HHS Public Access

Author manuscript

Res Autism Spectr Disord. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Res Autism Spectr Disord. 2015 October 1; 18: 73–82. doi:10.1016/j.rasd.2015.08.001.

Synergic effect of GSTP1 and blood manganese concentrations in Autism Spectrum Disorder

Mohammad H. Rahbar[a,b,c,*], **Maureen Samms-Vaughan**[d], **Jianzhong Ma**[e], **Jan Bressler**[a,e], **Aisha S. Dickerson**[c], **Manouchehr Hessabi**[c], **Katherine A. Loveland**[f], **Megan L. Grove**[e], **Sydonnie Shakespeare-Pellington**[d], **Compton Beecher**[g], **Wayne McLaughlin**[g,h], and **Eric Boerwinkle**[a,e]

[a]Division of Epidemiology, Human Genetics, and Environmental Sciences (EHGES), University of Texas School of Public Health at Houston, Houston, TX 77030, USA

[b]Division of Clinical and Translational Sciences, Department of Internal Medicine, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030,USA

[c]Biostatistics/Epidemiology/Research Design (BERD) Component, Center for Clinical and Translational Sciences (CCTS), University of Texas Health Science Center at Houston, Houston, TX 77030, USA

[d]Department of Child & Adolescent Health, The University of the West Indies (UWI), Mona Campus, Kingston, Jamaica

[e]Human Genetics Center, University of Texas School of Public Health at Houston, Houston, TX 77030, USA

[f]Department of Psychiatry and Behavioral Sciences, University of Texas Medical School at Houston, Houston, TX 77054, USA

[g]Department of Basic Medical Sciences, The University of the West Indies, Mona Campus, Kingston, Jamaica

[h]Caribbean Genetics (CARIGEN), The University of the West Indies, Mona Campus, Kingston, Jamaica

Abstract

We used data from 100 age- and sex-matched case-control pairs (age 2–8 years) from Jamaica to investigate whether there is an interaction between glutathione-S-transferase (GST) genes and blood manganese concentrations (BMC) in relation to Autism Spectrum Disorder (ASD). Our findings, indicate that among children who had the Ile/Ile genotype for GST pi 1 (*GSTP1*), those with BMC ≥ 12μg/L had about 4 times higher odds of ASD than those with BMC < 12μg/L,

[*]**Corresponding Author:** Mohammad H. Rahbar, PhD, University of Texas Health Science Center at Houston, Biostatistics/ Epidemiology/Research Design Component of Center for Clinical and Translational Sciences, 6410 Fannin Street, UT Professional Building Suite 1100.05, Houston, TX 77030, USA. Mohammad.H.Rahbar@uth.tmc.edu, Phone: (713)500-7901, Fax: (713)500-0766.

(*P*=0.03) under a co-dominant genetic model. After adjusting for potential confounders, among the subgroup of children with genotype Ile/Ile, those with BMC ≥ 12μg/L had about six times higher odds of ASD than those with BMC < 12μg/L, (*P*=0.04). The results were similar when a recessive genetic model was used. These findings suggest a possible synergic effect of BMC and *GSTP1* in ASD. Since our analysis included a variety of genetic models and was not adjusted for multiple testing, replication in other populations is warranted.

Keywords

Manganese; Autism Spectrum Disorder (ASD); Glutathione S-transferase (*GST*) genes; Oxidative stress; Interactions; Jamaica

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that impairs social interaction and communication (Genuis, 2009; Gillberg, 2013; Volkmar & Chawarska, 2008). The etiology of ASD is not well understood but there is a general agreement among scientists that ASD is possibly caused by an interplay between genes and environmental factors (Hallmayer et al., 2011) during crucial stages in brain development (Landrigan, Lambertini, & Birnbaum, 2012).

Manganese (Mn) is a naturally occurring trace element essential for human health and development (ATSDR, 2012b; EPA, 2007). Neurotoxic effects of excess exposure to manganese are well documented (ATSDR, 2012b; Bhang et al., 2013; Bouchard, Laforest, Vandelac, Bellinger, & Mergler, 2007; Bouchard et al., 2011; Claus Henn et al., 2010; Ericson et al., 2007; Khan et al., 2011; Khan et al., 2012; Kim et al., 2009; Menezes-Filho, Novaes, Moreira, Sarcinelli, & Mergler, 2011; Takser, Mergler, Hellier, Sahuquillo, & Huel, 2003; Roels et al., 2012; Wasserman et al., 2011; Wright, Amarasiriwardena, Woolf, Jim, & Bellinger, 2006; Zoni, Albini, & Lucchini, 2007). For example, it has been shown that excess manganese exposure has adverse effects on neuromuscular (ATSDR, 2012b) and cognitive function (Zoni et al., 2007) in adults. Other studies have also shown adverse neurological effects in children exposed to higher levels of manganese including increased behavioral problems (Bouchard et al., 2007; Ericson et al., 2007; Khan et al., 2011), reduced verbal and full-scale intelligence quotient (IQ) scores (Bouchard et al., 2011; Kim et al., 2009; Menezes-Filho et al., 2011; Wasserman et al., 2011; Wright et al., 2006), diminished attention (Takser et al., 2003), and lower academic achievement (Bhang et al., 2013; Khan et al., 2012).

Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism as well as in cellular response to xenobiotics, cytokines, and bacterial invasion (Ray, Huang, & Tsuji, 2012). Oxidative stress results from an imbalance in the production of ROS (Tamai et al., 2011). Some studies have identified increased blood managese concentrations as a possible factor associated with oxidative stress (Erikson, Dobson, Dorman, & Aschner, 2004; Fernsebner, Zorn, Kanawati, Walker, & Michalke, 2014; Wu et al., 2010). On the other hand, Mn is an essential cofactor for metalloenzyme superoxide dismutase, which protects cells against antioxidant processes (Hope et al., 2006; Koh et al., 2014; Rucker,

Thadhani, & Tonelli, 2010). Cells are also protected from oxidative stress by intracellular antioxidants like glutathione along with antioxidant enzymes like manganese superoxide dismutase (MnSOD) (Tamai et al., 2011). In addition, serum MnSOD level is often used as a biomarker for oxidative stress in assessment of disease (Tamai et al., 2011). Some studies reported that dietary Mn does not appear to markedly alter circulating Mn levels or expression of leucocyte MnSOD (Hope et al., 2006). On the other hand, chronic Mn accumulation in the mitochondria appears to affect MnSOD activity, resulting in increased oxidative stress in the mitochondria (Koh et al., 2014). Glutathione S-transferase (GST) genes, including glutathione S-transferase pi (*GSTP1*), glutathione S-transferase mu 1 (*GSTM1*), and glutathione S-transferase theta 1 (*GSTT1*), encode enzymes that can protect cells from oxidative stress; polymorphisms in these genes may influence enzymatic activity (Hayes & Strange, 1995). For example, it has been reported that *GSTP1* is an important phase II enzyme that can protect cells from oxidative stress (Li et al., 2013). James et al. (2004) reported that ASD may be caused by impaired capacity for methylation and increased oxidative stress (James et al., 2004). On the other hand, polymorphisms in the GST genes have been associated with ASD (Frustaci et al., 2012; James et al., 2006; Schmidt et al., 2011).

Several studies have examined the relationship between ASD and manganese exposure as measured by air distribution (Windham, Zhang, Gunier, Croen, & Grether, 2006), tooth enamel (Abdullah et al., 2012), hair (Abdullah et al., 2012; Adams, Holloway, George, & Quig, 2006; Al-Ayadhi, 2005; Blaurock-Busch, Amin, & Rabah, 2011; Blaurock-Busch, Amin, Dessoki, & Rabah, 2012; De Palma, Catalani, Franco, Brighenti, & Apostoli, 2012), urine (Blaurock-Busch et al., 2011), red blood cells (Jory & McGinnis, 2008), and whole blood (Rahbar et al., 2014b), but their findings are conflicting. For example, based on data from 109 pairs of sex- and age-matched ASD cases and typically developing (TD) controls from our Jamaican Autism Study, we have previously reported a lack of an additive effect of BMC in ASD (Rahbar et al., 2014b). However, none of the aforementioned studies, including our own, have tested for gene-environment interactions. As Palmer (2010) highlighted, without testing for potential gene-environment interactions, lack of an addtive effect of an environmental toxin in ASD should not be interpreted as unrelated (Palmer, 2010). As shown in our earlier report, Jamaican soils have high managese levels (Lalor, 1996), and Jamaicans consume large amounts of vegetables and fruits that contain manganese (Howe, Fung, Lalor, Rattray, & Vutchkov, 2005) as well as seafood that has been associated with higher blood manganese concentrations in young children (Rahbar et al., 2014b). In the present research, we investigated the possible synergistic effect of BMC and each of three GST genes (*GSTP1*, *GSTM1*, and *GSTT1*) in relation to ASD in Jamaican children.

2. Methods

2.1. Genera l description

The Jamaican Autism Study was designed as a case-control study to investigate the potential role of environmental exposures and glutathione S-transferase genes (*GSTT1*, *GSTM1*, and *GSTP1*), separately and interactively, in risk of ASD. This study enrolled Jamaican children

with ASD 2–8 years old along with 1:1 age- (within 6 months) and sex-matched typically developing controls from December 2009-May 2012. After provision of written consent, we administered the Autism Diagnostic Interview-Revised (ADI-R) (Rutter, Le, & Lord, 2003) and Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) to suspected ASD cases, previously identified using Diagnostic Statistical Manual of Mental Disorders (DSM-IV-TR) criteria (American Psychiatric Association., 2000) and the Childhood Autism Rating Scale (CARS) (Schopler, Reichler, DeVellis, & Daly, 1980), to confirm the diagnosis. For typically developing controls, we ruled out symptoms of ASD using the Social Communication Questionnaire (SCQ) Lifetime form (Rutter, Bailey, & Lord, 2003) with a cut-off score of 6, which is one standard deviation above the mean SCQ score for typically developing children (Mulligan, Richardson, Anney, & Gill, 2009). Details on the recruitment and assessment procedures of this study have been reported previously (Rahbar et al., 2012a; Rahbar et al., 2012b; Rahbar et al., 2013; Rahbar et al., 2014a; Rahbar et al., 2014b; Rahbar et al., 2014c; Rahbar et al., 2015).

Information was also collected on demographics and socioeconomic status (SES), parents' education, and potential nutritional exposures to heavy metals. The types of nutritional exposures included consumption of seafood, fruits, and vegetables. Details regarding these exposure variables were also reported previously (Rahbar et al., 2012a; Rahbar et al., 2014c).

At the end of the interview and other assessments, approximately 5mL of venous whole blood and 2mL of saliva were also collected from each child for assessment of trace metals and genetic analysis (Rahbar et al., 2014c). Trace metal analysis was carried out at Michigan Department of Community Health (MDCH) while genetic analysis was performed at the University of Texas School of Public Health at Houston. This study was approved by the Institutional Review Boards of MDCH, the University of Texas Health Science Center at Houston (UTHealth), and the University of the West Indies (UWI), Mona campus, in Kingston, Jamaica.

2.2. Assessment of manganese exposure

For this study, venous whole blood samples were diluted and analyzed using a PerkinElmer Elan DRCII inductively-coupled plasma mass spectrometer (PerkinElmer, Waltham, MA). Assays for blood manganese were performed at the MDCH Trace Metals Laboratory, a facility certified by the Centers for Disease Control and Prevention (CDC) for trace metal analyses. All BMC in our samples are above the limit of detection of 1μg/L.

2.3. Genetic analysis

Saliva was collected with Oragene Discover DNA Collection Kits for Research (OGR-500; DNA Genotek, Inc.; Kanata, Ontario, Canada). If the child had difficulty in spitting 2 mL of saliva, then the Oragene Discover OGR-575 for Assisted Collection with sponges was used (DNA Genotek). Saliva and whole blood samples (plasma, buffy coat, and red blood cell aliquots) were shipped from UWI to UTHealth for genetic analyses and details regarding DNA isolation and genotyping of *GSTT1*, *GSTM1*, and *GSTP1* were reported previously (Rahbar et al., 2014c; Rahbar et al., 2015).

2.4. Statistical analysis

Since this is a matched case-control study, we used conditional logistic regression (CLR) to compare various characteristics of ASD case and TD control groups. Specifically, we used univariable CLR to compare demographic and socioeconomic characteristics of ASD case and TD control groups. We also used univariable CLR to detect possible associations between ASD case status and genotypes of GST genes as well as with the levels of BMC. In a study on children (12 months of age) from Mexico City, Claus Henn et al. (Claus Henn et al., 2010) reported a nonlinear association between blood manganese concentrations and concurrent mental development scores (Claus Henn et al., 2010). On the other hand two studies from Taiwan and China demonstrated that cord blood manganese concentrations above the 75th percentile were associated with adverse cognitive, language, and overall quotients of the Comprehensive Developmental Inventory for Infants and Toddlers (Lin et al., 2013) and lower neonatal behavioral neurological assessments (Yu, Zhang, Yan, & Shen, 2014). Thus, for these analyses we categorized BMC into two levels [BMC <12 μg/L and BMC ≥12 μg/L], and herein will be called binary manganese levels. The choice of this cutoff point at the 75th percentile for BMC reflects a tradeoff between statistical power and biological significance. Although BMC=12 is 20% lower than the recommended cutoff value for elevated BMC=15(ATSDR, 2012b; ATSDR, 2012a), it allows analysis of a larger sample of children (i.e., 25%) in the higher level of manganese exposure. The variants in *GSTM1* and *GSTT1* examined here are insertion-deletion polymorphisms and the homozygous deletions or null genotypes indicate that activities or functionality of these genes are reduced or interrupted completely. Since the genotyping assay does not distinguish between a normal homozygote (I/I) and a heterozygote (I/D), we considered only a recessive model for both using a binary variable to represent their genotypes (I/I or I/D) and DD (null). For the *GSTP1*Ile105Val polymorphism, there are three common genotypes (Ile/Val, Ile/Ile, Val/Val) and the replacement of adenine by guanine at nucleotide 562 results in the change of amino acid from isoleucine to valine at codon 105 of the *GSTP1* protein. Since there is no consensus regarding the effect *of GSTP1* in the literature, not only for the genetic models but also for the direction of the effect (Ramprasath et al., 2011; Safarinejad, Shafiei, & Safarinejad, 2011; Serajee, Nabi, Zhong, & Huq, 2004; Tamer et al., 2004), we started our analysis with the co-dominant (Ile/Ile or Ile/Val or Val/Val) model for *GSTP1*, followed by the recessive model (Ile/Ile vs. Val/*).

Subsequently, we used multivariable CLR to assess potential gene-environment interactions between the GST genes and the binary manganese levels in relation to ASD in the absence and presence of potential cofounding variables. Since the classical definition of confounders does not apply in the presence of interactions, selection of potential confounders was based on the information we gained in our previous published work related to BMC and ASD (Rahbar et al., 2014b). Specifically, in our previous study based on additive models (no interaction term in the model) we showed that for assessing the role of manganese in ASD, the potential confounders include: paternal age, parental education, place of child's birth (Kingston parish vs. other parishes), consumption of root vegetables ("yam, sweet potato, or dasheen"), "cabbage", saltwater fish, and cakes/buns (Rahbar et al., 2014b). In the present study, we adjusted for these confounders while performing CLR except for paternal age because reliable estimates for parameters could not be obtained when this variable was in

the model, probably due to its frequency of missing values (13/200). Since we found a significant interaction between BMC and *GSTP1* genotypes in relation to ASD, we used the CONTRAST statement in PROC Logistic in SAS (Kleinbaum & Klein, 2010) to test whether there is a significant difference in ASD risk between two groups of children with BMC < 12 and BMC ≥ 12, with various genotypes *of GSTP1*. We calculated unadjusted and adjusted matched odds ratios (MOR) for groups of children with different *GSTP1* genotypes. All statistical tests were conducted at 5% level of significance without making any adjustments for multiple comparisons. All analyses were performed using the SAS software (SAS Institute, 2011).

3. Results

Consistent with a reported 4:1 male/female ratio for ASD, the percentage of boys in our sample was 85%, while 99% of TD controls and 93% of cases were Afro-Caribbean. As this study was matched on age, the mean age of children with ASD and TD controls was similar (68.4 months and 69.3 months, respectively). In TD children, the frequency of the *GSTM1* null genotype was 26.0% and the frequency of the *GSTT1* null genotype was 22.0% with no significant deviation from Hardy-Weinberg equilibrium for the *GSTP1* polymorphism (P = 0.69) (Rahbar et al., 2014c). Our analysis also indicated there were no significant genotype frequency differences between ASD cases and TD controls for *GSTM1*, *GSTP1*, and *GSTT1* (all $P > 0.21$). Other characteristics of study participants by ASD case status are shown in Table 1.

A comparison of socioeconomic status (SES) and sociodemographic factors between ASD cases and TD controls revealed that a significantly higher proportion of ASD cases belonged to a higher SES group [Matched Odds Ratio (MOR) = 3.58, 95% CI (1.90, 6.80), $P < 0.01$]. Similar comparisons between ASD cases and TD controls with respect to dietary consumption revealed that a significantly lower proportion of ASD cases reported eating cabbage [MOR = 0.15, 95% CI (0.06, 0.38), $P < 0.01$] and saltwater fish [MOR = 0.40, 95% CI (0.18, 0.91), $P = 0.03$]. Additionally, fewer ASD cases reported eating certain fruits and vegetables. Comparisons of other variables related to sociodemographic factors and dietary consumption between children with and without ASD are displayed in Table 2.

Our CLR analyses for identifying factors associated with binary blood manganese levels (BMC < 12μg/L and BMC ≥ 12μg/L) revealed the following results. Although not statistically significant, children in the BMC ≥ 12μg/L group were less likely to report eating cabbage than those in the BMC < 12μg/L group [MOR = 0.46, 95% CI: 0.16, 1.31; P = 0.14]. Similarly, children in the BMC ≥ 12μg/L group were more likely to report eating saltwater fish than those in the BMC < 12μg/L group, [MOR = 2.33, 95% CI: 0.60, 9.02; P = 0.23]. Associations of other exposure variables with blood manganese levels are reported in Table 3.

From our previous work we reported no association between BMC and ASD when BMC was analyzed as a continuous variable (Rahbar et al., 2014b). When we analyzed the association between our binary BMC variable and ASD in this research using CLR, we still found no significant association between these variables (univariable and multivariable $P >$

0.30). However, in co-dominant genetic models using CLR with ASD status as a dependent variable and the aforementioned binary BMC variable and each of the three genotypes *of GSTP1* as independent variables, the interaction between binary BMC and *GSTP1* was significant ($P = 0.02$) but became marginally significant ($P = 0.08$) when we adjusted for the aforementioned potential confounders. Using the interactive model, based on the co-dominant model, we found that among individuals with genotype Ile/Ile, the odds of ASD was significantly higher for children with BMC ≥ 12μg/L than for those with BMC < 12μg/L, (Unadjusted MOR = 3.9, 95% CI: 1.15–13.26; $P = 0.03$). When adjusted for the aforementioned potential confounders, this association was still significant (Adjusted MOR = 5.93, 95% CI: 1.06–33.29; $P = 0.04$). Using the recessive model for *GSTP1*, we found similar results. Specifically, among children with genotype Ile/Ile, those with BMC ≥ 12μg/L had a significantly higher odds of ASD than those with BMC < 12μg/L, (Unadjusted MOR = 4.02, 95% CI: 1.19–13.64; $P = 0.03$). When adjusted for the aforementioned potential confounders, the association was also statistically significant (Adjusted MOR = 5.92, 95%CI: 1.05–33.32; $P = 0.04$). Other associations between binary BMC and ASD status for other combinations of the *GSTP1* genotypes based on both co-dominant and recessive genetic models for *GSTP1* are displayed in Table 4.

4. Discussion

In this study, we have investigated the possible interactive effects of three GST genes (*GSTT1, GSTM1, and GSTP1*) and a binary BMC in relation to ASD in Jamaican children. Using univariable and multivariable CLRs, we investigated the additive and interactive effects of genotypes of the GST genes and the binary BMC in ASD. We did not find any statistically significant associations between ASD status and binary BMC, or with *GSTT1* or *GSTM1* genotypes. However, our findings revealed a significant interaction between binary BMC and *GSTP1* genotype in relation to ASD (i.e., for the recessive model: unadjusted $P = 0.01$ and adjusted $P = 0.03$; for the co-dominant model: unadjusted $P = 0.02$ and adjusted $P = 0.08$). Specifically, based on the recessive genetic models, in children with genotype Ile/Ile, those with BMC ≥ 12μg/L had about four times higher odds of ASD than those with BMC < 12μg/L. Similarly, in a co-dominant model, in individuals with genotype Ile/Ile, the odds of ASD was nearly 4 times higher among those with BMC ≥ 12μg/L compared to children with BMC < 12μg/L. For both the recessive and co-dominant genetic models, after adjusting for potential confounders that included parental education, place of child's birth (Kingston parish vs. other parishes), consumption of root vegetables ("yam, sweet potato, or dasheen"), saltwater fish, and cakes/buns, among children with genotype Ile/Ile, those with BMC ≥ 12μg/L had about six times higher odds of ASD than those with BMC < 12μg/L.

Based on an additive model (i.e., no interactions terms in the model) we have previously reported no association between BMC modeled as a continuous variable and ASD in Jamaican children in both univariable and multivariable models (Rahbar et al., 2014b). In this study, we also did not find an association between our binary BMC and ASD in an additive model. These results are consistent with several other studies that found no association between ASD status and manganese levels in red blood cells (Jory & McGinnis, 2008), hair, and urine (Blaurock-Busch et al., 2011). However, after finding an interaction between binary BMC and *GSTP1*, our findings provide new insight regarding a possible

synergic effect of BMC and *GSTP1* in ASD. As Palmer has highlighted, without testing for gene-environment interactions between environmental toxins and genes, lack of an additive effect should not be interpreted as no association between the environmental toxins studied and ASD (Palmer, 2010). To our knowledge, we are the first to report on interaction between BMC and *GSTP1* in relation to ASD.

In this study, we used 100 pairs of ASD cases and their age- and sex matched TD controls. We used conditional logistic regression models to account for potential correlation between ASD cases and TD controls due to matching. We adjusted for potential confounders that were determined from our earlier report (Rahbar et al., 2014b), except for paternal age due to its frequency of missing values (13/200). However, we believe that the adjusted effects reported for various associations have already taken into account most potential effects of SES as well as various food items consumed by the groups of children compared.

Manganese is ubiquitous in all human tissues, thus various factors, including nutritional intake, can influence biological measures (Santamaria & Sulsky, 2010). Additionally, higher levels of manganese have been reported to be associated with adverse neuromuscular (ATSDR, 2012b) and cognitive affects (Zoni et al., 2007) in adults, as well as with reduced IQ (Bouchard et al., 2011; Kim et al., 2009; Menezes-Filho et al., 2011; Wasserman et al., 2011; Wright et al., 2006) and increased behavioral problems (Bouchard et al., 2007; Ericson et al., 2007) in children. Mechanisms for Mn-induced neurotoxicity are varied with mitochondrial inactivation as the primary route of action (Gavin, Gunter, & Gunter, 1990). This results in enhanced production of reactive oxygen species in the affected areas of the brain, that could lead to oxidative stress (Gavin, Gunter, & Gunter, 1999).

Chronic Mn accumulation in the mitochondria has been shown to affect MnSOD activity and could result in an abnormal response to the mechanism that blocks oxidative stress in the mitochondria and protects against endothelial dysfunction (Koh et al., 2014). However, presence or absence of properly functioning GST genes may influence the occurrence of oxidative stress, and subsequently influence the occurrence of mitochondrial damage, decrease effectiveness of antioxidants, and increase susceptibility to neurological and muscular diseases (Pagano et al., 2014). Studies have suggested an association with mitochondrial disorders as well as with ASD (Oliveira et al., 2005) and have shown that children with ASD were more likely to have mitochondrial dysfunction (Giulivi et al., 2010). However, these studies were unable to pinpoint the cause of the mitochondrial dysfunction. Our results suggest that the *GSTP1* gene may play a significant role in the pathway to oxidative stress and mitochondrial dysfunction in children with ASD. However, further investigation in other populations is needed to support this suggestion.

Previously, we have reported that the mean blood manganese concentration in Jamaican TD children was 10.5μg/L (SD = 2.70μg/L) which is similar to that of children in the US or Canada (Rahbar et al., 2014b). We have also reported that consumption of some seafood is associated with BMC in Jamaican children. Our recently reported findings also indicate that about 25% of children in Jamaica have genotype Ile/Ile for *GSTP1*. For this subgroup of children we have shown that children with BMC ≥ 12μg/L may be at 4–6 times higher risk of ASD compared with those with exposure to BMC < 12μg/L. Since genetic risk factors are

not modifiable, we recommend implementation of interventions focused on dietary and environmental factors that could help to moderate exposure to excessive levels of manganese in Jamaican children as we have also recommended in our previous report (Rahbar et al., 2014b). However, we emphasize that regulating dietary intake of manganese should be discussed by qualified health professionals.

5. Limitations

We acknowledge several limitations in this study. First, although we used a culturally sensitive food frequency questionnaire that had been previously utilized in Jamaica, there is potential for recall bias from respondents. Although we used BMC as our biomarker of exposure, we acknowledge that tissue markers of exposure, such as nervous tissue, may be better indicators of long term exposures. Our data collection method also could not account for timing of manganese exposure via food consumption, so responses may only represent recent exposures. However, since Jamaican soils contain a unique distribution of a variety of trace elements, including manganese, that can accumulate in crops and seafood (Howe et al., 2005; Lalor, 1996) and because the Jamaican population consumes large amounts of locally grown fruits and vegetables along with large amounts of seafood (Howe et al., 2005), the residents may have continuous exposure to manganese, resulting in bioaccumulation (St-Pierre et al., 2001). Hence, our manganese levels could represent a long term exposure. Nevertheless, we acknowledge that BMC measurements for children represent “current” exposure, implying that the manganese exposure for ASD cases may reflect BMC after the child is already diagnosed with ASD. In addition, our BMC data were not differentiated by type of manganese exposures (i.e., organic and inorganic), so we could not provide any discussion regarding this distinction based on our data. We also lack information on maternal diet or manganese levels during pregnancy. Despite our initial effort to keep the age of matched TD control children within six months of ASD cases, for 12.8% of pairs the age of the TD controls was within 7–15 months. However, we do not believe this difference would likely have any significant impact on the findings reported here. Additionally, although we have conducted multiple testing for three different genes and a number of different genetic models, the reported p-values were not adjusted for multiple comparisons, the results reported here should be considered as preliminary, and replication in other populations is warranted.

6. Conclusions

In this article, we have shown that among Jamaican children with *GSTP1* genotype Ile/Ile, those with higher BMC (BMC ≥ 12μg/L) had about 4–6 times higher odds of ASD than those with BMC < 12μg/L under both a recessive and co-dominant genetic model. These findings suggest a possible synergic effect of manganese and *GSTP1* in ASD. Although replication of our findings in other populations is warranted, these results support our previous recommendation that because we cannot regulate genetic factors, intervention for reducing risk of ASD should emphasize reducing modifiable risk factors and moderating dietary and environmental exposures such as manganese in Jamaican children. However, since appropriate quantities of manganese are essential for healthy development, we

emphasize that regulating dietary intake of manganese should be supervised and discussed by qualified health professionals.

Acknowledgements

This research is co-funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institutes of Health Fogarty International Center (NIH-FIC) by a grant (R21HD057808) as well as National Institute of Environmental Health Sciences (NIEHS) by a grant (R01ES022165) awarded to University of Texas Health Science Center at Houston. We also acknowledge the support provided by the Biostatistics/Epidemiology/Research Design (BERD) component of the Center for Clinical and Translational Sciences (CCTS) for this project. CCTS is mainly funded by the NIH Centers for Translational Science Award (NIH CTSA) grant (UL1 RR024148), awarded to University of Texas Health Science Center at Houston in 2006 by the National Center for Research Resources (NCRR) and its renewal (UL1 TR000371) by the National Center for Advancing Translational Sciences (NCATS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD or the NIH-FIC or NIEHS or the NCRR or the NCATS. Finally, we acknowledge a special discount received from the Western Psychological Services (WPS) (Torrance, CA) for the purchase of ADOS, ADI-R, and SCQ forms that were used for this study.

References

Abdullah MM, Ly AR, Goldberg WA, Clarke-Stewart KA, Dudgeon JV, Mull CG, et al. Heavy metal in children's tooth enamel: related to autism and disruptive behaviors? J.Autism Dev.Disord. 2012; 42:929–936. [PubMed: 21735299]

Adams JB, Holloway CE, George F, Quig D. Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. Biol.Trace Elem.Res. 2006; 110:193–209. [PubMed: 16845157]

Al-Ayadhi LY. Heavy metals and trace elements in hair samples of autistic children in central Saudi Arabia. Neurosciences.(Riyadh.). 2005; 10:213–218. [PubMed: 22473261]

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Text Revision (DSM-IV-TR). Fourth Edition. Washington, DC: American Psychiatric Publishing, Inc.; 2000.

Agency for Toxic Substances and Disease Registry (ATSDR). Public Health Statement - Manganese. 2012a. Retrieved from http://www.atsdr.cdc.gov/ToxProfiles/tp151-c1-b.pdf

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for Manganese. 2012b. Retrieved from http://www.atsdr.cdc.gov/ToxProfiles/tp151.pdf

Bhang SY, Cho SC, Kim JW, Hong YC, Shin MS, Yoo HJ, et al. Relationship between blood manganese levels and children's attention, cognition, behavior, and academic performance-A nationwide cross-sectional study. Environ.Res. 2013; 126:9–16. [PubMed: 23790803]

Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T. Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism. Maedica.(Buchar). 2012; 7:38–48. [PubMed: 23118818]

Blaurock-Busch E, Amin OR, Rabah T. Heavy metals and trace elements in hair and urine of a sample of arab children with autistic spectrum disorder. Maedica.(Buchar). 2011; 6:247–257. [PubMed: 22879836]

Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ.Health Perspect. 2007; 115:122–127. [PubMed: 17366831]

Bouchard MF, Sauve S, Barbeau B, Legrand M, Brodeur ME, Bouffard T, et al. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ.Health Perspect. 2011; 119:138–143. [PubMed: 20855239]

Claus Henn B, Ettinger AS, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, Hernandez-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology. 2010; 21:433–439. [PubMed: 20549838]

De Palma G, Catalani S, Franco A, Brighenti M, Apostoli P. Lack of correlation between metallic elements analyzed in hair by ICP-MS and autism. J.Autism Dev.Disord. 2012; 42:342–353. [PubMed: 21503799]

Environmental Protection Agency (EPA). Manganese Toxicity and Exposure Assessment for Children's Health (TEACH) Chemical Summary. 2007. Retrieved from http://www.epa.gov/teach/chem_summ/manganese_summary.pdf

Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T, Robertson RT. Prenatal manganese levels linked to childhood behavioral disinhibition. Neurotoxicol.Teratol. 2007; 29:181–187. [PubMed: 17079114]

Erikson KM, Dobson AW, Dorman DC, Aschner M. Manganese exposure and induced oxidative stress in the rat brain. Sci Total Environ. 2004; 334–335:409–416.

Fernsebner K, Zorn J, Kanawati B, Walker A, Michalke B. Manganese leads to an increase in markers of oxidative stress as well as to a shift in the ratio of Fe(II)/(III) in rat brain tissue. Metallomics. 2014; 6:921–931. [PubMed: 24599255]

Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla BB, et al. Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. Free Radic.Biol Med. 2012; 52:2128–2141. [PubMed: 22542447]

Gavin CE, Gunter KK, Gunter TE. Manganese and calcium efflux kinetics in brain mitochondria. Relevance to manganese toxicity. Biochem.J. 1990; 266:329–334. [PubMed: 2317189]

Gavin CE, Gunter KK, Gunter TE. Manganese and calcium transport in mitochondria: implications for manganese toxicity. Neurotoxicology. 1999; 20:445–453. [PubMed: 10385903]

Genuis SJ. Is autism reversible? Acta Paediatr. 2009; 98:1575–1578. [PubMed: 19843021]

Gillberg, C. How Severe is Autism - Really?. The International Meeting for Autism Research (IMFAR), 12th Annual Meeting May 2–4, 2013; Donostia, San Sebastián Basque, Spain. 2013.

Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, Hertz-Picciotto I, et al. Mitochondrial dysfunction in autism. JAMA. 2010; 304:2389–2396. [PubMed: 21119085]

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. Genetic heritability and shared environmental factors among twin pairs with autism. Arch.Gen.Psychiatry. 2011; 68:1095–1102. [PubMed: 21727249]

Hayes JD, Strange RC. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. Free Radic.Res. 1995; 22:193–207. [PubMed: 7757196]

Hope S, Daniel K, Gleason KL, Comber S, Nelson M, Powell JJ. Influence of tea drinking on manganese intake, manganese status and leucocyte expression of MnSOD and cytosolic aminopeptidase P. Eur.J Clin.Nutr. 2006; 60:1–8. [PubMed: 16118651]

Howe A, Fung LH, Lalor G, Rattray R, Vutchkov M. Elemental composition of Jamaican foods 1: a survey of five food crop categories. Environ.Geochem.Health. 2005; 27:19–30. [PubMed: 15688127]

James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. American Journal of Clinical Nutrition. 2004; 80:1611–1617. [PubMed: 15585776]

James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am.J Med Genet.B Neuropsych ia tr.Genet. 2006; 141B:947–956.

Jory J, McGinnis WR. Red-cell trace minerals in children with autism. American Journal of Biochemistry and Biotechnology. 2008; 4:101–104.

Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F, et al. Manganese exposure from drinking water and children's classroom behavior in Bangladesh. Environ.Health Perspect. 2011; 119:1501–1506. [PubMed: 21493178]

Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, et al. Manganese exposure from drinking water and children's academic achievement. Neurotoxicology. 2012; 33:91–97. [PubMed: 22182530]

Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, et al. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. Neurotoxicology. 2009; 30:564–571. [PubMed: 19635390]

Kleinbaum, DG.; Klein, M. Logistic regression: a self-learning text. Springer; 2010.

Koh ES, Kim SJ, Yoon HE, Chung JH, Chung S, Park CW, et al. Association of blood manganese level with diabetes and renal dysfunction: a cross-sectional study of the Korean general population. BMC.Endocr.Disord. 2014; 14:24. [PubMed: 24606630]

Lalor G. Geochemical mapping in Jamaica. Environ.Geochem.Health. 1996; 18:89–97. [PubMed: 24194384]

Landrigan PJ, Lambertini L, Birnbaum LS. A research strategy to discover the environmental causes of autism and neurodevelopmental disabilities. Environ.Health Perspect. 2012; 120:a258–a260. [PubMed: 22543002]

Li T, Zhao XP, Wang LY, Gao S, Zhao J, Fan YC, et al. Glutathione S-transferase P1 correlated with oxidative stress in hepatocellular carcinoma. Int J Med Sci. 2013; 10:683–690. [PubMed: 23569432]

Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH, et al. In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ.Res. 2013; 123:52–57. [PubMed: 23578827]

Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J.Autism Dev.Disord. 2000; 30:205–223. [PubMed: 11055457]

Menezes-Filho JA, Novaes CO, Moreira JC, Sarcinelli PN, Mergler D. Elevated manganese and cognitive performance in school-aged children and their mothers. Environ.Res. 2011; 111:156–163. [PubMed: 20943219]

Mulligan A, Richardson T, Anney RJ, Gill M. The Social Communication Questionnaire in a sample of the general population of school-going children. Ir.J.Med.Sci. 2009; 178:193–199. [PubMed: 18651205]

Oliveira G, Diogo L, Grazina M, Garcia P, Ataide A, Marques C, et al. Mitochondrial dysfunction in autism spectrum disorders: a population-based study. Dev.Med.Child Neurol. 2005; 47:185–189. [PubMed: 15739723]

Pagano G, Talamanca AA, Castello G, Cordero MD, d'Ischia M, Gadaleta MN, et al. Oxidative stress and mitochondrial dysfunction across broad-ranging pathologies: toward mitochondria-targeted clinical strategies. Oxid.Med Cell Longev. 2014; 2014:541230. [PubMed: 24876913]

Palmer RF. Conclusions should be based on appropriate methodologies. Pediatrics. 2010:126. [PubMed: 19969619]

Rahbar MH, Samms-Vaughan M, Ardjomand-Hessabi M, Loveland KA, Dickerson AS, Chen Z, et al. The role of drinking water sources, consumption of vegetables and seafood in relation to blood arsenic concentrations of Jamaican children with and without Autism Spectrum Disorders. Sci.Total Environ. 2012a; 433:362–370. [PubMed: 22819887]

Rahbar MH, Samms-Vaughan M, Dickerson AS, Loveland KA, Ardjomand-Hessabi M, Bressler J, et al. Role of fruits, vegetables, and seafood consumption in blood cadmium concentrations of Jamaican children with and without Autism Spectrum Disorder. Research in Autism Spectrum Disorders. 2014a; 8:1134–1145. [PubMed: 25089152]

Rahbar MH, Samms-Vaughan M, Dickerson AS, Loveland KA, Ardjomand-Hessabi M, Bressler J, et al. Blood Manganese Concentrations in Jamaican Children with and without Autism Spectrum Disorder. Environ Health. 2014b; 13:69. [PubMed: 25149876]

Rahbar MH, Samms-Vaughan M, Loveland KA, Ardjomand-Hessabi M, Chen Z, Bressler J, et al. Seafood Consumption and Blood Mercury Concentrations in Jamaican Children With and Without Autism Spectrum Disorders. Neurotox.Res. 2013; 23:22–38. [PubMed: 22488160]

Rahbar MH, Samms-Vaughan M, Loveland KA, Pearson DA, Bressler J, Chen Z, et al. Maternal and paternal age are jointly associated with childhood autism in Jamaica. J.Au tism Dev.Disord. 2012b; 42:1928–1938.

Rahbar MH, Samms-Vaughan M, Ma J, Bressler J, Loveland KA, Ardjomand-Hessabi M, et al. Role of Metabolic Genes in Blood Arsenic Concentrations of Jamaican Children with and without Autism Spectrum Disorder. Int J Environ.Res Public Health. 2014c; 11:7874–7895. [PubMed: 25101770]

Rahbar MH, Samms-Vaughan M, Ma J, Bressler J, Loveland KA, Hessabi M, et al. Interaction between GSTT1 and GSTP1 allele variants as a risk modulating-factor for autism spectrum disorders. Research in Autism Spectrum Disorders. 2015; 12:1–9. [PubMed: 25685181]

Ramprasath T, Senthil MP, Prabakaran AD, Gomathi P, Rathinavel A, Selvam GS. Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione-S-transferases) variants and their association to CAD in patients with type-2 diabetes. Biochem.Biophys.Res.Commun. 2011; 407:49–53. [PubMed: 21352813]

Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal. 2012; 24:981–990. [PubMed: 22286106]

Roels HA, Bowler RM, Kim Y, Claus HB, Mergler D, Hoet P, et al. Manganese exposure and cognitive deficits: a growing concern for manganese neurotoxicity. Neurotoxicology. 2012; 33:872–880. [PubMed: 22498092]

Rucker D, Thadhani R, Tonelli M. Trace element status in hemodialysis patients. Semin.Dial. 2010; 23:389–395. [PubMed: 20557491]

Rutter, M.; Bailey, A.; Lord, C. The Social Communication Questionnaire. Los Angeles, CA: Western Psychological Services; 2003.

Rutter, M.; Le, CA.; Lord, C. Autism Diagnostic Interview-Revised (ADI-R). Los Angeles, CA: Western Psychological Services; 2003.

Safarinejad MR, Shafiei N, Safarinejad SH. Glutathione S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) and prostate cancer: a case-control study in Tehran, Iran. Prostate Cancer Prostatic.Dis. 2011; 14:105–113. [PubMed: 21243008]

Santamaria AB, Sulsky SI. Risk assessment of an essential element: manganese. J.Toxicol.Environ.Health A. 2010; 73:128–155. [PubMed: 20077284]

SAS Institute. SAS® 9.3. Cary NC: SAS Institute, Inc.; 2011.

Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011; 22:476–485. [PubMed: 21610500]

Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J.Autism Dev.Disord. 1980; 10:91–103. [PubMed: 6927682]

Serajee FJ, Nabi R, Zhong H, Huq M. Polymorphisms in xenobiotic metabolism genes and autism. J Child Neurol. 2004; 19:413–417. [PubMed: 15446388]

St-Pierre A, Normandin L, Carrier G, Kennedy G, Butterworth R, Zayed J. Bioaccumulation and locomotor effect of manganese dust in rats. Inhal.Toxicol. 2001; 13:623–632. [PubMed: 11452358]

Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology. 2003; 24:667–674. [PubMed: 12900080]

Tamai T, Uto H, Takami Y, Oda K, Saishoji A, Hashiguchi M, et al. Serum manganese superoxide dismutase and thioredoxin are potential prognostic markers for hepatitis C virus-related hepatocellular carcinoma. World J Gastroenterol. 2011; 17:4890–4898. [PubMed: 22171130]

Tamer L, Calikoglu M, Ates NA, Yildirim H, Ercan B, Saritas E, et al. Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology. 2004; 9:493–498. [PubMed: 15612961]

Volkmar FR, Chawarska K. Autism in infants: an update. World Psychiatry. 2008; 7:19–21. [PubMed: 18458791]

Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Ahsan H, Levy D, et al. Arsenic and manganese exposure and children's intellectual function. Neurotoxicology. 2011; 32:450–457. [PubMed: 21453724]

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK. Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay area. Environ.Health Perspect. 2006; 114:1438–1444. [PubMed: 16966102]

Wright RO, Amarasiriwardena C, Woolf AD, Jim R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology. 2006; 27:210–216. [PubMed: 16310252]

Wu HJ, Seib KL, Srikhanta YN, Edwards J, Kidd SP, Maguire TL, et al. Manganese regulation of virulence factors and oxidative stress resistance in Neisseria gonorrhoeae. J Proteomics. 2010; 73:899–916. [PubMed: 20004262]

Yu XD, Zhang J, Yan CH, Shen XM. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. Environ.Res. 2014; 133:232–238. [PubMed: 24971720]

Zoni S, Albini E, Lucchini R. Neuropsychological testing for the assessment of manganese neurotoxicity: a review and a proposal. Am.J.Ind.Med. 2007; 50:812–830. [PubMed: 17918193]

Highlights

- We studied the synergistic effect of blood manganese levels (Mn) and *GSTP1* in ASD.
- Odds of ASD was higher in children with Mn ≥12μg/L and *GSTP1* Ile/Ile genotype.
- Children with Mn ≥12μg/L and *GSTP1* Ile/Ile genotype had 4 times higher odds of ASD.
- Children with Mn ≥12μg/L and Ile/Ile genotype had 6 times higher adjusted odds of ASD

Table 1

Characteristics of children and their parents by ASD case status (100 matched pairs)

Variables	Categories	ASD Case (n=100) N (%)	TD Control (n=100) N (%)	*P*-value*
Child's sex	Male	85 (85.0)	85 (85.0)	1.00
Age of child (months)	Age < 48	16 (16.0)	13 (13.0)	0.64
	48 ≤ age < 72	46 (46.0)	47 (47.0)	
	Age ≥ 72	38 (38.0)	40 (40.0)	
Child's race	Afro-Caribbean	93 (93.0)	99 (99.0)	0.25
Place of child's birth	Kingston parish	19 (19.0)	70 (70.0)	<0.01
	Other parishes	81 (81.0)	30 (30.0)	
Maternal age[a] (at child's birth)	< 35 years	76 (76.0)	84 (88.4)	0.02
	≥ 35 years	24 (24.0)	11 (11.6)	
Paternal age[b] (at child's birth)	< 35 years	47 (48.5)	66 (71.7)	<0.01
	≥ 35 years	50 (51.5)	26 (28.3)	
Maternal race	Afro-Caribbean	93 (93.0)	99 (99.0)	0.25
Paternal race[c]	Afro-Caribbean	94 (94.9)	99 (99.0)	0.67
Maternal education[d] (at child's birth)	Up to high school †	53 (53.0)	75 (76.5)	<0.01
	Beyond high school††	47 (47.0)	23 (23.5)	
Paternal education[e] (at child's birth)	Up to high school †	53 (54.6)	85 (87.6)	<0.01
	Beyond high school††	44 (45.4)	12 (12.4)	
Assets owned	TV	99 (99.0)	94 (94.0)	0.10
	Refrigerator	97 (97.0)	85 (85.0)	<0.01
	Freezer [f]	12 (12.0)	19 (19.0)	0.17
	Living room set	85 (85.0)	47 (47.0)	<0.01
	Washing machine	76 (76.0)	53 (53.0)	<0.01
	Cars or other vehicle	68 (68.0)	37 (37.0)	<0.01
	Telephone/Cell phone	99 (99.0)	99 (99.0)	1.00

Variables	Categories	ASD Case (n=100) N (%)	TD Control (n=100) N (%)	*P*-value*
GSTP1	Ile/Ile	30 (30.0)	25 (25.0)	0.56
	Ile/Val	52 (52.0)	52 (52.0)	
	Val/Val	18 (18.0)	23 (23.0)	
GSTM1	DD [g]	27 (27.0)	26 (26.0)	0.87
	I/I or I/D [h]	73 (73.0)	74 (74.0)	
GSTT1	DD [g]	30 (30.0)	22 (22.0)	0.21
	I/I or I/D [h]	70 (70.0)	78 (78.0)	

**P*-values are based on Wald's test in conditional logistic regression models that compares the distribution of independent variables between and ASD case and TD control groups

†Up to high school education means attended Primary/Jr. Secondary, and Secondary/High/Technical schools

††Beyond high school education means attended a Vocational, Tertiary College, or University

[a]Maternal age was missing for 5 TD controls

[b]Paternal age were missing for 3 ASD cases and 8 TD controls

[c]Paternal race was missing for 1 ASD case

[d]Maternal education was missing for 2 controls

[e]Paternal education was missing for 3 cases and 3 controls

[f]Assets owned (freezer) was missing for 1 control family

[g]DD indicates the null alleles for *GSTT1* and *GSTM1*

[h]I/I or I/D indicate the homozygote (I/I) or a heterozygote (I/D) for *GSTT1 and GSTM1*

P-values are unadjusted for multiple testing

Table 2

Association between potential confounders and ASD case status using Conditional Logistic Regression (CLR) based on 200 children (100 matched-pairs).

Variables	Category		ASD Case N (%)	TD Control N (%)	Matched OR (MOR)	95% CI for MOR†	*P*-value
Parental education levels[a] (at child's birth)	At least one of the parents had education beyond high school		64 (66.0)	30 (31.6)	3.50	(1.84, 6.65)	<0.01
Socioeconomic status (SES)	High SES (own a car)		68 (68.0)	37 (37.0)	3.58	(1.90, 6.80)	<0.01
Source of drinking water[b]	Piped water		94 (94.0)	95 (96.0)	0.67	(0.19, 2.36)	0.53
Source of water for cooking[c]	Piped water		94 (94.0)	95 (96.0)	0.67	(0.19, 2.36)	0.53
Fruits and vegetables consumption[d]	Root Vegetables	A. Yam, sweet potato, or dasheen	73 (73.0)	82 (82.8)	0.52	(0.25, 1.07)	0.08
		B. Carrot or pumpkin	86 (86.0)	98 (99.0)	0.08	(0.01, 0.59)	0.01
	Leafy Vegetables	A. Lettuce	47 (47.0)	62 (62.6)	0.57	(0.33, 0.97)	0.04
		B. Callaloo, broccoli, or pakchoi	72 (72.0)	94 (95.0)	0.18	(0.07, 0.46)	<0.01
		C. Cabbage	66 (66.0)	94 (95.0)	0.15	(0.06, 0.38)	<0.01
	Legumes	String beans	33 (33.0)	42 (42.4)	0.68	(0.39, 1.18)	0.17
	Fruits	Tomatoes	62 (62.0)	85 (85.9)	0.23	(0.10, 0.51)	<0.01
		Ackee	58 (58.0)	92 (92.9)	0.06	(0.01, 0.23)	<0.01
		Avocado	29 (29.0)	68 (68.7)	0.19	(0.09, 0.38)	<0.01
		Green banana	67 (67.0)	90 (90.9)	0.27	(0.13, 0.57)	<0.01
		Fried plantains	70 (70.0)	89 (89.9)	0.17	(0.06, 0.48)	0.03

Res Autism Spectr Disord. Author manuscript; available in PMC 2016 October 01.

Variables	Category	ASD Case N (%)	TD Control N (%)	Matched OR (MOR)	95% CI for MOR†	*P*-value
Seafood consumption	High seafood consumption (More than 6 meals per week)	26 (26.0)	45 (45.0)	0.41	(0.21, 0.77)	<0.01
	Ate saltwater fish	77 (77.0)	89 (89.0)	0.40	(0.18, 0.91)	0.03
	Ate fresh water fish (Pond fish, tilapia)	46 (46.0)	52 (52.0)	0.75	(0.41, 1.38)	0.36
	Ate sardine, mackerel (Canned fish)	75 (75.0)	92 (92.0)	0.26	(0.11, 0.64)	<0.01
	Ate tuna (Canned fish)	31 (31.0)	44 (44.0)	0.55	(0.30, 1.02)	0.06
	Ate salt fish (Pickled mackerel)	70 (70.0)	93 (93.0)	0.15	(0.05, 0.42)	<0.01
	Ate shellfish (Lobsters, crabs)	7 (7.0)	14 (14.0)	0.42	(0.15, 1.18)	0.10
	Ate shrimp	19 (19.0)	27 (27.0)	0.62	(0.31, 1.24)	0.17
Grains consumed	Rice [e]	98 (98.0)	99 (100.0)	NR	NR	0.99
	Fried dumpling	78 (78.0)	93 (93.0)	0.29	(0.12, 0.71)	<0.01
	Boiled dumpling	74 (74.0)	98 (98.0)	0.08	(0.02, 0.32)	<0.01
	White bread	47 (47.0)	76 (76.0)	0.31	(0.17, 0.58)	<0.01
	Whole wheat bread	73 (73.0)	62 (62.0)	1.58	(0.89, 2.81)	0.12
	Cakes/buns [f]	83 (83.0)	98 (99.0)	0.06	(0.01, 0.44)	<0.01
	Porridge, like cornmeal, oatmeal [g]	85 (85.0)	97 (98.0)	0.07	(0.01, 0.54)	0.01
	Cereal [h]	74 (74.0)	91 (91.9)	0.31	(0.14, 0.68)	<0.01

*NR = Not reported due to unstable estimates caused by a limited number of observation in at least one of the cells

†If a 95% CI for the MOR does not include one, then we conclude a significant association at 5% level

[a]Parental education levels were missing for 3 cases and 5 controls

Res Autism Spectr Disord. Author manuscript; available in PMC 2016 October 01.

[b] Source of drinking water was missing for 1 control

[c] Source of water for cooking was missing for 1 control

[d] Fruits and vegetables consumption was missing for 1 control

[e] Rice consumption was missing for 1 control

[f] Cakes/buns consumption was missing for 1 control

[g] Porridge consumption was missing for 1 control

[h] Cereal consumption was missing for 1 control

P-values are unadjusted for multiple testing

Res Autism Spectr Disord. Author manuscript; available in PMC 2016 October 01.

Table 3

Factors associated with blood manganese levels (BMC < 12μg/L and BMC ≥ 12μg/L) using CLR for all participants (200 children or 100 matched pairs)

Variables	Category		BMC < 12 N (%)	BMC ≥ 12 N (%)	Matched OR (MOR)	95% CI for MOR[†]	*P*-value
Paternal age[a] (at child's birth)	More than 35 years		52 (38.8)	24 (43.6)	2.00	(0.60, 6.64)	0.26
Maternal age[b] (at child's birth)	More than 35 years		27 (19.7)	8 (13.8)	0.57	(0.17, 1.95)	0.37
Parental education levels[c] (at child's birth)	At least one of the parents had education beyond high school		74 (54.8)	20 (35.1)	0.50	(0.18, 1.33)	0.17
Source of drinking water[d]	Piped water		132 (94.3)	57 (95.0)	2.00	(0.37, 10.92)	0.42
Source of water for cooking[e]	Piped water		132 (94.3)	57 (95.0)	2.00	(0.37, 10.92)	0.42
Fruits and vegetables consumption[f]	Root vegetables	A. Yam, sweet potato, or dasheen	111 (79.3)	44 (74.6)	0.44	(0.14, 1.44)	0.18
		B. Carrot or pumpkin	130 (92.9)	54 (91.5)	0.60	(0.14, 2.51)	0.48
	Leafy vegetables	A. Lettuce	75 (53.6)	34 (57.6)	1.10	(0.47, 2.59)	0.83
		B. Callaloo, broccoli, or pakchoi	116 (82.9)	50 (84.8)	0.86	(0.29, 2.55)	0.78
		C. Cabbage	117 (83.6)	43 (72.9)	0.46	(0.16, 1.31)	0.14
	Legumes	String beans	55 (39.3)	20 (33.9)	0.56	(0.19, 1.66)	0.29
	Fruits	Tomatoes	100 (71.4)	47 (79.7)	0.88	(0.32, 2.41)	0.80
		Ackee	105 (75.0)	45 (76.3)	1.20	(0.37, 3.93)	0.76
		Avocado	66 (47.1)	31 (52.5)	1.25	(0.49, 3.17)	0.64
		Green banana	109 (77.9)	48 (81.4)	1.00	(0.32, 3.10)	1.00

Variables	Category	BMC < 12 N (%)	BMC ≥ 12 N (%)	Matched OR (MOR)	95% CI for MOR†	*P*-value
	Fried plantains	112 (80.0)	47 (79.7)	1.50	(0.42, 5.32)	0.53
Seafood consumption	High seafood consumption (More than 6 meals per week)	43 (30.7)	28 (46.7)	1.22	(0.51, 2.95)	0.66
	Ate saltwater fish	113 (80.7)	53 (88.3)	2.33	(0.60, 9.02)	0.23
	Ate fresh water fish (Pond fish, tilapia)	62 (44.3)	36 (60.0)	1.43	(0.54, 3.75)	0.47
	Ate sardine, mackerel (Canned fish)	115 (82.1)	52 (86.7)	0.60	(0.14, 2.51)	0.48
	Ate tuna (Canned fish)	51 (36.4)	24 (40.0)	1.17	(0.39, 3.47)	0.78
	Ate salt fish (Pickled mackerel)	113 (80.7)	50 (83.3)	1.00	(0.35, 2.85)	1.00
	Ate shellfish (Lobsters, crabs)	11 (7.9)	10 (16.7)	0.75	(0.17, 3.35)	0.71
	Ate shrimp	27 (19.3)	19 (31.7)	1.33	(0.46, 3.84)	0.59
Grains consumed	Rice [g]	138 (98.6)	59 (100.0)	NR	NR	NR
	Fried dumpling	120 (85.7)	51 (85.0)	0.57	(0.17, 1.95)	0.37
	Boiled dumpling	117 (83.6)	55 (91.7)	2.00	(0.50, 8.00)	0.33
	White bread	86 (61.4)	37 (61.7)	1.22	(0.51, 2.95)	0.66
	Whole wheat bread	102 (72.9)	33 (55.0)	0.55	(0.20, 1.48)	0.23
	Cakes/buns [h]	128 (91.4)	53 (89.8)	0.50	(0.09, 2.73)	0.42
	Porridge, like cornmeal, oatmeal [i]	128 (91.4)	54 (91.5)	0.75	(0.17, 3.35)	0.71
	Cereal [j]	116 (82.9)	49 (83.0)	0.86	(0.30, 2.55)	0.78

*NR = Not reported due to unstable estimates caused by a limited number of observation in at least one of the cells

†If a 95% CI for the MOR does not include one, then we conclude a significant association at 5% level

Res Autism Spectr Disord. Author manuscript; available in PMC 2016 October 01.

[a] Paternal age was missing for 6 with BMC<12 cases and 5 with BMC≥12

[b] Maternal age was missing for 3 with BMC<12 and 2 with BMC≥12

[c] Parental education levels were missing for 5 with BMC<12 and 3 with BMC≥12

[d] Source of drinking water was missing for 1 with BMC<12

[e] Source of water for cooking was missing for 1 with BMC<12

[f] Fruits and vegetables consumption was missing for 1 with BMC≥12

[g] Rice consumption was missing for 1 with BMC≥12

[h] Cakes/buns consumption was missing for 1 with BMC≥12

[i] Porridge consumption was missing for 1 with BMC≥12

[j] Cereal consumption was missing for 1 with BMC≥12

P-values are unadjusted for multiple testing

Table 4

Association of increased blood manganese concentrations (BMC) with ASD status among children with different *GSTP1* genotypes based on co-dominant and recessive genetic models using conditional logistic regression models. (200 children or 100 matched pairs)

Genetic Model	Higher BMC	Ref. BMC	Genotype (s)	Unadjusted			Adjusted[a]		
				Matched Odds Ratio	95% CI for MOR	*P* value	Matched Odds Ratio	95% CI for MOR	*P* value
Co-dominant	BMC ≥ 12μg/L vs.	BMC < 12μg/L at	*GSTP1* (Ile/Ile)	3.9	(1.15, 13.26)	0.03	5.93	(1.06, 33.29)	0.04
	BMC ≥ 12μg/L vs.	BMC < 12μg/L at	*GSTP1* (Ile/Val)	0.81	(0.31, 2.12)	0.66	1.15	(0.19, 6.82)	0.88
	BMC ≥ 12μg/L vs.	BMC < 12μg/L at	*GSTP1* (Val/Val)	0.35	(0.08, 1.52)	0.16	0.43	(0.06, 2.86)	0.38
Recessive	BMC ≥ 12μg/L vs.	BMC < 12μg/L at	*GSTP1* (Val/*)	0.62	(0.27, 1.34)	0.26	0.67	(0.19, 2.38)	0.54
	BMC ≥ 12μg/L vs.	BMC < 12μg/L at	*GSTP1* (Ile/Ile)	4.02	(1.19, 13.64)	0.03	5.92	(1.05, 33.32)	0.04

[a] Adjusted for potential confounders including parental education, place of child's birth (Kingston parish vs. other parishes), consumption of root vegetables ("yam, sweet potato, or dasheen"), salt water fish, and cakes/buns.

Note: All missing data are the same as those reported in the footnote of Tables 2 and 3.

P-values are unadjusted for multiple testing