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Maternal and offspring xenobiotic metabolism haplotypes and the risk of childhood acute lymphoblastic leukemia

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Abstract

Discovering genetic predictors of childhood acute lymphoblastic leukemia (ALL) necessitates the evaluation of novel factors including maternal genetic effects, which are a proxy for the intrauterine environment, and robust epidemiologic study designs. Therefore, we evaluated five maternal and offspring xenobiotic metabolism haplotypes and the risk of childhood ALL among 120 case-parent triads. Two of the five haplotypes were significantly associated with risk: *GSTM3*/*GSTM4* (*P*=0.01) and *GSTP1* (*P*=0.02). The *EPHX1* haplotype was marginally associated with risk (*P*=0.05), whereas haplotypes in *CYP1B1* and *GSTA4* were not. Our results suggest genetic variation in xenobiotic metabolism is important in childhood ALL etiology.

Keywords

Acute lymphoblastic leukemia; case-parent triad; haplotypes; xenobiotic metabolism

INTRODUCTION

Leukemia accounts for one out of three malignancies of childhood, and approximately 3,500 new cases are diagnosed in the United States (US) per year [1]. The most common subtype is acute lymphoblastic leukemia (ALL), representing 80% of leukemia cases in the US. In spite of its prevalence and clinical significance, the etiology of ALL is largely unknown, and only about <5% of cases can be attributed to previously identified risk factors [2]. As the

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AUTHORS' CONTRIBUTIONS

D.N. and P.J.L. contributed equally to this work in the analysis and preparation of the manuscript. P.J.L. also contributed largely to the conception and design of the study. M.F.O. was responsible enrollment of study participants and manuscript preparation. M.E.S. was responsible for the conception and design of the study and preparation of the manuscript. All authors have given final approval of the submitted manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

incidence of ALL peaks between ages two and four years, evaluating early life exposures, especially those that occur *in utero*, may be important.

In 1–2% of incident cases there is a family history of leukemia and other cancers, suggesting a genetic component to its etiology in a subset of patients [3, 4]. Additionally, previous population-based genome-wide association studies (GWAS) and candidate gene studies have identified a few genetic variants and pathways of interest. One potentially important genetic pathway that is suspected in playing a role in the etiology of childhood ALL is xenobiotic metabolism [5–8]. When an individual is exposed to various foreign chemicals, enzymes involved in xenobiotic metabolism are responsible for the elimination of these compounds through Phase I (e.g., oxidation) and Phase II (e.g., conjugation) reactions. In some cases, this metabolic process may result in "bioactivation", in which the metabolite becomes reactive and potentially more toxic leading to leukemogenesis [9].

Because of their potential role in leukemogenesis, variants of xenobiotic metabolism genes, including glutathione S-transferase M1 (*GSTM1*), glutathione S-transferase T1 (*GSTT1*), (*GSTP1*), and cytochrome P450 1A1 (*CYP1A1*), have been assessed in the context of childhood ALL risk [10]. The findings from these past studies have been equivocal, which may be attributed to several factors including: 1) Most population-based studies of childhood ALL utilize a case-control design, which may be subject to population stratification bias [11]; 2) To our knowledge, there have been no studies evaluating "maternal genetic effects", a proxy for the intrauterine environment, which may be important in the development of ALL; and 3) Few studies have assessed xenobiotic metabolism haplotypes (i.e., a combination of multiple single nucleotide polymorphisms [SNPs]) and risk of ALL, which may better detect weak associations from common alleles within genes [12]. Additionally, some key genes in the xenobiotic metabolism pathway have not been explored.

We used a case-parent triad study design, incorporating an extension of the log-linear model, to evaluate the association between childhood ALL and maternal and offspring haplotypes of six important xenobiotic metabolism genes: cytochrome P450 1B1 (*CYP1B1*), epoxide hydrolase 1 (*EPHX1*), glutathione S-transferase A4 (*GSTA4*), glutathione S-transferase M3 (*GSTM3*), glutathione S-transferase M4 (*GSTM4*), and glutathione S-transferase P1 (*GSTP1*). The case-parent triad study design is robust to bias from population stratification and can be used to estimate both maternal and offspring haplotypes [13, 14].

MATERIALS AND METHODS

Study Population

The study population included 120 complete and incomplete ALL case-parent triads diagnosed and recruited from the Childhood Cancer Epidemiology and Prevention Center at Texas Children's Hospital (Houston, TX) between 2003 and 2010. Cases of ALL were 0 to 14 years of age at diagnosis. Cases provided blood samples and parents provided saliva samples as sources of DNA for genotyping. Participation of both parents was not a requirement for enrollment or analysis [15]. Demographic and clinical information were abstracted from medical records. The study protocol was approved by the Baylor College of Medicine Institutional Review Board.

Haplotype Selection and Genotyping Methods

Ten SNPs in six genes of the xenobiotic metabolism pathway (*CYP1B1*, *EPHX1*, *GSTA4*, *GSTM3*, *GSTM4*, and *GSTP1*) were selected based on *a priori* knowledge of putative function or previous evidence of association with ALL or cancer risk [16–20]. Rather than evaluating the SNPs individually, we opted to employ a haplotype approach, based on

findings from other studies utilizing case-parent triads [21, 22]. For *CYP1B1*, *EPHX1*, *GSTA4*, and *GSTP1*, two SNPs for each gene were selected to be analyzed as a haplotype. One SNP was chosen for both *GSTM3* and *GSTM4* and grouped together as a haplotype because the two genes are located at 1p13.3 (Table 1) [23].

DNA was extracted from peripheral blood lymphocytes and saliva using the QIAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genotyping was performed using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA) in the Human Genetics Center at The University of Texas School of Public Health according to the manufacturer's protocol.

Statistical Analysis

The characteristics of cases and parents were summarized using counts and proportions. For each analyzed polymorphism, samples for which a genotype could not be assigned and triads that had genotype combinations that were inconsistent with Mendelian inheritance were determined and excluded if missing more than a certain percentage. For each subject, the number of genotyping failures (i.e., genotypes that could not be assigned) was determined. These analyses were performed using Intercooled Stata, version 12.1 (StataCorp LP, College Station, TX).

An extension of the log-linear model was used to assess the association between ALL and both offspring and maternal haplotypes. This was done using HAPLIN version 4.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin/), running under R version 2.14.0 (R Foundation for Statistical Computing) [24]. HAPLIN estimates the relative risk (RR) of a single- or double-dose of each haplotype using a maximum likelihood approach. In some instances, the phase information is unknown and constructed using the family information. Incomplete triads are included and missing information is imputed using the expectation maximization algorithm [24]. A threshold of 10% for haplotype frequency was used as our sample size ($N = 120$) was small compared to other case-parent triad assessments of haplotypes and to avoid inclusion of relatively rare haplotypes [21, 22]. For each haplotype, a RR and 95% confidence interval (95% CI) is estimated. The RR is a comparison of the specific haplotype compared to the reference group of the remaining haplotypes. The single-dose RR is the change for an individual carrying one copy (heterozygotes of the haplotype), and the double-dose RR compares homozygotes of the haplotype to the remaining subjects. In order to explore dose-response effects, we also calculated multiplicative risk models, where the RR represents the increase or decrease in risk with each additional copy of the haplotype. In addition, *P*-values for the overall effect of all haplotypes (i.e. both maternal and offspring effects) in each gene was determined using a likelihood ratio test to compare the log-linear model between a full model, which included terms for the effects of all haplotypes in each gene, with null models that included no effects.

RESULTS

Genotyping was completed on DNA samples from 120 families (276 subjects). Call rates for the 10 genotypes were >95%. Following quality control, 8 subjects (3%) were excluded for failure on more than 5 of the 10 (50%) genotypes that comprised the haplotypes of the six genes, and 2 families (2% of triads) were excluded from the analysis for concerns about handling errors. This resulted in 42 complete triads, 72 dyads (65 mother-child dyads and 7 father-child dyads), and 4 monads.

Demographic characteristics of the cases are presented in Table 2. Sixty-five (54%) were male. Fifty-seven subjects were Non-Hispanic White (48%), whereas 46 were Hispanic (39%). A majority of the cases (56%) was diagnosed at <4 years of age.

Table 3 provides the estimates of the frequencies of the offspring and maternal haplotypes, relative risks (RR), and 95% confidence intervals (CI). Table 3 also provides the *P*-value for the overall effect of all haplotypes of each gene. Two of the genes exhibited significant overall haplotype effects (*GSTM3*/*GSTM4*, *P* =0.01 and *GSTP1*, *P* =0.02), and one neared statistical significance (*EPHX1*, *P*=0.05). However, there were no significant overall haplotype effects of *CYP1B1* (*P*=0.62) and *GSTA4* (*P*=0.50).

There were significant associations in three of the five offspring haplotypes (Table 3). Specifically, there were significant associations between risk of ALL and offspring haplotypes in *EPHX1*, *GSTM3/GSTM4*, and *GSTP1*. For instance, a single dose of the T-g haplotype of *EPHX1* was inversely associated with risk for ALL (RR = 0.33, 95% CI: 0.15, 0.71). A single- and double-dose of the c-A haplotype of *EPHX1* was associated with an increased risk for ALL (RR = 2.64, 95% CI: 1.15, 6.01 and RR =5.84, 95% CI: 1.75, 20.1; respectively). A heterozygote of the g-C haplotype in *GSTP1* was associated with an increased risk for childhood ALL (RR = 2.67, 95% CI: 1.27, 5.54). Lastly, a single-dose of the G-G haplotype of *GSTM3*/*GSTM4* was inversely associated with disease risk (RR = 0.46, 95% CI: 0.23, 0.93). There was no evidence of an association between ALL and offspring haplotypes for *CYP1B1* or *GSTA4*.

There was no evidence of a statistical association between childhood ALL and maternal haplotypes of *CYP1B1*, *EPHX1*, and *GSTA4*. However, maternal haplotypes in *GSTM3*/*GSTM4* and *GSTP1* were associated with ALL risk. Specifically, a single- and double-dose of the a-c haplotype of *GSTM3*/*GSTM4* was associated with an increased risk for ALL (RR = 2.38, 95% CI: 1.04, 5.46; and RR = 10.40, 95% CI: 2.36, 47.60; respectively). A double-dose of the *GSTP1* g-C haplotype was also associated with a higher ALL risk (RR = 3.69, 95% CI: 1.32, 10.00).

The results using multiplicative risk models (i.e., dose-response models) were largely consistent with the single-dose and double-dose models (Table 4). Specifically, the *GSTP1* haplotypes remained significant when using the multiplicative risk models (*P*=0.02). The overall effect of the *EPHX1* haplotypes became stronger (*P*=0.004), while the overall effect of the *GSTM3*/*GSTM4* haplotypes lessened (*P*=0.18). However, the maternal a-c haplotype in *GSTM3/GSTM4* was significantly associated with ALL risk (RR = 2.31, 95% CI: 1.13, 4.71).

DISCUSSION

To our knowledge, no other studies reported to date have examined the association between both offspring and maternal haplotypes in the xenobiotic metabolism pathway and risk of childhood ALL. We conducted an analysis of five haplotypes comprising six genes (*GSTA4*, *GSTP1*, *EPHX1*, *GSTM3*, *GSTM4*, and *CYP1B1*) using a case-parent triad design. There was evidence of an association between childhood ALL and maternal haplotypes of *GSTM3/GSTM4* and *GSTP1*. Additionally, offspring haplotypes in *EPHX1*, *GSTM3/GSTM4*, and *GSTP1* were associated with childhood ALL risk. However, maternal and offspring haplotypes of *CYP1B1* and *GSTA4* were not associated with childhood ALL risk.

The *mu* class glutathione S-transferases (GSTs) are responsible for detoxification of electrophilic compounds, including environmental carcinogens [17, 19]. *GSTM3* and *GSTM4* were analyzed together as they are both found on chromosome 1. Variation in these genes, to our knowledge, has not previously been examined in relation to risk for childhood ALL. Previous literature has assessed the function of another member of the GST family, *GSTM1*, and found an increased risk of ALL in those with the *GSTM1* null variant

compared to those without the deletion [25]. The maternal haplotype of the single- and double-dose of both minor allelic haplotypes were associated with a considerable increase in the risk of childhood ALL, which may suggest that the GST *mu* family of enzymes are important in the etiology of ALL during intrauterine development. This study examined intronic regions of *GSTM3* and *GSTM4*, so these haplotypes may be in proximity of a functional variant. Also, because the *mu* family (M1–M5) of enzymes have an overlap of substrates they catalyze [26], the interactions between the genes should be explored further.

We also examined the GST *pi* class, which performs similar detoxification functions of the *mu* class. The SNP rs1695 encodes an amino acid change of Ile105Val in exon 5. This variant is associated with lower mean enzymatic activity against certain electrophilic carcinogenic compounds, like benzo[a]pyrene [20]. Results from studies regarding the influence of this SNP and a *GSTP1* haplotype on the risk of ALL are mixed, but this may be due to analyzing the SNP individually, study designs that may be subject to population stratification bias, or not accounting for the maternal haplotype [16, 18, 19]. *GSTP1* rs1138272 encodes an Ala114Val variant, but it was not frequent enough in our case population (i.e., <10%) to be included in the analysis [18]. Other studies have examined methylation on the silencing activity of GSTP1 in prostate carcinogenesis, which may be implicated in the etiology of ALL as lowered enzymatic activity may be correlated with leukemogenesis [27]. Our study also demonstrated that maternal haplotypes of *GSTP1* were associated with ALL. The GSTP1 enzyme is expressed in certain tissues, including the placenta, suggesting that these haplotypes may be important in the context of maternal exposures and childhood ALL risk [28]. We observed increased risks of ALL in both offspring and maternal effects, suggesting that ALL risk in this population is a product of both offspring and maternal genetic variation.

In our data, offspring haplotypes of *EPHX1* were associated with risk of ALL. Epoxide hydrolases are a family of enzymes responsible in the first phase of the xenobiotic metabolism (i.e., Phase I reactions) [29]. They metabolize procarcinogens, including polycyclic aromatic hydrocarbons, benzene, aromatic amines, and aflatoxin [29]. The enzymes can potentially either deactivate or activate carcinogenic compounds; whereby more active enzymes may activate procarcinogens and faulty enzymes may fail to detoxify carcinogens [30]. The haplotype under examination is a combination of the following SNPs: rs1051740 and rs2234922. *EPHX1* rs1051740 is responsible for a missense mutation resulting in a Tyr113His in exon 3. This mutation is associated with a 50% lower enzymatic activity of EPHX1 [31]. When analyzed individually, this variant was associated with increased risk of ALL and other cancers sites (e.g., ovarian) [31, 32]. It is likely that the mutation produces enzymes that fail to detoxify carcinogenic epoxides which can lead to cellular DNA damage and induce leukemogenesis. *EPHX1* rs2234922 codes a His139Arg missense mutation in exon 4 and is associated with a 25% increase in EPHX1 activity [33]. Previous studies have found no associations when analyzed individually [31, 34]. An additional study found no association with an *EPHX1* haplotype consisting of different SNPs [16]. However, another study reported a protective effect of the haplotype, consistent with our current results [34]. There was no evidence for maternal genetic variation of *EPHX1* and risk of ALL in this the current study population.

An important limitation of this study is the sample size. This may have influenced our ability to detect modest associations. Also, this study combined all ALL subtypes (B and T cell) because of the fewer number of subjects, which may differ in etiology. Finally, we did not have an even distribution among dyads (i.e., there were more mother-child dyads than father-child dyads), which may have limited our power to detect some maternal genetic effects. However, this study has several strengths, namely, the use of the case-parent triad design, which allows an estimate of maternal genetic effects, while adjusting for offspring

genetic effects, and is robust to population stratification bias from admixed study populations [13, 35]. Additionally, we evaluated the effects of both offspring and maternal haplotypes on the risk of ALL and identified several significant associations.

In conclusion, we observed that both offspring and maternal haplotypes in genes in the xenobiotic metabolism pathway are associated with childhood ALL. The effects of maternal haplotypes suggest that xenobiotic metabolism may be important *in utero* and that maternal exposures during pregnancy may be important in childhood ALL risk. Additionally, the effects of offspring genetic variants suggest that an individual's haplotype are important in the risk of ALL as well. In the future, it will be important to interrogate gene-environment interactions because of the role that these detoxification genes play in modifying potential carcinogens.

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ABBREVIATIONS AND UNITS

ALL	Acute lymphoblastic leukemia
CI	confidence interval
RR	relative risk

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Table 1

Xenobiotic metabolism genes included in the assessment of maternal and offspring haplotypes

Gene name	Location	Gene symbol	RefSNP	Amino Acid Change	Alleles[1]
Cytochrome P450 1B1	2p22.2	*CYP1B1*	rs1056836	Leu432Val	C/g
	2p22.2		rs1800440	Asn453Ser	A/g
Epoxide hydrolase 1	1q42.12	*EPHX1*	rs1051740	Tyr113His	T/c
	1q42.22		rs2234922	His139Arg	A/g
Glutathione S-transferase A4	6p12.2	*GSTA4*	rs316133	Intronic	G/c
	6p12.2		rs3756980	Intronic	T/c
Glutathione S-transferase M3	1p13.3	*GSTM3*	rs1571858	Intronic	G/a
Glutathione S-transferase M4	1p13.3	*GSTM4*	rs1010167	mRNA-Untranslated region	G/c
Glutathione S-transferase P1	11q13.2	*GSTP1*	rs1138272	Ala114Val	C/t
	11q13.2		rs1695	Ile105Val	A/g

[1] Lower case is minor allele of the SNP

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Table 2

Characteristics of childhood acute lymphoblastic leukemia cases, Childhood Cancer Epidemiology and Prevention Center, 2003–2010

Characteristic	No.	%
Families Included	118	
Triads	42	35.6
Dyads	72	61.0
Monads	4	3.4
Case sex		
Male	65	55.1
Female	53	44.9
Race/ethnicity		
Non-Hispanic White	57	48.3
Non-Hispanic Black	6	5.1
Hispanic	46	39.0
Other	9	7.6
Age at diagnosis (years)		
<4	66	55.9
4–7	33	28.0
7–14	19	16.1

Table 3

Offspring and maternal xenobiotic metabolism haplotypes and the risk of childhood ALL, single and double dose haplotype effects

Gene	Haplotype	Frequency (95% CI)	Offspring haplotype relative risk[1] (95% CI)		Maternal haplotype relative risk[1] (95% CI)		*P*-value[2]
			Single-dose	Double-dose	Single-dose	Double-dose	
CYP1B1[3]	C-A	0.35 (0.24, 0.48)	1.17 (0.55, 2.51)	1.43 (0.46, 4.43)	1.49 (0.70, 3.09)	2.80 (0.99, 7.70)	0.62
	g-A	0.43 (0.32, 0.56)	0.78 (0.39, 1.55)	0.77 (0.27, 2.17)	1.40 (0.65, 2.99)	1.58 (0.55, 4.50)	
	C-g	0.21 (0.12, 0.33)	1.08 (0.53, 2.22)	1.51 (0.35, 6.52)	0.60 (0.31, 1.22)	0.80 (0.21, 2.93)	
	c-A	0.20 (0.12, 0.31)	**2.64 (1.15, 6.01)**	**5.84 (1.75, 20.10)**	1.99 (0.91, 4.49)	2.81 (0.79, 10.10)	0.05
EPHX1[4]	T-A	0.56 (0.43, 0.68)	1.80 (0.65, 5.07)	2.18 (0.62, 7.83)	0.48 (0.24, 0.98)	0.46 (0.17, 1.23)	
	T-g	0.24 (0.13, 0.37)	**0.33 (0.15, 0.71)**	0.14 (0.01, 1.26)	0.88 (0.39, 1.92)	0.77 (0.13, 4.65)	
	c-c	0.20 (0.12, 0.32)	0.47 (0.22, 1.01)	0.16 (0.02, 1.62)	1.19 (0.57, 2.42)	1.42 (0.31, 6.57)	0.50
GSTA4[5]	c-T	0.24 (0.15, 0.35)	1.94 (0.92, 4.23)	2.79 (0.75, 10.30)	0.63 (0.32, 1.25)	0.72 (0.22, 2.37)	
	G-T	0.55 (0.43, 0.67)	1.46 (0.61, 3.61)	1.60 (0.53, 4.91)	1.96 (0.73, 5.43)	2.12 (0.65, 6.92)	
	a-c	0.10 (0.05, 0.19)	0.90 (0.37, 2.22)	1.45 (0.26, 7.85)	**2.38 (1.04, 5.46)**	**10.40 (2.36, 47.60)**	**0.01**
GSTM3/GSTM4[6]	G-c	0.22 (0.13, 0.34)	1.15 (0.53, 2.51)	3.11 (0.89, 10.10)	0.88 (0.42, 1.86)	1.32 (0.39, 4.36)	
	a-G	0.17 (0.09, 0.28)	1.88 (0.80, 4.45)	2.19 (0.42, 10.70)	0.61 (0.30, 1.24)	1.56 (0.47, 5.29)	
	G-G	0.50 (0.37, 0.62)	**0.46 (0.23, 0.93)**	0.50 (0.18, 1.38)	0.78 (0.37, 1.64)	0.82 (0.29, 2.37)	
GSTP1[7]	A-C	0.80 (0.68, 0.89)	--	REF[8]	--	REF[8]	**0.02**
	g-C	0.20 (0.11, 0.32)	**2.67 (1.27, 5.54)**	3.66 (0.89, 13.70)	1.22 (0.68, 2.21)	**3.69 (1.32, 10.00)**	

[1] Comparison of the specific haplotype compared to the reference group of the remaining haplotypes

[2] Likelihood ratio test P-value of overall effect

[3] Haplotype of SNP rs1056836 and rs1800440

[4] Haplotype of SNP rs1051740 and rs2234922

[5] Haplotype of SNP rs316133 and rs3756980

[6] Haplotype of SNP rs1571858 and rs1010167

[7] Haplotype of SNP rs1138272 and rs1695

[8] With two haplotypes, the model compares the g-C/A-C (single-dose) and g-C/g-C (double dose) against the reference A-C/A-C

Table 4

Offspring and maternal xenobiotic metabolism haplotypes and the risk of childhood ALL, dose-response haplotype effects

Gene	Haplotype	Frequency (95% CI)	Offspring haplotype relative risk (95% CI)	Maternal haplotype relative risk (95% CI)	*P*-value[2]
			Dose-response[1]	Dose-response[1]	
CYP1B1[3]	C-A	0.36 (0.25, 0.47)	1.00 (REF)	1.00 (REF)	0.38
	g-A	0.43 (0.32, 0.55)	0.85 (0.51, 1.40)	0.78 (0.48, 1.26)	
	C-g	0.21 (0.13, 0.32)	1.02 (0.53, 1.95)	0.56 (0.30, 1.02)	
	c-A	0.20 (0.12, 0.31)	1.55 (0.87, 2.69)	1.63 (0.97, 2.76)	**0.004**
EPHX1[4]	T-A	0.56 (0.43, 0.68)	1.00 (REF)	1.00 (REF)	
	T-g	0.24 (0.14, 0.37)	**0.39 (0.19, 0.78)**	1.15 (0.34, 4.98)	
	c-c	0.20 (0.12, 0.32)	**0.52 (0.27, 0.99)**	0.98 (0.51, 1.84)	0.14
GSTA4[5]	c-T	0.24 (0.16, 0.36)	1.16 (0.67, 1.98)	0.76 (0.46, 1.25)	
	G-T	0.55 (0.43, 0.66)	1.00 (REF)	1.00 (REF)	
	a-c	0.10 (0.05, 0.18)	1.30 (0.65, 2.63)	**2.31 (1.13, 4.71)**	0.18
GSTM3/GSTM4[6]	G-c	0.23 (0.15, 0.35)	1.60 (0.86, 2.89)	1.11 (0.63, 1.96)	
	a-G	0.18 (0.10, 0.28)	1.55 (0.80, 3.02)	0.99 (0.53, 1.85)	
	G-G	0.48 (0.36, 0.60)	1.00 (REF)	1.00 (REF)	
GSTP1[7]	A-C	0.79 (0.67, 0.88)	1.00 (REF)	1.00 (REF)	**0.02**
	g-C	0.21 (0.12, 0.33)	**1.83 (1.03, 3.19)**	**1.80 (1.06, 3.05)**	

[1] Relative risk represents the increase or decrease in risk with each additional copy of the haplotype

[2] Likelihood ratio test P-value of overall effect

[3] Haplotype of SNP rs1056836 and rs1800440

[4] Haplotype of SNP rs1051740 and rs2234922

[5] Haplotype of SNP rs316133 and rs3756980

[6] Haplotype of SNP rs1571858 and rs1010167

[7] Haplotype of SNP rs1138272 and rs1695