

Letter to the Editor

Role of local CpG DNA methylation in mediating the 17q21 asthma susceptibility gasdermin B (*GSDMB*)/*ORMDL3* sphingolipid biosynthesis regulator 3 (*ORMDL3*) expression quantitative trait locus

To the Editor:

The 17q21 asthma susceptibility locus, which was identified through genome-wide association studies, contains the zona pellucida binding protein 2 (*ZBP2*), gasdermin B (*GSDMB*), and *ORMDL3* sphingolipid biosynthesis regulator 3 (*ORMDL3*) genes.¹ The single nucleotide polymorphisms (SNPs) associated with asthma in this region are correlated with increased expression of both *GSDMB* and *ORMDL3*.² However, genetic risk factors alone cannot account for the overall risk for asthma or explain the observed increases in asthma prevalence over the past 3 decades. Indeed, epidemiologic studies support a substantial role for environmental factors in the development of asthma, such as early-life exposure to tobacco smoke and viral respiratory tract infections, both of which have also been shown to modify the genetic risk attributable to the 17q21 locus.^{3,4}

One possible mechanism by which environmental exposures can alter genetic risk is through epigenetic modifications, such as DNA methylation. One recent small study demonstrated an association between asthma and differential methylation of *ORMDL3* from peripheral blood leukocytes in children; however, these findings have not been replicated.⁵ Furthermore, gene expression of *GSDMB* and *ORMDL3* has been associated with CpG methylation in a larger population.⁶

To understand better the relative contribution of CpG methylation and asthma-associated SNPs in the *cis* regulation of 17q21 candidate gene expression, we performed an integrative genomic analysis in 2 large and racially diverse cohorts collected as part of the Asthma Biorepository for Integrative Genomic Exploration (Asthma BRIDGE) study.⁷ Genotype, gene expression, and methylation data were available for 293 and 264 nonoverlapping subjects with data from whole blood (WB) and CD4⁺ T cells, respectively. Detailed methods and characteristics of the study population (see Table E1 in this article's Online Repository at www.jacionline.org), as well as an overview of the main findings (see Fig E1 in this article's Online Repository at www.jacionline.org) can be found in this article's Online Repository.

Our analysis of *cis*-regulatory variants was limited to within 50 kb of *ZBP2*, *GSDMB*, and *ORMDL3* because those further away have not been associated with asthma risk. The 17q21 region under study showed a remarkable degree of architectural similarity between the WB and CD4⁺ cohorts both with respect to correlation of minor allele frequency (Pearson correlation coefficient, 0.941; 95% CI, 0.928-0.952; $P < 2.2 \times 10^{-16}$) and pairwise linkage disequilibrium (LD [r^2]; Pearson correlation coefficient, 0.928; 95% CI 0.927-0.928; $P < 2.2 \times 10^{-16}$), despite being compromised of different proportions of European American, African American, and Hispanic subjects (see Table E1).

We first confirmed the association between SNP genotype and target gene expression through expression quantitative trait locus (eQTL) mapping. Of the 1638 SNP-gene expression probes pairs

tested, 117 SNPs were significantly associated with *ORMDL3* and 127 were significantly associated with *GSDMB* in both WB and CD4⁺ T cells at a false discovery rate (FDR) of less than 0.05. Fig 1, A, shows the overlap of eQTL SNPs across tissues and genes and demonstrates that a plurality of SNPs ($n = 73$) was associated with expression of both genes in both cohorts. Most significant eQTLs identified were in strong LD (data not shown) and included the most commonly replicated asthma-susceptibility SNP (rs7216389; Fig 1, B) and the putative functional variants affecting CTCF (rs12936231) and transcription factor (rs8076131 and rs4065275) binding (see Table E2 in this article's Online Repository at www.jacionline.org).^{2,8}

We next evaluated the effect of methylation on gene expression for CpG sites within 50 kb of the target genes, a total of 265 CpG-gene expression probe pairs. Five CpG sites (cg12655416, cg22144450, cg18711369, cg10909506, and cg26162295) were associated significantly with *ORMDL3* expression in both the WB and CD4⁺ T-cell cohorts. These 5 sites and a sixth (cg24910161) were also associated with *GSDMB* expression in both groups, with increased methylation at these sites associated with decreased expression of *ORMDL3* and *GSDMB* (Fig 2). Furthermore, we found evidence of strong correlation among these 6 CpG sites, 2 of which are upstream of gasdermin A (*GSDMA*) and 4 of which are within the *ORMDL3* gene body.

Given that both genotype and methylation were associated with gene expression at this locus, we next evaluated the relationship between 17q21 SNPs and CpG sites. Testing of 9207 CpG-SNP pairs within 50 kb of each other across the region identified 1505 significant associations, representing various combinations between 19 CpG sites and 310 SNPs present in both the WB and CD4⁺ cohorts. Notably, these 310 methylation quantitative trait locus (mQTL) SNPs included all 171 replicated eQTL SNPs in our cohorts. The 6 expression-associated CpG sites were associated with 196 mQTL SNPs, most of which were eQTL SNPs as well (164/196 [84%]). Thus there was a strong association between SNPs and CpG sites associated with gene expression for the 17q21 locus. Importantly, these associations were robust to several sensitivity analyses, including tests for confounding by means of residual population stratification and asthma affection status (by using repeated analysis restricting either to subjects from only the major racial subgroup in each cohort or those with asthma, respectively). To determine whether our observed associations extended to neighboring genes, we expanded the region under study to include IKAROS family zinc finger 3 (*IKZF3*), *GSDMA*, and proteasome S36 subunit, non-ATPase 3 (*PSMD3*), but no additional associations were detected.

In light of the considerable associations among SNPs, CpGs, and gene expression, we then performed causal inference testing (CIT)⁹ to evaluate whether the observed SNP eQTL effects could be attributed to the associated methylation marks or whether genetic and epigenetic marks affect target gene expression independently. We limited this analysis to the subset of SNPs and CpG sites significantly associated with gene expression probes for *GSDMB* and *ORMDL3* in both cohorts. Of 1343 gene expression probe-SNP-CpG combinations tested, 862 (64%) supported methylation as a potential mediator of the eQTL effect (overall CIT FDR < 0.05 in both cohorts). These associations included all 127 SNPs and 6 CpG sites associated with *GSDMB*, as well

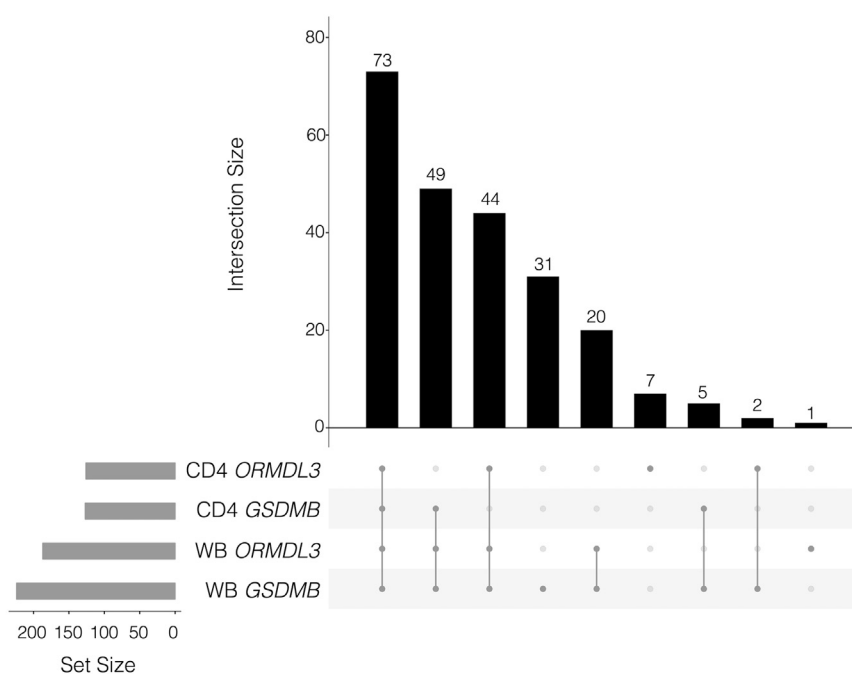
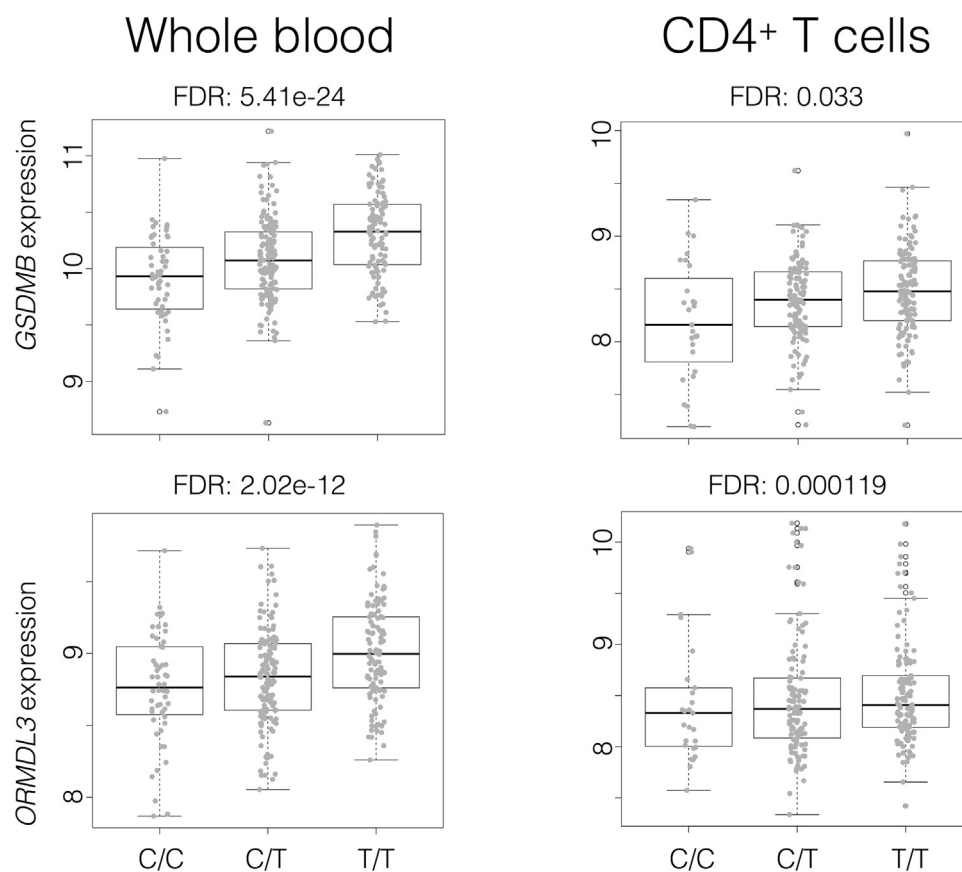
A**B**

FIG 1. Summary of 17q21 eQTL analyses. **A**, Main bar graph (black bars) denotes counts of SNPs associated significantly (FDR < 0.05) with *ORMDL3* or *GSDMB* expression in CD4⁺ T cells or WB. The vertical gray line and dot graph directly underneath denote the count of significant SNPs common to combinations of tested

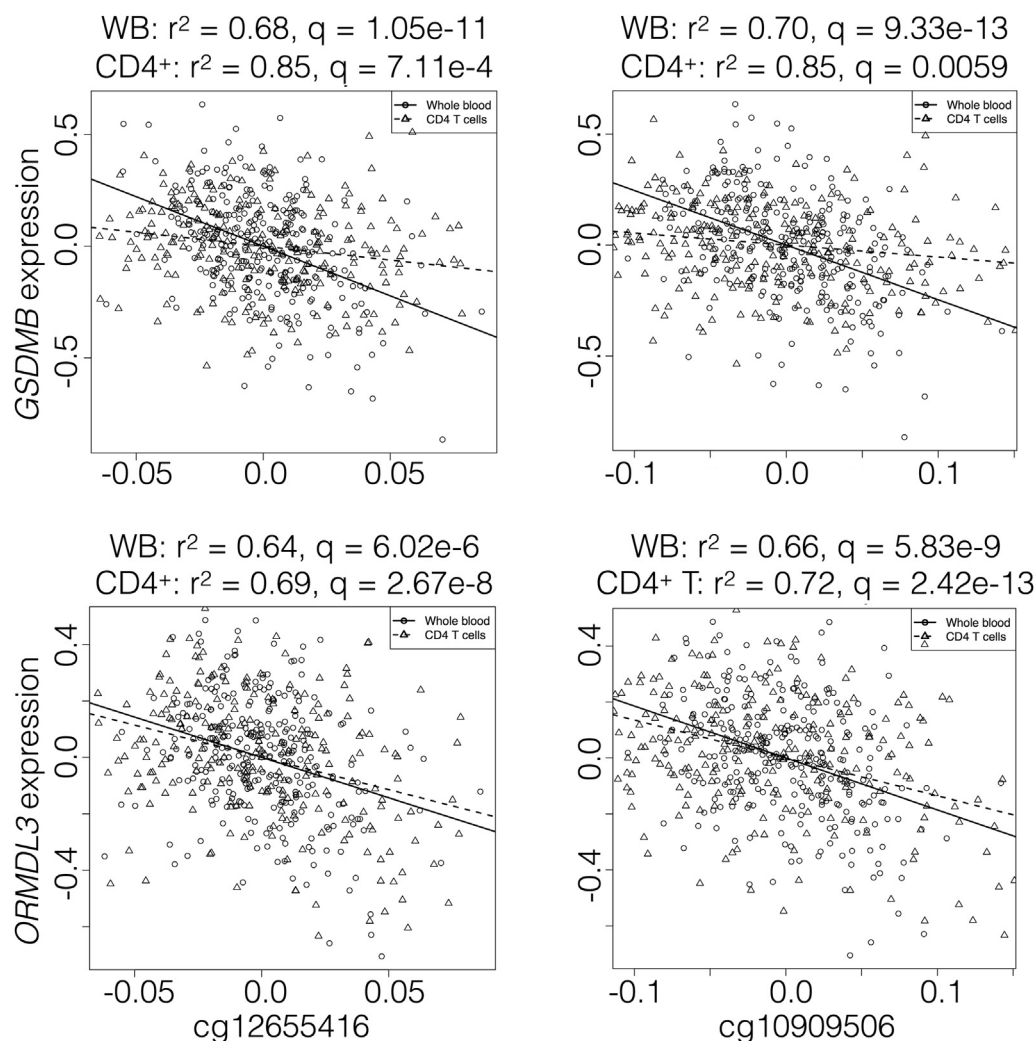


FIG 2. Correlation of CpG methylation with *GSDMB* and *ORMDL3* gene expression. Scatter plots of residuals, after adjusting for covariates, for gene expression of *GSDMB* (top panel) and *ORMDL3* (bottom panel) and methylation of selected CpG sites in WB (circles) and CD4⁺ T cell (triangles) cohorts. Linear regression fits for WB (solid lines) and CD4⁺ T-cell cohorts (dashed lines) are provided.

as all 117 SNPs and 5 CpG sites associated with *ORMDL3*. Thus, regardless of which SNP was considered by the CIT, its effects on target gene expression could be mediated by at least 1 associated CpG site. This is exemplified for the 3 putative functional variants (rs12936231, rs8076131, and rs4065275) and the most replicated asthma-susceptibility variant (rs7216389) in Table E3 in this article's Online Repository at www.jacionline.org. In addition, Fig E2 in this article's Online Repository at www.jacionline.org demonstrates how the correlation between *ORMDL3* gene expression and cg12655416 methylation differs by rs12936231

genotype. Furthermore, CIT suggests that CpG methylation influences gene expression independently of genotype, as shown in Table E3.

Given the strong correlations of methylation at the tested CpG sites and the extensive LD at 17q21, CIT could not localize the putative regulatory signals to one specific SNP-CpG combination. Nonetheless, our results demonstrated a higher percentage of eQTL SNPs, showing evidence of mediation through CpG sites within the *ORMDL3* gene body (cg10909506, cg12655416, cg18711369, and cg22144450) compared with those upstream

groups, with combinations indicated by connected circles. The gray bar graph on the bottom left shows the number of eQTLs for the specified groups. The plot was generated with the upset function from the UpSetR R package. **B.** Box plots for gene expression for *GSDMB* (top) and *ORMDL3* (bottom) according to rs7216389 genotype in WB (left; C/C [n = 52], C/T [n = 131], T/T [n = 105]) and CD4⁺ T cells (right; C/C [n = 27], C/T [n = 115], T/T [n = 122]).

of *GSDMA* (cg24910161 and cg26162295). Indeed, the 4 CpG sites found within *ORMDL3* were found to mediate the observed eQTL associations.

In conclusion, our population-based integrative genomics analysis of the 17q21 asthma-susceptibility locus illustrates the complex relationship between sequence variation, CpG methylation, and target gene expression. In 2 well-powered independent cohorts we show that (1) local CpG methylation mediates a proportion, but not all, of the functional effects of *cis*-acting asthma-susceptibility regulatory variants on *GSDMB* and *ORMDL3* gene expression and that, in addition to these effects, (2) residual differences in CpG methylation not explained by SNP genotype also influence target gene expression. *In vitro* studies that dissociate and isolate the genetic and epigenetic determinants of *GSDMB* and *ORMDL3* expression could confirm these observations, could determine whether these findings are applicable to other cells and tissues associated with asthma, and would motivate epigenetic manipulation of this locus as a potential therapeutic strategy.

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METHODS

Genotype, gene expression, and methylation data

Asthma BRIDGE was a multicenter North American initiative to establish a cohort of asthmatic patients and healthy subjects for the study of integrative genomics.^{E1,E2} The current study includes Asthma BRIDGE participants for whom genotype, methylation, and gene expression data were available. Asthma BRIDGE recruited subjects from 6 cohorts participating in the EVE Consortium for an asthma genome-wide association study^{E1,E2}: the Chicago Asthma Genetics study, the Childhood Asthma Management Program, the Childhood Asthma Research and Education Network, the Children's Health Study, the Genomic Research on Asthma in the African Diaspora study, and the Mexico City Childhood Asthma Study. Written informed consent was obtained from all subjects before enrollment in the study in accordance with local ethics committees at each institution.

All blood samples were processed by using a standardized protocol by all of the centers involved in the study. WB or CD4⁺ T cells were collected, depending on the capabilities of the study center at which the subjects were enrolled. For WB, samples collected in EDTA tubes had DNA for methylation extracted by using the PureGene Blood Kit (Qiagen, Germantown, Maryland), and samples collected in RNA PaxGene tubes had RNA isolated for gene expression studies by using the PreAnalytix PAXgene Blood RNA Kit (Qiagen). For CD4⁺ T cells, blood was collected in BD Vacutainer CPT tubes with sodium heparin (Fisher Scientific, Hampton, NH). PBMCs were stimulated with 25 μ L of PHA (at 5 μ g/mL; Sigma-Aldrich, St Louis, Mo) for 24 hours at 37°C. CD4⁺ T cells were isolated by using CD4 MicroBeads (Miltenyi Biotech, Cambridge, Mass), followed by DNA and RNA extraction with the AllPrep DNA/RNA Mini Kit (Qiagen) to be used for methylation and gene expression studies. We analyzed WB and CD4⁺ T-cell samples as independent cohorts because there is no subject overlap between the groups. Demographic and clinical characteristics are summarized in Table E1.

Genome-wide SNP genotype data were already available for all Asthma BRIDGE participants.^{E1} A common set of SNP genotypes was obtained by means of imputation in each cohort with MaCH (version 1.0)^{E3} and the 1000 Genomes Project EUR reference phased haplotypes based on phase 1 low-coverage data (20101123 release). SNPs with a minor allele frequency of less than 1%, a *P* value for the Hardy-Weinberg equilibrium test of less than .001, and/or an imputation quality score of less than 0.3, were excluded. Methylation and gene expression profiling were attempted on all subjects without regard to phenotype or endotype and collected simultaneously. Poorly performing samples were removed from all analyses. Genome-wide gene expression data generated at the Channing Division of Network Medicine were obtained from peripheral CD4⁺ lymphocytes (*n* = 471) and WB samples (*n* = 335) using the Illumina Human HT-12 v4 array (Illumina, San Diego, Calif). Genome-wide CpG methylation signatures for 450,000 sites were generated at the University of Southern California Epigenome Center by using Illumina HumanMethylation450 arrays in subjects from peripheral CD4⁺ lymphocytes (*n* = 297) and WB (*n* = 565). Subjects in the Childhood Asthma Management Program were excluded from analysis because their methylation data were generated by using an earlier Illumina methylation BeadChip. Genotype, methylation, and gene expression data were available for 293 and 264 subjects in the WB and CD4⁺ T-cell cohorts, respectively.

For the 17q21 gene locus, we restricted our analysis to a region within 50 kb of the gene boundaries of *ZBP2*, *GSDMB*, and *ORMDL3* as mapped to the hg19 build (chr17:37974455-38130456). From the imputed SNPs, we limited the analysis to the 356 SNPs in this region with a minor allele frequency of greater than 5% in Asthma BRIDGE. Seven gene expression probes were included: 3 for *ZBP2* (ILMN_2383638, ILMN_1788040, and ILMN_1666357), 3 for *GSDMB* (ILMN_2347193, ILMN_2260756, and ILMN_1666206) and 1 for *ORMDL3* (ILMN_1662174). Forty-five CpG sites were included in the analysis that did not have a known SNP underlying probe sequences and were not located within repeat regions (cg03293732, cg13878456, cg16293631, cg27613455, cg15637191, cg00716716,

cg15636887, cg14585353, cg05330360, cg16810031, cg09639931, cg11212589, cg21499348, cg27435903, cg21300187, cg10057218, cg16822095, cg05725940, cg13212159, cg05842113, cg12655416, cg22144450, cg18711369, cg10909506, cg02305874, cg13476672, cg04145193, cg16638648, cg01482279, cg05271360, cg14647739, cg09155575, cg04308185, cg10444806, cg08932654, cg02965290, cg02642223, cg09772097, cg19422138, cg24092444, cg06532257, cg04756853, cg24910161, cg2616229, and cg08995595).

Association tests

Linear regression models, as implemented with the R package *limma*, were used for all analyses, adjusting for age, sex, ethnicity, and study site as covariates. For analyses involving gene expression, methylation, or both, the respective technical processing batch was also included as a covariate. To account for unknown confounders, we generated gene expression and methylation principal components (PCs) for each cohort separately by using all subjects with available data. Gene expression PCs were generated with all 47,009 available probes. Methylation PCs were generated by using 470,870 autosomal CpG probes. PCs accounting for at least 1% of the variance were included in all analyses. Only additive genetic models were considered in all analyses that included genotype.

For eQTL analysis, we considered SNPs within 50 kb of the target gene boundaries (for *ZBP2* [3 probes], *GSDMB* [3 probes], and *ORMDL3* [1 probe]), resulting in 1638 SNP-probe pairs tested. For mQTL analysis, all SNP-CpG site pairs within 50 kb of each other (9207 total) were analyzed. CpG sites within 50 kb of the gene boundaries of *ZBP2*, *GSDMB*, and *ORMDL3* were analyzed for an association between methylation and expression of the gene, resulting in 265 CpG-probe pairs tests. To correct for multiple comparisons testing, we generated FDRs and declared statistical significance at an FDR of less than 0.05.

CIT

CIT was implemented by using the R package *cit*.^{E4} CIT consists of 4 component tests that evaluate the following: (1) the association between gene expression and genotype; (2) the association between methylation and gene expression adjusted for genotype; (3) the association between genotype and methylation adjusted for gene expression; and (4) the independence of genotype from gene expression adjusted for methylation. All 4 conditions must be met to provide statistical support for mediation. Gene expression probes that were replicated in both cohorts for associations with both genotype and methylation were included. For a given probe, we tested all possible combinations of replicated SNPs and CpG sites with which it was associated. To account for potential confounders, we used as inputs for CIT the residuals for gene expression and methylation after adjusting for covariates and PCs. The FDR was generated by using a permutation-based approach, and results were considered statistically significant at an FDR of less than 0.05. All analyses were conducted with R version 3.3 software.

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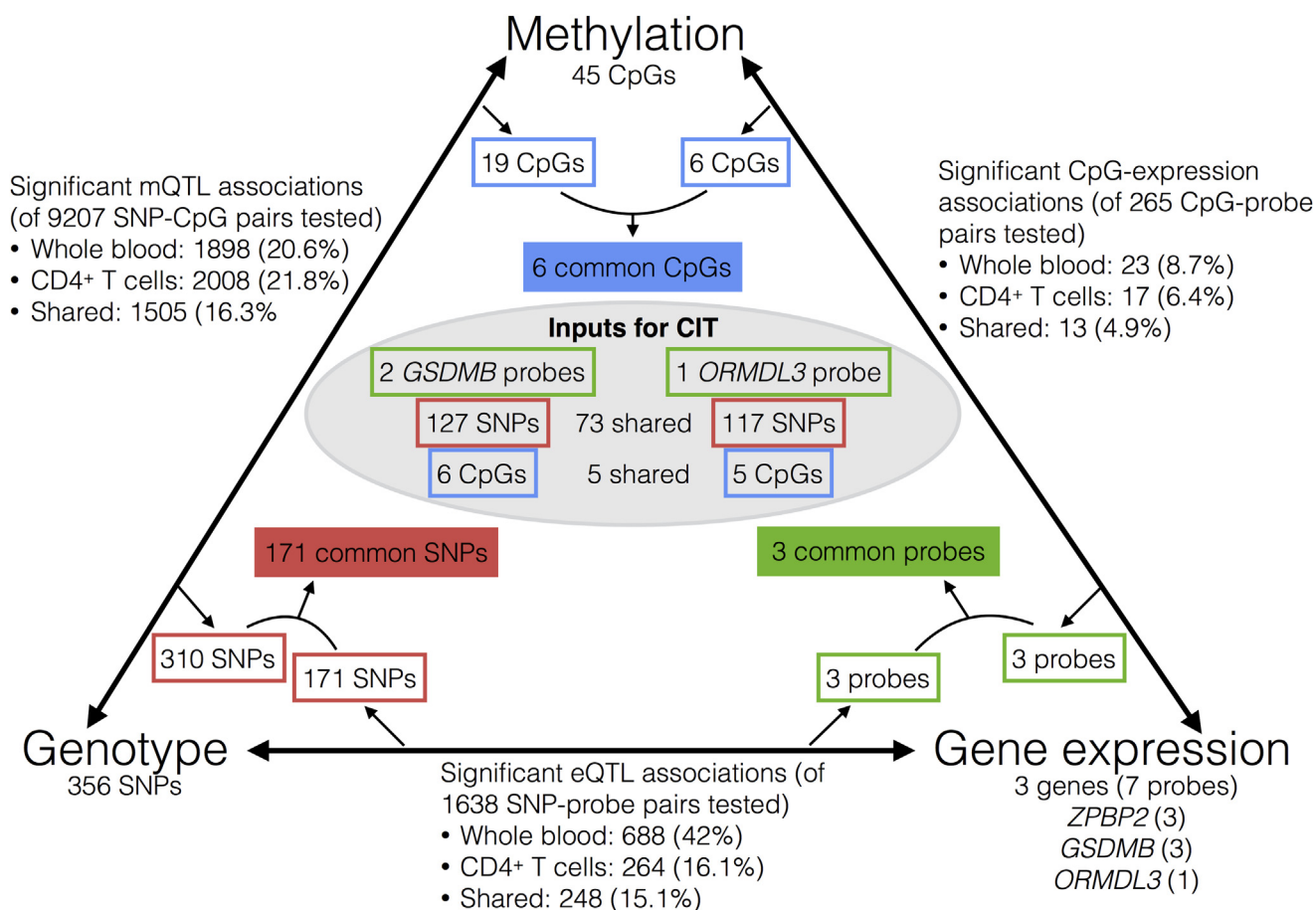


FIG E1. Summary of 17q21 univariate eQTL, mQTL, and methylation-expression analyses. The number of CpG methylation marks, SNPs, and gene expression probes used in association analyses are indicated below their respective labels. *Connecting arrows* indicate the analysis type and show the pairs of elements (SNPs, CpGs, and expression probes) tested. For each analysis, counts and proportions of significant tests in the WB and CD4⁺ T-cell cohorts and of results common to both cohorts are provided lateral to the *connecting arrows*. Counts of unique CpG sites (*blue boxes*), SNPs (*red boxes*), and gene expression probes (*green boxes*) that replicated in both cohorts are provided medial to the *connecting arrows*. Replicated elements (ie, SNPs, CpG marks, and expression probes) that are common across analyses and considered for downstream CIT are provided in *red, blue, and green boxes*, respectively.

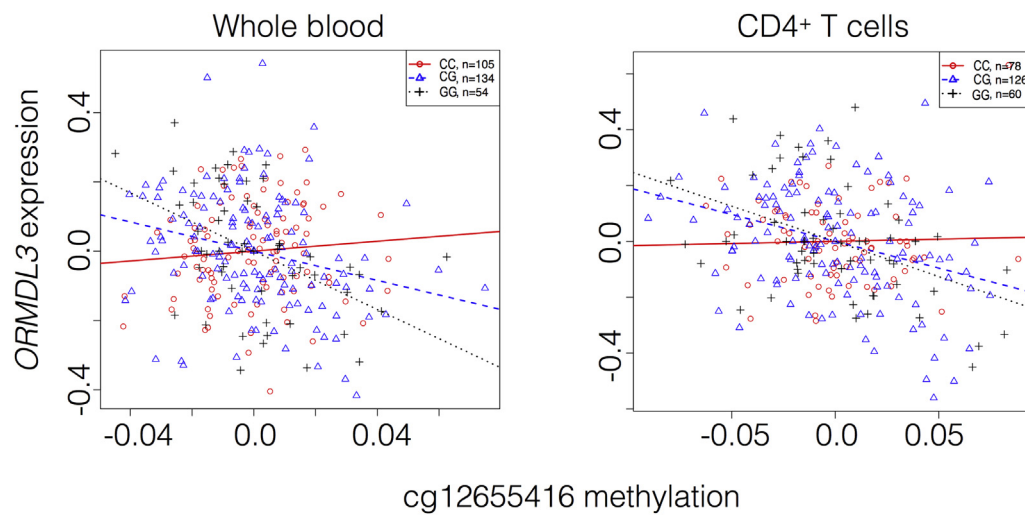


FIG E2. Correlation of cg12655416 methylation and *ORMDL3* gene expression stratified by genotype. Scatter plots of residuals after adjusting for covariates for *ORMDL3* gene expression and cg12655416 methylation in WB (*left*) and CD4⁺ T-cell (*right*) cohorts are shown. Linear regression fits shown for rs12936231 genotype are specified in the box in the upper right hand corner.

TABLE E1. Population characteristics

	WB (n = 293)	CD4 ⁺ T cells (n = 264)
Age (y), mean $\pm$ SD	23.6 $\pm$ 4.9	27.7 $\pm$ 16.3
Male sex, no. (%)	135 (46.1)	135 (51.1)
Asthma, no. (%)	199 (67.9)	215 (81.4)
Allergies, no. (%)	179 (61.1)	201 (76.1)
Hay fever, no. (%)	101 (34.5)	148 (56.1)
Ethnicity		
European	61	78
Hispanic/Latino	193	28
Black or African American	0	133
Multiple ethnicities	34	23
Other/uncertain*	5	2
Sites		
Children's Health Study (CHS)	187	0
Mexico City Childhood Asthma Study (MCCAS)	106	0
Chicago Asthma Genetics (CAG)	0	25
Childhood Asthma Research and Education Network (CARE)	0	129
Genomic Research on Asthma in the African Diaspora (GRAAD)	0	110

*Also includes American Indian and Southeast Asian.

TABLE E2. Results from regression analysis for selected eQTL SNPs

Gene	SNP	SNP position [†]	Alleles	WB (n = 293)				CD4 ⁺ T cells (n = 264)			
				Estimate	SE	Pr(> t)	FDR	Estimate	SE	Pr(> t)	FDR
<i>GSDMB</i> *	rs12936231	38029120	G/C	0.216	0.018	3.12E-26	2.37E-24	0.056	0.022	0.012	0.065
	rs8067378	38051348	G/A	0.216	0.018	3.12E-26	2.37E-24 [‡]	0.075	0.022	9.06E-04	9.16E-03
	rs12453507	38053207	G/C	0.213	0.018	6.48E-26	2.62E-24	0.086	0.023	2.88E-04	4.76E-03 [‡]
	rs1008723	38066267	T/G	0.216	0.018	9.03E-27	2.37E-24 [‡]	0.054	0.024	0.023	0.111
	rs4795401	38067533	G/A	0.220	0.018	4.65E-27	2.37E-24 [‡]	0.073	0.026	0.006	0.040
	rs7216389	38069949	C/T	0.211	0.018	2.25E-25	5.41E-24	0.075	0.026	0.005	0.033
	rs4065275	38080865	A/G	0.210	0.018	1.28E-25	3.49E-24	0.058	0.021	0.006	0.042
	rs8076131	38080912	G/A	0.155	0.021	2.64E-12	1.58E-11	0.057	0.024	0.016	0.084
	rs56199421	38090808	C/T	0.191	0.019	3.98E-21	7.49E-20	0.086	0.021	7.51E-05	1.40E-03 [‡]
	rs56301252	38109155	T/G	0.145	0.021	7.77E-11	3.88E-10	0.091	0.024	1.42E-04	2.47E-03 [‡]
<i>ORMDL3</i>	rs12936231	38029120	G/C	0.142	0.018	1.46E-13	1.01E-12	0.162	0.025	3.26E-10	1.33E-07
	rs2290400	38066240	C/T	0.146	0.018	9.24E-15	7.28E-14 [‡]	0.168	0.025	2.11E-10	1.51E-07 [‡]
	rs56380902	38066372	C/T	0.146	0.018	9.24E-15	7.28E-14 [‡]	0.172	0.025	8.91E-11	1.51E-07 [‡]
	rs7216389	38069949	C/T	0.139	0.018	3.02E-13	2.02E-12	0.144	0.030	3.72E-06	1.19E-04
	rs9303279	38073968	G/C	0.156	0.021	6.22E-13	4.01E-12	0.167	0.025	1.63E-10	1.15E-07 [‡]
	rs4065275	38080865	A/G	0.144	0.018	1.62E-14	1.26E-13	0.130	0.024	1.79E-07	1.95E-05
	rs8076131	38080912	G/A	0.061	0.020	2.69E-03	7.35E-03	0.124	0.027	9.20E-06	2.43E-04
	rs12603332	38082807	T/C	0.144	0.018	1.09E-14	8.53E-14 [‡]	0.123	0.024	6.11E-07	4.35E-05

Significant associations (FDR < 0.05) are marked in boldface.

*For *GSDMB*, results are shown for probe ILMN_1666206.[†]Location based on hg19 build.[‡]Identifies the top 3 hits for each gene in the specified cohort.

TABLE E3. FDRs (*q* values) from CIT for *GSDMB* and *ORMDL3* gene expression with selected CpG sites and SNPs

Gene	CpG	SNP	WB (n = 293)					CD4 ⁺ T cells (n = 264)				
			CIT	Exp assoc SNP	Exp assoc CpG gvn SNP	CpG assoc SNP gvn Exp	SNP ind of Exp gvn CpG	CIT	Exp assoc SNP	Exp assoc CpG gvn SNP	CpG assoc SNP gvn Exp	SNP ind of Exp gvn CpG
<i>GSDMB</i> *	cg10909506	rs4065275	1.54E-03	3.90E-06	8.70E-04	5.32E-06	6.62E-04	1.75E-02	4.50E-03	7.21E-03	5.04E-06	5.87E-03
		rs7216389	1.59E-03	3.90E-06	8.01E-04	5.32E-06	7.82E-04	1.71E-02	6.64E-03	4.29E-03	5.04E-06	6.30E-03
	cg12655416	rs4065275	1.14E-04	3.90E-06	6.74E-06	5.32E-06	9.78E-05	9.82E-03	4.50E-03	1.18E-04	5.04E-06	5.22E-03
		rs7216389	1.15E-04	3.90E-06	6.74E-06	1.45E-05	8.98E-05	1.19E-02	6.64E-03	2.43E-05	5.33E-05	5.22E-03
	cg18711369	rs4065275	3.29E-03	3.90E-06	2.34E-03	5.32E-06	9.37E-04	7.70E-02	4.50E-03	4.78E-02	5.04E-06	2.63E-02
		rs7216389	1.28E-03	3.90E-06	7.07E-04	5.32E-06	5.67E-04	4.53E-02	6.64E-03	1.94E-02	5.04E-06	1.99E-02
	cg22144450	rs4065275	6.55E-04	3.90E-06	6.74E-06	2.88E-05	6.15E-04	1.69E-02	4.50E-03	6.41E-03	5.04E-06	6.12E-03
		rs7216389	6.29E-04	3.90E-06	6.74E-06	1.45E-05	6.04E-04	1.45E-02	6.64E-03	2.74E-03	5.04E-06	5.22E-03
	cg24910161	rs4065275	2.76E-02	3.90E-06	1.77E-02	5.32E-06	9.99E-03	3.13E-02	4.50E-03	2.03E-02	5.04E-06	6.74E-03
		rs7216389	2.75E-02	3.90E-06	1.79E-02	5.32E-06	9.76E-03	2.09E-02	6.64E-03	9.13E-03	5.04E-06	5.22E-03
	cg26162295	rs4065275	3.95E-01	3.90E-06	2.80E-01	5.32E-06	1.59E-01	3.04E-02	4.50E-03	1.93E-02	5.04E-06	6.80E-03
		rs7216389	2.64E-01	3.90E-06	1.75E-01	5.32E-06	1.09E-01	2.05E-02	6.64E-03	9.38E-03	5.04E-06	4.56E-03
	<i>ORMDL3</i> cg10909506	rs12936231	3.09E-03	3.90E-06	7.86E-04	5.32E-06	2.30E-03	5.27E-05	2.96E-05	1.22E-05	5.04E-06	5.87E-06
		rs4065275	5.85E-03	3.90E-06	1.72E-03	5.32E-06	4.13E-03	4.82E-05	2.96E-05	1.22E-05	5.04E-06	1.36E-06
		rs7216389	3.55E-03	3.90E-06	1.03E-03	5.32E-06	2.52E-03	1.02E-04	8.44E-05	1.22E-05	5.04E-06	3.56E-08
		rs8076131	1.54E-03	1.53E-03	6.74E-06	5.32E-06	6.11E-06	4.69E-05	2.96E-05	1.22E-05	5.04E-06	5.21E-08
	cg12655416	rs12936231	4.11E-03	3.90E-06	1.41E-03	5.32E-06	2.69E-03	1.15E-04	2.96E-05	1.22E-05	5.04E-06	6.86E-05
		rs4065275	1.27E-02	3.90E-06	5.75E-03	5.32E-06	6.94E-03	7.86E-05	2.96E-05	1.22E-05	5.04E-06	3.17E-05
		rs7216389	6.86E-03	3.90E-06	3.30E-03	5.32E-06	3.56E-03	9.82E-04	8.44E-05	1.22E-05	8.63E-04	2.23E-05
		rs8076131	1.55E-03	1.53E-03	6.74E-06	5.32E-06	1.59E-05	6.32E-05	2.96E-05	1.22E-05	5.04E-06	1.63E-05
	cg18711369	rs12936231	1.14E-04	3.90E-06	6.74E-06	5.32E-06	9.77E-05	4.72E-04	2.96E-05	1.22E-05	5.04E-06	4.25E-04
		rs4065275	2.74E-04	3.90E-06	6.74E-06	5.32E-06	2.58E-04	7.59E-05	2.96E-05	1.22E-05	5.04E-06	2.90E-05
		rs7216389	1.16E-04	3.90E-06	6.74E-06	5.32E-06	1.01E-04	1.12E-04	8.44E-05	1.22E-05	5.04E-06	9.95E-06
		rs8076131	1.54E-03	1.53E-03	6.74E-06	5.32E-06	2.65E-07	5.42E-05	2.96E-05	1.22E-05	5.04E-06	7.30E-06
	cg22144450	rs12936231	6.22E-04	3.90E-06	3.77E-05	5.32E-06	5.75E-04	5.61E-04	2.96E-05	1.22E-05	5.04E-06	5.14E-04
		rs4065275	7.78E-04	3.90E-06	4.40E-05	5.32E-06	7.25E-04	1.70E-04	2.96E-05	1.22E-05	5.04E-06	1.23E-04
		rs7216389	6.03E-04	3.90E-06	1.90E-05	5.32E-06	5.75E-04	2.10E-04	8.44E-05	1.22E-05	5.71E-05	5.67E-05
		rs8076131	1.57E-03	1.53E-03	6.74E-06	3.78E-05	3.30E-06	1.06E-04	2.96E-05	1.22E-05	5.04E-06	5.96E-05
	cg26162295	rs12936231	3.14E-01	3.90E-06	2.23E-01	5.32E-06	1.18E-01	5.68E-01	2.96E-05	4.46E-01	5.04E-06	2.20E-01
		rs4065275	5.38E-01	3.90E-06	4.02E-01	5.32E-06	2.26E-01	3.58E-01	2.96E-05	2.52E-01	5.04E-06	1.41E-01
		rs7216389	3.24E-01	3.90E-06	2.26E-01	5.32E-06	1.26E-01	8.76E-02	8.44E-05	5.36E-02	5.04E-06	3.58E-02
		rs8076131	1.98E-03	1.53E-03	3.01E-04	5.32E-06	1.52E-04	1.49E-01	2.96E-05	9.31E-02	5.04E-06	6.11E-02

CIT, Overall permutation-based FDR for CIT; CpG assoc SNP gvn Exp, permutation-based FDR from CIT for association between CpG and SNP given gene expression; Exp assoc CpG gvn SNP, permutation-based FDR from CIT for association between gene expression and CpG given SNP; Exp assoc SNP, permutation-based FDR from CIT for association between gene expression and SNP; SNP ind of Exp gvn CpG, permutation-based FDR from CIT for equivalence test of SNP being independent (ind) of gene expression given CpG.

*For *GSDMB*, results are shown for probe ILMN_1666206.