Review Article

Chronic effects of air pollution on respiratory health in Southern California children: findings from the Southern California Children's Health Study

Zhanghua Chen[1], Muhammad T. Salam[1], Sandrah P. Eckel[2], Carrie V. Breton[1], Frank D. Gilliland[1]

[1]Department of Preventive Medicine, Division of Environmental Health, [2]Department of Preventive Medicine, Division of Biostatistics, Keck School of Medicine, University of Southern California, Los Angeles, CA 90032, USA

Correspondence to: Frank D. Gilliland, MD, PhD. Southern California Environmental Health Sciences Center, Department of Preventive Medicine, University of Southern California, Keck School of Medicine, University of Southern California, 2001 N. Soto Street, Los Angeles, CA 90032, USA. Email: gillilan@usc.edu.

Abstract: Outdoor air pollution is one of the leading contributors to adverse respiratory health outcomes in urban areas around the world. Children are highly sensitive to the adverse effects of air pollution due to their rapidly growing lungs, incomplete immune and metabolic functions, patterns of ventilation and high levels of outdoor activity. The Children's Health Study (CHS) is a continuing series of longitudinal studies that first began in 1993 and has focused on demonstrating the chronic impacts of air pollution on respiratory illnesses from early childhood through adolescence. A large body of evidence from the CHS has documented that exposures to both regional ambient air and traffic-related pollutants are associated with increased asthma prevalence, new-onset asthma, risk of bronchitis and wheezing, deficits of lung function growth, and airway inflammation. These associations may be modulated by key genes involved in oxidative-nitrosative stress pathways via gene-environment interactions. Despite successful efforts to reduce pollution over the past 40 years, air pollution at the current levels still brings many challenges to public health. To further ameliorate adverse health effects attributable to air pollution, many more toxic pollutants may require regulation and control of motor vehicle emissions and other combustion sources may need to be strengthened. Individual interventions based on personal susceptibility may be needed to protect children's health while control measures are being implemented.

Keywords: Air pollution; traffic pollution; asthma; genetic susceptibility; respiratory disease

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Introduction

Adverse health effects of exposures to regulated air pollutants have been widely studied (1-6). Other pollutants including volatile organic compounds and ultrafine particles may also have adverse health effects (7), but these pollutants have not been as extensively studied. There is growing evidence for adverse respiratory health effects from ambient air pollutants and near-source local air pollutants such as automobile tailpipe emissions, a major source of air pollution in Southern California and many regions in the world. Recent studies have shown that both ambient air pollutants and near source exposure to traffic-related pollutants are associated with increased incidence of asthma (8-11), lung function deficits (12-14), and airway inflammation (15,16). Traffic-related combustion tailpipe emissions contain high concentrations of reactive gases and high concentrations of ultrafine particles among other toxic compounds. It should be noted that levels of the most toxic of these combustion products are not regulated in the current criteria framework, although the regulated ambient air pollutant levels decreased over the last decades (17).

Because children are more sensitive to the effects of

Table 1 Data collection variations among different cohorts in the Children's Health Study

Variables	Cohort A	Cohort B	Cohort C	Cohort D	Cohort E
Year start	1993	1993	1993	1996	2002
Year end	1995	1998	2001	2004	2016
No. of subjects	938	937	1,806	2,081	5,603
School grade	10	7	4	4	K
Communities*	town code: 1-12				town code: 1-3, 6-9, 11-16
Lung function	All years	All years	All years	All years	Starting from 2007
Fe_{NO}	N/A	N/A	N/A	N/A	Starting from 2005

*Town names represented by the town code: 1, Alpine; 2, Lake Elsinore; 3, Lake Gregory; 4, Lancaster; 5, Lompoc; 6, Long Beach; 7, Mira Loma; 8, Riverside; 9, San Dimas; 10, Atascadero; 11, Santa Maria; 12, Upland; 13, Glendora; 14, Anaheim; 15, San Bernardino; 16, Santa Barbara.

air pollution than adults, due to rapidly growing and developing lungs and immune systems, research about the long-term impact of air pollution on the growth of lung function and respiratory illnesses is important to guide air pollution regulation and early prevention of respiratory diseases in the future. The Children's Health Study (CHS) is one of the largest and most comprehensive investigations of the long-term consequences of air pollution on the respiratory health of children. The CHS has also studied the effects of air pollution on genetic and epigenetic variations in genes in oxidative/nitrosative stress pathway, and how the genetic and epigenetic variations in this pathway influence respiratory health outcomes. Results from the CHS have shown that both ambient air pollution (8,14,16,18-22) and traffic-related pollution (3,9,13,15) have adverse health effects. Additionally, children's vulnerability to air pollution may be increased by higher level of parental stress (23), inadequate antioxidant defenses including low levels of vitamins A and C (24), and variations in the expression or function of antioxidant and inflammatory genes, such as glutathione-S-transferases (*GST*s) (25-27), arginases (*ARG1* and *ARG2*) (28), and tumor necrosis factor-α (*TNF-α*) (29). In addition to the findings reviewed in 2003 (30), we will summarize more recent findings from the CHS to highlight the heavy burden to children's respiratory health of current air pollution levels, even though these levels are often below national air quality standard.

The Children's Health Study (CHS)

The CHS study design has been described in detail in previous publications (13,30-33). Briefly, more than 11,000 school children were selected from classrooms in 16 communities in multiple waves of subject recruitment starting in 1993 to maximize the differences in regional air pollution concentrations and mixtures (*Table 1*). Beginning from study entry and continuing until high school graduation, yearly questionnaires assessed the development of respiratory symptoms and current activity patterns. Lung function was measured annually through spirometry. School absences were actively ascertained to evaluate the effects of pollution on acute respiratory illnesses. Outdoor concentrations of ozone (O_3), particulate matter (PM) of less than 2.5 μm and less than 10 μm aerodynamic diameter ($PM_{2.5}$ and PM_{10}, respectively), and nitrogen dioxide (NO_2) were measured continuously at central monitoring stations within each community. Several metrics of traffic-related pollution have been used, including (I) proximity of the residence to the nearest freeway or roadway; (II) average number of vehicles traveling within 150 m of the residence each day; (III) model-based estimates of traffic-related air pollution at the residence or school derived from dispersion models (CALINE) (8,12,34) and land-use regression exposures models (13).

For the cohort of kindergarten and first grade student recruited in 2003 to study the relationship between air pollution and airway inflammation (Cohort E, *Table 1*), exhaled nitric oxide (Fe_{NO}) was collected using both an offline breath collection technique according to American Thoracic Society (ATS) guidelines [ATS 1999; ATS/European Respiratory Society (ATS/ERS) 2005] in the initial years of the study, and an online Fe_{NO} collection in subsequent study years (32).

Participants provided DNA beginning in 1998 using standard buccal cell collection procedures (35). Genomic DNA was isolated using a Puregene DNA isolation kit

(Gentra Systems, Minneapolis, MN). Each genotype was validated by using PCR/restriction fragment length polymorphism methods (36). A nested case-control sample of 769 asthmatics and 1007 controls, who were either Hispanic white (n=817) or non-Hispanic white (n=959), were selected into CHS genome-wide association study. The genotyping was performed at the USC Epigenome Center using the Illumina HumanHap550, HunmanHap550-Duo or Human610-Quad BeadChip microarrays.

Main findings

Air pollution associations with asthma occurrence

Ambient air pollution has been associated with asthma prevalence and incidence in the CHS. In Cohort C,D, higher local NO_2 concentrations were associated with higher asthma prevalence [odds ratio (OR), 1.83; 95% confidence interval (CI): 1.04-3.22; per interquartile range (IQR) =5.7 ppb NO_2] after adjusting for sex, race, Hispanic ethnicity, cohort, and community (8) and higher risk of new-onset asthma [hazard ratio (HR), 1.29; 95% CI: 1.07-1.56; per IQR of 6.2 ppb NO_2] after adjusting for Hispanic ethnicity, medical insurance, cohort, community, and relative humidity (9). In Cohort A-D, Regional O_3 was associated with asthma incidence, but this association was modified by exercise (11). Specifically, the relative risk of asthma incidence associated with high regional O_3 was three times higher among children playing three or more team sports, compared to children playing no sports after adjusting for ethnicity and community with baseline strata for age and sex (OR, 3.3; 95% CI: 1.9-5.8). A statistically significant positive association between number of team sports played and asthma incidence was observed only in communities with high O_3 (means of O_3 concentrations in high and low pollution communities =59.6 ppb and 40.0 ppb, respectively) (OR, 1.4; 95% CI: 1.0-2.1). However, in the subsequent Cohort E, regional NO_2 and O_3 did not appear to be associated with asthma incidence after additionally adjusting for non-freeway traffic-related pollution at home and school (HR, 1.37; 95% CI: 0.69-2.71; and HR, 1.01; 95% CI: 0.49-2.11, respectively) (10).

Across CHS cohorts, several metrics of traffic-related pollution have shown adverse effects on asthma prevalence and incidence, independent of regional ambient air pollution. In Cohort C and D, the risk of life-time asthma was 1.9-fold among children with closer residential distance to a freeway (below 25th percentile) compared to children with farther residential distance from a freeway (above 75th percentile) after adjusting for sex, race, Hispanic ethnicity, cohort, and community (OR, 1.89; 95% CI: 1.19-3.02) (8). An IQR [2.3 ppb nitrogen oxide (NO_x)] increase of CALINE estimated freeway-related NO_x was associated with more than 2-fold increased risk of lifetime asthma (OR, 2.22; 95% CI: 1.36-3.63) (8). In Cohort A-D, children with residences within 75 m of a major roadway had a 29% increased risk of lifetime asthma and a 50% increased risk of prevalent asthma after adjusting for age, sex, race community, and language of the questionnaire (English/Spanish) (3). In Cohort E, after adjustment for race/ethnicity and for baseline hazards strata of age at study entry and sex, and random effects of school and community, an IQR (8 ppb NO_x) increase in CALINE estimated non-freeway traffic-related pollutions near home and school were both associated with a 1.5-fold increased risk of new-onset asthma, and these results were robust to adjustment for ambient NO_2 (OR, 1.46; 95% CI: 1.16-1.84) for home; and OR, 1.45; 95% CI: 1.03-2.06 for school) (10). Recent results further suggested that the effect of traffic-related pollution on the risk of new-onset asthma can be modified by parental stress levels. After adjusting for race/ethnicity and community with baseline strata for age and sex, an IQR increase of non-freeway traffic-related pollution (21 ppb of NO_x) was associated with a 1.5 times (HR, 1.51; 95% CI: 1.16-1.96) higher hazard of incident asthma for children with high parental stress versus a 1.1 times (HR, 1.05; 95% CI: 0.74-1.49) higher hazard of incident asthma for children with low parental stress (23), where parental stress was assessed by perceived stress scale (PSS >4) (37). These results from the CHS are consistent with a growing body of evidence from international studies indicating that that exposure to vehicle emissions increases the risk of new-onset asthma (4,38,39).

Air pollution effects on children with asthma

Air pollution may play a role in the exacerbation of existing asthma. In the CHS, children with physician-diagnosed asthma had more chronic lower respiratory tract symptoms including bronchitis and phlegm production if they lived in communities with higher levels of NO_2, PM_{10}, and $PM_{2.5}$ (40). Two pollutant models showed that within-community variations in organic carbon (OC) (OR, 1.41 per ppb; 95% CI: 1.12-1.78) and NO_2 (OR, 1.07 per ppb; 95% CI: 1.02-1.13) had robust positive associations with the risk of bronchitis symptoms after adjusting for age, maternal and

child's smoking history, sex, race, community and other pollutants including PM, O_3, organic and inorganic acid, and elemental carbon (EC) (18).

Air pollution was also associated with acute respiratory symptoms including wheezing and asthma medication use. Amongst fourth-grade school children, an IQR (13.39 μg/m^3) increase in monthly average PM_{10} was associated with almost a 3-fold higher monthly prevalence of wheezing during the spring and summer months after adjusting for age, sex, race/ethnicity, community, home characteristics, and secondhand tobacco smoke (OR, 2.91; 95% CI: 1.46-5.80), but this association was not significant during the fall and winter months (19). Pollutants primarily produced by photochemistry were associated with asthma medication use. IQR increases in monthly average O_3 (27.83 ppb), nitric acid (HNO_3) (1.64 ppb), and acetic acid (2.66 ppb) levels were associated with 80% (OR, 1.80; 95% CI: 1.19-2.70), 80% (OR, 1.80; 95% CI: 1.23-2.65) and 60% (OR, 1.57; 95% CI: 1.11-2.21) more monthly prevalence of asthma medication use (19). Associations between air pollutants and asthma medication use were stronger among children who spent more time outdoors (OR, 3.07; 95% CI: 1.61-5.86 for O_3; OR, 1.93; 95% CI: 1.18-3.15 for HNO_3; and OR, 2.38; 95% CI: 1.37-4.14 for acetic acid, respectively), compared to children who spent less time outside. Recent findings suggest that traffic-related pollution was also associated with children's wheezing (41). Among kindergarten and first grade (Cohort E) children aging 4.4- to 8.9-year-old who were diagnosed with asthma, per increase of 9 minutes in school commuting time was significantly associated with 50% increase (OR, 1.54; 95% CI: 1.01-2.36) of prevalence of severe wheezing using the criteria from the International Study of Asthma and Allergies in Childhood (ISAAC) (42) after adjusting for age, sex, race, community, mode of travel to school, and modeled residential traffic-related pollution. This association was more striking among asthmatic children with commuting times 5 minutes or longer (OR, 1.97; 95% CI: 1.02-3.77). Other effects of air pollution on asthmatic children include increased emergency department visits or hospitalizations (43), and higher school absence rates (44).

Taken together, these results from the CHS demonstrate that the effects of ambient air pollution and traffic-related air pollution on childhood asthma pose a large burden to public health and the economy. According to the CHS estimates, the successful improvement in O_3 levels in Southern California during the year 1990 to 1999 reduced more than 2.8 million school absences, which saved more than $220 million (45). On the other hand, asthma burden attributable to air pollution in two California communities was $18 million yearly during 1996 to 2004, and half of this cost was due to traffic-related pollution (46).

Air pollution and lung function

The deficit in the growth of lung function is another chronic health effect of air pollution. Following children from age 10 to 18 years, deficits in the growth of forced expiratory volume in one second (FEV_1) were associated with exposure to higher levels of NO_2, $PM_{2.5}$, EC, and acid vapor after adjusting for sex, Hispanic ethnicity, log-transformed height, BMI, BMI squared, present asthma status, child's smoking history, secondhand tobacco smoke, community, exercise or respiratory tract illness on the day of the test, and indicator variables for field technician (P=0.005, 0.04, 0.007, and 0.004, respectively) (14). Deficits in the growth of forced vital capacity (FVC) were associated with exposure to NO_2 and acid vapor (P=0.05 and 0.03, respectively), and deficits in the growth of maximal midexpiratory flow rate (MMEF) were associated with exposure to NO_2 and EC (P=0.02 and 0.04, respectively). Similar associations were also observed for FEV_1 attained at the age of 18 years (14). For example, the estimated proportion of 18-year-old subjects with a low FEV_1 (defined as a ratio of observed to expected FEV_1 of less than 80%) in the community with highest level of $PM_{2.5}$ was 4 times more than the community with the lowest level of $PM_{2.5}$ (7.9% *vs.* 1.6%, P=0.002).

Exposures to traffic-related pollution were associated with lung development as well. After adjusting for height, height squared, BMI, BMI squared, present asthma status, community, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the last year and field technician, children who lived within 500 m of a freeway had significant deficits in FEV_1 and MMEF growth from age 10 to 18 compared to children who lived more than 1,500 m from a freeway (P=0.01 and 0.03, respectively) (12). Joint models revealed that adverse effects of traffic exposures on the growth of FEV_1 were independent of regional air pollutions (NO_2, Acid vapor, PM_{10}, $PM_{2.5}$, and EC). In another cross-sectional analysis of children with mean age of 11.2 years, residential proximity to a freeway was shown to be inversely associated with the reduction in FVC after adjusting for log-transformed height and height squared, BMI and BMI squared, age, sex, race/ethnicity, community, respiratory illness on the day of the test and

field technician (13). Living within 500 m of a freeway was associated with 2% deficit in FVC (P=0.009). Additionally, higher model-based estimate of near-roadway (freeways, highways and large surface streets) NO_x was associated with deficits in FEV_1 and FVC (P=0.005 and 0.048, respectively) (13). Consistent with our previous findings, near-roadway NO_x and regional air pollutants (O_3, $PM_{2.5}$, PM_{10} and NO_2) had independent inverse association with deficits in FEV_1 and FVC. There was an evidence that associations between residential near-roadway NO_x and deficits in FEV_1 and FVC might be modified by parental stress (both interaction P<0.01) (47). Significant inverse associations were only observed among children from high-stress households (parental PSS >4) after adjusting for log height and log height squared, BMI and BMI squared, age, sex, race/ethnicity, community, respiratory illness on the day of the test and field technician, but not among children from low-stress households (parental PSS ≤4). However, no interactions were found for air pollution with sex and asthma status.

Air pollution and airway inflammation

Airway inflammation is a potential mechanism underlying the effects of air pollution on asthma exacerbations (48). The exhaled nitric oxide fraction (Fe_{NO}) is a noninvasive marker of aspects of airway inflammation that has been developed and validated in the past decade (49,50). Children with Fe_{NO} in the highest quartile at the start of follow-up (>14.8 ppb at 50 mL/s) had more than a 2-fold increased risk of new-onset asthma compared to children with Fe_{NO} in the lowest quartile (<7.8 ppb at 50 mL/s) after adjusting for race/ethnicity, lifetime wheeze and community with baseline strata for age and sex (HR, 2.1; 95% CI: 1.3-3.5) (51). In the CHS, both regional air pollution and traffic-related pollution were associated with higher Fe_{NO}. Among children ages 7 to 11 years old, daily 24-h cumulative lagged averages of $PM_{2.5}$ (over 1-8 days), PM_{10} (over 1-7 days) and O_3 (over 1-23 days) were significantly associated with 17.4% (P<0.01), 9.3% (P<0.05) and 14.3% (P<0.01) higher Fe_{NO} levels over the IQR (7.5 μg/m^3, 12.97 μg/m^3, and 15.42 ppb for $PM_{2.5}$, PM_{10}, and O_3, respectively) of each pollutant, respectively, after adjusting for age, sex, race/ethnicity, community, asthma, asthma medication use, history of respiratory allergy, time of Fe_{NO} collection, BMI, secondhand tobacco smoke, parental education, language of the questionnaire (English/Spanish), season and whether Fe_{NO} testing was conducted outdoors (16). These associations did not significantly vary by sex, asthma, and respiratory allergy status. However, results suggested that the effects of air pollutants were relatively larger in the warm season compared to the cold season. Longitudinal analysis showed increases of long-term (annual average) exposures of NO_2 and $PM_{2.5}$ (scaled to IQR of 1.8 ppb and 2.4 μg/m^3, respectively) were associated with 2.29 ppb (P=0.02) and 4.94 ppb (P=0.005) increase in Fe_{NO} after adjusting for age, sex, race/ethnicity, asthma, asthma-medication use, history of respiratory allergy, day of Fe_{NO} collection, season, and short-term (lags of up to 60 days prior to the day of Fe_{NO} test) effects of the same air pollutant (52).

From a set of traffic-related pollution metrics, only the length of road in a circular buffer around the residence was found to be positively associated with Fe_{NO} (15). This association was restricted to children with asthma, and was strongest in the 50 m buffer, the smallest buffer considered. Specifically, a 100 m increase in the length of road in a 50 m buffer around subject's home was associated with a 46.7% (95% CI: 14.3-88.4%) higher Fe_{NO} in children with asthma and 0.2% lower (95% CI: −5.5-5.3%) Fe_{NO} in children without asthma after adjusting for age, sex, race/ethnicity, community, asthma, asthma-medication use, rhinitis history, BMI percentile, secondhand tobacco smoke, parental education, month and hour of Fe_{NO} collection and outdoor testing. Our future work will investigate the longitudinal relationships between traffic-related pollutions and Fe_{NO}, as well as whether Fe_{NO} influences the relationship between air pollution and asthma incidence.

Genetic susceptibility and gene-environmental interaction

In the past 10 years, the CHS has revealed a great amount of evidence for genetic influence on the association between air pollution and respiratory illness (*Table S1*). The associated genes include GSTs (encoded by *GSTM1*, *GSTP1*, and *GSTT1*), microsomal epoxide hydrolase (*EPHX1*), catalase (*CAT*), myeloperoxidase (*MPO*), heme oxygenase 1 (*HMOX-1*), tumor necrosis factor (*TNF*), arginases (encoded by *ARG1* and *ARG2* genes), inducible nitric oxide synthase (iNOS, encoded by *NOS2*), and transforming growth factor β1 (*TGFβ1*).

Incomplete combustions from smoking and fossil fuels contain high levels of polyaromatic hydrocarbons (PAHs), which can lead to oxidative stress and has been shown to relate to asthma and wheeze (53-55). Thus, genes involved in xenobiotic-induced oxidative stress were of great interest.

GSTM1 null and *GSTP1* (rs1695) A/A genotype were shown to enhance nasal allergic responses with increased IgE levels (56). In the CHS (*Table S1*), *GSTP1* rs1695-G and the upstream promoter single-nucleotide polymorphism (SNP) rs6591255-A allele were both associated with increased occurrences of lifetime asthma and wheezing (25,57). There was a significant interaction between *in utero* exposure to maternal smoking and rs1695 genotype on the association with wheeze (25). Compared to children with no exposure and rs1695 A/A genotype, children exposed to *in utero* maternal smoking and having rs1695 A/G or G/G genotypes had a 2-fold increased risk of early-onset asthma, current wheezing and medication use for wheeze after adjusting for age, sex, ethnicity, community, gestational age, and secondhand tobacco smoke (OR, 2.0; 95% CI: 1.1-3.3; OR, 1.9; 95% CI: 1.3-2.6); and OR, 1.9; 95% CI: 1.2-2.8, respectively). In contrast, children carrying rs1695 A/G or G/G genotypes was found to be associated with 40% lower risk of new onset asthma compared to children with rs1695 A/A genotype after adjusting for ethnicity and community (HR, 0.6; 95% CI: 0.4-0.8) (27). The opposite direction of associations between rs1695 genotype with wheezing and asthma incidence suggests rs1695 might have pleiotropic effect on asthma traits.

EPHX1 is also involved in the xenobiotic metabolism, but less studied. We found *EPHX1* SNPs rs1051740 and rs2234922 were associated with several asthma outcomes (57) (*Table S1*). After adjustment for age, sex, race/ethnicity, *in utero* maternal smoking, number of smokers at home, community, parental education, health insurance and parental history of asthma, children with rs1051740 C/C genotype had a 49% reduced risk of late onset asthma (OR, 0.51; 95% CI: 0.29-0.88) compared to children with T/T genotype. Children with rs2234922 A/G genotype had 42% (OR, 1.42; 95% CI: 1.14-1.76); 45% (OR, 1.45; 95% CI: 1.12-1.89) and 58% (OR, 1.58; 95% CI: 1.19-2.10) increases of lifetime, current and late onset asthma compared to children with A/G genotype. The association between *EPHX1* phenotypes and the risk of asthma varied by the *GSTP1* rs1695 genotype and residential proximity to a major road. Among children with rs1695 G/G genotype, those had high *EPHX1* activity phenotypes were of a 4-fold increased risk of lifetime asthma compared to children with low/intermediate *EPHX1* activity phenotypes (OR, 4.0; 95% CI: 1.97-8.16). This association was not significant among children with rs1695 A/A or A/G genotypes. Association between high *EPHX1* activity and the increased risk of lifetime asthma was also found among children who lived within 75 m of a major road. Children having high *EPHX1* activity phenotype and rs1695 G/G genotype who lived within 75 m of a major road had a 9-fold increased asthma risk compared to those having low/intermediate *EPHX1* activity and rs1695 A/A or A/G genotypes, and living more than 75 m of a major road (OR, 8.91; 95% CI: 2.40-33.12). No significant association was found for children living at least 75 m far from a major road.

For lung function, variation in the GST mu family (*GSTM2-5*) locus was found to be associated with lower FEV_1 and MMEF (26) (*Table S1*). Two haplotypes of *GSTM2* (one showed risk effect and one showed protective effect) were significantly associated with 8-year growth of FEV_1 and FVC after adjusting for height, height squared, 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, *GSTM1* null genotype, and field technician (all $P<0.02$). Significant associations were only found among children exposed to *in utero* maternal smoking. One haplotype of *GSTM3* was associated with slower growth of MMEF compared with children with other haplotypes ($P=0.002$). One haplotype of *GSTM4* was associated with decreased growth in FEV_1 ($P=0.01$), FVC ($P=0.03$), and MMEF ($P=0.05$) from age 10 to 18. For respiratory illness-related absences, minor alleles in SNPs of *GSTP1* including rs6591255-A, rs1695-G, and rs749174-T were associated with a protective effect for respiratory illness-related absences after adjusting for age, sex, race, community, asthma status, family income, health insurance, secondhand tobacco smoke, *in utero* maternal smoking, and BMI (OR, 0.61; 95% CI: 0.43-0.87 for Hispanic White; and OR, 0.86; 95% CI: 0.71-1.04) for non-Hispanic White) (58). Additionally, the protective effect was restricted among children unexposed to *in utero* maternal smoking.

Catalase (encoded by *CAT*), myeloperoxidase (encoded by *MPO*), and heme oxygenase (encoded by *HMOX-1*) are enzymes in the oxidative stress defense pathway (59,60). Among children in the CHS (*Table S1*), we found there was an epistatic interaction of *CAT* (rs1001179) and *MPO* (rs2333227) for their association with respiratory-related school absences after adjusting for age, sex, race, community, family income, health insurance, secondhand tobacco smoke, *in utero* maternal smoking, BMI, cat or dog ownership, and asthma status (61). Children had *CAT* (rs1001179) G/G genotype and at least one A allele of *MPO* (rs2333227) had 35% higher risk of respiratory-related school absences compared to children with *CAT* (rs1001179) G/G genotype and *MPO* (rs2333227) G/G genotype (OR,

1.35; 95% CI: 1.03-1.77). The epistatic interaction was significant among children living in communities with high O_3 level, but was not evident in communities with low O_3 level. The number of $(GT)_n$ repeats of the *HMOX-1* gene showed a bimodal distribution with two peaks being 23 and 30 repeats among Hispanics and non-Hispanic whites (22). Among non-Hispanic whites, children carrying at least one *HMOX-1* "short" alleles (<23 repeats) were associated with 36% lower risk of new-onset asthma compared to children who had no "short" allele controlling for communities with age- and sex-specific baseline hazard (HR, 0.64; 95% CI: 0.41-0.99) (22). This association was differentiated by ambient ozone level (interaction P=0.003). Children having at least one "short" allele of *HMOX-1* and residing in the low ozone communities had 56% lower risk of asthma incidence than those having no "short" allele of *HMOX-1* and living in the low O_3 communities (HR, 0.44; 95% CI: 0.23-0.83). No significant association between *HMOX-1* and asthma risk was found among Hispanics, suggesting differential asthma risk of this genetic variant by race/ethnicity.

TNF mediates asthma occurrence by initiating airway inflammation and generating airway hyperreactivity (62-64). We previously found DNA sequence variant in rs1800629 modified the association between secondhand smoking and risk of respiratory illness-related school absences (65). In the following work, we found more direct associations between *TNF* variant and respiratory illness (*Table S1*). Among children of age 8-11 years old, rs1800629 G/G genotype was associated with 20-30% reduced risk of lifetime asthma (OR, 0.8; 95% CI: 0.7-0.9), life-time (OR, 0.8; 95% CI: 0.7-0.9) and current wheezing (OR, 0.7; 95% CI: 0.6-0.9), and medication for wheezing (OR, 0.7; 95% CI: 0.5-0.8) compared to G/A or A/A genotypes after adjusting for age, sex, race/ethnicity, town, lifetime residence, grade, secondhand tobacco smoke, and *in utero* maternal smoking (66). The protective effects of the G/G genotype on ever wheezing, current wheezing and medication use for wheeze were two times larger in magnitude for children who lived in low ozone (annual average <50 ppb) communities compared to others who lived in high ozone (annual average ≥50 ppb) communities (all interaction P<0.04). No significant interaction was found for rs1800629 with ozone for the association with asthma prevalence. The difference in the rs1800629 G/G genotype effect between low and high ozone exposure was stronger in the *GSTM1* null compared with the *GSTM1* present group. Similarly, the difference in the protective effect of rs1800629 G/G genotype between low and high ozone exposure was larger among children with *GSTP1* (rs1695) A/A genotype than children with rs1695 A/G or G/G genotypes. The interaction between the rs1800629 G/G genotype and O_3 was also found in the association with bronchitic symptoms among asthmatic children (29). The rs1800629 G/G genotype was associated with 47% reduced risk of bronchitic symptoms for asthmatic children who were exposed to low ambient O_3 after adjusting for age, sex, ethnicity, grade, secondhand tobacco smoke, lifetime residence, and community (OR, 0.53; 95% CI: 0.31-0.91). The protective effect was not found among children living in high O_3 communities.

Arginases play an important role in asthma pathogenesis through nitrosative stress-mediated airway inflammation (64,67-69). CHS results showed both *ARG1* and *ARG2* were globally associated with asthma prevalence (28) (*Table S1*). Compared to the most common *ARG1* haplotype that carried the wild-type allele for seven tagged SNPs, one ARG1 haplotype carrying the variant allele (T) for rs2749935 was associated with a 45% reduced risk of asthma after adjusting for age, sex, ethnicity, child's atopic status, parental history of asthma, parental education, secondhand tobacco smoke, *in utero* maternal smoking, health insurance, and community (OR, 0.55; 95% CI: 0.36-0.84). Each variant allele (G) of *ARG2* SNP rs3742879 was associated with a 31% increase in asthma risk (OR, 1.35; 95% CI: 1.04-1.76). Atopy and ambient O_3 modified the association between one *ARG1* haplotype and the risk of asthma (interaction P=0.04 and 0.02, respectively). This particular *ARG1* haplotype was associated with reduced asthma risk among atopic children or children living in high O_3 communities, but was not associated among non-atopic children or children living in communities with low level of O_3. No significant interactions were found for *ARG2* haplotypes or SNPs with atopy and O_3 in the association with asthma risk. In addition to the observed associations for genetic variations of *ARG*, epigenetic variations in *ARG* were also investigated for its role in modulating Fe_{NO} levels in children. In the CHS, DNA methylation in *ARG2* was significantly associated with airway inflammation among children with mean age of 9 years old (70). A 1% increase in average DNA methylation of *ARG2* was associated with a 2.3% (95% CI: −4.0% to −0.6%) decrease in Fe_{NO} after adjusting for age, sex, race, plate, town, month of DNA collection, asthma medication use, and parental education. This association was more striking among asthmatic children than children without asthma (interaction P=0.01). A similar

interaction was also found for *ARG1*, though little association existed between DNA methylation of *ARG1* and Fe_{NO}.

Another gene involved in the nitrosative stress is *NOS2*, which produces NO in response to environmental stimuli (71-74). CHS results showed seven SNPs in the promoter region of *NOS2* were globally associated with an increased risk of new-onset asthma (P=0.002) and a lower growth of FEV_1 (P=0.02) (75) (*Table S1*). Further analysis indicated that a pair of "yin-yang" haplotypes of these seven SNPs contributed to the association. One copy of the "yin" haplotype (h0111101) was associated with a 49% increased risk of new-onset asthma compared with children without this haplotype controlling for communities with age- and sex-specific baseline hazard (HR, 1.49; 95% CI: 1.03-2.14), and this association was dose-dependent. In contrast, the "yang" (h1000010) haplotype was associated with 34% (HR, 0.66; 95% CI: 0.49-0.88) reduced risk of new-onset asthma and 48.9 mL (95% CI: 11.6-86.2 mL) higher 8-year FEV_1 growth. Interestingly, the increased risk of new-onset asthma for the "yin" haplotype was only found among children who had *GSTM1* null genotype (interaction P=0.002). However, the protective effect of the "yang" haplotype did not vary by the *GSTM1* genotype. To investigate *NOS2* associations with airway inflammations, we found $PM_{2.5}$, DNA methylation in iNOS were jointly associated with Fe_{NO} after adjusting for age, sex, ethnicity, asthma, respiratory allergy, parental education, community, month of Fe_{NO} collection, *NOS2* promoter haplotypes and experimental plate (76). Among children at the highest 10^{th} percentile of iNOS methylation (>56.6%), higher ambient $PM_{2.5}$ was associated with higher Fe_{NO} (P=0.0002); whereas such an association was not significant among children at lower methylation levels.

Because TGF-β1 is involved in airway inflammation (77,78) and remodeling (79,80), the functional polymorphisms in the *TGFB1* gene may play a role in asthma occurrence. We found children with the SNP rs4803457 T/T genotype had a 1.8-fold increased risk of early persistent asthma (asthma as diagnosis before age 3 years with at least one episode of wheeze or asthma medication use after starting first grade) compared to children with C/C or C/T genotypes after adjusting for age, sex, ethnicity, atopic status, parental history of asthma, family income, parental education, *in utero* maternal smoking, number of smokers at home, insurance, and community (OR, 1.81; 95% CI: 1.11-2.95) (81) (*Table S1*). This association was varied by the residential proximity to a freeway (interaction P=0.02). The T/T genotype was associated with more than 3-fold increased risk of lifetime asthma among children living within 500 m of a freeway. However, such an association was not significant among children who lived more than 500 m from a freeway. *In utero* exposure to maternal smoking was previously found to be associated with higher risk of asthma (82). We additionally found such an association can vary by *TGFB1* genotypes (interaction P=0.1) (81). The association between *in utero* exposure to maternal smoking and increased risk of early persistent asthma was only observed among children with T/T genotype (OR, 3.15; 95% CI: 0.81-12.26), but not among children with C/C or C/T genotypes (OR, 0.97; 95% CI: 0.57-1.66).

Discussion

Although air pollution levels have decreased over the last decades (*Figure 1*), the CHS found both regional and traffic-related pollutants are associated with increased asthma prevalence and new-onset asthma, increased risk of both chronic and acute respiratory symptoms for children with asthma, slower lung function development, and higher airway inflammation. Effects of traffic-related pollutions are independent of effects of regional pollutions. The mechanisms underlying the observed associations may involve multiple genetic influences, gene-environmental interactions, and the interactions between air pollution and other exposures such as *in utero* maternal smoking and parental stress.

The CHS results provide evidence that air pollution is a major challenge to public health with respect to childhood respiratory illnesses, especially for countries whose air quality is worse than in the United States. Substantial lifelong adverse effects are a real threat if children's exposures are not reduced. Many approaches can be applied to reduce air pollution exposures including both primary and secondary strategies (30).

"Primary strategies" which reduce the release of air pollutants are critical for the reduction of regional ambient air pollution levels and local traffic-related pollutant levels. Such strategies require the stringent control of automobile and truck emissions. Even under current regulatory levels of air pollutants, adverse effects of air pollution occur for many respiratory illnesses including asthma, low lung function growth, and airway inflammation. These results suggest stricter regulatory standards are needed to prevent adverse health outcomes in the US, Europe and other developed nations. Additional pollutants which are not

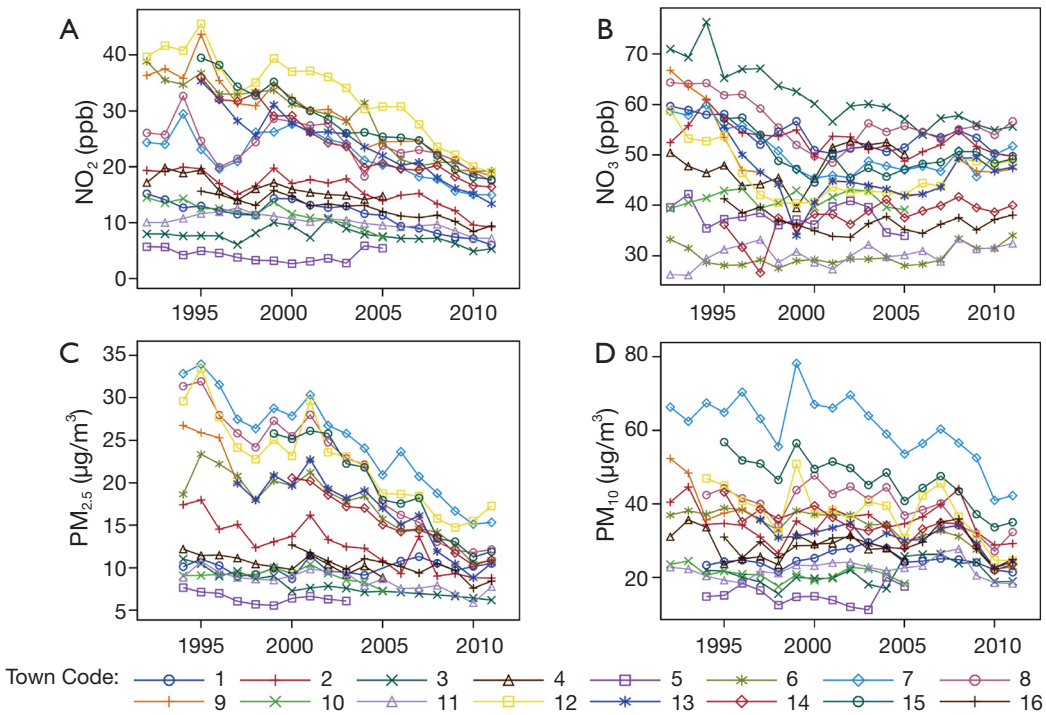

Figure 1 Regional air pollution trends from year 1992 to year 2010 in 16 Southern California communities from the Children's Health Study. Four air pollutants levels are presented: (A) NO_2; (B) O_3; (C) $PM_{2.5}$; and (D) PM_{10}. Town names represented by the town code: 1, Alpine; 2, Lake Elsinore; 3, Lake Gregory; 4, Lancaster; 5, Lompoc; 6, Long Beach; 7, Mira Loma; 8, Riverside; 9, San Dimas; 10, Atascadero; 11, Santa Maria; 12, Upland; 13, Glendora; 14, Anaheim; 15, San Bernardino; 16, Santa Barbara.

included in the current standard need to be targeted in the future based on the growing knowledge of their detrimental impact on public health, including ultrafine particles and PAHs. Traffic-related pollutions are major risk factors, but there are no federal regulatory standards for traffic-related pollutions as for regional air pollutions by NAAQS that was put in place as mandated by the 1970s Clean Air Act. Regulation of traffic-related pollutants would be appropriate to protect children's respiratory health.

Given the limitations in the current regulations and the long time necessary to revise regulations, "secondary strategies" to reduce exposure or to decrease personal susceptibility may also be required. Such strategies could include citing schools and parks away from roads with high traffic volumes; issuing warnings to the public with recommendations for reducing outdoor activity on high pollution days; and minimizing commuting time on roads especially for school commutes.

The strengths of CHS are the long-term, prospective follow-up of five large cohorts of children, with exposure and outcome data collected consistently. We have used central air monitors to measure regional pollution, and different traffic metrics to estimate traffic-related pollutions. Target genes, GWAS, and DNA methylation data are available for assessing the genetic and epigenetic associations with respiratory health outcomes.

However, we acknowledge that some challenges exist for our future studies. First, although different air pollution exposure models such as dispersion model and land use regression models, and air monitors have been used to estimate and measure ambient and traffic-related pollutions, incorporating activity patterns in time and space (home, school, commute, and workplace) (11) in estimating risk estimate remains a challenge particularly for investigating chronic health effects where personal monitoring (especially in children) is infeasible. Second, identification of the factors involved in asthma etiology has remained a big challenge because of complex interplay between environmental and genetic factors. While candidate gene approach has showed promising interactive effects of ambient air and traffic-related pollution on respiratory health, GWAS efforts has not yielded new susceptibility loci for air pollution mediated

effects. Additionally, much of the variability of asthma cannot be explained by known asthma-related SNPs. Third, use of epigenetics as a mediating factor of ambient air and traffic-related pollution with health outcomes has received interest in scientific community, but there are challenges ahead with evaluating pollution effects in biological samples with mixed cell populations from surrogate tissues (rather than the tissue of interest, lung or airway in this instance, which is infeasible in children or in population-based study), and that epigenetic variation occurring with short-term exposure making it difficult to use these variations for long-term effects.

In conclusion, air pollution has important adverse effects on respiratory illnesses, which may be mediated in part by genes, tobacco smoke exposures and parental stress. Future research is warranted to better define the long-term effects of air pollution including the relationship between early life exposure to air pollution and health outcomes after into adulthood. Individual interventions based on personal susceptibility may be needed to efficiently prevent adverse effects attributable to air pollution while control measures are being implemented. Lastly, more aggressive air pollution regulations are needed to achieve improved public health benefits for future generations of children.

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Table S1 Summary of genetic and epigenetic associations with respiratory health outcomes

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Li *et al.* 2008 (25)	Retrospective cohort	A-D	2,122 non-Hispanic white, 960 Hispanic white	4th, 7th, and 10th grade	NA	*GSTP1*	rs1695 (Ile105Val)	A/G (Ile/Val) or G/G (Val/Val)	A/A (Ile/Ile)	Lifetime asthma	1.2 (1.0-1.5)	NA	NA	NA
Li *et al.* 2008 (25)	Retrospective cohort	A-D	2,122 non-Hispanic white, 960 Hispanic white	4th, 7th, and 10th grade	NA	*GSTP1*	rs6591255	A/A or A/T	T/T	Lifetime, early-onset, and late-onset asthma, current wheezing, medication for wheeze	1.4 (1.0-1.8), 1.4 (1.0-1.8), 1.4 (1.0-1.9), 1.3 (1.0-1.6), and 1.6 (1.2-2.0), respectively	*In utero* exposure to maternal smoking (yes/no)	Early-onset asthma, current wheezing and medication for wheeze	2.0 (1.1-3.3), 1.9 (1.3-2.6), and 1.9 (1.2-2.8), respectively
Li *et al.* 2008 (25)	Retrospective cohort	A-D	2,122 non-Hispanic white, 960 Hispanic white	4th, 7th, and 10th grade	NA	*GSTP1*	rs6591255-rs4147581-rs1695-rs749174	h1000 (1 copy)	h0100 (2 copies)	Lifetime asthma	1.3 (1.0-1.7)	In utero exposure to maternal smoking (yes/no)	Current wheezing and medication for wheeze	2.1 (1.4-3.0) and 2.0 (1.2-3.1)
Li *et al.* 2008 (25)	Retrospective cohort	A-D	2,122 non-Hispanic white, 960 Hispanic white	4th, 7th, and 10th grade	NA	*GSTP1*	rs6591255-rs4147581-rs1695-rs749174	h0010 (1 copy)	h0100 (2 copies)	Current wheezing	0.7 (0.5-0.9)	In utero exposure to maternal smoking (yes/no)	Current wheezing and medication for wheeze	1.9 (1.3-2.6) and 1.9 (1.2-2.8)
Islam *et al.* 2009 (27)	Prospective cohort	A-D	1,064 non-Hispanic white, 546 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*GSTP1*	rs1695 (Ile105Val)	A/G (Ile/Val) or G/G (Val/Val)	A/A (Ile/Ile)	New-onset asthma	0.6 (0.4-0.8)	Joint effect of ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)], and number of team sports played (0, 1, and ≥2)	New-onset asthma	6.15 (2.2-7.4)
Islam *et al.* 2009 (27)	Prospective cohort	A-D	1,064 non-Hispanic white, 546 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*GSTP1*	rs6591255	A/A or A/T	T/T	New-onset asthma	1.4 (1.1-1.9)	NA	NA	NA

Table S1 (*continued*)

Table S1 (*continued*)

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/ haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Islam *et al.* 2009 (27)	Prospective cohort	A-D	1,064 non-Hispanic white, 546 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*GSTM1*		With null genotype (homozygous deletion polymorphisms)	Without null genotype	New-onset asthma	1.6 (1.2-2.2)	NA	NA	NA
Salam *et al.* 2007b (57)	Retrospective cohort	A-D	1,823 non-Hispanic white, 825 Hispanic white	4th, 7th, and 10th grade	NA	*EPHX1*	rs1051740 (Tyr113His)	C/C (His/His)	T/T (Tyr/Tyr)	Late onset asthma	0.51 (0.29-0.88)	NA	NA	NA
Salam *et al.* 2007b (57)	Retrospective cohort	A-D	1,823 non-Hispanic white, 825 Hispanic white	4th, 7th, and 10th grade	NA	*EPHX1*	rs2234922 (His139Arg)	A/G (His/Arg)	A/A (His/His)	Lifetime, current and late onset asthma	1.42 (1.14-1.76), 1.45 (1.12-1.89), and 1.58 (1.19-2.10)	NA	NA	NA
Salam *et al.* 2007b (57)	Retrospective cohort	A-D	1,823 non-Hispanic white, 825 Hispanic white	4th, 7th, and 10th grade	NA	*EPHX1*	Diplotype of rs1051740 and rs2234922	rs1051740T-rs2234922G (1 copy)	rs1051740C-rs2234922A (2 copies)	Lifetime and late onset asthma,	1.32 (1.04-1.67) and 1.61 (1.19-2.18)	NA	NA	NA
Salam *et al.* 2007b (57)	Retrospective cohort	A-D	1,823 non-Hispanic white, 825 Hispanic white	4th, 7th, and 10th grade	NA	*EPHX1*	Phenotypes	High	Low	Lifetime, current and late onset asthma	1.51 (1.14-1.98), 1.50 (1.08-2.09), and 1.84 (1.28-2.64)	GSTP1 rs1695 (G/G)	Lifetime, current, early persistent, and late onset asthma	4.01 (1.97-8.16), 4.42 (1.65-11.83), 3.89 (1.32-11.43), and 4.40 (1.56-12.45), respectively
Salam *et al.* 2007b (57)	Retrospective cohort	A-D	1,823 non-Hispanic white, 825 Hispanic white	4th, 7th, and 10th grade	NA	*EPHX1*	Phenotypes	High	Low	Lifetime, current and late onset asthma	1.51 (1.14-1.98), 1.50 (1.08-2.09), and 1.84 (1.28-2.64)	Distance of residence from a major road (<75 m and ≥75 m)	Lifetime and late-onset asthma	3.24 (1.75-6.00) and 4.85 (2.22-10.61)
Breton *et al.* 2009 (26)	Prospective cohort	C,D	1,398 non-Hispanic white, 710 Hispanic white	4th grade	8 years	*GSTM2*	rs2073483-rs574344-rs655315-rs619686-rs12024479	h00101 (≥1 copy)	Other haplotypes	8-year growth in FEV_1 and MMEF	–38.6mL and –71.0 mL/s (both P=0.02)	In utero exposure to maternal smoking (yes/no)	8-year growth in FEV_1 and FVC	–127.6 (–205.6, –49.5) and –169.7 (-258.6, -80.9)

Table S1 (*continued*)

Table S1 (*continued*)

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Breton *et al.* 2009 (26)	Prospective cohort	C,D	1,398 non-Hispanic white, 710 Hispanic white	4th grade	8 years	*GSTM2*	rs2073483-rs574344-rs655315-rs619686-rs12024479	h10000 (≥1 copy)	Other haplotypes	8-year growth in MMEF	109.4 mL/s (P=0.001)	NA	NA	NA
Breton *et al.* 2009 (26)	Prospective cohort	C,D	1,398 non-Hispanic white, 710 Hispanic white	4th grade	8 years	*GSTM3*	rs11101993-rs11101996-rs1537236-rs7483-rs10735234-and rs1799735	h001001 (≥1 copy)	Other haplotypes	8-year growth in MMEF	-164.9 mL/s (P=0.002)	NA	NA	NA
Breton *et al.* 2009 (26)	Prospective cohort	C,D	1,398 non-Hispanic white, 710 Hispanic white	4th grade	8 years	*GSTM4*	rs12745189-rs668413-rs1010167-rs560018-rs650985-rs506008- and rs521999	h1101000 (≥1 copy)	Other haplotypes	8 -yr growth in FEV_1, FVG, and MMEF	–51.3 mL (P=0.01), –44.4 mL (P=0.03), and –69.1 mL/s (P=0.05), respectively	NA	NA	NA
Wenten *et al.* 2009b (58)	Retrospective cohort	D	718 non-Hispanic white, 414 Hispanic white	4th grade	NA	*GSTP1*	rs6591255	T/A or A/A	T/T	Respiratory-related school absences	0.66 (0.49-0.90) among Hispanic whites only	NA	NA	NA
Wenten *et al.* 2009b (58)	Retrospective cohort	D	718 non-Hispanic white, 414 Hispanic white	4th grade	NA	*GSTP1*	rs4147581	G/G or C/G	C/C	Respiratory-related school absences	1.30 (1.01-1.67) among Hispanic whites only	NA	NA	NA
Wenten *et al.* 2009b (58)	Retrospective cohort	D	718 non-Hispanic white, 414 Hispanic white	4th grade	NA	*GSTP1*	rs1695 (Ile105Val)	G/G (Val/Val) or A/G (Ile/Val)	A/A (Ile/Ile)	Respiratory-related school absences	0.81 (0.67-0.97) among non-Hispanic white; and 0.66 (0.50-0.87) among Hispanic whites	NA	NA	NA
Wenten *et al.* 2009b (58)	Retrospective cohort	D	718 non-Hispanic white, 414 Hispanic white	4th grade	NA	*GSTP1*	rs6591255-rs4147581-rs1695-rs749174	h1001 (≥1 copy)	Other haplotypes	Respiratory-related school absences	0.61 (0.43-0.87) among Hispanic whites; 0.86 (0.71-1.04) among non-Hispanic whites	In utero exposure to maternal smoking (yes/no)	Respiratory-related school absences	0.78 (0.62-0.97) among non-Hispanic whites; 0.64 (0.45-0.91) among Hispanic whites

Table S1 (*continued*)

Table S1 (*continued*)

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/ haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Wenten *et al.* 2009a (61)	Retrospective cohort	D	1,136 non-Hispanic white and Hispanic white	4th grade	NA	Epistatic interaction between *CAT* and *MPO*	CAT rs1001179- and MPO rs2333227	rs1001179 G/G, and rs2333227 G/A or A/A	rs1001179 G/G and rs2333227 G/G	Respiratory-related school absences	1.35 (1.03-1.77)	Ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)]	Respiratory-related school absences	0.42 (0.20-0.89)
Islam *et al.* 2008 (22)	Prospective cohort	A-D	1,125 non-Hispanic white, 586 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*HMOX-1*	Microsatellite polymorphism	At least one "short" allele [<23 $(GT)_n$ repeats]	No "short" allele	new-onset asthma	0.64 (0.41-0.99) among non-Hispanic whites only	Ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)]	New-onset asthma	0.44 (0.23-0.83)
Islam *et al.* 2008 (22)	Prospective cohort	A-D	1,125 non-Hispanic white, 586 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*CAT*	rs1001179	C/T or T/T	C/C	new-onset asthma	1.9 (1.1-3.6) among Hispanic whites only	NA	NA	NA
Li *et al.* 2006 (66)	Retrospective cohort	A-D	2,182 non-Hispanic white, 989 Hispanic white, and 528 others	4th, 7th, and 10th grade	NA	*TNF*	rs1800629 (G-308A)	G/G	G/A or A/A	Life-time asthma, life-time and current wheezing, and mediation for wheezing	0.8 (0.7-0.9), 0.8 (0.7-0.9), 0.7 (0.6-0.9), and 0.7 (0.5-0.8), respectively	Joint effect of ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)] and GSTM1 genotype	life-time and current wheezing, and mediation for wheezing	0.5 (0.3-0.8), 0.4 (0.2-0.6), and 0.3 (0.1-0.6), respectively
Li *et al.* 2006 (66)	Retrospective cohort	A-D	2,182 non-Hispanic white, 989 Hispanic white, and 528 others	4th, 7th, and 10th grade	NA	*TNF*	rs1800629 (G-308A)	G/G	G/A or A/A	Life-time asthma, life-time and current wheezing, and mediation for wheezing	0.8 (0.7-0.9), 0.8 (0.7-0.9), 0.7 (0.6-0.9), and 0.7 (0.5-0.8), respectively	Joint effect of ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)] and GSTP1 rs1695 (Ile105Val)	life-time and current wheezing, and mediation for wheezing	0.6 (0.3-0.9), 0.5 (0.3-0.9), and 0.4 (0.2-0.8), respectively
Lee *et al.* 2009 (29)	Retrospective cohort	A-D	145 non-Hispanic whites, 65 Hispanic white, 24 others	4th, 7th, and 10th grade	NA	*TNF*	rs1800629 (G-308A)	G/G	G/A or A/A	bronchitic symptoms among asthmatic children	No significant main effect, OR 0.81 (0.55–1.21)	ambient ozone [low (<50 ppb) and high (mean ≥50 ppb)]	Bronchitic symptoms among asthmatic children	0.53 (0.31-0.91)

Table S1 (*continued*)

Table S1 (*continued*)

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Salam *et al.* 2009 (28)	Retrospective cohort	A-D	2010 non-Hispanic white, 936 Hispanic white	4th, 7th, and 10th grade	NA	*ARG1*	rs2608981-rs3895535-rs2608937-rs2749935-rs2781659-rs2246012	h000100	h000000	lifetime asthma	0.55 (0.36-0.84)	Joint effect of Children's atopy status (yes/no) and ambient ozone [low (mean 38.4 ppbs) and high (mean 55.2 ppbs)]	Lifetime asthma	0.12 (0.04-0.43)
Salam *et al.* 2009 (28)	Retrospective cohort	A-D	2,010 non-Hispanic white, 936 Hispanic white	4th, 7th, and 10th grade	NA	*ARG2*	rs12885261-rs7144243-rs3759757-rs4902501-rs7156352-rs4902503-rs7140310-rs742869-rs3742879-rs10483801	h1000000010	h1000100000	Lifetime asthma	1.35 (1.04-1.76)	NA	NA	NA
Salam *et al.* 2009 (28)	Retrospective cohort	A-D	2,010 non-Hispanic white, 936 Hispanic white	4th, 7th, and 10th grade	NA	*ARG2*	rs12885261-rs7144243-rs3759757-rs4902501-rs7156352-rs4902503-rs7140310-rs742869-rs3742879-rs10483801	h0111010100	h1000100000	Lifetime asthma	0.50 (0.28-0.92)	NA	NA	NA
Breton *et al.* 2011 (70)	Retrospective cohort	E	333 non-Hispanic white, 607 Hispanic white	Kindergarten, first grade	NA	*ARG2 methylation*	CpG islands	chr14:68,086,547-68,086,554		Fe_{NO} (collected in year 2004-2007)	–2.3% (–4.0%, –0.6%) for 1% increase in DNA methylation	Children's asthma status (yes/no)	Fe_{NO} (collected in year 2004-2007)	–8.7% (–14.6% to –2.4%)
Islam *et al.* 2010 (75)	Prospective cohort	A-D	1,042 non-Hispanic white, 554 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*NOS2*	rs4795080-rs2779253-rs1889022-rs10853181-rs2531866-rs1014025-rs2531872	h0111101 (1 copy)	Other haplotypes	New-onset asthma	1.49 (1.03-2.14)	GSTM1 genotype	New-onset asthma	1.89 (1.34-2.60)

Table S1 (*continued*)

Table S1 (*continued*)

References	Study design	Study Cohorts	Sample size and ethnicity	Age (year in school)	Follow-up years	Gene	SNPs	Associated genotype/haplotype	Reference genotype/ haplotype	Outcomes	OR/HR/beta (95% CI/P values)[a]	Effect modifier	Outcomes for effect modifications	OR/HR/beta (95% CI) of strongest contrast[a]
Islam *et al.* 2010 (75)	Prospective cohort	A-D	1,042 non-Hispanic white, 554 Hispanic white	4th, 7th, and 10th grade	2 to 8 years	*NOS2*	rs4795080-rs2779253-rs1889022-rs10853181-rs2531866-rs1014025-rs2531872	h1000010	Other haplotypes	New-onset asthma	0.66 (0.49-0.88)	NA	NA	NA
Islam *et al.* 2010 (75)	Prospective cohort	C,D	1,398 non-Hispanic white, 710 Hispanic white	4th grade	8 years	*NOS2*	rs4795080-rs2779253-rs1889022-rs10853181-rs2531866-rs1014025-rs2531872	h1000010	Other haplotypes	8-year growth in FEV_1	48.90 (11.60-86.20)	NA	NA	NA
Salam *et al.* 2012 (76)	Retrospective cohort	E	333 non-Hispanic white, 607 Hispanic white	Kindergarten, first grade	NA	*NOS2*	rs4795080-rs2779253-rs1889022-rs10853181-rs2531866-rs1014025-rs2531872	h1000010	h000000	Fe_{NO} (collected in year 2004-2007)	No significant main effect, beta 0.030 (–0.032-0.091)	7-day average $PM_{2.5}$	Fe_{NO} (collected in year 2004-2007)	0.044 (0.005-0.082)
Salam *et al.* 2012 (76)	Retrospective cohort	E	333 non-Hispanic white, 607 Hispanic white	Kindergarten, first grade	NA	*iNOS methylation*	iNOS promoter	iNOS (23150425)-F: TTAGGGTTAGGTA AAGGTATTTTTGTTT		Fe_{NO} (collected in year 2004-2007)	No significant main effect, beta –0.025 (–0.080-0.030)	7-day average $PM_{2.5}$	Fe_{NO} (collected in year 2004-2007)	0.044 (0.013-0.075)
Salam *et al.* 2007a (81)	Retrospective cohort	A-D	2,096 non-Hispanic white, 927 Hispanic white	4th, 7th, and 10th grade	NA	*TGFB1*	rs1800469 (C-509T)	T/T	C/C	Early persistant asthma	1.81 (1.11-2.95)	Joint effect of residential distance from nearest freeway (<500 m, 500-1,000 m, 1,001-1,500 m, >1,500 m) and *in utero* exposure to maternal smoking (yes/no)	Lifetime asthma	10.02 (1.25-80.40)
Salam *et al.* 2007a (81)	Retrospective cohort	A-D	2,096 non-Hispanic white, 927 Hispanic white	4th, 7th, and 10th grade	NA	*TGFB1*	Diplotype rs4803457 and rs1800469 (C-509T)	rs4803457-T/ rs1800469-T (2 copies)	0/1 copy	Early persistant asthma	1.83 (1.12-2.98)	Joint effect of residential distance from nearest freeway (<500 m, 500-1,000 m, 1,001-1,500 m, >1,500 m) and *in utero* exposure to maternal smoking (yes/no)	Lifetime asthma	3.11 (1.30-7.46)

[a], odds ratio (OR) and 95% confidence interval (CI) are presented for retrospective cohort study with categorical outcomes; hazard ratio (HR) and 95% CI are presented for prospective study with categorical outcomes; regression coefficient (beta) and P values are presented for continuous outcomes.