July Article of the Month

Fetal and Postnatal Metal Dysregulation in Autism

Authors: Manish Arora, Abraham Reichenberg, Charlotte Willfors, Christine Austin, Chris Gennings, Steve Berggren, Paul Lichtenstein, Henrik Anckarsa¨ter, Kristiina Tammimies & Sven Bo¨lte

ABSTRACT

Background:

Autism Spectrum Disorder (ASD) encompasses a group of developmental disorders which affects a child's behavior and ability to socially interact with others. ASD can impact a person's emotional, social, and communication skills to a varying degree. The Centers for Disease Control and Prevention (CDC) has found that 1 in 68 children are estimated to have been diagnosed with ASD, and there is some evidence that ASD develops immediately before, during, and after birth[1].

While much is still unknown about the causes of ASD, recent findings have given scientists a window into what factors may contribute to ASD risk. There are several known genetic factors/markers that increase the likelihood that a child will have ASD. For instance, a child is at greater risk of being diagnosed with ASD if he or she has a sibling with ASD, if the child has older parents, and if that child is a boy[2]. However, if genetics alone explained 100% of ASD cases, then we would expect each pair of identical twins (who share identical genes) to present with the same ASD profile. This is not always the case, which indicates that environmental exposures also factor into ASD risk.

The scientific community has only just begun to identify certain environmental factors that may contribute to the development of ASD. Possible factors of interest include prenatal and early life exposures to metal toxicants (such as lead), deficiencies of essential elements (such as zinc), and exposures to and/or deficiencies of elements such as manganese which is an essential nutrient but may also have neurotoxic effects. A child may be exposed to higher or lower levels of harmful or essential elements, and/or have problems in the way his/her body regulates metals (called *metal dysregulation*) which can lead to the greater uptake of harmful exposures or the lessened uptake of essential nutrients.

Previous studies frequently estimated metal or elemental exposures in children via blood samples or other biomarkers *after* they had already been diagnosed with ASD. Also many studies had recruited patient series rather than individuals from the general population, and many studies did not account for genetic factors when assessing metal exposure contributions to ASD risk. These issues have limited the interpretation of the findings and made the findings difficult to generalize to the general population.

Objective:

This study aimed to test whether pre- and post-natal exposure to metals or deficiency of essential elements increased the risk of ASD in identical and fraternal twins.

This involved 3 investigations:

1. Comparison of metal distributions in typically developing and ASD twins;
2. Identification of critical time periods during which developing children may be more vulnerable to harm from these factors; and
3. Examination of potential correlation between metals/essential elements and ASD traits and clinical severity.

Methods:

Twin pairs were recruited from nation-wide twin registries and by advertisements in Sweden.

They were screened for ASD, and 32 twin pairs were ultimately included in the study—17 pairs were identical (monozygotic or MZ), and 15 pairs were fraternal (dizygotic or DZ).

In addition, 19 twin pairs were categorized as "non-ASD" and 13 identified as "ASD".

ASD pairs were also categorized as "concordant" (both twins have ASD) or "discordant" (only one twin has ASD).

Some metals and elements that have entered the body eventually get stored in teeth and bones, accumulating over time. It is possible to examine the uptake of certain metals and elements for an individual via assessment of their shed teeth (the "baby" teeth that fall out). This "tooth-matrix" biomarker can reveal fetal and early post-natal exposure to multiple metals and elements with detailed and precise measurements regarding the timing of the exposures. Shed teeth were collected from the 32 twin pairs and examined for the 3 investigations:

1. Differences in lead, manganese, zinc, and 7 other metal/essential element levels among ASD and non-ASD pairs were observed and compared.
2. Statistical models were used to analyze the time-series data of exposures in order to determine critical windows of susceptibility to metals and elements.
3. Lastly, the tooth-matrix biomarkers were correlated with the presence and severity of ASD traits as measured approximately 10 years later using established clinical assessments.

Results:

Of the 10 metals/elements measured in the shed teeth, the results provided focus primarily on lead, manganese, and zinc. Results are provided for each of the 3 investigations:

1. Differences in lead, manganese, zinc, and 7 other metal/essential element levels among ASD and non-ASD pairs were observed and compared.

Each of the <u>non-ASD MZ twins</u> shared similar lead levels. They also had similar manganese and zinc levels. Among this group, manganese levels in teeth declined rapidly over the prenatal period to birth and continued to decrease at a slower rate post-natally. Zinc levels were steady pre-natally in this group, and then showed a marked decreased around birth. Lastly, there was no typical distribution of lead in teeth related to developmental age among this group.

Among ASD discordant MZ twins, manganese levels in teeth were lower in in the affected twin pre- and post-natally. In the non-affected twins, there was a drop in zinc levels around birth, but in the affected twins this drop occurred earlier during the prenatal period and subsequently zinc levels showed a marked increase post-natally surpassing the levels in their non-affected twin. Lead levels were generally higher in the affected twin and this difference was greatest after birth.

In ASD discordant DZ twins, similar differences in zinc and manganese levels and time-related trends between the affected and non-affected twins.

In ASD concordant MZ twins, the differences in metal distribution among twins were smaller than those observed in discordant pairs. This indicates that when only one twin in a pair (whether MZ or DZ) is affected by ASD, metal dysregulation may possibly be a factor.

2. Statistical models were used to analyze the time-series data of exposures in order to determine critical windows of susceptibility to metals and elements.

Comparing ASD discordant twins with non-ASD pairs revealed a consistently higher lead level for ASD cases, specifically between 10 to 20 weeks after birth. The greatest difference was observed at 15 weeks after birth, when lead levels in ASD cases were 1.5 times higher than in the non-affected twins. Manganese levels were lower in the ASD cases, particularly from 10 weeks before birth to birth, and from 5 to 20 weeks after birth. The greatest differences were observed at both 7 weeks before birth and at 15 weeks after birth when cases had 2.5 times lower manganese than the non-affected twins. In addition, zinc levels were 1.28 times lower in ASD cases than in non-ASD cases at 8 weeks before birth.

Comparing ASD discordant twin pairs with ASD concordant twins, similar patterns were observed as seen when comparing ASD discordant versus non-ASD pairs, including significantly higher lead levels in cases.

Among ASD discordant twin pairs, broad pre- and post-natal differences between the affected and the non-affected twins were found for 6 of the 10 metals examined, including higher levels of tin and strontium, and lower levels of chromium, in ASD affected twins. The researchers identified periods where the greatest differences were observed for these metals as well.

3. Lastly, the tooth-matrix biomarkers were correlated with the presence and severity of ASD traits as measured approximately 10 years later using established clinical assessments.

Lead and manganese showed statistically significant associations with ASD and autistic traits 10 years post-exposure.

Manganese was negatively associated with autistic traits (strongest association observed at 15 weeks after birth) and ASD severity (strongest association at 12 weeks after birth), meaning that lower levels of manganese were associated with more traits and greater ASD severity.

Lead concentrations were positively associated with autistic traits and ASD severity, meaning that higher levels of lead corresponded with more traits and greater ASD severity. Lead's association with ASD severity was statistically significant from 10 weeks before birth to 30 weeks after birth, with the strongest association 5 weeks before birth.

Zinc and other metals analyzed were not significantly associated with measures of ASD traits and severity.

Conclusion:

The findings of this study suggests that prenatal and early childhood metal dysregulation during critical windows of development may have a role in the development and severity of ASD later in life.

POLICY IMPLICATIONS

This study adds significant new understanding to the potential role of metal dysregulation in ASD etiology, and underscores the importance of environmental health research. Increased support for environmental health research is critical for increased insight into complex etiologies, and yields information with potentially actionable steps to reduce risk.

Currently, the task of eliminating childhood lead exposure falls to 9 federal government agencies, most notably, the U.S. Environmental Protection Agency, the Consumer Product Safety Commission, the U.S. Food and Drug Administration and the U.S. Department of Housing and Urban Development. Each agency is charged with regulating the multiple pathways of lead poisoning whether it be through lead paint, ambient air, water, soil, food or consumer products.

However, as evidenced by the Flint water crisis, our regulatory agencies at both the federal and state level need to be doing more. The federal government has taken steps to combat this issue under the direction of the President's Taskforce on Environmental Health Risks and Safety Risks to Children which published a comprehensive report in November 2016 entitled, *Key Federal Programs to Reduce Childhood Lead Exposures and Eliminate Associated Health Impacts*. This report charts the current actions and activities undertaken by several government agencies to address lead exposures and evaluates the gaps in federal efforts to do so. It also outlines future programs and initiatives to redress some of these shortcomings[3]. Furthermore, this body of work is ongoing, as the taskforce continues to gather feedback from various stakeholders and affected communities to develop a revised plan.

This new approach and policy shift needs to be adopted at the state and local levels as well. Many state legislatures have introduced, and some have enacted, new requirements and preemptive measures by mandating the replacement of lead service lines and requiring lead testing in schools. These actions at all levels of government are a positive step in the right direction.

However, the risk of lead exposure is not spread evenly throughout our communities in the United States. Environmental health hazards, including the incidence of lead poisoning, disproportionately affects low-income communities and communities of color[4]. Government institutions and agencies must not only recognize the existence of these environmental disparities but also pursue policies that help safeguard the wellbeing of our country's most vulnerable populations by addressing the underlying causes of environmental health inequities.

The results of this study also indicate that children have a heightened risk of developing ASD if they are deficient in essential nutrients, specifically zinc and manganese. This presents troubling possibilities for children and pregnant women who do not have access to key nutrients. Prenatal and early infant public health programs are vital to protecting young children. It is imperative that governments at both the

federal and state levels support robust public health campaigns and invests in prenatal care programs that provide education, resources, and health testing for new and soon-to-be mothers. Additionally, proper education is key, as exposure to even essential, beneficial elements such as manganese at high levels may be toxic.

Nutrition programs such as SNAP and WIC through the USDA provide education and supplemental food to low-income families, pregnant women and children[5],[6]. Adequate nutrition for both pregnant women and children is integral to their health as children who are deficient in essential nutrients like zinc will actually absorb more lead into their bodies.

REFERENCES

[1]Autism Spectrum Disorder. Updated March 28, 2016. *Centers for Disease Control and Prevention*. Retrieved on June 20, 2017 from https://www.cdc.gov/ncbddd/autism/facts.html

[2] Autism Spectrum Disorder. October 2016. *National Institute of Mental Health.* Retrieved on June 20, 2017 from https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd/index.shtml

[3] Key Federal Programs to Reduce Childhood Lead Exposures and Eliminate Associated Health Impacts. November 2016. *President's Task Force on Environmental Health Risks and Safety Risks to Children.* Retrieved on June 30, 2017 from here.

[4] Recommendations for Blood Lead Screening of Medicaid-Eligible Children Aged 1—5 Years: an Updated Approach to Targeting a Group at High Risk. 2009. *Centers for Disease Control and Prevention*. Retrieved on June 22, 2017 from https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5809a1.htm

[5] Supplemental Nutrition Assistance Program. Updated January 30, 2017. *United States Department of Agriculture: Food and Nutrition Service*. Retrieved on June 30, 2017 from https://www.fns.usda.gov/snap/supplemental-nutrition-assistance-program-snap

[6] Women, Infants, and Children (WIC). Updated May 3, 2017. *United States Department of Agriculture: Food and Nutrition Service*. Retrieved on June 30, 2017 from https://www.fns.usda.gov/wic/women-infants-and-children-wic

Article found in Nature Communications.