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## Genome-Wide Expression Profiles Identify Potential Targets for Gene by Environment Interactions in Asthma Severity

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### Abstract

**Background**—Gene by environment interaction ( $G \times E$ ) studies utilizing GWAS data are often underpowered after adjustment for multiple comparisons. Differential gene expression, in response to the exposure of interest, may capture the most biologically relevant genes at the genome-wide level.

**Methods**—We used differential genome-wide expression profiles from the Home Allergens and Asthma Birth cohort in response to Der f 1 allergen (sensitized vs. non-sensitized) to inform a  $G \times E$  study of dust mite exposure and asthma severity. Polymorphisms in differentially expressed genes were identified in GWAS data from CAMP, a clinical trial in childhood asthmatics. Home dust mite allergen ( $< \text{or} \geq 10 \mu\text{g/g}$  dust) was assessed at baseline, and ( $\geq 1$ ) severe asthma exacerbation (emergency room (ER) visit or hospitalization for asthma in the first trial year) served as the disease severity outcome. The Genetics of Asthma in Costa Rica (GACRS) study, and a Puerto Rico/Connecticut asthma cohort were used for replication.

**Results**—IL-9, IL-5 and PRG2 expression was up-regulated in Der f 1 stimulated PBMCs from dust mite sensitized individuals (adj.  $p$  value  $< 0.04$ ). IL-9 polymorphisms (rs11741137, rs2069885, rs1859430) showed evidence for interaction with dust mite in CAMP ( $p = 0.02$  to  $0.03$ ), with replication in GACRS ( $p = 0.04$ ). Subjects with the dominant genotype for these IL-9 polymorphisms were more likely to report a severe asthma exacerbation if exposed to elevated dust mite.

**Conclusions**—Genome-wide differential gene expression in response to dust mite allergen identified IL-9, a biologically plausible gene target that may interact with environmental dust mite to increase severe asthma exacerbations in children.

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## INTRODUCTION

Elevated indoor allergen levels have been associated with the development of allergic sensitization and asthma in children<sup>(1–4)</sup>, and are known to exacerbate symptoms in subjects with asthma<sup>(5, 6)</sup>. Interventions to decrease the burden of indoor allergens have been shown to decrease asthma morbidity<sup>(7)</sup>. Although indoor allergens are one of the strongest, most consistent environmental risk factors associated with asthma severity<sup>(8)</sup>, very little is known about genetic modifiers of an asthmatic's response to allergens. Genetic heterogeneity may explain why some children experience greater asthma morbidity in response to allergen exposures than others, even after accounting for underlying allergen sensitization.

The most common indoor allergen associated with asthma morbidity is dust mite<sup>(9)</sup>. 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 Genetics of Asthma in Costa Rica Study (GACRS)) found that dust mite exposure may modify the effects of polymorphisms in TGFB1<sup>(10)</sup> and IL-10<sup>(11)</sup> on asthma exacerbations. Other research (also conducted in CAMP) has shown that polymorphisms in PY12 (a receptor involved in leukotriene signaling) may modify the effect of environmental dust mite exposure on lung function<sup>(12)</sup>. While 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, where genome wide responses to network perturbations are utilized to understand underlying pathophysiology. For gene by 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.

In this work we stimulated PBMCs with dust mite allergen (Der f 1) and examined differential gene expression profiles from mite sensitized vs. non-sensitized individuals. In two separate studies of children with asthma (CAMP and GACRS), we examined the interaction between polymorphisms in the differentially expressed genes, environmental dust mite exposure and severe asthma exacerbations. For significant gene by environment interactions, we performed a meta-analysis including CAMP, the GACRS and a third cohort of asthmatics 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<sup>(13)</sup>.

Briefly, between September 1994 and June 1996, families from metropolitan Boston (MA) were recruited at a major Boston hospital during the immediate post-natal 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, skin testing to common allergens was conducted (n=208) as described previously<sup>(14)</sup> 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 Der p or Der f allergen were  $\geq 0.35$  IU/ml. Gene expression responses in PBMCs following mite allergen stimulation (Der f 1) were assessed for Der f 1 sensitized vs. non-sensitized subjects. These differential expression profiles were used to select genes for gene by environment interaction models in children with asthma.

CAMP, a clinical trial of asthma treatment and lung function in children with mild to moderate asthma (ages 5 to 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.<sup>(15)</sup> 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 eight participating institutions approved the study and parents or guardians of the subjects gave informed consent.

The GACRS<sup>(16)</sup> was used to replicate findings from the CAMP gene by environment interaction analyses. Details on subject recruitment and study protocols have been published elsewhere.<sup>(16)</sup> In brief, children 6 to 14 years of age were included if they had asthma (a physician's diagnosis of asthma and two or more respiratory symptoms or asthma attacks in the previous year) and a high probability of having six 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 by environment interactions (observed in CAMP) by combining data from CAMP and the GACRS with 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 supplementary methods file.

### Differential Gene Expression

In the HAA cohort, PBMCs from n=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 following stimulation. Genome wide gene expression responses were measured on the Illumina HumanHT-12 v4 platform. Expression levels were normalized using quantile normalization, and data were log2 transformed prior to analysis. Subjects sensitized to Der f were randomly distributed across in vitro transcription and microarray batches, minimizing potential for bias due to batch effects. The limma package (R statistical software) was used to identify differential gene expression in Der f sensitized vs. non-sensitized subjects' PBMCs following Der f 1 allergen stimulation.

### Genotyping

Genotyping of the top differentially expressed genes IL-9, IL-5 and PRG2 (along with a 5kb flanking region around each gene) was included in the CAMP and the GACRS cohorts as part of a GWAS analysis. For CAMP, 530 white children were genotyped using the HumanHap550 Genotyping BeadChip or Infinium HD Human610-Quad BeadChip by Illumina, Inc (San Diego, CA). IL9, IL-5 and PRG2 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 The GACRS (replication cohort), was conducted using the Illumina HumanOmniExpress-12v1 platform by Illumina Inc. (San Diego CA). Genotyping of 560 children in the Puerto Rico/Conn cohort was done using the HumanOmni2.5 Bead Chip platform by Illumina Inc. (San Diego, CA).

### Exposure and Outcome Variables

Dust mite allergen exposure was assessed in both the CAMP and the GACRS cohorts by extracting and analyzing a house dust sample (integrated across multiple rooms) from each child's home<sup>(11)</sup>.

Der p 1 allergen levels were analyzed using an ELISA, as previously described<sup>(11)</sup>, and a similar methodology was used for Der f 1 allergen. Mite allergen exposure level was categorized as high if either mite allergen (Der p 1 or Der f 1) was  $\geq 10\mu\text{g/g}$  house dust. If both Der p 1 and Der f 1 levels were  $< 10\mu\text{g/g}$  then mite allergen exposure was categorized as low.

In the CAMP cohort, the severe asthma exacerbation outcome was defined as having  $\geq 1$  emergency room (ER) visit or hospitalization for asthma in the first year of the clinical trial. In the GACRS, the severe asthma exacerbation outcome was defined as having had at least one hospitalization for asthma in the past year. One year after the start of the CAMP clinical trial, research assistants obtained spirometry measurements. Methacholine challenge data

were gathered 8 months after the start of the CAMP trial. Decrease in FEV1 (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% drop in FEV1 was used as an outcome in linear regression models. In the GACRS, spirometry measures and log<sub>10</sub> methacholine dose required for a 20% drop in FEV1 (with methacholine concentration expressed in μmol) were gathered at or within four weeks of the dust mite allergen exposure assessment. Dust mite allergen sensitization (to Der p and/or Der f), used as a covariate in all gene by environment interaction models, was ascertained in both the CAMP and the GACRS studies by skin prick testing (SPT). Sensitization to either Der p and/or Der f allergen were grouped together as one “dust mite sensitization” covariate, since the majority of children with a positive SPT response to dust mite are sensitized to both allergens (80% of dust mite sensitized children in CAMP, 73 % in GACRS). This overlap in Der p/Der f sensitization is consistent with immunological reports showing IgE cross reactivity between the two allergens<sup>(17)</sup> (which is likely due to a cross reactive IgE binding epitope on both Der p 1 and Der f 1 allergens)<sup>(18)</sup> For the Puerto Rico/Conn cohort, home Der p 1 allergen levels and Der p sensitization were assessed as described previously.<sup>(19)</sup>

## Gene by environment interaction models

For the analysis of severe asthma exacerbations ( $\geq 1$  in the past year), logistic regression models were used to study interactions between dust mite allergen exposure level ( $\geq 10 \mu\text{g/g}$ ) and polymorphisms in IL-9, IL-5 and PRG2. These models included terms for the main effects of SNP genotype (assuming a dominant model), and dust mite allergen exposure, as well as a multiplicative interaction term (SNP dominant genotype\*mite allergen exposure). Models were adjusted for treatment group, age, sex, and dust mite sensitization. Linear regression models were used for the analysis of airway responsiveness, and included the same terms for the main effects, interaction, and covariates. Models for percent predicted FEV1 were adjusted for treatment group. Parallel logistic and linear regression models were created in the GACRS study if the coefficient for the gene by environment interaction in CAMP had a p value of less than 0.05. Covariates in the GACRS models included, age, sex, dust mite sensitization and inhaled corticosteroid use. Meta-analysis was conducted using Comprehensive Meta Analysis v2 statistical software (Engelwood, NJ).

## RESULTS

## Differential Gene Expression

Forty one percent of the children with gene expression data in stimulated PBMCs were sensitized to Der f dust mite allergen, 61% were male, and 26% reported a maternal history of asthma. Subjects were age 12 years, on average, at the time of PBMC collection and stimulation with Der f 1 allergen (Table 1). Overall, three genes (IL-9, IL-5, and PRG2) were differentially expressed in Der f sensitized vs. non-sensitized subjects after adjustment for multiple comparisons. Expression of these genes was upregulated in Der f sensitized individuals, with a positive log fold change of 1.6 for IL-9, 0.8 for PRG2 and 0.3 to 0.5 for IL-5 (Table 2). Another top ranked gene in the differential expression analysis of borderline statistical significance after adjustment for multiple comparisons ( $p=0.09$ ) was CLDN9 (a junction protein in epithelial cells).

## Gene by Environment Interactions

**Cohorts**—Subjects in the CAMP and the GACRS were similar in terms of age (average 9 years), maternal history of asthma (25% to 30%), and sex (60% males). However, both dust mite exposure and sensitization rates were higher in the GACRS than in CAMP. Forty three percent of the GACRS subjects were exposed to dust mite allergen  $\geq 10$   $\mu\text{g/g}$  (vs. 19% in CAMP), and 80% of the Costa Rica subjects were sensitized to dust mite (vs. 50% in CAMP). Costa Rica study subjects tended to have higher levels of lung function as compared to CAMP subjects. (Table 1)

**Severe Asthma Exacerbation Outcome**—In CAMP, three SNPs in IL-9 (rs11741137, rs2069885, rs1859430) showed significant interactions (at the  $p < 0.05$  level) with environmental dust mite allergen to increase the odds of a severe asthma exacerbation ( $\geq 1$  ER visit or hospitalization for asthma in the first year of the trial) (Table 3). The IL-9 polymorphisms rs11741137 and rs2069885 are in linkage disequilibrium ( $R^2 = 99\%$ ), and are therefore capturing the same interaction effect. The SNP rs2069885 is a missense polymorphism (resulting in a threonine to methionine amino acid substitution), rs11741137 is a downstream gene variant with no known function, and rs1859430 is intronic (Table 4). Twenty seven percent of CAMP subjects had the dominant genotype for rs11741137 (T allele) /rs2069885 (A allele), while 38% had the rs1859430 polymorphism (A allele).

In CAMP, 29% of the children who had at least one copy of the IL-9 SNP rs11741137 (T allele) (or rs2069885 (A allele)) who were also exposed to elevated levels of dust mite allergen had severe asthma exacerbations ( $\geq 1$ ) within the past year (Figure 1). In contrast, 9% of the children who were exposed to high levels of mite allergen, but who did not have a copy of the IL-9 SNP rs11741137 (or rs2069885) had severe asthma exacerbations ( $\geq 1$ ). Children with elevated exposure to dust mite and a dominant IL-9 SNP genotype (T allele for rs11741137 or A allele for rs2069885) had nearly fourfold increased odds of at least one severe asthma exacerbation (OR=3.8, 95% CI 0.5 to 26.3), as compared to subjects with elevated dust mite exposure who did not have rs11741137/rs2069885 polymorphism. For children exposed to low levels of dust mite, the dominant genotype (rs11741137/rs2069885) was associated with a 30% lower odds of severe asthma exacerbation (OR=0.7, 95% CI 0.4 to 1.3). Similar effects were observed for rs1859430 in IL-9. Among children exposed to elevated mite allergen, one copy of the A allele for (rs1859430) was associated with threefold increased odds of severe asthma exacerbation (OR=3.0, 95% CI 0.5 to 19.0). In children exposed to low mite levels of dust mite, carriers of the A allele (rs1859430) had a 30% reduced odds of severe asthma exacerbation (OR=0.7, 95% CI 0.4 to 1.2).

**Replication**—The estimated interaction of rs11741137/rs2069885 in IL-9 with dust mite allergen was replicated in the GACRS ( $p = 0.04$  for interaction term), where children with the dominant genotype who were exposed to elevated mite allergen, had the highest frequency of severe asthma exacerbations. Compared to CAMP, similar trends for interaction of the rs1859430 genotype with mite allergen exposure level were also observed for the severe asthma exacerbations in the GACRS (Figure 2). However, the interaction term for this SNP was not replicated at the  $p < 0.05$  level ( $p = 0.13$ ). Overall, the frequency of IL-9 genotype for

rs11741137 (T allele carrier)/rs2069885 (A allele carrier) was 19% in the GACRS, and rs1859430 (A allele) had a prevalence of 27%.

**Meta-analysis**—We conducted a meta-analysis of the associations between dust mite exposure and severe asthma exacerbations stratified by IL-9 genotype (rs11741137/rs2069885), which included a third cohort of asthmatics from San Juan, Puerto Rico and Hartford, Conn (Figure 3a and 3b). A comparison of the three cohorts (CAMP, GACRS and Puerto Rico/Conn Cohort) is shown in Supplemental Table 1. The cohorts were similar in terms of age and gender, although severe asthma exacerbations were more prevalent in the Puerto Rico/Conn cohort. Minor allele frequency for rs11741137/ rs2069885 was 10% in the Puerto Rico/Conn. cohort, and 15% of study subjects reported at least one severe asthma exacerbation (hospitalization for asthma) within the past year.

In our meta-analysis of three cohorts (CAMP, GACRS and Puerto Rico/Conn), we observed an inconsistent effect of dust mite exposure in subjects without rs2069885, with a summary odds ratio centered around the null (Figure 3a). For subjects without the polymorphism, individual CAMP and GACRS analyses showed a borderline protective effect of elevated dust mite exposure, whereas in the Puerto Rico/Conn cohort, subjects without rs2069885 showed increased risk of severe asthma exacerbations in response to dust mite. For subjects with the IL-9 polymorphism rs2069885, the trend for increased severe asthma exacerbations with higher dust mite exposure was seen in all three cohorts (showing a consistent direction of effect across all three studies, with a pooled OR=2.5 (95%CI 0.6 to 10.2) (Figure 3b).

**Lung Function**—There were no interactions between polymorphisms in IL-9, IL-5 or PRG2 and dust mite allergen exposure that were significant at  $p=0.05$  for the lung function outcomes in CAMP. However, for airway hyper-responsiveness the interaction term for the dominant genotype $\times$ dust mite exposure for IL-5 SNP rs2069812 (A allele) was borderline significant at  $p=0.06$ , and the  $p$  value for the interaction of dust mite exposure with the IL-9 SNP rs31564 (G allele) was also somewhat suggestive ( $p=0.11$ ). Among subjects exposed to higher levels of dust mite, polymorphisms in IL-5 (rs2069812) and IL-9 (rs31564) appeared to have a potential protective effect;those with the polymorphism required higher doses of methacholine to achieve a 20% decline in FEV1. Subjects with one copy of the IL-5 rs2069812 SNP who are also exposed to high levels of dust mite had a least square mean log PC20 of 0.33; however, subjects without the SNP appeared to have more reactive airways (a lower least square mean log PC20 of -0.01 in response to higher levels of dust mite). Similar effects were observed for the IL-9 SNP rs31564 (those with the SNP who were exposed to high dust mite had a least square mean log PC20 of 0.27, while those without the SNP in the high dust mite exposure category had a least square mean of log PC20 of 0.07).

Polymorphisms in PRG2 did not interact with mite allergen exposure to influence severe asthma exacerbations in for asthma in CAMP. None of the polymorphisms studied were associated with percent predicted FEV1.

## DISCUSSION

In this study, we used genome wide differential expression profiles, in response to our exposure of interest (dust mite allergen), to select targets for gene by environment interactions. This integrative approach allowed us to test novel genes, all with potential biological relevance, for interaction with environmental dust mite allergen in a cohort of children with asthma. Of the top differentially expressed genes, IL-9 showed the most evidence for interaction with environmental dust mite allergen to alter severe asthma exacerbations in children.

Differential gene expression analysis, the first step in our approach, revealed three top genes for testing in gene by environment interaction models. As this was done in a cohort separate from our  $G \times E$  study, issues with statistical dependence (that would arise if gene expression and  $G \times E$  analysis were done in the same subjects) are not problematic. We examined expression levels in PBMCs, which capture cellular responses from both the innate and adaptive arms of the immune system. Dust mite allergen-stimulated PBMCs from sensitized subjects showed increased expression of IL-9, IL-5 and PRG2 (in comparison to un-sensitized subjects' stimulated cells) after adjustment for multiple comparisons. While IL-5 is part of classic immunological models of asthma and allergic disease, fewer studies have examined the role of IL-9 or PRG2 in asthma pathogenesis. These two genes, uncovered here using differential expression profiling, have not been considered in standard candidate gene by environment interaction studies of asthma, which typically rely on established biological pathways implicated in disease.

Immunological studies by specific cell type have identified sources of the cytokines IL-9 and IL-5. IL-9 is produced by mast cells, Th2 and Th9 cells<sup>(20)</sup>, while IL-5 is mainly produced by mast cells and Th2 lymphocytes<sup>(21)</sup>. PRG2, or natural killer cell activator, was also differentially expressed in our experiments; however the main cellular source of PRG2 mRNA levels from PBMCs is unclear. To our knowledge, this is the only study thus far to identify differential expression of IL-9 and PRG2 in mite allergen (Der f 1) stimulated PBMCs from individuals sensitized to Der f allergen. By utilizing a PBMC in vitro system for our gene expression experiments, we were able to capture expression signatures from adaptive immune cells that may not have been detectable if these cells had been cultured alone (without the presence of antigen presenting cells).

In our gene by environment interaction models, subjects with the IL-9 polymorphism rs11741137/rs2069885, who were also exposed to elevated levels of environmental dust mite allergen, had the highest frequency of severe asthma exacerbations. However, this risk allele seemed to have little influence on asthma severity when dust mite allergen exposure was low. This interaction was replicated in a second asthmatic cohort with higher dust mite exposure levels and prevalence of mite sensitization, and a meta-analysis of three cohorts revealed the same direction of effect for dust mite exposed subjects with the rs11741137/rs2069885 polymorphism. Replication of the gene by environment interaction for IL-9 polymorphisms strengthens the evidence for this gene's potential involvement in the pathogenesis of asthma severity, in the context of elevated allergen exposure levels. After accounting for dust mite allergen sensitization, the IL-9 rs11741137/rs2069885 dominant

genotype may identify the asthmatic population most susceptible to mite allergen exposure and therefore most likely to benefit from an intervention to reduce allergen levels.

The possible role of IL-9 in asthma severity, identified here as part of a gene by environment interaction, suggests that this cytokine may be a potential target for asthma treatment. Data from preliminary studies also indicate that IL-9 may be a viable target for asthma therapy. One possible anti-IL-9 agent, Sialostatin L, rendered almost complete abrogation of airway hyper-responsiveness and airway eosinophilia in a murine model of experimental asthma.<sup>(22)</sup> Results of a small 36-patient clinical trial in humans suggest that administration of an anti-IL-9 monoclonal antibody may decrease asthma exacerbations.<sup>(23)</sup> Additional data are available on the potential role of IL-9 in asthma pathogenesis. IL-9 has been shown to promote mast cell recruitment to the lung, increase mast cell activity, and enhance airway remodeling in a murine model of asthma.<sup>(24)</sup> This same report identified mast cells as the main expressers of IL-9 receptor in human asthmatic lung tissue.<sup>(24)</sup> IL-9 production from bronchial alveolar lavage lymphocytes increases following an inhaled allergen challenge in atopic asthmatic patients<sup>(25)</sup> and IL-9 has been shown to upregulate expression of eotaxin in cultured human airway smooth muscle cells.<sup>(26)</sup> Data on the specific effects of IL-9 polymorphisms on immune signaling and inflammation are limited. However, SNPs within the IL-9 gene could potentially impact the functionality of this cytokine, and may alter some of the biological effects described above.

Other genetic association studies have examined IL-9 signaling and asthma-related phenotypes, but these analyses did not consider environmental exposure to dust mite allergen. A link between an IL-9 G-T repeat polymorphism and atopic asthma (with sensitization to dust mite) was shown in a family based analysis<sup>(27)</sup>. Additional reports have demonstrated sex-specific effects of the IL-9 gene. One of these studies showed a sex-specific protective effect for wheeze in girls with a particular haplotype in the IL-9 receptor gene<sup>(28)</sup>, and another identified associations (in male subjects only) between the IL-9 polymorphisms rs2069885 and rs2069882 and phenotypes for lung function and multiple-allergen sensitization, respectively<sup>(29)</sup>. Lastly, a study in infants showed effect modification by sex for the association between the IL-9 polymorphism rs2069885 and susceptibility to severe RSV bronchiolitis, where the variant appeared protective in girls, but was linked with increased risk in boys<sup>(30)</sup>. In the present study, tests for the interaction between sex and the IL-9 polymorphism rs2069885 in the CAMP cohort were not significant for lung function phenotypes (percent predicted FEV1, airway hyper-responsiveness) or for the severe asthma exacerbation outcome ( $p > 0.5$  for interaction terms).

While this integrative gene by environment interaction study had many strengths including selection of genes using expression profiling, as well as replication of findings in a second cohort, there were also some study limitations. Gene expression profiles were based on only one time point (72 hours after dust mite allergen stimulation), and therefore may not have fully captured biologically relevant genes whose expression may have peaked at an earlier or later time point. We did not have data on how polymorphisms in IL-9, IL-5 and PRG2 may impact Der f 1 induced expression of these genes (genotyping data was unavailable in HAA study) or on how these SNPs may impact the function of downstream protein products. While we had gene expression profiles in response dust mite allergen Der f 1, we did not

conduct a parallel set of Der p 1 PBMC stimulation experiments, to determine the extent to which dust mite allergen expression profiles overlap. Lastly, we utilized only genetic polymorphisms available to us through GWAS studies in the CAMP and the GACRS cohorts. Other polymorphisms that were not genotyped as part of these studies may also be important for interaction with environmental dust mite exposure to alter asthma severity.

In summary, this integrative approach to gene by environment interactions in asthma severity utilized genome wide expression data to detect a biologically plausible gene target (IL-9) that may interact with environmental dust mite exposure to increase asthma severity in children. Future studies should consider gene expression profiles in response to other allergens known to exacerbate asthma. Eventually,  $G \times E$  studies such as this one may help identify children who would benefit most from interventions to decrease home aeroallergen levels.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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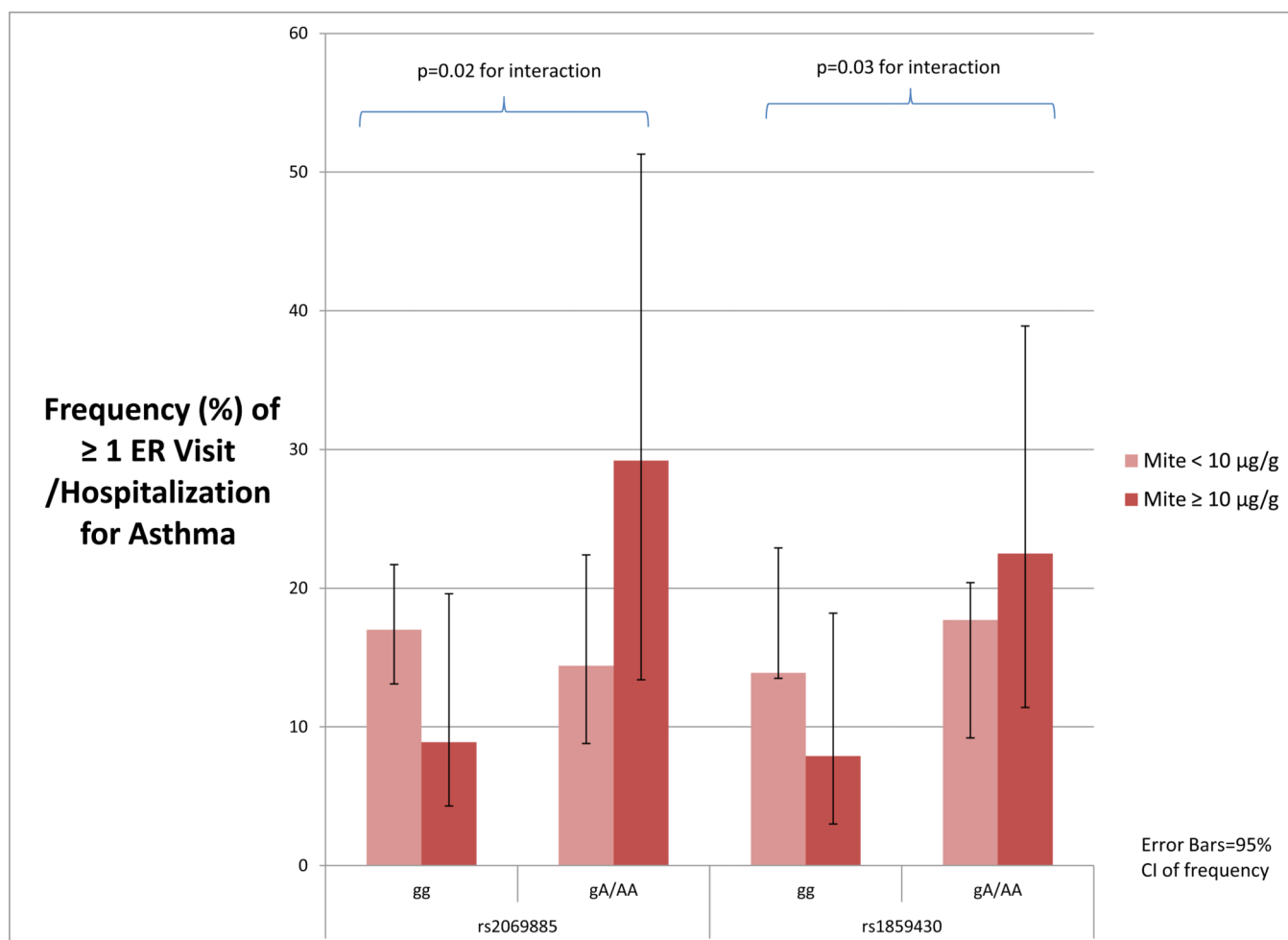
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**Figure 1.**

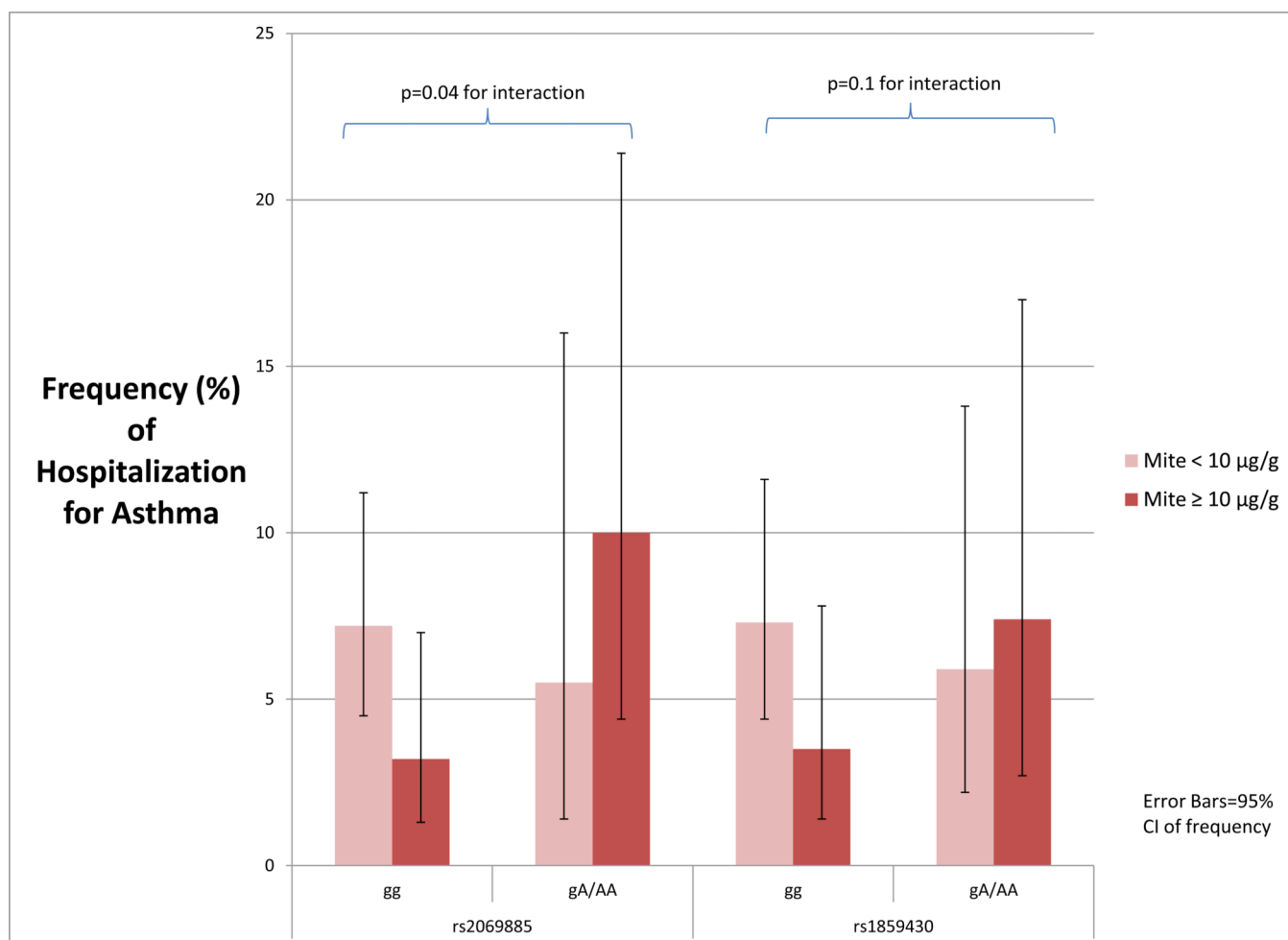
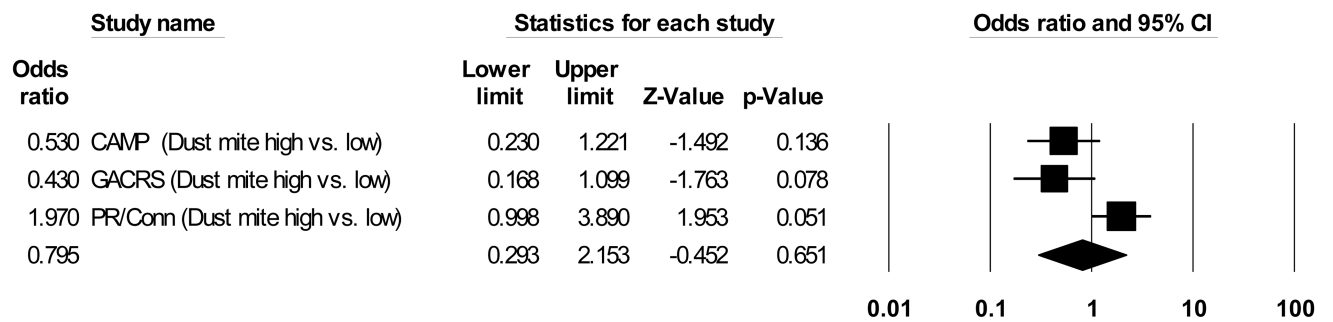


Figure 2.

## Meta Analysis



## Meta Analysis

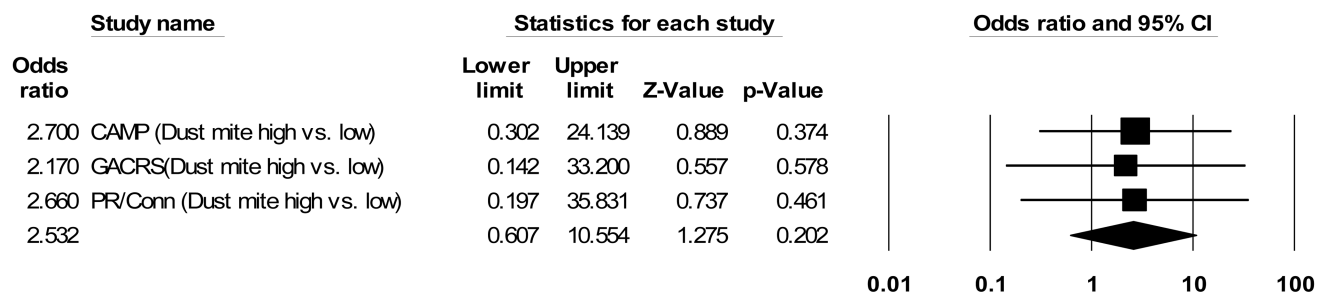


Figure 3.

**Table 1**

Characteristics of subjects for differential gene expression and gene by environment interaction studies

| Variable                                           | Gene Expression Study | Gene by Environment Interaction Study (Asthma Cohorts) |                         |
|----------------------------------------------------|-----------------------|--------------------------------------------------------|-------------------------|
|                                                    | HAA (n=80)            | CAMP (n=519)                                           | GACRS (n=558)           |
| Age (years) <sup>a</sup>                           | 11.9 (0.27)           | 8.9 (2.1)                                              | 8.9(1.8)                |
| Male Gender (n,%)                                  | 49 (61%)              | 309 (60%)                                              | 329 (60%)               |
| Maternal Asthma (n,%)                              | 21(26%)               | 127 (25%) *                                            | 169 (30%) *             |
| Current Asthma Diagnosis (n, %)                    | 10 (13%)              | 519 (100%)                                             | 558 (100%)              |
| Dust Mite Allergen sensitization (n,%)             | 33(41%) <sup>b</sup>  | 256 (50%) <sup>*c</sup>                                | 447 (80%) <sup>*c</sup> |
| Dust Mite Allergen (≥10µg/g)                       | --                    | 97(19%) *                                              | 240 (43%) *             |
| Severe Asthma Exacerbation <sup>d</sup>            | --                    | 84(16%) *                                              | 33 (6%) *               |
| Pre-bronchodilator FEV1(% predicted) <sup>a</sup>  | --                    | 94(14) *                                               | 100 (17) *              |
| Bronchodilator Response % change FEV1 <sup>a</sup> | --                    | 11(10) *                                               | 5.1(7.5) *              |
| Airway Hyperresponsiveness <sup>e</sup>            | --                    | 0.10 (1.18)                                            | 0.42 (0.29)             |

<sup>a</sup> Mean(standard deviation);<sup>b</sup> Sensitization to Der f<sup>c</sup> Sensitization to Der p or Der f<sup>d</sup> ER Visit/Hospitalization (≥ 1 in the past year) for asthma in CAMP; Hospitalization for asthma (≥1 in the past year) in GACRS;<sup>e</sup> Log dose for PC20 (mg/ml of methacholine) used in the CAMP study; Log<sub>10</sub> dose for PC20 (µmol of methacholine) used in the Costa Rican study.

\* p&lt;0.05 for comparison between the two asthma cohorts for responses with same units

**Table 2**

Differentially expressed genes in mite allergic (vs. non-allergic) individuals in the Home Allergens Cohort

| HUGO gene | LOG fold change | P-value      | Adjusted P value   |
|-----------|-----------------|--------------|--------------------|
| IL-9      | 1.5522564       | 4.259282e-09 | <b>0.000201481</b> |
| IL-5*     | 0.3171111       | 2.087846e-06 | <b>0.035029155</b> |
| PRG2      | 0.8325971       | 2.891444e-06 | <b>0.035029155</b> |
| IL-5*     | 0.5041824       | 2.962046e-06 | <b>0.035029155</b> |
| CLDN9     | -0.2174724      | 9.495920e-06 | 0.089838995        |

\* Two probes for IL-5 were in the top portion of the differentially expressed gene list

**Table 3**

Interaction between polymorphisms in mite-allergen induced genes and environmental dust mite exposure

| Gene | SNP        | Severe Asthma Outcome <sup>a</sup> |                       | Airway Hyperresponsiveness (AHR) |                     | Percent Predicted FEV1 |                     |
|------|------------|------------------------------------|-----------------------|----------------------------------|---------------------|------------------------|---------------------|
|      |            | CAMP                               | Costa Rica            | CAMP                             | CAMP                | CAMP                   | CAMP                |
|      |            | Interaction P value                | Interaction P value * | Interaction P value              | Interaction P value | Interaction P value    | Interaction P value |
| IL-9 | rs11741137 | <b>0.02</b>                        | <b>0.04</b>           | 0.91                             |                     | 0.99                   |                     |
|      | rs2069885  | <b>0.02</b>                        | <b>0.04</b>           | 0.93                             |                     | 0.99                   |                     |
|      | rs1859430  | <b>0.03</b>                        | 0.10                  | 0.22                             |                     | 0.74                   |                     |
|      | rs2069882  | 0.09                               | ---                   | 0.27                             |                     | 0.96                   |                     |
|      | rs31564    | 0.40                               | ---                   | 0.11                             |                     | 0.53                   |                     |
|      | rs31563    | 0.51                               | ---                   | 0.43                             |                     | 0.66                   |                     |
| PRG2 | rs490358   | 0.30                               | ---                   | 0.28                             |                     | 0.79                   |                     |
|      | rs10792094 | 0.98                               | ---                   | 0.87                             |                     | 0.18                   |                     |
| IL-5 | rs743562   | 0.18                               | ---                   | 0.48                             |                     | 0.39                   |                     |
|      | rs739719   | 0.70                               | ---                   | 0.98                             |                     | 0.70                   |                     |
|      | rs2069812  | 0.35                               | ---                   | 0.06                             |                     | 0.64                   |                     |

<sup>a</sup> Each model contains variables for dominant SNP genotype, mite exposure  $\geq 10$   $\mu\text{g/g}$  dust, interaction term (SNP genotype  $\times$  mite exposure), dust mite allergy, asthma treatment (treatment group for CAMP cohort; ICS use for Costa Rica), age and sex

\* One sided p value used for replication

**Table 4**

Polymorphisms Tested in Gene by Environment Interaction Models

| Gene | SNP        | Base<br>change | Minor Allele Freq |            | Note                                                            |
|------|------------|----------------|-------------------|------------|-----------------------------------------------------------------|
|      |            |                | CAMP              | Costa Rica |                                                                 |
| IL-9 |            |                |                   |            |                                                                 |
|      | rs11741137 | C→T            | 0.14              | 0.09       | Downstream gene variant                                         |
|      | rs2069885  | G→A            | 0.14              | 0.09       | Non-synonymous variant, T (ACG) threonine--> M (ATG) methionine |
|      | rs1859430  | G→A            | 0.21              | 0.15       | Intron Variant                                                  |
|      | rs2069882  | T→C            | 0.23              |            | Intron variant                                                  |
|      | rs31564    | T→G            | 0.34              |            | Intron variant                                                  |
|      | rs31563    | C→T            | 0.13              |            | Upstream gene variant                                           |
| PRG2 | rs490358   | G→A            | 0.27              |            | Synonymous variant                                              |
|      | rs10792094 | G→T            | 0.13              |            | Intron variant                                                  |
| IL-5 | rs743562   | C→T            | 0.38              |            | Downstream gene variant                                         |
|      | rs739719   | C→A            | 0.13              |            | Downstream gene variant                                         |
|      | rs2069812  | G→A            | 0.36              |            | Upstream gene variant                                           |