Genetic Variation in the Glutathione Synthesis Pathway, Air Pollution, and Children's Lung Function Growth

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Rationale: Glutathione plays an important role in antioxidant and inflammatory processes in the lung. Alterations in glutathione metabolism are a central feature of several chronic lung diseases.

Objectives: To determine whether sequence variation in genes in the glutathione synthesis pathway alters susceptibility to air pollution effects on lung function.

Methods: In this prospective study, 14,821 lung function measurements were taken on 2,106 children from 12 Southern California cities. Tagging single-nucleotide polymorphisms in glutathione metabolism pathway genes *GSS*, *GSR*, *GCLM*, and *GCLC* were genotyped by GoldenGate assay (Illumina, San Diego, CA). Mixed regression models were used to determine whether particular haplotypes were associated with FEV₁, maximal mid-expiratory flow rate, and FVC and whether any of the genetic associations varied with levels of exposure to air pollutants.

Measurements and Main Results: We found that variation in the *GSS* locus was associated with differences in susceptibility of children for lung function growth deficits associated with NO₂, PM₁₀, PM_{2.5}, elemental carbon, organic carbon, and O₃. The negative effects of air pollutants were largely observed within participants who had a particular *GSS* haplotype. The effects ranged from -124.2 to -149.1 for FEV₁, from -92.9 to -126.7 for FVC, and from -193.9 to -277.9 for maximal mid-expiratory flow rate for all pollutants except O₃, which showed a larger decrease in lung function in children without this haplotype.

Conclusions: Variation in *GSS* was associated with differences in susceptibility to adverse effects of pollutants on lung function growth.

Keywords: *GSS*; glutathione; lung function; oxidative stress; air pollution

Glutathione (γ -glutamyl-cysteinyl-glycine, GSH) is the most abundant intracellular antioxidant thiol and plays a vital role in the lung, defending airway epithelium from damage in response to oxidants and inflammation (1–3). Changes in the levels of GSH in the lung have been associated with pulmonary diseases such as idiopathic pulmonary fibrosis, acute respiratory distress syndrome, cystic fibrosis, and asthma (1, 4, 5).

At the molecular level, increased reactive oxygen species levels have been implicated in initiating inflammatory responses in the lungs through the activation of transcription factors, signal transduction, chromatin remodeling, and gene expression of proinflammatory mediators (6). Lung epithelium is constantly

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Air pollution has been associated with deficits in lung function development and respiratory illness in children and adults. However, relatively little is known about whether genetic variation contributes to underlying differences in susceptibility to air pollution.

What This Study Adds to the Field

Genetic variation in the glutathione synthesis pathway was associated with differences in the susceptibility of children to the harmful effects of several air pollutants on lung function growth.

exposed to oxidants in the ambient air, including oxygen, ozone, nitrogen dioxide, particulate matter, and cigarette smoke.

Exposure to such environmental oxidants can cause oxidant–antioxidant imbalance, which contributes to inflammation and respiratory diseases such as chronic obstructive pulmonary disorder and asthma (1, 4, 5). Exposure to ambient air pollutants and cigarette smoke has also been associated with depletion of intracellular GSH in animals and humans (5, 7–9). Such acute depletion is typically followed by a later rebound increase in GSH in epithelial cells as an adaptive response to oxidant stress (2).

GSH exists primarily in two redox forms, that is, reduced GSH and oxidized glutathione disulfide (GSSG). Reduction of GSH is catalyzed by GSH reductase (encoded by *GSR*). In response to oxidative stress, *de novo* synthesis of GSH from its amino acid constituents is an essential adaptive response. GSH synthesis is catalyzed by GSH synthetase (encoded by *GSS*) and glutamate cysteine ligase (GCL), which itself is a heterodimer (encoded by *GCLC* and *GCLM*). The rate of GSH synthesis is controlled by GCL, typically because cellular levels of the amino acid cysteine are rate-limiting (1). Polymorphisms in glutathione genes have been shown to affect GSH synthesis (2, 10) and have been associated with reduction in lung function (11, 12).

Given the known role of glutathione genes in oxidative stress, we hypothesize that genetic variation in genes in the glutathione metabolic pathway may influence the association between ambient air pollutant exposures and lung function growth in children. In this study, we investigated whether genetic variation in glutathione genes *GSS*, *GSR*, *GCLC*, and *GCLM* was associated with lung function growth in healthy children, using data collected on 2,106 children over an 8-year time period as part of the Children's Health Study (CHS) (13). Because glutathione plays a critical role in antioxidant defense and exposure to ambient air pollutants is known to increase oxidative stress, we evaluated whether genetic variation in these genes altered the susceptibility to air pollutant–induced deficits in lung function.

Some of the results of these studies have been previously reported in the form of an abstract (14).

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METHODS

Study Subjects

Two cohorts of fourth-grade children, one in 1993 (cohort 1, $n = 1,759$) and the second in 1996 (cohort 2, $n = 2,004$), were enrolled and monitored for 8 years, through twelfth grade in public high school. Children were recruited from schools in 12 southern California communities as part of an investigation of the long-term effects of air pollution on children's respiratory health (13, 15). Pulmonary function and questionnaire data were obtained annually by trained field technicians at the schools. Details of the testing protocol have been previously published (15, 16). Questionnaire details are provided in the online supplement. Of the 3,677 children initially recruited, complete genetic information was available for 2,106 (56%).

The study protocol was approved by the institutional review board for human studies at the University of Southern California (Los Angeles, CA), and consent was provided by a parent or legal guardian for all study subjects.

Air Pollution

The CHS has collected data on major air pollutants from central monitoring stations in each of the original 12 study communities from 1994 to the present. Each station measured average hourly levels of ozone (O_3), nitrogen dioxide (NO_2), and particulate matter with an aerodynamic diameter of less than $10\ \mu m$ (PM_{10}). Stations also collected 2-week integrated-filter samples for measuring the mass and chemical makeup of particulate matter with an aerodynamic diameter of less than $2.5\ \mu m$ ($PM_{2.5}$). Levels of elemental carbon and organic carbon were also measured, using method 5040 of the National Institute for Occupational Safety and Health (16). We computed annual averages on the basis of average levels in a 24-hour period in the case of PM_{10} and nitrogen dioxide, and a 2-week period in the case of $PM_{2.5}$, elemental carbon, and organic carbon. For ozone, we computed the annual average of the levels obtained from 10 A.M. to 6 P.M. (the 8-h daytime average). We also characterized every study participant's exposure to traffic-related pollutants by assessing the child's residential proximity to the nearest freeway (17).

Laboratory Methods

Buccal cells were collected from participants and used as the source of DNA for genotyping assays. DNA was extracted with a Gentra Pure-gene DNA isolation kit (Qiagen, Valencia, CA). Fifty-four tagging single-nucleotide polymorphisms (SNPs) in *GSS*, *GSR*, *GCLC*, and *GCLM* genes were genotyped, using the GoldenGate platform (Illumina Inc, San Diego, CA).

SNP Selection and Genotyping for Illumina Assay

Details of the SNP selection process are provided in the online supplement. SNPs were genotyped with the BeadArray platform, a highly accurate, high-throughput assay (Illumina, Inc). Fifty-four SNPs were used in this analysis across the 4 genes, with a squared correlation between haplotypes (R^2_h) of greater than 0.95 for each gene. RS numbers, gene location, minor allele frequencies, and Hardy-Weinberg equilibrium values are shown in Table E1 in the online supplement and the *GSS* locus is shown in Figure E1. Haplotype frequencies of unphased *GSS*, *GSR*, *GCLC*, and *GCLM* SNPs for Hispanic and non-Hispanic white children are shown in Table E2. For *GSS* and *GCLM*, two haplotype blocks, and for *GSR*, three haplotype blocks, were initially determined by the Gabriel method (18). Within each locus, haplotype blocks were combined into one block in the presence of strong linkage disequilibrium (i.e., high multiallelic D'). For *GCLC*, nine haplotype blocks were initially determined; however, we lacked SNPs in three of these, and the rest could be combined into three distinct haplotype blocks, given the high degree of linkage disequilibrium.

Statistical Methods

The data consisted of 14,821 pulmonary function tests recorded from 2,106 participants during 8 years of follow-up. Three measures of pulmonary function were evaluated: FVC, FEV_1 , and maximal mid-expiratory flow rate (MMEF, also known as FEF_{25-75}).

A hierarchical mixed effects model was used to relate 8-year growth in each lung function measure to haplotypes with a basic model structure that has been previously described (19). Growth patterns in lung function were modeled using linear splines with knots at ages 12, 14, and 16 years (17), parameterized so that 8-year growth in lung function was estimated jointly with other model parameters. Random effects for the intercept and 8-year growth parameters were included at the subject level. Likelihood ratio tests in combination with haplotype analyses were used to test the global significance of each gene in its entirety, and by haplotype block, in relation to 8-year lung function growth. Whether genetic variation altered the effects of several air pollutants and traffic exposures on lung function growth, including average annual levels of NO_2 , PM_{10} , $PM_{2.5}$, elemental carbon, organic carbon, O_3 , and distance to freeway, was also evaluated. Interactions between these air pollutants and haplotypes were tested using likelihood ratio tests comparing models with and without the appropriate interaction terms for evidence of statistical significance. In stratified analyses, haplotype was categorized as having at least one copy of the "010000" haplotype versus no copies of this haplotype.

We allowed for separate growth curves by sex, race, ethnicity, cohort, and baseline asthma status at baseline across the age range of 10 to 18 years with knots at ages 12, 14 and 16. The model also included adjustments for height, height squared, body mass index (BMI), BMI squared, current asthma status, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the last year, glutathione S-transferase mu 1 (*GSTM1*) genotype, and indicator variables for the field technician.

To address potential confounding by population stratification, four coefficients of ancestry variables were also included in the model (20, 21). These variables were constructed from four principal components derived from a set of 233 unlinked ancestry informative markers that were selected to differentiate four parental populations (African, European, American Indian, and East Asian). Controlling for these ancestry variables provided adjustment for ancestral history beyond adjustment for typical self-reported racial and ethnic categories. A sensitivity analysis evaluating the effects of air pollutants on lung function growth only within non-Hispanic white subjects was also conducted.

Regression procedures in SAS version 9.1 (SAS Institute Inc, Cary, NC) were used to fit all models. Associations denoted as statistically significant were those that yielded a P value less than 0.05, assuming a two-sided alternative hypothesis.

RESULTS

Over 8 years of follow-up, an average of seven pulmonary function tests were performed per child. Eight-year growth in FVC, FEV_1 , and MMEF averaged 1,569 ml, 1,367 ml, and 1,442 ml/second in girls and 2,848 ml, 2,441 ml, and 2,512 ml/second in boys. Approximately one-third of the sample members were of Hispanic origin (Table 1). By design, participants differed from nonparticipants in their ethnic composition and cohort from which they were recruited. Genetic analyses were restricted to non-Hispanic white and Hispanic white groups only. Slight differences in age and sex were also observed.

Of the four genes involved in glutathione synthesis that were evaluated, only *GSS* was significantly associated with lung function (FEV_1 and MMEF) in global haplotype analyses (Table E3). Therefore, further investigation of haplotypes was conducted only for *GSS*. Four primary haplotypes were identified within the *GSS* locus (Table E2). When we evaluated the association between *GSS* haplotypes and lung function, two complementary haplotypes, "101111" and the most common haplotype, "010000," were both associated with FEV_1 (Table E4 and Table 2) in opposite directions. Haplotype "010000" was associated with a 39.6 ml, 29.1 ml, and 51.0 ml/s reduction in 8-year growth of FEV_1 , FVC, and MMEF, respectively (Table 2). These associations appeared stronger in non-Hispanic white children than in Hispanic white children, although tests of the interaction did not achieve statistical significance (Table E5).

TABLE 1. BASELINE CHARACTERISTICS FOR THE 2,106 FOURTH-GRADE CHILDREN'S HEALTH STUDY PARTICIPANTS IN LUNG FUNCTION ANALYSES COMPARED WITH NONPARTICIPANTS

Variable	Participants (<i>n</i> = 2,106)		Nonparticipants (<i>n</i> = 1,657)		<i>P</i> Value
	<i>n</i>	%	<i>n</i>	%	
Sex					
Boys	1,022	48.5	858	51.8	0.05
Ethnicity					
Non-Hispanic white	1,397	66.3	653	39.4	<0.0001
Hispanic white	709	33.7	407	24.6	
Black	0	0	193	11.7	
Asian	0	0	174	10.5	
Other	0	0	230	13.9	
Ever diagnosed with asthma	310	15.0	208	13.1	0.10
Cohort					<0.0001
Recruited in 1993	911	43.3	848	51.2	
Recruited in 1996	1,195	56.7	809	48.8	
Height (cm), mean (SD)	139.8 (6.8)		140.0 (7.6)		0.32
BMI (kg/m ²), mean (SD)	18.3 (3.5)		18.5 (3.7)		0.08
Age (yr), mean (SD)	10.0 (0.6)		10.1 (0.7)		0.01

Definition of abbreviation: BMI = body mass index.

We next evaluated whether genetic variation in the *GSS* gene altered the susceptibility to air pollutant-induced decrements in lung function. We previously reported that ambient air pollutants, particularly traffic-related pollutants, are associated with deficits in lung function growth in children (16). These results are summarized again in Table E6 for the specific population in this study. NO₂, PM_{2.5}, PM₁₀, organic carbon, and elemental carbon, which are all highly correlated pollutants in our study communities, were associated with decreases in average growth of FEV₁, FVC, and MMEF over an 8-year period whereas no effect was observed for O₃ (16). In analyses stratified by *GSS* haplotype, the negative effects of the correlated pollutants were observed largely within participants who had the "0100000" haplotype. The magnitude of effects within this haplotype ranged from −124.2 to −149.1 for FEV₁, from −92.9 to −126.7 for FVC, and from −193.9 to −277.9 for MMEF for all pollutants except ozone (Table 3). Tests of interaction were marginally significant for NO₂ and FEV₁, elemental carbon and FEV₁ and MMEF, and statistically significant for NO₂ and O₃ with MMEF. Figures 1a and 1b illustrates the difference in magnitude of air pollution effects on 8-year growth in FEV₁ within each haplotype. Figure 1 clearly demonstrates that having a particular haplotype affects an individual's response to air pollution on lung function. Effects of pollutants on FVC and MMEF were similar (Table 3).

TABLE 2. ASSOCIATION OF *GSS* HAPLOTYPE H0100000* VERSUS ALL OTHER HAPLOTYPES WITH 8-YEAR GROWTH: CHILDREN'S HEALTH STUDY, FOURTH-GRADE COHORTS

Lung Function	β [†]	95% CI		<i>P</i> Value
FEV ₁	−39.6	−71.1	−8.0	0.01
FVC	−29.1	−63.6	5.5	0.10
MMEF	−51.0	−108.7	6.7	0.08

Definition of abbreviations: BMI = body mass index; CI = confidence interval; MMEF = maximal mid-expiratory flow rate; SNP = single-nucleotide polymorphism.

* Haplotype was defined by the following SNPs, in order: RS6087649, RS1801310, RS2273684, RS6060124, RS6060127, RS3761144, and RS3761143, where "1" is the variant and "0" is the common variant allele.

[†] Models are adjusted for height, sex, BMI, whether the child ever had asthma, respiratory illness at time of testing, exercise, smoking, ethnicity, cohort, town, field technician, *GSTM1* genotype, and ancestry indicators (*q* factors).

In two pollutant models evaluating both NO₂ and O₃ simultaneously, the effects of NO₂ and O₃ on 8-year lung function growth within genotype strata remained consistent, suggesting that these are independent effects (Table 4). In addition, when models were restricted to non-Hispanic white subjects only, similar results were observed (Table E7). No interactions with traffic metrics such as distance to freeway were observed.

DISCUSSION

GSH plays a vital role in the lung, defending airway epithelium from damage in response to oxidants and inflammation (1, 2). However, little is known about the role of glutathione, or about the genes that regulate it, with respect to normal lung function development in children exposed to air pollution. In this study, we observed that variation in the glutathione synthesis gene, *GSS*, was associated with differences in susceptibility to the harmful effects of ambient air pollution on children's lung function growth.

We have previously demonstrated a negative association between air pollutants and lung function growth (16). Whereas the average levels of ozone were not significantly correlated across communities with any other study pollutant, the correlations between other pairs of pollutants were all significant. Thus, nitrogen dioxide and the particulate matter pollutants can be regarded as a correlated suite of pollutants with a similar pattern relative to each other across the 12 communities. In this study, we observed that the negative effects of this suite of air pollutants on children's lung function growth largely occurred in children with the "0100000" haplotype (prevalence of 48%) but not in children with other *GSS* haplotypes. In single-pollutant models, an opposite trend was observed for ozone. The effects of ozone on lung function growth were most detrimental in children who did not have the "0100000" haplotype. Results remained consistent in models that jointly adjusted for O₃ and NO₂ (as a surrogate for our correlated pollutants).

The *GSS* gene, which encodes human glutathione synthetase, contains 12 coding and 1 noncoding exon and is located at chromosome 20q11.2 (10). Mutations in *GSS* have been shown to affect stability, catalytic capacity, and substrate affinity of the enzyme (10). The substrate of *GSS* can be used for two different reactions, making GSH and making γ-glutamylcyclotransferase. Variation in *GSS* could alter the enzymatic reactions *in vivo*

TABLE 3. DIFFERENCE IN AVERAGE GROWTH IN LUNG FUNCTION OVER THE 8-YEAR STUDY PERIOD FROM THE LEAST TO THE MOST POLLUTED COMMUNITY, USING SINGLE-POLLUTANT MODELS AND BY GSS HAPLOTYPE

Outcome	Pollutant	Haplotype 0100000 (<i>n</i> = 1,010)				Other Haplotypes (<i>n</i> = 1,096)				Interaction <i>P</i> Value
		β^*	95% CI	<i>P</i> Value		β^*	95% CI	<i>P</i> Value		
FEV ₁	Elemental carbon	-135.9	-208.5	-63.4	0.0002	-12.1	-131.4	107.3	0.84	0.10
	Organic carbon	-158.7	-252.9	-64.4	0.001	-49.1	-212.5	114.3	0.56	0.36
	NO ₂	-145.3	-227.7	-62.9	0.001	0.8	-132.6	134.3	0.99	0.08
	O ₃	25.9	-102.4	154.3	0.69	-76.6	-224.3	71.1	0.31	0.11
	PM ₁₀	-149.1	-242.3	-55.8	0.002	-52.8	-213.4	107.8	0.52	0.45
	PM _{2.5}	-124.2	-203.1	-45.3	0.002	-49.0	-181.9	83.9	0.47	0.46
FVC	Elemental carbon	-115.6	-196.4	-34.8	0.01	-75.5	-201.4	50.4	0.24	0.71
	Organic carbon	-126.7	-231.7	-21.6	0.02	-127.9	-299.7	43.9	0.14	0.82
	NO ₂	-117.0	-210.2	-23.7	0.01	-59.3	-200.3	81.7	0.41	0.59
	O ₃	0.1	-130.0	130.1	1.00	-17.2	-174.6	140.3	0.83	0.83
	PM ₁₀	-115.1	-222.4	-7.8	0.04	-129.0	-298.0	40.1	0.13	0.71
	PM _{2.5}	-92.9	-186.0	0.2	0.05	-106.8	-246.9	33.2	0.13	0.70
MMEF	Elemental carbon	-209.7	-344.2	-75.2	0.002	16.0	-197.7	229.6	0.88	0.09
	Organic carbon	-200.7	-408.3	7.0	0.06	-127.9	-299.7	43.9	0.14	0.40
	NO ₂	-277.9	-429.9	-125.9	0.0003	50.1	-188.2	288.5	0.68	0.03
	O ₃	136.5	-80.7	353.7	0.22	-200.3	-466.9	66.2	0.14	0.01
	PM ₁₀	-200.5	-404.0	3.0	0.05	-43.1	-331.3	245.0	0.77	0.41
	PM _{2.5}	-193.9	-352.2	-35.6	0.02	-70.9	-309.1	167.3	0.56	0.44

Definition of abbreviations: BMI = body mass index; CI = confidence interval; MMEF = maximal mid-expiratory flow rate; PM₁₀ and PM_{2.5} = particulate matter with an aerodynamic diameter of less than 10 and 2.5 μ m, respectively; ppb = parts per billion.

* Values represent the differences in the estimated rate of 8-year growth at the lowest and highest observed levels of the indicated pollutant. Differences are scaled to range across the 12 study communities in the average level of each pollutant from 1994 through 2000 as follows: 36.3 ppb O₃ (measured from 10 A.M. to 6 P.M.), 33.9 ppb NO₂, 52.2 μ g of PM₁₀ per cubic meter, 22.2 μ g of PM_{2.5} per cubic meter, 1.2 μ g of elemental carbon per cubic meter, and 10.5 μ g of organic carbon per cubic meter. Models are adjusted for height, sex, BMI, whether the child ever had asthma, respiratory illness at time of testing, exercise, smoking, ethnicity, cohort, town, field technician, *GSTM1*, and ancestry indicators (*q* factors).

in favor of creating γ -glutamylcyclotransferase, thereby decreasing availability of the antioxidant GSH. A wide-scale reduction in available GSH would have long-term implications for antioxidant defense in the developing lungs of children under normal conditions, which would be magnified only under conditions of higher oxidative stress, such as when exposed to higher air pollutant levels.

Exposure to air pollutants has been associated with an initial depletion of intracellular GSH followed by a later rebound increase in GSH as an adaptive response to oxidant stress (2). Genetic variation in GSH genes that diminishes GSH synthesis might exacerbate air pollutant-induced lung injury by delaying this ability to rebound in response to stimuli. The "0100000" haplotype is the most common haplotype of *GSS*, occurring in 48% of the children. In our population, this haplotype can be distinguished from all others by evaluating the SNP RS1801310. However, the RS1801310 SNP is an intronic tag SNP whose functionality is currently unknown. Further investigation of genetic variation in *GSS* in relation to this haplotype may shed light on which particular regions of the gene or nearby genes in

linkage disequilibrium are most relevant in association with air pollution-induced deficits in lung function.

The observed association in our data that haplotype "0100000" appears protective against the effects of ozone on MMEF is puzzling. The biological mechanisms through which air pollutants trigger oxidative stress pathways are not well characterized. Ozone and other pollutants may trigger different biological responses to exposure that are reflected, in part, by our observed differences in lung function effects by *GSS* haplotype. In support of our ozone results, an experiment in mice known to be deficient in GSH demonstrated that these mice had less ozone-induced lung injury than did wild-type mice (22). Although the mouse model involved knockout of *GCLM* rather than the *GSS* gene, genetic variation in *GSS* that reduces GSH may also lead to similar results, as our data suggest.

A strength of this study was the long-term, prospective nature of the data with consistent follow-up and measurement of exposure and outcome data. However, certain limitations should also be considered. Given the complex modeling framework for these analyses, we are limited in our power to detect sig-

Figure 1. Community-specific average growth in FEV₁ (a and b) over 8 years plotted against average NO₂ and O₃ levels, by *GSS* haplotype. Gray squares and the dashed trend line indicate clusters of children with at least one copy of the *GSS* haplotype "0100000," and solid triangles with the solid trend line indicate children without this haplotype.

TABLE 4. DIFFERENCE IN AVERAGE GROWTH IN LUNG FUNCTION OVER THE 8-YEAR STUDY PERIOD FROM THE LEAST TO THE MOST POLLUTED COMMUNITY, USING TWO-POLLUTANT MODELS AND BY GSS HAPLOTYPE

Outcome	Pollutant	Haplotype 0100000				Other Haplotypes				Interaction P Value
		β^*	95% CI		P Value	β^*	95% CI		P Value	
FEV ₁	NO ₂	-143.9	-226.5	-61.4	0.001	-14.7	-151.9	122.5	0.83	0.17
	O ₃	14.0	-79.7	107.8	0.77	-86.5	-239.1	66.2	0.27	0.15
FVC	NO ₂	-118.0	-214.3	-21.8	0.02	-68.0	-213.3	77.2	0.36	0.66
	O ₃	-10.9	-119.9	98.0	0.84	-37.0	-198.8	124.9	0.65	0.81
MMEF	NO ₂	-265.7	-418.2	-113.1	0.001	4.6	-241.6	250.7	0.97	0.08
	O ₃	118.5	-54.8	291.8	0.18	-202.5	-478.5	73.6	0.15	0.02

Definition of abbreviations: BMI = body mass index; CI = confidence interval; MMEF = maximal mid-expiratory flow rate; ppb = parts per billion.

* Values represent the differences in the estimated rate of 8-year growth at the lowest and highest observed levels of the indicated pollutant. Differences are scaled to range across the 12 study communities in the average level of each pollutant from 1994 through 2000 as follows: 36.3 ppb O₃ (measured from 10 A.M. to 6 P.M.) and 33.9 ppb NO₂. Models are adjusted for height, sex, BMI, whether the child ever had asthma, respiratory illness at time of testing, exercise, smoking, ethnicity, cohort, town, field technician, *GSTM1*, and ancestry indicators (*q* factors).

nificant interactions because we are, in effect, testing a three-way interaction between air pollutant, age (for growth curve of lung function), and haplotype.

Confounding by population admixture is often a concern with genetic studies. We controlled for admixture by adjusting for ancestry variables in addition to typical adjustment for self-reported race and ethnicity. The ancestry variables provided better control for genetic descent of four distinct groups: African, European, American Indian, and East Asian. Adjusting for these variables did not appreciably change our results. In addition, a sensitivity analysis of our main results only among non-Hispanic white subjects supported our main results.

The observed effects could also be explained by underlying associations of the exposures and outcome to unmeasured confounding variables. Although we adjusted for known potential confounders including personal characteristics, the possibility of confounding by other factors still exists. For example, dietary intake of antioxidants may modulate effects of air pollution (23). However, we do not have information about dietary supplementation. Thus, if supplementation with antioxidants differed both by community (and thus air pollution level) as well as by lung function, residual confounding of our association may be present.

Use of residential address to assess air pollution exposure may result in misclassification of exposure, because activity patterns outside the home were not explicitly monitored. However, for the age range (10–18 yr) of our participants, we and others have found use of residential address to be a reasonable proxy for overall exposure. The catchment areas for the respective elementary schools participating in our study tended to represent small and well-defined neighborhood-scale areas in generally suburban areas. Written questionnaires documented their respective general patterns of activity, including how subjects got from home to school and whether weekday and weekend mornings and afternoons were spent indoors or outdoors. On the basis of these limited responses, exposure assignments based on home locations were judged to be generally representative of subjects' cumulative exposure.

Over the 8-year follow-up period, approximately 10% of study subjects were lost to follow-up each year. Attrition is a potential source of bias in a cohort study if loss to follow-up is related to both exposure and outcome. However, we did not see evidence that the loss of subjects was related to either baseline lung function or exposure to air pollution (16, 17).

CONCLUSIONS

We have shown that susceptibility to health effects of air pollution on lung function growth is associated with genetic

variation in the *GSS* gene, a gene involved in glutathione production and oxidative injury. Clinically important deficits in lung function at the age of 18 years were observed among a subset of study participants with a particular pattern of genetic variation. Given the prevalence of this haplotype, affecting 48% of the study population, and the importance of lung function as a determinant of morbidity and mortality during adulthood, continued emphasis on the identification of strategies for reducing levels of urban air pollutants for the most susceptible populations is warranted.

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