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

Kathy T. Schroer, BS, Jocelyn M. Biagini Myers, PhD, Patrick H. Ryan, PhD, Grace K. LeMasters, PhD, David I. Bernstein, MD, Manuel Villareal, MD, James E. Lockey, MD, MS, Tiina Reponen, PhD, Sergey Grinshpun, PhD, and Gurjit K. Khurana Hershey, MD, PhD

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 *GST-P1* Ile^{105} genotype may be overwhelmed by multiple environmental exposures that converge on oxidative stress pathways. *(J Pediatr 2009;154:401-8)*

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 human beings, the glutathione S-transferase (*GST*) class of multifunctional enzymes are divided into 8 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 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, or mold uniquely modifies wheezing and persistent

CCAAPS	Cincinnati Childhood Allergy and Air Pollution Study	LUR	Land-use regression
CI	Confidence interval	OR	Odds ratio
DEP	Diesel exhaust particle	ROS	Reactive oxygen species
ETS	Environmental tobacco smoke	SPT	Skin prick test

From Division of Asthma Research (K.S., J.M., G.K.), Cincinnati Children's Hospital Medical Center, Molecular and Developmental Biology (K.S.), University of Cincinnati and Cincinnati Children's Hospital Medical Center, Environmental Health (J.M., P.R., G.L., J.L., T.R., S.G.), Internal Medicine (D.B., M.V.), and the Department of Pediatrics (G.K.), University of Cincinnati, Cincinnati, OH.

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Reprint requests: Gurjit K. Khurana Hershey, MD, PhD, Division of Asthma Research, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., MLC 7037, Cincinnati, OH 45229-3039. E-mail: Gurjit.Hershey@cchmc.org.

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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 with 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 1 atopic parent. A complete description of the study's recruitment, methods, and objectives has been published.[17] Briefly, infants with at least 1 atopic parent (on the basis of allergy skin prick testing) were enrolled between 2001 and 2003 in a 7-county area of Cincinnati, Ohio. Families were recruited on the basis of 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 2 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 with a nylon bristle cytology brush. Genomic DNA was isolated with the Zymo Research Genomic DNA II Kit (Orange, California). Genotyping was accomplished with the LightTyper platform (Roche Diagnostics, GmbH, Mannheim, Germany). The polymerase chain reaction 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 with 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 noncarriers 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 1 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 with 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 m of each sampling site was collected in a geographic information system. 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 $\mu g/m^3$ (range = 0.23-0.88). The level of 0.5 $\mu g/m^3$ was chosen to determine high versus low exposure on the basis of 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 1 smoker (person that smoked 1 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.

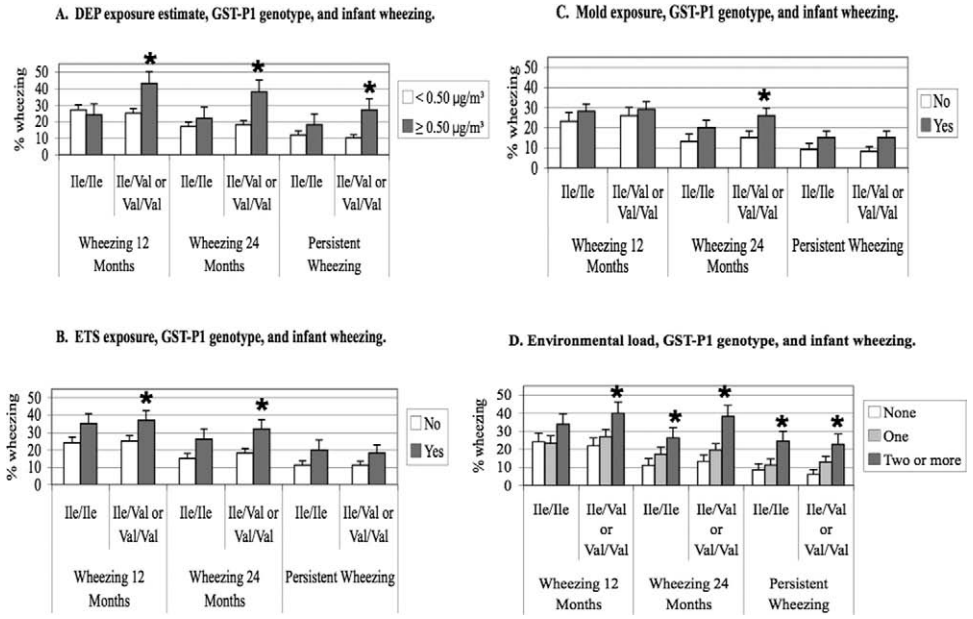

Figure 2. Individual environmental exposures, *GST-P1* genotype, and infant wheezing. **A,** High DEP estimate exposure levels were significantly associated with wheezing at 12 months, 24 months, and with persistent wheezing only in infants carrying the Val^{105} allele. **B,** ETS exposure was significantly 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 2 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 2 or more exposures. **P* value $< .05$.

Statistical Analysis

Racial differences for demographics, environmental exposures, health outcomes, and genotype and allele frequencies were compared by use of the χ^2 statistic. Unadjusted odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by use of 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. Because of 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 with conditional logistic regression adjusting for daycare attendance, race, and sex. Because 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 ($P = .46$), ETS ($P = .46$), and mold ($P = .44$) with wheezing at 12 months of age. Figure 2 therefore presents 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 .20. All analysis was performed with SAS software (version 8.2 for Windows; SAS Institute, Cary, North Carolina).

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% vs 26.7%; $P < .001$), have higher exposure to DEP ≥0.50 $\mu g/m^3$ ($P < .001$), and have visible mold in their homes ($P = .01$). ETS exposure did not significantly differ between the racial groups. Wheezing at 24 months of age (34.6% vs 17.2%) and persistent wheezing (24.6% vs 10.9%) were significantly increased in non-Caucasian versus Caucasian infants.

Effect of Exposure on Wheezing Phenotypes

Exposure to DEP ≥0.50 $\mu g/m^3$ 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.

Table I. Demographics, exposures, and health outcomes of infants

	Total	Caucasian	Non-Caucasian	*P* value
Sex				.76
Male	298 (52.3)	244 (52.6)	54 (50.1)	
Female	272 (47.7)	220 (47.4)	52 (49.1)	
Family income				<.001
<$40000	196 (34.7)	123 (26.7)	73 (69.5)	
≥$40000	369 (65.3)	337 (73.3)	32 (30.5)	
Daycare attendance at 12 months				.96
Yes	43 (7.6)	35 (7.6)	8 (7.7)	
No	524 (92.4)	428 (92.4)	96 (92.3)	
Daycare attendance at 24 months				.30
Yes	70 (12.4)	54 (11.7)	16 (15.4)	
No	497 (87.6)	409 (88.3)	88 (84.6)	
DEP estimate (binary)				<.001
<0.50 $\mu g/m^3$	469 (82.3)	396 (85.3)	73 (68.9)	
≥0.50 $\mu g/m^3$	101 (17.7)	68 (14.7)	33 (31.1)	
ETS exposure				.11
Yes	158 (28.5)	121 (27.0)	37 (34.9)	
No	396 (71.5)	327 (73)	69 (65.1)	
Mold exposure				.01
Yes	297 (56.5)	258 (59.2)	39 (43.3)	
No	229 (43.5)	178 (40.8)	51 (56.7)	
Environmental load*				.09
None	178 (32.2)	144 (31.0)	34 (32.1)	
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				.34
Yes	145 (27.5)	116 (26.6)	29 (31.5)	
No	383 (72.5)	320 (73.4)	63 (68.5)	
Wheezing at 24 months				<.001
Yes	99 (19.9)	72 (17.2)	27 (34.6)	
No	399 (80.2)	348 (82.8)	51 (65.4)	
Persistent wheezing				<.001
Yes	60 (12.9)	43 (10.9)	17 (24.6)	
No	404 (87.1)	352 (89.1)	52 (75.4)	
Total†	570	464	106	

*DEP estimate ≥ 0.50 $\mu g/m3$, ETS exposure, and mold exposure.
†The total n is different from the n for each individual demographic because not all data were available for each subject.

GST-P1 Alleles in Study Subjects

The *GST-P1* allele frequencies were significantly different ($P = .002$) 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 1 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% vs 14.7%; $P = .02$). *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 (≥0.50 $\mu g/m^3$) was associated with an increased risk for wheezing among carriers of the Val^{105} allele (Figure 2, *A*). 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 ($P = .01$), 24 months ($P = .002$), and with persistent wheezing ($P = .003$) (Figure 2, *A*). Similarly, carriers of the Val^{105} allele were significantly more likely to wheeze at 12 and 24 months if they were exposed to ETS ($P = .05$ and $P = .01$, respectively) compared with those infants with the protective Ile/Ile genotype (Figure 2, *B*). Carriers of the Val^{105} allele exposed to visible mold were also significantly more likely to wheeze at 24 months of age ($P = .04$) (Figure 2, *C*).

The effect of each exposure (DEP, ETS, and visible mold) was independently evaluated with respect to wheezing

Table II. Adjusted ORs for infant wheezing, environmental exposures, and *GST-P1* genotype

	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 exposure									
DEP estimate ≥0.50 $\mu g/m^3$	0.89	0.41-1.96	.78	1.16	0.47-2.82	.75	2.13	1.11-4.07	.02
Ile/Val or Val/Val genotype	0.94	0.61-1.44	.78	1.03	0.61-1.72	.93	0.92	0.52-1.61	.76
Daycare attendance*	1.48	0.76-2.88	.25	1.86	1.03-3.38	.04	1.13	0.52-2.48	.76
Non-Caucasian race	1.21	0.73-2.00	.46	2.29	1.31-3.99	<.01	2.57	1.33-4.98	.01
Male sex	1.41	0.96-2.09	.08	1.61	1.02-2.55	.04	1.90	1.07-3.38	.03
DEP* Ile/Val or Val/Val genotype	2.52	0.91-7.01	.08	2.23	0.72-6.92	.16	NS		
ETS exposure									
ETS exposure	1.78	1.17-2.71	.01	2.06	1.27-3.35	<.01	1.70	.94-3.07	.08
Ile/Val or Val/Val genotype	1.09	0.73-1.61	.68	1.21	0.75-1.93	.43	0.91	0.52-1.59	.73
Daycare attendance*	1.50	0.77-2.93	.23	1.72	0.95-3.12	.08	1.06	0.48-2.31	.89
Non-Caucasian race	1.21	0.73-1.99	.46	2.36	1.36-4.10	<.01	2.60	1.35-5.02	<.01
Male sex	1.50	1.01-2.23	.05	1.73	1.08-2.77	.02	1.98	1.11-3.52	.02
ETS * Ile/Val or Val/Val genotype	NS			NS			NS		
Mold exposure									
Mold exposure	1.31	0.87-1.97	.20	2.18	1.30-3.63	<.01	2.57	1.33-4.96	.01
Ile/Val or Val/Val genotype	1.09	0.73-1.64	.67	1.25	0.77-2.03	.38	0.95	0.52-1.73	.87
Daycare attendance*	1.68	0.82-3.46	.16	1.75	0.93-3.32	.08	1.27	0.55-2.93	.57
Non-Caucasian race	1.37	0.81-2.34	.24	3.06	1.68-5.60	<.01	3.64	1.75-7.59	<.01
Male sex	1.34	0.89-2.01	.16	1.74	1.07-2.82	.02	2.00	1.08-3.69	.03
Mold* Ile/Val or Val/Val genotype	NS			NS			NS		
Environmental load									
Environmental load†									
None	Ref			Ref			Ref		
One	1.21	0.75-1.95	.43	1.82	1.00-3.32	.05	2.05	0.96-4.41	.06
Two or more	2.07	1.24-3.46	.01	3.57	1.92-6.64	<.01	3.86	1.78-8.37	<.01
Ile/Val or Val/Val genotype	1.15	0.78-1.69	.50	1.30	0.81-2.08	.28	0.96	0.55-1.70	.89
Daycare attendance*	1.52	0.78-2.97	.22	1.78	0.98-3.24	.06	1.13	0.51-2.50	.75
Non-Caucasian race	1.22	0.74-2.00	.44	2.46	1.40-4.30	<.01	2.83	1.45-5.52	<.01
Male sex	1.43	0.96-2.12	.08	1.73	1.08-2.76	.02	2.05	1.14-3.68	.02
Total load* Ile/Val or Val/Val genotype	NS			NS			NS		

NS, Not significant at the 0.20 level and therefore removed from model (interaction only); *Ref,* Referent category.
*Daycare attendance at 12 months for 12-month wheezing, attendance at 24 months for 24-month wheezing, and persistent wheezing.
†ETS exposure, DEP estimate ≥0.50 $\mu g/m^3$, and/or mold exposure.

at 12 months, 24 months, and persistent wheezing after adjusting for genotype, daycare attendance, race, and sex (Table II). High DEP exposure (≥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 (≥0.50 $\mu g/m^3$) and *GST-P1* genotype on wheezing (DEP-*GST-P1* interaction, $P = 0.08$; Table II). 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 II). 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 II). 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 II). Thus, all 3 exposures were significant in a univariate model.

Multiple Exposures Associated with Wheezing

We next adjusted for all 3 exposures simultaneously, as well as genotype, daycare attendance, race, and sex (Table III). In this model, high DEP exposure (≥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

To evaluate the relationship of total environmental "load" with wheezing, the additive or synergistic effect of

Table III. Adjusted associations as estimated ORs 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 sex	1.47	0.97-2.23	.07	1.92	1.16-3.17	.01	2.13	1.15-3.97	.02
Non-Caucasian race	1.20	0.69-2.07	.52	2.54	1.36-4.75	.00	2.91	1.36-6.21	.01
Daycare attendance*	1.88	0.91-3.91	.09	1.81	0.95-3.47	.07	1.36	0.66-2.79	.41
DEP estimate exposure $\geq$0.50 $\mu g/m^3$	0.92	0.40-2.14	.85	1.93	1.06-3.53	.03	2.13	1.03-4.41	.04
ETS exposure	1.73	1.11-2.70	.02	1.90	1.13-3.18	.02	1.49	0.76-2.81	.22
Mold exposure	1.22	0.79-1.86	.37	2.12	1.25-3.60	.01	2.47	1.27-4.80	.01
Ile/Val or Val/Val *GST-P1* genotype	0.94	0.59-1.47	.77	1.25	0.76-2.07	.38	0.94	0.52-1.73	.85
Ile/Val or Val/Val *GST-P1** DEP estimate $\geq$0.50 $\mu g/m^3$	2.09	0.69-6.30	.19	NS			NS		

NS, Not significant at the 0.20 level and therefore removed from model (interaction only); *Ref,* Referent category.
*Defined as daycare attendance for the first year of life for wheezing at 12 months, attendance during the second year of life for wheezing at 24 months of age, and lifetime attendance for persistent wheezing.

having none, 1, or 2 or more environmental exposures (DEP, ETS, or mold) was investigated. One third (32.2%) of CCAAPS infants were not exposed to any of the 3 environmental exposures whereas 43.2% were exposed to 1 pollutant, and 25.6% were exposed to 2 or more. Carriers of the Val^{105} allele were found to be at risk for wheezing if they were exposed to 2 or more pollutants compared with unexposed infants at 12 months ($P = .02$), 24 months ($P < .01$), and with persistent wheezing ($P < .01$). In addition, infants homozygous for the Ile^{105} allele exposed to 2 or more air pollutants were at significantly increased risk for wheezing compared with those who were not exposed at 24 months ($P = .03$) and with persistent wheezing ($P = .01$) (Figure 2, *D*). 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 II).

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 support that DEP, ETS, 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 3 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 more than 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] Although 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 contrib-

utor 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 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 3 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 2. 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, 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.

REFERENCES

1. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998;351:1225-32.
2. Ryan PH, LeMasters GK, Biswas P, Levin L, Hu S, Lindsey M, et al. A comparison of proximity and land use regression traffic exposure models and wheezing in infants. Environ Health Perspect 2007;115:278-84.
3. World Health Organization (www.who.int). WHO Regional Office for Europe, 2004; Health aspects of air pollution. Results from the WHO project "Systemic review of health aspects of air pollution in Europe." Available from: www.euro.who.int/document/E83080.pdf.
4. Biagini JM, LeMasters GK, Ryan PH, Levin L, Reponen T, Bernstein DI, et al. Environmental risk factors of rhinitis in early infancy. Pediatr Allergy Immunol 2006;17:278-84.
5. Huang MF, Lin WL, Ma YC. A study of reactive oxygen species in mainstream of cigarette. Indoor Air 2005;15:135-40.
6. Riedl M, Diaz-Sanchez D. Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 2005;115:221-8; quiz 9.
7. Iossifova YY, Reponen T, Bernstein DI, Levin L, Kalra H, Campo P, et al. House dust (1-3)-beta-D-glucan and wheezing in infants. Allergy 2007;62:504-13.
8. Gilliland FD, Gauderman WJ, Vora H, Rappaport E, Dubeau L. Effects of glutathione-S-transferase M1, T1, and P1 on childhood lung function growth. Am J Respir Crit Care Med 2002;166:710-6.
9. Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA. Polymorphism at the glutathione S-transferase GSTP1 locus. A new marker for bronchial hyperresponsiveness and asthma. Am J Respir Crit Care Med 2000;161:1437-42.
10. Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B, et al. Nomenclature for human glutathione transferases. Biochem J 1992;282(Pt 1):305-6.
11. Pemble SE, Taylor JB. An evolutionary perspective on glutathione transferases inferred from class-theta glutathione transferase cDNA sequences. Biochem J 1992;287 (Pt 3):957-63.
12. Fryer AA, Hume R, Strange RC. The development of glutathione S-transferase and glutathione peroxidase activities in human lung. Biochim Biophys Acta 1986; 883:448-53.
13. Kano T, Sakai M, Muramatsu M. Structure and expression of a human class pi glutathione S-transferase messenger RNA. Cancer Res 1987;47:5626-30.
14. Doull IJ, Lawrence S, Watson M, Begishvili T, Beasley RW, Lampe F, et al. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. Am J Respir Crit Care Med 1996;153:1280-4.
15. Ahmad H, Wilson DE, Fritz RR, Singh SV, Medh RD, Nagle GT, et al. Primary and secondary structural analyses of glutathione S-transferase pi from human placenta. Arch Biochem Biophys 1990;278:398-408.
16. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998;19:275-80.
17. LeMasters GK, Wilson K, Levin L, Biagini J, Ryan P, Lockey JE, et al. High prevalence of aeroallergen sensitization among infants of atopic parents. J Pediatr 2006;149:505-11.
18. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
19. Martuzevicius D, Grinshpun SA, Reponen T, Gorny RL, Shukla R, Lockey J, et al. Spatial and temporal variations of PM2.5 concentration and composition throughout an urban area with high freeway density-the Greater Cincinnati study. Atmospheric Environment 2004;38:1091-105.
20. Hu S, McDonald R, Martuzevicius D, Biswas P, Turner J, Kelly A, et al. UNMIX calculations of ambient PM2.5 near an interstate highway in the greater Cincinnati area. Atmospheric Environment 2005;40:378-95.
21. Cho SH, Reponen T, LeMasters G, Levin L, Huang J, Meklin T, et al. Mold damage in homes and wheezing in infants. Ann Allergy Asthma Immunol 2006;97:539-45.
22. Gilliland FD, Li YF, Gong H Jr, Diaz-Sanchez D. Glutathione-S-Transferase M1 and P1 Prevent Aggravation of Allergic Responses by Secondhand Smoke. Am J Respir Crit Care Med 2006.
23. Gilliland FD, Rappaport EB, Berhane K, Islam T, Dubeau L, Gauderman WJ, et al. Effects of glutathione S-transferase P1, M1, and T1 on acute respiratory illness in school children. Am J Respir Crit Care Med 2002;166:346-51.
24. Vachier I, Damon M, Le Doucen C, de Paulet AC, Chanez P, Michel FB, et al. Increased oxygen species generation in blood monocytes of asthmatic patients. Am Rev Respir Dis 1992;146:1161-6.
25. Owen S, Pearson D, Suarez-Mendez V, O'Driscoll R, Woodcock A. Evidence of free-radical activity in asthma. N Engl J Med 1991;325:586-7.
26. von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. J Allergy Clin Immunol 2002;109:S525-32.
27. Jussila J, Komulainen H, Huttunen K, Roponen M, Halinen A, Hyvarinen A, et

al. Inflammatory responses in mice after intratracheal instillation of spores of Streptomyces californicus isolated from indoor air of a moldy building. Toxicol Appl Pharmacol 2001;171:61-9.
28. Jaakkola JJ, Jaakkola N, Ruotsalainen R. Home dampness and molds as determinants of respiratory symptoms and asthma in pre-school children. J Expo Anal Environ Epidemiol 1993;3(Suppl 1):129-42.
29. Nel AE, Diaz-Sanchez D, Li N. The role of particulate pollutants in pulmonary inflammation and asthma: evidence for the involvement of organic chemicals and oxidative stress. Curr Opin Pulm Med 2001;7:20-6.
30. Leem JH, Kim JH, Lee KH, Hong Y, Lee KH, Kang D, et al. Asthma attack associated with oxidative stress by exposure to ETS and PAH. J Asthma 2005; 42:463-7.
31. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445-600.
32. De Sario M, Di Domenicantonio R, Corbo G, Forastiere F, Pistelli R, Rusconi F, et al. Characteristics of early transient, persistent, and late onset wheezers at 9 to 11 years of age. J Asthma 2006;43:633-8.

50 Years Ago in *The Journal of Pediatrics*

Is There a Rheumatic Constitution?

Diamond EF. J Pediatr 1959;54:341-7

The mystery of widespread occurrence of streptococcal pharyngitis and yet relatively uncommon and unpredictable occurrence of rheumatic fever has intrigued the medical community for a century. Observant clinicians wondered whether gross physical characteristics might distinguish a rheumatic constitution—much as the prematurely gray, blue-eyed Nordic type is known to have a proclivity for pernicious anemia. By the 1950s, numerous authors had reported a preponderance of certain physical characteristics in children with rheumatic fever, spurring Diamond to formally evaluate a roster of Chicago children with rheumatic fever (sample size, 76-204, depending on factor studied). He sought a simple preponderance of certain features and used a formalized control group for assessing other characteristics.

Unlike previously reported associations, Diamond found no preponderance in rheumatic children of race, hair or eye color, asthenic or pyknic body build, skin pallor, blood type, or salivary non-secretion of ABO substance (ie, probable secretion of Lewis substance). Diamond found a single significant constitutional factor—hyperextensibility of metacarpophalangeal joints—significantly more frequently in active and inactive rheumatic groups compared with control subjects. Diamond speculated that this finding may be an expression of an underlying biochemical abnormality of connective tissue.

In *The Journal* in 2002, Barron et al (J Pediatr 2002;141:421-5) reported a case-control study performed in children referred to Johns Hopkins Hospital for chronic fatigue and found hyperextensibility of joints 3.5 times more frequently in these patients than in control children referred to the hospital's dermatology clinic. One wonders whether this finding in patients with chronic fatigue and rheumatic fever is merely deconditioning or whether it belies an underlying genetic disorder.

Recent geographically confined outbreaks of rheumatic fever and use of modern molecular microbiology have focused attention on rheumatogenic strains of group A streptococcus. Family size, however, also has been a risk factor for rheumatic fever. It seems that the duration of the unsolved mystery alone and evidence from several corners suggest that there is room to consider relevant contributions to rheumatogenicity from the microbe, the host, and the environment.

Sarah S. Long, MD
Section of Infectious Diseases
St. Christopher's Hospital for Children
Philadelphia, Pennsylvania
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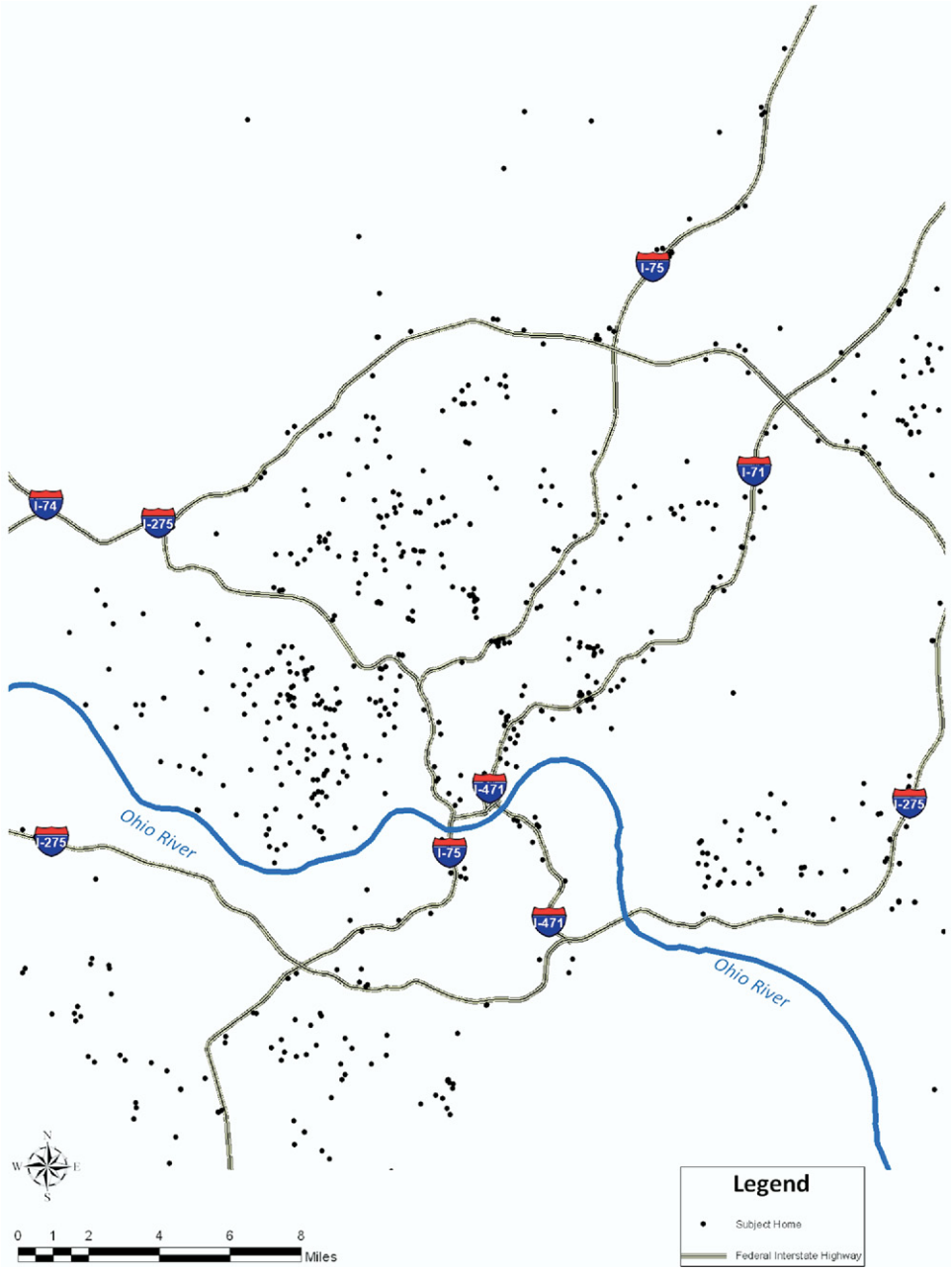

Figure 1. Geographic location of CCAAPS infants' homes. Infants lived within a 7-county area of Cincinnati, Ohio. Families were recruited on the basis of the proximity of their home residence to truck and bus traffic by geocoding residential addresses located on infant birth records.