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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 hypothesized that *GSTP1*, *GSTM1*, exercise and ozone exposure have inter-related effects on asthma pathogenesis.

Methods—We examined associations of the well characterized null variant of *GSTM1* and four SNPs that characterized common variation in *GSTP1* with new-onset asthma in a cohort of 1,610 school children. Children's exercise and ozone-exposure status were classified using participation in team sports and community-specific ozone levels, respectively.

Results—A two SNP model (rs6591255, rs1695 [Ile105Val]) best captured the association between *GSTP1* and asthma. Compared to children with common alleles for both the SNPs, the risk of asthma was lower for those with the *Val* allele of Ile105Val (HR 0.60, 95% CI 0.4, 0.8) and higher for the variant allele of rs6591255 (HR 1.40, 95%CI 1.1–1.9). Asthma risk increased with level of exercise among ile^{105} homozygotes but not among those with at least one val^{105} allele (interaction p-value=0.02). Risk was highest among ile^{105} homozygotes who participated in ≥3 sports in the high-ozone communities (HR: 6.15, 95%CI: 2.2–7.4). *GSTM1* null was independently associated with asthma and showed little variation with air pollution or *GSTP1* genotype. These results were consistent in two independent fourth-grade cohorts in the study population recruited in 1993 and 1996.

Conclusion—Children who inherit a val^{105} 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 increased risk of asthma.

Keywords

Oxidative stress; Candidate gene; Asthma genetics; Gene-environmental interaction; Air pollution

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COMPETING INTERESTS
None

INTRODUCTION

Asthma is a complex multifactorial disease with airway oxidative stress being a cardinal feature and an important pathway in asthma pathogenesis.[1] Although a growing body of evidence supports a joint effect of air pollutants and genetic variants in asthma pathogenesis,[2] there have been few large prospective studies of new onset asthma that have both genetic and environmental data needed to address these etiologic determinants with adequate power. To address this gap, we examined data on key genetic variants among children who participated in 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] In the present study, we wanted to further explore the impact of variants of oxidant defense genes in the context of these environmental and behavioral factors.

The choice of glutathione *S*-transferases (GST) as key oxidant defense genes was based on biological knowledge and prior study results. The lung has multiple anti-oxidative defenses including the GSTs. [6] The GSTs, a supergene family of phase II conjugating enzymes, are essential for glutathione homeostasis [7] and cytoprotection from the byproducts of oxidative stress.[8] Maintenance of glutathione homeostasis by GSTs is a determinant of cellular response to oxidative stress. [9,10] The GST superfamily includes a number of sub-classes including GSTP1 and GSTM1 which are expressed in the lungs [6] and have been implicated in asthma pathogenesis, oxidant defenses, xenobiotic metabolism and detoxification of hydroperoxides. [11] The gene deletions of *GSTM 1* [null-genotype] has been reported to be associated with increased risk of asthma and lower lung function.[12,13] A functional sequence variant in *GSTP1* at codon 105 (Ile105Val- rs1695)[14,15] has been associated with asthma in some [13,16–21], but not all studies. [22–24] This variant has been reported to be both protective [16–18,21] and a risk factor [13,19,20,25] in asthma. The inconsistency in results 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*. [19,20]

In addition to genetic variants, environmental factors such as ambient ozone are also determinants of oxidative stress in the lungs. [26] Because elevated ozone exposure is associated with increased risk of new-onset asthma in exercising children [5] and GSTs are involved in the oxidative pathway, we hypothesized 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 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 behavioral factors in asthma etiology.

METHODS

Subjects and materials

Children in this analysis participated in the CHS cohorts that have been described previously. [3,4] Briefly, fourth-, seventh- and tenth-grade children were enrolled into the study in each of the 12 southern California communities, selected primarily on the basis of different ambient pollution levels. Study subjects were followed annually until high school graduation. Each participant completed an annual self-administered questionnaire regarding socio-demographic, health and household characteristics and a brief exposure history to relevant asthma risk factors.

This analysis included 1,610 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. (Details of the study design are presented in the 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. Children were also interviewed annually about the use of inhaled medications. We defined a restricted group of new-onset cases with recent inhaler use for sensitivity analyses.

Ambient Air Pollution

Air quality monitoring stations were established in each of the 12 study communities beginning in 1994. Each station measured average hourly levels of ozone (O_3), nitrogen dioxide (NO_2), particulate matter (PM_{10}: with an aerodynamic diameter of less than 10 μm and two-week integrated $PM_{2.5}$: with an aerodynamic diameter of less than 2.5 μm) and two-week integrated levels of acid vapor (both weak [formic and acetic] and strong [hydrochloride and nitric]). For ozone, we computed the annual average of the ozone levels obtained from 10 a.m. to 6 p.m. (the 8-hour daytime average) in each community over the period of follow-up. To assess the effects of ambient ozone on genetic susceptibility, we classified the community-level exposure as low (range: 28.6–45.5ppb) or high (range: 46.5–4.9) with six communities in each group, as described previously. [5]

Other Covariates

Personal information such as ethnicity, birth weight, gestational age, maternal smoking during pregnancy, history of allergy and family history of asthma was available from the questionnaire data. We categorized BMI into age- and sex-specific percentiles based on the Centers for Disease Control (CDC) BMI growth charts using one-month age intervals (http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/sas.htm).

Participants with BMI at or above the 85th percentile were classified as overweight. These personal characteristics and household and indoor exposures (pets, pests, humidifier use and household smoking) were considered as potential effect modifiers as well as confounders in this analysis. (for details see Online Supplement).

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 the *GSTP1* and *GSTM1* association in our final models. [27] As described in our earlier publication, we categorized *HMOX1* alleles with 23 or less $(GT)_n$ repeats as ‘short’ (S). [27]

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 characterized Multi-Ethnic Cohort [28] (details are presented in the Online Supplement). Two haplotype blocks with substantial inter-block correlations (0.92) were identified for Hispanic whites (Figure E1A and E1B), and two htSNPs were selected for each of the blocks (rs6591255 [SNP1] and rs4147581 [SNP2] for the first block with R_h^2=0.87; Ile105Val [SNP3] and rs749174 [SNP4] for the second block with R_h^2=0.91). The four htSNPs defined a single haplotype block among non-Hispanic whites and accounted for 83% of the haplotype variation in the *GSTP1* locus (Figure E2). 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-rcf.usc.edu/~stram/tagSNPs.html).

Genotyping

Buccal cells were collected using standard protocols, and genomic DNA was isolated using the PUREGENE™ DNA isolation Kit (Gentra Systems, Minneapolis, MN). Single nucleotide polymorphisms (SNP) of *GSTP1* were identified using allele-specific MGB probes on an ABI PRISM™ 7700 Sequence Detector (Applied Biosystems, Foster City, CA). The deletion polymorphism of *GSTM1* was determined using TaqMan methodology as described previously. [12] We repeated 10% of the samples in each batch for inter- and intra-plate validity. In the presence of any discrepancy, the entire batch was re-genotyped (for details see Online Supplement). Genotyping of *HMOX1* and CAT-262C>T (rs1001179) has been described previously.[27]

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 polymorphisms and new onset asthma. The additive genetic model was considered for all the *GSTP1* SNPs (Coding scheme: Wild type homozygous=0, heterozygous=1 and variant homozygous=2). We initially performed ethnicity-specific analysis and formally tested for heterogeneity by ethnicity. Because the associations of the variants were consistent in both Hispanic and non-Hispanic groups (see online Table E2) and the lowest p-value for test 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 known functional effects. [14,15,29] We selected the best model based on the likelihood ratio tests (LRT 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 proportional hazard model based on whether their inclusion changed the effect estimate of the polymorphisms 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 interaction terms. In the presence of statistically significant heterogeneity among subgroups, a stratified analysis was performed. We conducted a sensitivity test to assess independent effect of *GSTP1* and *GSTM1* using models that included the functional polymorphisms of *HMOX1* and *CAT*, which have been shown to be associated with asthma risk. [27]

To assess whether the results could be replicated in independent groups of children, we performed stratified analyses for the two fourth-grade cohorts in the study population independently recruited in 1993 and 1996 and the seventh- and tenth-grade cohorts recruited in 1993. The fourth-grade cohorts had the same age structure and air pollution exposure profiles, having been recruited from the same study communities and schools.

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 Cox proportional hazards model to this time dependent data with random effects of the communities (details in Online Supplement). [30] 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, *GSTP1* variant and ozone level. From our initial analysis, we noted that the choice of fixed or random effect of the communities does not substantially affect the estimates for the gene effects (data not shown). 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 level of ambient ozone.

All analyses, except the hierarchical two-stage model, were conducted using SAS software version 9 (SAS Institute, Cary, NC). The hierarchical two-stage model was conducted in R-program using COXP procedure. [30] All hypothesis testing was conducted assuming a 0.05 significance level and a two-sided alternative hypothesis.

RESULTS

Participant Characteristics

The majority of the 1,610 children who were asthma- and wheeze-free at study entry and with genotype information for all the four *GSTP1* SNPs included in this analysis cohort, were under 10 years of age and non-Hispanic white (Table 1). Distributions for the socio-demographic and environmental factors at study entry differed between Hispanic and non-Hispanic white children (see Online Supplement Table E3). Compared to Hispanic children, non-Hispanic white children were more likely to have a history of atopy, exposure to second hand smoke (SHS) and *in* utero exposure to smoking, exposure to pets and pests, health insurance and parents with at least college education and annual family income more than $50,000. The Hispanic children were more likely to be overweight, boys and less than 10 years of age compared to the non-Hispanic whites.

The crude incidence rate of asthma did not differ between non-Hispanic white (IR=16.1/1000 person-year) and Hispanic (IR = 16.6/1000 person-year) children. A number of baseline characteristics showed some variation between those with and without genetic data (see Online Supplement Table E4); however, except for current maternal smoking and smokers at home, the magnitude of difference between the two groups was unlikely to have any substantial effect on the overall association. Furthermore, none of these factors were risk factors for newly diagnosed asthma in either of the populations. [27]

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 Online Supplement Table E5). The ‘C’ allele of SNP2 was the common allele in Hispanic whites (63%) but not in non-Hispanic whites (49%). The haplotype frequencies showed little variation between ethnic groups; except for TCGC which was common in Hispanics (22%) but not in non-Hispanic whites (1%). Except for SNP1 and SNP3 (Ile105Val), all SNPs showed moderate to high degrees of linkage disequilibrium (LD) with each other (Table 2). The overall LD pattern was similar in both ethnic groups except for SNP1 and SNP3 (see Online Supplement Table E6). Among non-Hispanic whites SNP1 and SNP3 were at high LD, but not among Hispanic whites. Approximately 48% of the children had the *GSTM1*-null genotype and the distribution was similar in Hispanic (45.3%) and non-Hispanic Whites (49.8%).

Association of *GSTP1* and *GSTM1* in 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 for asthma decreased by 40% (HR 0.60, 95% CI 0.4–0.8) among children with SNP3 ‘G’ (val^{105}) and

increased by 40% among children with SNP1 variant allele (A) (HR:1.40, 95% CI 1.1–1.9). This pattern is consistent with the haplotype analysis where reduced risk of asthma was observed for TC**G**C and AC**G**T (HR 0.64 and 0.76) and increased risk of asthma for **A**CAC (HR 1.33) compared to the **T**C**A**C haplotype (although the haplotype associations were not statistically significant). We also observed an association between *GSTM1* and new-onset asthma. Children who had the *GSTM1* null genotype were at 1.6 fold (95%CI: 1.2–2.2) increased risk of developing new-onset asthma compared to those without the null genotype. Both *GSTM1* and *GSTP1* contributed to the increased risk of new onset asthma (Table 3). The point estimates for asthma risk for *GSTP1* SNPs were similar among children with and without *GSTM1*-null genotype (see Online Supplement Table E7).

These associations were not substantially affected by adjustment for parents' educational attainment, family income, birth weight, gestational age, overweight, health insurance, parental history of asthma, pets, humidifier use, other household characteristics, or exposure to indoor-combustion sources including secondhand smoke (see Online Supplement Table E8 Model 1). The relationship between the variants and new-onset asthma varied little in areas of high and low ambient ozone or $PM_{2.5}$. (see Online Supplement Table E8: Models 2 and 3). To assess the potential effects of asthma misclassification, we conducted sensitivity analyses in which 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 polymorphisms (Online Supplement Table E8 Model 4). To assess the effect of *GSTP1* and *GSTM1* on asthma risk independent of *HMOX1* and *CAT*, we adjusted the joint-effect model of *GST* variants for the variants of *HMOX1* and *CAT* [27]. In both ethnic specific analysis (data not shown), as well as ethnic groups combined analysis (Online Supplement Table E8 Model 5), the estimates of the GST polymorphisms essentially remained unchanged in models that included *HMOX1* and *CAT* polymorphisms.

GSTP1, Team Sports and Ozone

We found evidence that the effect of SNP3 (*Ile105Val*) on new-onset asthma differed by the number of team sports in which children participated (Table 4, interaction p-value=0.02), after adjustment for SNP1 and *GSTM1* status. Children without a protective val^{105} 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 val^{105} allele (Table 4, p-trend=0.41). A marginally significant interaction between SNP1 and sports participation was observed (p-value= 0.08). The increased risk of asthma associated with the variant allele 'A' of SNP1, was observed among children, independent of team-sports participation. 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: 1.1–5.7). Unlike the *GSTP1* SNPs, the association between *GSTM1* and new onset asthma was independent of team sports participation (interaction p-value=0.23).

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 six-fold elevated risk of asthma (HR: 6.15, 95% CI: 2.2–7.4) in children who played >2 team sports and were Ile^{105} homozygotes was observed in the high-ozone communities (three-way [ozone-sports-SNP3] interaction p-value=0.10).

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 val^{105} 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 sports,[5] also depended upon a child's *Ile105Val* genotype. Playing multiple team sports was associated with increased risk of asthma only among children who were homozygous for Ile^{105} and the risk in this group was highest for those living in the high ozone communities. These observations reflect the complex inter-relationship of asthma risk with increasing doses of ozone (resulting from increasing ventilation associated with vigorous exercise) and anti-oxidant defenses (*GSTP1* genotype). We also observed that children lacking GSTM1 were at an increased risk for new onset asthma, but this risk did not vary by sports participation or air pollution exposure.

The relationship between oxidant-defense genes, oxidative stress, and asthma is supported by the growing body of evidence for gene-pollution interaction in asthma pathogenesis. [2] The 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. [31] 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 the GSTM1 enzyme activity and their increased susceptibility to asthma has been previously reported [13,32]. Furthermore, in the CHS we have observed that the null genotype is associated with reduced lung function growth during adolescence. [12] 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. [33] In this current prospective analysis, we observed that *GSTM1* is a determinant of asthma risk during adolescences, irrespective of *in utero* exposure to maternal smoking. The *GSTM1* null genotype appears to be associated with a detrimental effect of on respiratory health; however, its effects may vary with age.

In cross-sectional analysis of this cohort at study entry, [25] we also observed that a joint-effects model of the two functional *GSTP1* polymorphisms, SNP1 and SNP3 (*Ile105Val*), best explained the association between *GSTP1* and asthma during childhood. The variant 'A' allele of SNP1 corresponds to a haplotype of the promoter region that is associated with reduced GSTP1 activity, [29] thus the association with increased risk of asthma is biologically plausible. The observed association between new onset asthma and SNP1 is consistent with our earlier observation of associations between this polymorphism and early- and late-onset asthma. [25]

Our finding that the val^{105} confers protection against new-onset asthma is consistent with most of the previous publications [16–18,21,34,35]; however, increased risk [13,19,20,25] and no association [22–24,32,34] have also been reported (see Online Figure E3). We have previously reported that val^{105} 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. [25] Differences in the etiology of asthma by age of onset [36] and atopic status [20] have been postulated as possible explanation for the observed discrepancies across studies and both of these factors are possible explanation for the observed differences in asthma-val^{105} association in CHS. Although atopy is one etiologic 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 hypothesized age dependent pattern of risk associated with the val^{105} variant is likely to be more complex. Further research is needed to characterize the age-dependent effects of the val^{105} on the development of asthma and to determine the mechanism for any age-depended 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 six fold increased risk of asthma for children who were homozygous for Ile^{105}, 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 behavior on asthma risk. A similar modifying role of air pollution on the association between *Ile105Val* and asthma has been reported from a study involving South Korean fourth-ninth grade students.[17] In this study, compared to children with at least one val^{105} allele in the low pollution area, Ile^{105} homozygotes were at four and five fold increased risk of asthma in moderate and high pollution communities, respectively.

The strengths of our study originates from the prospective assessment of asthma and air pollution among school-age children with available genetic data. We had a large sample that allowed us to conduct ethnicity specific analyses. In both Hispanic and non-Hispanic whites, the distribution (Table E5), LD structure (Table E6) and association with asthma (Table E2) 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 risk and SNP3 decreased risk of asthma) in both ethnic groups (See Online Table E2) with modest differences in the magnitudes of the HRs. In the 'joint effect' model, the HRs for all the four *GSTP1* SNPs showed little differences between ethnic groups except for SNP1. Although the HR for SNP1 was 0.94 (95%CI: 0.4–2.0) in Hispanic whites and 1.36 (95%CI:0.8–2.4) in non-Hispanic whites, the CIs are wide and the estimates are not statistically significantly different (interaction p-value>0.15) and should not be over interpreted. We utilized joint model of the identified functional *GSTP1* model and *GSTM1* throughout our analysis. Furthermore, the four SNPs of *GSTP1* captured >80% variability of the *GSTP1* locus.

A potential limitation of our study is the accuracy of self-reported new-onset asthma assignment. However, our exclusion of any child with a history of wheezing at study entry from the statistical analyses is likely to have minimized misclassification. A recent study noted that children as young as 7 years of age can provide information regarding their asthma with acceptable level of validity and reliability. [37] Furthermore, unless the diagnostic accuracy varied by genotype, error in determining asthma status would likely attenuate the risk estimates, and therefore would not explain our observed associations. To further investigate the potential misclassification of new onset asthma, we conducted analyses restricting cases to those who recently used inhaled medication and found little change in the risk estimates (Table E8: Model 4). Because the associations with genotypes were apparent among the group of cases for which the diagnosis of asthma was most certain, our results are unlikely to be explained by misclassification of outcome. Some stratified analyses had small numbers of cases in categories and the associated estimates need to be interpreted in this context.

We considered the potential effects of selection bias, as genetic data was available from about two-thirds of the initial cohort. Demographic and socioeconomic factors, exposure to maternal smoking during pregnancy and secondhand smoke after birth, and household factors showed modest differences between participants and non-participants (Table E4 of the Online Supplement). Furthermore, these factors were not risk factors of asthma [27] and adjustment for these factors did not explain our results (Table E8: Model 1), indicating that selection bias is unlikely to explain our results. Population admixture is also an unlikely explanation of our findings as the incidence rate of new onset asthma did not vary by ethnicity and the main effects of the SNPs were similar in the ethnic-specific analysis (Table E2).

Another potential concern is that the results were due to chance, as we fitted different genetic models to identify the best model for subsequent analytic use. 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. [25] The *GST* associations were independent of polymorphisms in *HMOX1* and *CAT* (see Online Supplement Table E8 Model 5) that are involved in the anti-oxidative pathway and have been shown to be associated with new onset asthma. Furthermore, to validate our finding, we performed stratified analyses for the two fourth-grade cohorts in the study population independently recruited in 1993 and 1996 and the seventh- and tenth-grade cohort (Table E9). The genetic effect estimates for each of these cohorts were consistent.

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 regulatory policy for ozone level may need to consider *GSTP1* genotypes to set standard that protect the most vulnerable members of our societies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Selected characteristics of children at study entry

	N (1,610)	%
Hispanic	546	33.9
Age Group		
7–9 Years	861	53.5
10–11 Years	345	21.4
>11 Years	404	25.1
Sex		
Girls	893	55.5
Boys	717	44.5
Overweight	209	13.0
Parental History of Asthma	221	13.7
History of Atopy	384	25.3
Household Secondhand Smoke Exposure	235	14.6
In utero Exposure to Smoking	220	13.7
Current Maternal Smoking	125	7.8
Pests of any Kind	1,213	75.3
Pets at Home	1,323	82.2
# 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 (in 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	1,138	70.7
Graduate	227	14.1

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

Table 2

Pairwise measures of linkage disequilibrium (r^2 and D') for the *GSTP1* htSNPs in CHS participants.

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	---

r^2: upper triangle area (in gray background)
D': lower triangle area

Table 3

Association between GSTP1 and GST M1 genotype and newly diagnosed asthma.

Models		**Genotype and Haplotype**[†]	**N (With asthma/ Without asthma)**	**HR (95% CI)**[*]	**P-Value**[§]
GSTP1	Joint	SNP1(A)	150/	1.23 (0.8–1.9)	0.03
		SNP2(G)	1474	0.85 (0.6–1.2)	
		SNP3(G)		0.54 (0.3–0.9) [//]	
		SNP4(T)		1.10 (0.6–1.9)	
	Functional	SNP1(A)	150/	1.40 (1.1–1.9) [//]	0.007
		SNP3(G)	1493	0.60 (0.4–0.8) [**]	
	Haplotype[‡]	TCAC	150/	1 (Ref)	0.14
		ACAC	1474	1.33 (0.8–2.2)	
		TGAC		0.90 (0.6–1.4)	
		TCGC		0.64 (0.3–1.2)	
		ACGT		0.76 (0.5–1.2)	
		Other		0.97 (0.5–1.9)	
GSTM1		Null	152/	1.61 (1.2–2.2) [**]	0.004
			1472		
Joint	*GSTP1*	SNP1(A)	144/	1.45 (1.1–1.9) [//]	0.007[††]
GSTM1		SNP3(G)	1453	0.61 (0.4–0.8) [**]	
and GSTP1	*GSTM1*	Null		1.52 (1.1–2.1) [//]	
Model					

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

[†] The letter within the parenthesis 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 summarized as 'Other'.

[§] The P-value is derived from likelihood ratio test (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.

Table 4

Association of GSTP1 genotypes with new-onset asthma according to participation in team sports*

Number of Sports Played†	GSTP1 Genotype				Interaction P-value§
	N (with asthma/ without asthma)	HR (95%CI)	N (with asthma/ without asthma)	HR (95%CI)	
	SNP3 (*Ile105Val*)				
	Ile/Ile		*Ile/Val or Val/Val*		
None	21/226	1(Ref)	36/365	0.90(0.5–1.6)	0.02
1–2	37/286	1.42(0.8–2.5)	33/444	0.73(0.4–1.3)	
>2	10/46	2.66(1.2–5.9)**	5/71	0.68(0.2–1.9)	
P-value‡		0.03		0.41	
	SNP1 (rs6591255 A>T)				
	AA		AT/TT		
None	20/254	1(Ref)	37/337	1.92(1.1–3.5) //	0.08
1–2	26/282	1.17(0.6–2.1)	44/448	1.87(1.0–3.4)	
>2	9/48	2.49(1.1–5.7) //	6/69	1.84(0.7–4.8)	
P-value‡		0.07		0.81	

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

†Children were categorized 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 Ile1905Val 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

Table 5

Effect of Ile105Val* polymorphism and participation in team sports on the risk of new onset asthma in high- and low-ozone communities.

Number of Sports Played	Low-Ozone Communities(n=6)†				High-Ozone Communities(n=6)†			
	Ile/Ile		*Ile/Val or Val/Val*		*Ile/Ile*		*Ile/Val or Val/Val*	
	N‡	HR(95% CI)	N	HR(95% CI)	N	HR(95% CI)	N	HR(95% CI)
None	10/93	1(Ref)	20/181	0.86 (0.4–1.9)	11/133	1(Ref)	16/184	0.98 (0.4–2.3)
1–2	21/142	1.37 (0.6–3.0)	19/205	0.69 (0.3–1.6)	16/144	1.37 (0.6–3.1)	14/239	0.80 (0.3–1.9)
>2	3/28	1.06 (0.3–4.0)	2/38	0.50 (0.1–2.4)	7/18	6.15 (2.2–7.4) §	3/33	1.06 (0.3–4.1)

*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 a.m. to 6 p.m. ozone levels. The ozone levels (10 a.m. to 6 p.m.) ranged from 46.5–64.9 ppb in the 'high'-ozone communities (mean=38.4ppb) and 28.6–45.5 ppb in the 'low'-ozone communities (mean=55.2 ppb).

‡Children with and without asthma.

§p-value<0.05.