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**A REVIEW OF MILES & TAKAHASHI STUDY AND RELATED  
LITERATURE ON AUTISM RISK FROM ANTENATAL  
RHO-D IMMUNE GLOBULIN**

**Sallie Bernard, Mark Blaxill, Lyn Redwood**

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## **Executive Summary**

### **Background**

Rho(D) immune globulin routinely given during pregnancy formerly contained mercury from thimerosal, raising concerns over a possible role in autism causation. A May 2007 paper by Miles & Takahashi reported no association. The conclusions contradict other studies on the subject. This review evaluates the Miles & Takahashi research, related documents, and other relevant literature and identifies alternate explanations for the reported observations.

### **Findings**

The review found deficiencies in sample quality, including small and unmatched controls and inadequate methods for determining mercury exposure from RhIg brands. Poor sample recruitment design likely produced under-representation of mothers receiving RhIg, the key exposure variable. Alterations in sample composition during implementation, contravening accepted research standards, were detected, as were factual errors on vaccines, RhIg, and mercury. The lead author has many undisclosed conflicts of interest. These problems may underlie the negative finding on association between RhIg and autism. Additional calculations of the data, not done by Miles & Takahashi, show a 71% higher rate of Rh immune globulin exposure in children with autism relative to unaffected siblings, in contradiction to the original findings but consistent with other studies.

### **Conclusions**

The Miles & Takahashi conclusions are questionable based on research quality issues. Recalculation of the data shows an increased risk of autism from Rh immune globulin. Definitive conclusions await higher quality studies.

## Introduction

Mercury is a neuro- and immunotoxin that is particularly harmful to the fetus and infant. An organic mercury preservative called thimerosal, about 50% ethylmercury by weight, has been a common component of vaccines and other injected biologics such as immune globulins. In 1999, the U.S. Public Health Service and the American Academy of Pediatrics announced that the amount of mercury in infant vaccines exceeded some government safety guidelines, and they asked for the eventual elimination of mercury from routine vaccines given to infants and pregnant women.<sup>1</sup> Use of thimerosal above trace amounts in many routinely recommended U.S. vaccines and immune globulins for infants and pregnant women has been discontinued, the primary exception being the influenza vaccine, which is now recommended for all pregnant women and infants.

A number of parents of children with autism have asserted that their child's disability resulted from exposure to mercury from vaccines or immune globulins. The immune globulin most often singled out is Rho-D immune globulin (RhIg), given to pregnant women with Rh negative blood type (Rh-). Most but not all brands of RhIg contained thimerosal in varying amounts. In 1990, the American College of Obstetricians & Gynecologists (ACOG) made formal recommendations that Rh-pregnant women be routinely given RhIg at 28 weeks gestation and in cases of obstetric complications and invasive procedures like amniocentesis which might result in maternal-fetal blood mixing. Prior to this guideline, RhIg was only routinely given postpartum.<sup>2</sup>

A paper by Judith H. Miles and T. Nicole Takahashi on the risk of autism in offspring of Rh- mothers and from fetal exposure to mercury from RhIg injections was published in the May 2007 issue of the *American Journal of Medical Genetics*.<sup>3</sup> Accompanying the *AJMG* publication were a press release (Figure 1) from the University of Missouri, which is the institution with which the investigators are affiliated, and many interview placements for the lead author in the media.<sup>4,5</sup> Prior to the release of the paper, a poster presentation was given at a conference in October 2005 which covered preliminary results of the same study (Figure 2), and the final results of the study were also presented in January 2007 at a symposium convened in Missouri sponsored by State Senator John Loudon.<sup>6</sup> All of the presented versions of the study concluded that there is no increased risk of autism from exposure to mercury in

RhIg during pregnancy and hence, there is unlikely to be an association between thimerosal in vaccines and autism risk.

This review paper analyzes the various documents related to this study. The review examines the quality of the study itself and the representation of the results in various venues. The review encompasses relevant scientific literature on Rh, RhIg, thimerosal, and autism and considers reasons why the Miles & Takahashi findings are inconsistent with those of other research efforts. The review includes additional calculations of autism-RhIg risk derived from the study data but not included in the authors' findings. These new calculations are more consistent with findings of other studies.

## Methods

A review was made using Pubmed of the general literature on human blood types, Rh factor, Rh status, Rh incompatibility and subsequent disease. This literature is briefly summarized and used to understand the details of the autism-specific research. A comprehensive review was made of the published papers on Rh and autism, the most relevant of which are summarized and used for comparison purposes. Published and unpublished information on historical RhIg use was compiled for assessment of the probability of the observed results occurring and generation of likely alternative explanations. Finally, the authors' personal experience in the autism parent community, gained through years of collaboration and advocacy as parents of children with autism engaged in the community, was utilized to generate hypotheses around parental behavior and clinic characteristics.

## Findings

### Literature Review

**Rh Blood Type** – An individual's blood type can be Rhesus-Positive (Rh+) or Rhesus-Negative (Rh-) depending on whether a specific protein is or is not present on the surface of red blood cells. Rh factor blood type is inherited and the gene is recessive. Prevalence of either type varies worldwide and in the U.S. by ethnicity, ranging from less than 1% for Native Americans to 35% for people of the Basque region in Spain. Babies of mothers who are Rh- and receive an Rh+ gene from their fathers are Rh+, and the pregnancy is characterized by maternal-fetal Rh incompatibility. If an Rh- mother has

already had a prior pregnancy with an Rh+ fetus, and has another Rh+ baby, the baby is susceptible to Rh Hemolytic Disease (RhHD) if the first baby's blood inadvertently mixed with the mother's blood and the mother produced antibodies against Rh+ blood cells, which the mother's immune system recognizes as "foreign". Production of these antibodies is called sensitization or isoimmunization. RhHD can be inconsequential, producing mild anemia and jaundice, or severe, resulting in long term morbidity or death.<sup>7,8</sup>

***Rh Immune Globulins*** - Clinical trials of RhIg given to mothers postpartum to prevent isoimmunization were undertaken in the early 1960s. RhIg destroys Rh+ blood cells that may have entered the maternal circulation from the fetus and prevents production of Rh antibodies by the mother that may harm an Rh+ fetus in subsequent pregnancies. In 1968, the first brand of RhIg, RhoGAM®, was licensed by Ortho Diagnostics, now a division of Johnson & Johnson, and postnatal administration became routine practice in the U.S. RhHD was greatly reduced but not eliminated. Additional RhIg administered during pregnancy was observed to prevent some of these residual cases, and antenatal use at 28

weeks gestation and after invasive obstetric procedures became common by the latter 1980s. These practices were made routine in 1989-1990 with U.S. Public Health Service and ACOG guidelines.<sup>7</sup> With advancing technology, invasive diagnostic procedures during pregnancy have increased in frequency<sup>9</sup> which would have lead to multiple exposures to thimerosal during pregnancy for some Rh- women.<sup>10</sup>

Although RhoGAM® was the first licensed RhIg, since the 1970's other brands were widely sold in the U.S. Each of these brands had a different mercury concentration and dosage level. Brands sold during the 1990's and their average mercury content per recommended dose are shown in Table I. The actual mercury content was variable as the pre-filled syringe volume varied by lot to prevent the immune response to 15 mL or less of Rh+ RBC's per dose. The only way to accurately determine mercury exposure levels for an individual is through ascertainment of lot number and fill volume from the FDA.<sup>11,12</sup>

**Table I. RhIg Brands and Mercury Content 1990-2001<sup>11,12</sup>**

Brand	Manufacturer	% Concentration Mercury	Mercury (mcg) per Milliliter	Avg Rec'dDose Vol	Comments
RhoGAM	Ortho, subsidiary of Johnson & Johnson	0.003 (see comments)	15 (see comments)	0.6-1.0	Manufactured without mercury April 2001
Gamulin	Armour Pharmaceuticals	0.01	50	1.0-2.0	Withdrawn 1994
HypRho-D	Miles, subsidiary of Bayer	0.01	50	1.0	Discontinued 1996, replaced by BayRho-D
BayRho-D	Bayer	0.01	0	1.0	Licensed 1996, replaced HypRho-D

***Autism Rh Studies*** - There are only two studies besides Miles & Takahashi's which have examined RhIg administration and autism, one by Holmes *et al* (2003)<sup>13</sup>, of which one of the current co-authors was also a co-author, and the other by Geier & Geier (2007)<sup>14</sup>. Additional studies sometimes cited in reviews of Rh-related autism risk factors, Juul-Dam *et al* (2001)<sup>15</sup>, Zandi *et al* (2006)<sup>16</sup>, and Mason-Brothers *et al* (1990)<sup>17</sup> investigated maternal-fetal Rh incompatibility and not exposure to RhIg or incidence of maternal Rh- blood type. The significance of Rh incompatibility is addressed later.

Both the Holmes and Geier findings are contradictory to those of Miles & Takahashi. The latter found approximately the same rate of Rh- blood type and RhIg exposure in their autism and control groups, while the former reported significantly increased RhIg exposure in autism mothers relative to controls.

Holmes *et al* was focused on explaining the level of mercury in first baby haircuts and collected data on RhIg exposure as part of a total exposure assessment and not as part of a direct investigation of RhIg exposure risk. Consequently, they did not collect data on the rate of Rh-

negative blood type in autism or control mothers, and they did not attempt to determine whether the RhIg given contained mercury. They only compared the percentage of mothers receiving RhIg injections and the average number of injections received between autism and control groups. Autism children were patients at a single practice and control families were recruited via internet and newsletter alerts. Eligible control mothers had only typically developing children. Birth years of the autism mothers were 1985-1999 and those of the control mothers were 1990-1999, with the average birth year of both groups being 1994, so that virtually all births in both groups fell within the period of interest when RhIg with thimerosal was commonly or routinely given antenatally.<sup>2, 18, 19</sup> Besides birth year, children of cases and controls were matched on gender and the geographic representation was similar. No information was provided on the ethnicity of the sample. The autism sample size was 94 and the control sample size was 45. Data were collected using a structured telephone survey and RhIg exposure was based on mothers' recall.

The investigators found a 5-fold increase in RhIg exposure in their autism group relative to controls. They reported that 46% of the autism and 9% of the control mothers received RhIg. They found a statistically significant increase in the number of RhIg injections per autism mother, 0.52 versus 0.09 in the controls, or a nearly 6-fold increase. Approximately 12% of the autism group received more than one injection, while none of the control mothers received multiple doses.

The Geier & Geier study was targeted specifically at an assessment of mercury exposure risk from RhIg administration. The authors limited their sample to non-Jewish Caucasian women, so ethnicity and gender were consistent between cases and controls. The patient group consisted of 53 mothers of a child with autism seen in the authors' clinic in 2005 and 2006 whose child's birth year fell between 1987 and 2001, the period of interest for RhIg with thimerosal. The median birth year was 1997. The control group was comprised of 926 pregnant women seen in the same clinic from 1980 to 1989, prior to when antenatal RhIg became routine. The sample was consecutively seen, so none were lost to follow up. The investigators looked at the medical records of the clinic, which specializes in prenatal genetic testing, to calculate rates of Rh-negative blood type, so mothers' recall was not a factor. They report on RhIg exposure in the autism group and the presence of thimerosal in RhIg but do not describe how they determined this information.

The Geiers found that 28.3% of the autism mothers were Rh- and all of these received an antenatal dose of RhIg with thimerosal; one was given multiple injections. The incidence of Rh-negative blood type was 14.4% among control mothers, giving an odds ratio for Rh-blood type in the autism group of 2.35 times relative to controls. The rate of antenatal exposure to RhIg in controls was not provided but presumably, given pregnancy dates prior to 1987, it was lower than 14.4%, so *de facto* - but not stated by the authors - the relative risk of autism from exposure to RhIg with thimerosal would be higher than 2.35.

### Review of Miles & Takahashi Research & Related Statements

An in-depth examination of the Miles & Takahashi research and media statements reveals pervasive deficiencies. The shortcomings, summarized in Table II and detailed in the subsections following, have been grouped into 7 categories: conflicts of interest, autism sample selection bias, poorly chosen control groups, incorrect determination of RhIg exposure, omission of standard toxicological methodologies, sample alterations, and errors, ambiguities, and misrepresentations.

***Conflicts of Interest*** - The Miles & Takahashi work reflects several conflicts of interest, which appear to have affected study design and interpretation.

The 2005 poster lists "Johnson & Johnson Pharmaceutical Research" (J&J) as the supporter of the study, and the *AJMG* paper disclosed "Johnson and Johnson Company" as a funding source. J&J is the largest manufacturer of RhIg products. J&J has a direct financial interest in ongoing legal proceedings regarding thimerosal in Rho D immune globulin therapies and autism.<sup>20</sup> Analyses have shown that journal research funded by industry is more likely to present results favorable to the manufacturer's interests.<sup>21</sup> RhIg research sponsored by J&J therefore deserves special scrutiny for bias and conflict of interest. Notably, the University of Missouri press release omitted mention of industry sponsorship. In addition, the *AJMG* paper failed to alert the reader that J&J was the manufacturer of the product under study, saying only that "RhIg was first licensed by Ortho Clinical Diagnostics under the brand name Rhogam [sic]", and never offering that Ortho is a J&J subsidiary.

**Table II. Summary of Deficiencies of  
Miles & Takahashi Research and Its Representation**

<b>Deficiency</b>	<b>Description</b>
<b>Conflicts of interest</b>	The study was funded by the largest manufacturer of the product under examination, the manufacturer's role was not made clear, the primary author has served as a paid consultant in autism-related litigation involving RhIg, and the authors' research interests focus on genetic causes of autism to the exclusion of environmental ones such as mercury.
<b>Autism sample selection bias</b>	Unacknowledged but probable sample selection bias, due to the genetics orientation of the clinic from which it was drawn and the sample selection process, resulted in under-representation of Rh- and RhIg-exposed mothers of children with autism, leading to the observed negative findings on RhIg risk.
<b>Poorly chosen control groups</b>	The control groups used to calculate autism risk from RhIg with thimerosal are inadequate. Depending on the group, they are: too small to provide statistical power, unmatched on key characteristics, reflect selection bias, and overlap with the autism population.
<b>Incorrect RhIg exposure determination</b>	The method used to determine thimerosal exposure from RhIg is incorrect given the multiple brands and formulations available and practice terminology. The high rate of exposure to the one thimerosal-containing brand suggests an unreported higher mercury exposure rate in autism.
<b>Omission of standard toxicological methodologies</b>	Primarily an investigation of mercury toxicity, the study ignores dose-response effects and does not assess or control for exposure from other mercury sources, including vaccines.
<b>Sample alterations</b>	The study sample was altered and not just added to during implementation. Mothers and children who were part of the initial case and control samples in 2005 were no longer part of the final study, suggesting manipulation of the study protocol in violation of standard research practice.
<b>Errors, ambiguities, misrepresentations</b>	The poster, paper, and press statements contain misstatements, overreaching claims, deceptions, omissions, and errors, raising questions of competence and objectivity relative to the study subject.

In addition to the undisclosed direct sponsorship, the lead author has acted as a paid expert consultant for J&J in legal proceedings against the company brought by a family of a child with autism.<sup>20</sup> Dr. Miles failed to disclose her paid consulting work in both the paper and the press release.

Dr. Miles contends that J&J did not influence her research.<sup>6</sup> Whether by intent or subconsciously, the manufacturer's hand is evident in the study design. The survey questionnaire only asks about one brand, "Rhogam" (see Figure 2), and adequate procedures to elicit other brand usage were not instituted, so all exposures reported were for "Rhogam" (see Exposure Determination section below).

Dr. Miles has other conflicts. She is a geneticist by training and a past board member of the American Society for Human Genetics. She favors genetic explanations for autism, perhaps based on a desire to promote funding for such investigations, and dismisses environmental theories. Her clinic's website states that

"Dr. Miles' research interests are the delineation of the clinical and genetic heterogeneity within the autism behavioral diagnosis and how this information can be used to improve diagnosis, find specific genetic and epigenetic causes and to direct treatment choices which will improve outcomes." Consistent with this orientation, the beginning statement in the *AJMG* abstract that "causes of autism are considered largely genetic" ignores the scientific literature over environmental factors and the likely interplay of genetic susceptibilities and environmental agents in autism etiology.<sup>22,23</sup>

***Autism Sample Selection Bias*** - Miles & Takahashi accuse the Holmes clinic (the Geier study had not been released at the time of the *AJMG* publication, so it was not addressed) of "without doubt creating an ascertainment bias toward Rh- mothers who received RhIg" because it espoused a particular treatment for mercury (chelation) and relied on parent self-report of Rh- status. They contrast these problems with their own clinic, which they promote as "free of ascertainment biases" since it "espoused no specific treatment

paradigms” and relied on medical records rather than recall.

The Miles & Takahashi assessment of Rh-negative blood type incidence was based on mothers of individuals with autism seen at their clinic from 1995 to 2005. The authors focus on aspects of their *clinic* and the families who come there for *ascertainment*, and assure the reader that their center would not attract families biased toward or away from characteristics impinging on

Rh or RhIg status. They imply that because the ascertainment at their clinic is unbiased, then their study *sample* would not be biased. However, the study participants are a minority subset of the autism families seen at the clinic during this period, representing just 34% or 214 out of 626 individuals who qualified as having an autism spectrum disorder (ASD). The *AJMG* sample disposition is summarized in Table III.

**Table III. Miles & Takahashi Autism Sample Disposition**

- 765 consecutive individuals were evaluated at the clinic 1995-2005.
- 626 met autism spectrum disorder criteria.
- 525 individuals had “at least one available parent”.
- 305 of the 525 mothers were reached by phone, and 220, or 42%, could not be reached. These are the families designated as “lost to follow up”.
- 19 mothers of the 305 reached by phone refused to participate in the phone survey, leaving 286 mothers who presumably agreed to continue and were mailed forms for release of medical information.
- Of the 286 mothers sent release forms, 214 returned them, or 75%, and 72 did not respond, effectively declining to participate. The total declining, either by phone (19) or mail (72) is 91, or 30% of 305 mothers reached by phone.
- Thus, of 525 eligible families with an available parent, just 214 or 41% participated and the remaining 59% were refusals or no longer traceable by the clinic.

The final sample consisted only of those families who could be found for follow up and agreed to participate. While the authors note that participating, declining, and “lost to follow up” mothers were similar on standard demographic characteristics, no determination was made of differences in the perceptions of the clinic by those who participated and those who declined or were untraceable. No determination was made of the reasons why 220 families were no longer available to the researchers.

Rather than being representative, the autism sample is likely to be skewed in a manner related to the exposure variable. As noted above, this clinic portrays autism as a genetic condition and provides genetic-oriented services. It is part of the Division of Medical Genetics at the University of Missouri Hospital and openly states this affiliation. Its website, [www.genetics.missouri.edu/Autismhome.htm](http://www.genetics.missouri.edu/Autismhome.htm), states, “Our research is focused on delineation of the clinical and genetic heterogeneity within the autism behavioral diagnosis and how this information can be used to improve diagnosis, gene finding and treatment for children with autism.” The clinic communications materials mostly disregard environmental agents as

factors in autism causation or severity. As early as 2002, before study recruitment started, Miles publicly expressed her skepticism of a connection between thimerosal or vaccines and autism, stating as a co-author in a web document, “no scientific evidence for a relationship between vaccines and autism has been identified.”<sup>24</sup>

The *AJMG* paper cites a recent survey (Mercer, 2006) reporting that 40% of autism parents feel that vaccines played a role in their child’s autism. Many if not most parents in Missouri who suspect a connection would feel less welcome at a “genetics only” site that is hostile to their beliefs, and once diagnosed (ascertained) they would be motivated to take their child to other centers which they viewed as offering more effective treatment options based on an environmental causation approach. These families would be more likely to be lost to follow up. In addition, the study telephone survey explicitly asked about rhogam administration and receipt of vaccines and immunizations, and the mailed survey asked for the signed release forms to be sent to obstetricians, pediatricians, and clinics to obtain immunization histories. If they were concerned about research bias, environmentally-oriented families would

be more likely to decline participation in the research. It is these mothers who would also be more likely to have received RhIg with thimerosal and correspondingly, be Rh-negative. This bias would have artificially lowered the percentage of Rh- and RhIg-exposed mothers in the study. Direct evidence for this effect is the result reported for the 91 mothers who declined to participate: this group reported a higher percentage of Rh- blood type on the telephone, 19.8% compared to the 15.4% of the 214 participating mothers. The authors ascribe the higher rate to a recall effect but do not substantiate the claim.

Another indication of the bias toward excluding RhIg exposed mothers is that none of the 29 ASD mothers received multiple doses of RhIg, yet in the 1990s to the present, a significant fraction of pregnant Rh- women were given multiple RhIg injections. ACOG recommended RhIg in addition to the 28 weeks gestation dose in cases of abdominal trauma, invasive procedures, bleeding, or late delivery. "If RhoGAM is administered for one of the above indications early in pregnancy (before 26 to 28 weeks), there is an obligation to maintain a level of passively acquired anti-D by administration of RhoGAM at 12-week intervals."<sup>10</sup> In addition, ACOG stated, if a woman is given the standard injection at 28 weeks but the pregnancy "has gone past the due date, a doctor may suggest another dose of RhIg." About 16% of pregnant women experience bleeding,<sup>25</sup> about 10% of pregnancies go past 42 weeks gestation,<sup>26</sup> 6-7% of pregnant women experience complications from trauma and accidental injury,<sup>27</sup> and about 7% of pregnancies are subject to invasive procedures in the first trimester.<sup>28</sup> Given an n of 29, at least a few study mothers would be expected to have had a pregnancy that would warrant more than one injection.

**Poorly Chosen Control Groups** - The *AJMG* paper cited 3 control groups for the Rh typing comparison: a clinic-based chromosomal disorders group, a University of Missouri patient sample, and Missouri-Illinois Red Cross blood donors. It relied on one group for the RhIg analysis: a subset of the chromosomal disorders group. By contrast, in the 2005 poster version, siblings of children with autism were used, and clinic children with Down syndrome (DS) were separated from those with other genetic disorders to form a total of 3 control groups. All 3 groups were used for the Rh typing analysis; the sibling and DS groups were used for the RhIg analysis.

The authors reported incidences of Rh-negative blood type for the various case and control groups, summarized in Table IV. There is a 64% difference in the reported Rh-negative rate between the highest and lowest control groups (25.0 versus 15.2). There is a 62% difference

between the highest control group and the lowest case rate (25.0 versus 15.4), and a 28% difference between the highest case and the lowest control rate (19.5 versus 15.2). There is even a 27% difference in the autism group reported in the poster and *AJMG* versions (19.5 versus 15.4). Given this variability, it is hard to tell whether a real difference in Rh blood type exists between autism mothers and the non-autism population, or whether failure to detect a statistically significant difference is due to sampling problems of the autism group, as explained above, or to sampling problems of the controls. While some of these differences might seem minor, when attempting to calculate risk from a widely used product (~15% of pregnancies), even relative risk between 1.0 and 2.0 is meaningful.

**Table IV. Frequency of Rh-Negative Blood Type in Study Groups (%)**

<b><u>Controls</u></b>	
15.4	for the chromosomal disorders + Down syndrome group in <i>AJMG</i>
15.2	for University of Missouri patients in <i>AJMG</i>
17.7	for Red Cross donors in the Missouri-Illinois region in <i>AJMG</i>
25.0	for Down syndrome mothers in 2005 version
23.1	for genetic clinic mothers in 2005 version
23.3	for mothers of non-autistic siblings of autism cases in 2005 version
<b><u>Cases</u></b>	
15.4	actual in <i>AJMG</i>
18.0	expected if ethnicity-adjusted based on Red Cross data (given heavy Caucasian composition) in <i>AJMG</i>
19.5	in the 2005 version [ethnicity adjustment not provided]

In addition to these wide differences of Rh- status and population targets, the specific composition of the study control populations is problematic. The authors make no attempt to carefully match or delineate the control and autism groups on the critical characteristics of ethnicity, birth year, and gender. The single small control group for the RhIg analysis in the *AJMG* paper is inadequate and has probable diagnostic overlap with the autism group. Several control groups reflect selection bias.

**Ethnicity** - The incidence of Rh-negative blood type varies greatly among U.S. ethnic groups, as shown in Table V. Ethnic composition of a research sample can differ geographically or by sample recruitment practices. So, for example, a non-autism study among women giving birth at two hospitals in Indiana reported an Rh-rate of 11.7%,<sup>29</sup> and a study of patients undergoing genetic amniocentesis at a medical center in St. Louis was 15%.<sup>30</sup> Holmes *et al* reported a 9% Rh- incidence in their nationwide control group recruited through autism channels, and Geier & Geier reported 14.4% among non-Jewish Caucasians. If ethnic composition between case and control groups is not carefully matched, comparisons of Rh- prevalence will be inaccurate.

**Table V. Frequency of being Rh-negative for certain population\***

Population	% Rh-Positive	% Rh-Negative
Caucasian	85	15
African-American	92	8
Hispanic	92	8
Asian	99	1
Native American	99	1

Source: Reid ME, Lomas-Francis C.  
The Blood Group Antigen Facts Book.  
New York, NY: Academic Press, 1997.

\*Obtained from RhoGAM ®  
Ultra-Filtered website,  
[http://www.rhogam.com/English/Patients/rh\\_meaning.aspx](http://www.rhogam.com/English/Patients/rh_meaning.aspx)

The *AJMG* paper only provided the ethnic breakdown for the autism group. Ethnic composition was not provided in the paper or poster for any of the control groups, and Rh- rate by ethnic group was not provided for cases or controls outside of the Red Cross group.

**Birth year** - In calculating autism risk from RhIg with thimerosal, birth year is important because it is a surrogate for year of antenatal exposure, and antenatal RhIg usage has changed significantly over the past 40 years. Antenatal RhIg was not common until the mid 1980s and not routine until 1989-1990. The rate of prenatal invasive procedures warranting additional RhIg doses rose substantially between 1986 and 2002.<sup>9</sup> The average RhIg brand contained much more thimerosal in the early 1990s than the later 1990s. In April 2001, thimerosal RhIg production ceased, but older lots continued to be sold as shelf life was two years, making 2001 and 2002 transition years.

Miles & Takahashi provide no information on birth year for their autism group. They provide an average age and an age range, but do not provide the date at which the age was determined, whether it was when the child was first seen at the clinic or whether it was the age when the telephone interview was conducted with the mother. Birth date information was also lacking for the chromosomal disorder group in the *AJMG* paper and in the 2005 poster version for the autism, siblings, DS, and genetic clinic groups. Without birth year information, it is impossible to know if these groups received higher or lower amounts of mercury from RhIg.

In the analysis of autism risk from RhIg with thimerosal, Miles & Takahashi used a single control group in the *AJMG* paper, the chromosomal disorders group. For this analysis, both the control group and the autism group were restricted to birth years prior to 2002. The restriction led to the exclusion of 58% of the chromosomal disorders group, reducing the sample from the 65 mothers in the Rh blood type analysis to the 27 mothers remaining in the RhIg analysis. This reduction suggests that 58% of the chromosomal disorder children were born in 2002 or later. In contrast, the pre-2002 restriction led to only 6 of 214 autism mothers being excluded, meaning just 3% of the autism group had children born in 2002 or later. The mean age of the autism group was 7.2 years and the highest age was 23.5 years. The older age of the autism children relative to the much younger age of the chromosomal disorders children confirms how poorly matched the cases and controls were on the one key characteristic of birth year.

**Gender**- Gender is also relevant for matching, because autism is more common in males, with a ratio of 4:1, and because there is evidence that young males are more sensitive to mercury than young girls.<sup>31</sup> Investigations of mercury or autism should have control groups that are matched for gender.

Information on gender was provided for the autism group in the *AJMG* paper but not for the sibling, DS, genetic clinic, or genetic disorders groups in the paper or poster. Down syndrome is generally observed equally in males and females, and there is no evidence that one gender is over-represented in autism siblings, so obviously controls and cases were not matched on gender.

**Inadequate RhIg control group** – Despite having many other more suitable control populations, the *only* control group in the peer-reviewed *AJMG* paper used for the RhIg/thimerosal analysis is the chromosomal disorders group. The large University Hospital and Red Cross “general populations” were not part of this critical

analysis. The chromosomal disorders group used in this analysis had just 27 mothers and only 4 of them were given RhIg. This small number provides poor statistical power, especially since the composition of this control group in terms of ethnicity, birth year, and gender is unknown. The authors note the size limitation in a single sentence in the ninth paragraph of the Discussion section – “An additional constraint is the small age matched [sic] non-ASD control population” – but they never come to terms with its ramifications on the study’s validity.

*Case & control overlap* – Although these claims are subject to some controversy, some estimates attribute 5-14% of ASD cases to known genetic disorders and chromosomal anomalies.<sup>32</sup> Many of the chromosomal disorders children used for the *AJMG* RhIg analysis had DS. Emerging research from small studies is reporting meaningful comorbidity between ASD and DS, with 38-45% of DS children having ASD or ASD symptoms. The ASD symptoms in DS children are manifesting later than in non-DS ASD children, an average of 4-5 years versus 1-2 years.<sup>33</sup>

In scientific studies, cases and controls are not supposed to overlap; otherwise, a comparison is being made to the same group. Miles & Takahashi state that “autism was excluded” from their chromosomal disorders group. They never explain their exclusion methods. They never reconcile their methods with their description of case ascertainment procedures, which do not mention excluding children with comorbid DS or chromosomal disorders, nor with their control ascertainment procedures, which do not mention excluding dual-diagnosed children. As noted above, their chromosomal disorders group is heavily skewed to younger children, so it is possible that the DS children were too young to exhibit autism features.

*Selection bias* – The chromosomal disorders controls skew much more heavily to younger children than the cases, and the reason is never given. The autism sample universe was children consecutively entering the clinic from 1995 through 2005. Presumably, the investigators would have a similar span for their control group, since the autism clinic is contained within the genetics division. It would make sense for the genetics clinic group to be identified and recruited under the same process as the autism patients, in order for the control group to have validity as a matched population. The choice of only including a younger control group raises the concern again over a sampling selection bias, this time in controls.

Sample selection bias is inherent in the Red Cross donor group. The Red Cross actively recruits for type O

Rh-negative donors because this type is a “universal donor” compatible with any other blood type. Thus Rh-donors will be over-represented in this group. Blood donors represent just 5% or less of the U.S. population, and the Red Cross handles just 45% of the blood supply. The majority of donors are men, and the average age is 38 years.<sup>34,35</sup> The Red Cross sample included donors in 2005-2006. This group is very different from the autism mothers in the study – female, younger, and in most cases pregnant prior to 2002. Sometimes a large “general population” comparison sample can be impressive, but the quality of the controls in terms of matching to the cases is more important.

There is no description of the University of Missouri Hospital patients, the other “general population” control group.

*Incorrect RhIg Mercury Exposure Determination* – The *AJMG* paper states that all autism mothers received the RhoGAM® brand, and “when there was any question about the brand of RhIg used the hospitals or obstetrical offices were called to clarify the information.” They explain that since its launch in 1968 RhoGAM® has claimed “70-90% of the market” for RhIg. The market share claim is unlikely to be true for the period of interest, however. From 1996 onward, when many of the autism mothers in the study would have been pregnant, a brand of RhIg, BayRho-D, was sold in the U.S. that did not contain mercury. During 1996-2000, RhoGAM® had a 54% market share and BayRho-D had 41%, with other brands holding the remainder.<sup>36</sup> Miles & Takahashi probably misidentified the brand. The term “rhogam” is commonly used to refer to both the brand, RhoGAM®, and the generic RhIg product.<sup>37</sup> Medical staff will often write the word “rhogam” in records and refer to the RhIg product as “rhogam” in talking to patients, when in fact a different brand was administered. The only way to determine the real brand is to identify the lot number from the medical record and contact the manufacturer or FDA. Miles & Takahashi omitted this important step, relying only on mothers’ recollection of, and medical staff’s recording of, the word “rhogam”.

In her Missouri symposium presentation, Dr. Miles stated that the study designated a child as having been exposed to mercury from RhIg if the exposure occurred in an “antenatal” dose prior to 2002. In April 2001, J&J changed the formulation of RhoGAM® so it was mercury-free. The decision to define mercury exposure via RhIg based solely on a 2002 cut off is erroneous. Since the formulation change occurred just one quarter of the way into 2001, children born that year could have been exposed to a mercury-containing or a mercury-free formulation.

Thus, many children in the study designated as exposed to thimerosal from RhIg were likely not exposed. If in fact Miles & Takahashi are correct in their assertion that 100% of their autism mothers received thimerosal-containing RhoGAM®, then the implication is that autism mothers were more likely to be exposed to RhIg with thimerosal than would be expected from a pre-2002 cohort, and there is in fact an increased risk for autism from thimerosal-containing RhIg.

**Omission of Standard Toxicological Methodologies** -

The study definition of exposure is dichotomous, either exposed or not exposed, yet the amount of mercury per single RhIg injection could have varied from zero to 200 micrograms (Table I) and some women could receive multiple injections. Since the authors maintain only one thimerosal-containing RhoGAM® injection was given to their Rh- cases, the dose-response effect of exposure variation was not measured or considered.

The authors do not consider sources of mercury besides RhIg. Since effects may be cumulative or the result of “multiple hits” during gestation or infancy, the combined effect of RhIg thimerosal with mercury from maternal amalgams and from vaccines, food, air, and water to mother and infant should be evaluated. For example, two studies have found a higher rate of autism in locations with higher environmental mercury releases,<sup>38,39</sup> the Holmes study found an elevated autism rate in children with mothers who had more dental amalgams, and another study found a higher autism rate in children exposed to DTP with thimerosal relative to DTP without thimerosal.<sup>40</sup> Consideration of other exposures is especially warranted if there are age matching problems. Since the birth years of the cases appear to concentrate around 1990-2002, in large measure spanning the years of maximum exposure to thimerosal-containing infant vaccines, a bias in control groups to older or younger children would plausibly lead to a relatively lower or higher cumulative mercury exposure in cases. Following standard toxicological risk assessment approaches, the study design should have obtained data on other known exposures and controlled for them in the analysis.

**Sample Alterations** - The 2005 poster sample composition differs from that of the *AJMG* paper, suggesting changes were made to the protocol well into implementation. The 2005 sample consisted of 201 children with autism and 154 mothers of children with autism, meaning that as many as 47 of the mothers had more than one child with autism (201 – 154 = 47). The *AJMG* paper describes 230 children with autism and 214 mothers, so that only 16 mothers could have had more than one child with autism (230 – 214 = 16). The

elimination of 31 ‘multiplex’ families (47 – 16 = 31), means that a high proportion of the original sample, 62 of the original 201 children (31%) and 20% of the mothers was appreciably altered. It cannot be determined if similar alterations were made to the simplex families.

The removal of multiplex families would have interesting implications on the results, since multiplex families, as shown in the *AJMG* paper, Table III, were less likely to be Rh- (6.3% vs. 16.2% of simplex) and none received RhIg (0 out of 16; 2 expected). Yet, in the poster presentation version, when there were more multiplex families, the percent of Rh- mothers was higher than the 2007 final results, 19.5% vs. 15.4%.

The *AJMG* ASD sample likewise had fewer minority (non-Caucasian) families than the 2005 sample. The number dropped from an original 16 to just 9. Minorities have about half the Rh- rate as Caucasians, yet the 2005 Rh- results were higher, despite more minority representation (albeit still a small fraction of the total sample).

As noted above, the control groups were also altered between the 2005 poster and the journal version. The poster had three control groups for both the Rh typing and RhIg analyses: siblings, DS clients, and genetic clinic patients. The *AJMG* paper had 3 controls for the Rh blood type analysis – chromosomal disorders, hospital patients, and Red Cross donors – and the RhIg analysis had one control group – chromosomal disorders. No explanation has been given for why these control groups were removed or added. It is conceivable that, given the implications of the sibling data analyzed in the Risk Recalculation section below, the authors altered the original sample as the results were not in accord with the anticipated findings.

**Errors, Ambiguities, & Misrepresentations** - The poster, paper, and press statements contain a range of errors and omissions, misrepresentations of the study quality, and deceptive claims in reference to vaccines, thimerosal, and prenatal exposures. The nature and extent of these distortions, whether deliberate or not, raise questions of competence and objectivity relative to the study subject.

**Deceptive statements** – Deceptive statements on vaccines, thimerosal, and prenatal exposures all have the effect of minimizing perceptions of harm from any thimerosal exposure, in RhIg or elsewhere, in the past or present. They include the following:

- The press release headline, “Study Finds No Link Between Autism and Thimerosal in Vaccines.” It bears no relationship to the objectives of the study,

which is about thimerosal in RhIg, an immune globulin. Medical literature does not refer to immune globulins as vaccines.

- The conclusions of the abstract, "These findings support the consensus that exposure to ethylmercury in thimerosal is not the cause of the increased prevalence of autism." There is no consensus,<sup>22</sup> and this study did not look at postnatal vaccines containing ethylmercury.
- The press release and *AJMG* paper statement that "by 2002, all routinely recommended early childhood vaccines were thimerosal free." The influenza vaccine is a routinely recommended vaccine for infants and pregnant women and the vast majority of flu vaccine doses contain thimerosal. The claim tries to reassure the reader that thimerosal no longer poses a risk.
- The *AJMG* Introduction statement that "some low birth weight infants" were over the EPA limits for safe mercury exposure from routine infant vaccines in the first 6 months of life. This statement is false. Per calculations made by FDA, cumulative exposure at 6 months exceeded EPA – as well as WHO – limits for all infants, not just small babies.<sup>41</sup> From a daily dose perspective, all infants were many times over the limits if they received just one thimerosal-containing vaccine.<sup>42</sup>
- In the 2005 poster description of medical practice regarding the Rho D immunization procedure, "Rh immune globulin...[is] an immunization given to Rh- women *after delivery* [emphasis added] of an Rh+ baby to prevent blood incompatibility in future pregnancies." This statement misleads the reader that RhIg is an immunization, i.e., a vaccine, and that the exposure of interest is postnatal rather than *during* pregnancy.
- The *AJMG* Introduction statement that the "thimerosal is diluted before reaching the fetus and has been assumed to be innocuous". This is an egregious misstatement considering the placenta concentrates mercury resulting in an average 70% higher cord blood mercury than maternal and up to 3 times more mercury exposure to the fetus relative to the mother. The pharmacokinetic effects would be compounded given a recent EPA report estimating that one in six women of childbearing age already has mercury levels in her body that could cause neurodevelopmental injury to her unborn child.<sup>43</sup>
- The Discussion statement implying that RhIg was fully responsible for the reduction in RhHD, when it is well known that smaller family size is credited with a sizable portion of the decrease. The wording leads the reader to believe that *antenatal* RhIg was the critical factor in RhHD and isoimmunization reduction when the vast majority of the decline is attributable to *postpartum* RhIg. While postpartum RhIg was uniformly recognized as a medical breakthrough, routine antenatal RhIg was controversial based on cost-benefit considerations.<sup>7</sup> The authors' over-promotion of antenatal RhIg reflects a common technique employed to diminish product safety concerns by bolstering potential benefits in order to win the argument that "the benefits outweigh the risks".
- The Introduction statement that concerned families are being fueled by class action litigators. Currently there are no class action lawsuits filed nor are any expected to be filed regarding thimerosal.
- The Discussion statement that "Mercury is not on the list of agents that effect DNA methylation," which ignores research by Richard Deth and Jill James that mercury and especially thimerosal can alter DNA methylation pathways.<sup>44,45</sup>
- The use of the finding of no increased rate of maternal-fetal Rh incompatibility in autism relative to controls as a rationale for dismissing any role of Rh-related effects in autism. An earlier study<sup>14</sup> found a higher rate of Rh or ABO incompatibility but did not distinguish between Rh and ABO incompatibility, and the birth dates of the study group predated the routine use of antenatal RhIg.<sup>15</sup> Another study<sup>12</sup> found a higher Rh incompatibility rate relative to controls – 12% vs 3%. A later study has been in agreement on lack of risk from Rh incompatibility.<sup>13</sup> None of these efforts looked at rates of maternal Rh-negative blood type in the mother or antenatal exposure to RhIg. Of interest is that the incompatibility studies, Miles & Takahashi included, did not investigate the effect of complications from Rh sensitization as a factor. Complications include hyperbilirubinemia, anemia, and hydrops fetalis. The earlier studies hypothesized that the complications, rather than the incompatibility, might play a role in autism. Since inception of routine postnatal RhIg administration coupled with smaller family sizes leading to less sensitization, the percent of Rh- women producing Rh antibodies has fallen dramatically to less than 1%. None of the recent compatibility studies have investigated RhHD complications in autism

outcomes or determined how RhHD trends may impact assessments of expected rates of autism among Rh- mothers.

*Misrepresentation & errors* – Misrepresentations of the study's quality on sample selection and RhIg brand ascertainment have been noted above. Additionally, the *AJMG* abstract implies that the key analysis of thimerosal exposure between the autism and control groups was based on a large representative control group sample of the "general population". It states, "Rh- status is no higher in mothers of children with autism than in the general population, exposure to antepartum RhIg, preserved with thimerosal is no higher for children with autism and pregnancies are no more likely to be Rh incompatible." As previously described, the only control group for the critical RhIg/thimerosal comparison was with a small (n=27) chromosomal disorder group.

There are several errors regarding RhIg. The *AJMG* abstract says that RhIg contained thimerosal "until 2001". RhIg was made with thimerosal until April 2001. Since it has a two year shelf life, actual exposure to RhIg with thimerosal would have continued into 2002. The RhIg brand review in the Discussion says that BayRho "contained thimerosal at higher [mercury] concentration" than RhoGAM® when BayRho never contained mercury; rather its predecessor brand, HypRho-D, contained thimerosal and BayRho never did. No mention was made of the RhIg brand Gamulin by Armour Corporation, which had a significant market share in the 1990s and had the largest amount of mercury of any brand due to high content (0.01% mcg/mL) and volume (1.0-2.0 mL average dose) (see Table I).

### **Risk Recalculation Based on Equivalent Controls**

Our review of the Miles & Takahashi research has demonstrated how the autism sample may under-represent Rh- and RhIg-exposed mothers, and how a given control group may also reflect selection bias or be small, unmatched, and possibly overlapping. Among the groups for which data have been reported, the best comparison set, albeit not ideal for reasons of birth year

and gender matching, is the autism cases and the sibling controls, with the data derived from the 2005 poster. It is superior because comparison quality is independent of mothers not responding/lost to follow up and selection biases in controls; ethnic composition is equal; diagnostic overlap is eliminated; there is similar genetic susceptibility; and possible sample manipulation is minimized. This comparison would involve almost or all the same mothers in each group, since there were 224 siblings across 154 mothers, the majority of mothers would have had at least one non-ASD child. The poster says all the mothers' results were confirmed by medical record review, so data are not based on recall.

The relevant data and calculations for the sibling and case groups are presented in Table VI. For this analysis, it is important to distinguish between mothers and children, who can be those with an ASD or non-ASD siblings. In the autism group, 19.5% of the ASD children were born to Rh- mothers, equating to 39 out of the total of 201 ASD children. In the autism group, 12.9% of the ASD children were exposed to RhIg, equating to 26 out of the total of 201 ASD children. Given 26 exposed children out of 39 children, 13 ASD children of Rh-women were not exposed to RhIg, or 6.4% of 201 total ASD children. The RhIg exposed to unexposed rate ratio in ASD children is 0.129:0.064, or 2.02.

In the non-ASD sibling group, 23.3% of 224 siblings, or 52 children, were born to Rh- mothers. In the sibling group, 12.6% of 224 siblings, or 28 children, were exposed to RhIg, leaving 24 of the 52 children with an Rh- mother not exposed, or 10.7%. The rate ratio of exposed to unexposed siblings is 0.126:0.107, or 1.17.

Comparing the two exposure ratios of 2.0 and 1.17, the calculation shows that the autism children were 71% more likely to have been exposed to RhIg *in utero* than their non-ASD siblings. This increased risk for autism from RhIg is consistent with the findings of the two other published studies on RhIg administration by Holmes and Geier of 5.11 and 2.35 times greater risk respectively using different types of controls.

**Table VI. Frequency of RhIg Exposure Among ASD Children and Siblings Born to Rh-Mothers\***

Sample	Calculation	ASD Children		Non-ASD Siblings	
		%	#	%	#
Total (a)	n/a	100	201	100	224
Having Rh- mother (b)	$a\# \times b\% = b\#$	19.5	39	23.3	52
Having Rh- mothers + receiving antepartum RhIg – exposed (c)	$a\# \times c\% = c\#$	12.9	26	12.6	28
Having Rh- mothers + not receiving antepartum RhIg – unexposed (d)	$b\# - c\# = d\#$ $d\# \div a\# = d\%$	6.4	13	10.7	24
Exposed/unexposed rate ratio (e)	$c\% \div d\% = e$	2.02		1.17	
RhIg risk ratio (f)	$e(\text{ASD}) \div e(\text{sibling}) = f$	1.71			

\* Data obtained from Miles & Takahashi 2005 version shown in italics.

The limitation to this re-analysis of the Miles & Takahashi data is the high number of Rh- pregnancies in either group not given antepartum RhIg.

## Conclusions

The Miles & Takahashi research demonstrates pervasive flaws, including sample quality, exposure assessment, deviation from standard research practices, and numerous errors and inaccuracies. Its conclusions contradict other studies on the subject which have found an increased risk of autism from antenatal RhIg. Its own data indicate a similar if less pronounced increased risk of 71% when alternate statistical methods are applied to a comparison of ASD children with the more appropriately matched and larger control group of siblings.

Every study has limitations, but cumulatively as well as for the individual seven problem areas detailed in this review, the shortcomings in the Miles & Takahashi study raise questions of its validity. It is not unreasonable to conclude that a number of these problem areas could have had a material impact on the direction of the results on the key question of thimerosal risk in autism. The most problematic deficiencies, those that are likely to have distorted the findings on autism and RhIg from a true positive risk to the observed no risk, are: (a) bias in sample selection so RhIg-exposed mothers are undercounted and therefore a true increase in RhIg-exposure in autism mothers was not observed; (b) a single control group that is small, unmatched and misrepresented, which alters the conclusions from being “strong” or definitive as promoted to in actuality being quite weak, as well as invalidating the observed risk calculations; and (c) miscalculation of the thimerosal exposure from RhIg in the autism mothers, leading to a

logical assumption that autism mothers in fact had higher exposure to thimerosal RhIg.

The deficiencies of the research are compounded by the way in which it was promoted to the public. Some studies are exploratory and hypothesis driven. If presented as such they can be valuable in advancing science even if they do not reflect the most careful and rigorous study design. This research, however, despite serious flaws, was promoted widely and as definitive proof of no risk for autism from not just RhIg with thimerosal, but all thimerosal and all vaccines.

Dr. Miles has said that Johnson & Johnson did not influence her research.<sup>6</sup> Of greater relevance is why the largest manufacturer of RhIg, a multi-billion dollar company, chose to fund this particular effort rather than a more rigorous and standard product safety study. Safety study methodologies would involve larger samples and utilize a case-control design comparing rates of Rh- mothers of children with autism receiving RhoGAM® with those receiving BayRho-D, or, alternatively, a design comparing RhIg exposure among mothers of neurotypical children with that of mothers of children with autism, matched on birth year, gender, and ethnicity, with RhIg brands and dose volumes carefully tracked. Such efforts are warranted given the public’s understandable concerns over the safety of mercury in RhIg products given to pregnant women and the epidemic increase in autism resulting in a national public health crisis.

Credible conclusions on Rho-D immune globulins and autism await further studies using sound methodologies and adequate samples by unbiased investigators.

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## Figure 1. Press release of Miles & Takahashi AJMG paper

----- Original Message -----

**From:** Faddis, Jennifer "><<mailto:faddisj@missouri.edu>> <<mailto:faddisj@missouri.edu>>

**Sent:** Thursday, May 10, 2007 8:07 AM

**Subject:** Study Finds No Link Between Autism and Thimerosal in Vaccines (EMBARGOED)

Contact: Jennifer Faddis

Sr. Information Specialist

(573) 882-6217

FaddisJ@missouri.edu "><<mailto:FaddisJ@missouri.edu>> <<mailto:FaddisJ@missouri.edu>>

**EMBARGO DATE: May 16, 2007 at 12:01 a.m., Eastern**

### **Study Finds No Link Between Autism and Thimerosal in Vaccines** *Exposure to mercury preservatives before birth is not higher in children with autism*

COLUMBIA, Mo. – The increase in the number of diagnosed cases of autism in recent years has sparked concern that environmental toxins may cause this complex disorder. However, a new University of Missouri-Columbia study concludes that exposure to Rh immune globulin preserved with mercury-containing thimerosal before birth was no higher for children with autism.

“This study adds to the evidence that there is no casual association between thimerosal and childhood autism,” said Judith Miles, who is the William S. Thomson Endowed Chair of Autism and professor of pediatrics and pathology in the MU School of Medicine. “We conclude that there is no indication that pregnancies resulting in children with autism were more likely to be complicated by Rh immune globulin/thimerosal exposure.”

The study investigated thimerosal exposure during pregnancies that resulted in the birth of a child subsequently diagnosed with autism. Although experts anticipate that autism will be the first behavioral/psychiatric disorder for which major genes will be identified, there is still fierce debate that thimerosal, a preservative commonly used in vaccines and is almost 50 percent ethylmercury, is responsible for the rise in the disorder. Rh negative women are routinely treated with Rh immune globulin (RhIg) during the third trimester to prevent hemolytic disease, in which the mother’s immune system attacks fetal blood cells. Like many vaccines, RhIg manufactured in the United States contained thimerosal prior to 2001. Since young fetal brains are more susceptible to neurotoxic effects, researchers led by Miles, of the MU Thompson Center for Autism and Neurodevelopmental Disorders, assessed Rh status and thimerosal exposure of mothers of children with autism.

The study included 214 mothers of 230 children diagnosed with an autism spectrum disorder. Rh status, RhIg with thimerosal exposure and Rh incompatibility (in which the mother’s Rh status is different than the fetus’s) were established by reviewing medical records. The results showed that in children with autism, Rh negative status was no higher in their mothers than in the general population, that exposure to RhIg (preserved with thimerosal) before birth was no higher and that pregnancies were not more likely to be Rh incompatible.

“We hope this report of no association between autism, Rh negativity and thimerosal exposure during pregnancy will offset some of the decreased compliance with immunization recommendations which is known to increase morbidity and mortality from childhood infectious diseases,” Miles said.

Autism diagnoses have increased significantly during the past two decades, which coincides temporally with the addition of five pediatric vaccines to the immunization schedule, exposing children to increasing doses of ethylmercury, a known toxin. Though the vast majority of studies indicate no association between vaccines and autism, the FDA, CDC and American Academy of Pediatrics recommended that thimerosal be removed from all routinely recommended early childhood vaccines; this was accomplished by 2002.

Miles points out that even though RhIg and childhood vaccines are now free of thimerosal in the United States, it is important to analyze questions of safety since thimerosal continues to be used in many places around the world to preserve vaccines to help make them affordable.

Miles said that few studies have focused on pregnancies of Rh negative mothers who received RhIg during pregnancy, probably because the thimerosal is diluted before reaching the fetus and has been assumed to be innocuous. Nevertheless, there is a concern that even very small doses delivered when the brain is especially sensitive can be toxic. Numerous Internet sites and one research study assert that RhIg causes autism and that a high percentage of mothers of children with autism are Rh negative, neither of which was shown to be true in the current study. In addition, a recent study hypothesized that Rh incompatibility itself could disrupt fetal neurodevelopment, thus playing a role in autism, but the current study found no increase in the proportion of Rh incompatibility in mothers of autistic children. In response to the claim that only certain groups of children are at risk, the authors also analyzed specific autism spectrum disorder subgroups and found that none had significant increases in either Rh negativity or thimerosal exposure during pregnancy.

The study – “Lack of Association Between Rh Status, Rh Immune Globulin in Pregnancy and Autism” – was published in the May 2007 issue of the *American Journal of Medical Genetics*.



Figure 2. 2005 poster presentation

# Rh Immune Globulin in Pregnancy: Relationship to Autism Development

J.H. Miles, T.N. Takahashi, Division of Medical Genetics  
University of Missouri-Columbia

## Abstract

Autism is believed to be predominately a genetic disorder. However, recent increases in the prevalence of autism have raised the possibility that certain environmental exposures may cause or potentiate the development of autism. The most debated factor has been the increased exposure of children to mercury in the form of thimerosal, a preservative used to stabilize vaccines. Previous studies have concentrated on postnatal vaccines, however, we reasoned that exposure in pregnancy might have a greater effect on the developing nervous system. Since only Rh- women receive Rh immune globulin during pregnancy, any effect of Rh immune globulin exposure should be mirrored by an increased percentage of Rh-mothers of autistic children. We conducted a structured telephone survey to determine the blood type and Rh immune globulin exposure of mothers of autistic children and controls. We found no difference in the Rh- status of autism mothers compared to controls and found no evidence to suggest an etiologic role for thimerosal in the development of autism.

## Introduction

Autism is a neuropsychiatric disorder of childhood which has a complex and undoubtedly very heterogeneous etiology. Based on the high heritability factor (0.85 to 0.92), geneticists believe autism is predominately a genetic disorder. However, during the last 15 years there has been a dramatic increase in the apparent incidence of autism with increases in prevalence rates soaring from 1 in 2-5,000 to 1 in 2-500 children. It is unclear whether this is due to a true increase in the incidence of the disorder or to a combination of increased recognition of the symptoms and broadening of diagnostic criteria. Since there are no genetic epidemics, a major question is whether any environmental factors are responsible for the increased prevalence of autism. Because mercury is known to be neurotoxic in sufficiently high doses, it has been theorized that thimerosal, an organic mercury-based preservative used in vaccines, may be a cause for the increased prevalence of autism. Previous immunization studies investigating possible links with autism have focused on postnatal vaccines such as the Measles-Mumps-Rubella vaccine (MMR). This study focuses on prenatal thimerosal exposure in the form of Rh immune globulin or RhoGam, an immunization given to Rh- women after delivery of an Rh+ baby to prevent blood incompatibility in future pregnancies. Until 2001, RhoGam was manufactured with thimerosal as a preservative. The purpose of our study was to determine whether there is any evidence of a relationship between the development of autism and the exposure to Rh immune globulin/thimerosal during the mid trimester of pregnancy.

## Methods

### Subjects

Study subjects included mothers of 201 children with autism who were evaluated at the Missouri Autism Center between 1995 and 2003. Each child with autism met DSM-IV, CARS and our center based version of the ADI scoring protocol. Independent diagnostic evaluations were conducted by a child psychiatrist and a neuropsychologist. Subjects with a known syndrome or metabolic disorder were excluded from this study.

Control pregnancies consisted of 224 non-autistic siblings, 24 Down syndrome families and 13 families with other genetic disorders.

### Evaluation

A structured telephone survey was carried out to determine the blood type of autism mothers and control mothers. Results were verified by review of pregnancy, delivery and immunization histories. Each family was sent Release of Information forms to send to their obstetrician, birthing hospital and pediatrician or health clinic where immunizations were received.

### Structured Telephone Questionnaire

Questions	Answer	Level of certainty			
		Absolutely certain	Pretty sure	Think so	Don't know
Mother's ABO blood type	A B O				
Mother's Rh type	Rh- Rh+				
Father's ABO blood type	A B O				
Father's Rh type	Rh- Rh+				
History of Rh sensitization	Yes No				
Rhogam in pregnancy	Yes No				
Rhogam dose	Date Gest age Reason				
Did mother get any other vaccines in pregnancy?	Yes No Hep B Influenza Rubella				
Full siblings	Rhogam Yes No				
Maternal half sibs	Rhogam Yes No				
Immunizations	UTD Delay Unk				

## Results

- no significant difference in the Rh- status of autism mothers (19.5%, 30/154), Down syndrome mothers, (25%; 6/24) or genetic clinic mothers (23.1%; 3/13)
- exposure to Rh immune globulin during pregnancy was comparable for autism mothers (12.9%; 26/201) and Down syndrome mothers, (15%; 4/27)
- Rh immune globulin exposure during pregnancies of non-autistic sibs of children with autism was similar to the exposure in the autism pregnancies (12.9% vs 12.6%)

### Demographics of Autism Group (N=201)

Male:Female ratio	4:1 (161:40)
Mean age (SD)	8.0 (5.9)
Ethnicity	
Caucasian	92%
African American	3%
Asian	1.5%
Biracial/other	3.5%

	Autism	Non-autistic sibs	Down Syndrome control
Rh-	19.5%	23.3%	25%
Rh immune globulin exposure	12.9%	12.6%	15%

## Conclusions

- no indication that pregnancies of children with autism were any more likely to be complicated by Rh immune globulin/thimerosal exposure than those of controls
- no evidence of an etiologic role for thimerosal in the development of autism.

### Future studies:

- analysis of total thimerosal and organic mercury exposure for each autistic child and control.
- determine whether exposure to Rh immune globulin correlates with any specific subset of autism patients