Associations of Tumor Necrosis Factor G-308A with Childhood Asthma and Wheezing

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***Rationale*: Tumor necrosis factor (TNF) mediates a spectrum of airway inflammatory responses, including those to air pollutants, and is an asthma candidate gene. One TNF promoter variant (G–308A) affects expression of TNF and has been associated with inflammatory diseases; however, studies of asthma have been inconsistent. Because ozone produces oxidative stress, increased airway TNF, and inflammation, the associations of the –308 TNF polymorphism with asthma may vary by ozone exposure and variants of oxidant defense genes glutathione-S-transferase (GST) M1 and GSTP1.**

***Objectives*: To investigate the association of TNF G–308A with asthma and wheezing and to determine whether these associations vary with ozone exposure and GSTM1 and GSTP1 genotype.**

***Methods*: We studied associations of TNF–308 genotype with lifetime and current wheezing and asthma among 3,699 children in the Children's Health Study. We examined differences in associations with community ozone and by GSTM1 null and GSTP1 105 Ile/Val (A105G) genotype.**

***Results*: Children with TNF–308 GG had decreased risk of asthma (odds ratio, 0.8; 95% confidence interval, 0.7–0.9) and lifetime wheezing (odds ratio, 0.8; 95% confidence interval, 0.7–0.9). The protective effects of GG genotype on wheezing outcomes were of greater magnitude in lower compared with higher ozone communities. These findings were replicated in the two cohorts of fourth-grade children recruited in 1993 and 1996. The reduction of the protective effect from the –308 GG genotype with higher ozone exposure was most marked in the GSTM1 null and GSTP1 Ile/Ile groups.**

***Conclusions*: The TNF–308 GG genotype may have a protective role in asthma pathogenesis, depending on airway oxidative stress levels.**

Keywords: child; genetic epidemiology; lung

Asthma is a common complex disease with multiple determinants that include genetic variation, environmental exposures, and gene–environment interactions (1–4). Tumor necrosis factor (TNF)-α has a recognized role in asthma pathophysiology, and variation in the locus that affects expression of this cytokine, especially in response to inhaled air pollutants, may contribute to asthma occurrence (5–11).

TNF-α plays a central role in the initiation of airway inflammation and the generation of airway hyperreactivity (12–14). Genetic variants may affect TNF levels in the airways and thereby modulate asthma and wheezing occurrence. Many studies of respiratory conditions have focused on the G–308A polymorphism in the TNF gene 5′ untranslated region because of its associations with inflammation and diseases, including asthma (5, 9, 15–26). The studies of asthma have been inconsistent in the direction of effects, and some have found no significant association (24–26). A number of methodologic issues may contribute to these inconsistencies among studies. Alternatively, variation in asthma and wheezing may reflect differences in the magnitude of this inflammatory response, which depends on exposures that produce oxidative stress, airway antioxidant defenses, and TNF responses. Differences in genetic background and environmental exposures that interact with TNF could also contribute to differences in results among studies.

Ozone, a common air pollutant that has been associated with asthma incidence and exacerbation, is a strong oxidant that produces an inflammatory response (27–31). It has been suggested that ozone may modify the effect of the TNF−308 polymorphism on asthma and wheezing (32). The level of oxidative stress produced by ozone drives TNF-mediated inflammation; however, the level of oxidative stress is likely to depend on airway antioxidant defenses and ozone levels. Airway antioxidant defenses are mediated in part by enzymatic antioxidants, including glutathione-S-transferases (GSTs) (33). GSTs function in antioxidant defenses through reactive oxygen species metabolism, repair of reactive oxygen species damage, and detoxification of xenobiotics (34, 35). We investigated the modifying effects of GSTM1 and GSTP1 genotypes on TNF–ozone associations because these genes are expressed in the respiratory tract, are involved in antioxidant defenses, and have common functional alleles that result in the total absence or a marked alteration in the enzyme's activity (4, 33, 36–44).

To further investigate the role of TNF in asthma occurrence, we examined the associations TNF G–308A polymorphism with asthma and wheezing at entry into the Children's Health Study (CHS) (44, 45). The CHS is a population-based study of school-aged children from 12 southern California communities representing a wide range of exposures to ambient air pollution. We investigated the effects of the TNF−308 polymorphism on asthma and wheezing because we focused on variation in associations of this functional change with outcomes in high and low ambient ozone environments and in relationship with GSTM1 null and GSTP1 Ile105Val genotypes. Some of the results presented in this article have been reported in an abstract (46).

METHODS

The design and methods for the CHS have been described in detail (45, 47). The CHS recruited fourth-, seventh-, and tenth-grade students from schools in 12 southern California communities in 1993 and recruited a second group of fourth-grade students from the same schools in 1996. Information on sociodemographics, asthma risk factors, household

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exposures, asthma, and wheeze outcomes was collected by questionnaire completed at study entry by the parents or guardian of each CHS participant. An air pollution monitoring network was established in 1994, and levels were measured continuously in each of the 12 communities included in the CHS. Children and a sample of their parents provided buccal cells as the DNA resource for genotyping several years after study entry. The study protocol was approved by the institutional review board for human studies at the University of Southern California, and written, informed consent was provided by a parent or legal guardian for all participants.

Questionnaire

Children's asthma and wheezing histories were characterized using questionnaire responses. Asthma status was defined as any reported lifetime history of a physician diagnosis of asthma at study entry. Lifetime and past–12-mo wheezing symptoms were provided by the participant's parent or guardian at CHS entry. Risk factors and possible confounders were collected in the questionnaire, including gestational age, birth weight, race and Hispanic ethnicity, level of parental education, insurance coverage, *in utero* exposure to maternal smoking and second-hand exposure to tobacco smoke, and family history of asthma and allergy.

Air Pollution Data

Air pollution monitoring stations were established in each of the 12 study communities beginning in 1994 (45, 47). Each station measured average hourly levels of ozone. We computed the annual average of the ozone levels obtained from 10:00 A.M. to 6:00 P.M. (the 8-h daytime average) in each community in 1995. Community-level ozone exposures were grouped into low and high subcategories using a 50-ppb cutoff. The mean annual ambient ozone level was 37.5 ppb in low-ozone communities and 57.8 ppb in high-ozone communities.

DNA Collection and Genotyping

Participants and a sample of their parents provided samples of genomic DNA beginning in 1998 using standard buccal cell collection procedures (44). Because a number of subjects had left the study in the years between enrollment and DNA collection, TNF genotyping results were available for 3,699 children (87 individuals with DNA samples had undetermined TNF genotyping results). Among the 3,699 individuals with TNF genotyping results, 553 individuals had undetermined GSTM1 genotype, and 88 had undetermined GSTP1 genotype (26 missing results for both GSTs). The DNA samples not collected were largely the result of residential moves required from changes in parent employment. Children in the cohort who did not have genotyping results showed modest differences in socioeconomic-related characteristics from children who had the genotyping results (*see* Table E1 of the online supplement).

Buccal scrapes were collected using standard protocols, and genomic DNA was isolated using a Purgene DNA isolation kit (Gentra Systems, Minneapolis, MN). The polymorphisms were identified by real-time polymerase chain reaction (PCR) using allele-specific minor groove binder (MGB) probes on an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Each genotype was validated by using PCR/restriction fragment length polymorphism methods (48). The sequences of primers and probes we used is listed in Table E2.

Statistical Analysis

Unconditional logistic regression was used to estimate the association of the TNF–308 genotype with lifetime and current asthma and with wheezing outcomes. Genetic models were chosen based on available biological evidence from mechanistic and association studies. For TNF–308, we grouped genotypes into GG versus AG or AA because we hypothesized that the GG genotype is protective due to reduced inflammatory response. Confounding by *a priori* identified covariates was assessed by examining changes in TNF-effect estimates from the models with and without the potential confounder. To assess confounding by admixture, we examined genotype associations in child–parent trios using a logistic regression model that included indicators of parent mating type (49). In addition, we stratified our study population by non-Hispanic and Hispanic whites (two major ethnic groups) to assess whether effects of TNF and ozone differed by ethnicity. To determine whether our findings could be replicated in an independent group of subjects, we fit models in two independent groups of children, one group recruited in 1993 and another group of children recruited in 1996.

To investigate whether ambient ozone level modulated the associations of TNF genotype with asthma and wheezing outcomes, we fitted stratified models restricted to the 2,727 fourth graders who resided in communities with lower or higher ozone levels. We restricted this analysis to fourth graders because valid exposure data at the same age (approximate age, 10 yr) were available to estimate annual averages in the year before the questionnaire responses. Annual averages were also determined for the year before enrollment in the study. When considering the effects of GSTM1 genotype on the TNF associations, we stratified participants by GSTM1 genotype (null versus present) or GSTP1 105 genotypes using a dominant model (Ile/Ile vs. Ile/Val or Val/Val) and assessed TNF associations in low- and high-ozone areas.

To evaluate the effects of lifetime ozone exposure on lifetime history of asthma and wheezing, we restricted our analysis to a subgroup of 878 fourth graders who had lived in the same community from birth until study entry. All analyses were conducted using SAS software version 9.1 (SAS Institute, Cary, NC).

RESULTS

Selected characteristics for CHS participants with TNF genotyping information are presented in Table 1. The majority of the participants were non-Hispanic whites. More than 16% of

TABLE 1. SELECTED CHARACTERISTICS OF CHILDREN'S HEALTH STUDY PARTICIPANTS AND A SUBSET OF FOURTH GRADERS

	Fourth Graders (*n = 2,727*)		All CHS Participants	
	n	%	n	%
Demographic information				
Sex				
Girls	1,407	51.6	1,978	53.5
Boys	1,320	48.4	1,721	46.5
Age at study entry, yr				
8–9	1,939	71.2	1,939	52.5
10–11	787	28.8	787	21.3
12–13	1	0.0	552	14.9
14–18	0	0.0	421	11.3
Ethnicity				
Non-Hispanic White	1,574	57.7	2,182	59.0
Hispanic	772	28.3	989	26.7
African American	120	4.4	156	4.2
Asian	110	4.1	157	4.2
Other	151	5.5	215	5.8
Gestational age				
Full term	2,335	85.6	3,189	86.2
$<$ 4 wk early	197	7.2	254	6.9
$\geq$ 4 wk early	125	4.6	160	4.3
Missing	70	2.6	99	2.7
In utero exposure to maternal smoking				
Yes	445	16.8	603	16.9
Any lifetime SHS exposure				
Yes	866	33.1	1,230	34.6
Current SHS exposure				
Yes	473	17.9	650	18.1
Ozone exposure				
High ($\geq$ 50 ppb)	1,605	58.9	2,166	58.6
Lifetime residence				
Yes	878	32.2	1,271	34.4
Respiratory outcomes				
Ever asthma	403	15.1	551	15.2
Ever wheezing	886	34.3	1,222	34.8
Current wheezing*	493	22.5	704	23.6
Medication for wheezing*	296	14.9	401	14.9

Definition of abbreviations: CHS = Children's Health Study; SHS = second-hand smoke.

* Based on self-report for the past 12 mo.

TABLE 2. TNF G-308A GENOTYPES BY ETHNICITY IN CHILDREN'S HEALTH STUDY PARTICIPANTS (*n* = *3,699*)

		TNF (G-308A)					
		GG		GA		AA	
Ethnicity	Total	n	%	n	%	n	%
Non-Hispanic white	2,182	1,553	71.2	572	26.2	57	2.6
Hispanic	989	814	82.3	171	17.3	4	0.4
African American	156	122	78.2	33	21.2	1	0.6
Asian	157	137	87.3	18	11.5	1	1.3
Other	215	171	79.5	40	18.6	4	1.9

Definition of abbreviation: TNF = tumor necrosis factor.
Hardy-Weinberg equilibrium holds for TNF alleles in each ethnicity.

children in the study were exposed *in utero* to maternal smoking. Second-hand smoke exposure was common (about 33% lifetime and 18% current) in the study population. The average prevalence for lifetime asthma and wheezing was 15 and 34%, respectively. The TNF alleles were in Hardy-Weinberg equilibrium in each ethnic group and the GG genotype varied from 71.2% in non-Hispanic whites to 87.3% in Asians (Table 2). The GSTM1 null and the GSTP1 105 Ile/Ile genotypes were observed for 47.6 and 39.6% of children, respectively.

Children who were homozygous for the TNF–308 G showed reduced risk for asthma and wheezing (Table 3). For example, children with the GG genotype had a lower prevalence of lifetime asthma (odds ratio [OR], 0.8; 95% confidence interval [CI], 0.7–0.9) compared with children carrying at least one –308 A allele (e.g., GA or AA). Current and lifetime wheezing occurred less frequently in children who were –308 G homozygotes. The associations of the –308 A allele were replicated in the two cohorts of fourth graders independently recruited in 1993 and 1996 (Table E3). The effect estimates were qualitatively similar based on analyses of the 338 complete trios, indicating that our estimates for all CHS children are not due to bias from population stratification. The association for TNF did not substantially differ between non-Hispanic and Hispanic whites. GSTM1 and GSTP1 genotypes were not significantly associated with the outcomes in this analysis (Table E5).

We found that the protective effects of the GG genotype on wheezing outcomes were stronger for children living in low-ozone communities than in high-ozone communities (Table 4). Compared with children who had the GA or AA genotypes, those with the GG genotype had a marked reduction of ever wheezing with low ozone exposure (OR, 0.5; 95% CI, 0.4–0.7); however, this reduction was not observed in children living in high-ozone communities (OR, 1.0; 95% CI; 0.8–1.3). This difference in genotypic effects between low- and high-ozone environments was statistically significant (interaction p = 0.003). Similarly, the protective associations of the –308 GG genotype with current wheezing and medication for wheezing were of significantly greater magnitude for those exposed to lower compared with higher ozone (interaction p = 0.04 and 0.02 for current wheezing and medication for wheezing, respectively). The association of TNF with any lifetime history of asthma was similar in the low- and high-ozone communities (OR, 0.8 and 0.9; Table 4). We found no direct associations of ozone with asthma or wheezing outcomes (data not shown). In addition, we found no substantial differences in the effect of the –308 GG genotype in children in relation to exposure to the other monitored air pollutants in the CHS (PM_{10}, $PM_{2.5}$, NO_2, and acid vapor levels) or second-hand smoke exposure (data not shown). The variation in the protective effect of the –308 GG genotype on lifetime asthma and wheezing in high- and low-ozone communities was similar in analyses restricted to the subgroup of lifetime residents (data not shown). We found that the variations in the associations of the –308 A allele by community ozone were observed in the two cohorts of fourth graders independently recruited in 1993 and 1996 (Table E3).

To assess the role of GSTM1 null genotype on the protective effect of the –308 GG genotype on wheezing in low-ozone communities, we fitted models stratifying subjects by their GSTM1 genotypes (present or null, 352 excluded for missing genotypes). We found that the difference in the –308 GG genotype effect between low and high ozone exposure was more marked in the GSTM1 null compared with the GSTM1 present group (Table 5).

Similarly, the difference in the effect of TNF between low and high ozone exposure was greater in those with the GSTP1 Ile/Ile genotype compared with the GSTP1 Ile/Val and Val/Val genotypes (Table 6). We had insufficient sample size to jointly stratify on GSTM1 and GSTP1 genotype and examine the interaction of TNF genotype with ozone levels.

DISCUSSION

A growing number of studies have provided evidence that expression of genetic determinants of asthma and wheezing depends on exposure to environmental stressors (1, 2, 42–44). In this study, we show that the TNF−308 GG genotype is protective for asthma and wheezing but that protection depends on outdoor ozone levels in the community of residence. Children living in

TABLE 3. ASSOCIATIONS OF TNF G-308A WITH ASTHMA AND WHEEZING OUTCOMES IN CHILDREN AND TRIOS AT CHILDREN'S HEALTH STUDY ENTRY

		CHS			Complete Trios		
Outcomes	TNF	Ca/Co	OR	95% CI	Ca/Co	OR	95% CI
Ever asthma	GA/AA	399/2,331	1.0		29/102	1.0	
	GG	152/733	0.8	0.7–0.9	62/285	0.6	0.2–1.3
Ever wheezing	GA/AA	884/1,762	1.0		54/72	1.0	
	GG	338/523	0.8	0.7–0.9	132/210	0.5	0.3–1.1
Current wheezing	GA/AA	499/1,758	1.0		30/72	1.0	
	GG	205/523	0.7	0.6–0.9	80/210	0.6	0.3–1.6
Medication for wheezing	GA/AA	279/1,761	1.0		22/72	1.0	
	GG	122/523	0.7	0.5–0.8	54/210	0.5	0.2–1.4

Definition of abbreviations: Ca/Co = number of cases/number of controls; CHS = Children's Health Study; CI = confidence interval; OR = odds ratio; TNF = tumor necrosis factor.
Models are adjusted for age, sex, race/ethnicity, town, lifetime residence, grade, and smoking exposure (*in utero* and second-hand).

TABLE 4. THE ASSOCIATION OF TNF G-308A WITH ASTHMA AND WHEEZING OUTCOMES IN FOURTH-GRADE CHILDREN'S HEALTH STUDY PARTICIPANTS IN LOWER AND HIGHER OZONE COMMUNITIES*

		Low Ozone			High Ozone			
Outcomes	TNF	Ca/Co	OR	95% CI	Ca/Co	OR	95% CI	p Value for Interaction
Ever asthma	GA/AA	44/195	1.0		63/339	1.0		
	GG	122/726	0.8	0.5–1.1	174/1002	0.9	0.7–1.2	0.51
Ever wheezing	GA/AA	106/126	1.0		137/252	1.0		
	GG	256/575	0.5	0.4–0.7	387/745	1.0	0.8–1.3	0.003
Current wheezing	GA/AA	61/126	1.0		78/252	1.0		
	GG	147/574	0.5	0.4–0.8	207/743	0.9	0.6–1.2	0.04
Medication for wheezing	GA/AA	39/126	1.0		49/252	1.0		
	GG	84/574	0.4	0.3–0.7	124/745	0.9	0.6–1.2	0.02

For definition of abbreviations, *see* Table 3.

Models are adjusted for age, sex, race/ethnicity, town, lifetime residence, grade, and smoking exposure (*in utero* and second-hand).

* Two ozone strata were defined as less than and greater than 50-ppb ozone average.

high-ozone communities were not protected from increased asthma and wheezing by the GG genotype. These associations were replicated in two cohorts of fourth-grade children who were independently recruited in 1993 and 1996, indicating that the associations were unlikely to arise by chance. In addition to ozone, two common variants in unlinked genes involved in antioxidant defenses, GSTM1 and GSTP1, may affect the expression of the protective effect of the TNF–308 GG genotype, as indicated by the less marked protection in high-ozone communities among groups with the GSTM1 null or GSTP1 105 Val allele. We have previously reported that asthma occurrence may depend on epistatic relationships between antioxidant and inflammatory genes (4). We now provide evidence for a role of gene–environment interactions and genetic interactions in the occurrence of childhood asthma and wheezing.

Our results are consistent with the majority of studies reporting that the allele G of the TNF–308 polymorphism is associated with decreased risk of asthma and wheezing (9, 18, 20–23). However, a number of studies on the association of TNF–308 polymorphism with asthma have reported conflicting results (5, 9, 18, 20–26). Based on our findings, the inconsistency among studies may be due to differences in exposures or genetic background among the populations studied.

Although we did not directly study the mechanisms for the effects of the TNF–308 GG variant or the effects of ozone exposure on TNF-mediated asthma and wheezing in children, we speculate that TNF–308 GG promoter genotype is associated with less intense inflammatory responses to oxidant stressors and that this reduced responsiveness results in reduced risk for asthma and wheezing. TNF–308 genotype has been shown to influence the rate of transcription and protein translation of TNF with the G allele resulting in lower expression (50). High levels of TNF have been observed in the bronchoalveolar lavage fluid, serum, and bronchial submucosa of patients with asthma (51). Because TNF levels are associated with airway inflammation, increased bronchial hyperresponsiveness, and atopy, the GG allele has a biologically plausible role in the occurrence of asthma and wheezing. A modifying effect of ozone exposure on the effects of the TNF G–308A polymorphism on lung function has also been reported from studies of experimental ozone exposures in which the –308 G was associated with lung function changes after acute ozone exposure (52). However, this genetic association with lung function is in the opposite direction of the effect of the GG genotype on asthma and wheezing we report in this study. This difference may reflect a different mechanism producing the findings for lung function deficits than for asthma and

TABLE 5. THE ASSOCIATION OF TNF G-308A WITH WHEEZING OUTCOMES IN FOURTH-GRADE CHILDREN'S HEALTH STUDY PARTICIPANTS, STRATIFIED BY GSTM1 GENOTYPE AND OZONE EXPOSURE*

		GSTM1 Null				GSTM1 Present			
		Low Ozone			High Ozone	Low Ozone			High Ozone
Outcomes	TNF	Ca/Co	OR	95% CI	Ca/Co	Ca/Co	OR	95% CI	Ca/Co
Ever wheezing	GA/AA	47/57	1.0		50/111	50/58	1.0		63/105
	GG	107/245	0.5	0.3–0.8	152/292	126/275	0.5	0.4–0.8	176/332
					OR = 1.1 (0.7–1.5)				OR = 0.9 (0.6–1.2)
					$p = 0.009^{\dagger}$				$p = 0.10^{\dagger}$
Current wheezing	GA/AA	30/57	1.0		27/111	27/58	1.0		36/105
	GG	59/245	0.4	0.2–0.6	87/291	77/274	0.7	0.4–1.1	93/331
					OR = 0.9 (0.6–1.5)				OR = 0.7 (0.5–1.2)
					$p = 0.009^{\dagger}$				$p = 0.75^{\dagger}$
Medication for wheezing	GA/AA	18/57	1.0		17/111	17/58	1.0		21/105
	GG	33/245	0.3	0.1–0.6	48/292	45/274	0.5	0.3–1.0	54/332
					OR = 0.8 (0.5–1.4)				OR = 0.8 (0.4–1.3)
					$p = 0.02^{\dagger}$				$p = 0.42^{\dagger}$

For definition of abbreviations, *see* Table 3.

Models are adjusted for age, sex, race/ethnicity, town, lifetime residence, grade, and smoking exposure (*in utero* and second-hand).

* Two ozone strata were defined as less than and greater than 50-ppb ozone average (Figure 1).

† Testing of interaction between TNF and ozone within GSTM1 genotypes.

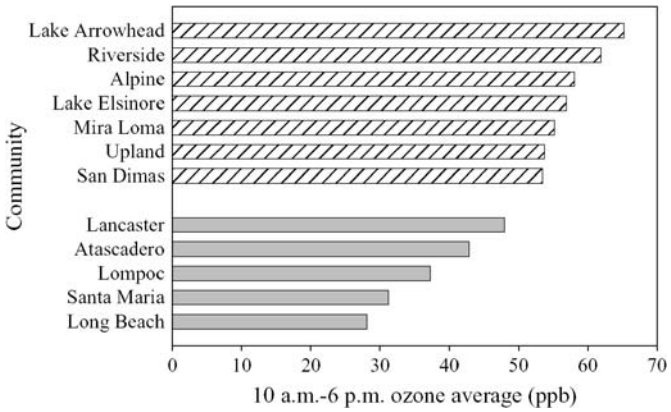

Figure 1. Average ozone in 12 Children's Health Study communities. *Hatched bars*: higher ozone; mean, 57.8 ppb. *Shaded bars*: lower ozone; mean, 37.5 ppb.

wheezing. For example, acute lung function deficits from ozone are not well correlated with cellular inflammatory responses, suggesting another function of TNF in producing deficits on ozone exposure. However, the reasons for the opposite effects on acute change in lung function are not clear and may warrant further study.

The protective effect of the TNF–308 GG genotype seems to depend on the level of oxidative stress, reflecting oxidant exposure and adequacy of antioxidant defenses. Oxidative stress is intimately involved in multiple inflammation processes that are regulated in part by cytokines such as TNF (53, 54). The TNF–308 A allele may alter gene expression and amplify the intensity of the inflammatory response to oxidants, leading to increased risk and severity of asthma and wheezing in susceptible groups. Ozone, an ambient photochemical oxidant, is capable of producing pulmonary inflammation and injury. Exposure to ozone results in the release of inflammatory cytokines, including TNF (8). Pretreatment of rats with antibody to TNF can reduce ozone-induced inflammation and lung damage, and acute ozone-induced airway hyperreactivity is reduced in TNF receptor−deficient mice (55). The findings in this study suggest that the effects of the –308 G on transcription are overcome by higher levels of oxidative stress. These results are consistent with studies of effects of GSTM1 and antioxidant vitamins in children exposed to high levels of ozone. We suggest that sequence variants that affect expression of genes participating in inflammatory pathways may show variable penetrance in the setting of high ozone exposure and low antioxidant defenses.

Interpretation of our results requires consideration of several limitations. We conducted a cross-sectional study of school-aged children. A limitation of this approach is the potential for selection bias. Because our study was based in a cohort, we were able to examine the potential for selection bias in the cross-sectional study. Of the total number of eligible subjects, approximately 40% did not provide buccal cell samples for this study, which may give rise to selection bias. Although those with and without genotypes did modestly differ in socioeconomic status and ethnicity, adjustment for these variables in the health models had little effect on estimates, making selection bias an unlikely explanation for our findings.

A second important limitation was the phenotypic definition of participants' asthma and wheezing status, which used parental report of physician-diagnosed asthma and wheezing symptoms. The use of physician diagnosis of asthma has been widely used in epidemiologic studies, and self-report has been found to accurately reflect physician diagnosis (56). Asthma is a variable clinical syndrome characterized by recurrent symptoms of wheezing, breathlessness, chest tightness, and coughing. There are no gold standard tests to diagnose asthma. Some researchers advocate using objective tests for detecting airway hyperresponsiveness or atopy to define asthma. However, the sensitivity of these tests is low, and studies have shown that these tests are not superior to clinical history information for asthma diagnosis. Another concern in using physician-diagnosed asthma to classify asthma status is that limited access to health care may result in underdiagnosis of asthma. In our sample, more than 80% of children had medical insurance, which suggests that access to medical care was not limited. To assess this potential source of misclassification of asthma status, we considered the effects of health insurance, income, and education on the risk estimates for wheezing and found little change in the adjusted risk estimates. Because asthma and wheezing status was defined without the knowledge of genotype, differential misclassification of asthma status by TNF genotype is probably not a major source of bias that accounts for our results. Therefore, although there is likely to be nondifferential misclassification of asthma and wheezing status, such misclassification of asthma and wheezing status

TABLE 6. THE ASSOCIATION OF TNF G-308A WITH WHEEZING OUTCOMES IN FOURTH-GRADE CHILDREN'S HEALTH STUDY PARTICIPANTS, STRATIFIED BY GSTP1 Ile105Val AND OZONE EXPOSURE*

		GSTP1 Ile/Ile (*n = 1,054*)							GSTP1 Ile/Val or Val/Val (*n = 1,605*)						
		Low Ozone				High Ozone			Low Ozone				High Ozone		
Outcomes	TNF	Ca/Co	OR	95% CI		Ca/Co	OR	95% CI	Ca/Co	OR	95% CI		Ca/Co	OR	95% CI
Ever wheezing	GA/AA	63/76	1.0			81/130	1.0		38/46	1.0			50/110	1.0	
	GG	150/348	0.6	0.3–0.9		227/431	1.1	0.8–1.7	101/216	0.5	0.4–0.8		153/302	0.9	0.6–1.2
					p = 0.04†							p = 0.07†			
Current wheezing	GA/AA	37/76	1.0			49/130	1.0		23/46	1.0			25/110	1.0	
	GG	86/348	0.5	0.3–0.9		122/431	1.2	0.7–2.0	58/215	0.5	0.3–0.8		84/300	0.7	0.5–1.1
					p = 0.06†							p = 0.28†			
Medication for wheezing	GA/AA	21/76	1.0			35/130	1.0		16/46	1.0			13/110	1.0	
	GG	52/348	0.4	0.2–0.8		76/431	1.3	0.7–2.6	30/215	0.5	0.3–0.9		47/302	0.6	0.4–0.9
					p = 0.02†							p = 0.53†			

For definition of abbreviations, *see* Table 3.

Models are adjusted for age, sex, race/ethnicity, town, lifetime residence, grade, and smoking exposure (*in utero* and second-hand).

* Two ozone strata were defined as less than and greater than 50-ppb ozone average (Figure 1).

† Testing of interaction between TNF and ozone within GSTP1 genotypes.

would not account for the genetic and environmental associations we observed.

In addition, ozone exposure assessment was based on ambient levels measured at central site monitors in each community. We dichotomized exposure into high- and low-ozone communities to account for uncertainties in indoor levels and time–activity patterns of the study participants. Although these estimates have associated measurement error, it is likely that the groups provided substantial contrasts in average ozone exposure. However, we only observed an association with ozone in a susceptible subgroup. We previously reported that children playing three or more team sports in a high-ozone environment had increased risk for developing asthma (29). Due to sample size limitations, we were unable to consider team sports as an additional effect modifier in this genetic analysis.

Other inflammatory exposures and conditions have the potential to confound the relationship between the TNF–308 polymorphisms and asthma. Some that have been well described in the literature include active personal smoking, exposure to second-hand smoke, indoor allergens, allergic diseases, and family history of asthma. Second-hand smoke and active smoking were taken into account by adjusting for these exposures; adjusting for cats, dogs, allergy, and family history of asthma individually and in combination resulted in negligible changes in the associations. Exposure to tobacco smoke was assessed using questionnaire responses about household sources and was not validated by objective measurements such as cotinine levels. However, the validity of exposure estimates based on questionnaire responses has been investigated and found to provide reasonably valid estimates of exposure for adjustment for confounding (57–59).

Conducting a candidate gene association study to assess the role of the TNF–308 polymorphism and susceptibility to asthma requires careful interpretation in light of the potential for population stratification and linkage disequilibrium in the chromosomal region that contains the TNF locus. In our analyses of asthma, TNF genotype, ozone, GSTM1, and GSTP1, we considered the effects of ethnicity and found the risk pattern of asthma and wheezing showed neither important confounding by ethnicity nor substantial differences in effects by ethnic status. We also examined the associations using parent–child trios and found associations consistent with the associations in all the children. Population stratification is unlikely to explain our findings. We note that the region spanning the TNF loci has long-range linkage disequilibrium (over 45 kb), and the associations with the –308 G allele may be due to other linked variants in this region (60). The TNF region is comprised of fewer than 20 common haplotypes. The –308 G variant occurs on two long-range haplotypes in populations with European ancestry, one of which is the most common haplotype in the linked region (60). Association studies have been conducted that have examined the TNF loci haplotypes and have found associations with haplotypes containing the –308 G variant (10). Based on the available information on locus region linkage structure, the associations with the –308 polymorphism reflect a comparison of haplotypes containing the –308 G variant to the remaining haplotypes across the 45-kb region and indicate that the –308 G variant or another variant on these haplotypes underlie the increased susceptibility for asthma and the loss of protection in high-ozone communities in the context of variant genotypes. Although studies of the TNF region using extended haplotypes capture the variation across the major histocompatibility complex region, splitting the –308 polymorphism across several haplotypes may require larger sample sizes to detect the same magnitude of effect for a functional polymorphism. Because the –308 polymorphism seems to be functional, our assessment of gene–environment interactions focused on assessing this genetic variant.

In conclusion, the protective association of TNF–308 GG genotype in children with low ozone exposures or protective GSTM1 or GSTP1 genotype suggests that this relatively common genetic polymorphism, or haplotype marked by this polymorphism, plays a protective role in asthma pathogenesis among children depending on airway oxidative stress levels.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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