Effects of Glutathione *S*-Transferase M1, Maternal Smoking during Pregnancy, and Environmental Tobacco Smoke on Asthma and Wheezing in Children

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The rise in childhood asthma prevalence suggests a role for environmental factors in the etiology of this evolving epidemic; however, genetics also influence the occurrence of asthma. Glutathione *S*-transferase (GST) M1 may play a role in asthma and wheezing occurrence among those exposed to tobacco smoke, as it functions in pathways involved in asthma pathogenesis such as xenobiotic metabolism and antioxidant defenses. Effects of GSTM1 genotype, maternal smoking during pregnancy, and childhood environmental tobacco smoke (ETS) exposure on asthma and wheezing were investigated in 2,950 children enrolled in 4th, 7th, and 10th grade classrooms in 12 Southern California communities. The effects of *in utero* exposure to maternal smoking on asthma and wheezing occurrence were largely restricted to children with GSTM1 null genotype. Among GSTM1 null children, *in utero* exposure was associated with increased prevalence of early onset asthma (odds ratio [OR] 1.6, 95% confidence interval [CI] 1.0–2.5), asthma with current symptoms (OR 1.7, 95% CI 1.1–2.8), persistent asthma (OR 1.6, 95% CI 1.1–2.4), lifetime history of wheezing (OR 1.8, 95% CI 1.3–2.5), wheezing with exercise (OR 2.1, 95% CI 1.3–3.3), wheezing requiring medication (OR 2.2, 95% CI 1.4–3.4), and emergency room visits in the past year (OR 3.7, 95% CI 1.9–7.3). Among children with GSTM1 (+) genotype, *in utero* exposure was not associated with asthma or wheezing. Our findings indicate that there are important long-term effects of *in utero* exposure in a genetically susceptible group of children.

Keywords: *In utero* exposure; tobacco smoke; GSTM1; asthma; wheeze; children

Over the last 25 years, asthma has emerged as an increasingly important public health problem in the industrialized world (1–3). Although a rapid rise in childhood asthma prevalence suggests a role for environmental factors in the etiology of this evolving epidemic, it is clear that genetics also influence the occurrence of asthma (4–6). The evidence that both genes and environment play etiologic roles suggests that the increase in asthma occurrence is likely to involve changes in specific exposures among the population of genetically susceptible individuals (7, 8).

The full spectrum of exposures and susceptibility genes involved in the pathogenesis of asthma and wheezing have yet to be established (6, 9). Tobacco smoke is an exposure of interest, especially among children, a group with high prevalence of asthma and increased sensitivity to air pollutants (10–16). An extensive body of evidence indicates that involuntary tobacco smoke exposure increases the prevalence of wheezing, cough, and phlegm, and that childhood household ETS exposures cause exacerbations in asthma (10–15). Fetal exposure to maternal smoking may contribute to the occurrence of asthma and wheezing; however, the evidence for independent effects of *in utero* exposure on the occurrence is still emerging (6, 10–15).

Susceptibility to the long-term adverse effects of *in utero* exposure on asthma and wheezing is likely to be modified by fetal tobacco smoke defenses such as xenobiotic detoxification systems, antioxidant responses, and damage repair mechanisms (17). A number of genes involved in xenobiotic detoxification systems, antioxidant responses, and damage repair mechanisms for tobacco smoke have been identified (18, 19). Glutathione *S*-transferase (GST) M1 enzyme product is involved in detoxification of both reactive tobacco metabolic intermediates and reactive oxygen species (20). GSTM1 has been extensively studied because the locus is polymorphic with a common null allele that results in a complete lack of the enzyme. The M1 null genotypes are homozygous for the null allele. The evidence indicates that the GSTM1 null genotype is associated with a small increase in risk of lung cancer and increased DNA damage among smokers. The GSTM1 enzyme product may also play a role in asthma and wheezing occurrence because xenobiotic metabolism and antioxidant pathways are involved in asthma pathogenesis (20–23). Although GSTM1 has the potential to explain a substantial portion of asthma occurrence at the population level, its role in asthma pathogenesis has not been extensively investigated, and no studies of the association of GSTM1 genotype and *in utero* exposure to maternal smoking with asthma occurrence have been reported.

The Children's Health Study (CHS) offers an opportunity to further investigate the effects of GSTM1 and involuntary tobacco smoke on the occurrence of asthma and wheezing during childhood. The CHS, which began in 1993, is a cohort study of the effects of air pollution on children's respiratory health (24). Participants include children enrolled as 4th, 7th, and 10th graders who attended public schools in 12 communities in Southern California. We used lifetime tobacco smoke exposure histories and parental reports of wheezing and physician-diagnosed asthma collected at cohort entry and GSTM1 genotypes obtained from buccal cell DNA to examine the relationships of GSTM1, maternal smoking during pregnancy, and childhood exposure to ETS with wheezing or asthma.

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METHODS

Participants

Of the 5,925 school-aged children with known asthma status recruited to the Children's Health Study (CHS), an ongoing study in 12 Southern California communities that began in 1993, 2,950 were included in this analysis, having provided buccal cell specimens as a source of germline DNA for genotyping. Details on the design, site selection, subject recruitment, and assessment of health effects are reported elsewhere (24). At study entry, a parent or guardian of each participating child provided written informed consent and completed a self-administered questionnaire on demographics, medical and family health history, indoor air exposures, and household characteristics. We recruited children for the genetic studies at schools and by mail during Years 6–10 of the study. Older children who were in grade 10 at enrollment, had already graduated from high school, or moved when sample collection started were not available for sample collection during school visits.

Procedures

Asthma and wheezing. Questionnaire responses by parents or guardians were used to categorize children's asthma status, age at asthma diagnosis, and wheezing history. Children were classified as having asthma if the adult completing the questionnaire reported that a doctor had "ever diagnosed the child as having asthma." We defined early age at diagnosis as age 5 years or younger. A child with persistent asthma was defined as any child who was diagnosed with asthma and who had wheezing or who took asthma medication in the year before study entry. A child with active asthma was defined as any child who was ill with asthma at any time in the 12 months before the date that the questionnaire was completed. A child was classified as having a lifetime history of wheezing if the adult completing the questionnaire responded affirmatively to the question "Has your child's chest ever sounded wheezy or whistling, including times when he or she had a cold?" Wheezing with/without colds was defined as any wheezing with/without colds in the 12 months before the questionnaire. Persistent wheezing was defined as wheezing for 3 or more days out of the week for a month or longer in the previous year. Wheezing with shortness of breath was defined as an episode of shortness of breath with wheezing in the last 12 months. Awakening at night by wheezing was defined as any episode of awakening at night by wheezing in the previous 12 months. Wheezing with exercise was defined as episodes of wheezing after he or she has been playing hard or exercising in the past 12 months. Any medication for asthma and treatment for wheezing were assessed for the 12 months before the interview. We also considered lifetime occurrence for each of the outcomes assessed for the previous 12 months.

Tobacco smoke exposure. Exposure to household ETS and exposure to maternal smoking *in utero* were characterized using the responses from the questionnaire completed by parents or guardians. Household smoking was defined as daily smoking inside the house by anyone living there. Information was collected about the current and past household smoking status of each participant's mother, father, other adult household members, and regular household visitors. *In utero* exposure to maternal smoking (yes or no) was assigned by responses to the question "Did your child's biological mother smoke while she was pregnant with your child? (Include time when she was pregnant but did not yet know she was.)"

Covariates. Ethnicity was grouped as non-Hispanic white, Hispanic, African American, Asian/Pacific Islanders, and all other ethnicities combined. Gestational age was categorized as full term, 1 to less than 4 weeks early, or 4 or more weeks early. Family history of atopy was defined as any biological parent having been diagnosed with hay fever or allergies. Family history of asthma was defined as any biological parent having been diagnosed with asthma at the time of study entry.

Laboratory methods. Buccal cells were collected from participants as a source of germline DNA for genotyping assays. Specimen processing and DNA extraction was performed using a PUREGENE DNA isolation kit (cat #D-5000; GENTRA, Minneapolis, MN) according to the manufacturer's recommendations. Genotypes for GSTM1 were determined using two methods. The first 698 samples were analyzed using polymerase chain reaction (PCR). All reactions were repeated at least once. The remaining GSTM1 genotypes were determined using real-time PCR by TaqMan 7700 (Applied Biosystems, Foster City, CA). A subset of participants was genotyped using both methods to assure consistency of genotype assignment by each method. Samples showing no signal or late cycle number for start of amplification were repeated and further analyzed with primers and probes for the actin gene to verify the presence of amplifiable DNA. Additional information on sample collection, processing, and genotyping methods are available online (*see* online data supplement).

Statistical Analyses

Logistic regression was used to assess the relationship between asthma and wheezing outcomes, GSTM1 genotype, ETS, and *in utero* exposure to maternal smoking. All odds ratios (ORs) were adjusted for town of residence, age, grade, race, sex, family history of asthma and atopy, and gestational age. We also assessed potential confounding by income and education, health insurance status, and housing characteristics using a 10% change in effect estimates as a criterion. The individual effects of GSTM1 genotype and two types of tobacco smoke exposure (*in utero* or current ETS exposure) on respiratory outcomes were assessed. The GSTM1 null genotypes are homozygous for the null allele, and the referent group consists of subjects heterozygous for the GSTM1 wild-type allele and homozygous for the M1 wild-type allele. The joint effects of GSTM1 genotypes (null [−] and present [+]) and tobacco smoke exposure (*in utero* or current ETS exposure) were assessed using four mutually exclusive levels: no tobacco smoke exposure and GSTM1 (+) (reference group), no tobacco smoke exposure and null GSTM1 (−), tobacco smoke exposure and GSTM1 (+), and tobacco smoke exposure and GSTM1 (−). Effect modification was assessed by fitting models with the appropriate interaction term and testing significance of the interaction term as well as likelihood ratio tests comparing full and reduced models. All analyses were conducted using SAS 8e Release 2 (SAS Institute, Cary, NC) (25).

RESULTS

The majority of participants were aged 10 years or less and non-Hispanic white (Table 1). Girls (53.1%) outnumbered boys (46.9%). A family history of asthma or atopy was common in this group of students. *In utero* exposure occurred among 16.2% of students and 16.7% were currently exposed to ETS. GSTM1 null genotype was observed in 45.2% of children. A physician diagnosis of asthma was reported by 15.6% of participants, but only 9.5% had active asthma. Thirty-five percent reported any lifetime history of wheezing, but only 4.6% had visited an emergency room for wheezing. Those who did not have genotyping data were older as a result of the study methods, more likely to be a member of a minority ethnic group, and had more tobacco smoke exposure and less asthma. The reasons for missing genotype data included an approximate 10% rate of decline to provide a sample, and the remaining children had moved or graduated and were no longer under active follow-up in school and not available for sample collection. Children from families with lower income and education were more likely to have moved and to be lost to follow-up, suggesting that the differences in smoking between those with and without genotyping were not related to GSTM1 genotype.

The GSTM1 null genotype frequency varied by ethnicity (Table 2). Among control subjects, African Americans had a lower proportion (27.4%) than non-Hispanic whites (45.8%). The proportions of control subjects with GSTM1 null are within the ranges reported in other populations (26). Among Asians, GSTM1 null was more common among cases than controls ($p = 0.04$); however, in multivariable models, the adjusted effect of GSTM1 null genotype among Asians was not statistically significant.

TABLE 1. SELECTED CHARACTERISTICS FOR PARTICIPANTS

	With Genotyping		No Genotyping	
	n	(%)	n	(%)
Total	2950		3166	
Demographic Information				
Sex				
F	1,567	53.1%	1,601	50.7%
M	1,383	46.9%	1,565	49.4%
Age at study entry, yr				
8–9	1,613	54.8%	1,223	39.2%
10–11	643	21.8%	675	21.5%
12–13	432	14.7%	555	17.6%
⩾14	258	8.8%	684	21.7%
Ethnicity				
Non-Hispanic Whites	1,779	61.2%	1,602	51.5%
Hispanics	712	24.5%	922	29.7%
African Americans	119	4.1%	212	6.8%
Asians	132	4.5%	162	5.2%
Others	166	5.7%	211	6.8%
Family history of asthma	554	20.1%	546	19.1%
Family history of atopy	1,380	50.8%	1,262	44.6%
Gestational Age				
Full term	2,534	88.2%	2,782	90.7%
< 4 wk early	205	7.1%	186	6.1%
⩾ 4 wk early	134	4.7%	100	3.3%
In utero exposure to maternal smoking	462	16.2%	653	21.5%
Current ETS exposure	482	16.7%	857	27.7%
Respiratory Outcomes*				
Asthma				
Ever asthma	451	15.6%	422	13.3%
Active asthma	257	9.5%	237	8.0%
Medication for asthma	305	11.1%	263	8.8%
Early onset asthma	297	10.8%	223	7.5%
Persistent asthma	390	13.5%	364	11.7%
Wheezing				
Ever wheezing	984	35.0%	957	31.8%
Wheeze with cold	511	21.9%	496	19.5%
Wheeze without cold	322	15.0%	282	12.1%
Persistent wheeze	175	8.7%	166	7.5%
Shortness of breath	256	12.3%	279	12.0%
Awakened at night	215	10.5%	216	9.5%
Wheeze with exercise	312	14.6%	303	12.9%
Medication for wheeze	335	15.5%	397	12.6%
Emergency room for wheeze	87	4.6%	98	4.6%

Definition of abbreviation: ETS = household environmental tobacco smoke.
Numbers may not add up because of missing values.
Percentage is reported among non-missing observations.
* All outcomes are for previous 12 months except ever and early onset asthma and ever wheezing.

In utero exposure to maternal smoking was associated with a broad spectrum of wheezing outcomes from a history of ever wheezing, wheeze with or without cold, shortness of breath, persistent wheezing, attacks of wheezing causing shortness of breath, wheezing with exercise, medication use, and emergency room visits (Table 3). *In utero* exposure was not as strongly associated with asthma outcomes. Furthermore, GSTM1 genotype and current ETS exposure were also not associated with asthma or wheezing outcomes. The estimated effects were not substantially changed in models that mutually adjusted for GSTM1 genotype, *in utero* and current ETS exposure, or additional personal and household characteristics. We also examined lifetime prevalence of wheezing outcomes and found the same patterns of effects.

Children who had the GSTM1 null genotype were at the greatest risk for adverse respiratory health effects when exposed to maternal smoking *in utero.* The effects of *in utero* exposure to maternal smoking on both current and lifetime asthma and wheezing outcomes were largest, and in general, restricted to children with GSTM1 null genotype (Table 4). For example, active asthma, early onset, and persistent asthma were associated with *in utero* exposure only in those with the GSTM1 null genotype. The GSTM1 null genotype was not

TABLE 2. DISTRIBUTION OF GSTM1 GNEOTYPE BY ETHNICITY AND CASE-CONTROL STATUS*

	GSTM1 (null)			
	Cases		Control Subjects	
Ethnicity	n	(%)	n	(%)
Non-Hispanic Whites	327	49.3%	481	45.8%
Hispanics	85	38.6%	194	42.7%
African Americans	12	29.3%	20	27.4%
Asians	13	65.0%	39	39.8%
Other	30	44.1%	51	54.3%

* Cases are defined as ever asthma or wheezing, whereas control subjects have neither.

TABLE 3. EFFECTS OF GSTM1, *IN UTERO* EXPOSURE TO MATERNAL SMOKING, AND CURRENT ETS EXPOSURE ON ASTHMA AND WHEEZE, ODDS RATIO AND 95% CONFIDENCE INTERVAL

	GSTM1 (null)		*In utero* Exposure		Current ETS	
Outcomes	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
	Univariate					
Asthma						
Ever asthma	1.0	(0.8, 1.2)	1.2	(0.9, 1.6)	1.2	(0.9, 1.5)
Active asthma	0.9	(0.7, 1.2)	1.2	(0.9, 1.7)	0.9	(0.6, 1.3)
Medication for asthma	1.0	(0.8, 1.3)	1.2	(0.9, 1.6)	1.0	(0.7, 1.4)
Early onset asthma	1.0	(0.8, 1.3)	1.2	(0.9, 1.7)	1.0	(0.7, 1.4)
Persistent asthma	1.0	(0.8, 1.3)	1.2	(0.9, 1.6)	1.1	(0.8, 1.4)
Wheezing						
Ever wheezing	1.0	(0.9, 1.2)	1.6	(1.3, 2.0)	1.2	(1.0, 1.5)
Wheeze with cold	1.0	(0.8, 1.2)	1.6	(1.2, 2.0)	1.2	(0.9, 1.6)
Wheeze without cold	1.1	(0.8, 1.4)	1.5	(1.1, 2.1)	1.0	(0.7, 1.4)
Persistent wheeze	0.9	(0.7, 1.2)	2.0	(1.4, 3.0)	1.1	(0.7, 1.7)
Attacks of wheezing						
Shortness of breath	1.0	(0.8, 1.3)	1.8	(1.3, 2.5)	1.1	(0.8, 1.6)
Awakened at night	1.0	(0.7, 1.3)	1.5	(1.0, 2.2)	1.2	(0.8, 1.8)
Wheeze with exercise	1.0	(0.8, 1.3)	1.6	(1.2, 2.2)	1.3	(0.9, 1.7)
Treatments for wheezing						
Medication for wheeze	1.1	(0.8, 1.3)	1.5	(1.1, 2.0)	1.1	(0.8, 1.5)
Emergency room for wheeze	1.2	(0.8, 1.9)	2.2	(1.3, 3.7)	1.4	(0.8, 2.3)
	Mutually adjusted*					
Asthma						
Ever asthma	1.0	(0.8, 1.3)	1.2	(0.9, 1.6)	1.1	(0.8, 1.5)
Active asthma	1.0	(0.8, 1.3)	1.3	(0.9, 1.8)	0.9	(0.6, 1.3)
Medication for asthma	1.1	(0.9, 1.4)	1.2	(0.8, 1.7)	0.9	(0.6, 1.3)
Early onset asthma	1.0	(0.8, 1.3)	1.2	(0.8, 1.7)	0.9	(0.7, 1.4)
Persistent asthma	1.1	(0.9, 1.3)	1.2	(0.9, 1.7)	1.0	(0.7, 1.4)
Wheezing						
Ever wheezing	1.1	(0.9, 1.4)	1.5	(1.2, 1.9)	1.1	(0.9, 1.4)
Wheeze with cold	1.0	(0.9, 1.3)	1.5	(1.1, 1.9)	1.1	(0.8, 1.4)
Wheeze without cold	1.1	(0.9, 1.5)	1.6	(1.0, 2.2)	0.9	(0.6, 1.3)
Persistent wheeze	0.9	(0.7, 1.3)	2.0	(1.3, 3.1)	0.9	(0.5, 1.4)
Attacks of wheezing						
Shortness of breath	1.1	(0.8, 1.4)	1.8	(1.3, 2.6)	0.9	(0.6, 1.4)
Awakened at night	1.0	(0.8, 1.4)	1.4	(0.9, 2.1)	1.1	(0.7, 1.7)
Wheeze with exercise	1.1	(0.8, 1.4)	1.5	(1.1, 2.1)	1.1	(0.8, 1.6)
Treatments for wheezing						
Medication for wheeze	1.1	(0.9, 1.4)	1.5	(1.1, 2.1)	1.0	(0.7, 1.4)
Emergency room for wheeze	1.3	(0.8, 2.0)	2.2	(1.3, 3.8)	1.1	(0.6, 1.9)

Definition of abbreviations: CI = confidence interval; ETS = household environmental tobacco smoke; OR = odds ratio.
* Models are adjusted by towns, age, grade, race, sex, family history of asthma and atopy, and gestational age. All outcomes are for the previous 12 months except ever and early onset asthma and ever wheezing. The reference groups for exposure main effects are GSTM1 (present), no *in utero* smoke exposure, and no current ETS exposure, respectively.

TABLE 4. ADJUSTED* ODDS RATIOS AND 95% CI FOR THE JOINT EFFECTS OF *IN UTERO* EXPOSURE TO MATERNAL SMOKING AND GSTM1 GENOTYPE ON ASTHMA AND WHEEZE, ODDS RATIO AND 95% CONFIDENCE INTERVAL

Outcomes	No *in utero* GSTM1 (+)	No *in utero* GSTM1 (−)		*In utero* GSTM1 (+)		*In utero* GSTM1 (−)	
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Asthma							
Ever asthma	Reference group	1.0	(0.8, 1.2)	0.9	(0.6, 1.4)	1.4	(0.9, 2.1)
Active asthma†	Reference group	0.8	(0.6, 1.1)	0.8	(0.5, 1.3)	1.7	(1.1, 2.8)
Medication for asthma†	Reference group	0.9	(0.7, 1.2)	0.7	(0.4, 1.2)	1.8	(1.1, 2.8)
Early onset asthma†	Reference group	0.9	(0.7, 1.2)	0.9	(0.7, 1.4)	1.6	(1.0, 2.5)
Persistent asthma†	Reference group	1.0	(0.8, 1.2)	0.9	(0.6, 1.4)	1.6	(1.1, 2.4)
Wheezing							
Ever wheezing	Reference group	1.0	(0.8, 1.2)	1.3	(1.0, 1.8)	1.8	(1.3, 2.5)
Wheeze with cold†	Reference group	1.0	(0.8, 1.2)	1.1	(0.8, 1.7)	1.8	(1.2, 2.7)
Wheeze without cold†	Reference group	1.0	(0.8, 1.3)	1.1	(0.7, 1.8)	2.3	(1.4, 3.5)
Persistent wheeze	Reference group	0.8	(0.6, 1.2)	1.6	(0.9, 2.8)	2.2	(1.3, 4.0)
Attacks of wheezing							
Shortness of breath	Reference group	1.0	(0.7, 1.3)	1.4	(0.9, 2.3)	2.3	(1.4, 3.8)
Awakened at night	Reference group	0.9	(0.7, 1.3)	1.1	(0.6, 1.9)	1.8	(1.0, 3.1)
Wheeze with exercise†	Reference group	0.9	(0.7, 1.2)	1.0	(0.6, 1.6)	2.1	(1.3, 3.3)
Treatments for wheezing							
Medication for wheeze†	Reference group	1.0	(0.7, 1.2)	1.0	(0.6, 1.5)	2.2	(1.4, 3.4)
Emergency room for wheeze†	Reference group	0.9	(0.5, 1.5)	1.0	(0.4, 2.4)	3.7	(1.9, 7.3)

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

* Models are adjusted for towns, age, grade, race, family history of asthma and atopy, gestational age, and current ETS exposure. All outcomes are for the previous 12 months except ever and early onset asthma, and ever wheezing.

† Significant interaction of *in utero* exposure to maternal smoking and GSTM1 ($p < 0.05$).

associated with asthma outcomes without *in utero* exposure. Notably, every wheezing endpoint assessed in this study was associated with *in utero* exposure in the GSTM1 null genotype group. Furthermore, effects among the *in utero* exposure and GSTM1 null genotype group appeared to be largest for wheezing outcomes with the greatest severity, emergency room visits, shortness of breath, wheezing without colds, medication use for wheezing, wheezing with exercise, and persistent wheezing. We found the same pattern of effects when we considered lifetime histories. *In utero* exposure was also associated with a history of ever wheezing in those with GSTM1 (+). Adjustment for family income, educational attainment, insurance, and housing characteristics resulted in little change in the effect estimates, and these covariates were not included in the final models. We found no statistically significant differences in these effects by ethnicity.

Exposure to ETS was not strongly associated with asthma or current or lifetime history of wheezing outcomes in children with either GSTM1 genotype (*see* Table E1 in the online data supplement). Those with GSTM1 null genotype but no ETS exposure again showed no associations with any of the outcomes of interest. Adjustment for family income, educational attainment, insurance, and housing characteristics resulted in little change in the effect estimates, and these covariates were not included in the final models.

DISCUSSION

We found that GSTM1 genotype modifies the effects of fetal tobacco smoke exposure on childhood asthma and wheezing. The adverse effects of *in utero* exposure to maternal smoking on a broad range of asthma and wheezing outcomes were largely restricted to children with GSTM1 null genotype. Our findings indicate that there are important long-term effects of *in utero* exposure in a genetically susceptible group of children.

A growing body of evidence supports an independent effect of *in utero* exposure to maternal smoking on wheezing and asthma occurrence during childhood (10, 11, 13, 27–31). Although variation in susceptibility for some of the adverse effects of tobacco smoke is well established among adults, less is known about the factors that influence susceptibility to prenatal tobacco smoke exposure (6, 11, 13, 16, 17, 32). Although it is clear that *in utero* exposure has direct effects on normal development consistent with its effects on birth weight, genetic variation may also contribute to the effects of *in utero* exposure on wheezing and asthma, as indicated by larger effects among children with a family predisposition for asthma (33, 34). Based on our findings, GSTM1 genotype may be an important susceptibility factor for childhood asthma after exposures during the fetal period. Because *in utero* exposure has adverse effects beyond wheezing and asthma occurrence, the joint effects of GSTM1 and *in utero* exposure on other health outcomes warrants additional study.

Our findings are consistent with *in utero* exposure increasing asthma occurrence by altering critical developmental pathways leading to lower lung function, increased bronchial hyperresponsiveness (BHR), and a permanent predisposition to asthma and wheezing (35). *In utero* exposure is associated with deficits in lung function at birth that may persist into young adulthood (36–41). The resultant persistent deficits in small airway function may predispose children to wheezing during respiratory infections or other events that produce inflammation, subsequent BHR, and variable airflow obstruction (42). Studies of neonates show that maternal tobacco smoke exposure during the *in utero* period is associated with increased BHR, especially in those with a family history of asthma (43). Animal studies also suggest that exposure during the period of lung development leads to BHR (44). Chronically increased BHR from *in utero* exposure may contribute to persistent wheezing and increased asthma predisposition and diagnosis (6, 43). Furthermore, *in utero* exposure may affect the development and maturation of the pulmonary immune system (33). Inappropriate persistence of a TH2-dominant response pattern appears to increase likelihood of allergic sensitization upon sufficient exposure to a variety of common

antigens (45). It is also possible that fetal ingestion of tobacco smoke products present in the amniotic fluid may have long-term effects on gut immune responses that appear to be important in allergic sensitization (46). Based on these findings, it is biologically plausible that toxins from *in utero* exposure to maternal smoking influence sensitization to common antigens, inflammation, decreased lung function, and increased BHR with variable obstruction to increase the occurrence of childhood wheezing and asthma.

A number of defenses exist to limit the damage from tobacco smoke. Phase II xenobiotic metabolizing enzymes play a central role in elimination of activated xenobiotics and in antioxidant defenses (47–52). GSTs are an important superfamily of Phase II enzymes that conjugate reactive intermediates with glutathione to produce less reactive water-soluble compounds that can be more easily excreted (20). Notably, the product of GSTM1 also functions in antioxidant defenses by detoxifying hydroperoxides from oxidant attack (20). Thus, GSTM1 may play a role because it is involved in critical pathways that modulate the effects of reactive intermediates and oxidative stress from tobacco smoke (52). GSTM1 may be especially important during the fetal period because the effects of a given level of tobacco-related toxins are greater in the fetus compared with the mother (16). It follows that variation in the amount or function of this enzyme modulates the amount of tobacco smoke-related toxins that influence asthma occurrence.

Our study has some limitations that influence the interpretation of our results. The findings are based upon cross-sectional data collected at cohort entry and are subject to the selection bias, information bias, and problems with temporality inherent in cross-sectional studies. The group of children with genotyping data included in this analysis did not differ from those without genotyping data on many demographic, medical history, and household exposure factors, but did show small differences in the proportion of those exposed to tobacco smoke and in family income (data not shown). The differences arose because children from lower income families with a higher prevalence of smoking were more likely to move and be lost to follow-up. Because the differences in distribution were modest and are probably not associated with the child's genotype, it is unlikely that selection of subjects biased the effect estimates for *in utero* or ETS exposure. However, parents or children may change their time-activity patterns to avoid ETS exposure. We lack data to directly assess changes in time-activity patterns after the diagnosis of asthma or onset of wheezing symptoms. We note that the proportion of children with asthma who were exposed to ETS in the past but not currently (40%) was approximately the same as that for children without asthma (43%), suggesting that adult smoking patterns did not differentially change over time. Differential participation by children with asthma who had different tobacco smoke exposure histories is unlikely to have been large enough to produce substantial bias because participation rates were high.

The possible effects of population stratification must be considered because we studied a multi-ethnic population using a population-based case-control design (53). Confounding from population stratification can occur when subpopulations such as ethnic groups have different disease risk and allele frequencies. We found that the GSTM1 null genotype varied by ethnicity from 27% to 46% and the ethnic variation in risk for the asthma and wheezing outcomes was less than twofold in our population. We included ethnicity in all models to account for possible confounding from population stratification. We did not have information on finer categories within our five categories of ethnicity, thus residual confounding is possible, although a bias that could explain our results is unlikely for the following reasons. First, in this analysis, ethnicity was, at most, a weak confounder, suggesting that population stratification did not introduce a large bias in our study. Second, based on the gene frequencies and the variation in risk among ethnic groups, the possible magnitude of confounding from population stratification is small. Moreover, estimates of interactions between exposures and genes are less sensitive to confounding by population stratification than those for gene effects.

In the present study, ETS exposure was at most weakly associated with asthma or wheezing occurrence overall or among the group with GSTM1 null genotype. We have previously reported that ETS exposure was associated with wheezing, but was not associated with physician-diagnosed asthma (54). Although the results appear to differ in the analyses based on the same study population, the effect estimates are similar in both studies (odds ratios ranging from 1.1–1.4) and to other results reported from meta-analyses of the effects of parental smoking on wheezing showing a summary odds ratio for ETS and wheezing of 1.2 (95% confidence interval 1.2–1.3) (10). The present study had lower power to detect small effects due to a reduced sample size with DNA available for genotyping (2,950 versus 5,762 children).

The smaller effect estimates for ETS may also have been the result of inaccurate retrospective recall of tobacco smoking that produced some misclassification of exposure status. Exposure to tobacco smoke was assessed using questionnaire responses about household sources and was not validated by objective measurements such as cotinine levels. All subjects in the study were older than 8 years of age at enrollment, and it is likely that those susceptible had already developed adverse respiratory outcomes by the time parents were questioned about *in utero* and environmental tobacco smoke exposure. However, the validity of exposure estimates based on questionnaire responses has been investigated and found to provide reasonably valid estimates of exposure (6, 11, 13). It is possible that parents of children with asthma may have underreported tobacco smoke exposure and biased our results toward the null. Because any recall bias would be independent of GSTM1 genotype, this factor is unlikely to explain the interaction between *in utero* exposure and GSTM1 genotype.

Assessment of *in utero* exposure may also have been imperfect. Although smoking is associated with an increasing social stigma, it seems unlikely that mothers would admit to smoking during pregnancy, but falsely deny smoking in the postnatal period. We were unable to investigate any dose–response relationships for *in utero* exposure because we lacked information on the intensity or duration of exposure. However, the dose to the fetus may be low, as pregnant women do not generally smoke as heavily as nonpregnant women, averaging 10 cigarettes per day (55). We also lack information on a number of potential confounders such as maternal nutritional status and intake of alcohol or other potentially toxic substances during pregnancy.

Finally, asthma was ascertained by parental report of physician-diagnosed asthma, so misclassification of asthma status or age at diagnosis may have arisen from imperfect parental recall of events, variation in access to medical care, differences in medical practice, or delay in diagnosis. More than 80% of participants had medical insurance, suggesting that any bias from differential access to care is likely to be small. We lack data to assess the magnitude of misclassification of asthma status from parental recall or medical practice; however, it is unlikely that our findings result from a spurious association that arose from consistent variations in medical practice across the

12 communities or from smokers overreporting asthma in their children.

A broad research program is needed to confirm and further investigate the relationships between *in utero* exposure, genetic variants, and asthma and wheezing occurrence. Additional population-based genetic epidemiologic studies of sufficient size are needed to replicate and expand our findings to additional genes and pathways. Prospective assessment of exposure and asthma and wheezing status might improve validity. Furthermore, the role of maternal genotype and exposures during pregnancy needs to be studied. Large studies will be necessary to assess gene–tobacco smoke and gene–gene interactions in relationship to asthma and wheezing occurrence.

We have identified a group of children who are at high risk for asthma and wheezing during childhood after *in utero* exposure to tobacco smoke. Our findings indicate that there are important long-term effects of *in utero* exposure in a genetically susceptible group of children. Because maternal smoking is common and the null genotype occurs in nearly half of the general population, this high-risk group may be an important target population for preventive intervention.

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