Associations Between Multiple Environmental Exposures and Glutathione S-transferase P1 on Persistent Wheezing in a Birth Cohort

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Abstract

Objective—To determine the impact of environmental exposures (diesel exhaust particle (DEP), environmental tobacco smoke (ETS), and mold) that may contribute to oxidative stress on persistent wheezing in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) birth cohort and to determine how the impact of these exposures is modified by the GST-P1 Ile105Val polymorphism.

Study design—A land-use regression model was used to derive an estimate of each child's DEP exposure. ETS exposure was determined by questionnaire data. Each child's home was evaluated for visible mold by a trained professional. Children in the CCAAPS cohort were genotyped for the GST-P1 polymorphism (N=570). Persistent wheezing was defined as wheezing at both 12 and 24 months.

Results—High DEP exposure conferred increased risk for wheezing phenotypes but only among the Val[105] allele carriers. Infants with multiple exposures were significantly more likely to persistently wheeze despite their genotype.

Conclusion—There is evidence for an environmental effect of DEP among carriers of the GST-P1 Val[105] allele in the development of persistent wheezing in children. The protective effect of the

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GST-P1 Ile[105] genotype may be overwhelmed by multiple environmental exposures that converge on oxidative stress pathways.

Keywords

The increasingly common occurrence of childhood wheeze and asthma, particularly in affluent westernized society, is well-documented.(1) Environmental factors associated with wheezing in early life include traffic exhaust exposure through diesel exhaust particles (DEP),(2,3) environmental tobacco smoke exposure (ETS),(4,5) and mold exposure.(3,6,7) The relationship between the glutathione S-transferase P1 (*GST-P1*) Ile105Val polymorphism and asthma has been reported in several populations, but these studies have not examined the interplay of the combined genetic and environmental factors on longitudinal wheezing status during early childhood.(8,9)

In humans, the glutathione S-transferase (*GST*) class of multifunctional enzymes are divided into eight families: Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta.(10,11) A single gene in the Pi subfamily, *GST-P1*, is the predominant cytosolic GST expressed in lung epithelium.(12) *GST-P1* is a 2.8 kb gene located on chromosome 11q13, a known "hot spot" for asthma-related genes.(13,14) A single nucleotide polymorphism at position 313 in *GST-P1* converts an adenine to a guanine (A→G).(15) The resulting isoleucine to valine substitution in codon 105 of exon 5 (Ile[105]→Val[105]) significantly lowers GST enzyme activity.(16)

Delineating the factors that are contributory or protective to persistent wheezing in early childhood is critical to advance our understanding of asthma. There is limited information about how genetic and environmental factors interact to influence longitudinal asthmatic/wheezing status over time. DEP, ETS, and mold exposures are common and each has been shown to aggravate respiratory symptoms. The gene-environment effect related to these individual and/or combined exposures has not been evaluated with regard to longitudinal wheezing status. The purpose of this study was to investigate whether exposure to DEP, ETS, and/or mold uniquely modifies wheezing and persistent wheezing in young children, especially among those with the *GST-P1* I105V polymorphism. Our study evaluates the modified effect of this polymorphism upon exposure to not only ETS and mold, but distinctively DEP exposure associated with traffic and their combined exposures utilizing the well-characterized Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) birth cohort.

METHODS

Study Participants

The CCAAPS study is a longitudinal birth cohort of high-risk children having at least one atopic parent. A complete description of the study's recruitment, methods, and objectives has been published.(17) Briefly, infants with at least one atopic parent (based on allergy skin prick testing) were enrolled between 2001 and 2003 in a seven county area of Cincinnati, Ohio. Families were recruited based on the proximity of their home residence to truck and bus traffic by geocoding residential addresses located on birth records (Figure 1; available at www.jpeds.com). All infants recruited for the CCAAPS study were carried to term (>35 weeks), and no premature infants were eligible. Parental asthma diagnosis history and shortness of breath symptoms were collected at the time of the parent SPT. Infant subjects were evaluated by skin prick testing with a panel of 15 aeroallergens and two foods (egg white and milk) at both 12 and 24 months of age. Annual questionnaires administered to parents with regards to infant respiratory symptoms were adapted from the International Study of Asthma and Allergies in Children (ISAAC).(18) At the time of recruitment, administered questionnaires

also collected information on household smoking habits and demographics. This study was approved by the Institutional Review Board.

DNA Collection and GST-P1 Gene Polymorphism Genotyping

Buccal cells were collected using a nylon bristle cytology brush. Genomic DNA was isolated using the Zymo Research Genomic DNA II Kit™ (Orange, CA). Genotyping was accomplished using the LightTyper platform (Roche Diagnostics, GmbH, Mannheim, Germany). The PCR primers (GST-P1 Forward: 5'-TGGACATGGTGAATGACGGCG-3' and GST-P1 Reverse: 5'-GGTCAGCCCAAGCCACCT-3') and hybridization probes (5'-LCR640-AGGGAGACGTATTTGCAGCGGAGG-3' and 5'-ACCCTGGTGCAGATGCTCACATAGTTGGTGTAGA-FL-3') were designed using the LightCycler Probe Design Software 2.0 (Roche Diagnostics, GmbH, Mannheim, Germany). Genotypes were confirmed by randomly re-genotyping 10% of the population. Genotypes were dichotomized to carriers and non-carriers of the Val^{105} allele.

Outcome Definitions

Parents were asked the following ISAAC adapted question: "In the past 12 months, have you ever noticed your child wheezing?" Infant wheezing at ages 12 and 24 months was defined as parental report of the child wheezing at the respective study visit one or more times in the past 12 months. Persistent wheezing was defined as parental report of the child wheezing at both the 12- and 24-month visits.

Environmental Exposure Definitions

The environmental exposures evaluated were DEP, ETS, and visible mold. Average daily levels of DEP at each infant's home were calculated using a land-use regression (LUR) model of exposure as previously described.(2) Briefly, ambient levels of fine particulate matter with aerodynamic diameter <2.5 µm ($PM_{2.5}$) were measured at 24 sampling sites located throughout the greater Cincinnati, Ohio metropolitan area. The $PM_{2.5}$ chemical composition has been previously described.(19) Elemental carbon was measured at these different monitoring sites and estimated source signatures in the airshed were determined to determine how much elemental carbon was attributable to traffic alone. This estimate was used to estimate truck and bus DEP exposure as previously reported.(20) Geographic, traffic, and land-use data within 400 meters (m) of each sampling site was collected in a geographic information system (GIS). From these data a LUR model with a coefficient of determination (R^2) of 0.75 was developed that included elevation, number of trucks within 400 m of the sampling site, and the length of bus routes within 100 m of the sampling site. The estimated model parameters were subsequently applied to the same geographic variables determined for each infant's home residence at the time of study enrollment when they were approximately seven months of age. This estimate was used to obtain unique estimates of their early life exposure to DEP. The median exposure to DEP was estimated to be 0.34 µg/m³ (range=0.23–0.88). The level of 0.5µg/m³ was chosen to determine high vs. low exposure based upon the distribution of estimated DEP and prior results indicating an approximate 2-fold increased risk for wheezing at 12 months at this exposure level, and this level represented the top quintile.(2) The LUR model was further evaluated deriving a LUR model with 6 sampling sites removed. The estimated DEP was subsequently compared with the sampled DEP and was generally found to slightly underpredict the sampled values (manuscript currently in review).

Infants were defined as exposed to household ETS if the parent reported at least one smoker (person that smoked one or more cigarettes per day) living in the infant's home. Infants were defined as exposed to mold if an in-home trained professional observed any visible mold, water damage, or moldy odor at the time of the home evaluation, generally before the infant's first birthday.(21) Infants in homes that did not meet any of these criteria were considered

unexposed to visible mold. Multivariate models were adjusted for race (Caucasian vs non-Caucasian) and sex. In analyses evaluating wheezing at 12 and 24 months, daycare attendance was also defined at 12 and 24 months.

Statistical Analysis

Racial differences for demographics, environmental exposures, health outcomes, and genotype and allele frequencies were compared using the chi-squared statistic. Unadjusted odds ratios (OR) and 95% Confidence Intervals (95% CI) were calculated using logistic regression to evaluate the univariate associations between outcomes, environmental exposures, and genotype. Three-way contingency tables were used to assess associations of environmental exposures, genotype, and health outcomes stratified by both genotype and exposure. Due to the significant difference in allele frequency between racial groups, a race-stratified analysis was also performed for Caucasians and non-Caucasians. The associations of DEP, ETS, and visible mold exposures and genotype with each outcome were evaluated using conditional logistic regression adjusting for daycare attendance, race, and sex. Since parent education, income, and health insurance were all highly correlated with race, we chose to adjust only for race. Race, however, was not found to be a significant covariate in adjusted models evaluating the independent associations of DEP (p=0.46), ETS (p=0.46), and mold (p=0.44) with wheezing at 12 months of age. Figure 2, therefore, present associations for racial groups combined between environmental exposures and *GST-P1* genotype. All possible gene-environment interactions to evaluate the effect modification of DEP, ETS, and mold exposure by genotype were evaluated in each model. An interaction was removed from the model if the p-value was greater than 0.20. All analysis was performed using SAS software (version 8.2 for Windows, SAS Institute, Cary, NC, USA).

RESULTS

Subjects, exposures, and health outcomes

Of the 570 study participants, 464 (81.4%) infants were Caucasian and 106 (18.6%) were non-Caucasian (Table I). Of the non-Caucasian infants, 86.8% were African Americans defined as both parents being African American. Non-Caucasian infants were significantly more likely than Caucasians to have a household income less than $40,000 (69.5% versus 26.7%; p<0.001), have higher exposure to DEP ≥0.50 µg/m^3 (p<0.001), and have visible mold in their homes (p=0.01). ETS exposure did not significantly differ between the racial groups. Wheezing at 24 months of age (34.6% versus 17.2%) and persistent wheezing (24.6% versus 10.9%) were significantly increased in non-Caucasian versus Caucasian infants.

Effect of exposure on wheezing phenotypes

Exposure to DEP ≥0.50 µg/m^3 increased the risk of wheezing at 24 months (OR=2.15, 95% CI=1.24–3.55) and persistent wheezing (OR=2.41, 95% CI=2.29–4.51) (data not shown). Similarly, ETS exposure was associated with wheezing at 12 months (OR=1.73, 95% CI=1.15–2.62), 24 months (OR=2.15, 95% CI=1.34–3.44), and persistent wheezing (OR=1.80, 95% CI=1.02–3.20). Mold exposure was significantly associated with infant wheezing at 24 months (OR=1.76, 95% CI=1.09–2.85) and persistent wheezing (OR=2.00, 95% CI=1.07–3.71). A comparison of the wheezing percentage among all infants versus those stratified by race revealed similar trends.

GST-P1 alleles in study subjects

The *GST-P1* allele frequencies were significantly different (p=0.002) among Caucasians (Ile=67.2%, Val=32.8%) and non-Caucasians (Ile=56.1%, Val=43.9%). Similar allelic differences were noted in other studies.(16) Overall, 81.4% of the children were homozygous

for the *GST-P1* Ile[105] allele, and 18.6% were carriers of the Val[105] allele. When children carrying at least one Val[105] allele (Ile/Val or Val/Val) were combined and compared with those homozygous for the Ile[105] allele, the non-Caucasian children were significantly more likely than Caucasians to be carriers of the Val[105] allele (21.9% versus 14.7%; p=0.02). *GST-P1* genotype data were in Hardy-Weinberg equilibrium when stratified by race and sex.

GST-P1 and environmental exposures

After stratifying by genotype, high DEP exposure ($\geq 0.50 \mu g/m^3$) was associated with an increased risk for wheezing among carriers of the Val[105] allele (Figure 2A). Again, racial groups were combined because race was not found to be a significant covariate in evaluating the independent associations of each environmental exposure with wheezing at 12 months of age. The Ile/Ile genotype conferred a protective effect against wheezing when infants were exposed to high amounts of DEP. This finding was consistent at 12 months (p=0.01), 24 months (p=0.002), and with persistent wheezing (p=0.003) (Figure 2A). Similarly, carriers of the Val[105] allele were significantly more likely to wheeze at 12 and 24 months if they were exposed to ETS (p=0.05 and p=0.01, respectively) compared with those infants with the protective Ile/Ile genotype (Figure 2B). Carriers of the Val[105] allele exposed to visible mold were also significantly more likely to wheeze at 24 months of age (p=0.04) (Figure 2C).

The effect of each exposure (DEP, ETS, and visible mold) was independently evaluated with respect to wheezing at 12 months, 24 months, and persistent wheezing after adjusting for genotype, daycare attendance, race, and sex (Table II). High DEP exposure ($\geq 0.50 \mu g/m^3$) conferred a significant risk for persistent wheezing (OR=2.13, 95% CI 1.11–4.07). There was a trend noted between high DEP exposure ($\geq 0.50 \mu g/m^3$) and *GST-P1* genotype on wheezing (DEP-*GST-P1* interaction, p=0.08; Table IIA). Similar interactions between ETS and visible mold exposures with the *GST-P1* Ile/Val or Val/Val genotypes were not observed and were subsequently removed from the models (Table IIB and C). ETS exposure was significantly associated with wheezing at both 12 (OR=1.78, 95% CI=1.17–2.71) and 24 months of age (OR=2.06, 95% CI=1.27–3.35), and an elevated risk was observed for persistent wheezing (Table IIB). Visible mold exposure significantly increased the risk of wheeze at 24 months (OR=2.18, 95% CI=1.30–3.63) and persistent wheezing (OR=2.57, 95% CI=1.33–4.96) (Table IIC). Thus, all three exposures were significant in a univariate model.

Multiple exposures associated with wheezing

We next adjusted for all three exposures simultaneously as well as genotype, daycare attendance, race, and sex (Table III). In this model, high DEP exposure ($\geq 0.50 \mu g/m^3$) was associated with wheezing at 24 months (OR=1.93, 95% CI=1.06–3.53) and with persistent wheezing (OR=2.13, 95% CI=1.03–4.41). Exposure to ETS was significantly associated with wheezing at 12 (OR=1.73, 95% CI=1.11–2.70) and 24 months (OR=1.90, 95% CI=1.13–3.18). Similarly, visible mold exposure was associated with wheezing at 24 months (OR=2.12, 95% CI=1.25–3.60) and persistent wheezing (OR=2.47, 95% CI=1.27–4.80) (Table III).

Environmental exposure load overwhelms genotype effect

In order to evaluate the relationship of total environmental "load" with wheezing, the additive or synergistic effect of having none, one, or two or more environmental exposures (DEP, ETS, or mold) was investigated. One-third (32.2%) of CCAAPS infants were not exposed to any of the three environmental exposures whereas 43.2% were exposed to one pollutant and 25.6% were exposed to two or more. Carriers of the Val[105] allele were found to be at risk for wheezing if they were exposed to two or more pollutants compared with unexposed infants at 12 months (p=0.02), 24 months (p<0.01), and with persistent wheezing (p<0.01). In addition, infants homozygous for the Ile[105] allele exposed to two or more air pollutants were at significantly increased risk for wheezing compared with those who were not exposed at 24 months (p=0.03)

and with persistent wheezing (p=0.01) (Figure 2D). The protective effect of the Ile/Ile genotype against wheezing previously observed with each of the individual exposures alone disappears when infants have multiple exposures. This trend is most evident with DEP exposure alone, but this same trend can be seen when infants are exposed to any one exposure (Figure 2). Environmental load was not significantly different between races (Table I). Clearly, long-term exposure to more pollutants places the infant at greater risk (almost 4 fold) of persistent wheezing irrespective *GST-P1* genotype (Table IID).

DISCUSSION

To our knowledge, this study is the first to investigate the impact of complex environmental exposures (DEP, ETS, and mold) along with genetics, specifically *GST-P1* on persistent wheezing in children. Our data supports that DEP, ETS, and/or mold exposure are risk factors for wheezing by 24 months of age. Furthermore, the presence of the Val[105] allele, which has been shown to significantly lower GST enzyme activity,(16) confers susceptibility to these environmental exposures compared with the Ile[105] allele. The Ile/Ile *GST-P1* genotype conferred protection against wheezing among the DEP exposed group, however, infants exposed to multiple environmental exposures were significantly more likely to persistently wheeze irrespective of genotype. Thus, the Ile[105] *GST-P1* genotype may confer protection from persistent wheezing, but strong environmental exposure converging on a similar pathway may overwhelm the genetic effect.

Other investigations have reported associations between asthma and the *GST-P1* polymorphism.(8,22,23) The Val[105] allele was shown to have a protective effect in children age 8 to 11 years against respiratory illness (23), and in adults age 20 to 34 years exposed to DEP and secondhand smoke against increased nasal allergic responses.(22) Contrary to these studies, our study examined infancy and early childhood, a period of time in which the lung undergoes critical development and asthma symptoms are just beginning to develop. Early childhood wheezing and early persistent wheezing may be a precursor to asthma in these young children. One limitation of this study is that it is difficult to definitively determine the cause of wheezing in this young age group. The use of the well-studied ISAAC-adapted questions was used to characterize wheezing as a precursor to asthma.

One mechanism by which environmental exposures may lead to lung injury is by inducing inflammatory cells to generate reactive oxygen species (ROS) leading to oxidative injury.(6, 24,25) DEP, ETS, and mold are three common environmental exposures that lead to increased generation of ROS and have been shown to cause respiratory symptoms.(2,4,21) DEP are respirable, with over 90% in the fine (0.1–2.5 µm) or ultrafine (<0.1 µm) size range. The DEP are composed of elemental carbon cores with large surface areas capable of binding organic polycyclic aromatic hydrocarbons and transition metals, which have the potential to induce ROS.(6) Cigarette smoke has also been shown to contain a high concentration of ROS.(5) Studies have demonstrated an association between ETS exposure during early childhood with the subsequent development of asthma.(26) Mold exposure has also been associated with increased intracellular levels of ROS(27) and respiratory illness in children.(28) Though the mechanism by which DEP, ETS, and mold may contribute to the development of asthma and asthma symptoms is unknown, there is mounting evidence implicating oxidative stress as a contributor to the airway inflammatory response.(29,30) GSTs can neutralize the electrophilic sites of reactive oxygen species (ROS) by conjugation to the tripeptide thiol, glutathione (GSH), which has an electron-donating capacity. The resulting product is more water-soluble promoting ROS detoxification and thereby protecting the lung from oxidative damage.(31) This may be one mechanism for the observed genetic effect of *GST-P1* in this study. We cannot rule out that the observed association between *GST-P1* and wheezing is due to a linked polymorphism in the same gene and/or another gene. Another limitation of the study is the

relatively small sample size of non-Caucasian children in the cohort (N=106). Race was not found to be a significant covariate in multivariate models evaluating independent associations of the three environmental exposures with wheezing at 12 months resulting in the combining of the racial groups. Although ideally it would be beneficial to stratify by race, the power of the study would have been severely compromised, particularly for African-Americans.

An important strength of this study is the longitudinal birth cohort design. In particular, DEP estimate exposure using multiple monitoring sites and a LUR model is unique to the CCAAPS cohort. Given the importance of early-life exposures, DEP, ETS, and mold exposure were determined through age two. Although we did collect data regarding treatment in the wheezing infants, we did not have complete data on all the children. In-utero smoke exposure was an independent risk factor for wheezing at 12 and 24 months but not persistent wheezing in only the Caucasian infants. There was no association among the non-Caucasian infants. We recognize that one limitation of the exposure assessment is that infants are not only exposed in the home. In order to address this, we adjusted for daycare attendance (also used as a proxy for exposure to respiratory infections). Future analyses will consider cumulative exposures and effect on asthma development as children age.

Overall, the relationship between DEP and wheezing was found to be stronger at 24 months of age than 12 months suggesting that longer exposure results in an elevated risk of wheezing. Interestingly, ETS exposure at 12 months of age was associated with wheezing at both 12 and 24 months of age suggesting that a shorter exposure time is needed to see an effect in infants. Since infants' lungs are still developing, household ETS exposure is generally much closer to the child's personal living space than is exposure to traffic exhaust and therefore highly likely to have a stronger impact during this sensitive stage of development. This reflects the findings of a recent study that reported exposure to parental smoking during the first year of life is associated with persistent wheezing.(32)

In conclusion, these data suggest that the *GST-P1* genotype should be considered when evaluating asthma/wheezing in young children exposed to high DEP levels, ETS, and/or visible mold. These data provide evidence that carriers of the Val[105] allele may be more susceptible to high DEP exposure with regard to the development of persistent wheezing. High environmental load converging on an oxidative stress pathway may overwhelm the genetic effect.

Abbreviations

CCAAPS, Cincinnati Childhood Allergy and Air Pollution Study; DEP, Diesel exhaust particle; ETS, Environmental tobacco smoke; ROS, Reactive oxygen species; SPT, Skin prick test.

Acknowledgments

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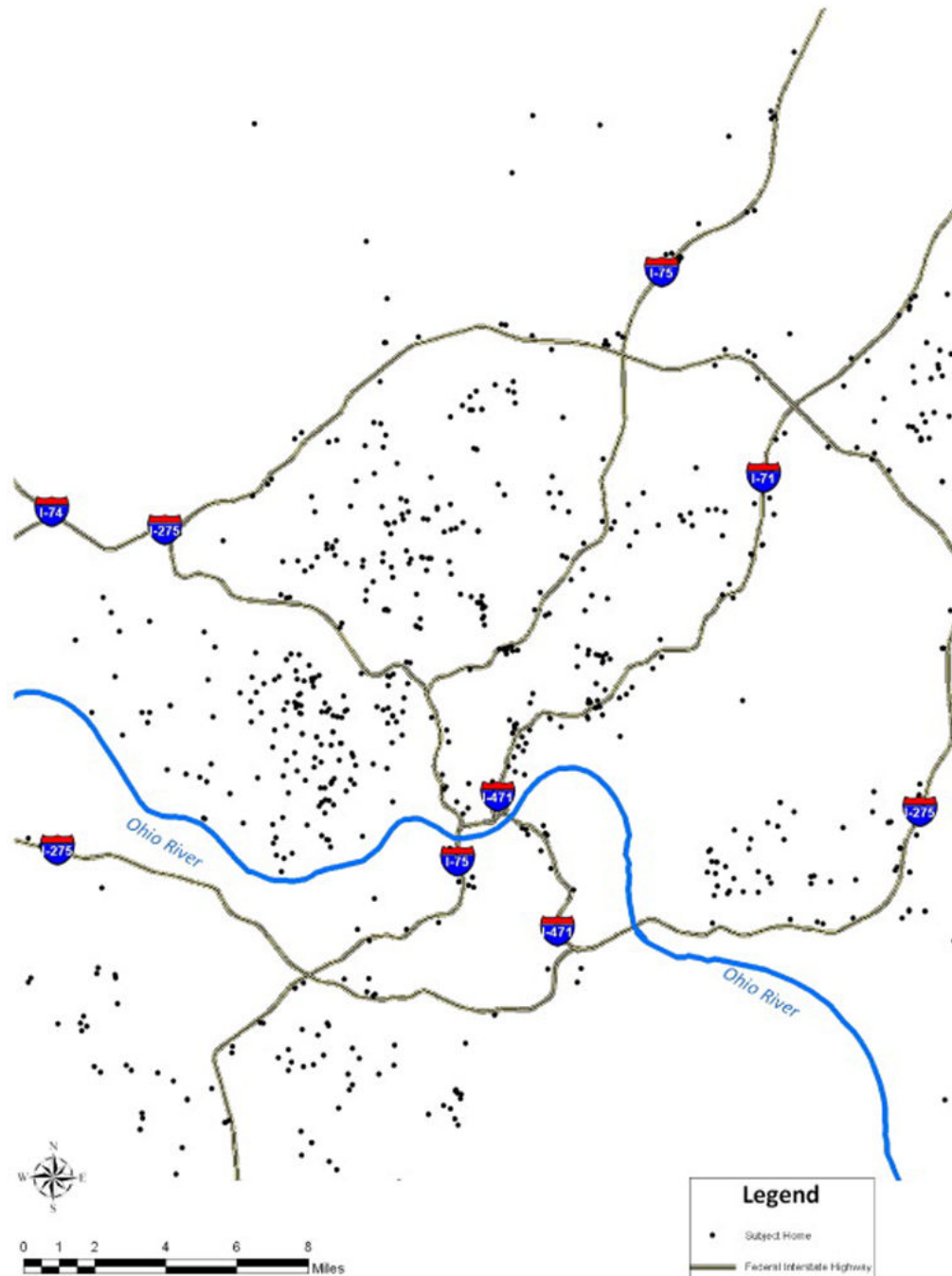
Figure 1.
Geographical location of CCAAPS infant's homes. Infants lived within a seven county area of Cincinnati, Ohio. Families were recruited based on the proximity of their home residence to truck and bus traffic by geocoding residential addresses located on infant birth records.

A. DEP exposure estimate, GST-P1 genotype, and infant wheezing.

C. Mold exposure, GST-P1 genotype, and infant wheezing.

B. ETS exposure, GST-P1 genotype, and infant wheezing.

D. Environmental load, GST-P1 genotype, and infant wheezing.
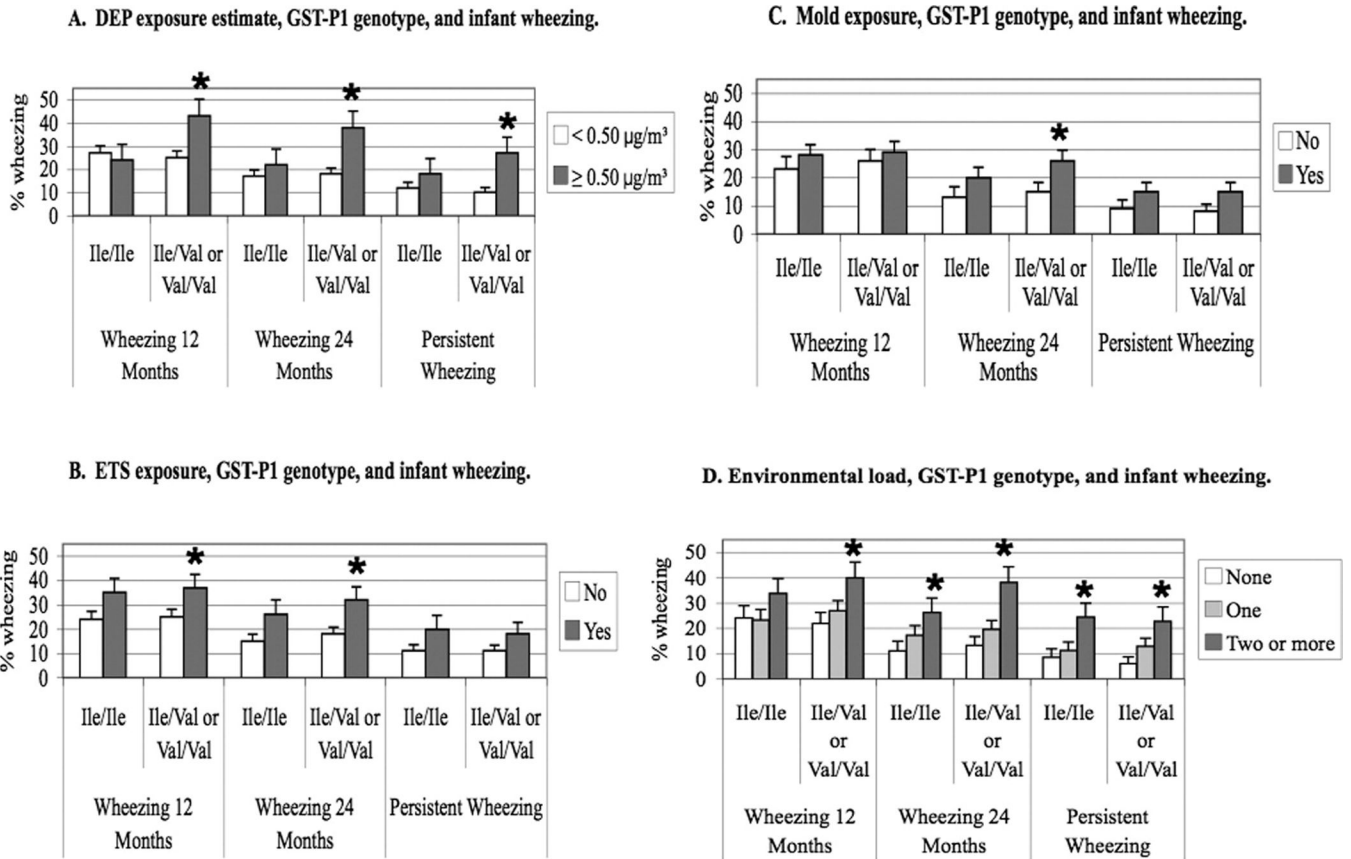
Figure 2.
Individual environmental exposures, *GST-P1* genotype, and infant wheezing. **A.** High DEP estimate exposure levels were significantly associated with wheezing at 12 months, 24 months, and with persistent wheezing only in infants carrying the Val[105] allele. **B.** ETS exposure was significantly associated with wheezing at 12 and 24 months only in infants carrying the Val[105] allele. **C.** Mold exposure was significantly associated with wheezing at 24 months in infants carrying the Val[105] allele. **D.** Environmental load, *GST-P1* genotype, and infant wheezing. At 12 months, only infants carrying the Val[105] allele and who were exposed to two or more exposures were significantly likely to wheeze. At 24 months and with persistent wheezing, all infants despite their genotype were significantly likely to wheeze when exposed to two or more exposures. * = p-value<0.05

Table I

Demographics, Exposures, and Health Outcomes of Infants.

	Total	Caucasian	Non-Caucasian	p-value
Gender				
Male	298 (52.3)	244 (52.6)	54 (50.1)	0.76
Female	272 (47.7)	220 (47.4)	52 (49.1)	
Family Income				
< $40,000	196 (34.7)	123 (26.7)	73 (69.5)	**<0.001**
≥ $40,000	369 (65.3)	337 (73.3)	32 (30.5)	
Daycare Attendance at 12 months				
Yes	43 (7.6)	35 (7.6)	8 (7.7)	0.96
No	524 (92.4)	428 (92.4)	96 (92.3)	
Daycare Attendance at 24 months				
Yes	70 (12.4)	54 (11.7)	16 (15.4)	0.30
No	497 (87.6)	409 (88.3)	88 (84.6)	
DEP Estimate (binary)				
< 0.50 µg/m^3	469 (82.3)	396 (85.3)	73 (68.9)	**<0.001**
≥ 0.50 µg/m^3	101 (17.7)	68 (14.7)	33 (31.1)	
ETS Exposure				
Yes	158 (28.5)	121 (27.0)	37 (34.9)	0.11
No	396 (71.5)	327 (73.0)	69 (65.1)	
Mold Exposure				
Yes	297 (56.5)	258 (59.2)	39 (43.3)	**0.01**
No	229 (43.5)	178 (40.8)	51 (56.7)	
Environmental Load[*]				
None	178 (32.2)	144 (31.0)	34 (32.1)	0.09
One	246 (43.2)	209 (45.1)	37 (34.9)	
Two or more	146 (25.6)	111 (23.9)	35 (33.0)	
Wheezing at 12 months				
Yes	145 (27.5)	116 (26.6)	29 (31.5)	0.34
No	383 (72.5)	320 (73.4)	63 (68.5)	
Wheezing at 24 months				
Yes	99 (19.9)	72 (17.2)	27 (34.6)	**<0.001**
No	399 (80.2)	348 (82.8)	51 (65.4)	
Persistent Wheezing				
Yes	60 (12.9)	43 (10.9)	17 (24.6)	**<0.001**
No	404 (87.1)	352 (89.1)	52 (75.4)	
Total n[†]	570	464	106	

[*] DEP estimate ≥ 0.50 µg/m^3, ETS exposure, and/or mold exposure

[†] The total n is different from the n for each individual demographic because not all data was available for each subject.

Table II

Adjusted Odds Ratios (OR) for Infant Wheezing, Environmental Exposures, and GST-P1 Genotype.

A. DEP Estimate Exposure

	Wheezing at 12 Months			Wheezing at 24 Months			Persistent Wheezing		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
DEP Estimate ≥ 0.50 µg/m³	0.89	0.41–1.96	0.78	1.16	0.47–2.82	0.75	2.13	1.11–4.07	0.02
Ile/Val or Val/Val Gentoype*	0.94	0.61–1.44	0.78	1.03	0.61–1.72	0.93	0.92	0.52–1.61	0.76
Daycare Attendance*	1.48	0.76–2.88	0.25	1.86	1.03–3.38	0.04	1.13	0.52–2.48	0.76
Non-Caucasian Race	1.21	0.73–2.00	0.46	2.29	1.31–3.99	<0.01	2.57	1.33–4.98	0.01
Male Gender*	1.41	0.96–2.09	0.08	1.61	1.02–2.55	0.04	1.90	1.07–3.38	0.03
DEP* Ile/Val or Val/Val Genotype	2.52	0.91–7.01	0.08	2.23	0.72–6.92	0.16	ns		

B. ETS Exposure

	Wheezing at 12 Months			Wheezing at 24 Months			Persistent Wheezing		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
ETS Exposure	1.78	1.17–2.71	0.01	2.06	1.27–3.35	<0.01	1.70	0.94–3.07	0.08
Ile/Val or Val/Val Genotype*	1.09	0.73–1.61	0.68	1.21	0.75–1.93	0.43	0.91	0.52–1.59	0.73
Daycare Attendance*	1.50	0.77–2.93	0.23	1.72	0.95–3.12	0.08	1.06	0.48–2.31	0.89
Non-Caucasian Race	1.21	0.73–1.99	0.46	2.36	1.36–4.10	<0.01	2.60	1.35–5.02	<0.01
Male Gender*	1.50	1.01–2.23	0.05	1.73	1.08–2.77	0.02	1.98	1.11–3.52	0.02
ETS* Ile/Val or Val/Val Genotype	ns			ns			ns		

C. Mold Exposure

	Wheezing at 12 Months			Wheezing at 24 Months			Persistent Wheezing		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Mold Exposure	1.31	0.87–1.97	0.20	2.18	1.30–3.63	<0.01	2.57	1.33–4.96	0.01
Ile/Val or Val/Val Genotype*	1.09	0.73–1.64	0.67	1.25	0.77–2.03	0.38	0.95	0.52–1.73	0.87
Daycare Attendance*	1.68	0.82–3.46	0.16	1.75	0.93–3.32	0.08	1.27	0.55–2.93	0.57
Non-Caucasian Race	1.37	0.81–2.34	0.24	3.06	1.68–5.60	<0.01	3.64	1.75–7.59	<0.01
Male Gender*	1.34	0.89–2.01	0.16	1.74	1.07–2.82	0.02	2.00	1.08–3.69	0.03
Mold* Ile/Val or Val/Val Genotype	ns			ns			ns		

D. Environmental Load

	Wheezing at 12 Months			Wheezing at 24 Months			Persistent Wheezing		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Environmental Load†									
None	ref			ref			ref		
One	1.21	0.75–1.95	0.43	1.82	1.00–3.32	0.05	2.05	0.96–4.41	0.06
Two or more	2.07	1.24–3.46	0.01	3.57	1.92–6.64	<.01	3.86	1.78–8.37	<0.01
Ile/Val or Val/Val Genotype*	1.15	0.78–1.69	0.50	1.30	0.81–2.08	0.28	0.96	0.55–1.70	0.89
Daycare Attendance*	1.52	0.78–2.97	0.22	1.78	0.98–3.24	0.06	1.13	0.51–2.50	0.75
Non-Caucasian Race	1.22	0.74–2.00	0.44	2.46	1.40–4.30	<0.01	2.83	1.45–5.52	<0.01
Male Gender*	1.43	0.96–2.12	0.08	1.73	1.08–2.76	0.02	2.05	1.14–3.68	0.02
Total Load* Ile/Val or Val/Val Geno	ns			ns			ns		

*
Daycare attendance at 12 months for 12 month wheezing; attendance at 24 months for 24 month and persistent wheezing.

ns: not significant at the 0.20 level and therefore removed from model (interaction only).

[†]ETS exposure, DEP estimate ≥ 0.50 μg/m^3 and/or mold exposure.

ref: referent category.

Table III

Adjusted Associations Estimated as Odds Ratios (OR) of Infant Wheezing with Demographic and Environmental Factors.

	Wheezing at 12 Months			Wheezing at 24 Months			Persistent Wheezing		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Male Gender	1.47	0.97–2.23	0.07	1.92	1.16–3.17	0.01	2.13	1.15–3.97	0.02
Non-Caucasian Race*	1.20	0.69–2.07	0.52	2.54	1.36–4.75	0.00	2.91	1.36–6.21	0.01
Daycare Attendance*	1.88	0.91–3.91	0.09	1.81	0.95–3.47	0.07	1.36	0.66–2.79	0.41
DEP Estimate Exposure ≥ 0.50 µg/m³	0.92	0.40–2.14	0.85	1.93	1.06–3.53	0.03	2.13	1.03–4.41	0.04
ETS Exposure	1.73	1.11–2.70	0.02	1.90	1.13–3.18	0.02	1.49	0.76–2.81	0.22
Mold Exposure	1.22	0.79–1.86	0.37	2.12	1.25–3.60	0.01	2.47	1.27–4.80	0.01
Ile/Val or Val/Val GST-P1 Genotype	0.94	0.59–1.47	0.77	1.25	0.76–2.07	0.38	0.94	0.52–1.73	0.85
Ile/Val or Val/Val GST-P1 *DEP Estimate ≥ 0.50µg/m³	2.09	0.69–6.30	0.19	ns			ns		

*
Defined as daycare attendance during the first year of life for wheezing at age 12 months, attendance during the second year of life for wheezing at 24 months of age and lifetime attendance for persistent wheezing.

ns: not significant at the 0.20 level and therefore removed from model (interaction only).

†ETS, mold and/or high DEP

ref: referent category