

ENVIRONMENTAL DETERMINANTS

The Role of *GSTP1* Polymorphisms and Tobacco Smoke Exposure in Children with Acute Asthma

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Background. The glutathione *S*-transferase enzymes (GSTs) play an important role in the detoxification of environmental tobacco smoke (ETS), which contributes to airway inflammation, a key component of asthma. Genetic variation in *GST* genes may influence individuals' ability to detoxify environmental pollutants. **Objective.** To examine the role of polymorphisms in *GSTP1* (Ile105Val and Ala114Val), alone and in combination with ETS exposure, on atopy and asthma severity. **Methods.** *GSTP1* Ile105Val and Ala114Val were genotyped and ETS exposure was assessed by parental questionnaire, which was validated by urinary cotinine measurements. Associations between ETS exposure, *GSTP1* polymorphisms, and their interaction on atopy and asthma severity were investigated. **Results.** For the functional *GSTP1* 105 SNP, those with the Ile/Ile genotype had odds for atopy of 2.77 ($p = .054$) when assessed by genotype alone, which increased to 9.02 ($p = .050$) when ETS was included, relative to individuals with other genotypes. Likewise, compared to children with other *GSTP1* 114 genotypes, those with Ala/Ala genotype had a 5.47-fold ($p = .002$) increased risk of atopy ($p = .020$) when assessed by genotype alone, increasing to 9.17-fold when ETS was included. The 105 Ile/Ile individuals all had the AA (105 Ile/Ile and 114 Ala/Ala) haplotype group; therefore, the odds for atopy were the same. Individuals without any *C haplotype (105 Val and 114 Val allele) who were exposed to ETS had a 9.17-fold increased risk of atopy when compared with individuals with at least one *C haplotype and not exposed to ETS ($p = .020$). **Conclusion.** There were significant interactions between *GSTP1* SNPs, atopy, and ETS exposure in this cohort.

Keywords asthma exacerbations, ETS, gene–environment interaction, glutathione, haplotype

INTRODUCTION

Asthma is a multifactorial disease and is influenced by both environmental and genetic factors (1). Genetic variation plays an important role in the development of atopy and asthma but environmental factors are equally important in determining whether susceptible children develop symptoms. The substantial increase in asthma prevalence in Western countries suggests a strong influence of environmental factors (2) and exposure to pollutants, in particular environmental tobacco smoke (ETS) has been shown to play a role (1). Reactive oxygen species (ROS) produced endogenously by metabolic reactions in the body or exogenously from air pollutants and cigarette smoke are one of the main causes of inflammation in the lung (3, 4). These ROS are detoxified by enzymatic antioxidants involved in defense against oxidative stress including the glutathione *S*-transferases (GSTs).

The *GST* genes encode a superfamily of enzymes comprising several classes including alpha (A), mu (M), pi (P), theta (T), and zeta (Z). Several of the *GST* genes

are polymorphic and result in altered levels and activities of the encoded enzymes. As GSTs play a vital role in detoxification, alterations in their activity or levels may adversely affect the ability of the body to detoxify foreign compounds (5, 6). Functional polymorphisms in the M, P, and T classes of GST have been associated with diseases such as asthma and cancer (7–13). The *GSTP1* gene, located on chromosome 11q13, contains two single nucleotide polymorphisms (SNPs), Ile105Val (rs1695) and Ala114Val (rs1138272), that have been associated with the incidence of asthma and risk of atopy in several studies (8, 9, 13–18). The majority of studies published to date have focused exclusively on the *GSTP1* Ile105Val substitution for several reasons. First, the Val 105 variant has a higher catalytic activity with some substrates (19); second, no additional effect of the Ala114Val substitution has been identified (8); third, Val 105 and Val 114 are in highly significant linkage disequilibrium (20); and lastly, the Val 114 variant does not appear to affect the function of the 105 variant (20). Studies of haplotypes are limited thus far, and only one study has shown that the AA haplotype group consisting of *GSTP1* 105 Ile/Ile and 114 Ala/Ala was associated with increased bronchial hyperresponsiveness in adults (16).

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There are a limited number of studies examining the *GSTP1* SNPs while investigating their interaction with environmental influences such as ETS (14). The aim of this study, therefore, was to examine the role of polymorphisms in the *GSTP1* gene, alone and in combination with ETS exposure, on asthma severity and atopy in a cohort of asthmatic children. The hypothesis of this study was that *GSTP1* 105 Ile/Ile and 114 Ala/Ala genotypes are associated with susceptibility to atopy and asthma severity, and that these associations are strengthened when gene/environment interactions are included, because of a genetically determined deficiency of GSTP1 enzyme involved in the detoxification of ROS.

METHODS

Study Population

Two hundred and twenty-one children aged 2–16 years were recruited from the Emergency Department at Princess Margaret Hospital, Perth, Western Australia, on presentation with an asthma exacerbation as part of the Perth Childhood Acute Asthma Study (PCAAS) as previously described (21, 22). Recruitment was conducted between July 2002 and November 2006. Informed consent was obtained from the parents or guardians and the study was approved by the Princess Margaret Hospital Ethics Committee. Clinical examinations were conducted and asthma diagnosis was made by an Emergency Department doctor based on previous history, hospital records, and the presence of wheeze with increased difficulty in breathing in accordance with previous and current published international guidelines (23). All children received inhaled salbutamol and ipratropium at 20-min intervals for the first hour and oral prednisolone (1 mg/1 kg). Supplemental oxygen was administered if saturations were less than or equal to 94%. Lung function testing using spirometry was done on children who could cooperate satisfactorily.

Phenotypic Data and Questionnaire

An asthma severity score was assigned to each subject based on a scoring system adapted from the National Institute of Health (22, 24). A score of 1–3 was given for five different parameters: oxygen saturation, auscultation, retractions, dyspnea, and respiratory rate (age specific) giving a rating of mild (5–7), moderate (8–11), or severe (12–15). The details of the acute asthma scoring system are shown in Table 1. Allergy testing by skin prick test (SPT) was performed to all children using 11 allergens, including cows' milk, egg white, dog hair and dander, cat pelt, cockroach, mixed grasses, rye grass, *Aspergillus fumigatus*, *Alternaria tenuis*, *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus* along with positive and negative controls. Atopy was defined as a positive SPT to at least one allergen with a wheal size greater than or equal to 3 mm. Peripheral blood and urine samples were collected within 24 hours of acute admission for

TABLE 1.—Asthma severity scoring system for children with acute asthma.

	1 point	2 points	3 points
Oxygen saturation	>95%	90–95%	<90%
Auscultation	Nil to mild end expiratory wheeze	Expiratory wheezing	Inspiratory and expiratory wheezing or diminished breath sounds
Retractions	None to intercostal	Intercostal and substernal	Intercostal, substernal and supraclavicular
Dyspnea	Speaks in sentences or coos and babbles	Speaks in partial sentences or utters short cries	Speaks in single words or short phrases or grunts
Respiratory rate (/min)			
2–5 years	<34	35–39	>39
6–8 years	<30	31–35	>35
9–12 years	<26	27–30	>30
>12 years	<23	24–27	>27

DNA isolation and measurement of cotinine levels. Blood and urine samples were collected and SPT performed only if the children were able and willing to cooperate.

Upon recruitment, the subject's parent(s) or guardian completed a questionnaire. The question "Does anyone in the household smoke now?" was used to define ETS exposure. To further investigate the nature of the exposure, outdoor and indoor ETS exposure was defined by "Does anyone in the household smoke in the house or car?" If parents answered yes to the first question but no to the last question, it was assumed that the exposure occurred outdoors (not in the house or car). Data from this questionnaire addressed the primary occupants of the household only and did not include other potential ETS exposure from sources such as extended family members, other caregivers or babysitters, and day care centers or schools.

Genotyping

DNA from all samples was extracted from peripheral blood using the salt precipitation method (25). Amplification by polymerase chain reaction (PCR) for *GSTP1* Ile105Val and Ala114Val SNPs was carried out in a total volume of 25 µL consisting of approximately 100 ng genomic DNA, 1× PCR buffer, 5 mM dNTPs (Ile105Val) or 1.25 mM dNTPs (Ala114Val), 25 ng of primers (Ile105Val F: 5' ACC CCA GGG GTC TCT GGG AA 3', R: 5' TGA GGG CAC AAG AAG CCC CT 3'; Ala114Val F: 5' CTG AAG TGG ACA GGA TTT GG 3', R: 5' GGA GCA GGC TGG AGG AAT 3'), 0.5 U of *Taq* DNA polymerase (Roche Diagnostic, Mannheim, Germany) (Ile105Val) or 0.5 U of *Tth* Plus (Fisher Biotech, Wembley, Australia) (Ala114Val), and sterile water. The PCR conditions consisted of an initial denaturation temperature of 95°C for 5 min, followed by 35 cycles at 94°C for 2 min, 59°C (Ile105Val) or 57°C (Ala114Val) for

30 s, and 72°C for 1 min. A final extension step at 72°C for 10 min terminated the process. Amplification was confirmed by electrophoresis on an ethidium bromide-stained 2% (w/v) agarose gel and visualization under UV illumination.

PCR amplicons were digested using the restriction enzymes *BsmAI* (New England Biolab, Beverly, MA, USA) and *AciI* (New England Biolab) for Ile105Val and Ala114Val, respectively. Restriction enzyme digestion of the amplicon for *GSTP1* Ile105Val and Ala114Val was carried out in a total volume of 10 μ L consisting of 5 μ L DNA amplicon, 1 $\times$ buffer '3' (New England Biolab), and 1.5 U of *BsmAI* (Ile105Val) or 5 U *AciI* (Ala114Val). Digested fragments were analyzed by electrophoresis on either 3% (w/v) or 2% (w/v) agarose gels containing ethidium bromide. For confirmation of genotyping, a random 10% of samples representing all three genotypes for each SNP were sequenced.

Generation of Haplotypes

Four haplotypes were constructed from the two linked SNPs: *GSTP1**A (105 Ile $\rightarrow$ 114 Ala), *GSTP1**B (105 Val $\rightarrow$ 114 Ala), *GSTP1**C (105 Val $\rightarrow$ 114 Val), and *GSTP1**D (105 Ile $\rightarrow$ 114 Val). Each person has two haplotypes thus there are ten possible haplotype groups (AA, AB, AC, AD, BB, BC, BD, CC, CD, and DD). Haplotypes for individuals homozygous for both *GSTP1* SNPs could be determined intuitively based on genotyping results. The program PHASE (version 2.0.2) was used to infer haplotypes for children heterozygous for both SNPs, with the phase certainty threshold set at the default (90%). The program PHASE implements a Bayesian statistical method for reconstructing haplotypes from population genotype data (26).

Cotinine Measurement

A commercial high-sensitivity salivary cotinine quantitative enzyme immunoassay kit (EIA) (Salimetrics, State College, PA, USA) was modified and used for the measurement of cotinine in urine samples. Validation to verify the performance of this kit was done by "spiking" urine samples with a known quantity of cotinine. ETS exposure assessed by questionnaire was validated by measuring urinary cotinine levels. Creatinine was measured by automated enzymatic assay utilizing creatinine anhydrolase and the Vitros 250 Chemistry System (Ortho_Clinical Diagnostics Inc., Rochester, NY, USA). Urine cotinine-to-creatinine concentration ratios were then determined. Children's exposure to ETS obtained through questionnaire was used for the subsequent analyses as the cotinine levels measured in the urine samples validated these data and urine samples available for cotinine measurements were limited.

Statistical Analysis

Hardy-Weinberg equilibrium for *GSTP1* Ile105Val and Ala114Val was calculated using exact Hardy-Weinberg equilibrium test available at Innate Immunity PGA

(www.innateimmunity.net). Linkage disequilibrium (D') and r^2 values for the *GSTP1* 105 Ile105Val and Ala114Val SNPs were calculated using Arlequin 3.1.1. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Results were reported as percentages for categorical outcomes whereas parametric tests were employed for continuous outcomes with means and confidence intervals (CI) reported. The cotinine levels in urine were positively skewed so geometric means (GM) and 95% CI were calculated after applying a logarithmic transformation that resulted in an approximately normal distribution. When one of the homozygous genotype frequencies was less than 10%, this genotype was pooled with the heterozygous group before analysis. Independent sample *t*-test was employed to validate the questionnaire ETS data by means of cotinine levels measured in urine. Logistic regression was used to estimate the odds ratio (OR) for asthma severity and atopy by comparing it to a baseline categorical outcome, with adjustment for age and gender where appropriate. *P* values of $<.05$ were considered statistically significant. Evidence for a linear trend on phenotype across the genotypes was obtained by entering genotype as a continuous covariate in logistic regression analysis (allele dose-dependant; expressed as P_{linear}).

RESULTS

Demographic and Phenotypic Data

The demographic and phenotypic data for the study population are shown in Table 2. There was no significant difference between atopic and non-atopic subjects in terms of gender ($p = .841$), asthma severity ($p = .247$), and ETS exposure ($p = .464$). A significant difference for age ($p = .014$) was found so all analyses were adjusted for age. *GSTP1* Ile105Val was genotyped in 202 DNA samples and Ala114Val in 200 samples. The Ile allele frequency for *GSTP1* Ile105Val was 69.8%

TABLE 2.—Demographic and phenotypic characteristics of the PCAAS cohort.

	Subjects ($n = 221$)
Mean age, years ($\pm$ SD)	6.54 (3.61)
Gender, n (%)	
Male	139 (62.9)
Atopy, n/n assessed (%)	118/143 (82.5)
Asthma severity, n/n assessed (%)	
Mild	38/218 (17.4)
Moderate	120/218 (55.1)
Severe	60/218 (27.5)
Mean asthma severity score ($\pm$ SD) ^a	9.93 ($\pm$ 2.31)
Mean cotinine levels in urine ($\pm$ SD) ^b	10.90 ($\pm$ 23.38)
Anyone in the household who smokes, n/n assessed (%)	81/173 (46.8)
Oxygen saturation %, mean ($\pm$ SD) ^c	93.8 ($\pm$ 3.35)
Spirometry completed, n (%) ^d	40 (17.17)

^aAsthma severity scores were recorded for 218 children.

^bCotinine levels were measured in 104 children.

^cOxygen saturation was measured in 220 children.

^dTotal of 233 children.

TABLE 3.—Association of *GSTP1* Ile105Val and Ala114Val with atopy.

<i>GSTP1</i> Gene	Genotypes	Atopics, n (%)	Non-Atopic, n (%)	OR	95% CI	P value
Ile105Val (n = 135)	Ile/Ile	64 (90.1)	7 (9.9)	4.83	1.09–21.45	.038*
	Ile/Val	44 (83.0)	9 (17.0)	2.15	0.48–9.77	.320
	Val/Val	6 (54.5)	5 (45.5)	1		
Ala114Val (n = 133)	Ala/Ala	97 (90.7)	10 (9.3)	5.47	1.86–16.09	.002*
	Ala/Val & Val/Val	16 (61.5)	10 (38.5)	1		

Note. Insufficient sample size for 114 Val/Val and Ala/Val & Val/Val were combined as reference category.

^aAnalyzed by logistic regression adjusted for age and gender.

* $p < .05$

and Ala allele frequency for Ala114Val was 89.6%. The genotype distribution of *GSTP1* Ile105Val and Ala114Val SNPs did not deviate from Hardy–Weinberg equilibrium ($p = .243$ and $.243$, respectively). The haplotype frequencies of *GSTP1**A, *GSTP1**B, *GSTP1**C, and *GSTP1**D were 69.3%, 20.3%, 9.9%, and 0.5%, respectively. The AA haplotype group (50%) was the most common followed by AB (26.2%) and AC (11.9%). Similar haplotype frequencies were reported in an unselected European adult population (16). Only one individual had either the AD or CD haplotype group and none had BD and DD reflecting the rarity of the *GSTP1**D haplotype. Linkage disequilibrium (D') and r^2 values for the Ile105Val and Ala114Val SNPs were 0.932 and 0.073, respectively.

Validation of ETS Assessments (Questionnaire Data) by Cotinine Levels

The results of the cotinine assays were consistent with parental reports of ETS exposure. Children with no reported ETS exposure had lower urinary cotinine levels (GM = 0.47 ng/mg creatinine, 95% CI 0.26–0.85) compared with children exposed to ETS (GM = 6.61 ng/mg creatinine, 95% CI 3.35–13.04) with $p < .001$. When further stratified by indoor or outdoor ETS exposure, children exposed to indoor ETS had much higher cotinine levels (GM = 14.44 ng/mg creatinine, 95% CI 6.90–30.22) compared with children exposed to outdoor ETS (GM = 3.66 ng/mg creatinine, 95% CI 1.17–11.40) with $p < .001$.

GSTP1 SNPs, Atopy, and ETS Exposure

Analysis of the associations between *GSTP1* Ile105Val and atopy showed that children with 105 Ile/Ile had higher odds for having atopy ($p = .038$) compared with children with the *GSTP1* 105 Val/Val genotype (Table 3). In an additive genetic model, there was a 2.21-fold increased odds of atopy for each additional Ile allele ($P_{\text{linear}} = .030$). Compared with individuals with *GSTP1* 114 Ala/Val & Val/Val, children with the Ala/Ala genotype had more than 5-fold higher odds of atopy ($p = .002$). When the allele dose-effect was examined, the Ala allele was associated with a 4.55-fold increased risk of atopy ($P_{\text{linear}} = .002$). There was no significant difference in the prevalence of atopy between individuals exposed to ETS

and individuals not exposed to ETS even after stratification of ETS exposure into outdoor and indoor exposure categories.

To investigate whether there was any interaction between each *GSTP1* SNPs and ETS exposure on atopy, subjects were classified into four groups (Figure 1). Inclusion of ETS exposure in the analyses showed that, compared with children possessing *GSTP1* 105 Ile/Val & Val/Val genotypes not exposed to ETS, those who had the Ile/Ile genotypes and exposed to ETS were 9.02 times (95% CI 1.00–81.70) more likely to be atopic ($p = .05$) (Figure 1A). In children exposed to ETS, comparison of 105 Ile/Ile with Ile/Val & Val/Val supported this

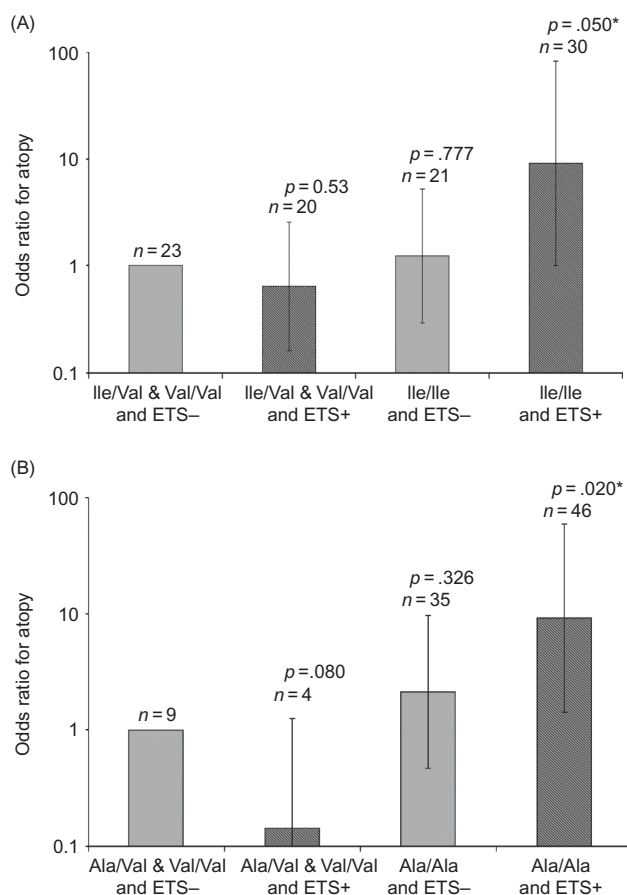


FIGURE 1.—Odds ratio for atopy in children stratified by genotypes and ETS exposure: (A) *GSTP1* Ile105Val genotypes and ETS exposure; (B) *GSTP1* Ala114Val genotypes and ETS exposure; * $p < .05$.

finding (OR = 13.66, 95% CI 1.3–143.41, $p = .029$). The interaction term for *GSTP1* Ile105Val and ETS was marginally significant ($p = .080$). Similarly, when ETS was included in the analysis, individuals who had the *GSTP1* 114 Ala/Ala genotype and were exposed to ETS had a 9.17-fold (95% CI 1.42–59.12) increased risk of atopy compared with those with Ala/Val & Val/Val genotypes and not exposed to ETS ($p = .020$) (Figure 1B). Comparison of 114 Ala/Ala with Ala/Val & Val/Val in children exposed to ETS also supported this finding ($p = .001$) although an odds ratio was not obtained as the sample size for Ala/Val and Val/Val was too small. The interaction term for *GSTP1* Ala114Val and ETS was significant ($p = .017$).

GSTP1 Haplotypes, Atopy, and ETS Exposure

The effects of *GSTP1* haplotypes on atopy were only investigated for the AA, AB, AC, BB, and BC haplotypes as the frequencies of other haplotypes were ≤ 3 (Table 4). The AA haplotype group had a 2.77-fold increased risk of atopy compared with the rest of the haplotype groups that approached statistical significance ($p = .054$). The frequencies of the AA haplotype group were identical to the frequencies of 105 Ile/Ile genotypes indicating the tight linkage disequilibrium between *GSTP1* 105 Ile/Ile and 114 Ala/Ala. The risk of atopy for the AA haplotype group increased to 9.02-fold in the presence of ETS compared with non-AA haplotype group not exposed to ETS (95% CI 1.0–81.69, $p = .050$). In contrast to the AA haplotype group, the AC haplotype group was associated with significantly lower odds for atopy indicating a protective effect with an OR of 0.25 ($p = .024$). Furthermore, although not significant, BC haplotype group had lower odds for atopy indicating a possible dominant protective effect of the *C haplotype. When compared with the rest of the haplotypes lacking the *C haplotype, children with at least one copy of the *C haplotype were five times less likely to be atopic (95% CI 0.07–0.58, $p = .003$). A combined analysis of ETS and the presence of at least one *C haplotype was performed to assess a possible dominant protective effect on atopy. Individuals with haplotypes lacking the *C haplotype who were exposed to ETS had an increased odds for atopy (OR: 9.17, 95% CI 1.42–59.12, $p = .020$) compared with individuals with at least

one *C haplotype and not exposed to ETS. This result was supported in the ETS-exposed group when individuals with at least one *C haplotype were compared with individuals lacking the *C haplotype ($p = .001$). An odds ratio was not obtained as the sample size for Ala/Val and Val/Val was too small. No association was found between the AB haplotype group and ETS exposure on atopy. The odds ratios for other haplotype groups (AC, BB, and BC) in combination with ETS exposure were not analyzed because of insufficient sample sizes.

GSTP1 SNPs, Asthma Severity, Haplotypes, and ETS Exposure

To analyze associations between the *GSTP1* SNPs, haplotypes, and asthma, the moderate and severe groups were combined for analysis and all comparisons were made between mild versus combined moderate and severe. For both *GSTP1* SNPs studied, no significant difference in asthma severity was found between genotypes and haplotypes or in combination with ETS exposure as determined by severity groups or scores. Asthma severity was also not associated with exposure to ETS, even after further classification of ETS into indoor and outdoor exposure.

DISCUSSION

This study of the role of polymorphisms in *GSTP1* in combination with ETS on asthma severity and atopy has shown evidence for gene/environment interactions for atopy, but not acute asthma severity. The *GSTP1* 105 Ile/Ile and 114 Ala/Ala genotypes were associated with an increased risk of atopy and the risks were markedly higher when ETS exposure was taken into account. The AA haplotype group (consisting of *GSTP1* 105 Ile/Ile and 114 Ala/Ala) was associated with atopy alone as well as in combination with ETS exposure. Conversely, having at least one *GSTP1**C haplotype (consisting of one 105 Val and one 114 Val allele) was associated with protection from atopy.

Our study of *GSTP1* SNPs Ile105Val and Ala114Val is unique, as we have examined relationships with acute asthma severity and atopy between genotypes and haplotypes and in combination with ETS exposure in a cohort of children with acute asthma. The majority of previous studies have focused only on the Ile105Val SNP as it has functional effects on the GSTP1 protein. In agreement with our results, associations between the 105 Ile allele and an increased risk of atopy have been reported in several studies (8, 9, 15–17, 27, 28) although one study demonstrated otherwise (13) and some found no associations (29, 30). Only a few of these studies examined environmental exposures such as ETS in combination with *GSTP1* alleles for effects on disease outcome. One major drawback of these studies was that potential exposure of subjects was only determined by assessing general environmental levels of pollutants or by speculating that a strong overall environmental exposure may have existed.

TABLE 4.—Association of haplotype groups with atopy ($n = 135$).

Haplotype Groups	Atopic ($n = 114$)	Non-atopic ($n = 21$)	OR ^a	95% CI	P value
AA	64 (90.1)	7 (9.9)	2.77	0.98–7.83	.054
AB	31 (91.2)	3 (8.8)	1.89	0.49–7.22	.354
AC	12 (66.7)	6 (33.3)	0.25	0.07–0.83	.024*
BB	3 (75.0)	1 (25.0)	0.57	0.05–6.46	.649
BC	3 (50.0)	3 (50.0)	0.21	0.06–1.88	.210
CC and CD	1 (50.0)	1 (50.0)			

^aOdds ratios were obtained by using the rest of the haplotypes groups as reference group (designated with an OR of 1—not shown in table) to compare with AA, AB, AC, BB, and BC haplotype groups. OR was adjusted by logistic regression for age and gender. * $p < .05$

In this study, a more accurate assessment of individual ETS exposure was obtained through questionnaires and validated by cotinine measurements.

In our study, apart from individual alleles, the AA haplotype group (which contains the 105 Ile and 114 Ala alleles) alone had a higher odds ratio for atopy although the association only approached significance. However, a higher odds ratio for risk of atopy was found when ETS exposure was included in the analyses, thus indicating a significant gene/environment interaction in this cohort. Supporting this finding, Hemmingsen et al. (2001) found that asthma was increased in the AA haplotype group although individual ETS exposure was not considered in their study (16). There are other haplotype studies that investigated the effects of *GSTP1* haplotypes from different polymorphisms (SNP 1: rs6591255; SNP 2: rs4147581; SNP 3: rs1695; SNP 4: rs749174) within the *GSTP1* gene on asthma outcomes (31, 32). However, there are limited studies on *GSTP1* 105 and 114 haplotype groups, which are the two most studied SNPs, and, to our knowledge, there are no reported studies that have included them in investigations of gene environment interactions with asthma and atopy. In addition, our results showed that the AC haplotype group (the *C haplotype contains the 105 Val and 114 Val allele) was associated with a significantly decreased risk of developing atopy, suggesting a protective effect. The AC and BC haplotype groups were also associated with lower odds ratios for atopy suggesting a dominant effect of the *C haplotype in masking the effect of the *A haplotype. A significant relationship was not found with the BC haplotype group, in contrast to the significant association found by Hemmingsen et al. (2001) in adults. This could possibly be because of a low number of individuals with this combination in our cohort. When further analyses were done to verify the hypothesis, individuals with at least one copy of the *C haplotype had a significantly decreased risk of atopy when compared with individuals without any *C haplotype.

Our results demonstrated that genotype/phenotype associations in atopy were modified by environmental exposures and, in our study cohort, significantly altered the odds ratio for atopy. These results, along with many other current studies examining genetic influences on atopy and asthma, highlight the importance of the environment on disease susceptibility and severity (33, 34). The complex pathophysiological relationship between GST, ETS exposure, and atopy remains unclear but it is known that environmental irritants cause inflammation and oxidative stress resulting in the formation of ROS and release of pro-inflammatory cytokines (27, 35, 36). Atopic individuals with an altered antioxidant defense against oxidative stress may have a decreased ability to detoxify environmental irritants and hence may be more vulnerable to airway inflammation (27, 37, 38). As GSTs are thought to influence the synthesis of eicosanoids, it has been proposed that individuals with the 105 Val/Val genotype may have an increased catalytic efficiency

toward lipid peroxides resulting in a decreased mobilization of arachidonic acid and reduced synthesis of pro-inflammatory eicosanoids, such as leukotrienes and prostaglandins (27, 39).

There were no associations between the *GSTP1* SNPs and haplotypes, and acute asthma severity in our cohort. *GSTP1* is highly expressed in the lung epithelium and is likely to play a pivotal role in the glutathione antioxidant pathway to control damage from ROS in the lungs. However, there are other major antioxidant proteins including glutamate cysteine ligase, glutathione reductase, glutathione peroxidase, glutathione synthase, manganese SOD, and catalase that are expressed in the lung and which play a role in managing ROS and maintaining healthy lungs (35, 39, 40). Therefore, it is likely that there is a level of redundancy in antioxidant defense systems. All of the children in our study were recruited when they were acutely asthmatic and their lungs would have been subject to overwhelming inflammation from an influx of inflammatory cells and cytokines. It is likely therefore that numerous genes would interact to modify the level and severity of inflammation during the course of an acute asthma episode.

One limitation of this study is the relatively small sample size, so the lack of association between asthma severity and *GSTP1* SNPs may be because of insufficient power. However, the sample size in this study is larger than those reported for other acute asthma studies (41, 42). In addition, we have previously published papers on other polymorphisms in asthma- and atopy-related genes in this cohort (21, 22, 43). Missing data including genotype data, asthma severity scores, SPT, and cotinine measurements on some of our subjects may also have limited the results of our study. SPT was only performed on those who consented to the procedure, and urine was collected only when full cooperation and participation was shown by the children. However, there were no differences in asthma severity or genotype frequencies for the subjects that completed SPT compared with those that did not consent to having SPT measured. Some of the questionnaire data on ETS exposure were incomplete possibly reflecting a negative bias in reporting by smoking parents. However, it is unlikely that the findings were affected, as the response rate was relatively high (78%) and the questionnaire data were consistent with levels of urinary cotinine. The significant associations found with the *GSTP1* SNPs may be because of linkage disequilibrium with other genes such as Clara cell secretory protein (CC16) and high-affinity IgE receptor (FC ϵ RI β) also located on chromosome 11q13 and previously associated with asthma (44–46). However, neither of these genes on 11q13 is as likely to interact with ETS as *GSTP1*.

In conclusion, the results of this study showed that *GSTP1* SNPs were associated with atopy in children with acute asthma and a significant gene/environment interaction between *GSTP1* polymorphisms and ETS exposure was demonstrated. The findings of this study further

emphasise the detrimental effects of passive smoke exposure on childrens' health and the need for continuing education to reduce the impact of tobacco smoke on the most susceptible individuals.

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DECLARATION OF INTEREST

The authors declare no competing interest.

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