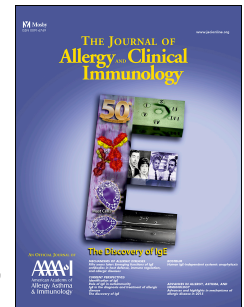


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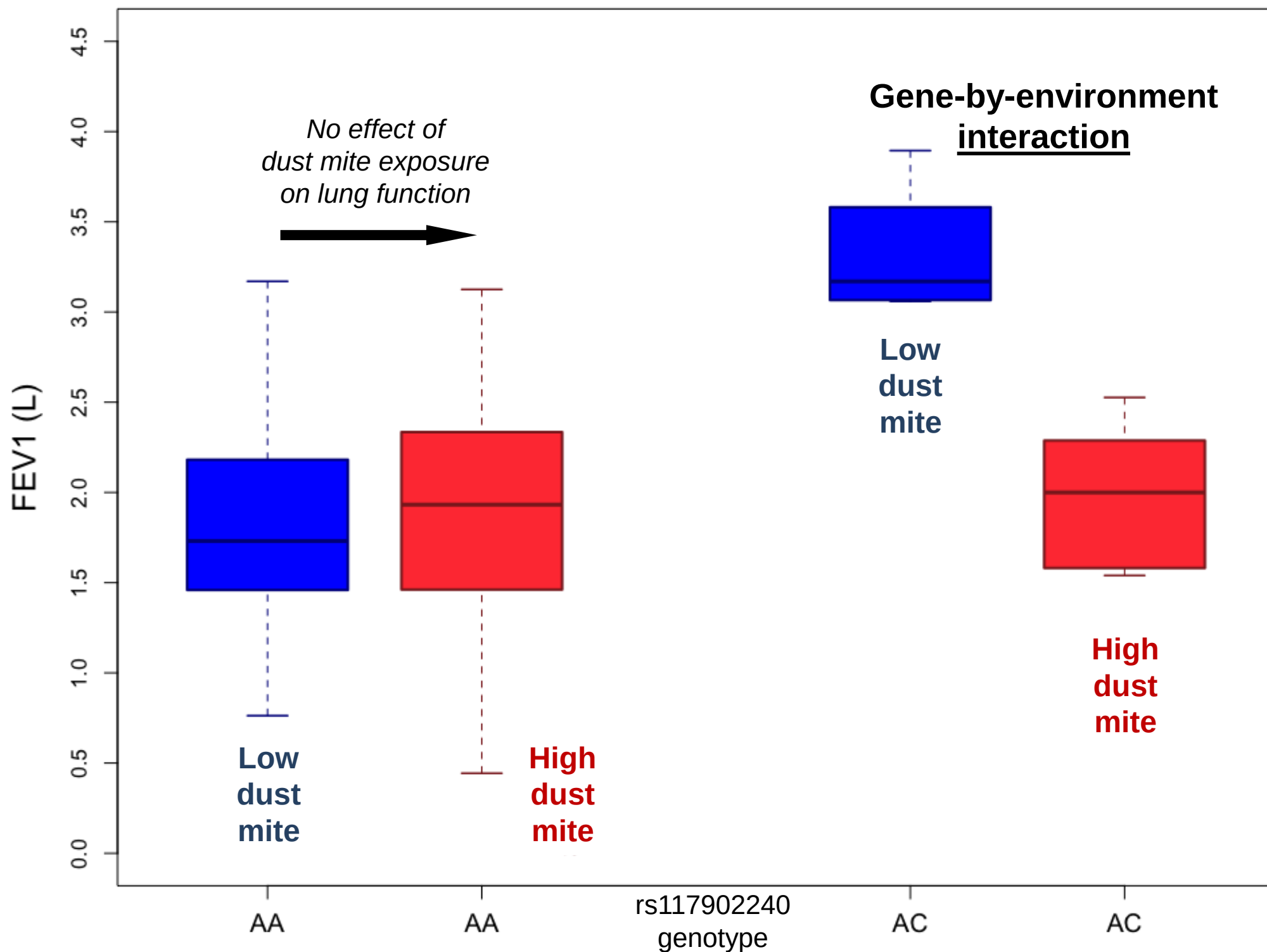
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Gene-by-environment interaction study:
rs117902240 and dust mite exposure on FEV₁ in children with asthma

[Discovered in Puerto Rican children; replicated in CAMP]



Genome-wide interaction study of dust mite allergen on lung function in children with asthma

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ABSTRACT

Background: Childhood asthma is likely the result of gene-by-environment (GxE) interactions.

Dust mite is a known risk factor for asthma morbidity. Yet, there have been no genome-wide GxE studies of dust mite allergen on asthma-related phenotypes.

Objective: To identify genetic variants whose effects on lung function in children with asthma are modified by level of dust mite allergen exposure.

Methods: A genome-wide interaction analysis of dust mite allergen level and lung function was performed in a cohort of Puerto Rican children with asthma (PRGOAL). Replication was attempted in two independent cohorts, the Childhood Asthma Management Program (CAMP) and the Genetics of Asthma in Costa Rica Study.

Results: SNP rs117902240 showed a significant interaction with dust mite allergen level on FEV₁ in PRGOAL (interaction $P=3.1 \times 10^{-8}$), and replicated in the same direction in CAMP White children and CAMP Hispanic children (combined interaction $P=0.0065$ for replication cohorts and 7.4×10^{-9} for all cohorts). Rs117902240 was positively associated with FEV₁ in children exposed to low dust mite allergen levels, but negatively associated with FEV₁ in children exposed to high levels. This SNP is on chromosome 8q24, adjacent to a binding site for CEBP β , a transcription factor that forms part of the IL-17 signaling pathway. None of the SNPs identified for FEV₁/FVC replicated in the independent cohorts.

Conclusions: Dust mite allergen exposure modifies the estimated effect of rs117902240 on FEV₁ in children with asthma. Analysis of existing data suggests this SNP may have transcription factor regulatory functions.

Capsule Summary: Dust mite allergen exposure modifies the effect of certain genetic polymorphisms on lung function in children with asthma.

Clinical Implications: The effect of certain genetic polymorphisms on lung function in children with asthma varies depending on their dust mite allergen exposure level. Future studies should assess whether personalized interventions for these children have a greater impact on lung function.

Key Words: Childhood asthma; lung function; dust mite allergen; genome-wide interaction study; gene-by-environment interaction; CEBP β

Abbreviations:

CEBP β	CCAAT/Enhancer binding protein beta
ChIP-seq	Chromatin immunoprecipitation and DNA sequencing
DNA	Deoxyribonucleic acid
ERK3	Extracellular signal-regulated kinase 3
FEV ₁	Forced expiratory volume in the first second
FVC	Forced vital capacity
GSK3 β	Glycogen synthase kinase 3 beta
GWAS	Genome-wide association study
GWIS	Genome-wide interaction study
GxE	Gene-by-environment interaction
IL	Interleukin
IRB	Institutional review board
P2RY12	Purinergic receptor P2Y, G-protein coupled, 12
PSU	Primary sampling unit
SNP	Single nucleotide polymorphism
TAK1	Transforming growth factor beta 1-activated kinase 1
TGF β 1	Transforming growth factor beta 1
TRAF6	Tumor necrosis factor receptor-associated factor 6

INTRODUCTION

Asthma, the most common non-communicable chronic illness among children¹, has an estimated heritability of up to 50-90%^{2,3}. Although dozens of genes that confer susceptibility to asthma or asthma morbidity have been recently identified⁴, single nucleotide polymorphisms (SNPs) in such genes account for as little as 10-50% of the disease's heritability. One potential reason for this "missing heritability" is gene-by-environment (GxE) interactions, as certain genetic variants may confer risk only in the presence of certain environmental exposures.

One of the best characterized environmental risk factors for asthma morbidity is exposure to indoor allergens. In particular, dust mite allergen is known to increase the risk of asthma exacerbations, and to increase airway responsiveness while reducing lung function⁵⁻⁷. The cysteine proteases in dust mites' fecal particles are potent inducers of allergic sensitization⁸, and exposure to house dust mite can also trigger a direct, non-allergic, inflammatory reaction⁹. Both interventions to reduce dust mite exposure^{10,11} and trials of immune therapy to induce dust mite tolerance¹²⁻¹⁴ have been shown to improve asthma symptoms or reduce morbidity from asthma.

Few studies have looked at the interaction between genetic variants and allergens on asthma.

Gene-by-allergen interactions have been reported for dust mite and several candidate genes for asthma, including *IL10*¹⁵, *TGFB1*¹⁶, or the purinergic receptor *P2RY12*¹⁷. More recently, a study using genome-wide expression *in vitro* responses to dust mite allergen reported that *IL9* may interact with environmental dust mite allergen and increase severe asthma exacerbations¹⁸.

In the present study, we performed a genome-wide study interaction study (GWIS) of house dust

105 mite allergen (*Dermatophagoides pteronyssinus*) level on lung function in Puerto Rican children
106 with asthma, with replication studies in two independent cohorts of children with asthma.

METHODS

Study population

As part of a case-control study of childhood asthma in Puerto Ricans (PRGOAL), we recruited 618 children with asthma (defined as physician-diagnosed asthma and at least one episode of wheeze in the prior year) living in Hartford (CT) and San Juan (PR). The details of subject recruitment have been published previously¹⁹⁻²¹.

In brief, from September 2003 to July 2008, flyers were distributed to all parents of children in grades K-8 in 15 public elementary/middle schools in Hartford, CT, that enroll a high proportion (42% to 94%) of Puerto Rican children. Of 640 children whose parents were interested in the study, 585 (91.4%) were eligible after screening; parents of 449 (76.7%) of these 585 children (including 267 children with asthma and 182 control subjects) agreed to participate. There were no significant differences in age, gender, or area of residence between eligible children who did (n=449) and did not (n=136) participate. From March 2009 to June 2010, children were recruited from households in metropolitan San Juan, PR, selected using a multistage probability sample design. Primary sampling units (PSUs) were randomly selected neighborhood clusters based on the 2000 U.S. census, and secondary sampling units were randomly selected households within each PSU. A household was eligible if ≥ 1 resident was a child 6-14 years old. In households with more than one eligible child, one child was selected for screening; in households with multiple eligible children, one child was randomly selected using Kish tables. Of 6,401 contacted households, 1,111 had ≥ 1 child within the study age range who met other inclusion criteria. In order to reach our target sample size (≥ 700 children), we attempted to enroll a random sample (n=783) of these 1,111 children. Parents of 105 (13.4%) of these households

refused to participate or could not be reached. There were no significant differences in age, gender, or area of residence between eligible children who did (n=678, including 351 children with asthma and 327 control subjects) and did not (n=105) participate. At both study sites, participants had to have four Puerto Rican grandparents and had to have lived in the same household for ≥ 1 year. Of the 618 children with asthma at both study sites, 520 (287 from San Juan and 233 from Hartford) had blood samples and sufficient DNA for genome-wide genotyping. Of these 520 children, 440 (84.6%, 249 from San Juan and 191 from Hartford) also had data on indoor dust mite allergen level and lung function, and were thus included in the current analysis.

Ethics statement

Written consent was obtained from parents of participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards (IRBs) of Connecticut Children's Medical Center (Hartford, CT [Protocol #135503]), the University of Puerto Rico (San Juan, PR [Protocol #0160507]), Brigham and Women's Hospital (Boston, MA [Protocol #2007P-001174], and the University of Pittsburgh (Pittsburgh, PA [Protocol #PRO10030498]).

Lung function

Spirometry was performed in participating children using an EasyOne spirometer (NDD Medical Technologies, Andover, MA). Subjects had to be free of respiratory infections for four or more weeks before testing, and they were also instructed (when possible) to avoid use of inhaled short- and long-acting bronchodilators for ≥ 4 and ≥ 12 hours before testing, respectively. Expiratory

maneuvers were judged acceptable if they met or exceeded American Thoracic Society criteria for children²². The best FEV₁ and FVC from each test were selected for data analysis.

Dust mite allergen measurement

Dust samples were obtained from three areas in the home: the one in which the child slept (usually his/her bedroom), the living room/television room, and the kitchen. The dust was sifted through a 50 μ -mesh metal sieve, and the fine dust was reweighed, extracted, and aliquoted for analysis of dust mite allergen (*D. pteronyssinus* [Der p 1], hereinafter referred to as “dust mite”) using monoclonal antibody Multiplex assays that used the same reagents as the established ELISA^{23,24}. Dust mite allergen level was categorized as $<2 \mu\text{g/g}$ vs. $\geq 2 \mu\text{g/g}$, because of known effects on allergic sensitization, distribution of allergen levels across the study cohorts (see Table 1), and ease of exposition⁶.

Genotyping

Details of genotyping in PRGOAL have been previously described^{21,25}. In brief, genotyping was performed using the Illumina HumanOmni2.5 BeadChip platform (Illumina, Inc., San Diego, CA), which includes ~2.5 million SNPs. Subjects with a call rate $<95\%$ were removed from the analysis. SNPs were removed if they were not in Hardy–Weinberg equilibrium ($P < 10^{-6}$) in control subjects, had minor allele frequency lower than 1%, or had a failure rate greater than 5%. After all subject and marker quality control steps were completed, we had ~1.83 million SNPs available for data analysis. In addition, SNPs not directly genotyped in the top region(s) of interest were imputed using all populations from the 1,000 Genomes Project (phase I v3)²⁶ as reference for phasing and imputation with SHAPE-IT²⁷ and IMPUTE2²⁸, respectively.

Replication populations

We attempted replication in two cohorts of children with asthma: the Childhood Asthma Management Program (CAMP) and the Genetics of Asthma in Costa Rica Study (GACRS). CAMP was a clinical trial of asthma therapy in 1,041 children. Details on study population, subject recruitment, and study procedures have been previously published^{29,30}. In this analysis, we included 497 non-Hispanic white children and 56 Hispanic children who had genome-wide genotypic data, Der p 1 level in house dust, and lung function. Genotyping in CAMP was performed using the Illumina HumanHap550v3 BeadChip, and imputation was performed based on the 1,000 Human Genomes Project²⁶ as previously described³¹. The GACRS was a cross-sectional study of 616 children with asthma and their parents. Details on study population, subject recruitment, and study procedures have been previously published³². In this analysis, we included 549 children who had genome-wide genotypic data, Der p 1 level in house dust, and lung function. Genotyping in the GACRS was performed using the Illumina BeadStation 500G platform, and imputation was performed based on the 1,000 Human Genomes Project²⁶, using the same methodology as in CAMP. In both CAMP and the GACRS, Der p 1 level in house dust was measured using the same approach as in the discovery cohort (PRGOAL, see above).

Statistical analysis

Our main outcomes were FEV₁ and FEV₁/FVC. FEV₁ was analyzed as absolute value in liters (adjusted for sex, age, and height); we decided not to utilize percent-predicted values because there are no validated predictive equations for Puerto Ricans or Costa Ricans. For CAMP, we used pre-randomization lung function in order to avoid any potential treatment arm effects. Dust

mite allergen level was dichotomized using the median level for our cohort (<2 vs ≥ 2 micrograms of Der p 1 per gram [$\mu\text{g/g}$] of dust), a cutoff that has been previously associated with allergic sensitization to dust mite⁶. All multivariate analyses were conducted under the assumption of an additive genetic model, and included the main effect of the SNP, the main effect of dust mite allergen level, an interaction term (*genotype*allergen*), age, gender, study site (two sites for PRGOAL and eight sites for CAMP), and cohort-specific principal components to account for potential population stratification (eight for PRGOAL, six for CAMP, four for the GACRS); models for FEV_1 also included height and height squared.

Genome-wide statistical significance was set at $P < 5 \times 10^{-8}$. The top five SNPs from the PRGOAL analysis for FEV_1 and FEV_1/FVC were carried forward for replication in CAMP and the GACRS. For each cohort, replication was defined as a one-tailed P-value < 0.05 , in the same direction of interaction as in the discovery cohort. Fisher combined p-values (sum of logs method) were obtained for all cohorts and for the replication cohorts only. All genome-wide interaction analyses for dust mite allergen were performed using PLINK v1.07³³. All other analyses were performed in R (version 3.1.3), using R-Studio (version 0.98.1103).

RESULTS

Table 1 summarizes the baseline characteristics of all cohorts. PRGOAL and CAMP White participants were slightly older than those in CAMP Hispanics and the GACRS. There was a higher proportion of boys in CAMP Hispanics (~69%) than in the other study groups (~56-60%). All children had similar lung function measures after adjusting for sex and age. Compared to PRGOAL, CAMP had a lower proportion of households with dust mite allergen level $>2 \mu\text{g/g}$, whereas the GACRS had a higher proportion of households with dust mite allergen $>2 \mu\text{g/g}$ than the other cohorts.

FEV₁

Interaction P-values for the GWIS of dust mite allergen in PRGOAL are shown in the Manhattan plot in **Figure 1**. The top five SNPs for FEV₁ are shown in **Table 2**. SNP rs117902240, located on chromosome 8q24.13, had a significant interaction with dust mite allergen level (P for interaction = 3.1×10^{-8}), while the P-values for the other four SNPs ranged from 1.3×10^{-7} to 5.4×10^{-7} . Minor allele frequencies for the top five SNPs ranged from 1.3% to 9%.

Table 3 shows the results of the replication efforts for FEV₁. Of the five SNPs carried forward for replication, SNP rs117902240 was associated with FEV₁ among non-Hispanic whites (P value = 0.05) and Hispanics (P = 0.01) in CAMP, but not in the GACRS. The combined P-value for all three replication cohorts (in CAMP and the GACRS) was 0.0048, and the combined P-value for interaction with dust mite in all four cohorts was 9.75×10^{-9} . Given differences in sample sizes and effect estimates among cohorts, we also combined P-values using a weighted approach: when weighted by the square root of the sample sizes, the combined P-value was 1.1×10^{-4} ; when

weighted by the effect sizes, the combined P-value was 3.0×10^{-11} . Of the remaining four SNPs, three were associated with FEV₁ in the GACRS (rs1404568 and rs35312258 in chr.3, and rs72716237 in chr.9), but the direction of the estimated interactions with dust mite allergen was opposite that found in PRGOAL.

Using imputed SNP data based on the 1,000 Human Genomes Project, we performed fine mapping of the region surrounding the top SNP (rs117902240): several SNPs showed $R^2 \sim 0.4$ with this SNP; the top interaction P value for these imputed SNPs was 2.6×10^{-6} (rs186935901 and rs10108856), with others ranging from 1.3×10^{-4} to 9.5×10^{-4} (**Figure 2**). **Figure S1 (online supplementary data)** shows the LD plots for the other SNPs: seven SNPs showed $R^2 \sim 0.75$ with rs72716237. SNPs rs13078996 and rs1404568 were in high LD with rs35312258; their interaction P values were 6.01×10^{-6} and 1.29×10^{-7} , respectively.

We then repeated the analysis for rs117902240 in PRGOAL, before and after stratification by dust mite allergen level. When evaluated in PRGOAL as a whole, rs117902240 was not significantly associated with FEV₁ ($\beta=0.07$ [SD=0.10], p-value=0.48). However, after stratifying by dust mite allergen level, SNP rs117902240 was *positively* associated with FEV₁ ($\beta=+0.84$ [0.15], $p=1.2 \times 10^{-7}$) in children exposed to *low* dust mite allergen levels, but *negatively* associated with FEV₁ in children exposed to *high* dust mite allergen levels ($\beta=-0.41$ [0.13], $p=0.003$).

Figure 3 shows the boxplot for FEV₁, stratified by dust mite level and rs117902240 genotype.

Table S1 (online supplementary data) shows further details for the stratified analysis of rs117902240 and the other top SNPs.

In an exploratory analysis, we assessed the biological plausibility of SNP rs117902240 as an allelic variant interacting with dust mite allergen. Using data from ENCODE³⁴ and is-rSNP³⁵, a software designed to predict whether a SNP has a regulatory effect on transcription factor binding sites *in silico*, we found that SNP rs117902240 is <1 kb away from a transcription factor binding island identified in ENCODE using ChIP-seq. Transcription factor CEBP β (CCAAT/Enhancer Binding Protein Beta) binds in this region in human lung fibroblasts (ENCODE accession ENCSR000EFM). CEBP β forms part of the signaling pathway of interleukin (IL)-17, IL-6, nuclear factor Kappa-B (NF- κ B), and interferon (IFN- γ) (see **Figure 4**)^{36,37}. Details for the other top SNPs may be found in the **online supplement**.

Finally, we explored SNPs within previously reported genes (*IL-9*, *IL-10*, *TGF- β 1*, *P2RY2*) for nominal association (one-tailed P-value<0.05) with FEV₁. Several SNPs within those genes showed nominal associations, either for the main effect of genotype or for the genotype*dust mite interaction (see **Table S2 in the online supplementary data**).

FEV₁/FVC

The top 5 SNPs for FEV₁/FVC are shown in **Table S3 (online supplementary data)** and the replication results are shown in **Table S4**. One SNP (rs115997623) was associated in the GACRS and one (rs78930606) in CAMP Whites, but both in opposite directions as in PRGOAL. None of the top SNPs for FEV₁/FVC had significant combined P-values for the replication cohorts.

DISCUSSION

In this study, we report that dust mite allergen level significantly modifies the estimated effect of one SNP (rs117902240) on FEV₁ in children with asthma. To our knowledge, this is the first study to examine a gene-by-allergen interaction on an asthma-related phenotype (in this case, lung function) using a genome-wide approach.

Dust mite exposure and sensitization have been widely reported as risk factors for asthma symptoms and exacerbations in affected individuals^{5,6,38,39}. Previous GxE studies of candidate genes have reported that dust mite exposure modifies the effects of *IL-9* and *IL-10* polymorphisms on asthma exacerbations^{15,18}, *TGF-β1* variants on asthma severity¹⁶, or purinergic receptor gene *P2RY12* haplotypes on lung function¹⁷. Our results extend current knowledge on the topic by identifying novel polymorphisms using a genome-wide approach.

Using data from ENCODE³⁴ and is-rSNP³⁵, we found that rs117902240 is closely adjacent to a transcription factor island that binds CEBPβ, which forms part of the IL-17 signaling pathway (**Figure 4**). IL-17A or IL-17F can stimulate the NF-κβ pathway, which in turn acts with CEBPβ to activate target gene transcription in the nucleus³⁷. IL-17 has been amply implicated in the pathogenesis of asthma⁴⁰⁻⁴², and studies in dust-mite sensitized subjects have shown that specific immunotherapy can lead to reductions in IL-17 levels that correlate with symptom reduction⁴³. However, CEBPβ also participates in several other pathways, including IL-4, IL-6, TNFα and IFN-γ signaling pathways, and it has been implicated in airway epithelium differentiation during lung development⁴⁴. Thus, functional studies will be needed to determine the precise role of CEBPβ in the interaction between dust mite and genotype on lung function in asthma.

Children with more severe or poorly controlled asthma have worse lung function⁴⁵. Moreover, poor lung function in children with asthma predicts exacerbations over subsequent follow-up⁴⁶, and is associated with worse outcomes in adulthood⁴⁷. Several studies (including GWAS and candidate-gene studies) have been performed to identify genetic variants associated with lung function in subjects with asthma⁴⁸⁻⁵⁰, regardless of allergen exposure. Of note, however, the top SNP reported here would have been missed in a “regular” GWAS, but had significant (and opposite) effects when stratified by level of dust mite allergen exposure. This has potential implications, both in terms of improving our understanding of the underlying mechanisms by which dust mite impacts lung function, but also in terms of developing new approaches to identify susceptibility variants for asthma-related phenotypes.

Our study has several strengths, including the overall sample size of ~1,550 children with asthma and data on dust mite allergen and lung function, and the biological plausibility of the top SNP based on ENCODE data. It also has several limitations: some of the SNPs examined had low minor allele frequencies and thus we had small sample size within some of the genotype*allergen level strata, which may have led to low power to detect modest interactions. *Post hoc* analysis based on results from SNP rs117902240 shows we had sufficient statistical power (see **Table S5 in the online supplementary data**) and the *SNP*dust mite exposure level* interaction replicated in the same direction in three out of four populations; nonetheless, results based on small strata must be interpreted with caution and should be further validated in future, prospective studies. In addition, we used statistical methods to adjust for population stratification but these may not be sufficient and some residual stratification may remain unaccounted for.

Moreover, the choice of method for combining P-values had an important impact on the results; different approaches yielded combined P-values ranging from 1.1×10^{-4} (weighted by sample size), to 7.4×10^{-9} (Fisher's unweighted method), to 3.0×10^{-11} (weighted by effect size). Finally, the timing and duration of dust mite exposure may be important factors in the pathogenesis of asthma, in addition to the level of the allergen⁵¹.

In summary, we show that dust mite allergen exposure modifies the estimated effect of a SNP on a key measure of lung function (FEV_1) in children with asthma. Analysis of existing data suggests possible transcription factor regulatory functions for this SNP; future studies should focus on the functional validation of these pathways.

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FIGURE TITLES AND LEGENDS

Figure 1 – Manhattan plot of GWIS interaction p-values in PRGOAL for FEV₁

Plot depicts the $-\log_{10}(\text{interaction P-value})$ for FEV₁ on the y-axis vs SNPs in each chromosome on the x-axis. Red line: genome-wide significance level ($P < 5 \times 10^{-8}$). Blue line: genome-wide suggestive level ($P < 1 \times 10^{-7}$).

Figure 2 – Regional association plot for SNP rs117902240

Regional association plot generated using the LocusZoom tool from the Abecasis lab at the University of Michigan (<http://locuszoom.sph.umich.edu/locuszoom/>)⁵² with data imputed from the 1,000 Genomes populations (phase I v3).

Figure 3 – FEV₁ stratified by rs117902240 genotype and dust mite allergen level

FEV₁ in PRGOAL stratified by dust mite allergen level (low in blue vs. high in red) and rs117902240 genotype (AA=no minor alleles vs. AC=one minor allele; there were no subjects homozygous for the minor allele [CC]). Model adjusted for age, sex, and height.

Figure 4 – CEBPβ in the IL-17 signaling pathway

IL-17 pathway (simplified). CEBPβ = CCAAT Enhancer Binding Protein Beta. CEBPβ is one of the mediators of IL-17A and IL-17F signaling pathways, as well as NF-κβ signaling^{36,37}. CEBPβ is also involved in IL-4, IL-6, and other signaling pathways (not shown).

TABLES**Table 1 – Participant characteristics**

	PRGOAL	CAMP Whites	CAMP Hispanics	GACRS
N	440	497	56	549
Age (years)	9.9 (2.6)	8.9 (2.1)	9.7 (2.1)	9.0 (1.8)
Male sex	246 (55.9%)	297 (59.8%)	39 (69.6%)	321 (58.5%)
Race/ethnicity	Puerto Rican	Caucasian	Hispanic	Costa Rican
FEV ₁ (L)	1.87 (0.65)	1.67 (0.47)	1.85 (0.50)	1.73 (0.49)
FEV ₁ /FVC (%)	81.5 (8.7)	79.4 (8.2)	79.3 (8.5)	82.4 (7.3)
Der p 1 $\geq$ 2 μ g/g	213 (48.4%)	108 (21.7%)	16 (28.6%)	504 (91.8%)

Numbers are means (SD) for continuous variables, or N (%) for binary variables. Final numbers included in the analysis may have varied slightly due to different missing rates for each SNP. Dust mite allergen dichotomized using the median level for our cohort (<2 vs $\geq$ 2 micrograms of Der p 1 per gram of dust).

Table 2 – GWIS results for dust mite allergen level and FEV₁ in PRGOAL

SNP	CHR	BP	A1	A2	BETA	P-VAL	MAF	n(A1)
rs117902240	8	122930641	C	A	-1.156	3.114E-08	0.015	4/7
rs1404568	3	143210189	G	A	0.391	1.294E-07	0.09	34/29
rs72716237	9	26458550	A	G	0.935	4.228E-07	0.013	8/5
rs7541272	1	108211005	G	A	0.476	4.447E-07	0.06	29/22
rs35312258	3	143193111	G	A	0.371	5.358E-07	0.09	33/31

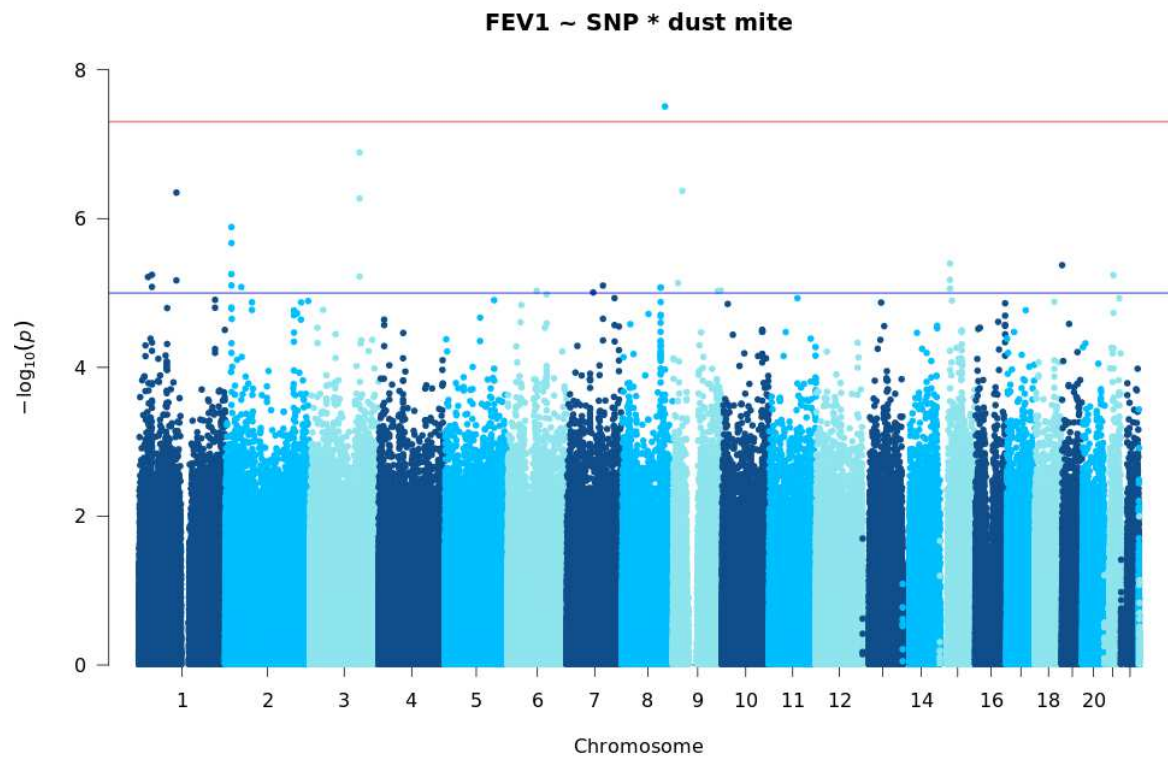
Models included the main effects of genotype (additive) and dust mite allergen level (dichotomized <2 vs ≥ 2 $\mu\text{g/g}$) and the interaction term (*genotype*dust mite allergen level*), and were additionally adjusted for age, sex, study site (San Juan, PR, or Hartford, CT), height, and height², and principal components.

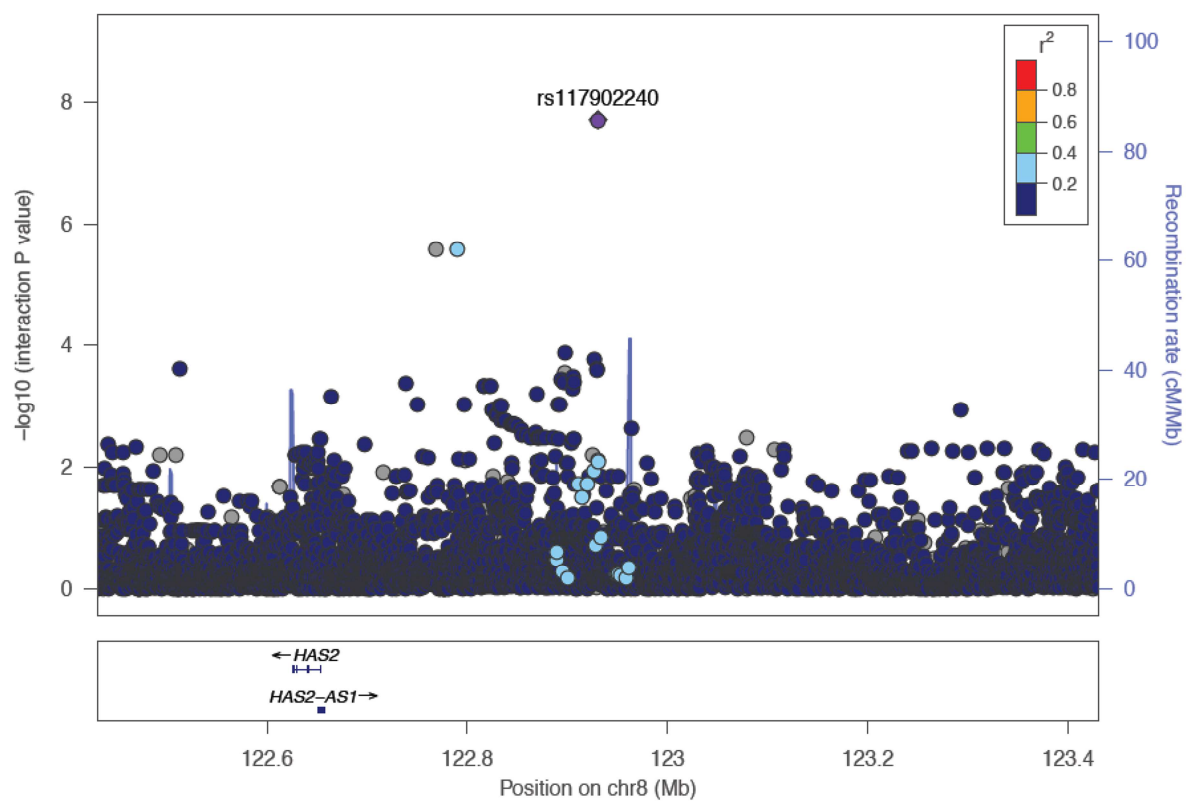
SNP=single nucleotide polymorphism; CHR=chromosome; BP=base position; A1=minor allele; A2=major allele; BETA=beta coefficient for interaction term; P-VAL=interaction p-value; MAF=minor allele frequency; n(A1)=number of subjects with at least one copy of the minor allele and low/high dust mite exposure.

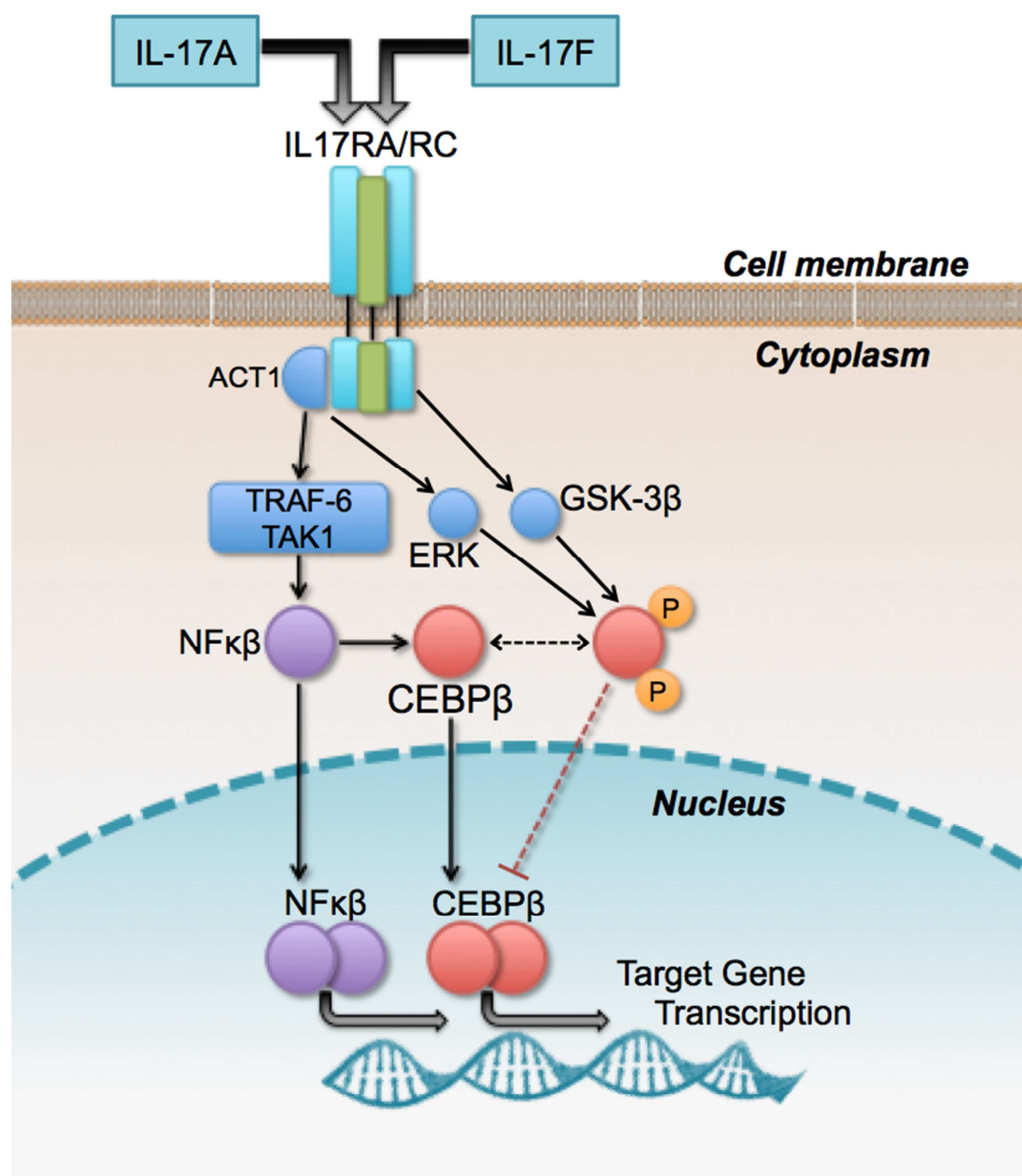
522 **Table 3 – GWIS replication results for FEV₁ in CAMP and the GACRS**

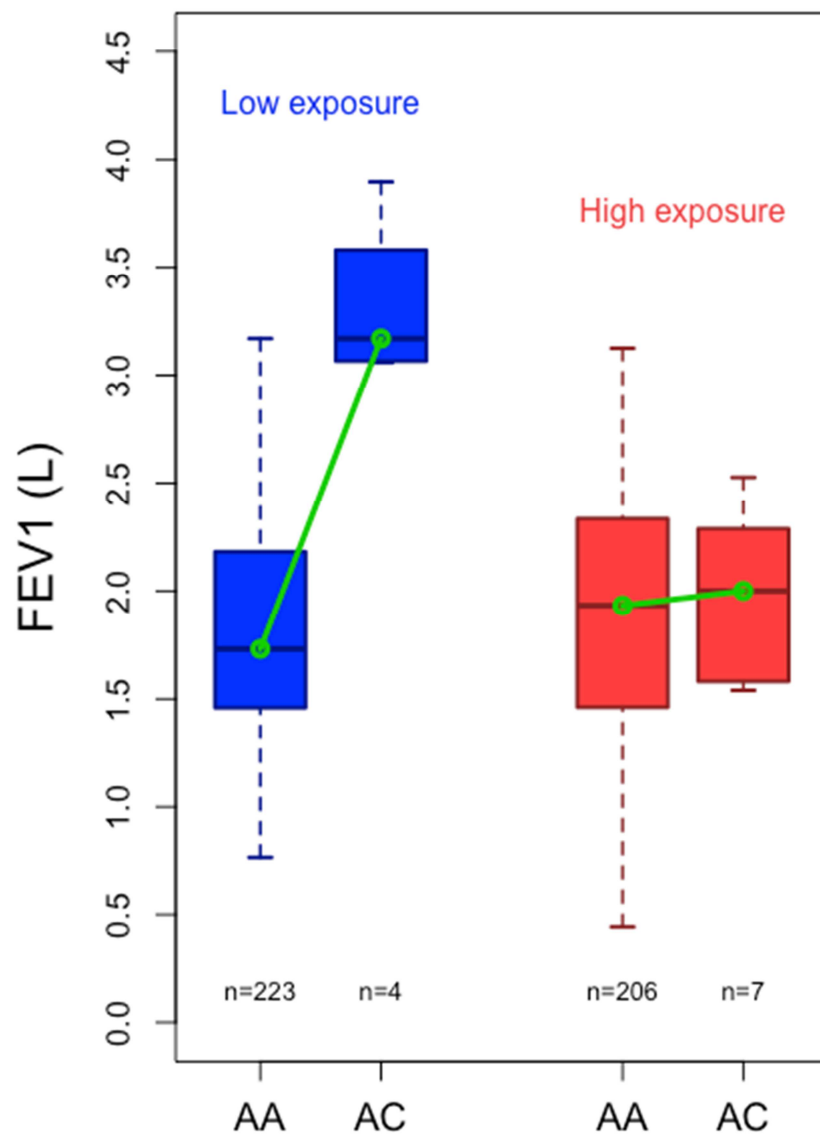
	CAMP Whites					CAMP Hispanics					GACRS					COMBINED P-VAL	
SNP	A1	BETA	<u>P-VAL</u>	MAF	n	A1	BETA	<u>P-VAL</u>	MAF	n	A1	BETA	<u>P-VAL</u>	MAF	n	All cohorts	Replication only
rs117902240	C	-0.43	0.051	0.01	8/1	C	-0.61	0.011	0.03	2/1	C	0.26	0.15	0.01	2/5	7.4E-09	0.0065
rs1404568	G	-0.0078	0.45	0.11	76/23	G	0.048	0.32	0.17	13/3	G	-0.12	0.048	0.18	20/167	1.6E-06	0.13
rs72716237	A	0.067	0.33	0.02	22/3	A	NA	NA	NA	NA	A	-0.38	0.046	0.01	2/14	1.3E-06	0.079
rs7541272	G	-0.105	0.25	0.02	16/3	G	NA	NA	NA	NA	G	0.24	0.21	0.02	1/20	4.1E-06	0.21
rs35312258	G	0.031	0.32	0.09	63/19	G	0.048	0.33	0.17	13/3	G	-0.12	0.046	0.17	19/159	3.8E-06	0.098

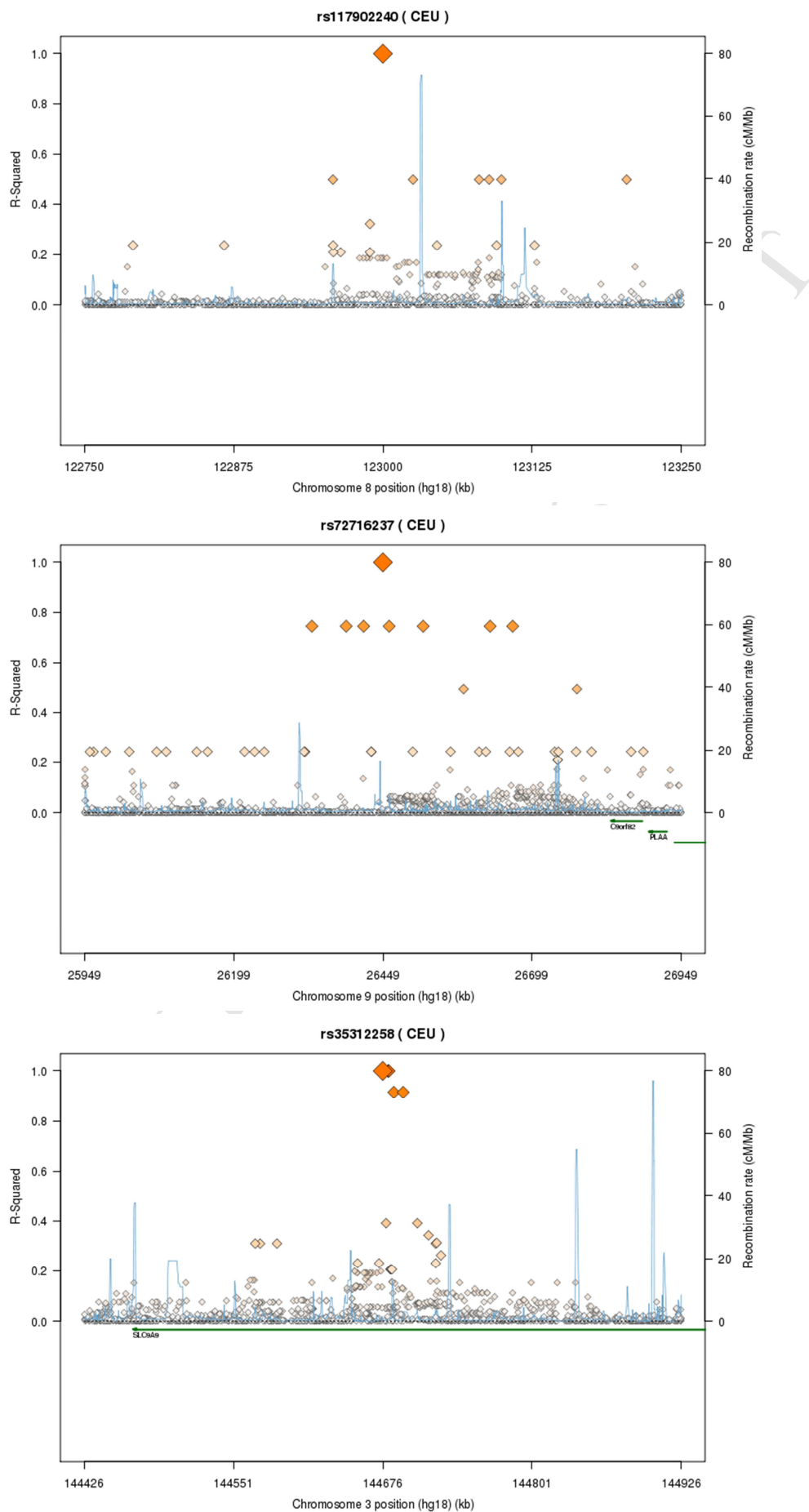
523 Models included the main effects of genotype (additive) and dust mite allergen level (dichotomized <2 vs ≥2 µg/g) and the interaction
 524 term (*genotype*dust mite allergen level*), and were additionally adjusted for age, sex, study site, height, height², and principal
 525 components. SNP=single nucleotide polymorphism; A1=minor allele; BETA=beta coefficient for interaction term; P-VAL=interaction
 526 p-value; MAF=minor allele frequency; n=number of subjects with at least one copy of the minor allele and low/high dust mite
 527 exposure; NA=not available (genotyped or imputed).











Genome-wide interaction study of dust mite allergen on lung function in children with asthma

Online Supplementary Data

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FEV₁ – Functional analysis of SNPs rs117902240, rs72716237, and rs35312258

Using data from ENCODE¹ and is-rSNP², a software designed to predict *in silico* whether a SNP has a regulatory effect on transcription factor binding sites, we found that while SNP rs117902240 in chromosome 8q24.13 is intergenic, it lies within <1 Kbp from a transcription factor binding island identified in ENCODE using ChIP-seq. Transcription factor C/EBP-beta (CEBPB) binds in this region in human lung fibroblasts (ENCODE accession ENCSR000EFM). CEBPB forms part of the signaling pathway of interleukin (IL)-17, nuclear factor Kappa-B (NF-κB), and interferon (IFN-)γ (see **Figure 3** in the main document)^{3,4}.

SNP rs72716237, in chromosome 9p21.2, lies right outside (<200bp) a transcription factor binding site for MAFK (ENCODE accession ENCSR000EFH), whose expression can be induced by transcription growth factor (TGF-)β⁵. MAFK regulates expression of NF-κB⁶ and NF-E2-related factor 2 (NRF2), implicated in antioxidant pathways⁷. Of note, MAFK and NRF2 may also regulate CFTR gene expression⁸.

SNP rs35312258, in chromosome 3q24, is an intronic SNP in gene SLC9A9 (solute carrier family 9, subfamily A, member 9), a Na⁺/H⁺ transmembrane exchanger that has been linked to autism and ADHD⁹, and more recently to increased arterial stiffness¹⁰.

Supplemental Figures – Titles and Legends**Figure S1** – Regional LD plots of the three top SNPs for FEV₁

Linkage disequilibrium (LD) plots generated at the SNP Annotation and Proxy Search (SNAP) website (<https://www.broadinstitute.org/mpg/snap/ldplot.php>) using data from the 1,000 Genomes Pilot 1.

Table S1 – Main effects of the three top SNPs on FEV₁, in PRGOAL as a whole and stratified by dust mite allergen level.

	rs117902240	rs72716237	rs35312258
Non-stratified	+0.07 (0.10) p=0.48	−0.04 (0.09) p=0.64	+0.03 (0.052) p=0.33
Low dust mite	+0.84 (0.15) p=1.2x10^{−7}	−0.39 (0.11) p=5.3x10 ^{−4}	−0.14 (0.052) p=0.0061
High dust mite	−0.41 (0.14) p=0.0032	+0.49 (0.15) p=0.0011	+0.21 (0.051) p=1.0x10^{−4}

Shown are the beta coefficients (standard error) and p-values for the additive, adjusted genotypic models in PRGOAL, for the whole cohort or stratified by low (<2mcg/g, n=227) or high (≥2 mcg/g, n=213) dust mite allergen level. **Positive association in bold**, *negative association in italics*. All models adjusted for age, sex, height, and height².

Table S2 – SNPs in IL-9, IL-10, *TGF- β 1*, or P2RY2 associated with FEV1

GENE	SNP	A1	Covariate ¹	BETA	P-VAL
IL9	rs2066758	G	Genotype main effect	0.1604	0.046
	rs4390174	G	Genotype*dust mite	0.082	0.039
IL10	rs3024498	G	Genotype main effect	0.076	0.042
	rs6673928	A	Genotype main effect	0.075	0.046
	rs62285907	G	Genotype*dust mite	0.16	0.0033
	rs58448575	A	Genotype*dust mite	-0.22	0.0075
	rs2276763	A	Genotype*dust mite	0.57	0.0077
	rs7646193	C	Genotype*dust mite	-0.19	0.011
	rs58448575	A	Genotype main effect	-0.19	0.031
P2RY2	rs62285878	A	Genotype*dust mite	-0.31	0.042
	rs116537786	A	Genotype*dust mite	0.21	0.044
	rs16863376	G	Genotype*dust mite	-0.29	0.047
	rs765281	G	Genotype*dust mite	-0.11	0.047
	rs79033882	G	Genotype*dust mite	-0.076	0.037

Models included the main effects of genotype (additive) and dust mite allergen level (dichotomized <2 vs $\geq$ 2 mcg/g) and the interaction term (*genotype*dust mite allergen level*), and were additionally adjusted for age, sex, study site (San Juan, PR, or Hartford, CT), height, and height², and principal components. SNP=single nucleotide polymorphism; A1=minor allele; BETA=beta coefficient; P-VAL=one-sided p-value.

Table S3 – GWIS results for dust mite allergen level and FEV₁/FVC in PRGOAL

SNP	CHR	BP	A1	A2	BETA	P-VAL	MAF
rs17048089	3	68923779	G	A	-0.067	4.01E-07	0.204
rs115997623	4	119115917	A	G	0.37	3.94E-07	0.011
rs10021113	4	159678591	A	G	0.36	1.51E-07	0.013
rs75863350	5	148423424	A	G	0.47	2.79E-07	0.013
rs78930606	6	119078652	C	A	-0.32	1.49E-07	0.018

Models included the main effects of genotype (additive) and dust mite allergen level (dichotomized <2 vs ≥2 mcg/g) and the interaction term (*genotype*dust mite allergen level*), and were additionally adjusted for age, sex, study site (San Juan, PR, or Hartford, CT), height, and height², and principal components. SNP=single nucleotide polymorphism; CHR=chromosome; BP=base position; A1=minor allele; A2=major allele; BETA=beta coefficient for interaction term; P-VAL=interaction p-value; MAF=minor allele frequency.

Table S4 – GWIS replication results for FEV₁/FVC in CAMP and the GACRS

FEV ₁ /FVC	CAMP Caucasians			CAMP Hispanics			GACRS			COMBINED P-VAL	
SNP	A1	BETA	<u>P-VAL</u>	A1	BETA	<u>P-VAL</u>	A1	BETA	<u>P-VAL</u>	All cohorts	Replication only
rs17048089	G	1.35	0.20	G	-1.077	0.39	G	-0.22	0.45	1.6E-05	0.35
rs115997623	T	0.64	0.44	T	NA	NA	T	-13.55	0.04	1.5E-06	0.10
rs10021113	T	NA	NA	T	NA	NA	T	NA	NA	NA	NA
rs75863350	A	NA	NA	A	NA	NA	A	NA	NA	NA	NA
rs78930606	C	10.99	0.037	C	NA	NA	C	NA	NA	9.9E-07	NA

Models included the main effects of genotype (additive) and dust mite allergen level (dichotomized <2 vs ≥2 mcg/g) and the interaction term (*genotype*dust mite allergen level*), and were additionally adjusted for age, sex, study site, and principal components. SNP=single nucleotide polymorphism; A1=minor allele; BETA=beta coefficient for interaction term; P-VAL=interaction p-value; MAF=minor allele frequency; NA=not available (genotyped or imputed). Combined P-values calculated only if at least two cohorts were significant.

Table S5 – Post hoc power calculations for interaction term based on results from SNP rs117902240

Alpha =	0.05	0.01	0.005	0.001
Power =	0.8975	0.7427	0.6630	0.4749

Shown are power calculations using different levels of significance. Calculations were performed using Quanto (<http://biostats.usc.edu/Quanto.html>), given a population mean FEV1 of 1.87 with SD=0.65 (see Table 1, main manuscript), an exposure proportion of 48.4% (Table 1), and risk allele frequency of 0.015 (rs117902240, Table 2), with N=440, and based on the observed main effects of the additive SNP and dust mite level as well as their interaction, using a 2-sided test (null: no significant interaction).

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