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Interaction between *GSTT1* and *GSTP1* allele variants as a risk modulating-factor for autism spectrum disorders

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Abstract

We investigated the role of glutathione S-transferase (*GST*) genes in Autism Spectrum Disorder (ASD). We used data from 111 pairs of age- and sex-matched ASD cases and typically developing (TD) controls between 2–8 years of age from Jamaica to investigate the role of *GST* pi 1 (*GSTP1*), *GST* theta 1 (*GSTT1*), and *GST* mu 1 (*GSTM1*) polymorphisms in susceptibility to ASD. In univariable conditional logistic regression models we did not observe significant associations between ASD status and *GSTT1*, *GSTM1*, or *GSTP1* genotype (all $P > 0.15$). However, in multivariable conditional logistic regression models, we identified a significant interaction between *GSTP1* and *GSTT1* in relation to ASD. Specifically, in children heterozygous for the *GSTP1* Ile105Val polymorphism, the odds of ASD was significantly higher in those with the null *GSTT1* genotype than those with the other genotypes [Matched Odds Ratio (MOR) = 2.97, 95% CI (1.09, 8.01), $P = 0.03$]. Replication in other populations is warranted.

Keywords

Autism spectrum disorder; Oxidative stress; glutathione S-transferase (*GST*) genes; Modulating-factor; gene-gene interaction

1. Introduction

Autism Spectrum Disorder (ASD) affects language development, communication, imagination, and social interactions. Repetitive, stereotyped behaviors are characteristic features of ASD (Rapin, 1997) that manifest in early childhood (Genuis, 2009; Volkmar,

Chawarska, & Klin, 2005). The etiology of ASD is believed to be multifactorial (Gardener, Spiegelman, & Buka, 2011), and researchers believe that ASD is caused by interplay among genes (Anderson et al., 2008; Anderson et al., 2009; Ashley-Koch et al., 2007; Bill & Geschwind, 2009; Bowers et al., 2011; Campbell, Li, Sutcliffe, Persico, & Levitt, 2008; Kim et al., 2008; Kumar & Christian, 2009; Ma et al., 2005) and between genes and environmental factors (Hallmayer et al., 2011; Herbert, 2010; Landrigan, 2010). Any individual factor in isolation, either genetic or environmental, is usually insufficient to explain the ASD phenotype (James, 2008).

Several previous studies have investigated the effect of oxidative stress on neuronal cell death or brain damage (Kern & Jones, 2006), and have linked oxidative stress, the imbalance between levels of reactive oxygen species (ROS), and antioxidant levels in the body with ASD (Chauhan & Chauhan, 2006; James, 2008; Main, Angley, O'Doherty, Thomas, & Fenech, 2012; McGinnis, 2004). It has also been shown that synthesis of glutathione, the major cellular antioxidant (Coles & Kadlubar, 2003; Maher, 2006), is impacted by factors including genetics, environmental exposures, or methionine metabolism (James, 2008). Levels of glutathione and the ratio of reduced (active) to oxidized (non-active) glutathione were lower in children with ASD compared to children without ASD (James et al., 2004; James et al., 2006; James, 2008), suggesting the involvement of oxidative stress in the disorder. Another study investigated the role of enzymes and metabolites involved in methionine metabolism and found decreased capacity for methylation may contribute to the development of ASD (James et al., 2004).

Members of the glutathione-S-transferase (*GST*) protein family play a major role in defense against oxidative stress by catalyzing the conjugation of glutathione to a variety of electrophilic toxins, which facilitates their excretion (Sharma, Yang, Sharma, Awasthi, & Awasthi, 2004). Some studies reported that mutations and dysfunction in the *GST* genes could result in oxidative damage (Sharma et al., 2004; Sharma, Kumar, & Saxena, 2010), which may contribute to the development of oxidative stress in children with ASD (Chauhan & Chauhan, 2006). In addition, enzymes generated by *GST* genes play an important role in detoxification of the products of oxidative stress and heavy metals (Garrecht & Austin, 2011). Several of the *GST* family subgroup genes, including *GST mu 1 (GSTM1)*, *GST pi 1 (GSTP1)*, and *GST theta 1 (GSTT1)*, are highly polymorphic (Loktionov et al., 2001). Sequence variation in these genes has also been associated with an increased or decreased risk for several cancers and chronic diseases, including colorectal cancer (Loktionov et al., 2001), esophageal cancer (Sharma et al., 2013), renal cell carcinoma (Cheng, You, & Zhou, 2012; Yang et al., 2013), acute leukemia (Ye & Song, 2005), prostate cancer (Harries, Stubbins, Forman, Howard, & Wolf, 1997; Kote-Jarai et al., 2001; Liu, Liu, Ran, Shang, & Li, 2013; Safarinejad, Shafiei, & Safarinejad, 2011; Taioli et al., 2011; Wei et al., 2013), type-2 diabetes mellitus (Dadbinpour, Sheikha, Darbouy, & Afkhami-Ardekani, 2013; Ramprasath et al., 2011), asthma (Tamer et al., 2004), and neurodevelopmental disorders such as ASD (Buyske et al., 2006). 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 (Ye, Song, Higgins, Pharoah, & Danesh, 2006).

The frequency of *GSTM1* and *GSTT1* null genotypes vary in different populations. For example, Hiragi *et al.* (2007) reported a frequency of 17% to 35% for the *GSTM1* null genotype and 22% to 44% for the *GSTT1* null genotype in Brazilians of African descent (Hiragi *et al.*, 2007). Chen *et al.* (1996) reported that the frequency of the *GSTT1* null genotype was significantly higher in African-Americans compared with whites (24.1% vs. 15.0%, $P = 0.019$), and the frequency of the *GSTM1* null genotype was significantly higher in whites compared with African-Americans in the US (53.5% vs. 27.6%, $P < 0.001$) (Chen, Liu, & Relling, 1996). A multi-institutional case-control study that included African-Caribbean men investigated the role of *GSTM1* and *GSTT1* deletions in prostate cancer and reported that the frequencies of the *GSTM1* and *GSTT1* null genotypes assessed at a study site in Jamaica were 26.2% and 35.2%, respectively, (Taioli *et al.*, 2011). Recently, Rahbar *et al.* reported that the frequencies of the null genotypes of *GSTM1* and *GSTT1* in Jamaican children were 26.0% and 22.0%, respectively (Rahbar *et al.*, 2014c). 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. Researchers have used different classifications (e.g., co-dominant, recessive, dominant, and additive models) for a variety of disease association studies (Ramprasath *et al.*, 2011; Safarinejad *et al.*, 2011; Sreeja *et al.*, 2008; Tamer *et al.*, 2004; Wei *et al.*, 2013). The frequency of the *GSTP1* polymorphism varies across different populations, as shown in the International Haplotype Map (HapMap) project (The International HapMap Consortium, 2003). Specifically, it has been reported that the allele frequency of Ile varies from 50% to 63% for the four African populations: African ancestry in the Southwest United States, Luhya in Webuye, Kenya, Maasai in Kinyawa, Kenya, and Yoruba in Ibadan, Nigeria (Lakkakula *et al.*, 2013). We recently reported that the allele frequency of Ile is 51% for the Jamaican population (Rahbar *et al.*, 2014c).

A study using a family-based association design (mother of ASD case and her parents) found that a haplotype consisting of two polymorphisms in the *GSTP1* gene (Ile105Val and Ala114Val) was significantly over-transmitted to the mothers [Odds Ratio (OR) = 2.67; 95% CI, 1.39–5.13] compared to two other haplotypes (Williams *et al.*, 2007). A subsequent genotype analysis identified the *GSTP1* Ile105Val allele as responsible for this effect. A case-control study reported an increased odds of ASD (OR = 2.02; 95% CI, 1.03–4.04) for individuals with the null polymorphism of *GSTM1* compared with unrelated unaffected controls (Buyske *et al.*, 2006). Additionally, a marginally increased ASD risk was observed for individuals with the *GSTM1* null polymorphism alone (OR = 1.37; 95% CI, 0.98–1.96), although in combination with a polymorphism in the reduced folate carrier gene, the OR was 3.78 (95% CI, 1.80–7.95) (James *et al.*, 2006). A limited number of studies have investigated the interactive effects of genetic factors in relation to ASD (Anderson *et al.*, 2008; Anderson *et al.*, 2009; Ashley-Koch *et al.*, 2007; Bill & Geschwind, 2009; Bowers *et al.*, 2011; Campbell *et al.*, 2008; Kim *et al.*, 2008; Kumar & Christian, 2009; Ma *et al.*, 2005). Bowers *et al.* (2011) highlighted that investigators usually do not examine interactions between genes because of the complexities involved to obtain measures of the effects (Bowers *et al.*, 2011). It is possible that individual effects of genes are not

statistically significant, but when these effects are investigated in an interactive model, the findings become statistically significant (Cordell, 2009).

Although limited information is available regarding the relationship between oxidative stress and ASD, some studies have reported on potential associations. For example, a study by Adams *et al.* (2011) reported that compared to TD children, children with ASD had significantly higher levels of oxidative stress markers ($P < 0.001$), including oxidized glutathione, the ratio of oxidized to reduced glutathione, and plasma nitrotyrosine (Adams *et al.*, 2011). Additionally, a more recent study from Saudi Arabia reported that children with ASD had lower mean levels of GST compared to TD controls (0.30 vs. 0.61 $\mu\text{mol}/\text{min}/\text{ml}$ plasma; $P < 0.001$) (Alabdali, Al-Ayadhi, & El-Ansary, 2014). The long term goal of our Jamaican Autism Project is to investigate the roles of exposures to environmental toxins (e.g., mercury, lead, arsenic, cadmium, and manganese) and *GST* genes (*GSTT1*, *GSTM1*, *GSTP1*), potential gene-gene interactions, and gene-environment interactions in relation to ASD. In this paper, we describe the genotype frequencies of the three aforementioned *GST* genes in Jamaican children with and without ASD. In addition, we investigate the role of these *GST* genes and their pair-wise gene-gene interactions in relation to ASD.

2. Materials and Methods

2.1. Study population

The Jamaican Autism Study is an age- and sex-matched case-control study that enrolled children between 2–8 years of age during December 2009-May 2012. Detailed information regarding the recruitment and assessment of ASD cases and typically developing (TD) controls is reported in our previous publications (Rahbar *et al.*, 2012b; Rahbar *et al.*, 2012a; Rahbar *et al.*, 2013; Rahbar *et al.*, 2014b; Rahbar *et al.*, 2014c; Rahbar *et al.*, 2014a). In brief, only children born in Jamaica were eligible to participate in this study. For enrolling ASD cases, children who were previously diagnosed as having ASD based on the Diagnostic and 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) and listed in the University of the West Indies' (UWI) Jamaica Autism Database were invited for reassessment of their ASD status. The Autism Diagnostic Observation Schedule (ADOS) (Lord, Rutter, DiLavore, & Risi, 2002) and the Autism Diagnostic Interview-Revised (ADI-R) (Rutter, Le, & Lord, 2003) were administered by a senior psychologist in the UWI Department of Child and Adolescent Health who was trained to administer these instruments to confirm the diagnosis of ASD. For each ASD case, an age- and sex-matched control was identified from schools, community churches, and well-child clinics. The criteria for matching required that the control be no older or younger than the matched case by six months. We administered the Lifetime form of the Social Communication Questionnaire (SCQ) (Rutter, Bailey, & Lord, 2003) to parents/guardians of potential control children to rule out symptoms of ASD. Potential controls with an SCQ score ≥ 6 (cutoff of 6 on the SCQ is one standard deviation above the mean score of TD school children) (Mulligan, Richardson, Anney, & Gill, 2009) were included in the control group. Additionally, children with any major

neurodevelopmental disability or major congenital malformation were excluded from the control group.

We also administered a pre-tested questionnaire to parents/guardians of both cases and controls to collect their demographic and socioeconomic (SES) information, such as ownership of a car by the family and parental level of education. For each child, we collected approximately 3 mL of venous whole blood and 2 mL of saliva for genetic analysis. All participating families provided written informed consent. This study was approved by the Institutional Review Boards of the University of Texas Health Science Center at Houston (UTHealth) and UWI, Mona campus in Kingston, Jamaica. The data presented here pertain to analysis of 111 matched case-control pairs (i.e., 222 children) for whom we had complete data.

2.2. Genotyping methods

Whole blood was collected in EDTA lavender top vacutainers for each participant at UWI. Blood components were centrifuged and separated into plasma, buffy coat, and red blood cell aliquots, then stored at -80°C for future use. Frozen specimens were sent to the UTHealth School of Public Health, Human Genetics Center using the CryOport high volume liquid nitrogen dry vapor shipper (CryOport, Inc.; San Diego, CA, USA). 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 samples were shipped from UWI to UTHealth at ambient temperature.

Genomic DNA was isolated from buffy coat using the Gentra PUREGENE Blood Kit (Qiagen, Inc.; Valencia, CA, USA) in accordance with the manufacturer's protocol. If a buffy coat was not available, then DNA was isolated from the saliva sample with the Gentra PUREGENE DNA Purification Kit (Qiagen protocol 400244 Rev A) according to the methods established by DNA Genotek (Dols, Chartier, & Lem, 2014). The *GSTT1* and *GSTM1* genotypes were detected using a multiplex polymerase chain reaction (PCR) and methods established by Li *et al.* (Li *et al.*, 2000). In this assay, the absence of a 130 bp product indicates that the individual is homozygous null for *GSTT1*, meaning this segment of DNA is absent in both the maternal and paternal chromosome. Similarly, the absence of a 230 bp product indicates homozygous deficiency for the *GSTM1* gene. As a quality control, a beta-globin amplicon of 280 bp was included in the assay. The *GSTP1* Ile105Val polymorphism (rs1695) was genotyped using the TaqMan Drug Metabolism SNP Genotyping Assay C_3237198_20 with the thermal cycler parameters recommended by Life Technologies (Grand Island, NY, USA). The sequence of primers and probes are available upon request. The ABI 7700 Sequence Detection System (Life Technologies) was used for ascertainment of the genotype calls.

2.3. Statistical analysis

Since the data are from a 1:1 sex- and age-matched case-control study, we used conditional logistic regression (CLR) models to compare ASD case and TD control groups with respect

to various characteristics including demographic and socioeconomic status. For the *GSTP1* polymorphism in the TD control group, Hardy-Weinberg equilibrium was tested using Pearson's Chi-square test. We used univariable CLR to assess potential effects of single *GST* genes on ASD status. Using multivariable CLR, we assessed potential interactive effects of *GST* genes (only two-way gene-gene interactions) on ASD status. When an interaction between two genes was included in the model, we reported the odds ratios by levels of the interacting genes. For the *GSTM1* and *GSTT1* genes, since the assay does not distinguish between a normal homozygote (I/I: no deletion in either the paternal or maternal chromosome) and a heterozygote (I/D: deletion in either the paternal or maternal chromosome, but not both), we considered only a recessive model using a binary variable to represent their normal genotype (I/I or I/D) versus the null (DD). For the *GSTP1* gene, all three genotypes, Ile/Ile, Ile/Val, and Val/Val, were available. 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 et al., 2011; Sreeja et al., 2008; Tamer et al., 2004), we analyzed the *GSTP1* gene using various genetic models, including the recessive (Ile/* vs. Val/Val), dominant (Ile/Ile, Val/*), additive (0, 1, and 2 copies of allele Val), and co-dominant (Ile/Ile or Ile/Val or Val/Val) models. The three *GST* genes, *GSTT1*, *GSTM1* and *GSTP1*, are located on chromosome 22, 11, and 1, respectively. Nevertheless, in order to rule out the possibilities of long-range disequilibrium among these genes that may be attributed to population admixture, selective pressure or a population bottleneck, we tested their pair-wise associations using logistic regression.

All statistical tests were conducted at 5% level of significance. However, in order to address the issue of multiple comparisons, we conducted 1000 permutations to rule out the role of chance in the results reported here. Each permutation involved random assignment of ASD status (i.e., ASD case or TD control) within each case-control pair with a probability of 0.5. Empirical p-values were calculated as the proportion of permutations that produced smaller p-values than the corresponding observed p-value from the original data. All analyses were performed using SAS software (SAS Institute Inc., 2011).

3. Results

As expected, due to matching by age, the mean ages were similar for the two groups of children when stratified by ASD status; children with an ASD had a mean age of 67.0 months, while the mean age for children in the TD control group was 68.0 months. Nearly all of the ASD cases (93.7%) and TD controls (99.1%) were Afro-Caribbean and 85.6% of the ASD cases and TD controls were male. Similarly, 96.4% of mothers and 97.3% of fathers were Afro-Caribbean. Our data indicate frequencies of 27.9% and 20.7% for the *GSTM1* and *GSTT1* null genotype, respectively, for TD Jamaican children. The allele frequency of Val for the *GSTP1* polymorphism was 46% and 49% for the ASD cases and TD controls, respectively. CLR analysis showed that there is no correlation between any two of the three *GST* genes ($P > 0.20$). There was no significant deviation from Hardy-Weinberg equilibrium in the TD controls ($P = 0.99$) for the *GSTP1* polymorphism. Demographic and other characteristics of the ASD cases and TD controls are reported in Table 1.

As shown in Table 2, in the univariable CLR analyses, we did not observe significant associations between ASD status and *GSTT1*, *GSTM1*, or *GSTP1* genotype in either the co-dominant model or any of the aforementioned reduced models (all $P > 0.15$). An additive model was also used in CLR for assessing the association between *GSTP1* and ASD, but no significant association was observed, ($P = 0.49$).

We then investigated the gene-gene interactions between any two *GST* genes. For the *GSTP1* gene, we considered not only the co-dominant model but also all the aforementioned reduced models. Under the co-dominant model, the genotype frequencies of the *GSTT1* null genotype (DD) and *GSTP1* Ile/Val was 18.0% in ASD cases compared to 9.8% in TD controls. Under the recessive model, the genotype frequencies of the *GSTT1* null genotype (DD) and *GSTP1* Ile/Ile or Ile/Val was 24.3% in ASD cases compared to 14.4% in TD controls. Additional information regarding the genotype frequencies of *GSTT1* and *GSTP1* under other models is provided in Table 3.

As shown in Table 4, in children heterozygous for the *GSTP1* Ile105Val polymorphism, there was a significantly higher odds of also having the *GSTT1* null genotype in ASD cases when compared to TD controls under the co-dominant model [Matched Odds Ratio (MOR) = 2.97, 95% CI: (1.09, 8.11); $P = 0.03$]. Though marginally significant, in the recessive models of *GSTP1* when *GSTP1* is Ile/Val or Ile/Ile, the odds of genotype DD for *GSTT1* was higher in the ASD group when compared with the TD control group [MOR = 2.14, 95% CI: (1.00, 4.71); $P = 0.06$]. No significant associations were observed when the dominant or additive models of *GSTP1* were employed (both $P > 0.05$).

As is shown in the last column of Table 4, our permutation tests showed that the empirical p-values were very close to the observed p-values reported earlier. Specifically, the observed significance of the effect of *GSTT1* when *GSTP1* was heterozygous held true in the permutation analysis, indicating that this finding is unlikely to be due to multiple testing.

4. Discussion

In this study, we reported the frequency of *GSTM1* and *GSTT1* null genotypes in Jamaican children with and without ASD. In children with ASD, we found a frequency of 29.7% and 28.8% for the null genotypes for *GSTM1* and *GSTT1*, respectively. In TD children, we found frequencies of 27.9% and 20.7% for the null genotypes for *GSTM1* and *GSTT1*, respectively. The frequency of the *GSTM1* null genotype from our study is similar to that previously reported (26.2%) for the Jamaican population by Taioli *et al.* (2011), but the frequency of the *GSTT1* null genotype reported earlier (35.2%) appears to be higher than in our study (Taioli *et al.*, 2011). Chen *et al.* (1996) reported that the frequency of the *GSTT1* null genotype was 24.1% in African-Americans in the US population; however, they reported that the frequency of the *GSTM1* null genotype in African-Americans (under 10 years of age) was 53.5% (Chen *et al.*, 1996; Chen *et al.*, 1997). In contrast, Li *et al.* (2000) reported a frequency for the *GSTM1* null genotype of 17.5%, and a frequency of 25.9% for the *GSTT1* null genotype in middle aged African-Americans (Li *et al.*, 2000). These are within the range of 17% to 35% for the *GSTM1* null genotype and 22% to 44% for the *GSTT1* null genotype reported in Brazilians of African descent (Hiragi *et al.*, 2007). Since over 90% of

the population in Jamaica is of African descent, it is not surprising that the frequency of the *GSTM1* and *GSTT1* null genotypes resemble those of the null genotypes in African-Americans in the US.

In this study, we also investigated the role of GST genes in relation to ASD. In univariable analyses, where the role of each GST gene (i.e., *GSTM1*, *GSTT1*, *GSTP1*) was individually assessed, we did not observe any significant associations between the GST genotypes and ASD. In contrast, a previous study that involved 70 ASD cases and 70 TD controls reported an increased ASD risk for individuals with the null polymorphism of *GSTM1* of 2.02 (95% CI 1.03–4.04) (Buyske et al., 2006). In multivariable analyses, when we included the interactions between GST genes we found evidence of significant interaction between *GSTP1* and *GSTT1* in relation to ASD. Specifically, for children who were heterozygous for the *GSTP1* Ile105Val polymorphism under the co-dominant genetic model, those with the null genotype (i.e., DD) in *GSTT1* have almost three times higher odds of ASD when compared to children with either the *GSTT1* II or I/D genotype. Though marginally significant, under the recessive model, in children with either the Ile/Ile or Ile/Val genotype, the odds of ASD were 2.14 times higher in those who also had the null *GSTT1* genotype than in those with the other two genotypes. To the best of our knowledge, we are the first to report significant interactions between *GSTT1* and *GSTP1* in relation to ASD. Other studies have reported interactive effects of GST genes with other genes in relation to ASD. For example, James *et al.* (2006) reported a marginally significant increased risk of ASD for individuals with the *GSTM1* null polymorphism alone (OR = 1.37; 95% CI, 0.98–1.96), although in combination with a polymorphism in the reduced folate carrier gene, the OR was 3.78 (95% CI, 1.80–7.95) (James et al., 2006). These results, along with our findings, demonstrate the importance of incorporating gene-gene interaction effects in genetic association studies of complex diseases such as ASD, since interaction may be detected even when there are no main effects identified for the individual genes that are tested for association. This should be done whenever the interaction effects are meaningful from a biological perspective and when the sample size is sufficiently large to ensure the desired statistical power (e.g., 0.80) for such analyses. In this study, with 111 matched-pairs (222 children) and at 5% level of significance, we have a power of at least 0.8 to detect odds ratios of 3.0 or higher between each pair of genotypes, assuming that the frequency of the null genotypes is between 0.2–0.3, and the correlation between matched ASD cases and TD controls is 0.35.

Our results suggest that the *GSTP1* and *GSTT1* genes have an interactive effect in relation to ASD and that the heterozygous genotype of *GSTP1* is the most deleterious. In the literature, we did not find a well-established genetic model for *GSTP1*. In fact, conflicting effects are reported for *GSTP1*, not only for the genetic models but also for the direction of influence [see <http://snpedia.com/index.php/Rs1695>]. For example, a study by Williams *et al.* (2007) indicated that the *GSTP1* Ile allele seems to be over-transmitted in mothers who have a child with ASD (Williams et al., 2007). Another study by Aynacioglu *et al.* (2004) reported that the Val/Val homozygous genotype appeared to be somewhat protective against developing asthma compared to the other two genotypes (Aynacioglu, Nacak, Filiz, Ekinci, & Roots, 2004). A recessive model of *GSTP1* was used in a study by Wei *et al.* (Wei et al., 2013),

where a significant association was found for low-stage prostate cancer in a meta-analysis. A dominant model was considered by Chen *et al.* (2010) and a significant association of *GSTP1* with hepatocellular carcinoma was observed (Chen et al., 2010). Using dominant and co-dominant models, Safarinejad *et al.* (2011) found an association between *GSTP1* and prostate cancer in an Iranian population, and they emphasized that the heterozygous *GSTP1* genotype Ile/Val is significantly associated with this disease (Safarinejad et al., 2011). Interestingly, Longo *et al.* (2013) recently reported an interaction between heterozygosity for the *GSTP1* Ile105Val polymorphism and exposure to pesticides in relation to Parkinson's disease (Longo et al., 2013). Our results provide further evidence in support of the role of the *GSTP1* Ile/Val genotype in susceptibility to a complex disease. Our findings also suggest that the deletion in the *GSTT1* gene on a *GSTP1* heterozygous genetic background (Ile/Val) may influence the production of *GST* related enzymes that could contribute to increasing lipid peroxidation and oxidative stress, resulting in neuronal cell death or brain damage (Kern & Jones, 2006) and ASD. Although it is difficult to demonstrate the biological plausibility for our finding of a gene-gene interaction involving the heterozygous *GSTP1* Ile/Val genotype, it should be noted that we observed a relatively large effect (i.e., MOR ~3.0). While the results from our permutation tests are reassuring that our observations are very unlikely to be due to chance, we not only recommend replication in other populations, but also detailed functional analyses of the *GST* genes in order to confirm the role and significance of the interaction between *GSTP1* and *GSTT1* in the pathogenesis of ASD. Additionally, future research should investigate differences in *GST* genotypes in relation to oxidative stress and ASD severity.

There are some limitations to our study. Because ASD cases obtained from the UWI Jamaica database were referred from several different locations in Jamaica, but the TD controls were recruited from the Kingston area, more controls were born in Kingston parish than cases. Thus, our results may not be generalizable to all of Jamaica. We also did not collect information on comorbid disorders in the ASD cases, which could have been potential confounding factors in this analysis. Additionally, because the Jamaican population is predominantly Afro-Caribbean (93.7% of ASD cases and 99.1% of TD controls were Afro-Caribbean), we were not able to test for genetic associations in more than one racial or ethnic group. Our results suggest that similar investigations should be conducted in different populations to confirm our findings.

5. Conclusions

In this study, we report the genotype frequencies for *GSTT1*, *GSTP1*, and *GSTM1* in Jamaican children. The frequency of the null genotypes of *GSTT1* and *GSTM1* are similar to those of African-Americans in the US. The *GSTP1* genotype frequency is closer to that of the African-American population than that of other African populations. Although in univariable analyses we did not find any significant associations between ASD and the *GSTP1*, *GSTT1*, and *GSTM1* genes individually, we found a significant interaction between *GSTP1* and *GSTT1* in relation to ASD. Specifically, for Jamaican children heterozygous for the *GSTP1* Ile105Val polymorphism, those with the null genotype (i.e., DD) in *GSTT1* have almost three times higher odds of ASD when compared to children with either the *GSTT1* I/I or I/D genotype. Our findings suggest that interaction between *GSTT1* and *GSTP1* may

influence individual susceptibility to ASD. Our findings reiterate that when searching for genetic risk factors related to ASD, simply checking for additive effects of individual genes could mask more complex interactive effects. In addition, our findings could help to identify a subgroup of children who may be at a higher risk of ASD based on their *GSTP1* and *GSTT1* genotypes. However, although this work may have the potential to lead to future applications in prevention and screening in a public health context, more studies confirming the observed gene-gene interaction are needed.

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Highlights

We investigated the role of *GSTP1*, *GSTT1*, and *GSTM1* polymorphisms in susceptibility to ASD

We identified a significant interaction between *GSTP1* and *GSTT1* in relation to ASD

Odds of ASD were higher in children with null *GSTT1* and *GSTP1* Ile105Val polymorphism

Table 1

Characteristics of children and their parents by ASD case status (111 matched pairs)

Variables	Categories	ASD Case (n=111) N (%)	TD Control (n=111) N (%)	P-value
Child's sex	Male	95 (85.6)	95 (85.6)	1.00
Child's age (months)	Age < 48	23 (20.7)	20 (18.0)	0.64
	48 age < 72	47 (42.3)	48 (43.2)	
	Age 72	41 (36.9)	43 (38.7)	
Maternal age^a (at child's birth)	Less than 35 years	85 (76.6)	93 (88.7)	0.03
	More than 35 years	26 (23.4)	13 (12.3)	
Paternal age^b (at child's birth)	Less than 35 years	53 (49.1)	76 (73.8)	< 0.01
	More than 35 years	55 (50.9)	27 (26.2)	
GST genes	Ile/Ile	33 (29.7)	29 (26.1)	0.76
	Ile/Val	55 (49.5)	56 (50.5)	
	Val/Val	23 (20.7)	26 (23.4)	
	<i>GSTM1</i> DD ^c	33 (29.7)	31 (27.9)	
	I/I or I/D ^d	78 (70.3)	80 (72.1)	
	<i>GSTT1</i> DD ^c	32 (28.8)	33 (20.7)	
	I/I or I/D ^d	79 (71.2)	88 (79.3)	0.17

^aMaternal age was missing for 5 controls

^bPaternal age was missing for 3 cases and 8 controls

^cDD indicates the null alleles for *GSTM1* and *GSTT1*

^dI/I or I/D indicate the homozygote (I/I) or a heterozygote (I/D) for *GSTM1* and *GSTT1*

Table 2

Association between genotypes and ASD case status using univariable conditional logistic regression models based on 111 matched pairs

Variables	ASD Case (%)	TD Control (%)	P-value	MOR	95%CI for MOR
<i>GSTT1</i>					
DD ^a vs. (I/I or I/D) ^b	32 (28.8)	23 (20.7)	0.16	1.5	(0.83, 2.82)
<i>GSTM1</i>					
DD ^a vs. (I/I or I/D) ^b	33 (29.7)	31 (27.9)	0.77	1.10	(0.61, 2.00)
<i>GSTP1</i> ^c					
Ile/Val vs. Ile/Ile	55 (49.6)	56 (50.4)	0.62	0.85	(0.45, 1.61)
<i>GSTP1</i> ^c					
Val/Val vs. Ile/Ile	23 (20.7)	26 (23.4)	0.50	0.76	(0.35, 1.66)
<i>GSTP1</i> (REC) ^d					
Val/Val vs. (Ile/Ile or Ile/Val)	23 (20.7)	26 (23.4)	0.62	0.85	(0.45, 1.62)
<i>GSTP1</i> (DOM) ^e					
Ile/Val vs. (Val/Val vs. Ile/Ile)	78 (70.3)	82 (73.9)	0.54	0.83	(0.45, 1.52)

^a DD indicates the null alleles for *GSTT1* and *GSTM1*

^b I/I or I/D indicate the homozygote (I/I) or a heterozygote (I/D) for *GSTT1* and *GSTM1*

^c *GSTP1* in the co-dominant (full) model (Ile/Ile, Ile/Val, Val/Val)

^d *GSTP1* (REC) = For *GSTP1* in the recessive model (Val/Val, Ile/Ile or Ile/Val)

^e *GSTP1* (DOM) = For *GSTP1* in the dominant model (Ile/Val or Val/Val, Ile/Ile)

Table 3

Joint distribution of genotypes *GSTT1* and *GSTP1* among ASD cases and TD controls based on 111 matched pairs

Models	<i>GSTT1</i>	<i>GSTP1</i>	ASD case n, (%)	TD Control n, (%)
<i>GSTP1</i> co-dominant model ^c	I/I or I/D ^b	Ile/Ile	26 (23.4)	24 (21.6)
	I/I or I/D ^b	Ile/Val	35 (31.5)	45 (40.5)
	I/I or I/D ^b	Val/Val	18 (16.2)	19 (17.1)
	DD ^a	Ile/Ile	7 (6.3)	5 (4.5)
	DD ^a	Ile/Val	20 (18.0)	11 (9.8)
	DD ^a	Val/Val	5 (4.5)	7 (6.3)
<i>GSTP1</i> recessive model (REC) ^d	I/I or I/D ^b	Ile/Ile or Ile/Val	61 (55.0)	69 (62.2)
	I/I or I/D ^b	Val/Val	18 (16.2)	19 (17.1)
	DD ^a	Ile/Ile or Ile/Val	27 (24.3)	16 (14.4)
	DD ^a	Val/Val	5 (4.5)	7 (6.3)
<i>GSTP1</i> dominant model (DOM) ^e	I/I or I/D ^b	Ile/Ile	26 (23.5)	24 (21.6)
	I/I or I/D ^b	Ile/Val or Val/Val	53 (47.7)	64 (57.7)
	DD ^a	Ile/Ile	7 (6.3)	5 (4.5)
	DD ^a	Ile/Val or Val/Val	25 (22.5)	18 (16.2)

^a DD indicates the null alleles for *GSTT1*

^b I/I or I/D indicate the homozygote (I/I) or a heterozygote (I/D) for *GSTT1*

^c *GSTP1* in the co-dominant model (Ile/Ile, Ile/Val, Val/Val)

^d *GSTP1* (REC) = For *GSTP1* in the recessive model (Val/Val, Ile/Ile or Ile/Val)

^e *GSTP1* (DOM) = For *GSTP1* in the dominant model (Ile/Val or Val/Val, Ile/Ile)

Association of GST genotypes (*GSTT1* and *GSTP1*) with ASD status based on the co-dominant model using conditional logistic regression models based on 111 matched pairs

Table 4

Variables										
<i>GSTP1</i>	Ile/Val	vs.	Ile/Ile	at	<i>GSTT1</i>	I/I or I/D ^b	MOR	95%CI for MOR	P-value	Empirical P-value ^e
<i>GSTP1</i>	Val/Val	vs.	Ile/Ile	at	<i>GSTT1</i>	I/I or I/D ^b	0.97	(0.39, 2.39)	0.94	0.942
<i>GSTP1</i>	Val/Val	vs.	Ile/Val	at	<i>GSTT1</i>	I/I or I/D ^b	1.43	(0.62, 3.32)	0.40	0.410
<i>GSTP1</i>	Ile/Val	vs.	Ile/Ile	at	<i>GSTT1</i>	DD ^a	1.46	(0.35, 6.03)	0.60	0.658
<i>GSTP1</i> co-dominant model ^c										
<i>GSTP1</i>	Val/Val	vs.	Ile/Ile	at	<i>GSTT1</i>	DD ^a	0.46	(0.09, 2.38)	0.35	0.350
<i>GSTP1</i>	Val/Val	vs.	Ile/Val	at	<i>GSTT1</i>	DD ^a	0.31	(0.08, 1.32)	0.11	0.101
<i>GSTT1</i>	DD ^a	vs.	I/I or I/D ^b	at	<i>GSTP1</i>	Ile/Ile	1.38	(0.40, 4.72)	0.61	0.632
<i>GSTT1</i>	DD ^a	vs.	I/I or I/D ^b	at	<i>GSTP1</i>	Ile/Val	2.97	(1.09, 8.11)	0.03	0.034
<i>GSTT1</i>	DD ^a	vs.	I/I or I/D ^b	at	<i>GSTP1</i>	Val/Val	0.65	(0.18, 2.38)	0.52	0.513
<i>GSTP1</i> REC	Val/Val	vs.	Ile/Ile or Ile/Val	at	<i>GSTT1</i>	I/I or I/D ^b	1.23	(0.56, 2.70)	0.61	0.630
<i>GSTP1</i> REC	Val/Val	vs.	Ile/Ile or Ile/Val	at	<i>GSTT1</i>	DD ^a	0.37	(0.10, 1.42)	0.15	0.141
<i>GSTP1</i> recessive model (REC) ^d										
<i>GSTT1</i>	DD ^a	vs.	I/I or I/D ^b	at	<i>GSTP1</i> REC	Ile/Ile or Ile/Val	2.14	(1.00, 4.71)	0.06	0.057
<i>GSTT1</i>	DD ^a	vs.	I/I or I/D ^b	at	<i>GSTP1</i> REC	Val/Val	0.65	(0.18, 2.36)	0.51	0.496

^aDD indicates the null alleles for *GSTT1*

^bI/I or I/D indicate the homozygote (I/I) or a heterozygote (I/D) for *GSTT1*

^c*GSTP1* in the full model (Ile/Ile, Ile/Val, Val/Val)

^d*GSTP1*(REC) = For *GSTP1* in the recessive model (Val/Val, Ile/Ile or Ile/Val)

^eEmpirical P-values are obtained from the permutations analyses