Genome-wide expression profiles identify potential targets for gene-environment interactions in asthma severity

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Background: Gene-environment interaction studies using genome-wide association study data are often underpowered after adjustment for multiple comparisons. Differential gene expression in response to the exposure of interest can capture the most biologically relevant genes at the genome-wide level.
Objective: We used differential genome-wide expression profiles from the Epidemiology of Home Allergens and Asthma birth cohort in response to Der f 1 allergen (sensitized vs nonsensitized) to inform a gene-environment study of dust mite exposure and asthma severity.
Methods: Polymorphisms in differentially expressed genes were identified in genome-wide association study data from the Childhood Asthma Management Program, a clinical trial in childhood asthmatic patients. Home dust mite allergen levels (<10 or ≥10 μg/g dust) were assessed at baseline, and (≥1) severe asthma exacerbation (emergency department visit or hospitalization for asthma in the first trial year) served as the disease severity outcome. The Genetics of Asthma in Costa Rica Study and a Puerto Rico/Connecticut asthma cohort were used for replication.
Results: *IL9*, *IL5*, and proteoglycan 2 expression *(PRG2)* was upregulated in Der f 1–stimulated PBMCs from dust mite–sensitized patients (adjusted $P < .04$). *IL9* polymorphisms (rs11741137, rs2069885, and rs1859430) showed evidence for interaction with dust mite in the Childhood Asthma Management Program ($P = .02$ to $.03$), with replication in the Genetics of Asthma in Costa Rica Study ($P = .04$). Subjects with the dominant genotype for these *IL9* polymorphisms were more likely to report a severe asthma exacerbation if exposed to increased dust mite levels.
Conclusions: Genome-wide differential gene expression in response to dust mite allergen identified *IL9*, a biologically plausible gene target that might interact with environmental dust mite to increase severe asthma exacerbations in children. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Dust mite allergen, asthma exacerbation, asthma severity, IL-9, gene expression*

Increased indoor allergen levels have been associated with the development of allergen sensitization and asthma in children[1-4] and are known to exacerbate symptoms in asthmatic patients.[5,6] Interventions to decrease the burden of indoor allergens have been shown to decrease asthma morbidity.[7] Although indoor allergens are one of the strongest and most consistent environmental risk factors associated with asthma severity,[8] very little is known about genetic modifiers of an asthmatic patient's response to allergens. Genetic heterogeneity might explain why some children experience greater asthma morbidity in response to allergen exposure than others, even after accounting for underlying allergen sensitization.

One of the most common indoor allergens associated with asthma morbidity is dust mite.[9] There have been relatively few studies on how genetic polymorphisms interact with environmental dust mite exposure to influence asthma severity in children. Studies thus far have mainly focused on individual candidate genes. For instance, previous reports (using data from both the Childhood Asthma Management Program [CAMP] and the Genetics of Asthma in Costa Rica Study [GACRS]) found that dust mite exposure might modify the effects of polymorphisms in *TGFB1*[10] and *IL10*[11] on asthma exacerbations. Other research (also conducted in CAMP) has shown that polymorphisms in *P2RY12* (a receptor involved in leukotriene signaling) might modify the effect of environmental dust mite exposure on lung function.[12] Although important insights can be gained by examining the interaction of candidate genes with environmental exposures, this type of restricted focus could miss important gene targets for interaction elsewhere in the genome.

Candidate gene work has begun to give way to systems biology approaches, in which genome-wide responses to network perturbations are used to understand the underlying pathophysiology. For gene-environment interaction models, this includes analyzing *in vitro* genome-wide expression data in response to environmental exposures of interest to select potential gene targets for interaction.

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Abbreviations used
CAMP: Childhood Asthma Management Program
GACRS: Genetics of Asthma in Costa Rica Study
HAA: Epidemiology of Home Allergens and Asthma
OR: Odds ratio
PRG2: Proteoglycan 2
SNP: Single nucleotide polymorphism

In this work we stimulated PBMCs with dust mite allergen (Der f 1) and examined differential gene expression profiles from mite-sensitized versus nonsensitized subjects. In 2 separate studies of children with asthma (CAMP and GACRS), we examined the potential for interaction between polymorphisms in the differentially expressed genes, and environmental dust mite exposure with severe asthma exacerbations as the main outcome of interest. For significant gene-environment interactions, we performed a meta-analysis, including CAMP, GACRS, and a third cohort of asthmatic patients from San Juan, Puerto Rico, and Hartford, Connecticut.

METHODS

Study populations

The Epidemiology of Home Allergens and Asthma (HAA) study, a longitudinal birth cohort study of the effects of environmental exposures on allergy and asthma risk in children, was used to examine gene expression responses to dust mite allergen stimulation *in vitro*. The HAA study was approved by the institutional review board of Brigham and Women's hospital. A detailed description of subject recruitment and study design has been published previously.[13]

Briefly, between September 1994 and June 1996, families from metropolitan Boston (Massachusetts) were recruited at a major Boston hospital during the immediate postnatal period of the index child's birth. After written informed consent was obtained from the child's primary caretaker, a series of home visits were made. Of the 505 children enrolled in the study, 430 (85%) were followed until age 12 years. At the home visit at age 12 years, skin testing to common allergens was conducted (n = 208), as described previously,[14] and blood samples were drawn to isolate PBMCs for stimulation with dust mite allergen (n = 80). Subjects were considered sensitized to dust mite allergen if specific IgE levels to *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* allergen were 0.35 IU/mL or greater. Gene expression responses in PBMCs after mite allergen stimulation (Der f 1) were assessed for Der f 1–sensitized versus nonsensitized subjects. These differential expression profiles were used to select genes for gene-environment interaction models in asthmatic children.

CAMP, a clinical trial of asthma treatment and lung function in children with mild-to-moderate asthma (ages 5-12 years), was used to study how polymorphisms in the differentially expressed genes interacted with environmental dust mite exposure to influence lung function and severe asthma exacerbations. Subjects in CAMP were randomly assigned to receive budesonide, nedocromil, or placebo and were followed every 2 to 4 months for 4 years to study the long-term use of the medications. Details of this study have been previously published.[15] For the current analysis, we included data from 530 white subjects with both genotype data and dust mite allergen measurements. The institutional review board at each of the 8 participating institutions approved the study, and parents or guardians of the subjects provided informed consent.

GACRS[16] was used to replicate findings from the CAMP gene-environment interaction analyses. Details on subject recruitment and study protocols have been published elsewhere.[16] In brief, children 6 to 14 years of age were included if they had asthma (a physician's diagnosis of asthma and ≥2 respiratory symptoms or asthma attacks in the previous year) and a high probability of having 6 or more great-grandparents born in the Central Valley of Costa Rica. Children completed a protocol, including a questionnaire, pulmonary function testing (n = 549), and methacholine challenge testing (n = 442). Blood samples were obtained from each child for DNA extraction. For the replication study, we used data from 558 children with genotype, dust mite allergen exposure, and severe asthma outcome data.

In addition to the replication study, we performed a meta-analysis on significant gene-environment interactions (observed in CAMP) by combining data from CAMP and GACRS with data from a third cohort of children with asthma based in San Juan, Puerto Rico, and Hartford, Connecticut (n = 618). Details of this third cohort are described in the Methods section in this article's Online Repository at www.jacionline.org.

Differential gene expression

In the HAA cohort PBMCs from 80 subjects (41% with mite allergen sensitization) were stimulated with 30 μg/mL dust mite allergen (Der f 1) for 72 hours. mRNA was extracted from cell pellets after stimulation. Genome-wide gene expression responses were measured on the Illumina HumanHT-12 v4 platform (Illumina, San Diego, Calif). Expression levels were normalized by using quantile normalization, and data were $\log_2$ transformed before analysis. Subjects sensitized to *D farinae* were randomly distributed across *in vitro* transcription and microarray batches, minimizing the potential for bias because of batch effects. The limma package (R statistical software) was used to identify differential gene expression in *D farinae*–sensitized versus nonsensitized subjects' PBMCs after Der f 1 allergen stimulation.

Genotyping

Genotyping of the top differentially expressed genes, *IL9*, *IL5*, and proteoglycan 2 (PRG2; along with a 5-kb flanking region around each gene), was included in the CAMP and GACRS cohorts as part of a genome-wide association study analysis. For CAMP, 530 white children were genotyped with the HumanHap550 Genotyping BeadChip or Infinium HD Human610-Quad BeadChip by Illumina. *IL9*, *IL5*, and *PRG2* single nucleotide polymorphisms (SNPs) included in subsequent analyses had a minor allele frequency of at least 5% and less than 1% genotype missingness. Genotyping of 558 children in GACRS (replication cohort) was conducted by using the Illumina HumanOmniExpress-12v1 platform. Genotyping of 560 children in the Puerto Rico/Connecticut cohort was done with the Illumina HumanOmni2.5 Bead Chip platform.

Exposure and outcome variables

Dust mite allergen exposure was assessed in both the CAMP and GACRS cohorts by extracting and analyzing a house dust sample (integrated across multiple rooms) from each child's home.[11] Der p 1 allergen levels were analyzed with an ELISA, as previously described,[11] and a similar methodology was used for Der f 1 allergen. Mite allergen exposure levels were categorized as high if the levels of either mite allergen (Der p 1 or Der f 1) was 10 μg/g house dust or greater. If both Der p 1 and Der f 1 levels were less than 10 μg/g, then mite allergen exposure was categorized as low.

In the CAMP cohort the severe asthma exacerbation outcome was defined as having 1 or more emergency department visits or hospitalizations for asthma in the first year of the clinical trial. In GACRS the severe asthma exacerbation outcome was defined as having had at least 1 hospitalization for asthma in the past year. One year after the start of the CAMP clinical trial, research assistants obtained spirometric measurements. Methacholine challenge data were gathered 8 months after the start of the CAMP trial. The decrease in FEV_1 (from baseline) was measured after inhalation of each methacholine solution in a series of challenges at increasing concentrations. The log of the methacholine dose required for a 20% decrease in FEV_1 was used as an outcome in linear regression models. In GACRS spirometric measures and $\log_{10}$ methacholine doses required for a 20% decrease in FEV_1 (with methacholine concentration expressed in micromoles) were gathered at or within 4 weeks of the dust mite allergen exposure assessment. Dust mite allergen sensitization (to *D pteronyssinus*, *D farinae*, or both), which is used as a covariate in all gene-environment interaction models,