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

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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.

Keywords: autism; mercury; environmental health; neurotoxin; neurodevelopment; blood

There is a marked increase in the diagnosis of autism. The question of what is (and is not) related to this increase is crucial to millions of persons affected by the disorder. This article reanalyzes an original data set regarding the relation between blood levels of mercury and diagnosis of an autism spectrum disorder (ASD) by Ip et al. based on our finding of discrepancies in the original article.[1]

A review of what is known about the neurotoxic effects of mercury is beyond the scope of this paper,[2] but the observable symptoms of acute mercury poisoning have been reported to match up with many of the problems observed in autism.[3] Furthermore, mercury poisoning has sometimes been presumptively diagnosed as autism of unknown etiology until the mercury poisoning has been uncovered.[4] Because there has been a several-fold increase in environmental mercury exposure, the hypothesis that the rise in autism could be related to an environmental increase in mercury levels is a reasonable one to pursue. Autism may result from a combination of genetic susceptibility (perhaps in the form of reduced ability to remove mercury or other neurotoxins from the system) and environmental exposure at key times in development.[5-7] This would mean a generalized increase in mercury levels would be expected to co-occur with a generalized increase in autism, but some people exposed to relatively high mercury would not be affected if, for example, their bodies were very efficient eliminators of such toxins. Only if an exposed infant or fetus also had a genetic susceptibility that makes one less able to remove mercury (or other heavy metals) would normal levels of mercury exposure lead to problems. Alternatively, it could be that genes that help detoxify get switched on and start to express themselves a little later than normal in those genetically predisposed to autism; or perhaps, autism results from some combination of these theories.

Nevertheless, if mercury does play any causal role in facilitating a diagnosis of autism, there would likely be at least some relation between high mercury measured in the blood and symptoms of autism even if ability to metabolize mediates the relationship between exposure and neural toxicity. This is because even if exposure is identical, those who remove mercury less effectively should still have higher levels in the blood. Interestingly, results of hair samples could be expected to be somewhat mixed. The level of mercury in hair may be better understood as an indication of how much mercury has been removed by the body as opposed to the level in the body.[6] If people are approximately equal in their ability to remove circulating mercury from the bloodstream, then these 2 indicators should match up closely, but if a person's ability to excrete is low, their hair samples might not be elevated even when their blood levels are high.

Fido and Al-Saad found that mercury levels in hair samples were higher in children diagnosed with autism.[8] These children were aged 4 to 7. In contrast, Kern et al. reported that mercury hair levels were not significantly different, but were lower at a marginally significant level.[9] Kern et al. used younger children, ages 1 to 6. Holmes et al. performed the

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most direct test of the hypothesis that autistic children may be deficient in terms of ability to remove mercury from circulation.[6] This study estimated mercury exposure of the mothers via a mercury exposure survey questionnaire. They then analyzed the first haircuts of the autistic children and a group of controls (the first haircuts would reflect mercury excretion in utero and very early life). In the autistic group, severity of autism was inversely related to hair mercury levels. This means that the more severe autistic cases actually had less excretion of mercury. Furthermore, among the normal children, hair levels of mercury were correlated to the mother's mercury exposure (as would of course be expected). But among the autistic children, there was no linear relation between the mother's mercury exposure and excretion of mercury in the hair. As the authors state, this pattern of results is easily understood if one considers "detoxification capacity of a subset of infants,"[6] (p 6) such that the bodies of those diagnosed with autism appeared to be less able to excrete and/or metabolize the mercury they were exposed to.

As the rise in autism is relatively recent, it is not surprising that research into the etiology has not kept pace. Indeed, there are few published articles that consider blood levels of children with mercury that utilize a control group; a psycInfo search using the words "autism," "mercury," and "blood" yields only one hit.[1] Given the high stakes involved, it is crucial that early reports of the connection between blood mercury levels and autism not be misstated. Even a small effect size would be of great theoretical and practical consequence.

In 2004, Ip et al. reported that no relationship existed between mercury blood levels and diagnosis of autistic spectrum disorder among a group of children with an average age of approximately 7 years. While attempting to estimate the effect size based on the Ip et al. statistics, we realized that the numbers reported by Ip et al could not be correct. The means and standard deviations reported in the 2004 article yielded an easily significant t value (autism mean = 19.53 nmol/L, SD = 5.6, n = 82; control mean = 17.68 nmol/L, SD = 2.48, n = 55 gives a $t = 2.283$, two-tailed $P = .024$ or one-tailed $P = .012$). Ip et al. wrote that the P value was "(P) = .15," [1(p432)] and that their data indicate "there is no causal relationship between mercury and as an environmental neurotoxin and autism." [1(p431)] After the error was brought to the attention of the authors, a new analysis was conducted by the original authors and they found the original t test to be in error and the P value to be a mistake (refer to Erratum, p. 1324). Based on their corrected analysis, the authors report the revised P value for their t test to actually be $P = .056$. We disagree on several grounds that these data indicate no significant effect exists, and report on a completely new reanalysis of the original data set.

Methods

Outliers were removed prior to statistical analysis. An outlier is defined as a score that is "substantially greater or less than the values obtained from any other individual." [10(p521)] Outliers have an unduly large influence on the outcome of a statistical test. What actually qualifies as an outlier differs depending on the research question and the statistician analyzing the results; however, values greater than 3 standard deviations either above or below the mean generally qualify as extreme cases.[11] Within the Ip et al. data, there were 2 such values that were not removed prior to our reanalysis. These 2 values were more than 3 standard deviations above the mean, and both of these values were far from any other score. (Other scores were within 3 points of the next individual; these 2 scores were each 15 or more points away from any other score in the distribution.) To avoid the appearance that these 2 outliers were removed to influence the statistical outcome as opposed to objective criteria for cleaning a data set, it should be noted that the biggest outlier of the 2 was an unusually high blood mercury level of 98, which was in the autistic group. To be clear—if anything, removal of the outliers resulted in a more conservative test as it actually decreased the mean difference between the 2 groups.

Results

Logistic regression was performed using blood mercury level as the predictor and the autistic/control group as the criterion. Results of this reanalysis indicate that blood mercury level can be used to predict autism diagnosis. Data included: $r = .20$, $r^2 = .04$, $F(1,133) = 5.76$, $P = .017$. This finding indicates that there is a statistically significant relationship between mercury levels in the blood and diagnosis of an autism spectrum disorder.

There was no difference in the mean hair levels where $t(135) = .24$ and one-tailed $P = .40$; this is essentially the same result reported in the original article. However, given that hair levels would normally be expected to be highly correlated to blood levels, it might be surprising that blood levels could predict an autism spectrum diagnosis, but that hair mercury levels could not. Indeed, hair and mercury levels for the full sample were correlated ($r = .86$, $P < .001$) indicating that about 75% of the variance in hair levels was accounted for by the mercury level in the blood. To us, the question turned to what the other 25% of the variance might be due, and whether the assumptions of the t test were violated. Although not the central focus of this report, these results could certainly help to inform future researchers of the nature of the relation between autism and mercury, and we include this information for completeness.

Exploratory Analysis. If one hypothesizes that persons with autism are less able to excrete mercury, especially when their blood levels get in the higher range, one might expect that the correlation between blood and hair levels would break down at the higher blood levels among the autism spectrum group (a type of heteroscedasticity).[5]

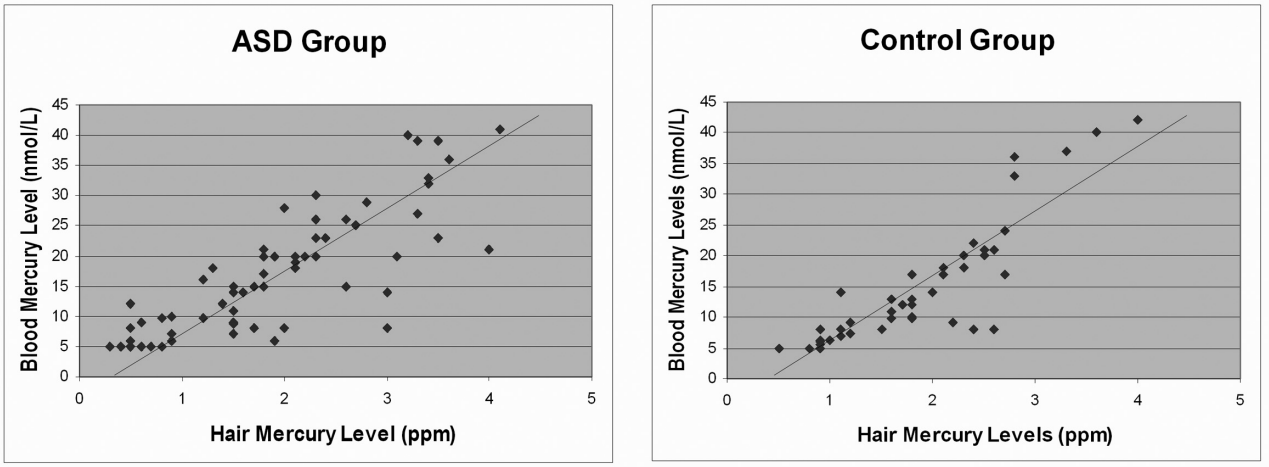

Figure 1. More variability is seen in the relation between circulating levels of mercury and hair levels (a mark of excretion) among those diagnosed with an autism spectrum disorder than among control group participants. Autistic participants are also significantly more likely to have lower hair excretion for the same blood levels. This is consistent with the idea that autism may be partly related to a lesser ability to rid the body of neurotoxins such as mercury.

Another way of looking at it, the relationship between blood level and hair excretion may be different for persons with autism than those without autism. Levine's test of equality of variance indicated the variance in hair mercury was not evenly distributed between the autism and control groups ($F = 5.98$, $P = .017$). We calculated the correlation for persons whose circulating levels of mercury were in the top quartile separately for the autism and control groups. The correlation between blood and hair levels of mercury was $r = .91$ for the control group (accounting for 84% of the variance). For the autistic group, the correlation was $r = .73$, meaning only about 55% of the variance in the hair mercury levels was attributable to the blood mercury level differences.

To check the hypothesis that hair excretion was overall lower than would otherwise be predicted based on a certain blood level in the autistic group, a best fit regression line was calculated ($y = 10.3$, $x = -2.48$) indicting that for each unit increase in hair level, blood level increased by 10.3 units. A t test on the residuals showed that autistic participants were significantly more likely to have lower hair mercury levels than would be predicted as a function of their blood levels, $t(133) = -2.92$, $P < .005$; see Figure 1). It should also be noted that the presence of unequal variances or nonrandom residuals (in this case, autistic persons are both more likely to have greater variability at high levels of circulating mercury and a lower hair value for a given blood level) are both violations of important assumptions of the t test; a t test of hair mercury is therefore probably not a valid means to predict autism diagnosis as a function of mercury exposure. We performed an analysis of covariance (ANCOVA) with autism diagnosis as the independent variable and hair mercury level as the dependent predictor using blood levels as a covariate. Results indicate that hair level may be related to diagnosis of autism, not as a predictor in terms of absolute value, but such that for equivalent circulating levels of mercury in the body, those with ASD excreted less than normal such that $F(1,134) = 3.9$ and $P = .05$. To sum, the relationship between blood levels of mercury and mercury excreted in the hair is reduced for those with autism compared with nonautistic persons; furthermore, the difference between autistic and nonautistic persons is most pronounced at high levels of mercury.

Discussion

In statistics, obtaining a probability value of $P < .05$ indicates that the obtained test statistic (based on one's sample) is extremely unlikely (less then 5% chance) to have been obtained by chance alone. By convention, this value is usually set at .05 (as a balance of type 1 and type 2 errors); however, this value is, in fact, arbitrary and statistical probability tables for hypothesis testing always include a range of probability values—not only probability at the .05 level. Given that this is the first direct test of this hypothesis and considering the potential importance of finding a relation between mercury blood levels and autism, it is just as important to avoid a false negative as a false positive. As the original authors have now currently calculated, the obtained difference suggests that there is probably a real difference (specifically that the chance that a real effect exists is about 94%, or, conversely, that the chance the null effect is true is less than

6%, which misses the conventional .05—or 5%—mark of statistical significance). Given the close value to conventional significance, most researchers would not call this a firm rejection of the hypothesis, but might say it was marginally significant. Most researchers facing a *P* value of .056 would not want to categorically state that results "indicate that there is no casual relation between mercury level . . . and autism."[1] It concerns us that the original authors would want to let this conclusion stand in light of the new *P* value (which differs markedly from the .15 previously reported in 2004).

Another issue to consider is the question of a one-tailed or a two-tailed hypothesis test. Usually, researchers use a two-tailed test, which tests if there is a "difference" between 2 groups. However, when the literature leads a researcher to propose a specific direction of the difference, a one-tailed test is called for, "Often a researcher begins an experiment with a specific prediction about the treatment effect. For example, a special training program is expected to increase student performance, or alcohol consumption is expected to slow reaction times . . . The result is a directional test, or what is commonly called a one-tailed test."[10(p246)]

Whether to use a one-tailed test or a two-tailed test can be decided based on considering what would happen if the results ended up in the opposite direction of what one suspects. In this case, it would mean that the blood mercury levels were lower in the autistic group. Would this support the original hypothesis? (No!) However, if this were to happen, that is, if the autistic group were significantly lower in their blood mercury levels than the normal group, the researchers would find themselves in the incongruous position of having to accept their hypothesis that autism is related to elevated levels of mercury in the blood! The key point here is that their hypothesis was directional, and a one-tailed test should have been used. In this case, the just missed significance of their new analysis using a two-tailed t-test ($P = .056$) would have reached a conventional level of statistical significance (with $P < .03$).

Although the statistics can be tedious, the bottom line is that only by an apparent error in the original data analysis was the original lack of effect found. The authors' revised calculation (*t* test) still has problems (two-tailed test for a directional hypothesis, not removing clear outliers). And finally, the willingness to characterize a *t* test with a .056 level of statistical significance as no effect is questionable, especially in this particular case.

Of utmost importance (which outweighs the discomfort of writing about an error made by colleagues whom we know are generally competent researchers) is that potential researchers who are trying to understand what is and is not behind the rise in autism are not misled by even the slightest misinformation. It is imperative that researchers, medical professionals, and the public at large have the full set of information. To put it in perspective, the connection between taking aspirin and prevention of heart attack has an effect size equal to .038 which represents an effect size approximately equal to what we find between circulating levels and ASD diagnosis in this age group.[12] Just as important is the fact that for those physicians in the aspirin group who did have a heart attack, the heart attack was less likely to be fatal. The effect size for this latter effect was .08 and did not represent a significant difference from the placebo group by traditional dichotomous significance testing.[13] Yet, this does not mean no effect exists or that the effect is not of practical importance. We would encourage all researchers to not only report whether a test of mercury and autism reaches significance with the sample size used, but to report the exact statistic and also effect sizes to help future researchers resolve all the factors involved in the etiology of autism.

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