, FAAP

N A J Med Sci. 2013;6(3):134-144. DOI: 10.7156/najms.2013.0603134

Abstract

Emerging research suggests that the timing of environmental factors in the presence of genetic predispositions has influenced the increase in autism spectrum disorders over the past several decades. A review of the medical literature suggests that autism may be impacted by environmental toxicants, breastfeeding duration, gut flora composition, nutritional status, acetaminophen use, vaccine practices and use of antibiotics and/or frequency of infections. The

author reports her retrospective clinical research in a general pediatric practice (Advocates for Children), which shows a modest trend toward lower prevalence of autism than her previous pediatric practice or recent CDC data. Out of 294 general pediatrics patients followed since 2005 there were zero new cases of autism (p value 0.014). Given the prevalence of autism for that cohort of 1 in 50 children in the United States, it is important to consider implementing strategies in primary care practice that could potentially modify environmental factors or affect the timing of environmental triggers contributing to autism.

218. [Autism: a novel form of mercury poisoning](#)

Medical Hypotheses (2001) 56(4), 462–471, 2001 Harcourt Publishers Ltd  
doi: 10.1054/mehy.2000.1281,

S. Bernard, A. Enayati, L. Redwood, H. Roger, T. Binstock

ARC Research, Cranford, New Jersey, USA

Summary Autism is a syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movements, and sensory dysfunction. Recent epidemiological studies suggest that autism may affect 1 in 150 US children. Exposure to mercury can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autism, and the similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Thimerosal, a preservative added to many vaccines, has become a major source of mercury in children who, within their first two years, may have received a quantity of mercury that exceeds safety guidelines. A review of medical literature and US government data suggests that: (i) many cases of idiopathic autism are induced by early mercury exposure from thimerosal; (ii) this type of autism represents an unrecognized mercurial syndrome; and (iii) genetic and non-genetic factors establish a predisposition whereby thimerosal's adverse effects occur only in some children.

219. [Autism and autoimmune disease: A family study](#)

John Money, Nanci A. Bobrow & Florence C. Clarke

Journal of autism and childhood schizophrenia volume 1, pages 146–160,  
Published: June 1971

Abstract

A family is presented to demonstrate the rare phenomenon of early infantile autism in the presence of autoimmune disease. The youngest son in the family has a multiple diagnosis of autism, Addison's disease, and moniliasis. The next

older brother has hypoparathyroidism, Addison's disease, moniliasis, and diabetes mellitus. The next older brother has hypoparathyroidism, Addison's disease, moniliasis, and alopecia totalis. The oldest son and first born child in this family of four is, along with the parents, symptom free. Whereas autism in the youngest son might be attributed to the traumatic family situation, in which there exists the constant threat of near-death, it might conceivably be attributed also to a primary effect of autoimmune impairment from the formation of autoantibodies affecting the central nervous system.

220. [Autistic children exhibit undetectable hemagglutination-inhibition antibody titers despite previous rubella vaccination](#)

J Autism Child Schizophr. 1976 Sep;6(3):269-74. doi:10.1007/BF01543467.

E G Stubbs

Abstract

The etiology of autism is unknown, but **autism has been associated with a number of diseases, including prenatal rubella**. Rubella vaccine challenge was used in an attempt to retrospectively diagnose prenatal rubella in autistic children. This test was selected because unresponsiveness of antibody titer has been reported as helpful in retrospective diagnosing of prenatal rubella. Fifteen autistic children and 8 controls matched for age were challenged with rubella vaccine. Rubella vaccine challenge did not differentiate autistic children from the control subjects. However, 5 of 13 autistic children had undetectable titers despite previous vaccine; all control subjects had detectable titers. **This finding of undetectable titers in autistic children suggests these children may have an altered immune response.**

221. [\[Autistic syndrome \(Kanner\) and vaccination against smallpox \(author's transl\)\]](#).

Eggers C.

Klin Padiatr. 1976 Mar;188(2):172-80. [Article in German]

Abstract

3-4 weeks following an otherwise uncomplicated first vaccination against smallpox a boy, then aged 15 months and last seen at the age of 5 1/2 years, gradually developed a complete Kanner syndrome. The question whether vaccination and early infantile autism might be connected is being discussed. A causal relationship is considered extremely unlikely. But **vaccination is recognized as having a starter function for the onset of autism.**

222. [Autistic disturbances of affective contact.](#)

Nervous Child 2, 217-250 (1943)

Kanner L.  
Johns Hopkins University

“Case 3. Richard M. was referred to the Johns Hopkins Hospital on February 5, 1941, at 3 years, 3 months of age, with the complaint of deafness because he did not talk and did not respond to questions.”

**“Following smallpox vaccination at 12 months, he had an attack of diarrhea and fever, from which he recovered in somewhat less than a week.”**

“In September, 1940, the mother, in commenting on Richard's failure to talk, remarked in her notes: I can't be sure just when he stopped the imitation of words sounds. It seems that he has gone backward mentally gradually for the last two years.”

Richard M:

November 1937 – Born

November 1938 – Vaccinated with Smallpox vaccine

September 1940 – Mother reports developmental regression beginning approximately two years previously

February 1941 – Referred to Hopkins for evaluation

1943 – Becomes the third child to be described as autistic by Leo Kanner in his disorder defining paper, the first paper published on autism.