

Glutathione-S-transferase (GST) P1, *GSTM1*, exercise, ozone and asthma incidence in school children

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ABSTRACT

Background: Because asthma has been associated with exercise and ozone exposure, an association likely mediated by oxidative stress, we hypothesised that glutathione-S-transferase (*GST*)P1, *GSTM1*, exercise and ozone exposure have interrelated effects on the pathogenesis of asthma.

Methods: Associations of the well characterised null variant of *GSTM1* and four single nucleotide polymorphisms (SNPs) that characterised common variation in the *GSTP1* locus with new onset asthma in a cohort of 1610 school children were examined. Children's exercise and ozone exposure were classified using participation in team sports and community annual average ozone levels, respectively.

Results: A two SNP model involving putatively functional variants (rs6591255, rs1695 (Ile105Val)) best captured the association between *GSTP1* and asthma. The risk of asthma was lower for those with the Val allele of Ile105Val (hazard ratio (HR) 0.60, 95% CI 0.4 to 0.8) and higher for the variant allele of rs6591255 (HR 1.40, 95% CI 1.1 to 1.9). The risk of asthma increased with level of exercise among *ile*¹⁰⁵ homozygotes but not among those with at least one val¹⁰⁵ allele (interaction p value = 0.02). The risk was highest among *ile*¹⁰⁵ homozygotes who participated in ≥3 sports in the high ozone communities (HR 6.15, 95% CI 2.2 to 7.4). *GSTM1* null was independently associated with an increased risk of asthma and showed little variation with air pollution or *GSTP1* genotype. These results were consistent in two independent fourth grade cohorts recruited in 1993 and 1996.

Conclusion: Children who inherit a val¹⁰⁵ variant allele may be protected from the increased risk of asthma associated with exercise, especially in high ozone communities. *GSTM1* null genotype was associated with an increased risk of asthma.

Airway oxidative stress is a cardinal feature and an important pathway in the pathogenesis of asthma.¹ Although a growing body of evidence supports a joint role for air pollutants and variants in genes involved in oxidant defences in asthma pathogenesis,² there have been few prospective studies of new onset asthma that have collected both the genetic and environmental data needed to address these determinants with adequate power. We addressed this gap by using data from the Children's Health Study (CHS),^{3,4} a longitudinal study of children's respiratory health. We have previously reported that children who exercise more in communities with high ozone levels are at increased risk of developing asthma.⁵ We have

now investigated the impact of variants of oxidant defence genes in the context of these environmental and behavioural factors.

The lung has multiple antioxidative defences, including the superfamily of glutathione S-transferases (GSTs)⁶ that are essential for glutathione homeostasis⁷ and cytoprotection from the byproducts of oxidative stress.⁸ The GST superfamily includes a number of subclasses including *GSTP1* and *GSTM1* which are expressed in the lungs⁶ and have been implicated in the pathogenesis of asthma.⁹ The deletion allele of *GSTM1* (null genotype) has been associated with an increased risk of asthma and lower lung function.^{10,11} A functional sequence variant in *GSTP1* at codon 105 (Ile105Val- rs1695)^{12,13} has been associated with asthma in some^{11,14–19} but not all studies.^{20–22} This variant has been reported to be both protective^{14–16,19} and a risk factor^{11,17,18,23} for asthma. The inconsistency may have several explanations, including differences in asthma pathogenesis in young children and adults, as well as the effects of other common variants in *GSTP1* coding and promoter regions or other GSTs, such as *GSTM1*.^{17,18}

Environmental factors such as ambient ozone are also determinants of oxidative stress in the lungs.²⁴ Because elevated ozone exposure is associated with an increased risk of new onset asthma in exercising children⁵ and GSTs are involved in the oxidative pathway, we hypothesised that the effects of genetic variants of GSTs on asthma are modified by children's participation in team sports in high and low ozone communities.

To investigate the joint effect of variants in GSTs (four single nucleotide polymorphisms (SNPs) of *GSTP1* and *GSTM1* null) and exercise on new onset asthma, we examined health, genetic and exposure data collected from Hispanic and non-Hispanic white children participating in the CHS. Our findings highlight the potential importance of genetic susceptibility, environmental exposure and behavioural factors in the aetiology of asthma.

METHODS

Subjects and materials

Children in this analysis participated in CHS cohorts that have been described previously.^{3,4} Each participant completed an annual self-administered questionnaire regarding sociodemographic, health and household characteristics and a brief exposure history to relevant asthma risk factors (further details of adjustment covariates are presented in the online supplement). This analysis

Table 1 Selected characteristics of the study participants at study entry

	No (%) (n = 1610)
Hispanic	546 (33.9)
Age groups (years)	
7–9	861 (53.5)
10–11	345 (21.4)
>11	404 (25.1)
Sex	
Girls	893 (55.5)
Boys	717 (44.5)
Overweight	378 (23.5)
Parental history of asthma	221 (13.7)
History of atopy	384 (25.3)
Household second hand smoke exposure	235 (14.6)
In utero exposure to maternal smoking	220 (13.7)
Current maternal smoking	125 (7.8)
Pests of any kind	1213 (75.3)
Pets at home	1323 (82.2)
No of team sports participation	
0	648 (41.0)
1	536 (33.9)
2	264 (16.7)
≥3	132 (8.3)
Children with health insurance	1323 (82.2)
Annual family income (US dollars)*	
≤ 14 999	177 (11.0)
15 000–49 999	586 (36.4)
≥ 50 000	609 (37.8)
Highest parental education level*	
Less than high school	211 (13.1)
College	1138 (70.7)
Graduate	227 (14.1)

*The numbers do not add up to the total due to missing data.

included 1610 children of Hispanic or non-Hispanic ethnicity who had no history of asthma or wheezing symptoms at study entry and who had GST genotype data (see online supplement).

New onset asthma

Children with no prior history of asthma or wheeze at study entry who subsequently reported physician diagnosed asthma at annual follow-up were classified as having new onset asthma.

Ambient air pollution

Outdoor levels of ozone (O₃), nitrogen dioxide (NO₂), particulate matter with an aerodynamic diameter of less than 10 μm (PM₁₀) or 2.5 μm (PM_{2.5}), acid vapour and elemental and organic carbon were measured continuously during the period of follow-up in each of the 12 communities at centrally located

Table 2 Pairwise measures of linkage disequilibrium (r² and D') for the *GSTP1* htSNPs

D'\r ²	SNP1(T) (rs6591255)	SNP2(C) (rs4147581)	SNP3(A) (rs1695)	SNP4(C) (rs749174)
SNP1(T)	–	0.49	0.36	0.63
SNP2(C)	–0.99	–	0.43	0.32
SNP3(105A)	0.60	–0.927	–	0.58
SNP4(C)	0.94	–0.95	0.91	–

r², upper triangle area (bold typeface). D', lower triangle area.
GST, glutathione-S-transferase; SNP, single nucleotide polymorphism.

air monitoring sites. Average pollutant levels varied substantially across these communities; however, no substantial year to year variation was observed within communities.²⁵ To assess the effects of ambient ozone on genetic susceptibility, we classified the community level exposure as low (range 28.6–45.5 ppb) or high (range 46.5–64.9 ppb) with six communities in each group, as described previously (see online supplement).⁵ Children's variation in dose of outdoor pollution was categorised using questionnaire responses about participation in team sports and pollution levels. Children were grouped into those who played no, one and two or more team sports.

Other oxidative stress genes

Based on previous findings in this cohort, we also considered the effect of a (GT)_n tandem repeat of the 5 flanking region of *HMOX1* and a functional polymorphism of *CAT* (CAT-262C>T: rs1001179) on *GSTP1* and *GSTM1* associations in our final models.²⁶ As described previously, we categorised *HMOX1* alleles based on functional studies with 23 or less (GT)_n repeats as “short” (S).²⁶

Identifying haplotype tagging SNPs (htSNPs) of *GSTP1*

Ethnic specific haplotype block structure for *GSTP1* was identified by using Haploview V.3.3, which was based on genotype data for 71 Hispanic and 71 non-Hispanic whites from the well characterised Multi-Ethnic Cohort (see online supplement).²⁷ The four htSNPs (rs6591255 (SNP1), rs4147581 (SNP2), Ile105Val (SNP3) and rs749174 (SNP4)) accounted for >80% of the haplotype variation in the *GSTP1* locus (see fig E2 online). Haplotype frequencies of unphased *GSTP1* SNPs were estimated separately for Hispanic and non-Hispanic white subjects using TagSNPs (the program is available at <http://www.hsc.usc.edu/~stram/>).

Statistical methods

We fitted Cox proportional hazards models with sex and age specific (integer age at study entry) baseline hazards to investigate the association between the genetic variants and new onset asthma. An additive genetic model was used for each SNP. Because the associations of the variants were consistent in both Hispanic and non-Hispanic groups (see table E2 online) and the lowest p value for tests for ethnic heterogeneity was >0.15, the results are presented for the combined population. All models were adjusted for community and ethnicity.

To capture the joint effect of the four *GSTP1* SNPs, we considered three different models: all four SNPs in a single model (joint model), two functional SNPs (functional model) and a haplotype model. The functional model included *Ile105Val* and *rs6591255* based on their functional effects.^{12 13 28} We selected the best model based on the likelihood ratio tests (comparing “base” (without any genetic information) and “full” models (genetic information added to the base model)). Joint effect models for *GSTP1* and *GSTM1* were estimated similarly.

Additional covariates were considered for inclusion in the final model based on whether their inclusion changed the effect estimates of the genetic variants by more than 10%. Possible heterogeneity of association by participation in team sports or *GSTM1* status was assessed by comparing appropriate models with and without appropriate interaction terms. In the presence of statistically significant heterogeneity among subgroups, a stratified analysis was performed.

Table 3 Associations between *GSTP1* and *GSTM1* genotypes and haplotypes with new onset asthma

Model	Genotype and haplotype [†]	No (with asthma/without asthma)	HR (95% CI)*	p Values	
GSTP1	Joint	SNP1(A)	150/1474	1.23 (0.8 to 1.9)	0.03
		SNP2(G)		0.85 (0.6 to 1.2)	
		SNP3(G)		0.54 (0.3 to 0.9) [¶]	
		SNP4(T)		1.10 (0.6 to 1.9)	
	Functional	SNP1(A)	150/1493	1.40 (1.1 to 1.9) [¶]	0.007
		SNP3(G)		0.60 (0.4 to 0.8)**	
	Haplotype [‡]	TCAC	150/1474	1 (Ref)	0.14
		ACAC		1.33 (0.8 to 2.2)	
		TGAC		0.90 (0.6 to 1.4)	
		TCGC		0.64 (0.3 to 1.2)	
ACGT			0.76 (0.5 to 1.2)		
Other			0.97 (0.5 to 1.9)		
GSTM1	Null	152/1472	1.61 (1.2 to 2.2)**	0.004	
Joint GSTM1 and GSTP1 model					
<i>GSTP1</i>	SNP1(A)	144/1453	1.45 (1.1 to 1.9) [¶]	0.007 ^{††}	
	SNP3(G)		0.61 (0.4 to 0.8)**		
<i>GSTM1</i>	Null		1.52 (1.1 to 2.1) [¶]		

*Hazard ratio (HR) and 95% CI adjusted for community of residence and ethnicity. Additive genetic coding was used for all SNPs.

[†]The letter in parentheses represents the variant allele.

[‡]The sequence of the GSTP1 haplotype is SNP1-SNP2-SNP3-SNP4. Haplotypes with less than 5% frequency in both the ethnic groups were summarised as "Other".

[§]The p value is derived from the LRT. The LRT was calculated by comparing models with and without the corresponding genetic data.

[¶]p value <0.05; **p value <0.001.

^{††}This LRT P value was calculated by comparing a model with SNP1 and SNP3 of *GSTP1* and *GSTM1* "null" to one with only *GSTM1* "null". All models were adjusted for ethnicity and community of residence.

GST, glutathione-S-transferase; LRT, likelihood ratio test; SNP, single nucleotide polymorphism.

To assess the effect of ambient ozone on the relationship between genetic variation in *GSTP1* and new onset asthma and account for the clustering effect of children in communities, we fitted a Cox proportional hazards model to these time dependent data with random effects for the communities (see online supplement).²⁹ In these models, the community specific average ozone levels were fitted as continuous variables along with the appropriate interaction terms for participation in team sports, genotype and ozone level. To allow more easily interpretable estimates of the effect of participation of team sports and genotype on risk of new onset asthma, we also performed stratified analysis using Cox proportional hazards models in communities with higher/lower levels of ambient ozone.

All analyses, except the hierarchical two stage model, were conducted using SAS software V.9.1 (SAS Institute, North Carolina, USA). The hierarchical two stage model was fitted using the R-program's COXP procedure.²⁹ All hypothesis testing was conducted assuming a 0.05 significance level and a two sided alternative hypothesis.

RESULTS

Participant characteristics

The majority of the children included in this analysis were less than 10 years of age and non-Hispanic white (table 1). Distributions for some sociodemographic and environmental factors at study entry differed between Hispanic and non-Hispanic white children (see online supplement and table E3 online). However, the crude incidence rate of asthma did not differ between non-Hispanic white children (IR = 16.1/1000 person-year) and Hispanic children (IR = 16.6/1000 person-year). There were some modest differences in baseline

characteristics between children with and without genetic data (see table E4 online).

Allele frequencies

All *GSTP1* SNPs were in Hardy-Weinberg equilibrium in both ethnic groups. The distribution of the *GSTP1* alleles was similar for both ethnic groups except for SNP2 (see table E5 in the online supplement). Except for SNP1 and SNP3 (Ile105Val), all SNPs showed moderate to high degrees of linkage disequilibrium -with each other (table 2 and see online supplement results).

Associations of *GSTP1* and *GSTM1* with new onset asthma

The two functional variants of *GSTP1* (SNP1 and SNP3) best defined the association between *GSTP1* and new onset asthma (table 3). In this "functional" SNP model, the risk of asthma decreased by 40% (hazard ratio (HR) 0.60, 95% CI 0.4 to 0.8) among children with SNP3 "G" (*val*¹⁰⁵) and increased by 40% among children with SNP1 variant allele (A) (HR 1.40, 95% CI 1.1 to 1.9). This pattern is consistent with the haplotype analysis where reduced risk of asthma was observed for TCGC and ACGT (HR 0.64 and 0.76) and increased risk of asthma for ACAC (HR 1.33) compared with the TCAC haplotype.

Children who had the *GSTM1* null genotype were at 1.6-fold (95% CI 1.2 to 2.2) increased risk of developing new onset asthma compared with those without the null genotype (table 3). The point estimates for asthma risk for *GSTP1* SNPs were similar among children with and without the *GSTM1* null genotype (see table E7 online).

These associations were not substantially affected by adjustment for possible confounders (see online table E8 model 1) and ambient pollutants (see online table E8: models 2 and 3). To

Table 4 Associations of *GSTP1* genotypes with new onset asthma, stratified by participation in team sports*

No of sports played†	GSTP1 genotype		No (with asthma/without asthma)	HR (95% CI)	Interaction p value§
	No (with asthma/without asthma)	HR (95% CI)			
	SNP3 (<i>Ile105Val</i>)				
	<i>Ile/Ile</i>		<i>Ile/Val or Val/Val</i>		
None	21/226	1 (Ref)	36/365	0.90 (0.5 to 1.6)	0.02
1–2	37/286	1.42 (0.8 to 2.5)	33/444	0.73 (0.4 to 1.3)	
>2	10/46	2.66 (1.2 to 5.9)¶	5/71	0.68 (0.2 to 1.9)	
p Value‡		0.03		0.41	
	SNP1 (rs6591255 A>T)				
	AA		AT/TT		
None	20/254	1 (Ref)	37/337	1.92 (1.1 to 3.5)¶	0.08
1–2	26/282	1.17 (0.6 to 2.1)	44/448	1.87 (1.0 to 3.4)	
>2	9/48	2.49 (1.1 to 5.7)¶	6/69	1.84 (0.7 to 4.8)	
p Value‡		0.07		0.81	

*All models are adjusted for ethnicity, community of residence, *GSTM1* and SNP1/SNP3.

†Children were categorised by the number of team sports played.

‡A test for a trend was performed using the likelihood ratio test comparing full (dummy variables for 1, 2 and 2+ sports participation) to base models stratified by *Ile105Val* status.

§Interaction p value was calculated from likelihood ratio test comparing full (main effect of sports, SNP and interaction term for sport category and SNP) to the base (main effect of sports and SNP only) models.

¶p value<0.05.

GST, glutathione-S-transferase; SNP, single nucleotide polymorphism.

assess the potential effects of asthma misclassification, we restricted the case definition of new onset asthma to those using inhaled medication and found no substantial differences in the magnitude of risk associated with the genetic variants (see online table E8 model 4). In both ethnic specific (data not shown) and combined analyses (see online table E8 model 5), the estimates of risk for the GST polymorphisms remained essentially unchanged after adjustment for *HMOX1* and *CAT* genotypes.

GSTP1, team sports and ozone

The effect of SNP3 (*Ile105Val*) on new onset asthma differed by participation in team sports (table 4, interaction p value = 0.02), after adjustment for SNP1 and *GSTM1* status. Children without a protective *val¹⁰⁵* allele showed an increased risk for new onset asthma with increasing participation in team sports (p trend = 0.03). In contrast, the number of team sports was unrelated to asthma risk for children with a *val¹⁰⁵* allele (table 4, p trend = 0.41). An increased risk of asthma associated with the variant allele “A” of SNP1 was observed among children, independent of participation in team sports. Among children without the variant allele of SNP1, an increased risk for asthma

was observed for those who played three or more team sports (HR 2.49, 95% CI 1.1 to 5.7). Unlike the *GSTP1* SNPs, the association between *GSTM1* and new onset asthma did not vary by team sports participation.

Although sample size was limited, we investigated the joint effects of *GSTP1* genotype and sport participation in high and low ozone communities (table 5). A sixfold elevated risk of asthma (HR 6.15, 95% CI 2.2 to 7.4) in children who played >2 team sports and were *Ile¹⁰⁵* homozygotes was observed in the high ozone communities.

DISCUSSION

We observed that functional variants of both *GSTP1* and *GSTM1* were associated with new onset asthma during adolescence. In addition to the well studied *val¹⁰⁵* variant, we found that a potentially functional SNP of *GSTP1*, located in the promoter region, was also associated with asthma and these two functional SNPs best explained the association between the *GSTP1* gene and new onset asthma during adolescence. Furthermore, the associated risk of new onset asthma among participants playing multiple team sports⁵ also depended upon a child's *Ile105Val* genotype. Playing multiple team sports was

Table 5 Joint associations of *GSTP1* *Ile105Val** genotype and participation in team sports on the risk of new onset asthma among CHS participants residing in high and low ozone communities

No of sports played	Low ozone communities (n = 6)†				High ozone communities (n = 6)†			
	<i>Ile/Ile</i>		<i>Ile/Val or Val/Val</i>		<i>Ile/Ile</i>		<i>Ile/Val or Val/Val</i>	
	No‡	HR (95% CI)	No	HR (95% CI)	No	HR (95% CI)	No	HR (95% CI)
None	10/93	1 (Ref)	20/181	0.86 (0.4 to 1.9)	11/133	1 (Ref)	16/184	0.98 (0.4 to 2.3)
1–2	21/142	1.37 (0.6 to 3.0)	19/205	0.69 (0.3 to 1.6)	16/144	1.37 (0.6 to 3.1)	14/239	0.80 (0.3 to 1.9)
>2	3/28	1.06 (0.3 to 4.0)	2/38	0.50 (0.1 to 2.4)	7/18	6.15 (2.2 to 7.4)§	3/33	1.06 (0.3 to 4.1)

*All models are adjusted for ethnicity, community of residence, *GSTM1* and SNP1. The effect of SNP3 was treated as “additive”.

†High and low ozone communities were defined according to average 10:00 to 06:00 ozone levels. The ozone levels (10:00 to 06:00) ranged from 46.5 to 64.9 ppb in the “high” ozone communities (mean 38.4 ppb) and from 28.6 to 45.5 ppb in the “low” ozone communities (mean 55.2 ppb).

‡Children with and without asthma.

§p value<0.05.

GST, glutathione-S-transferase; SNP, single nucleotide polymorphism.

associated with an increased risk of asthma only among children who were homozygous for *Ile¹⁰⁵*, and the risk in this group was highest for those living in the high ozone communities. These observations reflect the complex interrelationship of asthma risk with increasing doses of ozone (resulting from increasing ventilation associated with vigorous exercise) and antioxidant defences.

Our findings are supported by the growing body of evidence for gene–pollution interaction in the pathogenesis of asthma.² *GSTM1* and *GSTP1* are two important phase II enzymes that protect the airways from oxidative stress.⁸ Oxidative stress has been shown to be central to asthma pathogenesis.³⁰ Therefore, genetic variants that regulate the availability and functionality of the GST enzymes are expected to determine the dose of oxidative effects in the airway and associated injury. Individuals with the *GSTM1* null genotype completely lack *GSTM1* enzyme activity and their increased susceptibility to asthma has been previously reported.^{11–31} Furthermore, in the CHS we have observed that the null genotype is associated with reduced lung function growth during adolescence.¹⁰ In cross sectional analysis of childhood asthma, we observed that in utero exposure to maternal smoking was associated with increased risk of asthma/wheeze only among carriers of the *GSTM1* null genotype.³² In this current prospective analysis, we found that *GSTM1* is a determinant of asthma risk during adolescence, irrespective of in utero exposure to maternal smoking. Thus the detrimental effect of the *GSTM1* null genotype on respiratory health is apparent from early childhood into adolescence.

In cross sectional analysis of this cohort,²³ we also observed that a joint effects model of *SNP1* and *SNP3* best explained the association between *GSTP1* and asthma during early childhood. The variant “A” allele of *SNP1* corresponds to a haplotype of the promoter region that is associated with reduced *GSTP1* activity²⁸ and thus the association with increased risk of asthma is biologically plausible. The observed association is consistent with our earlier observation of associations between *SNP1* and early and late onset asthma.²³

Our finding that the *val¹⁰⁵* confers protection against new onset asthma is consistent with most of the previous publications^{14–16 19 33 34}; however, increased risk^{11 17 18 23} and no association^{20–22 31 33} have also been reported (see fig E3 online). We have previously reported that *val¹⁰⁵* is associated with an increased risk of early onset asthma (diagnosis by 3 years of age) but not with asthma onset after 3 years of age.²³ Differences in the aetiology of asthma by age of onset³⁵ and atopic status¹⁸ have been postulated as a possible explanation for the observed discrepancies across studies and both of these factors are possible explanations for the observed differences in asthma–*val¹⁰⁵* association in CHS. Although atopy is one aetiological factor whose contribution to asthma varies by age, we did not observe a difference in effect of the *Ile105Val* variant by atopy status, suggesting that the mechanism for the hypothesised age dependent pattern of risk associated with the *val¹⁰⁵* variant is likely to be more complex. Further research is needed to characterise the age dependent effects of the *val¹⁰⁵* on the development of asthma and to determine the mechanism for any age dependent differences in risk.

We previously reported that the risk of new onset asthma was associated with heavy outdoor exercise, especially in high ozone communities.⁵ The plausibility of a causal association is further strengthened by the observation that the risk of participation in team sports was related to increased genetic susceptibility to oxidative stress. The observed sixfold increased risk of asthma for children who were homozygous for *Ile¹⁰⁵*,

participated in ≥ 3 team sports and lived in high ozone communities demonstrates the potential importance of a combination of genetic variability, environmental ozone exposure and behaviour on asthma risk. A modifying role of air pollution on the association between *Ile105Val* and asthma has also been reported from a study involving South Korean children.¹⁵

The strengths of our study originate from the prospective assessment of asthma and air pollution among a large population based sample of children. Potential limitations include selection bias, confounding by admixture and outcome misclassification. We considered the potential effects of selection bias, as genetic data were available from about two-thirds of the initial cohort. Demographic and socioeconomic factors, exposure to maternal smoking during pregnancy and second hand smoke after birth, and household factors showed modest differences between participants and non-participants (see table E4 of the online supplement). Adjustment for these factors did not explain our results (see table E8 model 1 online), indicating that selection bias is unlikely to explain our results.

Population admixture is also an unlikely explanation for our findings as the incidence rate of new onset asthma did not vary by ethnicity and the associations of the SNPs were similar in ethnic specific analysis (see table E2 online). In both Hispanic and non-Hispanic whites, the distribution (see table E5 online), linkage disequilibrium structure (see table E6 online) and association with asthma (see table E2 online) of the *GSTP1* SNPs were largely consistent. The *GSTM1* “null” genotype and both *GSTP1* SNPs in the “functional” model showed a similar pattern (*SNP1* increased the risk and *SNP3* decreased the risk of asthma) in both ethnic groups (see table E2 online). In the “joint effect” model, the HRs for all four *GSTP1* SNPs showed little differences between ethnic groups except for *SNP1*. However, the CIs were wide and the estimates are not statistically significantly different (interaction p value > 0.15) and should not be over interpreted. We used joint models of the identified functional *GSTP1* model and *GSTM1* throughout our analysis. Furthermore, the four SNPs of *GSTP1* captured >80% of the variability of the *GSTP1* locus.

Our findings are also unlikely to be due to asthma misclassification as we excluded children with any history of wheeze/asthma at study entry, and the observed risk estimates were largely unchanged when we restricted our analysis to asthma cases with inhaler use (see discussion in the online supplement). Another potential concern is that the results were a result of chance. However, both *SNP1* and *SNP3* were identified a priori for the joint genetic models, based on the known functional effect of these SNPs, as well as our previous findings from cross sectional analysis of childhood asthma.²³ The GST associations were independent of *HMOX1* and *CAT* (see table E8 model 5 online) that are involved in the antioxidative pathway and have been shown to be associated with new onset asthma. Furthermore, to confirm our finding, we performed separate analyses for the two fourth grade cohorts in our study population independently recruited in 1993 and 1996 and the seventh and tenth grade cohort (see table E9 online). The genetic effect estimates for each of these cohorts were consistent across these groups.

We conclude that common functional variants of *GSTP1* and *GSTM1* null genotype modulate the risk of new onset asthma during adolescence. The role of regulation of expression and variation of the function of *GSTP1* in asthma pathogenesis needs further investigation, especially in the context of oxidative stress and age of diagnosis. Furthermore, the *GSTP1*–*Ile105Val*

may also influence asthma susceptibility for adolescents who participate in sports in communities with elevated ozone levels. These findings suggest that the regulatory policy for ozone level may need to consider *GSTP1* genotypes to set standards that protect the most vulnerable members of our societies.

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