

DNA methylation and childhood asthma in the inner city

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Background: Epigenetic marks are heritable, influenced by the environment, direct the maturation of T lymphocytes, and in mice enhance the development of allergic airway disease. Thus it is important to define epigenetic alterations in asthmatic populations.

Objective: We hypothesize that epigenetic alterations in circulating PBMCs are associated with allergic asthma.

Methods: We compared DNA methylation patterns and gene expression in inner-city children with persistent atopic asthma versus healthy control subjects by using DNA and RNA from PBMCs. Results were validated in an independent population of asthmatic patients.

Results: Comparing asthmatic patients (n = 97) with control subjects (n = 97), we identified 81 regions that were differentially methylated. Several immune genes were hypomethylated in asthma, including *IL13*, *RUNX3*, and specific genes relevant to T lymphocytes (*TIGIT*). Among asthmatic patients, 11 differentially methylated regions were associated with higher serum IgE concentrations, and 16 were associated with percent predicted FEV₁. Hypomethylated and hypermethylated regions were associated with increased and decreased gene expression, respectively ($P < 6 \times 10^{-12}$ for asthma and $P < .01$ for IgE). We further explored the relationship between DNA methylation and gene expression using an integrative analysis and identified additional

candidates relevant to asthma (*IL4* and *ST2*). Methylation marks involved in T-cell maturation (*RUNX3*), T_H2 immunity (*IL4*), and oxidative stress (catalase) were validated in an independent asthmatic cohort of children living in the inner city. **Conclusions:** Our results demonstrate that DNA methylation marks in specific gene loci are associated with asthma and suggest that epigenetic changes might play a role in establishing the immune phenotype associated with asthma. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: DNA methylation, atopic asthma, epigenetics, T_H2 immunity, inner city

Asthma can be inherited and is affected by environmental exposures. To date, genome-wide linkage and association studies have identified more than 100 asthma-associated gene variants.^{1,2} However, sequence variants explain less than 10% of the risk of having asthma,³ and several studies have shown that the risk of transmission of atopic disease from an affected mother is approximately 4 times higher than that from an affected father.⁴ Because epigenetic marks are also heritable and can account for parent-of-origin patterns of inheritance,⁵ it is logical to speculate that epigenetic marks play a role in the transmission of asthma.⁶ Furthermore, although it is well established that asthma risk and severity are affected by specific environmental exposures, the

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Abbreviations used

DMR:	Differentially methylated region
NK:	Natural killer
PC:	Principal component
PCA:	Principal components analysis
PEER:	Probabilistic estimation of expression residuals
RUNX3:	Runt-related transcription factor 3
WTA:	Whole transcriptome–amplified

epigenome can be altered by many of these environmental exposures, and these environmentally induced epigenomic changes often lead to rapid and persistent changes in gene expression.⁷ For example, *in utero* exposure to tobacco smoke is associated with childhood asthma, and this exposure can modify gene expression through DNA methylation.^{8,9} In mice we have demonstrated that *in utero* supplementation with methyl donors alters locus-specific DNA methylation and predisposes mice to allergic airway disease by directing the differentiation of T lymphocytes, skewing toward a T_H2 phenotype.¹⁰ Importantly, epigenetic mechanisms have been shown to specifically affect the expression of transcription factors involved in the development of mature T lymphocytes (T_H1, T_H2, and regulatory T cells),^{11–13} providing a potential mechanism that links heritability, the environment, immune biology, and asthma.^{14,15} In candidate gene studies DNA methylation has been shown to be related to childhood asthma in peripheral blood cells,^{16,17} buccal cells,¹⁸ or nasal epithelia.¹⁹ Thus epigenetic marks represent logical biological changes to pursue when considering the cause and pathogenesis of asthma.

Because black children and families living in poverty are at particularly high risk of asthma,²⁰ we have investigated the relationship between asthma and DNA methylation in African American children residing in the inner city. We hypothesize that epigenetic marks in circulating PBMCs are associated with allergic asthma.

METHODS**Study population**

Our study population consisted of inner-city children aged 6 to 12 years with atopy and persistent asthma (cases, n = 97) and without atopy or asthma (healthy control subjects, n = 97) recruited by 6 sites of the Inner-City Asthma Consortium from census tracts that contain at least 20% of households living at less than the US government–defined poverty level.²¹ All subjects reported being African American, Hispanic with Dominican/Haitian background, or both. The validation population consisted of 101 African Americans between 6 and 12 years of age with atopic asthma collected by the Inner-City Asthma Consortium independent of the primary study population. Please refer to the [Methods](#) section in this article's Online Repository at www.jacionline.org for more information on selection of the study population.

DNA methylation and gene expression data collection

DNA methylation in PBMCs was measured on Illumina's Infinium Human Methylation 450k BeadChip (Illumina, San Diego, Calif) and validated by using pyrosequencing with custom-designed primers (see [Table E1](#) in this article's Online Repository at www.jacionline.org). Gene expression was assessed on Nimblegen Human Gene Expression arrays (12x135k). Please refer to the [Methods](#) section in this article's Online Repository at www.jacionline.org for details of the protocols used for data collection.

Data quality controls

We first examined the quality control figures created by using the minfi R package for the 450k data.²² We observed a strong bimodal distribution of methylation values in the 450k data, as previously observed.²³ Further data quality for Illumina 450k and expression arrays was assessed by using principal components analysis (PCA). The principal components were examined for correlation with all clinical/demographic and laboratory data to identify observable batch effects or covariates that explained variation (see [Table E2](#) in this article's Online Repository at www.jacionline.org).²⁴ As a result of PCA, we removed one outlier sample from the 450k methylation data, whereas no outlier samples were identified in expression arrays. Although no laboratory variables were significantly correlated with principal components in the methylation data set, we included 4 laboratory variables in the expression analysis (labeling, date and concentration of whole transcriptome–amplified (WTA) RNA, and hybridization date) because of their significant correlation with principal components (PCs). Of all demographic and clinical data, age and sex are strongly associated with the top PCs in both methylation and expression data sets. Although race/ethnicity is not associated with the top PCs, we included these covariates in the statistical model because of their known associations with DNA methylation.²⁵

In addition to PCA, probabilistic estimation of expression residuals (PEER) factors²⁶ were estimated to account for unknown batch effects. The number of PEER factors included in downstream analyses was 1 for 450k and 5 for gene expression. The number of PEER factors was based on the inflection point seen on visual inspection of the weighting of the inferred factors.²⁶

Overview of statistical analyses

The goal of our analyses was to determine whether DNA methylation and gene expression changes are associated with asthma, IgE levels, and percent predicted FEV₁. Cases and control subjects were used to determine whether methylation marks were associated with asthma, whereas the other 2 outcomes (IgE level and FEV₁) were analyzed only within asthmatic patients. We first identified differentially methylated regions (DMRs) associated with atopic asthma. For asthma-associated DMRs, we explored whether the DMRs were influenced by the cell composition of PBMCs. Next, we explored the relationship between PBMC gene expression and asthma, IgE levels, and percent predicted FEV₁ using the same approach outlined above. Lastly, we investigated the relationship of DNA methylation to gene expression in PBMCs. DNA methylation–gene expression relationships were examined by using (1) plots of gene expression versus DMRs and analysis of enrichment for inverse correlations of DMRs and gene expression and (2) integrated analysis of single-probe DNA methylation and gene expression. The second approach focused on single methylation probes as opposed to DMRs because of the limitation of statistical methodologies; all available methods for integrative analysis are limited to analysis of 1 methylation and 1 expression probe in statistical models.

Our rationale for inclusion of DMRs in this analysis is 4-fold: (1) identification of regions as opposed to single CpGs is conceptually consistent with what is known about DNA methylation patterns in the human genome²⁷; (2) DMRs increase power to detect associations^{28,29}; (3) DMRs allow for analysis of all probes on the array, which, in turn, facilitates finding regions of interest; and (4) DMR analysis has been used successfully in patients with other diseases.^{30–32} The alternative to DMR analysis is to remove between 20,000 and 140,000 probes from the analysis depending on the required frequency and distance of a variant in the African American population from the CpG motif. It is known that single nucleotide polymorphisms contained in the Infinium probes can affect binding and result in spurious methylation measurements³³; however, our combined *P* value method³⁴ requires 2 or more adjacent probes and, as such, minimized this effect in the methylation analysis.

Statistical analyses of DNA methylation data

Data from the methylation array were normalized with the SWAN method³⁵ contained in the R package minfi,³⁶ and the normalized M-values were used in all downstream analyses. β values (which range from 0–1) of

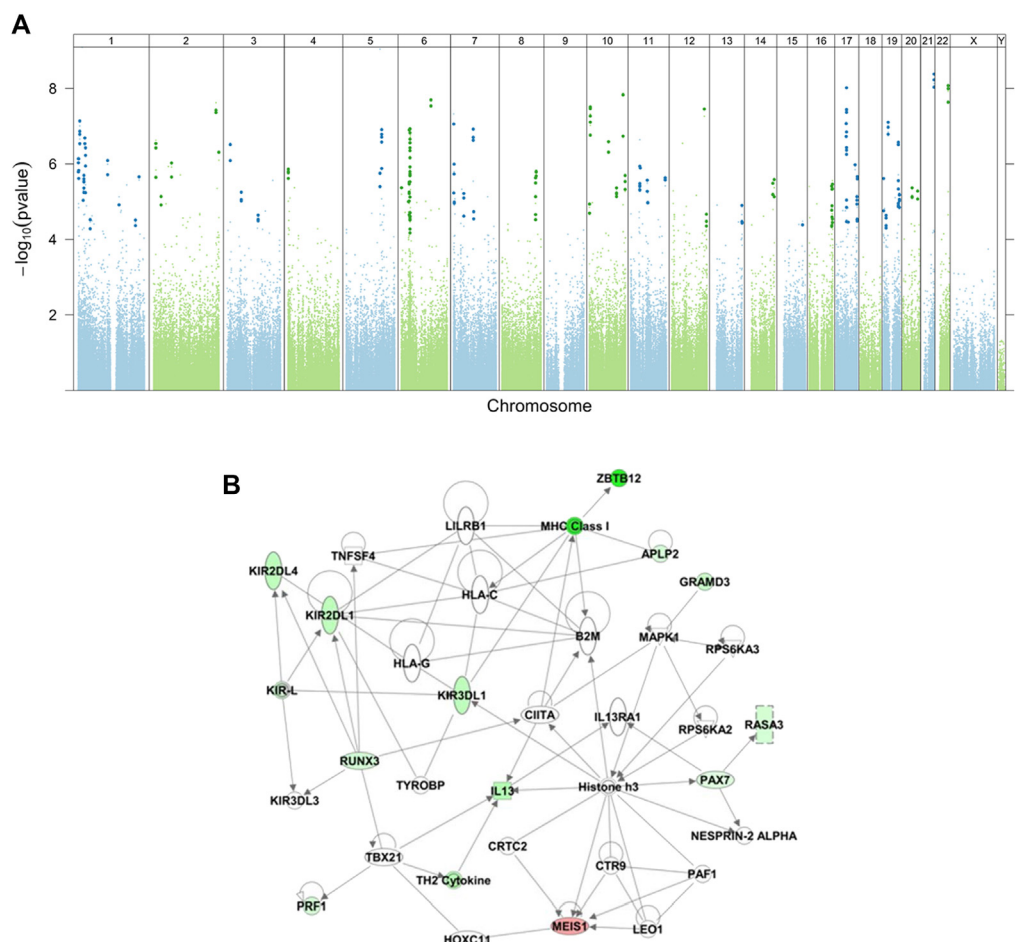


FIG 1. DMRs are associated with asthma after controlling for age, sex, race, technical variables, and batch effects. **A**, Manhattan plot of adjusted P values for disease status (asthma/control) from the linear model. Each dot represents a P value for a probe on the Illumina 450k array that has been adjusted by the significance of neighboring probes within 300 bases according to their correlation. Probes within statistically significant DMRs are identified by a darker and slightly larger symbol after adjustment for genome-wide comparisons. **B**, A representative transcriptional network of genes with associated DMRs from Ingenuity Pathway Analysis. Network analysis was performed with only direct interactions (solid lines) and networks with a score of greater than 20. Genes are intensity colored red (hypermethylated) or green (hypomethylated). Horizontal ellipse, Transcriptional regulator; square, cytokine; double circle, group/complex; triangle, phosphatase; vertical ellipse, transmembrane receptor; rectangle, ion channel.

all of the probes within each DMR are plotted in Figs 1, A, and 2, whereas average β values across the DMR are reported in Fig 3, A and B, and the supplemental tables. β values are biologically intuitive interpretations of percentage methylation changes on the 0- to 1-point scale corresponding to 0% to 100% methylation.

We performed methylation array analysis in 3 steps. In brief, these are (1) infer PEER factors, (2) fit linear models with limma,³⁷ and use comb-p³⁴ to identify DMRs from the P values reported by limma. Limma was used to fit linear models because it allows us to use the moderated t statistic, which has been shown to be more powerful in microarray experiments than the standard t statistic. Our models included a clinical covariate of interest (asthma, FEV₁ percent predicted, or IgE level), demographic covariates that are known to affect DNA methylation (age, sex, and race/ethnicity), and 1 PEER factor to account for unobserved batch effects (with details of statistical models included in Table E3 in this article's Online Repository at www.jacionline.org):

Methylation $\sim$ Asthma + Age + Sex + Race/ethnicity + Peer(1).

Methylation $\sim$ ln(IgE) + Age + Sex + Race/ethnicity + Peer(1).

Methylation $\sim$ FEV₁(% predicted) + Age + Sex + Race/ethnicity + Peer(1).

In these models *Peer(1)* refers to the first PEER component. Cases and control subjects were used in the first model, whereas cases only were used in the second and third models. DMRs were identified from linear model P values by using comb-p,³⁴ with a window size of 300 bases and containing a minimum of 2 probes.

The identification of DMRs is described in detail in Pedersen et al³⁴ and outlined in the diagram in Fig E1 in this article's Online Repository at www.jacionline.org. Comb-p takes as input unadjusted P values for each probe on the array from limma and calculates an adjusted P value for each probe that accounts for the local correlation and the P values of neighboring probes within 300 bases. Probe-level P values are then adjusted by using the Benjamini-Hochberg methodology,³⁸ resulting in multiple testing-corrected P values for individual probes that are influenced by their neighboring CpG motifs. Next, comb-p finds regions or DMRs and calculates P values for the regions based on correlation (SLK P value).³⁹ Finally, the region P values are adjusted for multiple testing by using the Sidak correction⁴⁰ based on the size of the region and number of possible regions of that size, such that larger regions undergo less stringent correction because there are fewer possible large regions (SLK-Sidak P value). DMRs were annotated with the CruzDB tool.⁴¹

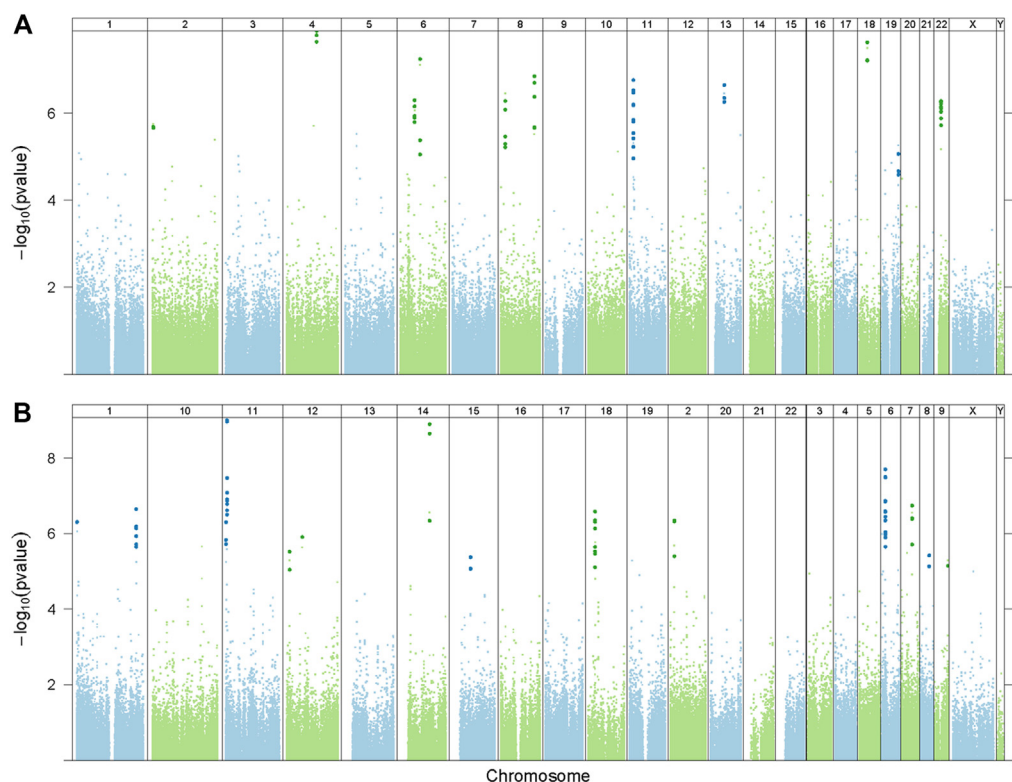


FIG 2. Differentially methylated marks are associated with higher total serum IgE concentrations (A) and percent predicted FEV₁ (B) after controlling for age, sex, race, technical variables, and batch effects among the asthmatic patients. Manhattan plots were constructed in the same fashion as in Fig 1, A. Probes within statistically significant DMRs are identified by a darker and slightly larger symbol after adjustment for genome-wide comparisons.

For validation, we analyzed pyrosequencing data from the discovery and validation cohorts using the 1-sided t test for cases versus control subjects and considered DMRs validated if they had P values of less than .05.

Estimate of cell proportions from DNA methylation data

We also analyzed Illumina 450k methylation data to estimate proportions of different mononuclear cell populations and contaminating granulocytes.⁴² Using our methylation β values and the 500 informative probes reported by Houseman et al,⁴² we estimated the coefficients for the 6 cell types: 5 monocyte cell types (B lymphocytes, CD4⁺ T lymphocytes, CD8⁺ T lymphocytes, monocytes, and natural killer [NK] cells) and granulocyte contamination. We then used penalized regression to regress out the cell types and obtain a β value that represents the average cell. This process is encapsulated by the single R function `adjust.beta` (<https://gist.github.com/brentp/5058805#file-houseman-r>). We then logit transformed the adjusted β value to determine the M value and ran the model to determine which of the methylation differences were independent of cell type differences.

Statistical analyses of gene expression data

Intensity data from the Nimblegen DEVA software were $\log_2$ transformed and normalized by using RMA with the oligo R package.⁴³ The R package `limma`³⁷ was used to fit linear models for expression data, and P values were based on the moderated t statistic. For expression analysis, we used a model that incorporates clinical covariate of interest (asthma, $\ln$ [IgE], or percent predicted FEV₁), demographic covariates that are known

to affect expression (age, sex, and race/ethnicity), observable batch effects from PCA (labeling, hybridization date, WTA date, and WTA RNA concentration), and 5 PEER factors. The models for expression are presented in Table E3.

Correlation analysis of DNA methylation and gene expression

To understand the relationship of DMRs with gene expression changes, we considered inversely correlated (canonical) versus directly correlated pairs, limiting the analysis to genes within 3 kb of a DMR. We calculated the enrichment of inversely correlated pairs in relation to all pairs using the binomial test.

Integrated analysis of DNA methylation and expression

To integrate the expression and methylation data, we used LCMix, which was shown to be effective at identifying target genes in real and simulated data sets by combining information from disparate data sets.⁴⁴ LCMix requires a 1:1 relation between the input data sets, and therefore we limited our analysis to expression probes that had a methylation probe upstream within 3 kb of the transcription start site (23,848 genes). We then determined the number of components for the marginal fits to methylation and expression using integrated classification likelihood-Bayesian information criterion,⁴⁵ as suggested in Dvorkin et al.⁴⁴ Using those component numbers, we then performed joint fit using the chained topology (methylation and expression), 2 hidden components, and the Pearson type VII family.

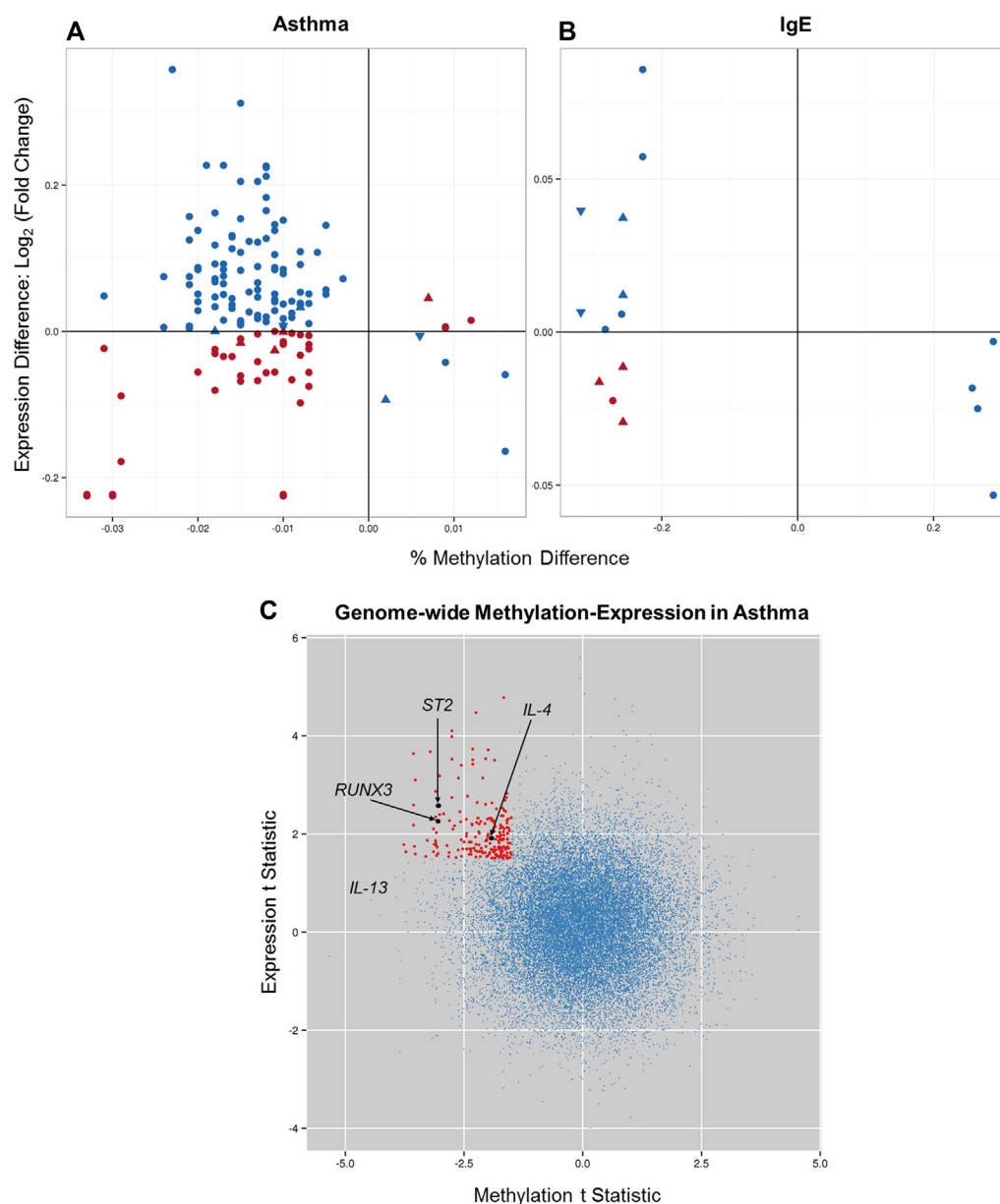


FIG 3. Expression changes in genes within 3 kb of the nearest DMR associated with asthma in the entire study population (A), IgE levels among asthmatic patients (B), and genome-wide methylation expression in asthmatic patients (C). Fig 3, A, *x-axis*, The methylation difference is represented by the mean percentage methylation difference in asthmatic patients compared with control subjects. Fig 3, A, *y-axis*, The expression difference is represented by the mean fold change in asthmatic patients compared with control subjects (on the log base 2 scale). Fig 3, B, The percentage methylation difference on the *x-axis* is represented by the mean correlation coefficient between IgE and methylation in asthmatic patients. The *y-axis* is represented by the slope of the IgE covariate in the linear model for expression in asthmatic patients. *Blue symbols* represent hypomethylated genes associated with increased gene expression, as well as some hypermethylated genes associated with decreased gene expression. *Red symbols* represent methylation changes that were not associated with expected gene expression differences. *Upward triangles* indicate DMR location upstream of the gene, *circles* represent DMRs in the gene body, and *downward triangles* refer to DMRs downstream of the gene. In Fig 3, C, the *t* statistic for expression change of all genes in the genome in asthmatic patients compared with control subjects from PBMCs is plotted against the *t* statistic for methylation changes in these genes (single CpG within the 3-kb promoter). *Red dots* represent the 207 genes with posterior probability from the joint model of methylation and expression of greater than 0.95 (corresponding to $q < 0.05$), a methylation *t* statistic of less than -1.5 , and an expression *t* statistic of greater than 1.5 (see Table E10, A). Additionally, 82 genes have posterior probability of greater than 0.95, a methylation *t* statistic of less than -1.5 , and an expression *t* statistic of less than -1.5 (*lower left quadrant*, see Table E10, B). No significant expression/methylation changes were identified in the *right 2 quadrants* on this plot.

TABLE I. Demographic and clinical features of asthmatic patients and control subjects

	Asthmatic patients (n = 97)	Control subjects (n = 97)	P value*	Validation asthmatic population (n = 101)
Site				
Boston	23 (23.7%)	31 (32.0%)	<.01†	13 (12.9%)
Dallas	8 (8.2%)	2 (2.1%)		10 (9.9%)
Denver	28 (28.9%)	13 (13.4%)		4 (4.0%)
Detroit	4 (4.1%)	6 (6.2%)		12 (11.9%)
New York	22 (22.7%)	14 (14.4%)		6 (5.9%)
Washington, DC	12 (12.4%)	31 (32.0%)		9 (8.9%)
Baltimore	—	—		28 (27.7%)
Chicago	—	—		10 (9.9%)
Cincinnati	—	—		9 (8.9%)
Age at recruitment (y)	9.0 (8.0-11.0)	9.0 (8.0-11.0)	.76‡	9.0 (8.0-11.0)
Sex (male)	56 (57.7%)	41 (42.3%)	.03†	64 (63.4%)
Participant race: African American (yes)	82 (84.5%)	86 (88.7%)	.40†	101 (100.0%)
Participant race: Hispanic or Latino (yes)	26 (26.8%)	15 (15.5%)	.05†	0 (0.0%)
How often exposed to smokers: daily	26 (26.8%)	25 (25.8%)	.85†	22 (21.8%)
Dog living in the home in the last 6 mo (yes)	24 (24.7%)	22 (22.7%)	.74†	24 (23.8%)
Cat living in the home in the last 6 mo (yes)	15 (15.5%)	24 (24.7%)	.11†	12 (11.9%)
Any water/dampness in the last 12 mo (yes)	31 (32.0%)	16 (16.5%)	.01†	30 (29.7%)
Gas stove, gas range, or gas oven (yes)	55 (56.7%)	69 (71.1%)	.04†	61 (60.4%)
Allergic to any indoor allergen (yes)	97 (100.0%)	0 (0.0%)	§	96 (95.1%)
Cockroach IgE (IU/mL)	0.34 (0.34-1.65)	0.34 (0.34-0.34)	§	0.62 (0.34-10.0)
Total IgE (kU/L)	408.5 (189.0-951.0)	29.0 (15.0-58.0)	§	481.0 (167.0-1264.0)
Baseline: FEV ₁ (% predicted)	90.9 ± 16.7	103.0 ± 10.3	§	93.9 ± 19.3
Baseline: FEV ₁ /FVC ratio	75.5 ± 9.7	84.4 ± 6.1	§	77.5 ± 10.2
Asthma medications				
Albuterol	95 (97.9%)	0 (0.0%)	§	101 (100.0%)
Inhaled steroids	84 (86.6%)	0 (0.0%)		84 (83.2%)
Montelukast	38 (39.2%)	0 (0.0%)		9 (8.9%)

Data are presented as means ± SDs, medians (IQRs), or numbers (percentages).

FVC, Forced vital capacity.

*P values were calculated with the use of the χ^2 test for categorical variables and the Wilcoxon rank sum test for continuous variables.

† χ^2 Test.

‡Rank sum test.

§By protocol design.

Pathway and network analysis

We used Ingenuity Pathway Analysis⁴⁶ to identify protein-protein networks and enriched biofunctions in the data sets (using the Fisher exact test).

RESULTS

The study groups (97 healthy control subjects and 97 patients with atopic asthma) were selected from a larger population of potential study subjects who were specifically screened for this project (see Table E4 in this article's Online Repository at www.jacionline.org). When compared with control subjects, asthmatic children were more often male, more often exposed to damp home conditions, and less often exposed to gas stoves (Table I). Per study design, all asthmatic patients were atopic and had significant airflow limitation, as indicated by their total IgE levels and spirometric parameters (Table I).

Comparing asthmatic patients with control subjects and adjusting for age, sex, race/ethnicity, and unknown batch effects (the statistical model is shown in Table E3),³⁴ we identified 81 DMRs (Fig 1, A). Seventy-three DMRs were hypomethylated in asthmatic patients compared with control subjects, and 8 were hypermethylated (Table II). Several genes involved in T-lymphocyte biology were hypomethylated in asthmatic patients, including *IL13*, runt-related transcription factor 3 (*RUNX3*), and specific genes involved in the maturation and function of NK cells

(*KIR2DL4*, *KIR2DL3*, *KIR3DL1*, and *KLRD1*) and T lymphocytes (*TIGIT*). Many of the genes associated with the 81 DMRs are components of regulatory networks that have been traditionally associated with asthma (Fig 1, B, and Table II, with remaining DMR regulatory networks presented in Fig E2 in this article's Online Repository at www.jacionline.org). Most DMRs are near genes that have not been previously implicated in asthma but are plausible asthma candidates, including alkaline phosphatase (*ALPL*), a gene with a role in modulating host-bacterial interactions by dephosphorylating LPS,⁴⁷ and Kruppel-like factor 6 (*KLF6*), a transcriptional activator with a recently established role in activation of inducible nitric oxide synthase 1 during influenza A virus infection⁴⁸ and activation of TGF- β during human respiratory syncytial virus infection.⁴⁹

To address the potential confounding effect of differential PBMC cell content, we used a regression calibration based on peripheral blood leukocyte cell-specific methylation to estimate the specific monocyte cell type and granulocyte contamination for each sample.⁴² Significant differences ($P = .004$) were observed between asthmatic and nonasthmatic groups only for the concentration of NK cells (see Table E5 in this article's Online Repository at www.jacionline.org). Fifty-five of the 81 DMRs remained significant after adjustment for cell mixture ($P < .05$), and an additional 8 approached statistical significance ($P < .1$, see Table E6 in this article's Online Repository at

TABLE II. DMRs associated with asthma at an SLK-Sidak *P* value of less than .05

Chromosome	Start	End	No. of probes	Asthma SLK <i>P</i> value	Asthma SLK-Sidak <i>P</i> value	Gene	Location	CpG island distance	Methylation difference (asthma-control [%])	FEV ₁ <i>P</i> value	IgE <i>P</i> value
chr1	1091164	1091325	4	5.59E-07	.00168	<i>CS444397</i>	1075	717	-0.012	.003504	
chr1	2220460	2220783	4	4.52E-07	.00068	<i>SKI</i>	Intron	0	-0.015		
chr1	6263726	6263914	4	3.79E-08	9.79E-05	<i>ACOT7</i>	Exon;intron;nc_exon;nc_intron	0	-0.011		
chr1	6277594	6277647	2	1.41E-07	.0013	<i>ACOT7</i>	Exon;intron;nc_exon;nc_intron	0	-0.02	.03596	
chr1	15264894	15265020	2	4.38E-10	1.69E-06	<i>LOC391005</i>	Intron;utr3	0	-0.029	.03778	
chr1	18883602	18883759	2	3.83E-06	.01177	<i>PAX7</i>	Intron	0	-0.017		
chr1	21750110	21750368	5	3.59E-06	.00673	<i>ALPL</i>	utr5_intron	23,263	-0.01		
chr1	21773573	21773847	3	3.79E-06	.00668	<i>ALPL</i>	Intron	0	-0.03		
chr1	25113451	25113525	3	1.63E-07	.00107	<i>RUNX3</i>	Intron	0	-0.018		
chr1	27555725	27556088	5	1.96E-07	.00026	<i>MAP3K6</i>	Exon;intron	0	-0.018		
chr1	45046081	45046267	3	1.74E-05	.04438	<i>TCTEX1D4</i>	-537	1,268	0.006	.01963	
chr1	110108029	110108165	3	5.01E-07	.00179	<i>EPS8L3</i>	utr5	23,218	-0.01	.01368	
chr1	152566740	152566865	2	5.46E-06	.021	<i>ATP8B2</i>	utr5_intron	807	0.012	.03256	
chr1	213807046	213807324	3	2.66E-05	.0453	<i>KCTD3</i>	34	0	0.002		
chr1	227230174	227230200	2	2.00E-06	.03665	<i>AX748369</i>	-110597	119,496	0.01		
chr2	8339686	8339783	3	9.66E-08	.00048	<i>LINC00299</i>	nc_intron	77,665	-0.019	.03115	
chr2	8340572	8340606	2	1.02E-06	.01438	<i>LINC00299</i>	nc_intron	76,842	-0.017		
chr2	27652710	27652915	3	7.47E-06	.01754	<i>C2orf16</i>	utr5	6,296	-0.013		
chr2	66518038	66518302	3	4.82E-07	.00089	<i>MEIS1</i>	Intron	1	0.009		
chr2	232101307	232101500	3	1.15E-08	2.90E-05	<i>NMUR1</i>	Exon	0	-0.018	.0002612	
chr2	242516227	242516284	2	5.56E-07	.00472	<i>AK097934</i>	45223	707	-0.025		
chr3	11626535	11626881	3	1.25E-07	.00018	<i>VGLL4</i>	Intron	0	-0.012		
chr3	52787406	52787560	4	5.39E-06	.01686	<i>ITIH1</i>	Exon;nc_exon	7,238	-0.013		
chr3	115495348	115495602	4	1.67E-05	.03144	<i>TIGIT</i>	Exon;utr5	16,590	-0.01		
chr4	1233848	1234086	7	5.29E-07	.00108	<i>AK125775</i>	nc_exon	0	-0.018		
chr5	125723585	125723747	3	3.32E-10	9.96E-07	<i>GRAMD3</i>	63253	63,229	-0.012	.03647	
chr5	132021512	132021960	6	5.34E-08	5.79E-05	<i>IL13</i>	Exon;intron;utr5	1,309	-0.008		
chr6	458210	458297	2	4.91E-06	.027	<i>EXOC2</i>	Intron	2,395	-0.014		
chr6	26334181	26334235	2	1.41E-07	.00126	<i>HIST1H3F</i>	utr3	0	-0.009		
chr6	29019737	29020145	10	8.39E-07	.001	<i>TRIM27</i>	-19990	0	0.017		
chr6	31954747	31955007	10	6.90E-06	.01281	<i>SLC44A4</i>	utr5	0	-0.015		
chr6	31975676	31976004	20	2.89E-08	4.28E-05	<i>C2;ZBTB12</i>	Exon;utr3;utr5_intron	0	-0.015	.01655	
chr6	109881430	109881854	3	1.42E-08	1.62E-05	<i>MICAL1</i>	Exon;intron	942	-0.008		
chr7	888576	888761	3	3.47E-08	9.11E-05	<i>GET4;SUN1</i>	Intron;nc_intron	0	-0.005		
chr7	2056125	2056406	3	7.89E-07	.00136	<i>MAD1; MAD1L1</i>	Intron	0	-0.016	.01102	
chr7	2695301	2695438	3	8.55E-06	.02983	<i>AMZ1</i>	utr5_intron	0	-0.013		
chr7	38323253	38323572	4	2.71E-06	.00412	<i>TCRg;TRGV9</i>	Exon;intron;nc_exon	5,468	-0.021		
chr7	73283658	73283902	4	9.19E-08	.00018	<i>RFC2</i>	utr3	0	-0.017		
chr7	74890962	74891132	3	1.05E-05	.0296	<i>POM121C</i>	Intron	0	-0.01	.00866	
chr8	125532162	125532491	6	1.73E-06	.00255	<i>TRMT12</i>	Exon;utr5	0	0.016		9.29E-08
chr8	127638762	127638859	4	8.24E-07	.00412	<i>FAM84B</i>	Exon;utr5	0	-0.008		.0131
chr10	848816	848923	3	8.24E-06	.0367	<i>LARP5</i>	Exon;utr3	1,721	-0.012		.03864
chr10	3813757	3814016	5	8.39E-09	1.57E-05	<i>KLF6</i>	Exon;intron	0	-0.021		
chr10	3814386	3814687	4	3.08E-08	4.96E-05	<i>KLF6</i>	Exon;intron	88	-0.024		
chr10	72030353	72030454	3	1.27E-07	.00061	<i>PRF1</i>	Exon	0	-0.015	.01763	
chr10	101814909	101815175	5	3.92E-06	.00713	<i>CPN1</i>	Exon;intron	0	-0.016		
chr10	126320834	126321170	4	2.03E-07	.00029	<i>FAM53B</i>	Intron	246	-0.016	.0125	
chr10	134072207	134072497	4	2.63E-06	.0044	<i>PWWP2B</i>	Intron;utr3_intron	0	-0.009	.00745	
chr11	34416873	34417133	7	7.88E-07	.00147	<i>CAT</i>	utr5	0	-0.018	.01748	
chr11	36377805	36377877	2	7.07E-07	.00475	<i>FLJ14213</i>	Intron;utr5_intron	21,831	-0.01	.006936	
chr11	62378748	62378810	3	1.62E-06	.01262	<i>SNHG1</i>	nc_intron	696	0.007		
chr11	64289922	64290199	3	3.71E-06	.00647	<i>SF1</i>	Exon;intron	0	-0.009	.02808	
chr11	129485687	129485739	3	2.10E-06	.01937	<i>APLP2</i>	Exon;utr5_intron	13,029	-0.007	.0003045	
chr12	122162107	122162366	2	3.43E-08	6.43E-05	<i>PITPNM2</i>	utr5_intron	38,347	-0.021		

(Continued)

TABLE II. (Continued)

Chromosome	Start	End	No. of probes	Asthma SLK <i>P</i> value	Asthma SLK-Sidak <i>P</i> value	Gene	Location	CpG island distance	Methylation difference (asthma-control [%])	FEV ₁ <i>P</i> value	IgE <i>P</i> value
chr12	130134393	130134600	4	1.33E-05	.03062	<i>GPRI33</i>	Intron;utr5_intron	0	−0.006		
chr13	113220890	113221025	3	1.19E-05	.04192	<i>TMCO3</i>	Intron	0	−0.015	.0001811	
chr13	113825056	113825282	3	1.34E-05	.02828	<i>RASA3</i>	Intron;utr5_intron	0	−0.007		
chr14	98857115	98857373	3	3.11E-06	.00583	<i>BCL11B</i>	−49540	0	−0.015		
chr14	104255101	104255221	3	2.80E-06	.01125	<i>INF2</i>	Intron;nc_intron;utr3_intron	0	−0.013		
chr15	89170960	89171237	2	2.72E-05	.0465	<i>BLM</i>	−11270	0	−0.013		.0374
chr16	84108978	84109249	3	1.59E-06	.00285	<i>KIAA0182</i>	93281	0	−0.01		
chr16	84233792	84234027	4	1.86E-05	.03767	<i>KIAA0182</i>	Intron;utr5_intron	0	−0.011		
chr16	85272036	85272335	4	1.63E-06	.00264	<i>BC041439</i>	39457	0	−0.012		
chr16	87075079	87075362	3	1.39E-05	.02358	<i>ZFPM1</i>	Intron	1,919	−0.003		
chr16	87085565	87085738	3	1.59E-06	.00444	<i>ZFPM1</i>	Intron	0	−0.018		
chr17	37379133	37379307	3	5.59E-08	.00016	<i>CNP</i>	Exon	0	−0.015	.00594	
chr17	38531660	38531955	12	1.59E-08	2.62E-05	<i>NBR2</i>	nc_intron	0	−0.021	.02746	
chr17	44652266	44652511	2	1.18E-05	.02301	<i>ABI3</i>	Exon;intron	0	−0.014	.002791	
chr17	70268887	70268897	2	9.75E-07	.04621	<i>SLC9A3R1</i>	Intron;nc_intron	11,350	−0.017	.005602	
chr17	77226056	77226266	4	4.00E-06	.00921	<i>TSPAN10</i>	nc_exon;utr3	0	−0.031		
chr17	77986792	77986955	4	1.35E-05	.03938	<i>HEXDC</i>	Exon;intron	0	−0.011	.0008078	
chr17	78474047	78474241	3	1.15E-06	.00288	<i>TBCD</i>	Intron	0	−0.013		
chr19	1207998	1208217	3	1.38E-05	.03013	<i>MIDN</i>	Exon	0	−0.007		
chr19	10523581	10523782	5	1.46E-05	.03466	<i>ATG4D</i>	Exon;intron;nc_exon;nc_intron;utr5	3,012	−0.008		
chr19	18121329	18121515	4	3.31E-08	8.64E-05	<i>MAST3</i>	Exon	0	−0.016		
chr19	55911909	55912349	9	1.02E-07	.00011	<i>SHANK1</i>	Exon;utr5	0	−0.008	7.29E-06	
chr19	60006690	60006895	6	3.44E-06	.00812	<i>KIR2DL4</i> ; <i>KIR2DS2</i> ; <i>KIR3DL1</i>	Intron;utr5	12,754	−0.012		
chr20	34937784	34937967	4	1.33E-06	.00353	<i>C20orf118</i>	17	11,603	−0.008		
chr20	55416108	55416384	3	3.23E-06	.00566	<i>RBM38</i>	Exon;utr3	0	−0.012	.0143	
chr21	46670037	46670291	4	1.79E-09	3.42E-06	<i>PCNT</i>	Exon;intron	0	−0.021	.00112	
chr22	45064135	45064392	4	3.11E-09	5.88E-06	<i>TTC38</i>	Exon;intron	0	−0.023		

www.jacionline.org). As expected, the DMRs associated with NK cells (*KIR2DL4*, *KIR2DL3*, *KIR3DL1*, and *KLRD1*) were no longer significant after adjustment for differences in mononuclear cell populations, whereas *RUNX3* ($P = .04$), *IL13* ($P = 2.8 \times 10^{-5}$), and many other genes remained significant (see Table E6). Disease and biofunction enrichment analysis of the 55 DMRs identified organismal development, oversecretion of mucus and inflammatory/respiratory disease, and airway hyperresponsiveness among the most significantly enriched categories ($P < .039$, Benjamini-Hochberg multiple comparison corrected; see Table E7 in this article's Online Repository at www.jacionline.org).

To determine whether specific measures of allergy or asthma are associated with DNA methylation, among asthmatic patients, we investigated the relationship between methylation marks and either the total serum IgE concentration or percent predicted FEV₁. Taking a genome-wide approach to DNA methylation and the serum concentration of IgE among asthmatic patients, we identified 11 IgE-associated DMRs (Fig 2, A, and see Table E8, A, in this article's Online Repository at www.jacionline.org), including a glutathione S-transferase (*GSTT1*). Focusing on the 81 asthma-associated DMRs (Fig 1, A), we found that 4 of these

DMRs were associated with serum IgE concentrations ($P < .05$, Sidak corrected; Table II), and only one of those DMRs (*TRMT12*) overlapped with the 11 IgE-associated DMRs in the genome-wide analysis (Fig 2, A). We also investigated the relationship between DNA methylation and percent predicted FEV₁ among asthmatic patients and found that 16 DMRs were identified in the genome-wide DNA analysis (Fig 2, B, and see Table E8, B), and 28 DMRs were associated with FEV₁ when the analysis was limited to the 81 asthma-associated DMRs (Fig 1, A).

We next evaluated the relationship between asthma-, IgE-, or FEV₁-associated DMRs and gene expression. Gene expression analysis identified several interesting genes associated with asthma (T-box transcription factor, arachidonate 15-lipoxygenase, and NK cell genes) or the serum concentration of IgE among asthmatic patients (arachidonate 15-lipoxygenase) but not with percent predicted FEV₁ (see Fig E3 and Table E9 in this article's Online Repository at www.jacionline.org). Correlation of methylation and expression revealed enrichment in expected relationships with hypomethylated genes associated with increased gene expression and hypermethylated genes associated with decreased gene

expression (binomial $P < 6 \times 10^{-12}$ for enrichment in inverse relationships in asthma-related DMRs [Fig 3, A] and $P < .01$ for IgE-related DMRs [Fig 3, B]); however, a number of DNA methylation changes were not associated with canonical gene expression differences (Fig 3, A and B). This was not the case for FEV₁ (see Fig E4 in this article's Online Repository at www.jacionline.org).

We also examined whether location of the DMR (upstream, gene body, or downstream of the gene) affected this relationship of methylation and expression. Most DMRs (85% [n = 69] for asthma, 82% [n = 9] for IgE, and 63% [n = 10] for FEV₁) are in gene bodies, and only a few (4 for asthma, 1 for IgE, and 5 for FEV₁) are upstream or downstream within 3 kb of the gene; therefore we were unable to detect any differences in methylation-expression relationships based on DMR location relative to gene. Of note, some DMRs are not associated with changes in expression (those close to zero on the y-axis in Fig 3, A and B, as well as a few intergenic DMRs that are >3 kb from any gene). However, among the DMRs within 3 kb of a gene, inverse correlations and gene expression are enriched in DMRs with higher statistical significance. We examined the percentage of inverse relationships in the 4 quartiles of DMRs ranked by adjusted (SLK-Sidak) P values and obtained the following results: 89% in the top quartile, 84% in the second quartile, 74% in the third quartile, and 55% in the bottom quartile. Additionally, the T_H2 immunity genes *IL13* and *RUNX3* are also among the genes with inverse correlation of DNA methylation and gene expression. This analysis demonstrates that these inverse relationships rank at the top of asthma-associated DMRs and adds further biological relevance of our results. The finding that many of the inverse relationships are within gene bodies is in agreement with a recent publication that showed prominent hypomethylation of gene bodies with both inverse and direct correlation to gene expression in cancer⁵⁰ and demonstrates that the relationship of gene body methylation and expression is more complex than previously thought.⁵¹

To further explore the relationship between DNA methylation and gene expression, we performed an integrative analysis of DNA methylation and expression using LCMix⁴⁴ and identified 2484 methylation-expression pairs (posterior probability > 0.95 from the joint fit corresponding to a local false discovery rate < 0.05), including *RUNX3* and *IL4*, the expression of which was related to asthma-associated DNA methylation marks (Fig 3, C, and see Table E10 in this article's Online Repository at www.jacionline.org). Among the genes with the most significant methylation-expression relationship is an additional asthma-associated gene, *ST2*, the receptor for IL-33.^{52,53} Importantly, *ST2* is one of the few genes that has consistently been associated with asthma in genome-wide association studies.^{54,55}

We selected 10 loci from the 81 asthma-associated DMRs for internal and external validation by using pyrosequencing and an independent population of asthmatic children compared with our original control samples (Table I). Five loci were selected based on the relevance to asthma (*IL13*, *RUNX3*, *CAT*, *PRF1*, and *TIGIT*), 4 were selected based on large methylation differences between asthmatic patients and control subjects from the original comparison (*ALPL*, *C2/ZBTB12*, *KLF6*, and *NBR2*), and 1 locus (*IL4*) was selected based on the asthma-specific methylation-expression matrix. All 10 loci (13 CpG motifs) were validated internally in the original study

population ($P < .05$, 1-tailed t test) comparing methylation changes detected by using the Illumina array and pyrosequencing assay (see Table E11 in this article's Online Repository at www.jacionline.org). As expected based on previous comparison of Illumina and pyrosequencing data,⁵⁶ there are some differences in percentage methylation change detected on the 2 platforms, but directionality and statistical significance are consistent. Five loci (*RUNX3*, *IL4*, *CAT*, *KLF6*, and *NBR2*, involving 8 CpG motifs) were validated externally in an independent population of children with asthma ($P < .05$, 1-tailed t test; see Table E11).

DISCUSSION

Our results demonstrate that methylation marks at specific loci are associated with asthma and define novel methylation-gene transcription relationships that might prove important in asthmatic patients. The relevance of epigenetic marks in asthmatic patients builds on our current understanding that the cause of asthma is associated with non-Mendelian inheritance, parent-of-origin patterns of inheritance, environmental exposures, and *in utero* exposures. Our findings that epigenetic marks in immune cells are associated with allergic asthma in inner-city children provide a provocative new direction for asthma research that is likely to have mechanistic, preventative, therapeutic, and public health implications and might establish novel biological insights into the development of asthma.

Our results suggest that epigenetic marks might prove important in understanding the pathogenesis of asthma. The asthma-associated DMRs appear to be biologically important; almost all were hypomethylated,⁵⁷ the majority were within gene bodies or CpG islands,⁵⁸ approximately 15% were in CpG island shores,⁵⁹ and asthma-associated DMRs were associated with gene expression. Moreover, we found that 3 genes previously associated with T_H2 immunity⁶⁰ and asthma pathogenesis,^{61,62} *IL4*, *IL13*, and *RUNX3*, are hypomethylated in asthmatic patients. Although IL-4 and IL-13 are classic T_H2 cytokines,^{60,61} *RUNX3* is a transcription factor known to regulate CD4/CD8 T-lymphocyte development by interacting with T-box transcription factor and silencing IL-4 expression.⁶³ Differential methylation of *RUNX3* has previously been associated with lung cancer,⁶⁴ as well as *in utero* exposure to cigarette smoke.⁶⁵ Moreover, the previously unrecognized asthma-associated epigenetic marks and methylation-expression relationships that we observed might identify novel genes and networks that are critically involved in the complex pathogenesis of this disease. Methylation changes in *KLF6* are of particular relevance given its role in activation of inducible nitric oxide synthase 1 during influenza A virus infection⁴⁸ and TGF- β during respiratory syncytial virus infection⁴⁹ and the importance of viral infections in childhood asthma.⁶⁶

Our results add to a growing body of evidence that methylation marks direct the expression of genes that are critical to the development of T_H2 immunity and allergic airway disease. *In vitro* development of mature T_H2 cells has been associated with hypomethylation of CpG motifs in the promoters of *IL4* and *IL13*^{11,12,67} and the RAD50-hypersensitive site in the T_H2 cytokine locus control region. Similarly, development of T_H1 immunity is also influenced by methylation. For example, cord blood CD4⁺ cells enhance development of the T_H1 lineage

through progressive demethylation of the *IFNG* promoter.⁶⁸ DNA methylation of promoter and enhancer elements of forkhead box protein 3 has been shown to influence the development of regulatory T cells.¹³ *In vivo* experimentally induced methylation changes in CD11c⁺ dendritic cells,⁶⁹ CD19⁺ B lymphocytes,⁷⁰ *Ifng* in CD4⁺ cells,⁷¹ and *Runx3* in splenocytes and lung tissue¹⁰ have been observed in murine models of allergic airway disease. Demethylation with 5-aza-deoxycytidine demonstrates the importance of methylation marks in the development of T_H2 immunity¹⁰ and allergic airway disease.⁷¹

Our results could potentially affect our approach to asthma prevention and treatment. Although mutations in epigenetic regulators and epigenetic signatures have been observed in hematologic malignancies and are being targeted therapeutically,⁷² interest in using pharmacologic modulation of epigenetic marks in patients with nonmalignant diseases is only beginning to emerge.⁷³ Because epigenetic mechanisms are dynamic and reversible, it is entirely conceivable that a T_H2-skewed phenotype could be reprogrammed therapeutically by altering the methylome with small-molecule inhibitors that target specific methylation sites in the genome.⁷³ Moreover, the pharmacologic agents used to treat airway disease might function through epigenetic mechanisms; theophylline has been shown to affect histone deacetylase activity and improve steroid responsiveness.⁷⁴

There are several limitations to our study. The main limitation is the use of PBMCs as “surrogate” tissue for clinically and biologically relevant changes that occur in the lung. Although nasal epithelial cells might better reflect changes in airway epithelia, as has recently been demonstrated for gene expression,⁷⁵ PBMCs are the most reasonable surrogate for examination of methylation changes related to immune phenotypes in asthmatic patients and are the most accessible and least invasive to collect in children. Moreover, atopic asthma can be considered a systemic immunologic derangement with a dramatic manifestation in the pulmonary airways, further supporting the significance of our findings in PBMCs. Methylation changes we identified in PBMCs are small in magnitude but might be reflective of larger changes in the lung. Alternatively, they might be small due to the presence of multiple cell types in PBMCs; however, further studies will be needed to demonstrate this. Second, although we were unable to evaluate the effect of exposures on the epigenome because of lack of exposure assessment in our study, this is an important future direction for the field. The third limitation is reliance on a statistical deconvolution method to assess differences in cell composition in the absence of white blood count data. The fourth limitation is the inability to detect differences in methyl- versus hydroxyl-methyl cytosine, which is believed to be a mark for demethylation,⁷⁶ as well as changes in DNA methylation over time, because they are dynamic and influenced by exposures. Finally, our association analysis is unable to distinguish between DNA methylation marks and gene expression being the cause or consequence of disease.

Despite these limitations, our findings that the epigenome is dysregulated in asthmatic patients provide a provocative new direction for this disease that might have important public health implications and might establish novel biological insights into the development of asthma. Although we identified strong associations between DNA methylation and gene expression, much more work will be needed to understand the

complexities of this important regulatory mechanism in asthmatic patients.

Key messages

- DNA methylation changes in genes with direct relevance to T_H2 immunity and asthma are associated with allergic asthma in African American inner-city children.
- DNA methylation changes are associated with changes in gene expression with enrichment in inversely correlated methylation-expression relationships.
- Our results suggest that the epigenome is dysregulated in asthmatic patients, and this observation provides a provocative new direction for this disease that might have important mechanistic, therapeutic, and public health implications.

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DNA Methylation and Childhood Asthma in the Inner-City

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SUPPLEMENTAL METHODS

Study Populations

Our study population consisted of inner-city children aged 6-12 years with atopy and persistent asthma (cases) and without atopy or asthma (healthy controls). The cases and controls were recruited by six sites of the Inner-City Asthma Consortium (Boston; Washington, DC; Denver; New York; Dallas; and Detroit) from census tracts that contain at least 20% of households below the U.S. government poverty level (1). Cases of asthma were required to meet the following criteria: 1) a physician diagnosis of asthma; 2) persistent or uncontrolled disease as defined by the National Asthma Education and Prevention Program (2); 3) physiologic evidence of asthma ($FEV_1 < 85\%$ predicted, or FEV_1/FVC ratio $< 85\%$ and bronchodilator responsiveness ($\geq 12\%$), or $PC_{20} < 8$ mg/ml of methacholine); and 4) positive prick skin-test to at least one of a panel of indoor aeroallergens (i.e. dust mite, cockroach, mold, cat, dog, rat, or mouse). Controls were required to have: 1) no medical history of asthma, rhinitis, sinusitis, and atopic dermatitis; 2) an $FEV_1 > 85\%$ predicted; and 3) no positive prick skin-tests. The validation population consisted of 101 African Americans between 6 and 12 years of age with atopic asthma (as defined above) that were collected by the Inner-City Asthma Consortium independent of the primary study population.

Nucleic Acid Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using the Ficoll density gradient separation. DNA and RNA were isolated simultaneously from the PBMCs using the AllPrep DNA/RNA kit (Qiagen, Germantown, MD), DNA and RNA samples were quantified and purity assessed using a NanoDrop spectrophotometer (Thermo Scientific,

Wilmington, DE). RNA integrity was determined using the Bioanalyzer (Agilent, Santa Clara, CA).

Global Methylation Assessment

To measure methylation at approximately 485,000 single CpG sites across the genome, we used Illumina's Infinium Human Methylation 450k BeadChip on bisulfite-treated samples. 0.85-1.00 µg DNA were bisulfite converted using the Zymo EZ DNA Methylation kit (Zymo Research, Orange, CA). Each conversion assay included a commercially available positive and negative control sample. Bisulfite converted samples formed the input for the Illumina Infinium Methylation assay using the Human Methylation 450k BeadChips (Illumina Inc, San Diego, CA). The labeling, hybridization, and scanning procedures were performed on the iScan system. All samples were assayed once (no technical replicates) with 194 arrays performed in 3 batches.

Pyrosequencing

Bisulfite-converted DNA was PCR amplified using primers designed in the Pyromark Assay Design Software (Qiagen, Germantown, MD). The PCR was performed with one biotinylated PCR primer, which facilitates the conversion of the PCR product to a single-stranded DNA template suitable for pyrosequencing. A sequencing primer was then added, which anneals to the single stranded DNA template. Pyrosequencing was performed on the PyroMark Q96 MD sequencer (Qiagen, Germantown, MD) using reagents and protocols supplied by the manufacturer. Each plate contained fully methylated and unmethylated DNA controls. Percent methylation was calculated from the peak heights of C and T using the Pyro Q-CpG software (Qiagen, Germantown, MD). PCR and sequencing were performed in duplicate for each sample and averaged values were used in statistical analysis. PCR and sequencing primers are listed in Supplemental Table 11.

mRNA Expression

RNA was reverse transcribed and amplified using the TransPlex Whole Transcriptome Amplification (WTA) Kit (Sigma-Aldrich, St. Louis, MO) followed by Cy3 labeling using Nimblegen one-color labeling kits, hybridization on Nimblegen Human Gene Expression arrays (12x135), washed on the Little Dipper station (SciGene, Sunnyvale, CA), and scanned using Nimblegen's scanner (Roche Nimblegen, Madison, WI). Images were processed using Nimblegen's DEVA software. All samples were assayed once with 194 arrays performed in 9 batches. All mRNA data met the quality control criteria established by the Tumor Analysis Best Practices Working Group (3) and are available in the Gene Expression Omnibus repository as GSE-40736.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Flow chart describing steps in the methodology for assessing significance of differential methylation and identification of differentially methylated regions (DMRs).

Supplemental Figure 2: Remaining two transcriptional networks with the score > 20 of DMRs associated with asthma that are not included in Figure 1. Legend: genes are intensity colored red (hypermethylated) or green (hypomethylated), horizontal ellipse = transcriptional regulator, square = cytokine, double circle = group/complex, triangle = phosphatase, vertical ellipse = transmembrane receptor, rectangle = ion channel.

Supplemental Figure 3: Gene expression changes associated with (A) asthma and (B) IgE. mRNA profiles from all 194 subjects were subject to hierarchical clustering based on the expression of transcripts that are differentially expressed at 20% FDR in asthmatics compared to controls (panel A) or associated with IgE at 20% FDR in asthmatics only (panel B) after adjusting for age, gender, race, technical variables, and batch effects. The distance metric is Euclidean with average linkage across genes. Legend: red = control subjects and green = asthmatic subjects in panel A; IgE concentration is depicted by the color gradient with red representing lowest IgE and green representing the highest IgE.

Supplemental Figure 4. Expression changes in genes within 3 kilobases of the nearest DMR associated with FEV₁ percent predicted. % methylation difference on the x-axis is represented by the mean correlation coefficient between FEV₁ % predicted and methylation in asthmatic subjects; y-axis is represented by the slope of FEV₁ % predicted covariate in the linear model for expression in asthmatic subjects. The blue symbols represent hypomethylated genes that were associated with increased gene expression as well as some hypermethylated genes associated with decreased gene expression. The red symbols represent methylation changes that were not associated with expected gene expression differences. Upward triangles indicate DMR location upstream of the gene, circles represent DMRs in the gene body, and downward triangles refer to DMRs downstream of the gene.

SUPPLEMENTAL TABLES

Supplemental Table 1: List of PCR and sequencing primers used in the pyrosequencing analysis of bisulfite-converted DNA. * denotes biotinylated primer while Y denotes CpG cytosines.

Chr	CpG base pair position	Nearest Gene	Forward Primer	Reverse Primer	Sequencing Primer	Sequence to Analyze
1	25113452	RUNX3	GGTGAGGTTGTGATTT AGATATAGTAGTTA	ACCCCACTTTCATCTAC TCACAAAAA*	AGTTATTGTTTATTTAG TAGTAGG	GTGGGGYGTGTTGTTT GAGGT
1	25113519	RUNX3	GGTGAGGTTGTGATTT AGATATAGTAGTTA	CAACCCCAAACCTCTA AAAAC*	AGATTTATATTAGATTT TTAATAGT	TTYGYGGAATYGTAGG YGGGTGT
1	25113525	RUNX3	GGTTGTGATTTAGATA TAGTAGTTATTGT*	CCCCACTTACAACCCT AAAAAA	ACAAACCACCCCAA	CCCRACCCRCCTAC RATTCC
3	115495348	TIGIT	GGAGGATGTTAGAGGA GTTTAGTTAATAG	ACACAAACTACATAAA ATAAACACAAAA*	ATAGAATTTAAAAATA ATAGATTGA	TTATAATTTAAAGAYG AGTGTATTTTAAATTAT AATTTTT
5	132021751	IL-13	TGTTTGAAAAAGTAGA GATTATGGTGTTAG	ATCAACAAAAACCCCA TAAACCCAATACC*	ATTATTTGGGTTTATAA AAGTTG	TTATAAGAYGTTAAGG TTATAAGTTATTTAGTT T
5	132037528	IL-4	GTTGATTGGTTTTAAGT GATTGATAATT	ACTAACCATAACAATAC TAACAAAAATATCT*	AATTTTTAATGTAAATT TATTTTTT	TTYGGTTTTAGTAATTT TAAATTTATAT
10	72030353	PRF1	GGGTTATAGTTTTAGT GGAGTTGA	ACACCTACACCCTCTA TAAAAAT*	GATTTTGGTTTTGGTTA TATG	GYGTTGGTAGTTAGAG TTTTGGGTT
11	34416961	CAT	GGGAGAGGTTTTTTAA TTGTTGGGTTAG*	CCCCCTCCAAAAAACT ACCTTCTAATTAAC	ACTACCTTCTAATTAA CTACT	CRACRCTAAACAAACC AAAATTAACAAACC
11	34417091	CAT	TGTTTGTTGAGGGTGG AGATTT	CTACTCCTTCCAATACT ACATCTAATC*	GTTGAGGGTGGAGATT TA	YGAGTYGAGGTTTTTT GTAGTGTTTT
1	21773846	ALPL	AGGTTTTTTGAAGTTAA GGAGTTAGTTAGAA*	CCCTCTATTCCCACCA ATCT	CCTAACCTTACTCACA	AAAAAAACCRCTCCCR AAAAAACCC

6	31975825	C2; ZBTB1 2	TTGAGGTGTAGTTTA ATGGTAGGT	ACACAAAAATCCACCC TTCAC*	GGAGTAGAGGTAGGGT	YGYGTTTTYGAGTGTA GGTTGAGGTGGT
10	3813789	KLF6	GGTTTTTTTTAGGGTT GGTGTAATG	ACACCAAAAACTCCCA CTTAA*	AGGGTTGGTGTAATGT A	GTGGYGTTTATTAGYG GT
17	38531866	NBR2	TGAGGGATAAGTGGT AAGAGTT	CTAACTAACCCAAACT ACTTCCT*	GGATAAGTGGTAAGAG TTAAT	TTTTTTGGYGAAAAYG YGGAGAAA

Supplemental Table 2: Correlation of clinical/demographic and laboratory data with principal components that explain >1% of variability in **(A)** methylation and **(B)** expression data.

(A)

PC	Variance Explained	Clinical/technical Variable	Variable Type	Number Observations	Correlation	Number ANOVA Groups	p	Bonferroni p value
1	0.194	gender	categorical	193	na	2	3.00E-161	1.80E-159
1	0.194	albuterol	categorical	193	na	2	0.0184	1.10
1	0.194	mice_home	categorical	193	na	2	0.0214	1.28
1	0.194	any_meds	categorical	193	na	5	0.0372	2.23
1	0.194	asthma	categorical	193	na	2	0.0407	2.44
1	0.194	any_ind_skin	categorical	193	na	2	0.0407	2.44
1	0.194	lnige	numeric	192	-0.145	na	0.045	2.70
1	0.194	signal_strength	numeric	193	-0.139	na	0.0544	3.26
1	0.194	total_IgE	numeric	192	-0.136	na	0.0605	3.63
1	0.194	dampness_home	categorical	193	na	2	0.0638	3.83
1	0.194	inh_steroid	categorical	193	na	2	0.0735	4.41
1	0.194	FEV1FVC	numeric	192	0.126	na	0.0807	4.84
2	0.088	FVC	numeric	192	-0.129	na	0.0737	4.42
2	0.088	dog_6mo	categorical	193	na	2	0.0776	4.66
2	0.088	age	numeric	193	-0.12	na	0.0968	5.81
3	0.069	DNA.conc.1st.elution	numeric	193	-0.205	na	0.00415	0.25
3	0.069	race_hispanic	categorical	41	na	2	0.0205	1.23
3	0.069	inh_steroid	categorical	193	na	2	0.027	1.62
3	0.069	PC20	numeric	30	-0.395	na	0.031	1.86
3	0.069	hyb_date	categorical	193	na	9	0.0426	2.56
4	0.036	DNA.extraction.date	categorical	193	na	50	0.0329	1.97
4	0.036	nasal_steroid	categorical	153	na	2	0.0367	2.20
4	0.036	total_IgE	numeric	192	-0.147	na	0.0423	2.54

4	0.036	cat_6mo	categorical	193	na	2	0.0511	3.07
4	0.036	around_smokers	categorical	193	na	4	0.0972	5.83
4	0.036	tech	categorical	193	na	2	0.0977	5.86
5	0.028	total_IgE	numeric	192	0.244	na	0.000664	0.04
5	0.028	DNA.conc.2nd.elution	numeric	182	-0.21	na	0.00445	0.27
5	0.028	montelukast	categorical	193	na	2	0.0142	0.85
5	0.028	barcode	numeric	193	0.172	na	0.0168	1.01
5	0.028	nasal_steroid	categorical	153	na	2	0.0674	4.04
5	0.028	skintest_source	categorical	193	na	2	0.0687	4.12
5	0.028	lnige	numeric	192	0.131	na	0.0703	4.22
5	0.028	FVC	numeric	192	0.13	na	0.0729	4.37
5	0.028	albuterol	categorical	193	na	2	0.0808	4.85
5	0.028	asthma	categorical	193	na	2	0.0939	5.63
5	0.028	any_ind_skin	categorical	193	na	2	0.0939	5.63
5	0.028	race_other	categorical	193	na	2	0.0997	5.98
6	0.017	age	numeric	193	0.229	na	0.00134	0.08
6	0.017	FVC	numeric	192	0.175	na	0.0153	0.92
6	0.017	hyb_date	categorical	193	na	20	0.0212	1.27
6	0.017	FEV1FVC	numeric	192	-0.142	na	0.0499	2.99
6	0.017	atopy_count	categorical	193	na	13	0.0618	3.71
6	0.017	race_other	categorical	193	na	2	0.0656	3.94
6	0.017	caretaker_smokes	categorical	193	na	2	0.0656	3.94
6	0.017	FEVpred	numeric	193	-0.129	na	0.0746	4.48
6	0.017	post_FEV1	numeric	81	0.198	na	0.0771	4.63
6	0.017	FEV2575pred	numeric	165	-0.138	na	0.0781	4.69
6	0.017	FEV1	numeric	193	0.121	na	0.0934	5.60
6	0.017	post_FEF2575	numeric	81	0.186	na	0.0957	5.74
7	0.011	Collection.Site	categorical	193	na	6	0.00294	0.18
7	0.011	post_FEF2575	numeric	81	0.28	na	0.0112	0.67
7	0.011	post_FEV1	numeric	81	0.269	na	0.0152	0.91
7	0.011	post_FEF2575pred	numeric	70	0.263	na	0.0277	1.66

7	0.011	race_hispanic	categorical	41	na	2	0.0371	2.23
7	0.011	post_FEV1pred	numeric	81	0.229	na	0.0399	2.39
7	0.011	FEVpred	numeric	193	0.137	na	0.0573	3.44
7	0.011	FEV1FVC	numeric	192	0.135	na	0.0615	3.69
7	0.011	post_FVC	numeric	81	0.204	na	0.0671	4.03
7	0.011	num_smokers_home	numeric	193	-0.12	na	0.0972	5.83

(B)

	Variance PC Explained	Clinical/technical Variable	Variable Type	Number Observations	Correlation	Number ANOVA Groups	p	Bonferroni p value
1	0.495	wta_conc	numeric	194	0.39	na	1.94E-08	2.08E-06
1	0.495	WTA.Date	categorical	194	na	18	2.91E-05	3.11E-03
1	0.495	Hyb.Date	categorical	194	na	9	0.00774	0.83
1	0.495	PC20	numeric	30	0.358	na	0.0519	5.55
1	0.495	race_aa	categorical	194	na	2	0.0621	6.64
1	0.495	Barcode.expr	numeric	194	-0.13	na	0.0712	7.62
1	0.495	Sample.TC	numeric	194	-0.127	na	0.0774	8.28
2	0.067	WTA.Date	categorical	194	na	18	8.41E-12	9.00E-10
2	0.067	Hyb.Date	categorical	194	na	9	2.10E-07	2.25E-05
2	0.067	FEV1FVC	numeric	193	-0.235	na	0.00101	0.11
2	0.067	wta_conc	numeric	194	-0.222	na	0.00185	0.20
2	0.067	skintest_source	categorical	194	na	2	0.0051	0.55
2	0.067	FEV2575pred	numeric	166	-0.216	na	0.00511	0.55
2	0.067	Sample.TC	numeric	194	-0.181	na	0.0115	1.23
2	0.067	gas_home	categorical	194	na	2	0.0136	1.46
2	0.067	total_IgE	numeric	193	0.169	na	0.0188	2.01
2	0.067	any_ind_skin	categorical	194	na	2	0.0218	2.33
2	0.067	asthma	categorical	194	na	2	0.0218	2.33
2	0.067	albuterol	categorical	194	na	2	0.0334	3.57
2	0.067	reverse_10per	categorical	81	na	2	0.0336	3.60

2	0.067	mouse_pos	categorical	194	na	2	0.0349	3.73
2	0.067	inh_steroid	categorical	194	na	2	0.0367	3.93
2	0.067	post_FEV1FVC	numeric	81	-0.229	na	0.0401	4.29
2	0.067	FEF2575	numeric	193	-0.142	na	0.0488	5.22
2	0.067	montelukast	categorical	194	na	2	0.0511	5.47
2	0.067	lnige	numeric	193	0.14	na	0.0522	5.59
2	0.067	PC20	numeric	30	-0.325	na	0.0798	8.54
2	0.067	roach_12mo	categorical	194	na	2	0.087	9.31
2	0.067	atopy_count	categorical	194	na	13	0.0995	10.65
3	0.055	Hyb.Date	categorical	194	na	9	1.74E-11	1.86E-09
3	0.055	WTA.Date	categorical	194	na	18	1.01E-05	1.08E-03
3	0.055	any_ind_skin	categorical	194	na	2	0.014	1.50
3	0.055	asthma	categorical	194	na	2	0.014	1.50
3	0.055	inh_steroid	categorical	194	na	2	0.0148	1.58
3	0.055	Labelling.Conc	numeric	190	0.174	na	0.0162	1.73
3	0.055	albuterol	categorical	194	na	2	0.0236	2.53
3	0.055	dog_6mo	categorical	194	na	2	0.037	3.96
3	0.055	labelling	categorical	194	na	3	0.0584	6.25
3	0.055	Collection.Site	categorical	194	na	6	0.0768	8.22
3	0.055	Barcode.expr	numeric	194	-0.121	na	0.0938	10.04
4	0.04	WTA.Date	categorical	194	na	18	1.85E-18	1.98E-16
4	0.04	Hyb.Date	categorical	194	na	9	1.36E-06	1.46E-04
4	0.04	wta_conc	numeric	194	-0.27	na	0.000142	0.02
4	0.04	lnige	numeric	193	0.215	na	0.00264	0.28
4	0.04	rodent_6mo	categorical	194	na	2	0.00581	0.62
4	0.04	total_IgE	numeric	193	0.154	na	0.032	3.42
4	0.04	Labelling.Conc	numeric	190	-0.137	na	0.0588	6.29
4	0.04	gender	categorical	194	na	2	0.0754	8.07
5	0.028	Hyb.Date	categorical	194	na	9	4.79E-06	5.13E-04

5	0.028	labelling	categorical	194	na	3	8.40E-05	8.99E-03
5	0.028	postFEV1_80per	numeric	30	0.435	na	0.0163	1.74
5	0.028	signal_strength	numeric	194	-0.151	na	0.0355	3.80
5	0.028	WTA.Date	categorical	194	na	18	0.0407	4.35
5	0.028	roach_IgE	numeric	192	-0.146	na	0.0431	4.61
5	0.028	cat_6mo	categorical	194	na	2	0.0581	6.22
5	0.028	rodent_6mo	categorical	194	na	2	0.0686	7.34
5	0.028	age	numeric	194	0.121	na	0.093	9.95
6	0.025	labelling	categorical	194	na	3	3.27E-21	3.50E-19
6	0.025	WTA.Date	categorical	194	na	18	7.58E-19	8.11E-17
6	0.025	Hyb.Date	categorical	194	na	9	0.000123	0.01
6	0.025	heat_source	categorical	194	na	6	0.000189	0.02
6	0.025	Barcode.expr	numeric	194	0.183	na	0.0107	1.14
6	0.025	gender	categorical	194	na	2	0.0465	4.98
6	0.025	wta_conc	numeric	194	0.136	na	0.0579	6.20
6	0.025	cat_6mo	categorical	194	na	2	0.096	10.27
7	0.021	Hyb.Date	categorical	194	na	9	1.09E-07	1.17E-05
7	0.021	Barcode.expr	numeric	194	-0.292	na	3.73E-05	3.99E-03
7	0.021	wta_conc	numeric	194	-0.244	na	0.000603	0.06
7	0.021	gender	categorical	194	na	2	0.00141	0.15
7	0.021	signal_strength	numeric	194	0.192	na	0.00747	0.80
7	0.021	Collection.Site	categorical	194	na	6	0.0114	1.22
7	0.021	labelling	categorical	194	na	3	0.0138	1.48
7	0.021	WTA.Date	categorical	194	na	18	0.0216	2.31
7	0.021	around_smokers	categorical	194	na	4	0.0362	3.87
7	0.021	nasal_steroid	categorical	154	na	2	0.0382	4.09
7	0.021	montelukast	categorical	194	na	2	0.0646	6.91
8	0.017	labelling	categorical	194	na	3	0.00114	0.12
8	0.017	Barcode.expr	numeric	194	0.214	na	0.0027	0.29

8	0.017	gender	categorical	194	na	2	0.0152	1.63
8	0.017	Hyb.Date	categorical	194	na	9	0.0167	1.79
8	0.017	post_FEV1pred	numeric	81	0.231	na	0.0382	4.09
8	0.017	dog_wheal	numeric	194	0.135	na	0.0613	6.56
8	0.017	WTA.Date	categorical	194	na	18	0.0711	7.61
8	0.017	dog_pos	categorical	194	na	2	0.1	10.70
9	0.014	WTA.Date	categorical	194	na	18	3.15E-06	3.37E-04
9	0.014	Hyb.Date	categorical	194	na	9	1.37E-05	1.47E-03
9	0.014	gender	categorical	194	na	2	7.88E-05	8.43E-03
9	0.014	Labelling.Conc	numeric	190	0.212	na	0.00326	0.35
9	0.014	Barcode.expr	numeric	194	0.203	na	0.00455	0.49
9	0.014	labelling	categorical	194	na	3	0.0135	1.44
9	0.014	dampness_home	categorical	194	na	2	0.0283	3.03
9	0.014	race_hispanic	categorical	41	na	2	0.0301	3.22
9	0.014	around_smokers	categorical	194	na	4	0.076	8.13
9	0.014	num_smokers_home	numeric	194	-0.126	na	0.0789	8.44
9	0.014	race_a_carrib	categorical	193	na	2	0.0914	9.78
10	0.012	WTA.Date	categorical	194	na	18	1.06E-09	1.13E-07
10	0.012	Collection.Site	categorical	194	na	6	8.16E-05	8.73E-03
10	0.012	Hyb.Date	categorical	194	na	9	0.000137	0.01
10	0.012	labelling	categorical	194	na	3	0.000296	0.03
10	0.012	wta_conc	numeric	194	0.226	na	0.0015	0.16
10	0.012	dog_6mo	categorical	194	na	2	0.0316	3.38
10	0.012	signal_strength	numeric	194	-0.146	na	0.0425	4.55
10	0.012	Barcode.expr	numeric	194	-0.142	na	0.0477	5.10
10	0.012	gas_home	categorical	194	na	2	0.0528	5.65
10	0.012	post_FEV1change	numeric	81	0.197	na	0.0781	8.36
10	0.012	gender	categorical	194	na	2	0.0983	10.52
10	0.012	Sample.TC	numeric	194	0.118	na	0.0999	10.69

Supplemental Table 3: List of statistical models used in the analyses included in the manuscript. **(A)** Methylation or expression values were fit to all variables shown in the model and p values for the covariate of interest were extracted. Models were run using limma.

(A)

Dependent Variable	Covariate	Model*
Methylation	Asthma (compared to controls)	asthma + age + gender + race(aa) + race(hispanic) + race(other) + peer(1)
	ln(IgE) (asthmatic only; either focused on 81-asthma DMRs or genome-wide)	ln(IgE) + age + gender + race(aa) + race(hispanic) + race(other) + peer(1)
	FEV1 % predicted (asthmatic only; either focused on 81-asthma DMRs or genome-wide)	FEV1(%pred) + age + gender + race(aa) + race(hispanic) + race(other) + peer(1)
Expression	Asthma (compared to controls)	asthma + age + gender + race(aa) + race(hispanic) + race(other) + labeling date + wta(conc) + peer(1) + peer(2) + peer(3) + peer(4) + peer(5)
	ln(IgE) (asthmatics only)	ln(IgE) + age + gender + race(aa) + race(hispanic) + race(other) + wta date + wta(conc) + hyb date + labeling + peer(1) + peer(2) + peer(3) + peer(4) + peer(5)
	FEV1 %predicted	FEV1 %pred + age + gender + race(aa) + race(hispanic) + race(other) + wta date + wta(conc) + hyb date + labeling + peer(1) + peer(2) + peer(3) + peer(4) + peer(5)

* aa refers to African American, wta concentration refers to concentration of the whole-transcriptome amplified RNA, pc refers to principal component and peer refers to estimated latent factors to account for unknown batch effects, ln refers to natural log

Supplemental Table 4: Population characteristics of screened participants*

	Enrolled (N=194)	Screened Not Enrolled** (N=121)	Total (N=315)	p-value †
Site: Boston	54 (27.8%)	30 (24.8%)	84 (26.7%)	0.21 ¹
Dallas	10 (5.2%)	3 (2.5%)	13 (4.1%)	
Denver	41 (21.1%)	23 (19.0%)	64 (20.3%)	
Detroit	10 (5.2%)	3 (2.5%)	13 (4.1%)	
New York	36 (18.6%)	36 (29.8%)	72 (22.9%)	
Washington D.C.	43 (22.2%)	26 (21.5%)	69 (21.9%)	
Age at recruitment (yr)	9.0 [8.0, 11.0]	9.0 [8.0, 10.0]	9.0 [8.0, 11.0]	0.78 ²
Gender (Male)	97 (50.0%)	55 (45.5%)	152 (48.3%)	0.43 ¹
Participant race: African American (Yes)	168 (86.6%)	92 (76.7%)	260 (82.8%)	0.02 ¹
Participant race: Hispanic or Latino (Yes)	41 (21.1%)	35 (28.9%)	76 (24.1%)	0.12 ¹

* Data are presented as mean $\pm$ standard deviations, medians [interquartile range] or numbers (%).

** Children did not meet entry criteria.

† P-values were calculated with the use of the Chi-Square¹ test for categorical variables and the Wilcoxon rank-sum² test for continuous variable

Supplemental Table 5: Estimated percentage of each of the six lymphocyte cell types averaged in asthmatic cases and non-asthmatic controls. p values were calculated using two-sided t-test between cases and controls.

Leukocyte cell type	Mean % cells in controls	Mean % cells in cases	p value
B lymphocytes	0.175	0.170	0.363
CD4+ T lymphocytes	0.375	0.360	0.235
CD8+ T lymphocytes	0.084	0.088	0.578
Granulocytes	0.111	0.107	0.775
Monocytes	0.127	0.133	0.248
Natural killer (NK) cells	0.128	0.142	0.004

Supplemental Table 6: Cell composition analysis of 81 asthma-associated DMRs. SLK p values before and after adjustment for cell composition are shown.

Chr	Start	End	Gene	Asthma SLK p	Adjusted Asthma SLK p
chr1	6263726	6263914	ACOT7	3.79E-08	4.91E-10
chr17	38531660	38531955	NBR2	1.59E-08	2.24E-08
chr10	3813757	3814016	KLF6	8.39E-09	9.18E-07
chr16	84108978	84109249	KIAA0182	1.59E-06	1.18E-06
chr1	2.27E+08	2.27E+08	AX748369	2.00E-06	1.46E-06
chr4	1233848	1234086	AK125775	5.29E-07	1.52E-06
chr8	1.26E+08	1.26E+08	TRMT12	1.73E-06	2.40E-06
chr6	29019737	29020145	TRIM27	8.39E-07	2.88E-06
chr16	87085565	87085738	ZFPM1	1.59E-06	3.59E-06
chr11	34416873	34417133	CAT	7.88E-07	3.95E-06
chr1	27555725	27556088	MAP3K6	1.96E-07	4.83E-06
chr1	21750110	21750368	ALPL	3.59E-06	5.60E-06
chr16	85272036	85272335	BC041439	1.63E-06	6.81E-06
chr10	1.02E+08	1.02E+08	CPN1	3.92E-06	1.70E-05
chr1	2.14E+08	2.14E+08	KCTD3	2.66E-05	2.34E-05
chr5	1.32E+08	1.32E+08	IL13	5.34E-08	2.88E-05
chr1	21773573	21773847	ALPL	3.79E-06	3.06E-05
chr21	46670037	46670291	PCNT	1.79E-09	3.13E-05
chr17	77226056	77226266	TSPAN10	4.00E-06	4.90E-05
chr10	3814386	3814687	KLF6	3.08E-08	0.0001007
chr7	888576	888761	GET4; SUN1	3.47E-08	0.0001262
chr6	31954747	31955007	SLC44A4	6.90E-06	0.0001517
chr1	18883602	18883759	PAX7	3.83E-06	0.0001672
chr6	1.1E+08	1.1E+08	MICAL1	1.42E-08	0.0001963
chr11	64289922	64290199	SF1	3.71E-06	0.001193
chr8	1.28E+08	1.28E+08	FAM84B	8.24E-07	0.001303
chr10	1.26E+08	1.26E+08	FAM53B	2.03E-07	0.001768
chr12	1.3E+08	1.3E+08	GPR133	1.33E-05	0.001777
chr1	15264894	15265020	LOC391005	4.38E-10	0.001866
chr19	1207998	1208217	MIDN	1.38E-05	0.002011
chr2	66518038	66518302	MEIS1	4.82E-07	0.00218
chr16	87075079	87075362	ZFPM1	1.39E-05	0.002293
chr12	1.22E+08	1.22E+08	PITPNM2	3.43E-08	0.002375
chr2	8340572	8340606	LINC00299	1.02E-06	0.003077
chr11	62378748	62378810	SNHG1	1.62E-06	0.003269
chr17	78474047	78474241	TBCD	1.15E-06	0.003873
chr7	2695301	2695438	AMZ1	8.55E-06	0.00455

chr20	34937784	34937967	C20orf118	1.33E-06	0.00589
chr7	73283658	73283902	RFC2	9.19E-08	0.00755
chr6	26334181	26334235	HIST1H3F	1.41E-07	0.007994
chr15	89170960	89171237	BLM	2.72E-05	0.008067
chr19	10523581	10523782	ATG4D	1.46E-05	0.009453
chr5	1.26E+08	1.26E+08	GRAMD3	3.32E-10	0.01069
chr7	74890962	74891132	POM121C	1.05E-05	0.01584
chr2	8339686	8339783	LINC00299	9.66E-08	0.01709
chr13	1.14E+08	1.14E+08	RASA3	1.34E-05	0.01873
chr1	6277594	6277647	ACOT7	1.41E-07	0.02326
chr2	2.43E+08	2.43E+08	AK097934	5.56E-07	0.02331
chr11	36377805	36377877	FLJ14213	7.07E-07	0.02544
chr19	18121329	18121515	MAST3	3.31E-08	0.03303
chr3	52787406	52787560	ITIH1	5.39E-06	0.03566
chr6	458210	458297	EXOC2	4.91E-06	0.03687
chr16	84233792	84234027	KIAA0182	1.86E-05	0.03963
chr13	1.13E+08	1.13E+08	TMCO3	1.19E-05	0.04193
chr1	25113451	25113525	RUNX3	1.63E-07	0.04432
chr7	38323253	38323572	TCRg;TRGV9	2.71E-06	0.05124
chr3	1.15E+08	1.15E+08	TIGIT	1.67E-05	0.05344
chr10	72030353	72030454	PRF1	1.27E-07	0.05713
chr22	45064135	45064392	TTC38	3.11E-09	0.06468
chr17	44652266	44652511	ABI3	1.18E-05	0.07629
chr1	1091164	1091325	CS444397	5.59E-07	0.07982
chr1	1.53E+08	1.53E+08	ATP8B2	5.46E-06	0.08959
chr10	1.34E+08	1.34E+08	PWWP2B	2.63E-06	0.09638
chr20	55416108	55416384	RBM38	3.23E-06	0.119
chr7	2056125	2056406	MAD1;MAD1L1	7.89E-07	0.1251
chr17	70268887	70268897	SLC9A3R1	9.75E-07	0.13
chr19	55911909	55912349	SHANK1	1.02E-07	0.1421
chr2	2.32E+08	2.32E+08	NMUR1	1.15E-08	0.1439
chr2	27652710	27652915	C2orf16	7.47E-06	0.1695
chr14	1.04E+08	1.04E+08	INF2	2.80E-06	0.1802
chr1	1.1E+08	1.1E+08	EPS8L3	5.01E-07	0.2224
chr1	45046081	45046267	TCTEX1D4	1.74E-05	0.2293
chr3	11626535	11626881	VGLL4	1.25E-07	0.2444
chr14	98857115	98857373	BCL11B	3.11E-06	0.262
chr17	77986792	77986955	HEXDC	1.35E-05	0.2629
chr17	37379133	37379307	CNP	5.59E-08	0.2754
chr19	60006690	60006895	KIR2DL4;KIR2DS2;KIR3DL1	3.44E-06	0.3652
chr1	2220460	2220783	SKI	4.52E-07	0.3677
chr10	848816	848923	LARP5	8.24E-06	0.3887
chr11	1.29E+08	1.29E+08	APLP2	2.10E-06	0.6786

chr6	31975676	31976004	C2;ZBTB12	2.89E-08	0.8111
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Supplemental Table 7: Disease and biofunction enrichment analysis of 55 DMRs that remain significant after adjustment for cell mixture. Analysis was performed in Ingenuity Pathway Analysis (IPA).

Categories	Diseases or Functions Annotation	p-Value	B-H p-Value	Molecules
Organismal Development	oversecretion of mucus	3.35E-04	3.90E-02	IL13,RUNX3
Cellular Assembly and Organization, Cellular Function and Maintenance	organization of nucleus	3.44E-04	3.90E-02	BLM,POM121/POM121C,SUN1
Cancer, Connective Tissue Disorders, Skeletal and Muscular Disorders	metastatic bone cancer	8.72E-04	3.90E-02	ALPL,CAT
Inflammatory Disease, Respiratory Disease	airway hyperresponsive ness	1.05E-03	3.90E-02	CAT,IL13,RUNX3
Hair and Skin Development and Function	pallor	1.49E-03	3.90E-02	BLM,KLF6,ZFPM1
Cell Cycle	arrest in interphase	1.81E-03	3.90E-02	BLM,CAT,KLF6,RUNX3,SUN1
Cell Death and Survival	cell death of connective tissue cells	2.05E-03	3.90E-02	BLM,CAT,IL13,MIC AL1,RUNX3,SUN1
Cancer, Organismal Injury and Abnormalities, Reproductive System Disease	prostate cancer	2.67E-03	3.90E-02	ALPL,CTBP1-AS2,KLF6,POM121/POM121C,RUNX3,SF1
Inflammatory Response	innate immune response	3.02E-03	3.90E-02	EXOC2,IL13,TRIM27
Cellular Development, Embryonic Development, Hematological System Development and Function, Hematopoiesis, Organismal Development	hematopoiesis in embryo	4.37E-03	4.23E-02	KLF6,ZFPM1
Cellular Movement, Hematological System Development and Function, Hypersensitivity Response, Immune Cell Trafficking	infiltration by eosinophils	5.19E-03	4.23E-02	IL13,RUNX3
DNA Replication, Recombination, and Repair	DNA damage	5.42E-03	4.23E-02	CAT,IL13,SUN1
Respiratory System Development and Function	respiration of mice	5.72E-03	4.39E-02	CAT,IL13
Cell Cycle	arrest in S phase	6.27E-03	4.56E-02	BLM,SUN1
Cellular Development, Hematopoiesis, Tissue Development	differentiation of bone marrow cells	7.18E-03	4.56E-02	IL13,RUNX3,ZFPM1
Cellular Development, Cellular Growth and Proliferation, Skeletal and Muscular System Development and Function, Tissue Development	proliferation of muscle cells	7.19E-03	4.56E-02	IL13,MEIS1,PAX7,SF1
Free Radical Scavenging, Small Molecule Biochemistry	production of hydrogen peroxide	7.44E-03	4.62E-02	CAT,IL13

				ALPL,BLM,CAT,GET4,IL13,MEIS1,PAX7,RASA3,RUNX3,TRIM27,ZFPM1
Cellular Development	differentiation of cells	7.53E-03	4.64E-02	
Cell Cycle	arrest in G2/M phase	7.85E-03	4.68E-02	BLM,CAT
Cell Death and Survival	killing of cells	7.93E-03	4.68E-02	EXOC2,GET4,IL13
Drug Metabolism, Molecular Transport, Small Molecule Biochemistry	concentration of glutathione	8.27E-03	4.68E-02	CAT,IL13
Cellular Compromise, Inflammatory Response	degranulation of cells	1.02E-02	5.03E-02	EXOC2,IL13,ZFPM1
Cardiovascular System Development and Function, Tissue Development	development of cardiovascular tissue	1.06E-02	5.03E-02	IL13,RASA3,RUNX3,ZFPM1
Cancer, Skeletal and Muscular Disorders	rhabdomyosarcoma	1.08E-02	5.03E-02	BLM,PAX7
Cardiovascular System Development and Function, Tissue Development	morphogenesis of cardiovascular tissue	1.22E-02	5.34E-02	IL13,ZFPM1
Tissue Morphology	abnormal morphology of vitelline vessel	1.33E-02	5.52E-02	KLF6,ZFPM1
Gastrointestinal Disease, Hepatic System Disease, Inflammatory Disease, Organismal Injury and Abnormalities	primary biliary cirrhosis	1.33E-02	5.52E-02	ALPL,IL13
Cell Death and Survival	cell death of fibroblasts	1.44E-02	5.62E-02	BLM,CAT,RUNX3
Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance	formation of cilia	1.49E-02	5.74E-02	MAST3,PCNT
Cellular Development, Tissue Development	differentiation of connective tissue	1.55E-02	5.94E-02	ALPL,CAT,IL13,RUNX3,ZFPM1
Cell Death and Survival	apoptosis of cerebral cortex cells	1.57E-02	5.98E-02	CAT,KLF6
Nutritional Disease, Organismal Injury and Abnormalities	wasting	1.57E-02	5.98E-02	ALPL,PAX7
Cellular Development, Hematological System Development and Function, Hematopoiesis	differentiation of dendritic cells	1.60E-02	6.04E-02	IL13,RUNX3
Hematological Disease, Immunological Disease, Organismal Injury and Abnormalities	leukocytosis	1.78E-02	6.32E-02	IL13,RUNX3
Cellular Development, Skeletal and Muscular System Development and Function, Tissue Development	differentiation of myoblasts	1.81E-02	6.32E-02	PAX7,TRIM27
Cellular Development	maturation of blood cells	1.86E-02	6.45E-02	IL13,RUNX3,ZFPM1
Cellular Development, Cellular Growth and Proliferation, Hematological System Development and Function	proliferation of myeloid cells	1.87E-02	6.46E-02	CAT,MEIS1
Skeletal and Muscular System Development and Function, Tissue Morphology	quantity of muscle cells	1.90E-02	6.51E-02	PAX7,RFC2

Embryonic Development, Tissue Morphology	abnormal morphology of yolk sac	1.93E-02	6.51E-02	KLF6,ZFPM1
Cell Signaling, Small Molecule Biochemistry	synthesis of nitric oxide	1.97E-02	6.51E-02	CAT,IL13,KLF6
Cell Death and Survival	apoptosis of connective tissue cells	2.10E-02	6.69E-02	BLM,IL13,RUNX3
Cancer, Organismal Injury and Abnormalities, Reproductive System Disease	prostatic carcinoma	2.10E-02	6.69E-02	KLF6,POM121/POM121C,RUNX3
Cellular Growth and Proliferation	cytostasis	2.12E-02	6.69E-02	BLM,KLF6,MEIS1
Cancer	hyperplasia of epithelial tissue	2.18E-02	6.69E-02	IL13,RUNX3
Cell Death and Survival	apoptosis of epithelial cells	2.19E-02	6.69E-02	CAT,IL13,RUNX3
Lymphoid Tissue Structure and Development	growth of lymphatic system component	2.21E-02	6.72E-02	BLM,IL13,MEIS1
Post-Translational Modification, Protein Degradation, Protein Synthesis	hydrolysis of protein	2.31E-02	6.86E-02	ATG4D,CPN1,IL13
Cell Death and Survival	cell death of epithelial cells	2.37E-02	6.91E-02	CAT,IL13,RUNX3,T RIM27
Cell-mediated Immune Response, Cellular Development, Cellular Function and Maintenance, Hematological System Development and Function, Hematopoiesis, Lymphoid Tissue Structure and Development	T cell development	2.45E-02	7.11E-02	BLM,IL13,RUNX3,Z FPM1
Cellular Development, Hematological System Development and Function, Hematopoiesis	maturation of lymphocytes	2.69E-02	7.39E-02	IL13,RUNX3
Cell Morphology, Hematopoiesis	abnormal morphology of hematopoietic progenitor cells	2.84E-02	7.73E-02	BLM,ZFPM1
Embryonic Development, Organismal Development, Tissue Development	morphogenesis of embryonic tissue	2.95E-02	7.90E-02	CAT,ZFPM1
Humoral Immune Response, Protein Synthesis	quantity of IgM	2.95E-02	7.90E-02	BLM,IL13
Organ Morphology, Skeletal and Muscular System Development and Function	abnormal morphology of muscle	3.06E-02	7.98E-02	ALPL,PAX7,SUN1
Protein Degradation, Protein Synthesis	degradation of protein	3.17E-02	8.18E-02	ATG4D,CAT,CPN1,I L13
Cancer, Organismal Injury and Abnormalities, Renal and Urological Disease	renal-cell carcinoma	3.18E-02	8.18E-02	ACOT7,KLF6,POM121/POM121C,RUNX3
Cell Death and Survival	apoptosis of fibroblast cell lines	3.21E-02	8.18E-02	BLM,MICAL1,SUN1

Cellular Development, Cellular Growth and Proliferation, Hematological System Development and Function	proliferation of immune cells	3.29E-02	8.29E-02	BLM,CAT,IL13,MEIS1,RUNX3
Immunological Disease	systemic autoimmune syndrome	3.49E-02	8.53E-02	ALPL,CAT,IL13,RUNX3,SLC44A4,TRIM27
Hematological Disease	anemia	3.50E-02	8.53E-02	BLM,CAT,ZFPM1
Embryonic Development, Organ Development, Organismal Development, Skeletal and Muscular System Development and Function, Tissue Development	development of striated muscle	3.52E-02	8.54E-02	PAX7,ZFPM1
Cell-To-Cell Signaling and Interaction, Molecular Transport, Small Molecule Biochemistry	secretion of neurotransmitter	3.52E-02	8.54E-02	IL13,SLC44A4
Cardiovascular System Development and Function, Cellular Development, Organismal Development, Tissue Development	endothelial cell development	3.70E-02	8.74E-02	IL13,RASA3,RUNX3
Cancer	tumorigenesis of tumor cell lines	3.76E-02	8.74E-02	KLF6,RUNX3
Tissue Morphology	quantity of cells	4.46E-02	9.93E-02	ALPL,BLM,CAT,IL13,PAX7,RFC2,RUNX3,SUN1
Cellular Development, Cellular Growth and Proliferation	proliferation of lymphoma cell lines	4.61E-02	1.01E-01	CAT,IL13
Cancer, Cell Death and Survival, Tumor Morphology	cell death of tumor cells	4.63E-02	1.01E-01	CAT,EXOC2,PAX7
Cell-To-Cell Signaling and Interaction, Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response	recruitment of neutrophils	4.92E-02	1.05E-01	CAT,IL13

Supplemental Table 8: Differentially methylated regions DMRs associated with (A) IgE at and (B) FEV₁ percent predicted at SLK-Sidak p <0.05 in asthma cases only.

(A) IgE

Chr	Start	End	Min raw p	# probes	SLK p	SLK-Sidak p	Gene	Location	CpG Island Distance	IgE correlation
chr4	1.12E+08	1.12E+08	0.001003	7	4.19E-09	7.58E-06	ENPEP	utr5	0	-0.283
chr18	30427081	30427225	0.001416	4	1.71E-08	5.75E-05	DTNA	utr5_intron	34877	-0.272
chr19	60579610	60579894	0.04203	2	8.76E-08	0.00015	TMEM190	122	1004	-0.292
chr8	1.26E+08	1.26E+08	0.006347	6	1.41E-07	0.000208	TRMT12	exon;utr5	0	0.288
chr22	22714104	22714573	0.00998	12	2.29E-07	0.000237	GSTT1	exon;intron;nc_exon;nc_intron;utr5	0	-0.228
chr13	51667662	51667952	0.006347	4	1.99E-07	0.000334	DKFZp434F1622	nc_intron	0	0.257
chr11	14950488	14950973	0.006347	12	6.82E-07	0.000682	CALCA	-80	0	-0.257
chr8	16904002	16904295	0.006347	6	7.41E-07	0.001228	FGF20;fgf-20	utr5	0	-0.259
chr6	53638461	53638587	0.01055	6	3.85E-07	0.001481	KLHL31	utr5	13426	-0.281
chr2	3728362	3728440	0.01728	3	1.35E-06	0.008375	ALLC	-227	0	-0.319
chr6	74161108	74161321	0.002895	3	3.91E-06	0.008866	DDX43;OOEP	utr5;utr5_intron	0	0.265

(B) FEV₁ % predicted

Chr	Start	End	Min raw p	# probes	SLK p	SLK-Sidak p	Gene	Location	CpG Island Distance	IgE correlation
chr18	14737927	14738439	0.004836	9	9.46E-07	0.000896	ANKRD30B	TSS+utr5+cds	0	-0.2
chr9	1.37E+08	1.37E+08	0.03159	2	5.05E-06	0.01139	COL5A1	intron	-1514	0.3
chr14	68164809	68164984	0.000219	3	2.26E-09	6.26E-06	RAD51B	intergenic	0	-0.3
chr14	68165322	68165432	0.004836	2	2.74E-07	0.001209	RAD51B	intergenic	162	-0.5
chr8	1.01E+08	1.01E+08	0.02653	3	3.72E-06	0.03846	RNF19A	intergenic	25741	0.2
chr15	43102080	43102235	0.02252	4	2.92E-06	0.009116	SORD	intergenic	-258	-0.2
chr7	99613378	99613494	0.004836	5	2.05E-06	0.008537	STAG3	exon+utr5;intron+exon+utr5	0	-0.1
chr2	27384387	27384674	0.00523	4	7.14E-07	0.001207	UCN	+utr5	0	-0.1
chr11	306087	306456	0.01067	4	1.19E-06	0.001569	IFITM1	intergenic	0	-0.1
chr11	2876627	2877013	0.0002	10	3.51E-07	0.000441	SLC22A18AS	intron	-2864	0.3
chr12	7483673	7483914	0.03159	3	5.02E-06	0.01005	CD163L1	intron	0	-0.1
chr12	40117798	40117825	0.01839	2	2.31E-06	0.0407	PDZRN4	TSS+exon+utr5;intron	-135707	0.2
chr6	31517387	31517736	0.001043	11	1.76E-08	2.45E-05	HCP5	intergenic	37491	-0.3
chr6	32665298	32665456	0.01176	5	1.24E-06	0.003786	HLA-DRB1	intron+cds	4989	0.4
chr1	1107335	1107488	0.01067	3	8.77E-07	0.002778	TTLL10	intron	1466	-0.1
chr1	2.22E+08	2.22E+08	0.004836	7	1.40E-06	0.001884	CCDC185	TSS+cds	0	-0.2

Supplemental Table 9: Gene expression changes associated with (A) asthma, (B) IgE in asthmatics, and (C) FEV1 percent predicted in asthmatics at Benjamini-Hochberg q value < 0.2.

(A) Asthma

Probe ID	Gene Symbol	Description	p value	q value	log2 (asthma/control)
NM_030760	EDG8	endothelial differentiation, sphingolipid G-protein-coupled receptor, 8	7.87E-08	0.00354	0.591
BC067781	EDG8	endothelial differentiation, sphingolipid G-protein-coupled receptor, 8	0.000000598	0.01346	0.477
NM_017731	OSBPL7	oxysterol binding protein-like 7	0.00000169	0.02536	0.258
NM_013351	TBX21	T-box 21	0.00000245	0.02763	0.372
NM_012445	SPON2	spondin 2, extracellular matrix protein	0.00000353	0.03066	0.358
NM_017931	FLJ20699	hypothetical protein FLJ20699	0.00000409	0.03066	0.4
AY372494	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	0.00000535	0.03444	0.482
NM_031950	KSP37	Ksp37 protein	0.0000067	0.03671	0.28
NM_002071	GNAL	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	0.00000734	0.03671	0.42
AB051543	SYNE1	spectrin repeat containing, nuclear envelope 1	0.0000103	0.04651	0.416
NM_001015002	LLGL2	lethal giant larvae homolog 2 (Drosophila)	0.0000124	0.05004	0.3
NM_032214	SLA2	Src-like-adaptor 2	0.0000133	0.05004	0.272
BC110613	KIR2DL3	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 3	0.0000176	0.06092	0.257
NM_001140	ALOX15	arachidonate 15-lipoxygenase	0.000021	0.06748	0.412
AY129023	N/A	Homo sapiens clone FP18821 unknown mRNA.	0.0000396	0.11895	0.285
NM_004131	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	0.0000436	0.12269	0.431
CR933676	SYNE1	spectrin repeat containing, nuclear envelope 1	0.0000529	0.13609	0.254
U73394	KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	0.0000603	0.13609	0.208
AK125378	RUSC1	RUN and SH3 domain containing 1	0.0000604	0.13609	0.308
NM_173854	SLC41A1	solute carrier family 41, member 1	0.0000585	0.13609	0.392
XM_932084	CEP78	centrosomal protein 78kDa	0.0000667	0.14313	0.3

BC095492	CHST12	carbohydrate (chondroitin 4) sulfotransferase 12	0.0000819	0.15555	0.143
NM_015570	AUTS2	autism susceptibility candidate 2	0.0000818	0.15555	0.258
AJ002102	KIR2DS2	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 2	0.0000829	0.15555	0.395
NM_007173	PRSS23	protease, serine, 23	0.0000864	0.15555	0.338
NM_000615	NCAM1	neural cell adhesion molecule 1	0.0000995	0.1601	0.329
AF276292	KIR2DL4	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	0.0000969	0.1601	-0.272
NM_017854	FLJ20512	hypothetical protein FLJ20512	0.0000966	0.1601	0.239
XM_927454	CEP78	centrosomal protein 78kDa	0.000112	0.17459	0.307
AF074087	KIR2DS2	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 2	0.000131	0.19721	0.265

(B) IgE

Probe ID	Gene Symbol	Description	p value	q value	IgE correlation
NM_001140	ALOX15	arachidonate 15-lipoxygenase	0.0000031	0.074	0.126
XM_931774	LOC643749	hypothetical protein LOC643749	0.00000329	0.074	-0.102
BC064693	AUTS2	autism susceptibility candidate 2	0.00000616	0.0925	0.096
BC101738	ZSCAN4	zinc finger and SCAN domain containing 4	0.0000115	0.1289	0.055
NM_001039702	THEDC1	thioesterase domain containing 1	0.0000158	0.1319	0.05
CR608350	CC2D1A	coiled-coil and C2 domain containing 1A	0.0000212	0.1319	0.068
NM_032214	SLA2	Src-like-adaptor 2	0.0000234	0.1319	0.074
AF290986	SLA2	Src-like-adaptor 2	0.0000217	0.1319	0.087
NM_001039799	FLJ41484	hypothetical protein LOC650669	0.0000335	0.1373	-0.092
NM_152891	PRSS33	protease, serine, 33	0.0000289	0.1373	0.104
NM_017854	FLJ20512	hypothetical protein FLJ20512	0.0000317	0.1373	-0.081
NM_017731	OSBPL7	oxysterol binding protein-like 7	0.0000452	0.1697	0.062

(C) FEV₁ % predicted

Probe ID	Gene Symbol	Description	p value	q value	FEV1 %pred correlation
NM_173626	SLC26A11	solute carrier family 26, member 11	3.75E-06	0.16877	0.177

Supplemental Table 10: List of genes with methylation and expression changes in asthma cases compared to controls identified using the LCMix method with posterior probability >0.95 (corresponds to $q < 0.05$). **(A)** 207 genes with methylation t statistic <-1.5, and expression t statistic > 1.5 and **(B)** 82 genes with methylation t statistic <-1.5, and expression t statistic <-1.5 These genes are selected from all 2484 genes with posterior probability >0.95 (Figure 3C).

(A)		Expression		Methylation			
Gene	ID	t statistic	p value	t statistic	p value	Distance to Gene	Posterior Probability
SPON2	NM_012445	4.78	3.53E-06	-1.66	0.0982	-408	0.964
SLA2	NM_032214	4.47	1.33E-05	-2.24	0.026	-120	0.971
KIR2DL4	U73394	4.11	6.03E-05	-2.75	0.00648	-12	0.973
KIR2DL4	AF276292	3.99	9.69E-05	-2.75	0.00648	-24	0.973
KIR2DS4	BC101977	3.73	0.000252	-2.31	0.0217	-149	0.972
GNLY	NM_006433	3.72	0.000268	-1.99	0.0483	-131	0.969
SLA2	AF290986	3.68	0.000304	-3.21	0.00157	-1112	0.974
KIR3DL2	BC107951	3.64	0.000355	-3.56	0.000468	-2	0.973
KIR3DL3	NM_153443	3.54	0.000514	-2.04	0.0426	-5	0.969
KIR2DL4	X99481	3.53	0.000529	-2.75	0.00648	-19	0.973
KIR2DS4	NM_012314	3.51	0.000556	-2.31	0.0217	-114	0.972
GPR56	NM_005682	3.51	0.000571	-1.85	0.0653	-41	0.967
KIR2DS4	AY102623	3.42	0.00076	-2.31	0.0217	-160	0.972
KLRD1	AF498040	3.40	0.000819	-2.55	0.0115	-577	0.973
FCRL6	AY212514	3.19	0.00169	-3.01	0.00296	-13	0.974
C1orf86	AK127994	3.15	0.00193	-2.10	0.0368	-647	0.970
SYCE1	BC034821	3.14	0.00196	-2.61	0.00969	-789	0.973
KLRD1	AJ000001	3.10	0.00222	-3.52	0.000536	-10	0.973
GNLY	BC023576	2.87	0.0046	-3.10	0.00226	-10	0.974
C15orf61	XM_096885	2.83	0.0052	-1.59	0.113	-517	0.962
LRRC2	NM_024750	2.77	0.00613	-2.42	0.0164	-1219	0.972
KIR2DL4	AF002256	2.74	0.00667	-2.75	0.00648	-28	0.973
ZDHHC9	BC000035	2.74	0.00667	-1.60	0.11	-35	0.962
DLK2	BC006425	2.69	0.00783	-1.63	0.104	-35	0.963
KIR2DL4	NM_002255	2.64	0.00892	-2.22	0.0277	-32	0.971
APOBEC3H	BC069023	2.63	0.00915	-1.91	0.0578	-130	0.968
PLA2G6	NM_001004426	2.60	0.00995	-2.04	0.0425	-51	0.970
KIR3DL2	NM_006737	2.59	0.0103	-3.56	0.000468	-25	0.973

IL1RL1	BC030975	2.59	0.0104	-3.04	0.00269	-296	0.974
CAMK1	NM_003656	2.53	0.0123	-1.69	0.0935	-207	0.964
AKAP8	BC037270	2.51	0.0128	-1.81	0.0712	-352	0.966
ATP4A	NM_000704	2.48	0.0139	-1.65	0.1	-1212	0.963
PYROXD2	NM_032709	2.46	0.0147	-1.62	0.107	-684	0.963
ABCB5	AY851364	2.46	0.015	-2.58	0.0105	-858	0.973
MXD4	BC002713	2.41	0.0169	-2.93	0.00385	-1203	0.974
FCRL6	AY654627	2.40	0.0175	-3.01	0.00296	-20	0.974
ALDOC	NM_005165	2.37	0.0187	-1.71	0.0888	-705	0.965
DLL1	NM_005618	2.37	0.0187	-1.68	0.0953	-65	0.964
GNLY	NM_012483	2.35	0.0199	-3.10	0.00226	-20	0.974
ZFAT	NM_001029939	2.34	0.0206	-1.50	0.134	-432	0.960
SPINK9	AY396740	2.33	0.021	-2.20	0.0291	-110	0.971
SH3BGR	NM_007341	2.32	0.0212	-1.96	0.0518	-58	0.969
NKG7	NM_005601	2.32	0.0212	-1.92	0.0561	-1330	0.968
RANBP17	NM_022897	2.32	0.0213	-2.21	0.0285	-1	0.971
NKG7	BC015759	2.32	0.0216	-1.92	0.0561	-1331	0.968
CTRL	NM_001907	2.31	0.0221	-1.56	0.12	-1282	0.961
ABCA2	AF178941	2.31	0.0222	-2.00	0.0466	-1502	0.969
FBF1	BC023549	2.30	0.0225	-2.45	0.0153	-2520	0.972
POLR1A	BC017115	2.28	0.0237	-1.60	0.112	-1330	0.962
IKZF2	AY008294	2.28	0.0238	-1.87	0.0626	-276	0.967
RUNX3	NM_001031680	2.28	0.0239	-3.07	0.00243	-1391	0.974
LEPREL4	NM_006455	2.27	0.0241	-1.92	0.0567	-566	0.968
CCBL1	NM_004059	2.27	0.0241	-2.81	0.00555	-991	0.973
SURF4	NM_033161	2.26	0.0252	-2.14	0.0333	-2305	0.970
XCL2	BC069360	2.25	0.0257	-2.57	0.011	-21	0.973
SLC22A17	NM_016609	2.24	0.0263	-1.87	0.0627	-2450	0.967
RPL3L	BC050413	2.24	0.0266	-2.23	0.0268	-270	0.971
CADM1	NM_014333	2.23	0.0272	-1.59	0.113	-825	0.962
TP53RK	BC019621	2.21	0.0281	-2.06	0.0412	-366	0.970
RCC2	NM_018715	2.21	0.0285	-2.33	0.0208	-551	0.972
CLIC3	BC007012	2.20	0.0288	-2.18	0.0307	-41	0.971
WNT5A	NM_003392	2.19	0.0298	-2.33	0.0211	-2920	0.972
DNAJC17	NM_018163	2.19	0.0301	-1.73	0.0858	-57	0.965

KIR3DL2	X93596	2.18	0.0303	-3.56	0.000468	-27	0.973
MXD4	NM_006454	2.17	0.0313	-2.70	0.00763	-15	0.973
JOSD1	NM_014876	2.16	0.0324	-1.84	0.0675	-1102	0.967
OTUD7A	NM_130901	2.14	0.0333	-1.69	0.0932	-280	0.964
ACOT7	AB074415	2.14	0.034	-1.59	0.113	-2596	0.962
OR10G4	NM_001004462	2.12	0.0354	-1.56	0.121	-141	0.961
SLC16A12	AK124901	2.12	0.0355	-1.73	0.0845	-2340	0.965
IKZF2	NM_016260	2.11	0.0364	-1.87	0.0626	-857	0.967
RBFOX3	NM_001025448	2.11	0.0364	-1.65	0.1	-1147	0.963
MYBL1	BC101186	2.10	0.0367	-3.14	0.00196	-1475	0.974
PYHIN1	BC020822	2.10	0.037	-2.73	0.00691	-714	0.973
TMTC1	AK125140	2.07	0.0397	-1.54	0.125	-2730	0.961
HLCS	BC060787	2.05	0.0414	-1.87	0.0633	-2855	0.967
NR5A2	U93553	2.05	0.0422	-2.19	0.0299	-493	0.971
RAB11FIP5	NM_015470	2.04	0.0423	-1.67	0.097	-194	0.964
ASCL4	NM_203436	2.03	0.0434	-1.75	0.0825	-91	0.965
PCBP4	BC017098	2.03	0.0439	-3.08	0.00239	-546	0.974
TSPY2	BC075016	2.02	0.0447	-1.58	0.116	-28	0.962
ZIM3	BC069071	2.02	0.0451	-2.17	0.0309	-103	0.971
TAF6	NM_005641	2.02	0.0453	-2.44	0.0156	-177	0.972
MEG3	CR615599	2.01	0.0462	-1.93	0.0555	-71	0.968
TSPY1	AY130858	2.01	0.0463	-1.58	0.116	-55	0.962
MAMDC4	NM_206920	1.99	0.0476	-1.99	0.0478	-104	0.969
NKAPL	NM_001007531	1.98	0.0491	-1.72	0.0872	-5	0.965
MACROD2	BC018687	1.98	0.0492	-1.66	0.0992	-674	0.963
FBF1	AK057261	1.96	0.052	-2.45	0.0153	-2522	0.972
CPS1	NM_001875	1.95	0.0522	-1.80	0.0735	-1400	0.966
ANKRD17	BC043394	1.95	0.0525	-2.00	0.0467	-2158	0.969
TMPRSS13	AY190317	1.95	0.0525	-1.76	0.0805	-900	0.965
MKS1	NM_017777	1.94	0.0538	-2.25	0.0253	-490	0.971
IL4	BC070123	1.94	0.0544	-1.94	0.0544	-51	0.968
RTKN2	BC025765	1.92	0.057	-1.74	0.0844	-1669	0.965
RAB11FIP5	BC035013	1.91	0.0582	-1.67	0.097	-196	0.964
PTCD1	BC103495	1.90	0.0589	-1.58	0.116	-92	0.962
NPHP4	AL117405	1.90	0.0596	-1.66	0.0981	-176	0.964

IER3IP1	NM_016097	1.89	0.0598	-1.83	0.069	-1033	0.967
TSPY2	NM_022573	1.89	0.0603	-1.58	0.116	-9	0.962
ANO1	BC027590	1.89	0.0608	-2.02	0.0451	-2972	0.969
PACRG	BC044227	1.89	0.0609	-1.71	0.0885	-155	0.965
KLRD1	BC028009	1.88	0.0613	-2.55	0.0115	-632	0.973
ZG16	XM_930062	1.88	0.0619	-1.70	0.0914	-64	0.964
TMEM237	BC029611	1.87	0.0628	-3.27	0.00128	-1841	0.973
HNRNPA0	NM_006805	1.87	0.0634	-3.10	0.00225	-205	0.974
METAP1	NM_015143	1.87	0.0635	-1.77	0.0788	-703	0.966
GCSH	NM_004483	1.87	0.0635	-2.02	0.0451	-315	0.969
PLA2G6	BC036742	1.86	0.0647	-2.04	0.0425	-58	0.969
FKBP11	BC048292	1.84	0.0677	-2.39	0.0181	-684	0.972
PDE6G	BX537414	1.84	0.0678	-2.50	0.0134	-1628	0.973
CYB561	NM_001017916	1.84	0.068	-2.34	0.0201	-45	0.972
FKBP11	NM_016594	1.84	0.0681	-2.39	0.0181	-674	0.972
ARHGEF28	XM_371755	1.82	0.0707	-1.57	0.118	-90	0.961
TMPRSS13	AB048796	1.82	0.0711	-1.76	0.0805	-902	0.965
PDE6B	BC000249	1.81	0.0717	-1.52	0.13	-59	0.960
GARNL3	NM_032293	1.81	0.0719	-1.95	0.0528	-30	0.968
MYPN	NM_032578	1.81	0.0719	-3.09	0.0023	-134	0.974
MAPT	AK055986	1.79	0.0749	-3.12	0.00207	-1291	0.974
MRAP	BC062721	1.79	0.0753	-2.24	0.0261	-966	0.971
PIK3R1	BC030815	1.78	0.0761	-3.76	0.000224	-62	0.973
GSG1	AK075288	1.78	0.0766	-2.18	0.0304	-282	0.971
FAM71A	NM_153606	1.77	0.079	-2.86	0.00477	-146	0.973
ITPR1	U23850	1.76	0.0806	-3.08	0.00239	-739	0.974
HES2	NM_019089	1.75	0.0821	-3.54	0.000501	-913	0.973
ENPEP	NM_001977	1.75	0.0825	-2.04	0.0431	-105	0.969
CCNL2	AY037150	1.74	0.0829	-1.66	0.0986	-7	0.963
KCNA6	NM_002235	1.74	0.0836	-1.58	0.115	-7	0.962
CCDC142	NM_032779	1.74	0.084	-1.75	0.0815	-760	0.965
ZC3H12D	XM_933939	1.73	0.0845	-1.86	0.0651	-1665	0.967
AKR1C3	NM_003739	1.73	0.0847	-3.06	0.00251	-1115	0.974
GYS1	NM_002103	1.73	0.0856	-1.77	0.0776	-1417	0.966
FBRSL1	AF490258	1.72	0.0867	-1.68	0.0947	-23	0.964

ZFYVE9	NM_007323	1.72	0.0868	-1.71	0.0896	-98	0.964
TBC1D9B	NM_015043	1.72	0.0871	-1.63	0.104	-1987	0.963
CCND3	AK055519	1.71	0.0881	-2.29	0.0233	-2196	0.971
TRPM6	AK057748	1.71	0.0887	-2.13	0.0342	-1006	0.970
IL18R1	BC093977	1.71	0.0888	-1.56	0.121	-568	0.961
LIPF	AL833751	1.71	0.0891	-1.61	0.109	-587	0.962
STPG1	NM_178122	1.70	0.0914	-2.52	0.0124	-1957	0.973
CSF2	NM_000758	1.70	0.0914	-2.35	0.0199	-133	0.972
GRHL1	NM_014552	1.70	0.0915	-2.38	0.0181	-101	0.972
GLYATL1	NM_080661	1.69	0.092	-2.44	0.0155	-456	0.972
HOXA5	NM_019102	1.69	0.0927	-1.76	0.0796	-108	0.965
APOBEC3H	NM_181773	1.69	0.0935	-1.91	0.0578	-176	0.968
SPATA2L	BC053588	1.68	0.0937	-1.93	0.0555	-33	0.968
ZBTB38	XM_929657	1.68	0.0939	-1.62	0.107	-839	0.963
OR2T5	NM_001004697	1.68	0.0944	-1.74	0.0832	-47	0.965
HIST1H2AA	NM_170745	1.68	0.0953	-1.83	0.0689	-2430	0.967
SPP2	NM_006944	1.67	0.0957	-2.62	0.00955	-398	0.973
LOC644248	XM_932073	1.67	0.0957	-1.88	0.0616	-530	0.967
EXTL3	BC006363	1.66	0.0983	-2.26	0.025	-1672	0.971
SHF	AK123658	1.65	0.0996	-1.69	0.0927	-1228	0.964
ABCC13	NM_172025	1.65	0.101	-1.68	0.0938	-132	0.964
KLHL17	NM_198317	1.65	0.102	-2.17	0.0314	-98	0.971
SFPQ	NM_005066	1.65	0.102	-2.35	0.0196	-2415	0.972
HHIP-AS1	XM_933718	1.64	0.102	-2.12	0.0355	-343	0.970
MPC2	NM_015415	1.64	0.102	-2.21	0.0285	-2715	0.971
MYBL1	XM_933460	1.64	0.103	-3.14	0.00196	-1915	0.974
LCN15	BC035124	1.64	0.103	-3.72	0.000263	-627	0.973
MMP8	BC074989	1.64	0.103	-1.86	0.0645	-832	0.967
GPR12	NM_005288	1.63	0.104	-1.88	0.0621	-2346	0.967
IKZF2	AY587063	1.63	0.104	-1.87	0.0626	-1597	0.967
AGT	BC011519	1.63	0.105	-1.72	0.0874	-183	0.965
L3MBTL2	BC017191	1.63	0.105	-1.71	0.0881	-14	0.965
TIGIT	AL833175	1.62	0.106	-1.52	0.13	-150	0.960
KLHL3	BC045683	1.62	0.106	-2.81	0.00544	-1742	0.973
SH3BGR	BC006371	1.62	0.107	-1.96	0.0518	-107	0.968

REEP4	BC050622	1.62	0.107	-2.05	0.0414	-960	0.970
C1orf86	NM_182533	1.62	0.108	-2.10	0.0368	-661	0.970
FBLN2	NM_001004019	1.61	0.108	-1.59	0.114	-175	0.962
OPN5	NM_001030051	1.60	0.111	-3.05	0.00265	-419	0.974
ILKAP	NM_030768	1.59	0.113	-3.52	0.000532	-953	0.973
CHST6	NM_021615	1.59	0.114	-1.97	0.0507	-1072	0.969
CSF2	BC108724	1.59	0.115	-2.35	0.0199	-146	0.972
H2AFX	NM_002105	1.58	0.115	-2.39	0.0179	-704	0.972
RIPK1	NM_003804	1.58	0.115	-1.72	0.0869	-17	0.965
IVL	NM_005547	1.58	0.115	-1.81	0.0716	-715	0.966
ZNF423	BC112315	1.58	0.116	-1.50	0.134	-2780	0.959
STMN3	BC009381	1.58	0.117	-1.83	0.0686	-526	0.967
C9orf40	BC039588	1.57	0.119	-1.66	0.0995	-68	0.963
C10orf62	NM_001009997	1.57	0.119	-3.07	0.00243	-89	0.974
ANKRD17	NM_032217	1.56	0.121	-2.00	0.0467	-1253	0.969
DIXDC1	BC064479	1.56	0.122	-1.74	0.0836	-7	0.965
WDR66	NM_144668	1.55	0.122	-1.60	0.112	-83	0.962
LOX	BC074820	1.55	0.122	-1.81	0.0721	-1189	0.966
KRTAP10-10	NM_181688	1.55	0.123	-1.72	0.0878	-191	0.965
DERL1	BC050691	1.54	0.124	-2.55	0.0115	-389	0.973
TMEM182	BC020898	1.54	0.124	-3.30	0.00114	-183	0.973
DHRS11	AB209247	1.54	0.124	-1.77	0.0789	-1291	0.966
CCSER1	NM_207491	1.54	0.125	-2.02	0.0448	-27	0.969
CFB	NM_001710	1.54	0.125	-2.15	0.0324	-3	0.970
LOXL3	NM_032603	1.54	0.126	-2.82	0.0053	-1866	0.973
PHLDB1	AK074070	1.53	0.127	-1.59	0.115	-29	0.962
ADAM32	BC026085	1.53	0.127	-1.69	0.0929	-12	0.964
ZNF764	NM_033410	1.53	0.127	-2.25	0.0259	-1571	0.971
POLR1A	NM_015425	1.52	0.129	-1.60	0.112	-2163	0.962
CCDC142	AK027303	1.52	0.129	-1.75	0.0815	-448	0.965
AGMO	BC108676	1.52	0.13	-2.66	0.00841	-688	0.973
FURIN	BC012181	1.52	0.13	-1.53	0.129	-2804	0.960
TXNDC2	NM_032243	1.51	0.132	-1.88	0.0619	-5	0.967
OR7A5	BC104809	1.51	0.133	-1.91	0.0572	-542	0.968
ZNF321P	NM_203307	1.51	0.133	-1.68	0.0955	-1383	0.964

GALR1	BC095530	1.51	0.134	-1.67	0.0962	-136	0.964
FAM183A	XM_933723	1.51	0.134	-1.84	0.0667	-331	0.967
FBLN2	BC051690	1.50	0.135	-1.59	0.114	-212	0.962
LRP11	BC025239	1.50	0.135	-1.58	0.117	-202	0.961
(B)		Expression		Methylation			
Gene	ID	t statistic	p value	t statistic	p value	Distance to Gene	Posterior Probability
SERPINA5	NM_000624	-1.51	0.134	-1.69	0.0932	-91	0.956
CHCHD3	NM_017812	-1.51	0.133	-2.09	0.0379	-1440	0.963
PREPL	BC013193	-1.51	0.132	-1.91	0.0572	-1112	0.961
AXL	BC032229	-1.52	0.13	-1.74	0.083	-80	0.958
GZMK	NM_002104	-1.52	0.13	-1.63	0.106	-111	0.955
HSPA6	BC035665	-1.52	0.13	-1.61	0.109	-230	0.954
ZNF274	BX641169	-1.53	0.128	-1.56	0.121	-1141	0.953
SEMA3G	BC098104	-1.53	0.127	-2.31	0.0217	-278	0.966
CLMN	NM_024734	-1.54	0.126	-1.97	0.0499	-920	0.962
PER3	NM_016831	-1.54	0.125	-2.06	0.0404	-28	0.963
MGAM	NM_004668	-1.54	0.125	-2.43	0.0158	-56	0.966
ZNF107	NM_001013746	-1.54	0.125	-2.38	0.0183	-41	0.966
EVI2A	NM_001003927	-1.56	0.121	-1.51	0.132	-511	0.951
PERP	NM_022121	-1.56	0.12	-2.51	0.0131	-2963	0.967
TMX4	BC033787	-1.56	0.119	-1.94	0.0539	-173	0.961
ZC3HAV1L	NM_080660	-1.58	0.117	-2.43	0.0162	-1575	0.966
TMEM168	BC030804	-1.59	0.115	-1.59	0.113	-77	0.954
PREPL	DQ023506	-1.59	0.114	-1.91	0.0572	-900	0.961
PTPRC	BC017863	-1.59	0.114	-1.52	0.13	-1551	0.952
MPC1L	XM_293325	-1.59	0.113	-2.61	0.00968	-232	0.967
BAIAP2- AS1	NM_001004336	-1.60	0.111	-1.78	0.0775	-1959	0.958
IL19	NM_153758	-1.62	0.107	-1.79	0.0743	-85	0.959
ALS2	NM_020919	-1.63	0.106	-1.54	0.125	-237	0.952
B3GALT5	BC104862	-1.63	0.105	-1.59	0.112	-2896	0.954
HYAL1	BC025774	-1.63	0.105	-1.53	0.128	-20	0.952
SLC25A25	BC103930	-1.63	0.104	-2.38	0.0183	-1213	0.966
LGALS1	NM_002305	-1.63	0.104	-1.52	0.129	-36	0.952
KCNJ15	BC013327	-1.64	0.102	-2.53	0.0123	-437	0.967

NOL9	BC105095	-1.67	0.0972	-1.52	0.13	-1990	0.951
NHLRC1	NM_198586	-1.67	0.0968	-2.07	0.0396	-2322	0.963
VWA3B	AK093084	-1.67	0.096	-1.61	0.11	-199	0.954
CNGA4	XM_927349	-1.68	0.0955	-2.18	0.0308	-2401	0.964
LAMTOR1	NM_017907	-1.68	0.0943	-2.20	0.0289	-1763	0.965
TIMP2	NM_003255	-1.68	0.0942	-1.62	0.106	-29	0.955
ESRRA	BC093720	-1.70	0.0908	-1.79	0.0743	-503	0.959
ANKRD44	BC016985	-1.70	0.0907	-1.56	0.121	-2285	0.953
CHN1	NM_001025201	-1.70	0.0901	-1.65	0.101	-2531	0.955
CRHR1-IT1	NM_152466	-1.71	0.0881	-1.68	0.0941	-1031	0.956
ACTL9	NM_178525	-1.72	0.0869	-1.71	0.0882	-1879	0.957
GUCY1A3	NM_000856	-1.72	0.0866	-2.61	0.00982	-1168	0.967
LRRC3	NM_030891	-1.74	0.0837	-1.55	0.122	-270	0.952
CAPZB	NM_004930	-1.74	0.0831	-1.80	0.074	-7	0.959
DDI1	NM_001001711	-1.76	0.0803	-2.76	0.00638	-66	0.967
TEKT4	NM_144705	-1.77	0.0783	-2.00	0.047	-22	0.962
NME6	NM_005793	-1.78	0.0766	-2.53	0.0121	-1163	0.967
MITF	BC065243	-1.81	0.0715	-1.70	0.0909	-201	0.957
ZEB1	AK091478	-1.82	0.0709	-2.10	0.0374	-5	0.963
H2AFZ	NM_002106	-1.82	0.07	-1.55	0.123	-496	0.952
MOSPD2	NM_152581	-1.83	0.0695	-1.85	0.0662	-110	0.960
GPIHBP1	NM_178172	-1.83	0.0682	-1.66	0.0983	-9	0.956
FKRP	BC002612	-1.87	0.0627	-1.76	0.0802	-1262	0.958
SH3BGRL3	NM_031286	-1.87	0.0626	-1.66	0.0981	-334	0.956
CEL	NM_001807	-1.90	0.0586	-1.51	0.133	-464	0.951
HMGB1	BC066889	-1.91	0.0581	-2.58	0.0106	-1434	0.967
HIATL1	NM_032558	-1.92	0.0562	-1.74	0.0832	-20	0.958
CYP46A1	AK090886	-1.94	0.0539	-1.86	0.0648	-1170	0.960
CNFN	BC101197	-1.95	0.0523	-1.63	0.105	-2274	0.955
RPL4	NM_000968	-1.96	0.0516	-1.55	0.124	-218	0.952
FXN	BC023633	-1.98	0.0497	-2.01	0.0458	-340	0.962
CCNJL	NM_024565	-1.98	0.0489	-1.83	0.069	-46	0.959
CLIC5	AK097048	-1.99	0.0484	-1.65	0.102	-1726	0.955
BTNL3	BC100800	-2.06	0.0403	-1.97	0.0499	-21	0.962
CEL	BC042510	-2.08	0.0386	-1.51	0.133	-479	0.951

SRP14	NM_003134	-2.10	0.0369	-1.84	0.068	-213	0.960
PSCA	BC023582	-2.11	0.0361	-3.08	0.00237	-113	0.968
RPS4X	BC100903	-2.11	0.036	-3.05	0.00259	-40	0.968
BTNL3	NM_006707	-2.14	0.034	-1.56	0.12	-995	0.953
ZEB1	NM_030751	-2.15	0.0331	-2.10	0.0374	-6	0.964
L3MBTL4	AK093255	-2.15	0.0325	-3.16	0.00185	-150	0.968
FLJ26850	NM_001001687	-2.20	0.029	-1.58	0.115	-138	0.954
SOX15	BC072003	-2.32	0.0215	-1.55	0.123	-1268	0.953
PTPRC	NM_080923	-2.35	0.0196	-1.52	0.13	-1550	0.952
IL13RA1	NM_001560	-2.37	0.0188	-1.66	0.0976	-146	0.956
FNIP1	AB075841	-2.39	0.0179	-2.50	0.0132	-854	0.967
FUT3	BC108675	-2.43	0.0162	-2.37	0.0186	-2103	0.966
CYP4F2	BC067437	-2.44	0.0157	-3.85	0.000161	-398	0.967
SLC44A1	BC049203	-2.46	0.0147	-1.73	0.0849	-17	0.958
HYAL1	NM_007312	-2.48	0.0141	-1.53	0.128	-16	0.952
LRRC18	AY358137	-2.50	0.0132	-1.53	0.129	-1044	0.952
NGFRAP1	NM_206915	-2.67	0.00821	-1.53	0.127	-191	0.952
IL13RA1	BC009960	-2.85	0.00486	-1.66	0.0976	-159	0.956
IL13RA1	U81380	-3.17	0.00177	-1.66	0.0976	-152	0.957

Supplemental Table 11. Internal (A) and external (B) validation of Illumina methylation array data by pyrosequencing analysis of bisulfite-converted DNA. Mean methylation changes, with standard deviations (SD), methylation differences, and one-sided t-test p values between cases and controls and for individual CpGs that overlap Illumina 450k CpGs within asthma DMRs (Supplemental Table 3) or methylation/expression changes (Supplemental Table 9). Significant changes ($p < 0.05$) are indicated in bold.

(A)

Illumina 450k asthma Associations				Illumina 450k Individual Probe Data					Derivation Cohort Pyrosequencing			
DMR position	Nearest Gene	DMR SLK-sidak p	LCMix Posterior Probability	Chr	CpG base pair position	Mean Cases	Mean Controls	Difference (cases-controls)	Mean Cases (SD)	Mean Controls (SD)	Difference (cases-controls)	One-tailed ttest p value
chr1:25113451-25113525	RUNX3	0.001069	0.974	1	25113452	84.7	86.0	-1.4	89.3 (6.8)	91.9 (6.9)	-2.6	5.49E-03
				1	25113519	83.6	86.0	-2.4	82.9 (6.0)	85.6 (6.0)	-2.8	9.42E-04
				1	25113525	91.5	92.9	-1.4	80.9 (5.8)	83.2 (5.0)	-2.3	1.99E-03
chr3:115495348-115495602	TIGIT	0.03144	0.973	3	115495348	79.1	80.1	-1.0	58.7 (5.5)	62.1 (3.9)	-3.4	8.62E-03
chr5:132021512-132021960	IL-13	5.79E-05	0.97	5	132021752	87.4	88.8	-1.4	85.6 (3.8)	86.9 (3.2)	-1.3	4.15E-03
NA	IL-4	NA	0.968	5	132037528	95.9	96.3	-0.4	82.0(4.3)	83.2(3.1)	-1.2	0.0263
chr10:72030353-72030454	PRF1	6.08E-04	NA	10	72030353	91.7	92.7	-1.0	84.5 (3.7)	85.7 (3.5)	-1.2	0.0198
chr11:34416873-34417133	CAT	0.00147	0.972	11	34416961	5.8	7.2	-1.4	3.2 (1.6)	3.7 (2.0)	-0.5	0.0211
				11	34417091	3.1	4.2	-1.1	2.1 (1.6)	2.6 (1.9)	-0.5	0.0283
chr1:21773573-21773847	ALPL	6.68E-03	NA	1	21773846	44.9	50.3	-5.4	11.5 (3.1)	13.1 (3.9)	-1.7	4.20E-04
chr6:31975676-31976004	C2; ZBTB12	5.79E-05	NA	6	31975825	75.1	79.1	-4.0	50.0 (4.6)	51.6 (4.0)	-1.6	9.70E-03
chr10:3813757-3814016	KLF6	5.79E-05	0.957	10	3813789	54.9	59.1	-4.2	34.0 (6.9)	36.4 (7.9)	-2.4	0.016
chr17:38531660-38531955	NBR2	2.62E-05	NA	17	38531866	74.3	79.1	-4.8	81.1 (6.8)	83.3 (6.5)	-2.2	0.0134

(B)

Illumina 450k asthma Associations				Illumina 450k Individual Probe Data					Replication Cohort Pyrosequencing			
DMR position	Nearest Gene	DMR SLK-sidak p	LCMix Posterior Probability	Chr	CpG base pair position	Mean Cases	Mean Controls	Difference (cases-controls)	Mean Cases (SD)	Mean Controls (SD)	Difference (cases-controls)	One-tailed ttest p value
chr1:25113451-25113525	RUNX3	0.001069	0.974	1	25113452	84.7	86.0	-1.4	82.0(7.9)	85.7(6.4)	-3.7	3.76E-04
				1	25113519	83.6	86.0	-2.4	77.4(6.4)	80.5(4.8)	-3.1	2.04E-04
				1	25113525	91.5	92.9	-1.4	75.5(7.0)	78.7(5.9)	-3.2	5.74E-04
chr3:115495348-115495602	TIGIT	0.03144	0.973	3	115495348	79.1	80.1	-1.0	61.2(5.3)	59.3(4.8)	1.8	8.72E-03
chr5:132021512-132021960	IL-13	5.79E-05	0.97	5	132021752	87.4	88.8	-1.4	83.4(3.0)	83.5(3.3)	-0.1	0.429
NA	IL-4	NA	0.968	5	132037528	95.9	96.3	-0.4	82.2(3.8)	83.2(3.1)	-1.0	0.036
chr10:72030353-72030454	PRF1	6.08E-04	NA	10	72030353	91.7	92.7	-1.0	86.2(3.0)	86.4(3.8)	-0.2	0.353
chr11:34416873-34417133	CAT	0.00147	0.972	11	34416961	5.8	7.2	-1.4	2.6(0.9)	3.7(1.8)	-1.1	1.94E-07
				11	34417091	3.1	4.2	-1.1	0.8(0.85)	1.5(1.6)	-0.7	1.76E-04
chr1:21773573-21773847	ALPL	6.68E-03	NA	1	21773846	44.9	50.3	-5.4	11.1(3.4)	11.8(3.0)	-0.6	0.095
chr6:31975676-31976004	C2; ZBTB12	5.79E-05	NA	6	31975825	75.1	79.1	-4.0	49.0(3.7)	49.3(3.4)	-0.25	0.321
chr10:3813757-3814016	KLF6	5.79E-05	0.957	10	3813789	54.9	59.1	-4.2	31.9(7.4)	33.7(6.7)	-1.8	0.045
chr17:38531660-38531955	NBR2	2.62E-05	NA	17	38531866	74.3	79.1	-4.8	78.9(7.8)	83.5(7.9)	-4.6	6.79E-05

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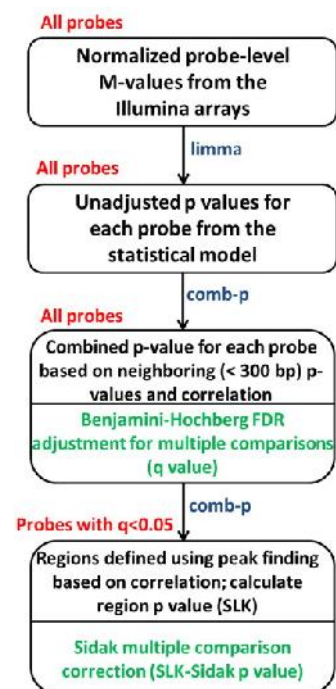


Fig E1

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DNA methylation and childhood asthma in the inner city

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2015.01.025>

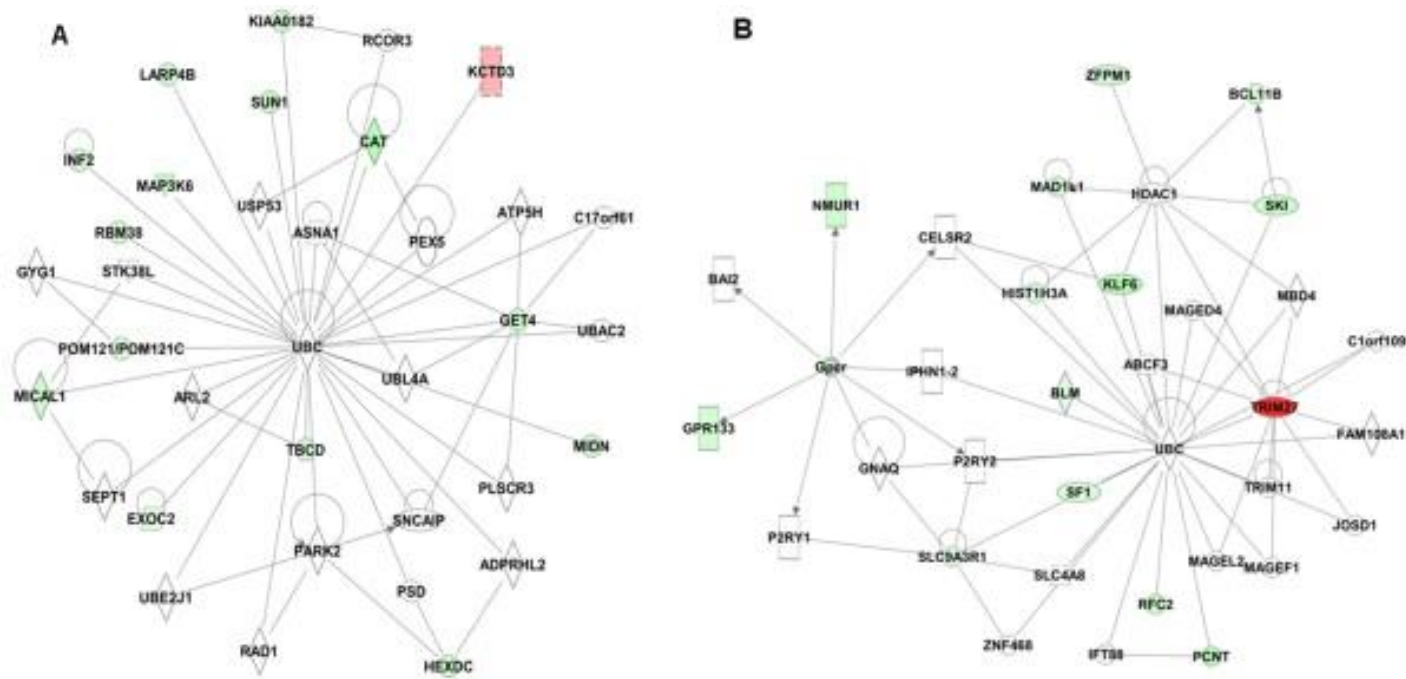


Fig E2

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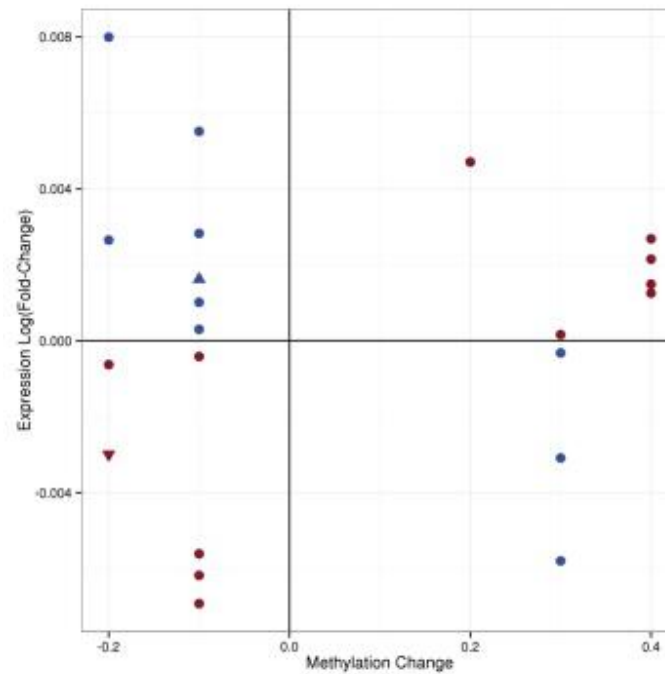


Fig E4

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