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Interaction between manganese and *GSTP1* in relation to autism spectrum disorder while controlling for exposure to mixture of lead, mercury, arsenic, and cadmium

Mohammad H. Rahbar[a,b,c,*], **Maureen Samms-Vaughan**[d], **MinJae Lee**[b,c], **MacKinsey A. Christian**[a,c], **Jan Bressler**[a,e], **Manouchehr Hessabi**[c], **Megan L. Grove**[a,e], **Sydonnie Shakespeare-Pellington**[d], **Charlene Coore Desai**[d], **Jody-Ann Reece**[d], **Katherine A. Loveland**[f], **Compton Beecher**[g], **Wayne McLaughlin**[g,h], and **Eric Boerwinkle**[a,e,i]

[a]Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas 77030, USA.

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

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

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

[e]Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas 77030, USA.

[f]Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, Texas 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

[i]Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas 77030, USA.

Abstract

[*]**Corresponding Author**: Mohammad H. Rahbar, PhD, 6410 Fannin Street, Suite 1100.05, UT Professional Building, Houston, TX 77030, USA. Phone: (713)500-7901. Fax: (713)500-0766. Mohammad.H.Rahbar@uth.tmc.edu.

Conflict of interest

The authors declare no conflict of interest.

Background: We previously reported a significant interactive association between polymorphisms of *GSTP1* and blood manganese concentrations (BMC) with autism spectrum disorder (ASD) in Jamaican children. In this paper, we investigate the same interactive association with ASD while adjusting for the mixture of four metals (lead, mercury, cadmium, and arsenic).

Method: We used data from 163 case-control pairs of children 2–8 years of age from our autism project in Jamaica, in which we collected blood for heavy metals analysis at enrollment. To minimize potential multicollinearity between concentrations of the four metals, we generated a mixture index using generalized weighted quantile sum regression, which was used in conditional logistic regression models to control for the four metals while assessing the interactive association between *GSTP1* and BMC with ASD.

Results: Similar to the findings we reported previously, we found that in co-dominant and dominant models for *GSTP1,* among children with the Ile/Ile genotype, those with BMC > 12μg/L had 4.6 and 4.27 times higher odds of ASD compared to those with BMC < 12μg/L (adjusted Matched Odds Ratio (MOR) = 4.6, 95% CI: 1.21 – 17.42 and adjusted MOR = 4.27, 95% CI: 1.15 – 15.85, respectively). In the co-dominant model, for children with the Ile/Val and Val/Val genotypes, the adjusted MORs were 1.26 (95% CI: 0.32, 5.01) and 0.26 (95% CI: 0.05, 1.42), respectively.

Conclusions: After adjusting for the mixture of four metals, the interactive association of BMC and *GSTP1* with ASD remained significant with similar magnitude of associations. Results should be interpreted cautiously.

Keywords

Autism spectrum disorder (ASD); *GSTPP*; Heavy metals; Interaction; Weighted quantile sum (WQS) regression

1. Introduction

Humans are regularly exposed to a large number of environmental chemicals with potentially toxic effects on human health. These exposures often do not occur in isolation, but as a mixture of chemicals; however limited information is published regarding the effects of exposure to mixtures of chemicals on human health, including neurodevelopment (Taylor et al., 2016; Grandjean & Landrigan, 2014; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). This area of research has been identified as a topic of importance by the National Institute of Environmental Health Sciences (NIEHS), which has held several workshops since 2011 and provided grant funding (National Institute of Environmental Health Sciences (NIEHS), 2017) for development of innovative research methods to account for mixtures of chemicals when investigating their adverse effects on human health (Taylor et al., 2016; Carlin, Rider, Woychik, & Birnbaum, 2013). Additionally, according to the U.S. Environmental Protection Agency (EPA), greater emphasis should be given to understanding the combined toxic effects of metals (Fairbrother, Wenstel, Sappington, & Wood, 2007).

A review of the literature on the effects of trace metals on human health revealed many studies with sufficient sample sizes that appropriately adjusted for potential confounding variables (e.g., demographic factors) (Wirth & Mijal, 2010). Some studies have controlled

for potential confounding by other trace metals individually or as a mixture (Jurasovic, Cvitkovic, Pizent, Colak, & Telisman, 2004; Lunyera & Smith, 2017; Hsueh et al., 2017; Tsai et al., 2017). For example, Jurasovic et al., reported that blood lead concentration was positively associated with the percentage of slow sperm when adjusted for potential confounding variables including age, smoking, alcohol, blood cadmium, and serum copper, zinc, and selenium (Jurasovic et al., 2004). In addition, Tsai et al. investigated the association of urine concentrations of some heavy metals with kidney function, and reported an association between chromium exposure and decreased kidney function after controlling for lead and cadmium (Tsai et al., 2017). However, to our knowledge, very limited studies may have utilized mixtures of heavy metals in relation to autism spectrum disorder (ASD).

Several studies have reported that heavy metals, such as lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) are associated with ASD (Fido & Al-Saad, 2005; Kern, Grannemann, Trivedi, & Adams, 2007; Windham, Zhang, Gunier, Croen, & Grether, 2006; Yorbik, Kurt, Hasimi, & Ozturk, 2010; Blaurock-Busch, Amin, Dessoki, & Rabah, 2012; Soden, Lowry, Garrison, & Wasserman, 2007; Clark, Vandermeer, Simonetti, & Buka, 2010; Obrenovich, Shamberger, & Lonsdale, 2011; Adams, Romdalvik, Ramanujam, & Legator, 2007; DeSoto & Hitlan, 2007; DeSoto & Hitlan, 2008; Lakshmi Priya & Geetha, 2010; Blaylock, 2009; Blaylock, 2012; Arora et al., 2017). However, most of these findings are based on additive models assessing a single exposure at a time, an approach that is unable to account for potential confounding and interactions among a mixture of chemicals that co-exist in the environment. Analysis of environmental exposure data without consideration of influences from a mixture of chemicals could result in misleading findings.

Glutathione-S-transferase (GST) enzymes catalyze the conjugation of glutathione (GSH) to xenobiotic compounds, including heavy metals and metalloids, and thus play an important role in protecting against oxidative stress (Sharma, Yang, Sharma, Awasthi, & Awasthi, 2004; Jan et al., 2015). Several studies have linked oxidative stress, the imbalance between levels of reactive oxygen species (ROS) and antioxidant levels in the body, with ASD (Chauhan & Chauhan, 2006). Levels of GSH, the major cellular antioxidant (Coles & Kadlubar, 2003), as well as the ratio of reduced to oxidized GSH were lower in children with ASD compared to children without ASD (James et al., 2006; James et al., 2004; James, 2008), suggesting the involvement of oxidative stress in the disorder. Other studies have linked markers of oxidative stress, such as increased lipid peroxidation (Ming et al., 2005) and altered vascular characteristics (Yao, Walsh, McGinnis, & Pratico, 2006) to ASD. It has been suggested that oxidative stress may play a role in ASD through induction of autoimmunity in some children with ASD (Mostafa, El-Hadidi, Hewedi, & Abdou, 2010). Therefore, it has been postulated that variants in the genes coding for these GST enzymes may be associated with the risk of ASD.

Since 2009, our research team at the University of Texas Health Science Center at Houston (UTHealth) has collaborated with faculty at the University of the West Indies (UWI) to investigate the role of five metals (lead, mercury, arsenic, cadmium, and manganese) and their potential interaction with three *GST* family genes (*GSTM1, GSTP1 and GSTT1*) in relation to ASD in Jamaica. So far, we have reported the absence of associations between ASD status and each of the five metals (lead, mercury, arsenic, cadmium, and manganese) in

additive models (Rahbar et al., 2012; Rahbar et al., 2013; Rahbar et al., 2014b; Rahbar et al., 2014c; Rahbar et al., 2014a). However, using data from 100 age- and sex-matched case-control pairs (age 2–8 years), we found a significant gene-environment interaction between *GSTP1* and blood manganese concentrations (BMC) indicating that among children who had the Ile/Ile genotype for *GSTP1*, those with BMC > 12μg/L had nearly six times higher odds of ASD compared to those with BMC < 12μg/L (adjusted MOR = 5.9, 95% CI: 1.06 – 33.29) after adjusting for parental education, place of child's birth, and consumption of root vegetables (yam, sweet potato, or dasheen), salt water fish, and cakes/buns in a co-dominant model (Rahbar et al., 2015).

However, due to limited sample size and the aforementioned covariates, we did not control for potential confounding effects of the other four metals (i.e., lead, mercury, cadmium, and arsenic) individually or as a mixture. The main objective of this research is to investigate whether the magnitude of the interactive associations of exposure to manganese and *GSTP1* in relation to ASD will remain similar to our previously reported findings after controlling for environmental exposure to a mixture of four metals (lead, mercury, cadmium, and arsenic) as well as the same covariates used in our previous paper (Rahbar et al., 2015) with a larger sample size.

2. Materials and Methods

2.1. General description

Since 2009, our team at UTHealth, in collaboration with a team of investigators at the UWI, has conducted two phases of an age- and sex-matched case-control study in which we enrolled Jamaican children ages 2–8 years. After obtaining written consent, to confirm the diagnosis of ASD, 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). For typically developing (TD) 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 TD children (Mulligan, Richardson, Anney, & Gill, 2009). At the time of enrollment, we administered a socioeconomic status (SES) questionnaire to the parents of each participant to obtain demographic data, such as age and race/ethnicity of the child and parents, educational level of the parents, and car ownership as an index of wealth. We also administered a food frequency questionnaire to collect data on possible exposures to heavy metals through food and water sources, especially the types and frequency of fruits, vegetables, and seafood consumed by the children on a weekly basis. Details on the recruitment and assessment procedures of this study have been reported previously (Rahbar et al., 2012; Rahbar et al., 2013; Rahbar et al., 2014a; Rahbar et al., 2014c; Rahbar et al., 2014d).

At the end of the interview and other assessments, approximately 5mL of venous whole blood and 2mL of saliva were collected from each child for assessment of trace metals and

genetic analysis (Rahbar et al., 2014d). Trace metal analysis was carried out at the Michigan Department of Health and Human Services (MDHHS) while genetic analysis was performed at the Human Genetics Center (HGC), School of Public Health, The University of Texas Health Science Center at Houston. This study was approved by the Institutional Review Boards of MDHHS, UTHealth, and the UWI, Mona campus, in Kingston, Jamaica. For this paper, we have pooled the data from both phases of this study (n=163 matched pairs).

2.2. Assessment of exposure to metals

The methods for analysis of blood metal concentrations by MDHHS have been described previously (Rahbar et al., 2013; Rahbar et al., 2014a; Rahbar et al., 2014b; Rahbar et al., 2014d; Rahbar et al., 2015). The half-life in blood varies among the different metals. For example, cadmium is eliminated from the body in two parts: a fast component and a slow component. In blood, the half-life of cadmium is estimated to be 75–128 days for the fast component and 7.416.0 years for the slow component (Jarup, Rogenfelt, Elinder, Nogawa, & Kjellstrom, 1983), however estimates of blood half-life are scarce in the literature. The half-life of cadmium is typically measured in the kidneys but is similar to that estimated in blood, ranging from 6 to 38 years (ATSDR, 2008). According to the Centers for Disease Control and Prevention (CDC), blood cadmium concentrations reflect recent and long-term exposures, and many studies found similar cadmium concentrations in both urine and blood (Centers for Disease Control and Prevention (CDC), 2016). Arsenic has a relatively short half-life in blood, ranging from 4–6 hours for inorganic arsenic and 20–30 hours for its methylated metabolites (Mayo Clinic, 2018). Therefore, arsenic concentrations in blood reflect exposures within about 2 days. While urine is typically preferred as the biomarker to measure arsenic, urine and blood arsenic concentrations have been reported as strongly correlated (Hall et al., 2006). The half-life of lead, mercury, and manganese in blood is estimated to be 28–36 days (ATSDR, 2007), 60–90 days (Clarkson, Magos, & Myers, 2003), and less than 74 days (Crossgrove & Zheng, 2004), respectively. We believe that the Jamaican community has continuous exposure to these five metals (i.e., lead, mercury, arsenic, cadmium, and manganese) through various sources including fruits and vegetables (Howe, Fung, Lalor, Rattray, & Vutchkov, 2005), as well as seafood (Rahbar et al., 2012; Rahbar et al., 2013) and water. It has been reported that continuous exposures such as these subsequently increase blood concentrations in humans through bioaccumulation (ATSDR, 2007). Therefore, although not perfect, we consider the blood concentrations of the five metals used in this study to be relatively reliable.

The limit of detection (LoD) was reported by MDHHS for each of the five metals (Pb, Hg, As, Cd, and Mn). Since analysis of metals for both phases of this study was carried out over the last 9 years, there have been changes in technology that affected the ability of instruments to detect metal concentrations in blood samples. Therefore, MDHHS reported different LoDs for each metal in phases 1 & 2. The LoDs for Pb, Hg, As, Cd, and Mn in phase 1 were 0.3μg/dL, 0.3μg/L, 1.0μg/L, 0.20μg/L, and 1.0μg/L respectively. In phase 2, the LoDs for Pb, Hg, As, Cd, and Mn were 0.25μg/dL, 0.25μg/L, 1.3μg/L, 0.13μg/L, and 2.5μg/L respectively. Blood metal concentrations were reported as undetectable if they were below the LoD. One hundred percent of samples had Pb and Mn concentrations that were above the LoD. However, over half of the samples (56.44%) had Cd concentrations below

the LoD and 8.28% and 16.26% of Hg and As concentrations were below the LoD, respectively.

2.3. Statistical Analysis

Descriptive analyses were conducted to compare demographic and socioeconomic characteristics of ASD cases and TD controls. As the distributions of blood lead, mercury, arsenic and cadmium concentrations are skewed, the data were transformed using the natural logarithm (ln) to produce approximately normal distributions. The means of the log transformed blood metal concentrations were transformed back to their original scale by applying the natural exponential function to calculate geometric means. However, BMC had an approximately normal distribution, so no transformation was made and only the arithmetic mean is reported.

For the *GSTM1* and *GSTT1* genes, since the assay does not distinguish between a normal homozygote (I/I) and a heterozygote (I/D), we considered only a recessive model using a binary variable to represent their genotype: I* and DD. For the *GSTP1* gene, all three genotypes, Ile/Ile, Ile/Val and Val/Val, were available. We analyzed the GSTP1 gene using different genetic models, including the dominant (Val/* vs Ile/Ile) and the co-dominant models. For the *GSTP1* polymorphism, we tested whether Hardy-Weinberg equilibrium expectations were met using the chi-square test in the TD control group (Rahbar et al., 2016; Rahbar et al., 2014d; Rahbar et al., 2015).

In this study, we used conditional logistic regression (CLR) models to assess the association between ASD status (ASD case or TD control) and various exposure variables including genotypes for *GSTP1, GSTM1,* and *GSTT1* (Rahbar et al., 2016; Rahbar et al., 2014d; Rahbar et al., 2015). Other variables included demographics and SES, parents' education, and potential nutritional exposures. The types of nutritional exposures included weekly consumption of seafood, fruits, and vegetables by the children, which were dichotomized (i.e. consumed or never consumed) to be consistent with our previous publication in terms of the way these variables were analyzed (Rahbar et al., 2015). Details regarding these exposure variables were also reported previously (Rahbar et al., 2012; Rahbar et al., 2014c).

We initially assessed pairwise correlations between all the metal concentrations using log transformed concentrations, if applicable. Then we used General Linear Models (GLMs) with the log-transformed (if applicable) metal concentrations as the dependent variable to investigate possible univariable associations with ASD. In all GLMs, we also controlled for the clustering effect of matching by including an appropriate number of dummy variables that represented the matched pairs (e.g., 162 dummy variables for 163 matched pairs). Given that we have already reported a significant interaction between Mn and *GSTP1* in relation to ASD when Mn was individually tested, (Rahbar et al., 2015), our goal is to test whether this interaction effect remains after controlling for concentrations of the other four metals (Pb, Hg, As, and Cd). In order to minimize any potential effects of multicollinearity due to possible correlation between/among concentrations of Pb, Hg, As, and Cd, we utilized mixture analysis. Specifically, we used generalized weighted quantile sum (WQS) regression (Czarnota, Gennings, & Wheeler, 2015) to generate a mixture of the four metals in ASD. Since the current version of the WQS model does not allow one to account for the statistical

interaction effect between the mixture of metals and other variables, we used WQS to generate a mixture of only the four metals that did not show statistical interactive effects with *GSTP1* based on our prior research (i.e., Pb, Cd, Hg, and As), keeping Mn outside the mixture. To minimize the influence of measurements below the LoD and/or skewed distributions of metals in our study, concentrations of each of the four metals that were below the LoD were replaced by the LoD for that metal divided by the square root of two (Hornung & Reed, 1990; Meeker, Sathyanarayana, & Swan, 2009), and then the metal concentrations were scored into quantiles (i.e., 0, 1, 2 and 3). Then in the WQS model, the relevant contribution of each of the four metal concentrations to the association between the WQS index and ASD was identified by estimated weights that are empirically determined through bootstrap sampling and assigned to each factor to reduce dimensionality of correlated data. Since the current version of WQS regression cannot account for the correlation within the clusters (i.e., pairs), we conducted bootstrap sampling at the individual level, instead of pair level and considered the variables on which the ASD cases and TD controls were matched (i.e., sex and age) for the estimation of weights. From a total of B=1,000 bootstrap samples of size 196 that were generated from the training dataset (60% of the total dataset), the unknown weights, which are constrained to be between 0 and 1 and add up to 1, were estimated for the model where sex and age and interaction between Mn and each *GSTP1* gene were included. The WQS was then generated based on the bootstrap samples in which the parameter estimate for the weighted index was significant.

Once we generated the WQS of the four metals, we categorized the mixture index as above or below the 75th percentile and this binary index variable is used in several analyses. We first assessed the univariable association between the mixture index of the four metals and ASD, as well as other various exposures, including sociodemographic and food frequency variables using GLMs. Then, we used the WQS mixture index as a variable in the conditional logistic regression (CLR) models to control for the effect of the mixture of the four metals along with other potential confounders while assessing the interaction effect between Mn and *GSTP1* on ASD. In this analysis, we considered the co-dominant and dominant genetic models previously used to assess the interaction of *GSTP1* genotype and Mn in relation to ASD after categorizing BMC into two levels (BMC < 12 μg/L and BMC >_12 μg/L), Although the recommended cutoff value for elevated BMC is 15 μg/L (ATSDR, 2012b; ATSDR, 2012a) the choice of 12 μg/L, which corresponds to the 75th percentile, allows greater statistical power by analyzing 25% of children in the high exposure group (Rahbar et al., 2015). The CONTRAST statement in PROC Logistic (Kleinbaum DG and Klein M. Logistic regression: a self-learning text. Springer New York 2010) in SAS version 9.4 (SAS Institute Inc., 2013) was used to test whether there was a significant difference in odds of ASD between children whose BMC was either <12 μg/L or >12 μg/L for each *GSTP1* genotype group considered.

To compare the performance of the WQS methods for mixtures with the traditional methods of adjusting for confounders, we also fit different CLR models that controlled for concentrations of the other four metals (Pb, Hg, As, and Cd) individually as well as controlling for all four metal concentrations simultaneously as separate variables in one model. We ran these CLR models both a) adjusted only for the other metal(s) and b) adjusted for the other metal(s) and other potential confounders. We refer to the former as

"unadjusted" and the latter as "adjusted." All analyses were performed using R (R Core Team, 2015) and SAS version 9.4 at a significance level of 0.05.

3. Results

The mean age of ASD cases and TD controls was 65.3 months and 65.7 months, respectively. About 80% of the ASD cases and TD controls were male. Nearly all of the ASD cases (94.5%) and TD controls (99.4%) were Afro-Caribbean. Similarly, 97.0% of mothers and 97.2% of fathers were Afro-Caribbean. A higher proportion of both the mothers (19.6%) and fathers (48.1%) of ASD cases were age 35 or greater at the time of the child's birth compared to that of the mothers (9.5%) and fathers (24.2%) of TD controls. Similarly, the mothers (88.9%) and fathers (88.2%) of ASD cases had higher education compared to the mothers (75.5%) and fathers (75.5%) of TD controls. ASD cases were also of a higher SES compared to TD controls, with 60.1% of case families owning a car versus 36.2% of control families. There were no significant differences in allele frequencies for *GSTP1, GSTM1*, or *GSTT1* between ASD cases and TD controls. Furthermore, the *GSTP1* allele frequency in the TD controls was in agreement with Hardy-Weinberg equilibrium expectations ($P = 0.81$). Descriptive characteristics of the study population are reported in Table 1.

The pairwise correlation coefficients between each of the five metals are reported in Table 2, both overall and stratified by each possible genotype of *GSTP1.* Although the pairwise correlations were generally low to moderate, most correlations indicated a positive relationship between each pair of metals. Hg was the most consistently correlated with the other metals across all possible genotypes, with a moderate correlation with As ($r = 0.44$ to 0.51), weaker correlations with Cd ($r = 0.13$ to 0.25) and Pb ($r = 0.10$ to 0.15), and low to no correlation with Mn ($r = -0.004$ to 0.12). Pb was also moderately correlated with Cd and As, however the correlations varied depending on the genotype. Cd showed consistently moderate correlations with the other metals, however over half of the sample had cadmium concentrations below LoD. In general, Mn had low to no correlation with the other metals.

In generating the WQS mixture index of the four metals (Pb, Hg, As, and Cd), the relative strength of contributions of each metal on ASD was assessed, and the mixture index was included as a variable in the models that examined interactive effects of Mn and *GSTP1* in relation to ASD. We found that Pb was the largest contributor to the mixture effect of four metals on ASD. About 61% of the total weights of WQS index were captured by Pb, and 18%, 13%, and 8% were captured by Hg, Cd, and As, respectively (data not shown).

Univariable analysis (Table 3) revealed that, compared to TD controls, ASD cases had lower geometric mean blood concentrations of Pb (2.11 μg/dL vs. 2.68 μg/dL; $P < 0.01$) and Hg (0.66 μg/L vs. 0.78 μg/L; $P < 0.05$). In contrast, no significant differences were detected between ASD cases and TD controls for the geometric mean blood concentrations of As or Cd, or the arithmetic mean blood concentration of Mn (all $P > 0.25$). ASD cases had a significantly lower mean mixture index of Pb, Hg, As, and Cd compared to controls (1.30 vs 1.60, $P < 0.01$).

We also investigated the possible associations between the mixture index of Pb, Hg, As, and Cd with various exposure variables using univariable GLMs (Table 4). Results suggest that the mixture index of the four metals is significantly higher among those with dietary exposure to certain fruits and vegetables, including yam, sweet potato, or dasheen ($P < 0.01$), string beans ($P = 0.05$), tomatoes ($P = 0.02$), and ackee ($P < 0.01$), compared to those who did not have these exposures. Similarly, results showed higher mixture index among those with dietary exposure to certain types of seafood compared to those who were not exposed. Specifically, the mean mixture index was significantly higher for those who ate salt water fish ($P = 0.04$) and salted fish ($P < 0.01$), and marginally higher for those who ate fresh water fish ($P = 0.08$), sardine or mackerel ($P = 0.07$), and shellfish ($P = 0.06$), compared to those who did not eat these types of seafood. No associations were detected between the mixture index and SES, maternal age, parental education levels, source of drinking water, consumption of other fruits, vegetables, or seafood, and genotypes of *GSTT1, GSTM1,* or *GSTP1.*

To be consistent with our previous publication, we first ran both the co-dominant and dominant genetic models without adjusting for the mixture of four metals as a potential confounder, making the increased sample size the only difference between the prior and current models (Table 5). In the co-dominant model, we found a significant interaction between BMC and *GSTP1* genotype (Ile/Ile) (P for the interaction term = 0.03). This interaction remained significant (P for the interaction term = 0.03) after adjusting for parental education, place of child's birth, and consumption of root vegetables (yam, sweet potato, dasheen), salt water fish, and cakes/buns, indicating that among children with the Ile/Ile genotype, those with BMC >12 μg/L had 4.54 times higher odds of ASD compared to those with BMC < 12 μg/L (MOR = 4.54, 95% CI: 1.20 – 17.25). We observed similar results based on the dominant genetic model. The interaction between BMC and *GSTP1* genotype (Ile/Ile) was significant in both unadjusted and adjusted models (P for the interaction term = 0.01 and 0.02, respectively). Based on the adjusted dominant model, among children with the Ile/Ile genotype, those with BMC > 12 μg/L had 4.23 times higher odds of ASD compared to those with BMC < 12 μg/L (MOR = 4.23, 95% CI: 1.14 – 15.69). Interactive effects (MORs) of the binary BMC and other *GSTP1* genotypes in relation to ASD status using both co-dominant and dominant genetic models are reported in Table 5.

After reinvestigating our previously published interaction between BMC and *GSTP1 g*enotype in relation to ASD status, while controlling only for the mixture index of Pb, Hg, As, and Cd, we found marginally significant interaction between BMC and *GSTP1* genotype (Ile/Ile) (P for the interaction term = 0.06) in the co-dominant genetic model. However, after adjusting for the additional covariates used in our previously reported models (Rahbar et al., 2015), including parental education, place of child's birth, and consumption of root vegetables (yam, sweet potato, dasheen), salt water fish, and cakes/buns, the interaction between BMC and *GSTP1* genotype (Ile/Ile) became significant (P for the interaction term = 0.03) in the co-dominant genetic model. Specifically, we found that among children with the Ile/Ile genotype, those with BMC >12 μg/L had 4.58 times higher odds of ASD compared to those with BMC <12 μg/L (MOR = 4.58, 95% CI: 1.21 – 17.42) after adjusting for the aforementioned covariates. Similar results were found using the dominant genetic model. In the interactive model only controlling for the mixture index of Pb, Hg, As, and Cd, there was

significant interaction between BMC and *GSTP1* genotype in relation to ASD status (*P* for the interaction term = 0.01). This interaction remained significant after controlling for the previously mentioned covariates (*P* for the interaction term = 0.02). Our results suggest that among those with the Ile/Ile genotype, children with BMC > 12μg/L had 4.27 times higher odds of ASD compared to those with BMC < 12μg/L (MOR = 4.27, 95% CI: 1.15 – 15.85), after adjusting for the aforementioned covariates. In the co-dominant model, for children with the Ile/Val and Val/Val genotypes, the adjusted MORs were 1.26 (95% CI: 0.32, 5.01) and 0.26 (95% CI: 0.05, 1.42), respectively. In the dominant model, for children with the Val/* (Ile/Val or Val/Val) genotype, the adjusted MOR was 0.67 (95% CI: 0.25, 1.83). Interactive effects of the binary BMC and other *GSTP1* genotypes in relation to ASD status using both co-dominant and dominant genetic models are reported in Table 6.

We also ran models adjusting for each metal individually and all four metals at once as separate variables in order to compare the WQS method with other traditional methods of making adjustments. We found similar results across all CLR models for both the co-dominant and dominant genetic models. In short, all interaction terms for BMC and *GSTP1* genotype (Ile/Ile) were either significant or marginally significant, ranging from $P = 0.01$ to $P = 0.09$ in unadjusted co-dominant and dominant models (Table 7.a.) and $P = 0.02$ to $P = 0.05$ in adjusted co-dominant and dominant models (Table 7.b.). Similarly, all adjusted co-dominant models showed that among children with the Ile/Ile genotype, children with BMC > 12μg/L had about 4.5 times higher odds of ASD compared to those with BMC < 12μg/L. All adjusted dominant models showed that among children with the Ile/Ile genotype, children with BMC > 12μg/L had about 4.2 times higher odds of ASD compared to those with BMC < 12μg/L. Interactive effects (MORs) of the binary BMC and other *GSTP1* genotypes in relation to ASD status using both codominant and dominant genetic models are reported in Table 7.a. and 7.b.

4. Discussion

In this paper, we reinvestigated our previously reported finding of a significant interaction between BMC and *GSTP1* genotype in relation to ASD status in Jamaican children (Rahbar et al., 2015) with a larger sample size and controlling for a mixture score that represents a combined exposure to Pb, Hg, As, and Cd. The main finding of this paper is that the interactive effects of BMC and *GSTP1* genotype remained similar to our previously reported findings after controlling for the mixture index of four metals (Pb, Hg, As, and Cd) and the same covariates that were used in our previously reported models, including parental education, place of child's birth, and consumption of root vegetables (yam, sweet potato, dasheen), salt water fish, and cakes/buns (Rahbar et al., 2015). In our previous study, based on a co-dominant genetic model, we found that among children with the Ile/Ile genotype, those with BMC > 12μg/L had nearly six times higher odds of ASD compared to those with BMC < 12μg/L (adjusted MOR = 5.9, 95% CI: 1.06 – 33.29) after adjusting for the aforementioned covariates (Rahbar et al., 2015). When we replicated the same analyses, with the only difference being the increased sample size (from 100 pairs to 163 pairs), the MOR was reduced to 4.54 (adjusted MOR = 4.54, 95% CI: 1.20 – 17.25). This is similar in magnitude to the MOR we reported in our analyses with the additional adjustment for the mixture score of Pb, Hg, As, and Cd (MOR = 4.58, 95% CI: 1.21 – 17.42), suggesting that

the increased sample size is responsible for the reduction in the MOR rather than the adjustment for the mixture score of the four metals as a potential confounder. Despite the 22% reduction in the MOR from 5.9 to 4.58, the magnitude of the interactive effect of *GSTP1* and BMC on ASD still remains sizeable and in the same direction compared to our previous analyses. Similar results were found for Ile/Ile using the dominant genetic model. Our results for other genotypes were also of similar in terms of magnitude and direction for both the co-dominant and dominant models in the present analyses compared to our previous work.

However, when we adjusted for the effects of the other metals as separate variables in our CLR models, we found results similar to those reported using WQS mixture score to adjust for the other four metals. Although in this particular example the metal concentrations were not strongly correlated enough to make a significant difference between the traditional adjustment methods for the metal concentrations and the adjustment using the WQS mixture score, we advocate the use of the WQS score as an indicator of the mixture of several metal exposures for adjustment in regression models. Some of the advantages of using the WQS methods include: 1) the reduced dimensionality of the data (i.e., fewer independent variables in the regression models), which results in a more efficient analysis, particularly when the sample size is limited; and 2) even when the pairwise correlations between the metals are considered low, the WQS method still incorporates these correlations in calculating the mixture score, resulting in a more reliable measure for controlling the potential cofounding effects of mixtures of metals.

The analysis and identification of important chemicals in a mixture is usually difficult due to collinearity effects of heavy metals (Czarnota et al., 2015). The ordinary regression models (traditional methods) could not address the collinearity and variance inflation due to mixtures (Carrico, Gennings, Wheeler, & Factor-Litvak, 2015) and can be difficult to interpret due to large variances and covariances (Oyeyemi, Ogunjobi, & Folorunsho, 2015). Shrinkage methods (e.g., Least Absolute Shrinkage and Selection Operator (LASSO) method) reduces the variability of the estimates by shrinking the coefficients (Oyeyemi et al., 2015), but this method is less accurate in the selection of potentially correlated metal mixtures (Czarnota et al., 2015). Recently, WQS regression analysis is one of the methods that help to address this issue (Czarnota et al., 2015).

5. Limitations

There are several limitations in this study. Some of these limitations have already been reported earlier (Rahbar et al., 2015). Previously reported limitations include the potential for recall bias and the use of blood concentrations of the metals, which accurately represent “current” exposures at the time of enrollment. Therefore, our analyses cannot establish causality or implicate metals in ASD etiology. However, as discussed in our previous manuscript (Rahbar et al., 2015), the Jamaican population may be continually exposed to manganese and the other four metals because of accumulation in crops and seafood, which are consumed regularly by much of the Jamaican population. Therefore, the blood concentrations of Jamaican children may represent continuous exposure levels with long-term duration. Additionally, our limited sample size may have caused increased standard

errors, resulting in wide confidence intervals that should be interpreted with caution. However, the confidence intervals in the present analysis were narrower compared to our previous findings. We also acknowledge that a large percentage of blood Cd concentrations were below LoD. Since these concentrations were scored into their quantiles for WQS estimation, rather than the actual concentrations of these metals, we believe that the estimates are not significantly affected by a large proportion of Cd data below the LoD.

There are also some limitations associated with the WQS method. Even though this paper is mainly focused on testing for the interactive effect of manganese and *GSTP1* in relation to ASD, while adjusting for a weighted index (WQS) of the other four metals and other covariates as potential confounders, estimating weights simultaneously using WQS based on all five metals including Mn and stratified by genotypes of *GSTP1,* would be of great interest. However, stratifying the samples by genotypes of *GSTP1,* which may vary within the case-control pairs, would be inappropriate for a matched case-control study. Additionally, given the smaller sample size of a stratified subsample, conducting estimation procedures using bootstrap samples may not be reliable. Despite these issues, we conducted stratified WQS analysis, but we were not able to evaluate a *GSTP1* genotype-specific mixture of all five metals because the models did not converge, possibly due to limited frequency of data within a case-control pair when stratified by genotypes of *GSTP1.* In order to estimate a weighted index in which the stratum-specific weights of exposure variables are determined through the optimization procedure that accounts for correlations within a matched-pair, it is imperative to develop a new statistical approach that can simultaneously establish these processes using the entire dataset, not subsamples. This should be considered as future directions for methods improvement related to the WQS procedure.

6. Conclusions

We previously reported a significant interaction between polymorphisms of *GSTP1* and BMC in relation to ASD in Jamaican children. In this paper we reassessed the aforementioned interactive association of BMC and *GSTP1* with ASD while adjusting for the mixtures of environmental exposures to lead, mercury, cadmium, and arsenic as well as other covariates that included parental education, place of child's birth, and consumption of root vegetables (yam, sweet potato, dasheen), salt water fish, and cakes/buns. We found that the interactive effects of *GSTP1* and BMC in relation to ASD remained similar to our previously reported findings after adjusting for the mixture score of the other four metals and the same covariates used in our previous investigation. Specifically, in both co-dominant and dominant models for *GSTP1,* our findings indicated that among children with the Ile/Ile genotype, those with BMC > 12 μg/L had about 4 times higher odds of ASD compared to those with BMC <12 μg/L. In the co-dominant model, for children with the Ile/Val and Val/Val genotypes, the aforementioned adjusted MORs were 1.26 and 0.26, respectively. Despite similar findings in both investigations, we recommend considering mixture analysis of environmental exposures when investigating their associations with ASD to avoid potential multicollinearity due to correlation between metal concentrations since such exposures do not occur in isolation.

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HIGHLIGHTS

- Children with BMC>12μg/L and *GSTP1* Ile/Ile genotype have 4 times higher odds of ASD
- Humans are also exposed to mixtures of other metals such as Pb, Hg, As, and Cd
- Generalized weighted quantile sum was used to control for mixture of these 4 metals
- Interaction between *GSTP1* and BMC was significant after adjusting for the mixture

Table 1.

Characteristics of children and their parents by ASD case status (163 matched pairs)

Variables	Categories	ASD Case (n=163) N (%)	TD Control (n=163) N (%)	*P*-value*
Child's sex	Male	130 (79.8)	130 (79.8)	NA
Child's age (months)	Age < 72	108 (66.3)	108 (66.3)	1.00
	Age ≥ 72	55 (33.7)	55 (33.7)	
Child's race	Afro-Caribbean	154 (94.5)	162 (99.4)	0.15
Maternal age[a] (at child's birth)	Age < 35	131 (80.4)	143 (90.5)	0.01
	Age ≥ 35	32 (19.6)	15 (9.5)	
Paternal age[b] (at child's birth)	Age < 35	82 (51.9)	116(75.8)	<0.01
	Age ≥ 35	76 (48.1)	37 (24.2)	
Maternal race	Afro-Caribbean	155 (95.1)	162 (99.4)	0.19
Paternal race[c]	Afro-Caribbean	155 (95.1)	159 (97.6)	0.77
Maternal education[d] (at child's birth)	Up to high school†	18 (11.1)	39 (24.5)	<0.01
	Beyond high school††	144 (88.9)	120 (75.5)	
Paternal education[e] (at child's birth)	Up to high school†	18 (11.8)	37 (24.5)	<0.01
	Beyond high school††	135 (88.2)	114(75.5)	
Socioeconomic status (SES)	Car ownership	98 (60.1)	59 (36.2)	<0.01
GSTP1	Ile/Ile	50 (30.7)	40 (24.5)	0.28
	Ile/Val	82 (50.3)	83 (50.9)	
	Val/Val	31 (19.0)	40 (24.5)	
GSTM1[h]	DD[f]	43 (26.5)	38 (23.8)	0.60
	I/I or I/D[g]	119(73.5)	122 (76.2)	
GSTT1[i]	DD[f]	48 (29.6)	33 (20.6)	0.09
	I/I or I/D[g]	114(70.4)	127 (79.4)	

*P-values are based on Wald's test in conditional logistic regression models

†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 was missing for 5 ASD cases and 10 TD controls

[c] Paternal race was missing for 1 ASD case and 1 TD control

[d] Maternal education was missing for 1 ASD case and 4 TD controls

[e] Paternal education was missing for 10 ASD cases and 12 TD controls

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

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

[h] *GSTM1* genotype was missing for 1 ASD case and 1 TD control

[i] *GSTT1* missing for 1 ASD case and 1 TD control

Table 2.

Pairwise correlation coefficients between all blood metals concentrations overall and by *GSTP1* genotype

Overall	**Log (Pb)**	**Log (Cd)**	**Log (Hg)**	**Log (As)**	**Mn**
Log (Pb)	1.00	0.15	0.13	0.16	0.03
Log (Cd)		1.00	0.19	0.27	0.08
Log (Hg)			1.00	0.45	−0.004
Log (As)				1.00	0.04
Mn					1.00
By GSTP1 (Ile/Ile)	**Log (Pb)**	**Log (Cd)**	**Log (Hg)**	**Log (As)**	Mn
Log (Pb)	1.00	0.05	0.10	0.09	−0.06
Log (Cd)		1.00	0.25	0.24	0.01
Log (Hg)			1.00	0.45	−0.07
Log (As)				1.00	−0.04
Mn					1.00
By GSTP1 (Ile/ Val)	**Log (Pb)**	**Log (Cd)**	**Log (Hg)**	**Log (As)**	Mn
Log (Pb)	1.00	0.18	0.12	0.23	0.08
Log (Cd)		1.00	0.19	0.33	0.14
Log (Hg)			1.00	0.44	−0.02
Log (As)				1.00	0.01
Mn					1.00
By GSTP1 (Val/Val)	**Log (Pb)**	**Log (Cd)**	**Log (Hg)**	**Log (As)**	Mn
Log (Pb)	1.00	0.13	0.15	0.05	0.01
Log (Cd)		1.00	0.13	0.16	0.04
Log (Hg)			1.00	0.51	0.12
Log (As)				1.00	0.26
Mn					1.00

Table 3.

Arithmetic and geometric mean blood metal concentration of children by ASD case status (163 matched pairs)

Variables	Arithmetic Mean ASD Cases	Arithmetic Mean TD Control	*P*-value	Geometric Mean[a] ASD Cases	Geometric Mean[a] TD Control	*P*-value[b]
Lead (μg/dL)	2.97	3.51	0.09	2.11	2.68	<0.01
Mercury (μg/L)	1.00	1.03	0.77	0.66	0.78	<0.05
Arsenic (μg/L)	3.09	3.08	0.93	2.48	2.60	0.25
Cadmium (μg/L)	0.23	0.21	0.21	0.17	0.17	0.98
Mixture index (Lead, Mercury, Arsenic, Cadmium)	1.30[c]	1.60[c]	<0.01	-	-	-
Manganese (μg/L)	10.68	10.69	0.98	NA	NA	NA

NA: not applicable, because distribution of blood manganese concentrations was normal

[a] Mean blood metal concentration indicates the geometric mean = Exp. [Mean (In metal concentration)]

[b] P-values are based on the log-transformed blood metal concentrations

[c] Mixture index is weighted for the mixture of only the four metals

Table 4.

Associations of various exposure variables with mixture index (lead, mercury, arsenic, cadmium) based on univariable General Linear Models (163 matched pairs)

Exposure variables	Category		Yes		No		P-value
			Mean* Mixture index	N	Mean* Mixture index	N	
Socioeconomic status	Own a car		1.39	157	1.50	169	0.36
Maternal age[a] (at child's birth)	More titan 35 years		1.29	47	1.48	247	0.24
Parental education levels[b] (at child's birth)	At least one of the parents had education beyond high school		1.46	282	1.50	18	0.89
Source of drinking water[c]	Piped water		1.45	302	1.54	23	0.67
Fruits and vegetables consumption[d]	Root vegetables	A. Yam, sweet potato, or dasheen	1.56	235	1.19	90	<0.01
		B. Carrot or pumpkin	1.45	286	1.49	39	0.83
	Leafy vegetables	A. Lettuce	1.50	157	1.41	168	0.43
		B. Callaloo, broccoli, or pak choi	1.50	256	1.29	69	0.14
		C. Cabbage	1.50	239	1.33	86	0.24
	Legumes	String beans	1.61	102	1.38	223	0.05
	Fruits	Tomatoes	1.56	222	1.24	103	0.02
		Ackee	1.59	222	1.18	103	<0.01
		Avocado	1.54	145	1.39	180	0.24
Seafood consumption	Ate salt water fish		1.53	236	1.24	90	0.04
	Ate fresh water fish (pond fish, tilapia)		1.58	134	1.36	192	0.08
	Ate sardine, mackerel (canned fish)		1.50	259	1.24	67	0.07
	Ate tuna (canned fish)		1.50	111	1.43	215	0.56
	Ate salted fish (pickled mackerel)		1.55	250	1.13	76	<0.01
	Ate shellfish (lobsters, crabs)		1.79	33	1.41	293	0.06
	Ate shrimp		1.43	59	1.46	267	0.86
Genes	GSTT1 (I*)[e]		1.45	241	1.47	81	0.88
	GSTM1 (I*)[e]		1.45	241	1.48	81	0.82
	GSTP1 (Ile/Ile)[f]		1.43	90	1.46	236	0.83
	GSTP1 (Val/Val)[f]		1.40	71	1.46	255	0.69
	GSTP1 (Ile/Val)·[f]		1.48	165	1.42	161	0.61

*Mixture index is weighted for the mixture of only the four metals

The "Yes" column includes participants who met the category specified in front of each exposure variable

The "No" column includes participants who did not meet the category specified in front of each exposure variable

[a]Maternal age was missing for 5 participants

[b] Parental education levels was missing for 26 participants

[c] Source of drinking water was missing for 1 participant

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

[e] I* indicate the homozygote (FI) or a heterozygote (FD) for *GSTT1 and GSTM1; genotypes* were missing for 4 participants

[f] *GSTPI* has 3 categories (Ile Ile, Ile/Val, VaFVal)

Table 5.

Association of elevated blood manganese concentrations (BMC) with ASD status among children with different GSTP1 genotypes based on co-dominant and dominant genetic models using conditional logistic regression models. (163 matched pairs) (Without adjusting for the four heavy metals

Genetic Model	Higher BMC		Ref. BMC		Genotype(s)	Unadjusted			Adjusted[a]		
						Matched Odds Ratio	95% CI for MOR	*P* value *b,c*	Matched Odds Ratio	95% CI for MOR	*P* value *d,e*
Co–dominant	BMC ≥ 12μg/L	vs.	BMC< 12μg/L	at	*GSTP1* (Ile/Ile)	2.45	(0.99, 6.09)	0.05	4.45	(1.20, 17.25)	0.03
	BMC ≥ 12μg/L	vs.	BMC< 12μg/L	at	*GSTP1* (Ile/Val)	0.72	(0.34, 1.49)	0.37	1.30	(0.33, 5.07)	0.71
	BMC ≥ 12μg/L	vs.	BMC< 12μg/L	at	*GSTP1* (Val/Val)	0.58	(0.20, 1.71)	0.32	0.27	(0.05, 1.43)	0.12
Dominant	BMC ≥ 12μg/L	vs.	BMC< 12μg/L	at	*GSTP1* (Val/*)	0.67	(0.36, 1.24)	0.21	0.69	(0.26, 1.84)	0.45
	BMC ≥ 12μg/L	vs.	BMC< 12μg/L	at	*GSTP1* (Ile/Ile)	2.54	(1.03, 6.30)	0.04	4.43	(1.14, 15.69)	0.03

[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;

[b] For the unadjusted co-dominant model, the interaction term had $P = 0.03$;

[c] For the unadjusted dominant model, the interaction term had $P = 0.01$;

[d] For the adjusted co-dominant model, the interaction term had $P = 0.03$;

[e] For the adjusted dominant model, the interaction term had $P = 0.02$.

Note: All missing data are the same as those reported in the footnote of Tables 2–4

Table 6.

Association of elevated blood manganese concentrations (BMC) with ASD status among children with different GSTP1 genotypes based on co-dominant and dominant genetic models using conditional logistic regression models including the mixture index of the four heavy metals. (163 matched pairs)

						Unadjusted[a]			Adjusted[b]		
Genetic Model	**Higher BMC**		**Ref. BMC**		**Genotype(s)**	**Matched Odds Ratio**	**95% CI for MOR**	***P* value *c,d***	**Matched Odds Ratio**	**95% CI for MOR**	***P* value *e,f***
Co-dominant	BMC > 12pg/L	vs.	BMC < 12pg/L	at	GSTP1 (Ile/Ile)	2.38	(0.95, 6.00)	0.07	4.58	(1.21, 17.42)	0.03
	BMC > 12pg/L	vs.	BMC < 12pg/L	at	GSTP1 (Ile/Val)	0.69	(0.33, 1.46)	0.34	1.26	(0.32, 5.01)	0.74
	BMC > 12pg/L	vs.	BMC < 12pg/L	at	GSTP1 (Val/Val)	0.62	(0.21, 1.85)	0.39	0.26	(0.05, 1.42)	0.12
Dominant	BMC > 12pg/L	vs.	BMC < 12pg/L	at	GSTP1 (Val/*)	0.67	(0.36, 1.26)	0.22	0.67	(0.25, 1.83)	0.44
	BMC > 12pg/L	vs.	BMC < 12pg/L	at	GSTP1 (Ile/Ile)	2.49	(0.99, 6.25)	0.05	4.27	(1.15, 15.85)	0.03

[a] Adjusted for the binary mixture index based on four metals (lead, mercury, cadmium, and arsenic)

[b] 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 as well as the binary mixture index based on four metals (lead, mercury, cadmium, and arsenic);

[c] For the unadjusted co-dominant model, the interaction term had P = 0.06 and the binary mixture index had MOR=0.56 with (P=0.04);

[d] For the unadjusted dominant model, the interaction term had *P* = 0.01 and the binary mixture index had MOR=0.57 with (P=0.04);[8]

[e] For the adjusted co-dominant model, the interaction term had *P* = 0.03 and the binary mixture index had MOR=1.18 with (P=0.73);

[f] For the adjusted dominant model, the interaction term had *P* = 0.02 and the binary mixture index had MOR=1.14 with (P=0.78). (In the unadjusted analyses for both co-dominant and dominant models, lower mixture index was significantly associated with ASD, but these findings were not observed in the adjusted models.)

Note: All missing data are the same as those reported in the footnote of Tables 2–4.

Table 7.a.

Association of elevated blood manganese concentrations (BMC) with ASD status among children with different GSTP1 genotypes based on co-dominant and dominant genetic models after controlling for each heavy metal (lead, mercury, cadmium, and arsenic) using conditional logistic regression models. (163 matched pairs)

Genetic Model	Higher BMC	Ref. BMC	Genotype(s)	Unadjusted (Lead)			Unadjusted (Cadmium)			Unadjusted (Mercury)			Unadjusted (Arsenic)			Unadjusted (four heavy metal)		
				Matched Odds Ratio	95% CI for MOR	*P* value *a, b*	Matched Odds Ratio	95% CI for MOR	*P* value *c, d*	Matched Odds Ratio	95% CI for MOR	*P* value *e, f*	Matched Odds Ratio	95% CI for MOR	*P* value *g, h*	Matched Odds Ratio	95% CI for MOR	*P* value *i, j*
Co-dominant	BMC ≥ 12¼g/L vs.	BMC ≥ 12¼g/L at	GSTP1 (Ile/Ile)	2.28	(0.93, 6.10)	0.07	2.46	(0.99, 6.14)	0.05	2.30	(0.92, 5.77)	0.08	2.36	(0.94, 5.87)	0.07	2.33	(0.90, 6.04)	0.08
	BMC ≥ 12¼g/L vs.	BMC ≥ 12¼g/L at	GSTP1 (Ile/Val)	2.67	(0.31, 1.43)	0.30	2.72	(0.34, 1.49)	0.37	0.73	(0.35, 1.52)	0.40	0.72	(0.35, 1.50)	0.38	0.68	(0.32, 1.46)	0.32
	BMC ≥ 12¼g/L vs.	BMC ≥ 12¼g/L at	GSTP1 (Val/Val)	0.69	(0.22, 2.13)	0.52	0.58	(0.19, 1.71)	0.32	0.60	(0.20, 1.79)	0.36	0.57	(0.19, 1.70)	0.32	0.70	(0.22, 2.19)	0.54
Dominant	BMC ≥ 12¼g/L vs.	BMC ≥ 12¼g/L at	GSTP1 (val/*)	0.68	(0.36, 1.29)	0.24	0.67	(0.36, 1.24)	0.21	0.69	(0.37, 1.28)	0.24	0.67	(0.36, 1.25)	0.21	0.69	(0.36, 1.32)	0.26
	BMC ≥ 12¼g/L vs.	BMC ≥ 12¼g/L at	GSTP1 (Ile/Ile)	2.50	(0.98, 6.38)	0.06	2.56	(1.03, 6.36)	0.04	2.40	(0.96, 6.00)	0.06	2.45	(0.99, 6.09)	0.06	2.45	(0.95, 6.32)	0.06

[a] For the unadjusted (lead) co-dominant model, the interaction term had $P = 0.07$

[b] For the unadjusted (lead) dominant model, the interaction term had $P = 0.02$.

[c] For the unadjusted (cadmium) co-dominant model, the interaction term had $P = 0.05$;

[d] For the unadjusted (cadmium) dominant model, the interaction term had $P = 0.01$

[e] For the unadjusted (mercury) co-dominant model, the interaction term had $P = 0.08$;

[f] For the unadjusted (mercury) dominant model, the interaction term had $P = 0.02$.

[g] For the unadjusted (arsenic) co-dominant model, the interaction term had $P = 0.06$;

[h] For the unadjusted (arsenic) dominant model, the interaction term had $P = 0.02$.

[i] For the unadjusted [four heavy metal (lead, mercury, cadmium, and arsenic)] co-dominant model, the interaction term had $P = 0.09$;

[j] For the unadjusted [four heavy metal (lead, mercury, cadmium, and arsenic)] dominant model, the interaction term had $P = 0.02$.

Note: All missing data are the same as those reported in the footnote of Tables 2–4.

Table 7.b.

Association of elevated blood manganese concentrations (BMC) with ASD status among children with different GSTP1 genotypes based on co-dominant and dominant genetic models after controlling for each heavy metal (lead, mercury, cadmium, and arsenic) using conditional logistic regression models. (163 matched pairs)

Genetic Model	Higher BMC		Ref. BMC		Genotype(s)	Adjusted * (Lead)			Adjusted * (Cadmium)			Adjusted * (Mercury)			Adjusted * (Arsenic]			Adjusted * (four heavy metal)		
						Matched Odds Ratio	95% CI for MOR	*P* value *a, b*	Matched Odds Ratio	95% CI for MOR	*P* value *c, d*	Matched Odds Ratio	95% CI for MOR	*P* value *e, f*	Matched Odds Ratio	95% CI for MOR	*P* value *g, h*	Matched Odds Ratio	95% CI for MOR	*P* value *i, j*
Co-dominant	BMC ≥ 12¼g/L	v s.	BMC ≥ 12¼g/L	a t	GSTP1 (Ile/Ile)	4.34	(1.14, 16.55)	0.03	4.54	(1.20, 17.19)	0.03	4.49	(1.18, 17.07)	0.03	4.82	(1.27, 18.32)	0.02	4.48	(1.17, 17.12)	0.03
	BMC ≥ 12¼g/L	v s.	BMC ≥ 12¼g/L	a t	GSTP1 (Ile/Val)	1.43	(0.36, 5.59)	0.61	1.32	(0.34, 5.18)	0.69	1.34	(0.33, 5.36)	0.68	1.24	(0.32, 4.92)	0.75	1.43	(0.35, 5.91)	0.62
	BMC ≥ 12¼g/L	v s.	BMC ≥ 12¼g/L	at	GSTP1 (Val/Val)	0.30	(0.06, 1.63)	0.16	0.27	(0.05, 1.44)	0.13	0.27	(0.05, 1.43)	0.12	0.24	(0.04, 1.44)	0.12	0.28	(0.05, 1.64)	0.16
Dominant	BMC ≥ 12¼g/L	v s.	BMC ≥ 12¼g/L	a t	GSTP1 (val/ *)	0.76	(0.28, 2.06)	0.59	0.69	(0.26, 1.86)	0.47	0.69	(0.25, 1.85)	0.46	0.67	(0.24, 1.84)	0.43	0.75	(0.26, 2.10)	0.58
	BMC ≥ 12¼g/L	v s.	BMC ≥ 12¼g/L	a t	GSTP1 (IIe/IIe)	3.98	(1.07, 14.82)	0.04	4.26	(1.15, 15.79)	0.03	4.25	(1.13, 15.91)	0.03	4.60	(1.23, 17.16)	0.02	4.28	(1.13, 16.16)	0.03

*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

[a]For the adjusted (lead) co-dominant model, the interaction term had $P = 0.05$;

[b]For the adjusted (lead) dominant model, the interaction term had $P = 0.04$.

[c]For the adjusted (cadmium) co-dominant model, the interaction term had $P = 0.03$;

[d]For the adjusted (cadmium) dominant model, the interaction term had $P = 0.02$.

[e]For the adjusted (mercury) co-dominant model, the interaction term had $P = 0.03$;

[f]For the adjusted (mercury) dominant model, the interaction term had $P = 0.03$.

[g]For the adjusted (arsenic) co-dominant model, the interaction term had $P = 0.02$;

[h]For the adjusted (arsenic) dominant model, the interaction term had $P = 0.02$.

[i]For the adjusted [four heavy metal (lead, mercury, cadmium, and arsenic)] co-dominant model, the interaction term had $P = 0.04$;

[j]For the adjusted [four heavy metal (lead, mercury, cadmium, and arsenic)] dominant model, the interaction term had $P = 0.04$.Note: All missing data are the same as those reported in the footnote of Tables 2–4.