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

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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

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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.