

The nasal methylome and childhood atopic asthma

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Background: Given the strong environmental influence on both epigenetic marks and allergic asthma in children, the epigenetic alterations in respiratory epithelia might provide insight into allergic asthma.

Objective: We sought to identify DNA methylation and gene expression changes associated with childhood allergic persistent asthma.

Methods: We compared genomic DNA methylation patterns and gene expression in African American children with persistent atopic asthma (n = 36) versus healthy control subjects (n = 36). Results were validated in an independent population of asthmatic children (n = 30) by using a shared healthy control population (n = 36) and in an independent population of white adult atopic asthmatic patients (n = 12) and control subjects (n = 12).

Results: We identified 186 genes with significant methylation changes, differentially methylated regions or differentially methylated probes, after adjustment for age, sex, race/ethnicity, batch effects, inflation, and multiple comparisons. Genes differentially methylated included those with established roles in asthma and atopy and genes related to extracellular matrix, immunity, cell adhesion, epigenetic regulation, and airflow obstruction. The methylation changes were substantial (median, 9.5%; range, 2.6% to 29.5%). Hypomethylated and hypermethylated genes were associated with increased and decreased gene expression, respectively ($P < 2.8 \times 10^{-6}$ for

differentially methylated regions and $P < 7.8 \times 10^{-10}$ for differentially methylated probes). Quantitative analysis in 53 differentially expressed genes demonstrated that 32 (60%) have significant methylation-expression relationships within 5 kb of the gene. Ten loci selected based on the relevance to asthma, magnitude of methylation change, and methylation-expression relationships were validated in an independent cohort of children with atopic asthma. Sixty-seven of 186 genes also have significant asthma-associated methylation changes in nasal epithelia of adult white asthmatic patients.

Conclusions: Epigenetic marks in respiratory epithelia are associated with allergic asthma and gene expression changes in inner-city children. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: DNA methylation, gene expression, microarray, atopic asthma, respiratory epithelia, epigenetic regulation, inner city

The increase in the prevalence, incidence, and severity of asthma over the last 20 years¹ provides strong evidence that exposures play an important role in this disease. Although common genetic variants explain only a small portion of asthma heritability,² epigenetic changes could potentially explain both the non-Mendelian³ and parent-of-origin⁴ patterns of inheritance that are

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Abbreviations used

ALOX15: Arachidonate 15-lipoxygenase

CAPN14: Calpain 14

DMP: Differentially methylated probe

DMR: Differentially methylated region

POSTN: Periostin

characteristic of asthma. Additionally, epigenetic marks can be influenced by the environment⁵; these marks have been shown to affect the expression of transcription factors that alter the maturation of T lymphocytes,^{6–8} and we have demonstrated a causal relationship between DNA methylation and both T_H2 immunity and allergic airway disease in mice.⁹

Recently, we have shown that DNA methylation marks in PBMCs are associated with allergic asthma¹⁰ and account for 13.5% of the variation in serum IgE concentrations.¹¹ However, the airway epithelium is the primary interface with the environment, interacts with allergens¹² and other environmental stimuli,¹³ and represents a potentially important mediator of allergic airway disease. Gene expression profiles of the airway epithelium in asthmatic patients have identified genes associated with exposure to endotoxin and house dust mite allergen,¹⁴ as well as cigarette smoke,¹⁵ asthma,¹⁵ and T_H2-high versus T_H2-low subphenotypes of disease.¹⁶ More recently, it has been demonstrated that gene expression in nasal epithelia is a proxy measure for gene expression of the lower airway epithelium.¹⁷ However, to date, no study has comprehensively characterized genome-wide DNA methylation patterns and associated changes in gene expression in nasal epithelia of patients with atopic asthma.

METHODS

Study populations

Our primary study population consisted of inner-city children aged 10 to 12 years with both atopy and persistent asthma (cases, $n = 36$) or without atopy or asthma (healthy control subjects, $n = 36$).¹⁰ Study subjects were recruited at 6 sites supported by the Inner City Asthma Consortium from census tracts that contain at least 20% of households at less than the US government poverty level (see Table E1 in this article's Online Repository at www.jacionline.org). We limited our study population to 36 cases and 36 control subjects with at least 80% ciliated epithelial cells visualized from slides obtained from nasal brushings and by means of expression of the *FOXJ1* gene (see Table E2 in this article's Online Repository at www.jacionline.org). The validation population consisted of 30 African American subjects aged 10 to 12 years with atopic asthma by using the same definition as the derivation cohort collected by the Inner City Asthma Consortium independent of the primary study population. The original 36 control samples were used in the validation analysis and will be referred to as shared control samples. The second validation population consisted of 24 white adults (age range, 24–74 years), 12 with and 12 without asthma, all with at least 80% ciliated epithelial cells visualized from slides obtained from nasal brushings recruited at National Jewish Health. In this cohort all asthmatic patients were also atopic, as assessed based on positive skin prick test responses for multiple allergens or RAST/Phadiatop tests. Control subjects were required to have IgE measurements less than 100 but did not have additional allergy testing performed.

DNA methylation and gene expression data collection

DNA methylation was measured on Illumina's Infinium Human Methylation 450k BeadChip (Illumina, San Diego, Calif) and validated internally and externally by using pyrosequencing with custom-designed primers

(see Table E3 in this article's Online Repository at www.jacionline.org). Gene expression was assessed on Agilent Human Gene Expression arrays (G3 SurePrint 8x60k; Agilent Technologies, Santa Clara, Calif). DNA methylation and gene expression array data have been deposited in the Gene Expression Omnibus (GSE65205).

Overview of statistical analyses

The goal of our analyses was to determine whether DNA methylation and gene expression changes in nasal epithelia are associated with atopic asthma. Overall study design and workflow are presented schematically in Fig E1 in this article's Online Repository at www.jacionline.org. We identified DNA methylation changes associated with asthma for both single CpG motifs (differentially methylated probes [DMPs]) and differentially methylated regions (DMRs). Although identification of DMPs is the most commonly used method for identification of methylation changes in Illumina arrays,¹⁸ our rationale for identification of DMRs is 3-fold: (1) identification of regions is conceptually consistent with what is known about DNA methylation patterns in the human genome¹⁹; (2) it increases power to detect associations²⁰; and (3) it has been used in other diseases.^{21,22} We also performed 2 exploratory analyses: (1) to determine whether any of the DMRs or DMPs identified in nasal epithelia were associated with nasal corticosteroid use among asthmatic patients and (2) to test whether any of the 81 DMRs we previously identified as associated with asthma in PBMCs¹⁰ are also associated with asthma in nasal epithelia.

Statistical analyses of DNA methylation data

Data from the methylation array were normalized by using the SWAN method (Illumina),²³ and the normalized M values were used in all downstream analyses, whereas β -values on the scale of 0% to 100% are used for tables and figures. Differences between cases and control subjects are reported as percentage methylation changes by using β -values. We filtered out probes on the 450k Illumina array with known single nucleotide polymorphisms in European (CEU) and African (YRI) populations within the CpG motif.

We performed the analysis to identify differential methylation in 3 steps. In brief, these are as follows: (1) infer probabilistic estimation of expression residuals factors to account for unobserved batch effects²⁴; (2) fit linear models with limma²⁵; and (3) use comb-p²⁶ with a window size of 300 bp to identify regions of sustained low P values or DMRs from the P values reported by using limma. DMPs were identified by calculating q values, corresponding to false discovery rate, from linear model P values by using the method of Benjamini and Hochberg.²⁷ After examination of quantile-quantile plots, we performed global adjustment for inflation of P values using standard methodology. Details of the analyses are outlined in the diagram in Fig E2 and the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analyses of gene expression data

The R package limma²⁵ was used to background correct, normalize (quantile), and fit linear models for expression data. P values were based on the moderated t statistic, and q values were calculated from P values by using the method of Benjamini and Hochberg.²⁷

Analysis of DNA methylation and gene expression

To understand the relationship of DMRs with gene expression changes, we considered inversely correlated (canonical) versus positively correlated pairs, limiting the analysis to genes within 5 kb of a DMR. We calculated the enrichment of inversely correlated pairs in relation to all pairs by using the binomial test. To integrate the expression and methylation data, we used a method derived from comb-p,²⁶ except instead of testing each methylation site for its relation to asthma status, we tested methylation regions and expression probes within 5 kb.

Additional methods are available in the Methods section in this article's Online Repository.

TABLE I. Demographic and clinical characteristics of asthmatic patients and control subjects*

	Patients with allergic asthma (n = 36)	Control subjects (n = 36)	P value†	Replication allergic asthma population (n = 30)
Site				
Boston	8 (22.2%)	15 (41.7%)	.07‡	—
Dallas	4 (11.1%)	1 (2.8%)		6 (20.0%)
Denver	7 (19.4%)	3 (8.3%)		—
Detroit	4 (11.1%)	1 (2.8%)		—
New York	6 (16.7%)	3 (8.3%)		3 (10.0%)
Washington, DC	7 (19.4%)	13 (36.1%)		—
Chicago	—	—		7 (23.3%)
Cincinnati	—	—		14 (46.7%)
Age at recruitment (y)	11.0 (10.0-12.0)	11.0 (10.0-12.0)	.52§	11.0 (10.0-12.0)
Male sex	19 (52.8%)	17 (47.2%)	.64‡	19 (63.3%)
Participant race: African American (yes)	33 (91.7%)	33 (91.7%)	>.99‡	30 (100.0%)
Participant race: Hispanic or Latino (yes)	7 (19.4%)	3 (8.3%)	.17‡	0 (0.0%)
How often exposed to smokers: daily	10 (27.8%)	8 (22.2%)	.59‡	8 (26.7%)
Dog living in the home in the last 6 mo (yes)	11 (30.6%)	13 (36.1%)	.62‡	9 (30.0%)
Cat living in the home in the last 6 mo (yes)	9 (25.0%)	12 (33.3%)	.44‡	3 (10.0%)
Any water/dampness in the last 12 mo (yes)	9 (25.0%)	4 (11.1%)	.13‡	10 (33.3%)
Gas stove, gas range, or gas oven (yes)	22 (61.1%)	24 (66.7%)	.62‡	12 (40.0%)
Allergic to any indoor allergen (yes)	36 (100%)	0 (0%)	¶	30 (100%)
Cockroach IgE (IU/mL)	0.34 (0.34-1.53)	0.34 (0.34-0.34)	¶	0.44 (0.34-25.2)
Total serum IgE (kU/L)	366.0 (185.0-785.0)	29.0 (16.5-49.5)	¶	519.5 (181.0-883.0)
Baseline FEV ₁ (% predicted)	88.8 ± 17.5	105.4 ± 11.2	¶	97.3 ± 19.3
Baseline FEV ₁ /FVC ratio	72.6 ± 10.1	84.6 ± 6.4	¶	79.6 ± 8.1
Asthma medications				
Albuterol	34 (94.4%)	0 (0%)	, ¶	30 (100.0%)
Inhaled steroids	29 (80.6%)	0 (0%)		22 (73.3%)
Nasal steroids	9 (25%)	0 (0%)		25 (83.3%)
Montelukast	15 (41.7%)	0 (0%)		11 (36.7%)

FVC, Forced vital capacity.

*Data are presented as means ± SDs, medians (interquartile ranges), or numbers (percentages).

†P values were calculated by using the χ^2 test (§) for categorical variables and the Mann-Whitney test (§) for continuous variables.

||Medication data were missing on 14 control subjects.

¶By protocol design.

RESULTS

There were no significant demographic differences between patients with allergic asthma (n = 36) and control subjects (n = 36), although collection site approached statistical significance (Table I). Per study design, all patients with allergic asthma were atopic based on positive skin prick test responses to at least 1 indoor allergen (data not shown) and total IgE concentrations and had airflow limitation, as indicated by spirometric measurements (Table I).

After performing global adjustment for inflation, removing probes with known European and African single nucleotide polymorphisms at the CpG motif, and adjusting for age, sex, race/ethnicity, and unknown batch effects, we identified 119 genome-wide significant DMRs associated with 118 unique genes (Fig 1, A, and Table II and see Table E4 in this article's Online Repository at www.jacionline.org) and 118 single CpG motifs (DMPs) associated with 107 unique genes (Fig 1, B, and Table II and see Table E5 in this article's Online Repository at www.jacionline.org). The median percentage methylation change between allergic asthmatic patients and control subjects was 6.8% (range, 2.6% to 28.8%) for DMRs and 13.6% (range, 5.2% to 29.5%) for DMPs. Thirty-nine genes were identified in both the DMR and DMP analysis, but a number of genes only have DMPs or DMRs because these 2 analytic approaches are complementary. Some genes/loci with a highly significant CpG (DMP) will not be represented in the DMR analysis because there are

no neighboring CpGs with low P values because the Illumina array does not interrogate all CpGs; sequence data would be needed to examine this further. On the other hand, DMR analysis identifies regions of sustained low P values in multiple CpG sites at which coverage on the Illumina array is available, but not all of these loci have 1 highly significant CpG (DMP). Representative histograms of percentage methylation estimates (β -values) in patients with allergic asthma and control subjects are shown in Fig E3 in this article's Online Repository at www.jacionline.org.

Among the 186 (DMR, DMP, or both) allergic asthma-associated differentially methylated genes in the nasal epithelia are genes with established roles in asthma and atopy: arachidonate 15-lipoxygenase (*ALOX15*), calpain 14 (*CAPN14*), histamine N-methyltransferase (*HNMT*), and periostin (*POSTN*; Fig 1, C). Moreover, components of the extracellular matrix (*COL16A1*, *COL5A2*, *COL5A3*, *ELN*, *HAS3*, and *MMP14*) and genes related to immunity (*IFNGR2*, *HLKA-DPA1*, *LAG3*, *NFIL3*, *PRF1*, and *TNFSF13*), cell adhesion (*CTNND1*, *EPPK1*, and *GJA4*), epigenetic regulation (*ATXN7L1*, *HIF0*, *HIST1H1D*, and *METTL1*), airway obstruction (*GABRG3*), obesity (*C1QTNF1* and *GPC4*), and autophagy (*AMBRA1*) are also differentially methylated in nasal epithelia of patients with atopic asthma compared with control subjects (Fig 1, C).

None of the allergic asthma-associated DMRs/DMPs were significantly associated with nasal corticosteroid use among asthmatic patients after adjustment for multiple comparisons.

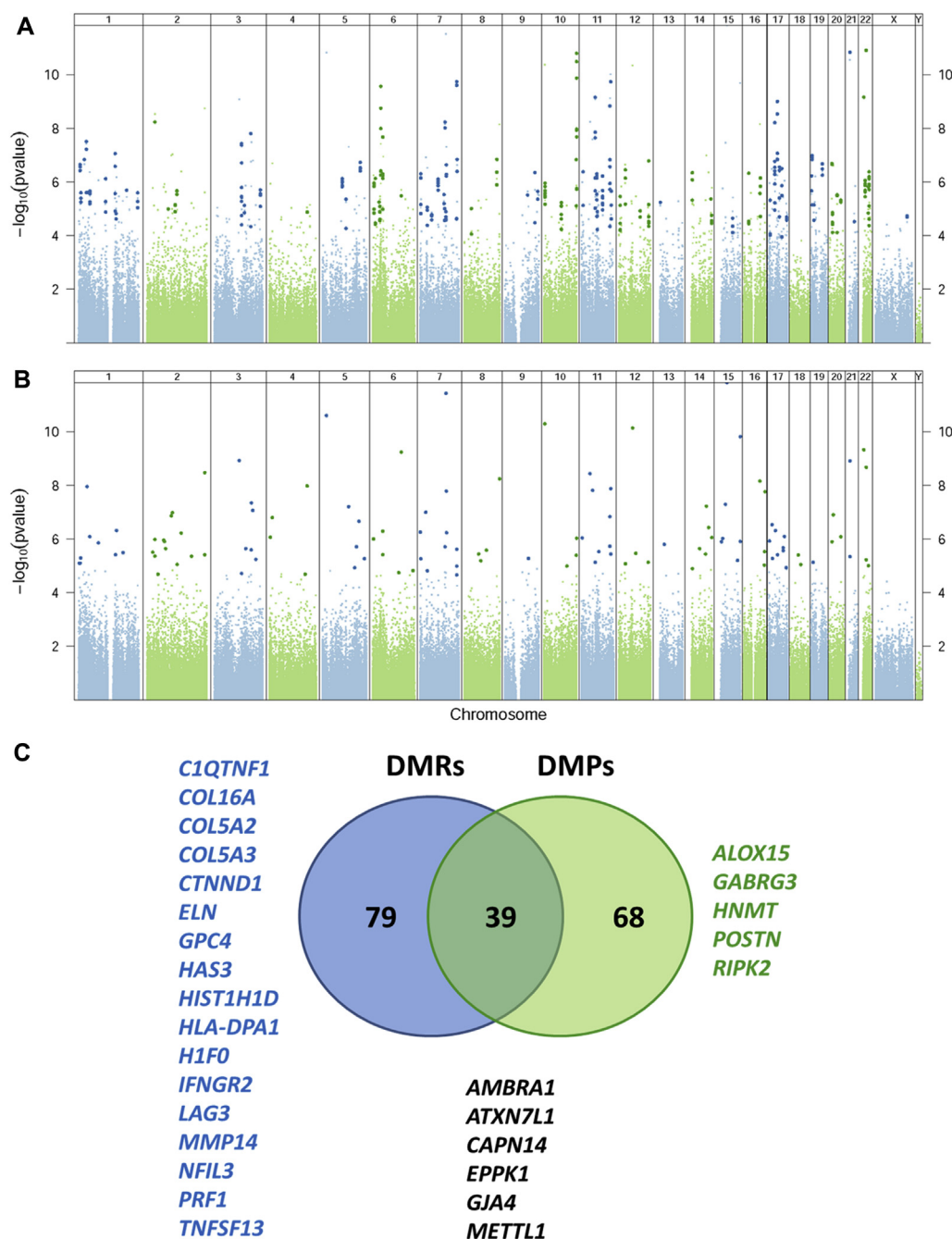


FIG 1. DMRs (Fig 1, A) and differentially methylated single-CpG probes (DMPs; Fig 1, B) in nasal epithelia are associated with asthma after controlling for age, sex, race/ethnicity, technical variables, and batch effects. **A**, Manhattan plot of adjusted P values for disease status (asthma/control) 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 after adjustment for genome-wide comparisons are identified by *darker larger symbols*. **B**, Manhattan plot of the false discovery rate-adjusted P values (q values) for disease status (asthma/control) from the linear model. Probes with q values of less than .05 are highlighted by *darker larger symbols*. **C**, Venn diagram depicting gene-based overlap of DMRs and DMPs. Representative genes of clinical/biological relevance to asthma that are discussed in the text are highlighted.

Moreover, the small degree of overlap in allergic asthma-associated epigenetic changes in PBMCs and nasal epithelia (3 of the 81 PBMC DMRs are significant in nasal epithelia at $q < 0.05$, see Table E6 in this article's Online Repository at www.jacionline.org) is not surprising given that PBMCs are a surrogate

for an asthma-associated immune response, whereas nasal epithelia are directly exposed to the environment that might influence DNA marks and the course of asthma. Moreover, the nasal epithelium represents a more pure cell population than PBMCs, and some of the methylation changes present in specific

TABLE II. Ten DMRs and 10 DMPs with the most pronounced DNA methylation changes in patients with allergic asthma compared with control subjects

Type	Chromosome	Start	End	Adjusted <i>P</i> value	Methylation difference (%)	Nearest gene	Gene distance	CpG island distance
DMR	chr11	36,030,085	36,030,086	.002895	−28.8%	<i>LDLRAD3</i>	0	63,631
DMR	chr7	1.06E+08	1.06E+08	1.32E-06	−27.3%	<i>ATXN7L1</i>	−4,084	3,152
DMR	chr12	58,162,286	58,162,287	2.12E-05	−27.0%	<i>METTL1</i>	63	2,286
DMR	chr10	4,386,801	4,386,802	2.28E-05	−26.1%	<i>LINC00703</i>	−39,635	−481,323
DMR	chr15	1.02E+08	1.02E+08	9.70E-05	−25.1%	<i>PCSK6</i>	0	51,207
DMR	chr22	19,471,092	19,471,093	4.13E-04	−24.4%	<i>CDC45</i>	0	3,281
DMR	chr3	1.02E+08	1.02E+08	3.78E-04	−23.2%	<i>LOC152225</i>	177,435	325,159
DMR	chr15	39,544,143	39,544,144	.01364	−22.9%	<i>C15orf54</i>	0	−328,383
DMR	chr15	45,449,436	45,449,437	6.83E-07	−22.7%	<i>DUOX1</i>	0	4,731
DMR	chr8	1.45E+08	1.45E+08	.003634	−21.5%	<i>EPPK1</i>	−17,562	3,314
DMP	chr16	88,558,222	88,558,223	1.43E-05	−29.5%	<i>ZFPM1</i>	0	0
DMP	chr11	36,030,085	36,030,086	6.23E-06	−28.8%	<i>LDLRAD3</i>	0	63,631
DMP	chr7	1.06E+08	1.06E+08	4.09E-09	−27.3%	<i>ATXN7L1</i>	−4,084	3,152
DMP	chr12	58,162,286	58,162,287	5.03E-08	−27.0%	<i>METTL1</i>	63	2,286
DMP	chr10	4,386,801	4,386,802	5.03E-08	−26.1%	<i>LINC00703</i>	−39,635	−481,323
DMP	chr15	1.02E+08	1.02E+08	2.39E-07	−25.1%	<i>PCSK6</i>	0	51,207
DMP	chr22	19,471,092	19,471,093	8.17E-07	−24.4%	<i>CDC45</i>	0	3,281
DMP	chr6	2,977,292	2,977,293	4.99E-04	−24.1%	<i>SERPINB6</i>	−4,893	5,330
DMP	chr3	1.02E+08	1.02E+08	8.15E-07	−23.2%	<i>LOC152225</i>	177,435	325,159
DMP	chr15	39,544,143	39,544,144	2.62E-05	−22.9%	<i>C15orf54</i>	0	−328,383

mononuclear cell subsets, such as T_H2 T-cell subsets, for example, might be diluted by the presence of other cell types. The 3 DMRs that are present in both cell types might be associated with genetic variants (methylation quantitative trait loci).²⁸

We also performed an enrichment analysis of methylation marks within areas of open chromatin, as assessed by means of DNase I hypersensitivity site analysis, in cell lines profiled by ENCODE.²⁹ This analysis identified the strongest enrichment of allergic asthma–associated DNA methylation changes in DNase I hypersensitivity sites in epithelial cells ($q < 6.57 \times 10^{-11}$), suggesting that our analysis identified DNA methylation changes in open areas of chromatin in the cell type of interest (see Fig E4 in this article's Online Repository at www.jacionline.org).

To begin to explore the influence of environmental exposures on the nasal epithelial epigenome, we identified 48 DMRs that are significantly associated with environmental tobacco smoke (ETS) after adjusting for age, sex, race/ethnicity, unknown batch effects, and allergic asthma. Forty-eight DMRs are in 46 unique genes (see Table E7 in this article's Online Repository at www.jacionline.org), one of which (*SFRP2*) overlaps with the analysis of direct cigarette smoke exposure on methylation of small airway epithelia that identified 3 genes after adjustment for genome-wide comparisons.³⁰

Gene expression analysis of nasal epithelia identified 53 differentially expressed genes (Fig 2, A, and see Table E8, A, in this article's Online Repository at www.jacionline.org). Among differentially expressed genes are genes associated with asthma and allergy: *ALOX15*, bradykinin B1 receptor (*BDKRB1*), cadherin 26 (*CDH26*), calpain 14 (*CAPN14*), cathepsins C and G (*CTSC* and *CTSG*), cystatin SN (*CST1*), gelsolin (*GSN*), mast cell carboxypeptidase *CPA3* and tryptases (*TPSAB1* and *TPSD1*), NADPH oxidase 1 (*NOX1*), neurotrophic tyrosine kinase receptor (*NTRK2*), plasminogen activator inhibitor 2 (*SERPINB2*), and von Willebrand factor (*VWF*). Moreover, a number of immune-related genes with high relevance to asthma (*CASP4*, *CCK*, *CCL5*, *CCL26*, *CD3G*, *CD55*, *CD6*, *CD74*,

CXCR6, *HLA-DMB*, *HLA-DOA*, *KLRB1*, and *TCRA*) are also differentially expressed in samples from patients with atopic asthma compared with those from control subjects.

Given the substantial number of immune-related differentially expressed genes, we examined the function, general tissue/cell expression patterns,³¹ and immune cell–specific expression patterns³² of all 53 differentially expressed genes to discern which genes were likely contributed by immune cells in the respiratory epithelium (see Table E8, B). This analysis revealed 11 of the 53 of the genes as highly likely to be contributed by immune cells: B cells (*HLA-DMB* and *HLA-DOA*), mast cells (*CPA3*, *CTSG*, *TPSAB1*, and *TPSD1*), natural killer cells (*KLRB1*), T cells (*CD3G*, *CD6*, and *TCRA*), and multiple immune cells (*CST1*). This is consistent with published findings in the lung epithelium; for example, differential expression of mast cell genes (*CPA3*, *TPSAB1*, and *TPSD1*) in the airway epithelium of asthmatic patients with different immune subtypes of asthma (T_H2 high and T_H2 low) is due to accumulation of intraepithelial mast cells with a unique protease phenotype in patients with T_H2-high asthma.³³ The remaining 42 genes are specifically expressed by epithelial cells, contributed both by epithelial and immune cells, or have unknown expression patterns.

The relationship between DNA methylation and gene expression revealed enrichment of hypomethylated genes associated with increased gene expression and hypermethylated genes associated with decreased gene expression (binomial $P < 2.8 \times 10^{-6}$ for enrichment in inverse relationships in asthma-related DMRs [Fig 2, B] and $P < 7.8 \times 10^{-10}$ for allergic asthma–associated DMPs [Fig 2, C]) within 5 kb of the transcription start site. However, a number of DNA methylation changes were not associated with canonical gene expression differences (Fig 2, B and C). Although these results are similar when we use a 2- or 3-kb distance, beyond 5 kb of the transcription start site, we begin to lose the enrichment in anticorrelated methylation-expression pairs. We did not observe an enrichment in negatively correlated methylation-expression

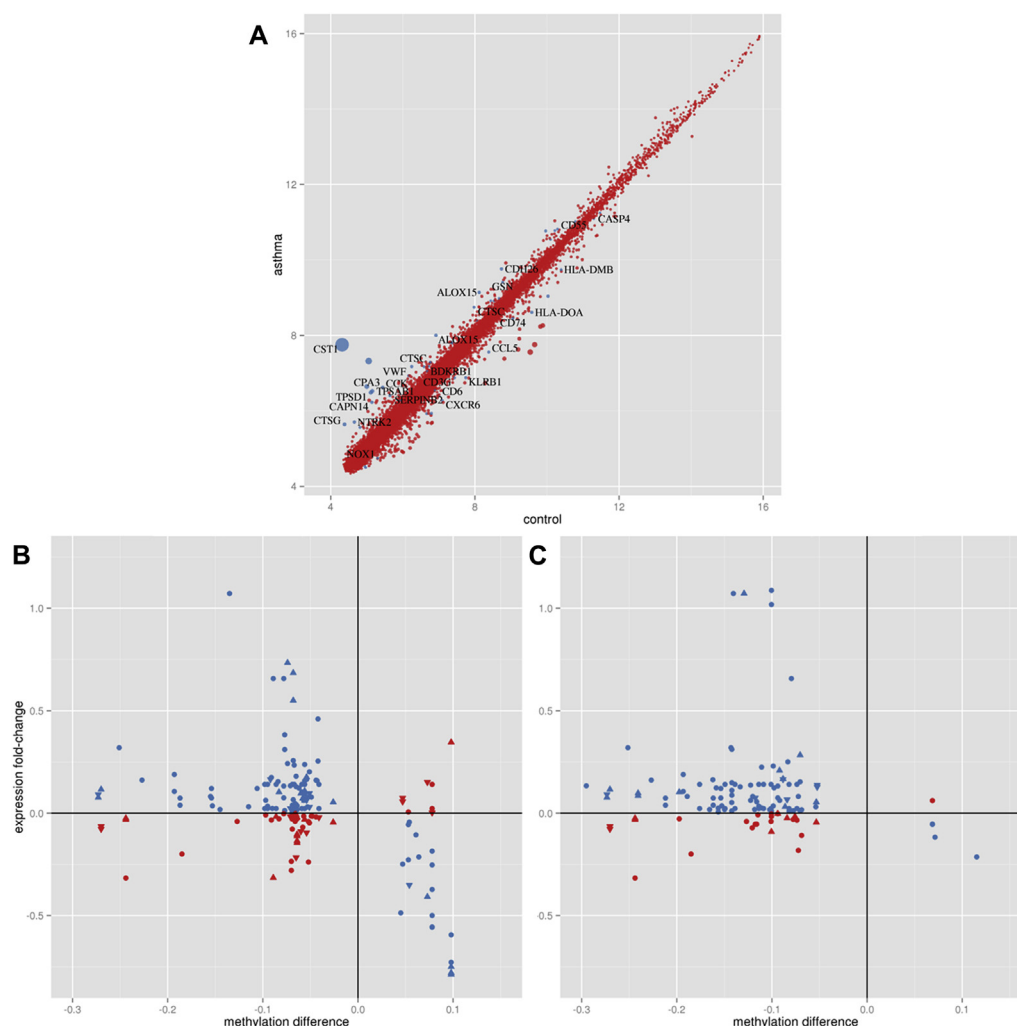


FIG 2. DNA methylation changes are associated with changes in gene expression in nasal epithelia. **A**, Asthma-associated gene expression changes after adjusting for age, sex, and race/ethnicity. *Blue dots* indicate 27 genes with q values of less than .05 for the asthma versus control comparison with high functional relevance to asthma and atopy. The size of the dot is proportional to asthma versus control fold change. **B** and **C**, Expression changes in genes within 5 kb of the nearest DMR (Fig 2, **B**) and DMP (Fig 2, **C**). In both panels the x-axis methylation difference is represented by the mean percentage methylation difference in asthmatic patients compared with control subjects. The y-axis expression difference is represented by the mean fold change in asthmatic patients compared with control subjects (on the $\log_2$ scale). *Blue symbols* represent hypomethylated genes that were 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/DMP location upstream of the gene, *circles* represent DMRs in the gene body, and *downward triangles* refer to DMRs/DMPs downstream of the gene.

relationships in gene promoters compared with gene bodies, as has been previously reported³⁴; however, this analysis was underpowered.

We implemented quantitative trait locus mapping to identify methylation marks that control gene expression (methyl-eQTL or quantitative trait region). We performed 2 analyses: one centered on differentially methylated genes (union of DMRs and DMPs from Fig 1; 186 unique genes), and the other focused on 53 differentially expressed genes (Fig 2, **A**). Of the 186 allergic asthma-associated differentially methylated genes, 158 have methylation and expression data within 5 kb, and of these, 16 (10%) have significant relationships of DNA methylation and gene expression within 5 kb (see Table E9, **A**, in this article's Online Repository

at www.jacionline.org). Of the 53 allergic asthma-associated differentially expressed genes, 32 (60%) have significant relationships of DNA methylation and gene expression within 5 kb (see Table E9, **B**). We observed a strong enrichment in significant methylation-expression pairs with 5 kb of differentially expressed genes compared with all methylation-expression pairs within 5-kb distance of each other in the genome (enrichment $P = 3.2 \times 10^{-6}$). Both analyses identified predominantly canonical inverse correlations of methylation and expression (15 of the 16 genes in the methylation-centered analysis and 32 of the 53 in the expression-centered analysis). Importantly, both analyses demonstrated that *ALOX15* has significant relationship of DNA methylation and gene expression.

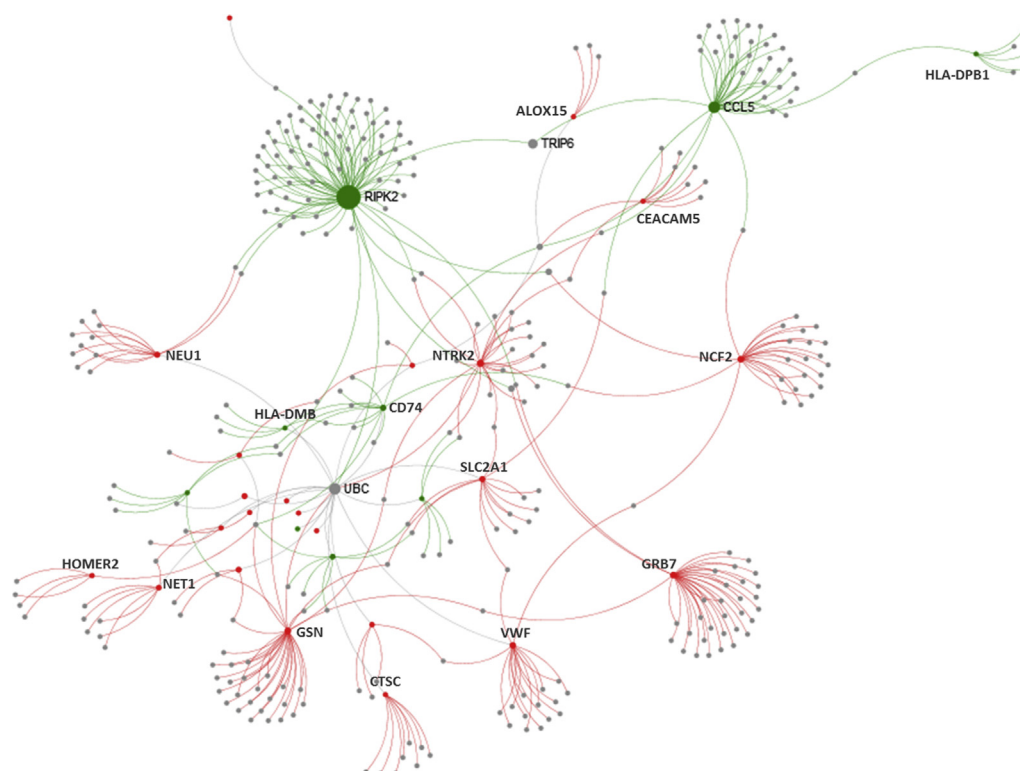


FIG 3. Protein-protein interactome analysis of 47 allergic asthma-associated genes with inverse relationship of methylation and expression. The interactome was created by using NetworkAnalyst³⁶ and the InnateDB protein-protein interaction data set. The nodes are colored based on their methylation and expression: *green nodes* are downregulated and hypermethylated, and *red nodes* are upregulated and hypomethylated. Node sizes are proportional to their betweenness centrality values.

TABLE III. Pyrosequencing and quantitative PCR validation of selected DNA methylation marks in the same asthmatic cases and control subjects and an independent population of asthmatic patients: Selected DMRs/DMPs from the Illumina analysis

DMR/DMP coordinates	Gene	Illumina-Adjusted <i>P</i> value	Illumina methylation change
chr17:4541333-4541334	<i>ALOX15</i>	7.82×10^{-4}	-10.1
chr6:33041220-33041697	<i>HLA-DPA1</i>	1.04×10^{-6}	9.8
chr1:35258778-35258933	<i>GJA4</i>	6.00×10^{-3}	-9.8
chr13:38172802-38172803	<i>POSTN</i>	7.25×10^{-4}	-11.6
chr11:36030085-36030086	<i>LDLRAD3</i>	6.23×10^{-6}	-28.8
chr7:105521115-105521116	<i>ATXN7L1</i>	4.09×10^{-9}	-27.3
chr12:58162286-58162287	<i>METTL1</i>	5.03×10^{-8}	-27
chr3:45984742-45985168	<i>CXCR6</i>	7.30×10^{-4}	5.9
chr17:34202460-34202461	<i>CCL5</i>	.017	1.9
chr11:88059526-88059527	<i>CTSC</i>	1.51×10^{-3}	-1.1

Upstream regulator analysis of the 47 allergic asthma-associated genes that demonstrate canonical inverse relationships between methylation and expression by using Ingenuity Pathway Analysis revealed a significant enrichment ($P < 1 \times 10^{-4}$) in cytokines (IL-13, IL-4, IL-6, IFN- γ , and others), as well as transcription factors (CIITA) and growth factors (TGF- β) known to regulate gene expression profiles in asthmatic patients (see [Table E10](#) in this article's Online Repository at www.jacionline.org).

Protein-protein interaction analysis of the 47 allergic asthma-associated genes with inverse relationships of methylation and expression revealed a network of proteins, with the largest hub being *RIPK2* ([Fig 3](#)).³⁵ *RIPK2* or *CARD3* is a component of signaling complexes in both the innate and adaptive immune pathways and is critical for NOD-mediated nuclear factor κ B activation and cytokine production,³⁶ and silencing of its expression attenuates allergic airway inflammation in mice.³⁷ Smaller hubs in the network include other proteins important in immunity and identified in our analysis. Taken together, these analyses support biological and disease relevance of our findings.

To determine the validity of these findings, we selected 10 loci for pyrosequencing and included an independent population of children with allergic asthma from the inner city ([Table I](#)). Ten loci were chosen to represent genes with known relevance to asthma, loci with the largest methylation changes and methylation marks that affect gene expression. Four allergic asthma-associated DMRs or DMPs were selected based on relevance to asthma (*ALOX15*, *HLA-DPA1*, *GJA4*, and *POSTN*), 3 were selected based on the extent of methylation differences between asthmatic patients and control subjects (*LDLRAD3*, *ATXN7L1*, and *METTL1*), and 3 additional loci were selected based on the allergic asthma-specific methylation-expression relationships (*CCL5*, *CTSC*, and *CXCR6*). Nine of these 10 loci were validated internally in the original study population ($P < .05$, 1-tailed *t* test), with the *CTSC* locus approaching statistical significance ($P = .12$, [Tables III-V](#) and see [Table E11](#) in this article's Online Repository at www.jacionline.org) if using nominal *P* values, and 8 of 10 loci

TABLE IV. Pyrosequencing and quantitative PCR validation of selected DNA methylation marks in the same asthmatic cases and control subjects and an independent population of asthmatic patients: Internal validation

Coordinates of Measured Cs	Gene	Methylation change	One-tailed <i>t</i> test	Expression fold change	One-tailed <i>t</i> test	Methylation-expression correlation	Correlation <i>P</i> value
chr17:4541333	<i>ALOX15</i>	−8.7	1.55×10^{-7}	2.0	0.0258	−0.39	5.53×10^{-4}
chr6:33041528	<i>HLA-DPA1</i>	9	2.10×10^{-4}	0.6	0.0361	−0.46	2.90×10^{-5}
chr1:35258850, 35258855	<i>GJA4</i>	−3.4, −7.8	6.0×10^{-5} , 1.74×10^{-3}	1.7	0.0913	−0.0061, −0.028	.9595, .810
chr13:38172802	<i>POSTN</i>	−8.9	1.70×10^{-4}	5.8	1.84×10^{-4}	−0.35	1.71×10^{-3}
chr11:36030086	<i>LDLRAD3</i>	−20.9	2.18×10^{-7}	1.2	0.263	−0.093	.424
chr7:105521115	<i>ATXN7L1</i>	−24.8	2.60×10^{-14}	0.9	0.375	0.048	.682
chr12:58162286	<i>METTL1</i>	−22.8	8.25×10^{-11}	0.8	0.214	0.054	.643
chr3:45984839, 45984841	<i>CXCR6</i>	5.7, 6.0	9.89×10^{-4} , 1.48×10^{-3}	0.5	0.0197	−0.32, −0.27	5.42×10^{-3} , .0193
chr17:34202460	<i>CCL5</i>	4.4	0.048	0.8	0.152	−0.29	9.94×10^{-3}
chr11:88059526	<i>CTSC</i>	−4.7	0.12	1.6	9.30×10^{-5}	−0.36	1.40×10^{-3}

TABLE V. Pyrosequencing and quantitative PCR validation of selected DNA methylation marks in the same asthmatic cases and control subjects and an independent population of asthmatic patients: External validation

Coordinates of Measured Cs	Gene	Methylation change	One-tailed <i>t</i> test	Expression fold change	One-tailed <i>t</i> test	Methylation-expression correlation	Correlation <i>P</i> value
chr17:4541333	<i>ALOX15</i>	−10.6	7.50×10^{-10}	1.5	0.0697	−0.092	.449
chr6:33041528	<i>HLA-DPA1</i>	8.1	1.27×10^{-3}	0.5	0.00109	−0.47	4.20×10^{-5}
chr1:35258850, 35258855	<i>GJA4</i>	−4.7, −13.7	2.03×10^{-8} , 2.26×10^{-8}	7.1	1.27×10^{-5}	−0.16, −0.13	.172, .303
chr13:38172802	<i>POSTN</i>	−13.6	6.5×10^{-9}	7.3	9.73×10^{-5}	−0.57	$<1 \times 10^{-5}$
chr11:36030086	<i>LDLRAD3</i>	−23.8	8.80×10^{-9}	1.5	0.0282	−0.13	.298
chr7:105521115	<i>ATXN7L1</i>	−31.1	4.91×10^{-20}	0.9	0.346	0.11	.347
chr12:58162286	<i>METTL1</i>	−27.1	6.02×10^{-14}	1.3	0.149	−0.076	.537
chr3:45984839, 45984841	<i>CXCR6</i>	4.2, 4.7	0.0227, 0.0188	0.7	0.0632	−0.30, −0.31	.0125, 9.49×10^{-3}
chr17:34202460	<i>CCL5</i>	7.8	1.6×10^{-3}	1.0	0.419	−0.25	.0393
chr11:88059526	<i>CTSC</i>	−14.5	8.2×10^{-5}	1.5	0.00232	−0.46	7.30×10^{-5}

were validated if stringent Bonferroni correction was applied. More importantly, all 10 loci validated in an independent population of children with allergic asthma residing in the inner city compared with control subjects from the original population (shared control design; $P < .05$, 1-tailed *t* test; Table II) and methylation levels in 2 sets of allergic asthmatic cases are comparable (see Table E11). Significance levels were very similar if adjustment for age, sex, and race/ethnicity was included, demonstrating the robustness of these associations with allergic asthma. We also examined expression of these 10 genes using quantitative PCR and showed that half of them have significant changes in patients with allergic asthma compared with control subjects and are also significantly inversely correlated with methylation in the original study population, as well as independent patients with allergic asthma (Tables III–V).

To further examine the validity and generalizability of our findings, we profiled nasal epithelia from adult white subjects, 12 atopic asthmatic patients, and 12 control subjects (cohort characteristics are shown in Table E12 in this article's Online Repository at www.jacionline.org) on Illumina methylation arrays. Using the same methods applied to our primary cohort, we identified 1766 DMPs and 370 DMRs, representing 1108 unique genes, significantly associated with asthma (genome-wide adjusted $P < .05$) after adjustment for age, sex, and batch effects. Of the 186 genes with significant methylation changes in the

primary cohort of atopic asthmatic African American children from the inner city, 67 (36%) have at least 1 genome-wide significant DMP, DMR, or both in adult white asthmatic patients (see Table E13 in this article's Online Repository at www.jacionline.org). Similarly, 56 (30%) genes also overlap with genes that have IL-13-responsive CpGs in cultured nasal epithelial cells from 57 unrelated adult lung donors.³⁸ These results demonstrate the external validity and generalizability of some of our findings to asthma, regardless of age, race/ethnicity, specific exposures, and conditions (cultured vs fresh cells and nasal vs lung epithelia).

DISCUSSION

Our findings demonstrate that methylation marks in the nasal epithelia of children with allergic asthma are associated with changes in gene expression. Moreover, the magnitude of these allergic asthma-associated epigenetic signals is substantial when compared with other diseases.^{39,40} These findings suggest that genes that have been previously reported to be differentially expressed in the airway epithelia of patients with allergic asthma^{14–16} might be regulated by epigenetic mechanisms.

The effect of aeroallergens¹² and other environmental stimuli¹³ on the respiratory epithelium is a key mediator of asthma. Gene expression profiles of the allergic asthmatic airway^{14–16} and

nasal¹⁷ epithelia demonstrate that these cells are involved in an active biological process. Our findings demonstrate a clear relationship between DNA methylation and gene expression in the nasal epithelia of young patients with allergic asthma by using a stringent analytic pipeline to ensure that methylation changes we identified are not false-positive results because of the influence of demographic factors or technical variables. More than half of the differentially expressed genes in our analysis have significant associations with methylation marks; this includes the asthma and allergy genes *ALOX15*,⁴¹ *CAPN14*,⁴² *CTSC*,⁴³ *CSTI*,⁴⁴ *GSN*,⁴⁵ *NTRK2*,⁴⁶ and *VWF*,⁴⁷ as well as a number of immune and extracellular matrix genes. Moreover, genes that have been identified previously as differentially expressed in the respiratory epithelia but did not reach statistical significance in our expression analysis, such as *POSTN*,^{15,17} are also differentially methylated. Finally, genes with the largest absolute changes in methylation observed in allergic asthma (*LDLRAD3*, *ATXN7L1*, and *METTL1*) have not previously been implicated in allergic asthma and represent biological candidates for future investigation. There is recent evidence that expression of *ALOX15* is regulated by demethylation of H3K27me3 at the *ALOX15* promoter after IL-4 treatment in epithelial cells, providing support for the specific genes identified in our analysis.⁴⁸

Importantly, the magnitude of DNA methylation changes at some of the loci is large, which is consistent with that observed in other diseased tissue,^{39,40} and these loci can become important therapeutic targets in the future, given additional evidence from cohorts with larger sample sizes. DNA methylation changes have been shown to drive tumor formation and malignant progression,⁴⁹ appear to be critical to disease pathogenesis, and represent novel therapeutic targets in cancer. DNA methyltransferase inhibitors, such as 5-azacitidine and decitabine, have been approved for the treatment of myelodysplastic syndrome^{50,51} and are being tested in patients with solid tumors.^{52,53} Methylation changes in nasal epithelia of patients with allergic asthma are, on average, approximately 10 times larger than DNA methylation changes in peripheral blood that are associated with childhood allergic asthma¹⁰ or other diseases of the airway, such as chronic obstructive pulmonary disease.¹⁸ The strength of the DNA methylation signal in the nasal epithelia of children with asthma was detectable even in the limited sample size of the study population. Moreover, many of the methylation marks were validated in an even smaller cohort of adult white asthmatic patients, demonstrating the generalizability of our findings to asthma. Given similarities in the nasal and airway/bronchial epithelial transcriptomes,¹⁷ it is logical to speculate that DNA methylation in nasal epithelia could be used as a biomarker of exposure or disease; however, further work in larger cohorts will be needed to explore this biomarker's potential.

The majority of allergic asthma-associated methylation marks we identified in the nasal epithelia are hypomethylated and within gene bodies. Our results are in concordance with previous reports that have shown hypomethylation of specific genes in asthmatic patients^{10,54-56} and patients with other airway diseases, such as chronic obstructive pulmonary disease.¹⁸ Similarly, cigarette smoke exposure is more often associated with hypomethylated regions in airway epithelia,³⁰ and large blocks of hypomethylation have been identified in patients with cancer.^{57,58} Although early studies focused specifically on promoter methylation as a mechanism of gene regulation,⁵² more recent research has demonstrated the significance of gene body methylation^{59,60} in

transcriptional regulation in both malignant³⁴ and nonmalignant²² diseases. Our study is the first to show an association of gene body methylation and gene expression in patients with allergic asthma.

There are several potential causes for these methylation changes. First, nasal epithelia are subject to a number of environmental exposures that are relevant to allergic asthma and are also known to shape the epigenome; these include cigarette smoke,^{30,61} air pollution,⁶² and farming aerosols.⁶³ Although we were unable to evaluate the effect of exposures on the epigenome because of the lack of exposure assessment in our study, this is an important future direction for the field. Second, epigenetic marks can influence the severity of allergic asthma. Previous work has shown association of DNA methylation in specific gene loci (*IL6* and inducible nitric oxide synthase) in the nasal epithelia⁵⁴ and buccal cells (*IFNG*).⁶⁴ Finally, microbial species are also known to influence the epigenome. The differential effect of rhinovirus infection in nasal epithelia from asthmatic patients (n = 6) compared with that from nonasthmatic subjects (n = 3) on methylation of a small noncoding RNA locus (*SNORA12*) has been recently demonstrated.⁶⁵

There are several limitations to our study. First, our study design precludes us from differentiating epigenetic marks associated with allergy versus those associated with asthma. However, because most children with asthma also are atopic, our findings among children with allergic asthma are generalizable to a larger population of children with asthma. Second, we were able to evaluate the effect of exposures on the epigenome in a limited fashion by using questionnaires and not exposure assessments. We did not collect longitudinal samples and were unable to examine longitudinal variation in DNA methylation caused by changes in exposure. The current study is an observational study with small sample size showing an association between methylation biomarkers and childhood persistent asthma, and further work will be required to address the stability of the biomarker over time. The relationship between the environment and epigenome, especially in relation to conditions like allergic asthma, remains a particularly important area of future research. Third, our small sample size precluded us from assessing methylation marks in relation to disease heterogeneity and asthma endotypes, and future work in larger cohorts will be required to address this question. Fourth, although all our subjects are of self-reported African American or Dominican/Haitian descent, there is substantial genetic admixture of African ancestry in these populations,^{66,67} and we did not examine the effect of genetics on DNA methylation.^{68,69} Fifth is the inability to detect differences in methyl- versus hydroxyl-methyl cytosine, which is thought to be a mark for demethylation.⁷⁰ Finally, our association analysis is unable to distinguish between DNA methylation marks being the cause or consequence of disease. Despite these limitations, our study identified substantial and consistent DNA methylation changes in the nasal epithelia of children with allergic asthma residing in the inner city compared with nondiseased control subjects, and these methylation marks were internally and externally validated in both children and adults. These methylation changes are associated with gene expression and need to be further evaluated in larger cohorts for their potential as biomarkers and therapeutic targets. The peculiar spatial and biological relation of epigenetic changes in the nasal epithelia of asthmatic patients suggests that these cells and these

mechanisms might provide insight into the cause and pathogenesis of this persistent public health concern.¹

Key messages

- Expression of genes related to extracellular matrix, immunity, cell adhesion, epigenetic regulation, and airflow obstruction are epigenetically regulated in nasal epithelia and associated with asthma.
- The methylation changes in nasal epithelia are substantial (median, 9.5%; range, 2.6% to 29.5%) and similar in magnitude to those observed in patients with other diseases.
- More than one third of methylation changes identified by using our analysis appear to be generalizable to atopic asthma regardless of age, sex, race/ethnicity, and specific exposures.

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The Nasal Methylome and Childhood Atopic Asthma Supplemental Materials

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SUPPLEMENTAL METHODS

Study Populations

Our primary study population consisted of inner-city children aged 10-12 years with both atopy and persistent asthma (cases, N=36) or without atopy or asthma (healthy controls; N=36). Study subjects were recruited by six sites supported by the Inner City Asthma Consortium (ICAC) from census tracts that contain at least 20% of households below the U.S. government poverty level(1). The cases and controls in the present study represent a subset of subjects from a larger population of 97 asthmatics and 97 controls that were included in our study of the relationship between the PBMC epigenome and asthma(2). The cases and controls in the present study did not differ demographically or clinically from the larger study population (Supplemental Table S1) except for age (current study limited to subjects 10-12 years of age). We limited our study population to 36 cases and 36 controls with at least 80% ciliated epithelial cells visualized from slides obtained from nasal brushings(3) and by expression of the FOXJ1 gene(4).

Cases 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(5); 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 skin prick test to at least one of a panel of indoor aeroallergens (dust mite, cockroach, mold, cat, dog, rat, 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 skin prick tests.

Nasal epithelial cells were collected from the posterior portion of the inferior turbinate with a cytology brush using a nasal illuminator as previously reported(3). The collected brush

was submerged in RLT Plus lysis buffer with β -mercaptoethanol and frozen at -80 °C until DNA and RNA extraction. All collected samples were screened by Taqman qPCR assays for expression of the ciliated cell marker FOXJ1 (relative to GAPDH in each sample, using the Δ Ct method(6)) to identify samples with the purest population of columnar epithelia (Supplemental Table S2). Samples with known predominant columnar and squamous cell population were included as positive and negative controls.

The validation population consisted of 30 African Americans aged 10-12 years with atopic asthma, using the same definition as the derivation cohort, collected by the ICAC independent of the primary study population. Nasal brushings were collected using the same techniques as described above and screened by hematoxylin and eosin stains of brush smears on microscope slides; only samples with greater than 80% columnar cells were included in the validation analysis.

The National Jewish Health adult population consisted of 12 atopic asthmatics and 12 controls. Asthmatic inclusion criteria were the following: (1) FEV1 > 55% predicted at visit 1; (2) positive methacholine challenge (PC20 <12 mg/ml) within last 6 months or demonstrated at visit 1, OR demonstrates improvement in FEV1 of > 12% and 200 mL; (3) no history of life threatening asthma; (4) no treatment with bronchial thermoplasty; (5) must be taking inhaled corticosteroid for at least 4 weeks prior to Visit 1; and (6) no leukotriene modifiers for 1 week. While atopy was not an inclusion criterion, all asthmatics were also atopic, as assessed by positive skin prick testing for multiple allergens or RAST/Phadiatop tests. Control inclusion criteria were the following: (1) normal lung function values at visit 1; (2) negative methacholine challenge (PC20 >25 mg/ml) within last 6 months or demonstrated at visit 1; and (3) IgE level at visit < 100; no active or history of lung disease.

Nucleic Acid Isolation

DNA and RNA were isolated simultaneously from the nasal epithelial cells 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).

Genome-wide 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 72 arrays performed in 2 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 0%, 50%, and 100% methylated 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 S3.

mRNA Expression

Samples with the RNA integrity number (RIN) >7 were used. Gene expression profiling was performed on the Agilent G3 SurePrint 8x60 human gene expression one-color microarray platform using Agilent's low input labeling kits for one-color assays, hybridization and wash reagents according to manufacturer's protocols. Arrays were scanned on the Nimblegen MS200 high-resolution microarray scanner (Roche Nimblegen, Madison, WI), and images were processed in the Agilent Feature Extraction software. Intensities were exported from the Feature Extraction software. All samples were assayed once with 72 arrays performed in 3 batches. All mRNA data met the quality control criteria established by the Tumor Analysis Best Practices Working Group (7).

Data Quality Controls

We initially examined the quality control figures created by the minfi R package for the 450k data(8). We observed a strong bi-modal distribution of methylation values in the 450k data as expected(9). Further data quality for Illumina 450k and expression arrays was assessed using

Principal Components Analysis (PCA). The principal components were examined for correlation with all laboratory data to identify observable batch effects(10). As a result of PCA, we removed 2 outlier samples from expression arrays while no outliers were detected in the Illumina 450k methylation dataset. No laboratory variables were significantly correlated with principal components in the methylation or expression datasets and we therefore did not include any laboratory variables in statistical models (Supplemental Table S14). Of the demographic variables, only age was strongly associated with top PCs in both methylation and expression datasets. Although gender and race/ethnicity were not associated with top PCs, we included these covariates in the statistical model because of their known associations with DNA methylation(11, 12).

Probabilistic estimation of expression residuals (PEER) factors(13) were included to account for unknown batch effects. The number of PEER factors included in downstream analyses was three for 450k and none for gene expression and was based on the inflection point seen upon visual inspection of the weighting of the inferred factors to ensure that all technical variation was captured with latent variables(13).

Statistical Analysis of DNA Methylation Data

Data from the methylation array were normalized using the SWAN method(14) contained in the R package minfi(8), and the normalized M-values were used in all downstream analyses. Beta-values (which range from 0 to 1) of individual probes are plotted in Figure 2B while average beta-values across the DMR are reported in Figure 2C. Beta values are biologically intuitive interpretation of percent methylation changes on the 0-1 scale corresponding to 0-100% methylation. It is known that SNPs contained in the Infinium probes can affect binding and result

in spurious methylation measurements and we therefore filtered out probes on the 450k Illumina array with known SNPs in European (CEU) and African (YRI) populations within the CpG motif(15).

We performed the analysis to identify differential methylation in three steps. In brief, these are: infer PEER factors, fit linear models with limma(16), and use comb-p(17) 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 asthma status (yes/no), demographic covariates that are known to affect DNA methylation (age, gender, and race/ethnicity), and three PEER factors to account for unknown batch effects. Explicitly, the model used is: $\text{methylation} \sim \text{asthma} + \text{gender} + \text{age} + \text{race_aa} + \text{race_hispanic} + \text{race_other} + \text{PEER1} + \text{PEER2} + \text{PEER3}$.

DMPs were identified by calculating q values, corresponding to false discovery rate (FDR), from linear model p-values using the method of Benjamini and Hochberg(18). DMRs were identified from linear model p-values using comb-p(17) with a window size of 300 bases. The identification of DMRs is described in detail in(17) and outlined in the diagram in Supplemental Figure S1. 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 using the Benjamini-Hochberg methodology(18), 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)(19). Finally, the region p-values are adjusted for multiple-testing using

the Sidak correction(20) based on the size of the region and the number of possible regions of that size such that larger regions undergo less stringent correction since there are fewer possible large regions (SLK-Sidak p-value). DMPs and DMRs were annotated using the CruzDB tool(21). Following examination of q-q plots, we performed global adjustment for inflation of p values using standard methodology(22).

Statistical Analyses of Gene Expression Data

Background-subtracted intensity data from the Agilent Feature Extraction software were $\log_2$ -transformed prior to any analysis. The R package limma(16) was used to background-correct, normalize (quantile) and then fit linear models for expression data; p-values were based on the moderated t-statistic. For expression analysis, we used a model that incorporates asthma and demographic covariates that are known to affect expression (age, gender, and race/ethnicity) and no PEER factors were included. Explicitly, the model used is: $\text{expression} \sim \text{asthma} + \text{gender} + \text{age} + \text{race_aa} + \text{race_hispanic} + \text{race_other}$. q-values were calculated from p-values using the method of Benjamini and Hochberg(18).

Analysis of DNA Methylation and Gene Expression

To understand the relationship of DMRs with gene expression changes, we considered inversely correlated (canonical) vs directly correlated pairs limiting the analysis to genes within 5kb of a DMR. We calculated the enrichment of inversely correlated pairs in relation to all pairs using the binomial test. This distance cutoff was used as a way to focus on the most proximal cis relationships of methylation and expression. We also considered 2kb, 3kb, and >5kb distances in our analysis.

To integrate the expression and methylation data, we used a method that is derived from the comb-p(17) pipeline except that, instead of testing each methylation site for its relation to asthma status, we tested methylation regions and expression probes within 5kb. We refer to this method as methylation-expression quantitative trait region (methyl-eQTR) mapping. For methylation-centered analysis, we tested all expression probes within 5kb of asthma-associated DMRs/DMPs. Similarly, for expression-centered analyses, we tested all methylation probes within 5kb of genes differentially expressed in asthma compared to controls. Methylation regions are defined by correlation as with A-clustering (23) and then the p-values for each probe within a region are combined using Stouffer-Liptak under the model: methylation $\sim$ expression + asthma + gender + age + race/ethnicity.

Pathway and Network Analysis

We used the Network Analyst tool to generate the protein-protein interactome. NetworkAnalyst uses a comprehensive high-quality protein-protein interaction (PPI) database based on InnateDB(24). The database contains manually curated protein interaction data from published literature as well as experimental data from several PPI databases including IntAct, MINT, DIP, BIND, and BioGRID. The database currently contains 14755 proteins and 145955 interactions for human, and 5657 proteins and 14491 interactions for mouse. We used Ingenuity Pathway Analysis (IPA)(25, 26) to identify enriched upstream regulators.

ENCODE data DNase I Hypersensitivity Site Analysis

We used the eFORGE tool (<http://www.ucl.ac.uk/cancer/medical-genomics/eforgeproject>) to identify over-representation of allergic asthma-associated methylation changes in DNase I hypersensitivity site (DHS) hotspots in specific cell lines in the

ENCODE data(27). For each eFORGE analysis, a random background set (pairing random probe sets to the input set adjusting for Gene and CpG Island annotation) is generated. Comparing DHS Hotspot overlap between input and background probes allows testing for the tissue-specific enrichment of EWAS probe sets using the binomial test.

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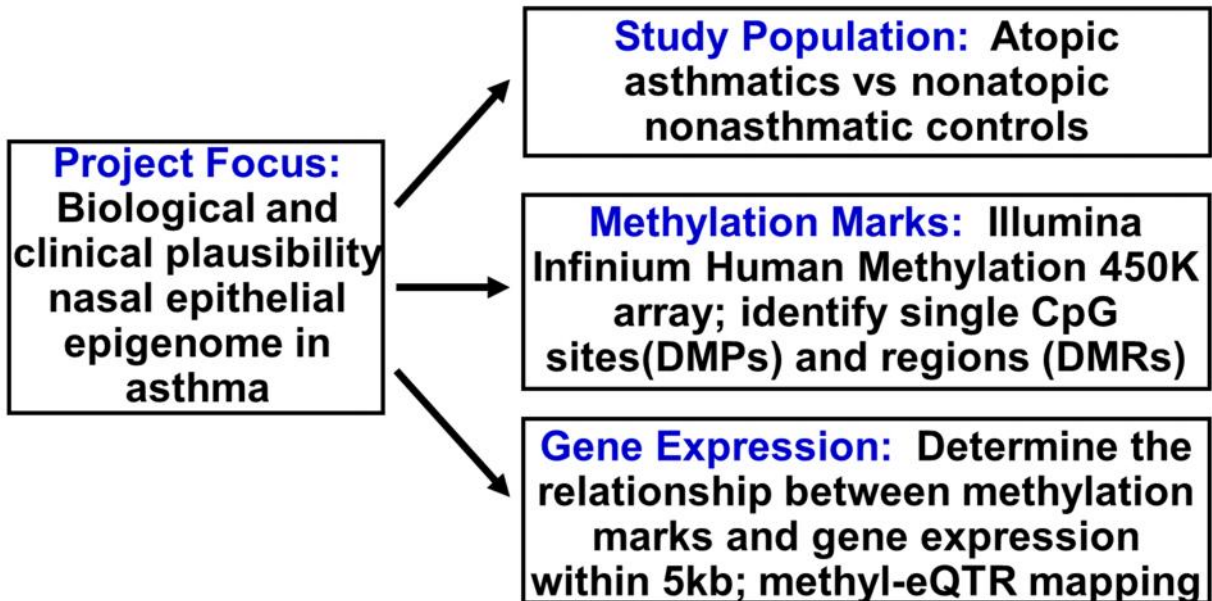
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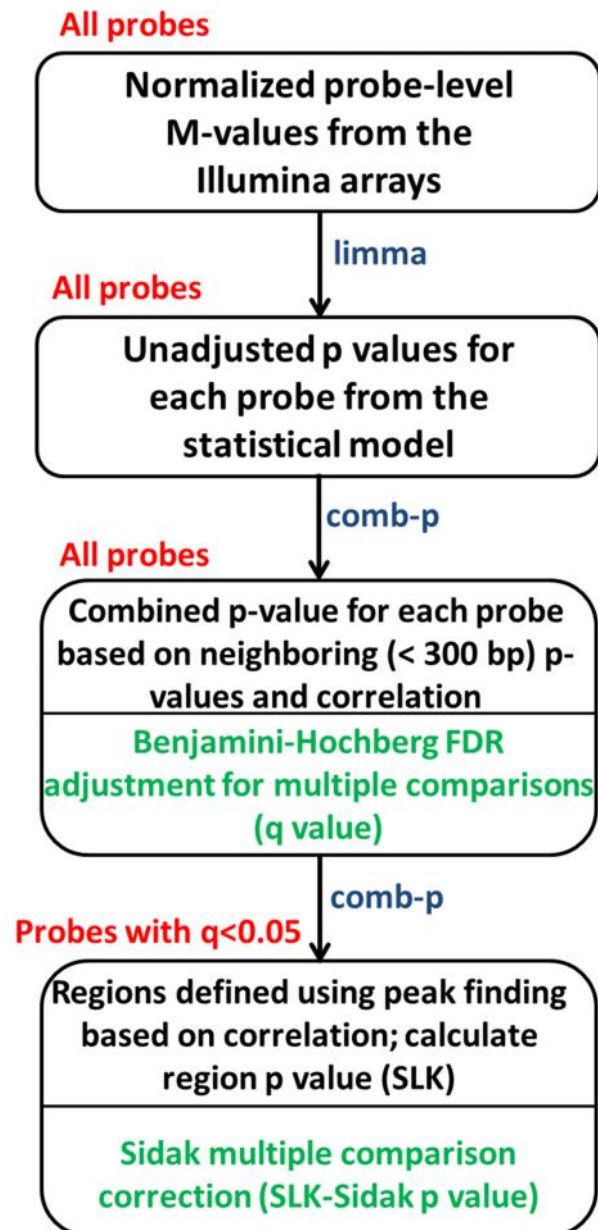
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SUPPLEMENTAL FIGURES

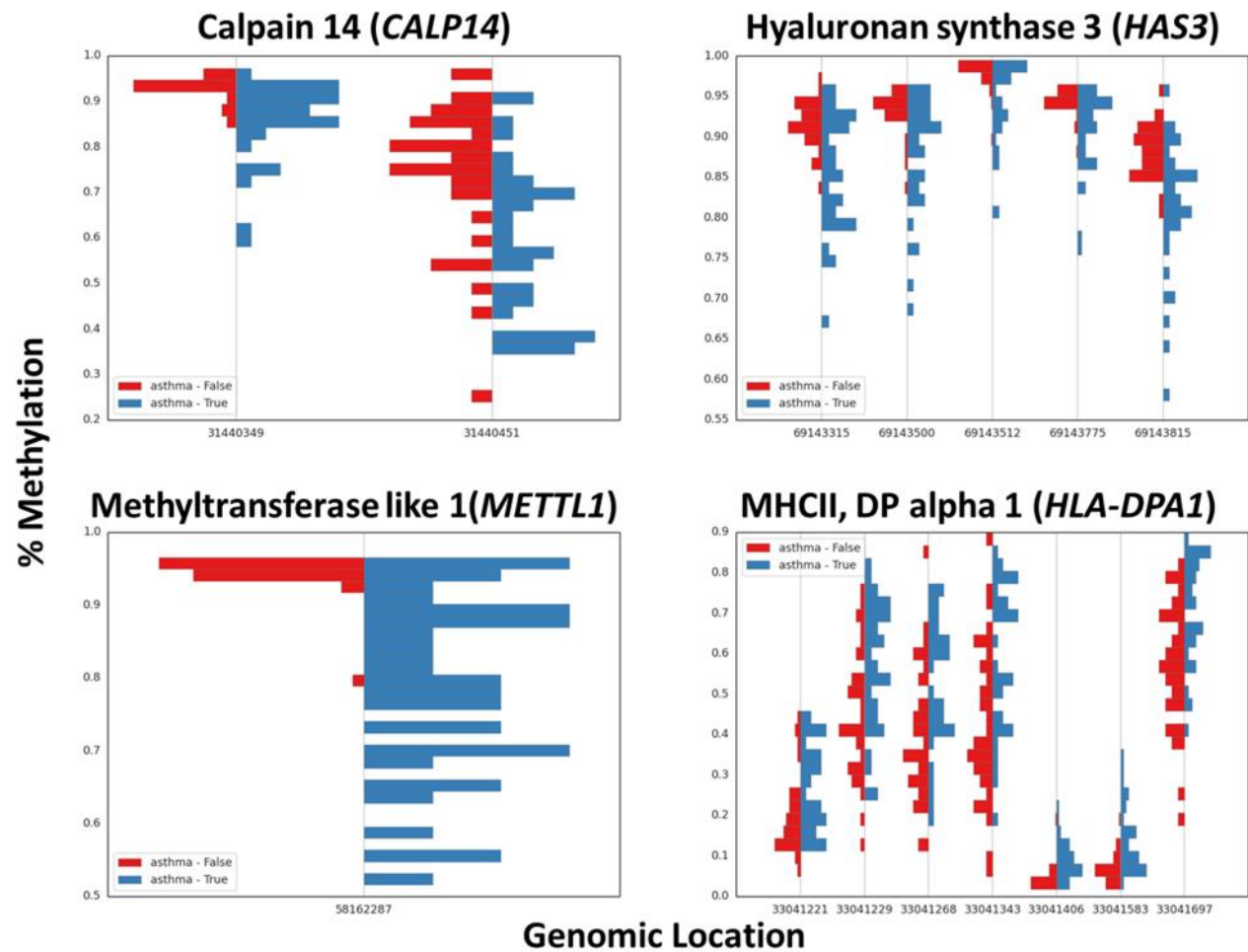
Supplemental Figure S1. Overview of the overall study design and workflow.



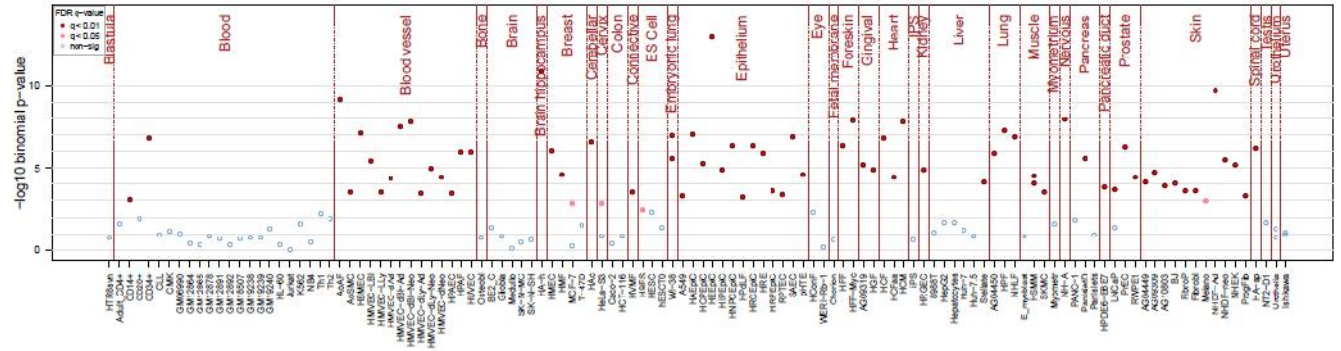
Supplemental Figure S2. Flow chart describing steps in the methodology for assessing significance of differential methylation and identification of differentially methylated regions (DMRs).



Supplemental Figure S3. Representative histograms of percent methylation estimates for individual probes in asthma associated differentially methylated probes (DMPs) and differentially methylated regions (DMRs).



Supplemental Figure S4. Enrichment of allergic asthma-associated DNA methylation changes in DNase I hypersensitivity sites (DHS) in ENCODE project cell lines. Enrichment analysis was performed using the eFORGE tool (<http://www.ucl.ac.uk/cancer/medical-genomics/eforgeproject>).



Supplemental Table S1. Demographic and clinical features of larger population of asthma subjects and controls

	Asthma Subjects (N=97)	Control (N=97)	p-value †
Site: Boston	23 (23.7%)	31 (32.0%)	<0.01 ¹
Dallas	8 (8.2%)	2 (2.1%)	
Denver	28 (28.9%)	13 (13.4%)	
Detroit	4 (4.1%)	6 (6.2%)	
New York	22 (22.7%)	14 (14.4%)	
Washington D.C.	12 (12.4%)	31 (32.0%)	
Baltimore	--	--	
Chicago	--	--	
Cincinnati	--	--	
Age at recruitment (yr)	9.0 [8.0, 11.0]	9.0 [8.0, 11.0]	0.762
Gender (Male)	56 (57.7%)	41 (42.3%)	0.031
Participant race: African American (Yes)	82 (84.5%)	86 (88.7%)	0.401
Participant race: Hispanic or Latino (Yes)	26 (26.8%)	15 (15.5%)	0.051
How often exposed to smokers: Daily	26 (26.8%)	25 (25.8%)	0.851
Dog living in the home in the last 6 months (Yes)	24 (24.7%)	22 (22.7%)	0.741
Cat living in the home in the last 6 months (Yes)	15 (15.5%)	24 (24.7%)	0.111
Any water/dampness in the last 12 months (Yes)	31 (32.0%)	16 (16.5%)	0.011
Gas stove, gas range or gas oven (Yes)	55 (56.7%)	69 (71.1%)	0.041
Allergic to any indoor allergen (Yes)	97 (100.0%)	0 (0.0%)	**
Cockroach IgE (IU/mL)	0.34 [0.34, 1.65]	0.34 [0.34, 0.34]	**
Total IgE (kU/l)	408.5 [189.0, 951.0]	29.0 [15.0, 58.0]	**
Baseline - FEV1(% predicted) ‡	90.9 ± 16.7	103.0 ± 10.3	**
Baseline - FEV1/FVC §	75.5 ± 9.7	84.4 ± 6.1	**
Asthma Medications: Albuterol	95 (97.9%)	0 (0.0%)	**
Inhaled Steroids	84 (86.6%)	0 (0.0%)	
Montelukast	38 (39.2%)	0 (0.0%)	

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

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

‡ FEV₁ denotes forced expiratory volume in one second

§ FVC denotes forced vital capacity

** By protocol design

Supplemental Table S2. qPCR results for FOXJ1 expression in epithelial cell samples. RIN refers to RNA integrity number, assessed on the Agilent Bioanalyzer.

Sample ID	Diagnosis	RIN	GAPDH Ct Mean	GAPDH Ct SD	Foxj1 Ct Mean	Foxj1 Ct SD	delta Ct
15-02-049-9-NAS	Case	10	26.508	0.030	27.662	0.066	1.154
Columnar cell control			27.321	0.031	28.573	0.032	1.252
15-08-028-9-NAS	Case	10	29.773	0.057	31.292	0.226	1.519
15-08-025-8-NAS	Control	10	28.179	0.012	29.747	0.064	1.568
15-02-016-9-NAS	Control	8.3	26.036	0.006	27.680	0.004	1.644
15-02-053-1-NAS	Control	10	27.178	0.005	28.881	0.133	1.703
15-02-040-6-NAS	Control	10	25.583	0.050	27.376	0.017	1.792
15-08-027-0-NAS	Control	10	26.414	0.190	28.245	0.011	1.830
15-06-006-7-NAS	Case	10	26.778	0.027	28.621	0.146	1.844
15-04-006-0-NAS	Control	10	27.747	0.027	29.600	0.235	1.853
15-07-018-0-NAS	Case	10	28.263	0.071	30.122	0.006	1.859
15-07-034-1-NAS	Control	10	27.222	0.001	29.101	0.016	1.879
15-02-019-5-NAS	Control	9.8	25.260	0.006	27.152	0.047	1.892
15-08-018-4-NAS	Case	9.3	26.968	0.001	28.871	0.037	1.903
15-02-017-6-NAS	Case	9.8	26.626	0.017	28.594	0.075	1.968
15-07-020-0-NAS	Case	7.6	26.725	0.032	28.731	0.027	2.006
15-08-013-2-NAS	Control	8.6	24.751	0.008	26.863	0.022	2.112
15-08-017-8-NAS	Control	9.4	26.031	0.003	28.192	0.009	2.161
15-02-047-5-NAS	Control	8.5	27.819	0.086	30.038	0.033	2.219
15-08-023-6-NAS	Control	9.9	26.959	0.044	29.267	0.010	2.308
15-02-020-2-NAS	Case	9.4	25.665	0.045	28.014	0.034	2.349
15-02-035-8-NAS	Control	10	25.801	0.014	28.153	0.096	2.352
15-07-005-3-NAS	Case	10	26.706	0.016	29.075	0.002	2.368
15-02-013-0-NAS	Control	9.5	25.682	0.085	28.057	0.102	2.375
15-08-008-1-NAS	Control	10	25.623	0.028	28.028	0.060	2.405
15-08-042-0-NAS	Control	10	27.498	0.022	29.927	0.078	2.429
15-07-021-6-NAS	Case	9.4	25.464	0.064	27.905	0.080	2.441
15-04-003-9-NAS	Case	10	26.733	0.010	29.206	0.097	2.473
15-08-012-1-NAS	Case	9.9	27.061	0.017	29.552	0.131	2.491
15-05-032-3-NAS	Case	10	26.953	0.002	29.453	0.009	2.500
15-02-038-9-NAS	Case	8.9	29.982	0.058	32.490	0.006	2.508

15-08-011-3-NAS	Case	9	25.304	0.024	27.876	0.066	2.572
15-05-022-0-NAS	Case	10	25.711	0.080	28.289	0.019	2.579
15-05-031-0-NAS	Control	9.8	28.038	0.031	30.641	0.029	2.603
15-02-030-4-NAS	Control	9.5	29.116	0.304	31.791	0.027	2.675
15-06-008-0-NAS	Control	10	25.376	0.005	28.072	0.175	2.696
15-07-013-3-NAS	Case	9	25.628	0.049	28.467	0.155	2.839
15-07-023-7-NAS	Case	10	26.025	0.044	28.946	0.043	2.921
15-07-030-2-NAS	Control	10	27.444	0.113	30.377	0.011	2.933
15-08-003-4-NAS	Control	8.5	25.229	0.015	28.168	0.012	2.939
15-08-035-5-NAS	Case	10	26.874	0.128	29.868	0.056	2.995
15-06-004-6-NAS	Case	10	26.521	0.023	29.519	0.003	2.998
15-02-015-3-NAS	Case	8	26.556	0.009	29.572	0.087	3.015
15-05-004-0-NAS	Case	9.1	25.161	0.021	28.177	0.044	3.016
15-08-036-4-NAS	Control	9.9	26.991	0.023	30.043	0.102	3.052
15-02-008-4-NAS	Control	10	25.068	0.061	28.120	0.040	3.052
15-02-005-0-NAS	Case	9.9	28.259	0.084	31.313	0.025	3.054
15-06-002-2-NAS	Case	8.9	28.054	0.056	31.158	0.062	3.104
15-02-039-1-NAS	Control	10	28.169	0.058	31.282	0.170	3.113
15-08-026-2-NAS	Control	9.4	26.180	0.005	29.321	0.017	3.141
15-08-034-0-NAS	Control	9.9	25.637	0.791	28.844	0.126	3.207
15-02-011-1-NAS	Case	10	27.483	0.084	30.736	0.063	3.253
15-02-026-0-NAS	Control	10	27.573	0.052	30.850	0.109	3.277
15-08-002-3-NAS	Case		24.774	0.036	28.098	0.076	3.324
15-06-007-9-NAS	Case	8.5	31.253	0.049	34.612	0.388	3.359
15-08-007-5-NAS	Case	8.5	25.222	0.032	28.605	0.056	3.383
15-02-010-7-NAS	Case	10	27.157	0.045	30.584	0.062	3.427
15-08-024-3-NAS	Control	9.6	24.996	0.021	28.440	0.098	3.443
15-04-009-4-NAS	Case	9.2	28.182	0.026	31.681	0.180	3.499
15-05-006-4-NAS	Control	10	26.917	0.094	30.419	0.080	3.502
15-02-028-7-NAS	Control	9.5	27.735	0.042	31.254	0.158	3.520
15-02-041-0-NAS	Control	10	28.558	0.004	32.140	0.232	3.582
15-04-001-8-NAS	Case	10	28.868	0.075	32.574	0.058	3.706
15-08-020-4-NAS	Control	9.4	25.822	0.007	29.660	0.091	3.838
15-05-012-6-NAS	Case	8	27.016	0.045	30.893	0.102	3.877

15-05-005-5-NAS	Case	10	27.130	0.023	31.134	0.031	4.004
15-05-017-4-NAS	Control	10	27.115	0.024	31.214	0.328	4.099
15-07-029-2-NAS	Control	10	26.957	0.020	31.106	0.064	4.149
15-05-037-5-NAS	Case	10	28.458	0.047	32.745	0.106	4.287
15-05-024-9-NAS	Case	10	26.986	0.029	31.597	0.092	4.611
15-04-005-6-NAS	Case	10	28.744	0.116	33.503	0.124	4.759
15-02-025-6-NAS	Control	10	27.910	0.024	32.949	0.030	5.039
15-02-014-8-NAS	Control	10	28.739	0.003	33.782	0.021	5.043
Squameous cell control			25.475	0.015	39.252	0.119	13.776

Supplemental Table S3. Sequence of primers used in pyrosequencing analysis of DNA methylation

DMR or Single CpG Genomic Coordinates	Gene	Coordinates of Measured Cs	Forward Primer	Reverse Primer	Sequencing Primer	Sequence to Analyze
chr17:4541333-4541334	ALOX15	4541333	ATTTTGTAGAATTTT AGTATTAGGGGTTA G*	AACCCACTATAAAC CTAACTTACT	ACCTCCTTCCTACA C	YAAAATAACTAC
chr6:33041220-33041697	HLA-DPA1	33041528	ATGGGTGGAGAGG AATTAG	ATCTCTTCCCCAAC ACCTT*	TTTGTTGTTGGGT AAAG	AGGAYGTTGGA AGGTGTTGGGG AAGAGATG
chr1:35258778-35258933	GJA4	35258850, 35258855	GGGAGGAGTTTTG GGTTTTT	ACCTCTAAACCCCT CAATCT*	ATTTTTTTTTTATG GAATTTTTGTA	ATAATTYGAGGY GGGAGGAATAT TATTGTTTT
chr13:38172802-38172803	POSTN	38172802	AGGAAAAAGAAAA ATGGTTTATAAAAT	CAAATAATTCCT TTTTACCCATAT	ATGAGTTAAGATT TGTATAATGA	TTGTTGGYGTTT ATAGGGTTAATA ATAAGTAGT
chr11:36030085-36030086	LDLRAD3	36030086	AGTTTGTGGGGTT AAGAAATGT	AAAACATAAACACC CAAAAAATTACCTA T*	AAATGTTTTTATTG TAAGATTGG	GTTTGTYGATAT ATAGGTAATTTT TTGGGTGT
chr7:105521115-105521116	ATXN7L1	105521115	TTGAAAAGAAGAG AAGAGGGAATGTT AGT	ACCTCCAAATAACT CCCAACAATCTA*	AATTTTGTAGTGG TTGAGGTA	YGGTGTGAGTAT TTTATTTTTATT T
chr12:58162286-58162287	METTL1	58162286	AGGGAATATAGATT TTTTAGGTTAAGTT	ATATACCTTCTACC ATCCTCCTATC*	ATATAGATTTTTTA GGTTAAGTTTT	AYGTAGGGAGA GGATAGGAGGA TGGA
chr3:45984742-45985168	CXCR6	45984839, 45984841	ATGAAGGGAGTGT GGGGTATT*	ACTCCCTCCTTCA AATCAAAATCCATA TA	ACCAACCCCCCCT ATAT	AACYCYTAAATC AAAAATAAATAA ATAACTA
chr17:34202460-34202461	CCL5	34202460	TAGATTGTTTGGTG AGAATGAGG	CACCACCCTAAATA AAAACACAACT	TCCCTTTTCATCTCT AC	ACACCRAAATAA AAAAAAAAAAAA ATAATCAA

chr11:88059526-88059527	CTSC	88059526	ATGTAAATAAAGAT TTTAGAAGGGATTG T	AAAACCTCTTTTAAA ACCACAACCA	GTTGTTATAGGTA GGTTTATATT	GTYGTAGGTTGT TTTGTTTTTTAGT TTT
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* denotes biotinylated primer

Y denotes measured cytosine

Supplemental Table S4. Differentially methylated regions (DMRs) associated with asthma in nasal epithelia.

Chr	Start	End	# Probes	SLK-Sidak p	% Methylation Difference	refGene Name	refGene Distance	refGene Feature	CpG Island	CpG Island Distance	CpG Island Feature
chr1	6341139	6341327	4	0.0007	-0.095	ACOT7	0	intron+cds	CpG: 21	0	island
chr1	9788516	9788769	4	0.0082	0.078	PIK3CD	0	exon+utr3	CpG: 32	-1523	shore
chr1	23888985	23889092	2	0.00092	-0.086	ID3	-2700	intergenic	CpG: 49	2773	shore
chr1	32169567	32169868	4	5.85E-05	-0.069	COL16A1	0	TSS+exon+utr5	CpG: 27	0	island
chr1	35258778	35258933	2	0.00599	-0.098	GJA4	0	intron+utr5	CpG: 29	0	island
chr1	46859670	46859845	6	0.00933	-0.056	FAAH	-93	intergenic	CpG: 71	0	island
chr1	1.12E+08	1.12E+08	4	0.00363	-0.067	ADORA3	0	intron	CpG: 32	0	island
chr1	1.51E+08	1.51E+08	5	0.00056	-0.059	FAM63A;FAM63A; FAM63A;FAM63A	0;0;0;0	TSS+exon+utr5;intron+utr5;intron+	CpG: 42	-1371	shore
chr1	1.55E+08	1.55E+08	3	0.02118	-0.041	EFNA4	1853	intergenic	CpG: 50	0	island
chr1	1.99E+08	1.99E+08	2	0.01128	0.078	PTPRC;PTPRC	0;0	nc_intron;intron	CpG: 21	520681	
chr1	2.44E+08	2.44E+08	5	0.00919	-0.071	ZBTB18	0	intron+utr5	CpG: 16	0	island
chr2	31440348	31440451	2	1.34E-05	-0.135	CAPN14	0	TSS+exon+utr5	CpG: 79	-16289	
chr2	85999497	85999628	2	0.03142	-0.074	ATOH8	0	intron	CpG: 166	17299	
chr2	98552471	98552472	1	0.04382	-0.127	TMEM131	0	intron	CpG: 146	-59261	
chr2	1.03E+08	1.03E+08	1	0.04186	-0.154	SLC9A4	0	exon+utr3	CpG: 111	-85881	
chr2	1.14E+08	1.14E+08	4	0.01533	-0.044	PSD4	0	intron+cds	CpG: 77	0	island
chr2	1.21E+08	1.21E+08	3	0.01985	-0.043	LINC01101	-45366	intergenic	CpG: 26	-10470	
chr2	1.9E+08	1.9E+08	4	0.02885	-0.052	COL5A2	0	TSS+exon+utr5	CpG: 26	-261519	
chr2	2.35E+08	2.35E+08	1	0.00077	-0.204	SPP2	174674	intergenic	CpG: 184	-244051	
chr3	1.02E+08	1.02E+08	1	0.00038	-0.232	LOC152225	177435	intergenic	CpG: 118	325159	
chr3	1.12E+08	1.12E+08	10	0.00024	-0.051	TAGLN3;TAGLN3	0;0	TSS+intron+exon+utr5;TSS+exon+utr5	CpG: 40	19390	
chr3	1.23E+08	1.23E+08	3	0.02329	-0.07	MYLK;MYLK;MYLK-AS1	0;0;0	intron;TSS+exon+utr5;nc_intron	CpG: 16	35115	
chr3	1.49E+08	1.49E+08	3	3.74E-05	-0.068	TM4SF1-AS1	0	nc_exon	CpG: 81	247604	
chr3	1.52E+08	1.52E+08	1	0.01471	-0.212	RP11-454C18.2	0	nc_intron	CpG: 101	-366404	
chr3	1.88E+08	1.88E+08	5	0.00835	-0.048	LPP-AS2	0	nc_exon	CpG: 67	-444	shore

chr4	1.54E+08	1.54E+08	2	0.02936	0.064	KIAA0922	0	intron	CpG: 23	532	shore
chr5	15927183	15927184	1	8.12E-06	-0.193	FBXL7;FBXL7	0;0	intron;intron+utr5	CpG: 34	-904	shore
chr5	80529066	80529340	6	0.00164	-0.057	CKMT2;RNU5E-1;RNU5D-1	0;0;0	TSS+intron+exon+utr5;nc_intron;nc_intron	CpG: 39	-67980	
chr5	96038613	96039066	3	0.00348	-0.097	CAST;CAST;CAST	0;0;0	intron+utr5;intron+exon+utr5;intron	CpG: 57	0	island
chr5	1.07E+08	1.07E+08	1	0.02238	-0.185	EFNA5	0	intron	CpG: 256	-145265	
chr5	1.54E+08	1.54E+08	5	0.00051	-0.066	FAXDC2	0	TSS+exon+utr5	CpG: 118	-6570	
chr6	5026163	5026552	5	0.00165	-0.058	RPP40	-21866	intergenic	CpG: 35	21791	
chr6	10521451	10521715	4	0.00216	-0.066	GCNT2	0	TSS+intron+exon+utr5	CpG: 43	94813	
chr6	26233351	26233442	2	0.04094	-0.092	HIST1H1D	997	intergenic	CpG: 28	-7255	
chr6	28351147	28351351	2	0.01681	-0.066	ZSCAN12	0	nc_intron	CpG: 25	0	island
chr6	29527869	29528119	4	0.02815	-0.089	UBD	-167	intergenic	CpG: 80	6036	
chr6	33041220	33041697	7	1.04E-06	0.098	HLA-DPA1;HLA-DPA1	0;0	intron+utr5+cds;TSS+intron+utr5+cds	CpG: 35	-6719	
chr6	41168800	41169171	7	0.0017	-0.065	TREML2	0	TSS+exon+utr5	CpG: 70	99457	
chr6	1.17E+08	1.17E+08	2	0.00336	-0.111	FAM26D	0	utr3+cds	CpG: 62	-12743	
chr7	2563477	2563909	5	0.00301	-0.042	LFNG	0	intron	CpG: 35	0	island
chr7	29233210	29233705	4	0.00404	-0.026	CHN2	-415	intergenic	CpG: 112	0	island
chr7	47344578	47344750	4	0.03606	0.061	TNS3	0	intron+cds	CpG: 52	50292	
chr7	73442281	73442531	6	0.00241	-0.065	ELN	0	utr5+cds	CpG: 51	-55567	
chr7	97663875	97663940	3	0.0293	-0.055	OCM2	-44459	intergenic	CpG: 51	62062	
chr7	1E+08	1E+08	3	0.0446	-0.051	MIR6875;TRIP6	0;0	nc_gene;intron+cds	CpG: 32	1478	shore
chr7	1.02E+08	1.02E+08	3	3.72E-05	-0.078	CUX1	0	intron	CpG: 20	129022	
chr7	1.05E+08	1.05E+08	5	0.03911	-0.053	ATXN7L1;ATXN7L1	0;0	TSS+intron+exon+utr5;intron	CpG: 27	95586	
chr7	1.06E+08	1.06E+08	1	1.32E-06	-0.273	ATXN7L1	-4084	intergenic	CpG: 88	3152	

chr7	1.07E+08	1.07E+08	4	8.46E-05	-0.083	DUS4L;DUS4L	0;0	nc_intron+nc_exon;intron+exon+utr5	CpG: 59	307	shore
chr7	1.5E+08	1.5E+08	3	2.43E-06	-0.145	ZNF862	0	intron	CpG: 91	7098	
chr7	1.51E+08	1.51E+08	3	0.0013	-0.06	RHEB	-872	intergenic	CpG: 188	0	island
chr8	29210372	29210592	3	0.0134	-0.054	DUSP4	-2105	intergenic	CpG: 28	0	island
chr8	1.34E+08	1.34E+08	4	0.00157	-0.077	TMEM71	0	TSS+exon+utr5	CpG: 53	-14326	
chr8	1.45E+08	1.45E+08	1	0.00363	-0.215	EPPK1	-17562	intergenic	CpG: 20	3314	
chr9	94183342	94183408	2	0.0157	-0.049	NFIL3	0	intron+utr5	CpG: 68	0	island
chr9	1.24E+08	1.24E+08	3	0.00073	-0.077	DAB2IP	0	intron	CpG: 46	0	island
chr9	1.36E+08	1.36E+08	3	0.01125	-0.09	ABO	0	intron	CpG: 145	0	island
chrX	1.33E+08	1.33E+08	3	0.03479	0.053	GPC4	0	intron	CpG: 233	-180	shore
chr10	4386801	4386802	1	2.28E-05	-0.261	LINC00703	-39635	intergenic	CpG: 75	-481323	
chr10	5488224	5488628	8	0.0033	-0.056	NET1;NET1;NET1	0;0;0	nc_exon;intron;TSS+exon+utr5	CpG: 18	0	island
chr10	72362451	72362866	7	0.02585	0.035	PRF1	0	TSS+exon+utr5	CpG: 25	1846	shore
chr10	1.34E+08	1.34E+08	3	0.00077	-0.066	PWWP2B;PWWP2B	0;0	intron;intron+utr3	CpG: 136	2017	shore
chr10	1.35E+08	1.35E+08	9	5.15E-06	-0.104	CYP2E1	0	TSS+utr5+cds	CpG: 119	-230	shore
chr11	7533816	7534329	3	0.00094	-0.068	PPFIBP2	-666	intergenic	CpG: 97	-225	shore
chr11	36030085	36030086	1	0.0029	-0.288	LDLRAD3	0	intron	CpG: 73	63631	
chr11	46534979	46534980	1	0.01223	-0.153	AMBRA1	0	intron	CpG: 45	-80217	
chr11	57267100	57267244	4	7.92E-05	-0.064	SLC43A1	0	intron	CpG: 27	0	island
chr11	57529254	57529614	5	0.00217	-0.068	TMX2-CTNND1;CTNND1	0;0	nc_intron;intron+exon+utr5	CpG: 36	8501	
chr11	63973845	63974229	7	0.00198	0.047	FERMT3	0	TSS+exon+utr5	CpG: 18	-600	shore
chr11	66103385	66103604	4	0.04243	-0.071	RIN1	0	intron+cds	CpG: 22	1106	shore
chr11	87908557	87908938	9	0.00825	-0.067	RAB38	0	TSS+exon+utr5	CpG: 33	0	island
chr11	1.18E+08	1.18E+08	2	7.57E-05	-0.142	SCN2B	-874	intergenic	CpG: 90	24017	
chr11	1.18E+08	1.18E+08	6	0.01235	-0.057	MPZL2	0	intron+utr5+cds	CpG: 22	-95059	
chr11	1.21E+08	1.21E+08	2	4.73E-07	-0.155	SORL1	0	intron+cds	CpG: 73	97903	

chr11	1.23E+08	1.23E+08	4	0.00609	-0.053	GRAMD1B;GRAMD1B	0;0	TSS+exon+utr5;intron	CpG: 27	-16931	
chr12	6881302	6881629	6	0.02943	0.073	LAG3	-40	intergenic	CpG: 44	-1226	shore
chr12	11002196	11002609	4	0.04834	-0.074	PRH1-PRR4	0	nc_intron	CpG: 107	126016	
chr12	28124010	28124233	3	0.00044	-0.089	PTHLH	0	intron+utr5	CpG: 95	237	shore
chr12	28124664	28124870	2	0.00265	-0.078	PTHLH	0	intron+exon+utr5	CpG: 95	891	shore
chr12	58162286	58162287	1	2.12E-05	-0.27	METTL1	63	intergenic	CpG: 91	2286	shore
chr12	89744470	89744524	3	0.04574	-0.069	DUSP6;DUSP6	0;0	intron;cds	CpG: 259	-644	shore
chr12	1.25E+08	1.25E+08	4	0.02923	-0.057	NCOR2	0	intron+utr5	CpG: 34	-10867	
chr12	1.25E+08	1.25E+08	4	0.00085	-0.049	NCOR2	0	intron+utr5	CpG: 34	0	island
chr13	21951378	21951631	2	0.01393	-0.106	ZDHHC20;ZDHHC20;MIPEPP3	0;0;0	nc_intron;intron;nc_intron	CpG: 29	0	island
chr14	23305779	23305957	4	0.01529	-0.054	MMP14	0	exon+utr5	CpG: 100	0	island
chr14	81610811	81610812	1	0.04282	-0.141	TSHR	0	exon+utr3	CpG: 132	-75573	
chr14	1.01E+08	1.01E+08	2	0.03757	0.054	DEGS2	0	intron	CpG: 41	-1848	shore
chr14	1.04E+08	1.04E+08	4	0.02887	0.045	EXOC3L4	0	TSS+utr5+cds	CpG: 258	-1777	shore
chr15	39544143	39544144	1	0.01364	-0.229	C15orf54	0	intron+utr5	CpG: 109	-328383	
chr15	45449436	45449437	1	6.83E-07	-0.227	DUOX1	0	intron	CpG: 18	4731	
chr15	69366046	69366554	4	0.04278	-0.041	MIR548H4	0	nc_intron	CpG: 38	0	island
chr15	1.02E+08	1.02E+08	1	9.70E-05	-0.251	PCSK6;PCSK6	0;0	intron;exon+utr3	CpG: 100	51207	
chr16	21170926	21171239	5	0.04076	-0.064	TMEM159	0	intron+utr5	CpG: 50	648	shore
chr16	67051004	67051005	1	0.00304	-0.094	CES4A	7345	intergenic	CpG: 79	2534	shore
chr16	67563388	67563418	2	0.0352	-0.064	FAM65A	0	intron+utr5	CpG: 74	0	island
chr16	69143314	69143815	4	0.00013	-0.091	HAS3	0	cds	CpG: 33	0	island
chr17	7461259	7461638	5	0.01304	-0.065	TNFSF13;TNFSF13;TNFSF12-TNFSF13	0;0;0	TSS+exon+utr5;nc_exon;intron	CpG: 108	-3028	
chr17	27044628	27045302	9	2.01E-06	-0.064	RAB34;RAB34;RAB34;NARR	0;0;0;0	TSS+intron+utr5+cds;TSS+exon+utr5;TSS+intron+exon+utr5;TSS+utr5+cds	CpG: 95	0	island

chr17	37894164	37894636	6	0.00013	-0.062	GRB7;GRB7	0;0	TSS+exon+utr5;intron+exon+utr5	CpG: 59	7154	
chr17	46632964	46633202	2	0.04272	-0.053	HOXB3	0	intron+exon+utr5	CpG: 36	752	shore
chr17	46646358	46646444	2	0.02512	-0.065	HOXB3	0	intron+utr5	CpG: 56	4248	
chr17	46654201	46654330	3	0.00174	-0.058	HOXB4	0	cds	CpG: 32	0	island
chr17	56401622	56402238	5	0.00071	-0.052	BZRAP1	0	intron+cds	CpG: 54	0	island
chr17	77030220	77030546	5	0.04108	-0.047	C1QTNF1;C1QTNF1;C1QTNF1	0;0;0	intron+utr5;nc_intron;TSS+exon+utr5	CpG: 79	9352	
chr19	940966	941243	3	0.00454	-0.115	ARID3A	0	intron	CpG: 130	0	island
chr19	3369477	3369820	5	0.00042	-0.084	NFIC	0	intron	CpG: 37	0	island
chr19	10077133	10077324	3	0.03006	-0.055	COL5A3	0	intron+cds	CpG: 36	0	island
chr19	41882231	41882327	4	0.00188	-0.066	TMEM91	0	intron+utr5	CpG: 23	-180	shore
chr20	11898477	11898557	3	0.00201	-0.068	BTBD3;BTBD3;BTBD3	0;0;0	TSS+exon+utr5;intron;intron+utr5	CpG: 104	26270	
chr20	13975852	13976190	8	0.04017	-0.069	MACROD2;SEL1L2	0;0	TSS+exon+utr5;nc_exon	CpG: 61	0	island
chr20	33759752	33760017	4	0.01266	-0.051	PROCR	0	utr5+cds	CpG: 30	-2386	shore
chr20	48998615	48998834	3	0.01104	0.059	RP11-290F20.2	-60736	intergenic	CpG: 15	38242	
chr21	16340624	16340722	2	1.43E-07	-0.187	NRIP1	0	exon+utr5	CpG: 148	-95774	
chr21	34776491	34776637	2	0.04039	-0.066	IFNGR2	0	intron	CpG: 99	0	island
chr22	19471092	19471093	1	0.00041	-0.244	CDC45	0	intron	CpG: 109	3281	
chr22	24890689	24890833	7	0.00517	-0.068	ADORA2A-AS1	0	nc_exon	CpG: 98	0	island
chr22	29458675	29458723	2	1.15E-07	-0.141	C22orf31	-768	intergenic	CpG: 54	-8568	
chr22	38201495	38201690	3	0.00448	-0.056	H1FO	0	cds	CpG: 89	0	island
chr22	41763092	41763512	10	0.0023	-0.047	TEF	0	TSS+exon+utr5	CpG: 93	-13918	

Supplemental Table S5. Differentially methylated probes (DMPs) associated with asthma in nasal epithelia.

Chr	Start	End	% Methylation Difference	p Value	q Value	refGene Name	refGene Distance	refGene Feature	CpG Island	CpG Island Distance	CpG Island Feature
chr1	3447347	3447348	-0.074	1.38E-06	0.00613	MEGF6	0	intron	CpG: 57	-101	shore
chr1	6341286	6341287	-0.114	1.30E-06	0.00594	ACOT7	0	cds	CpG: 21	0	island
chr1	8585851	8585852	-0.082	8.11E-07	0.00410	RERE	0	intron	CpG: 80	101512	
chr1	35258932	35258933	-0.176	4.29E-10	0.00001	GJA4	0	intron+utr5	CpG: 29	141	shore
chr1	46030073	46030074	-0.101	3.18E-08	0.00041	AKR1A1	0	intron	CpG: 27	13379	
chr1	82063628	82063629	-0.139	6.25E-08	0.00065	LPHN2	-202452	intergenic	CpG: 274	-202369	
chr1	1.53E+08	1.53E+08	-0.149	2.16E-07	0.00159	LCE3D	-263	intergenic	CpG: 27	64973	
chr1	1.57E+08	1.57E+08	-0.109	4.98E-08	0.00055	NTRK1	0	intron	CpG: 166	2060	shore
chr1	1.84E+08	1.84E+08	-0.162	5.38E-07	0.00304	NCF2	0	intron	CpG: 101	-66513	
chr2	22504249	22504250	-0.123	1.86E-07	0.00139	AC096570.1	-255100	intergenic	CpG: 183	-1103479	
chr2	31440348	31440349	-0.141	7.73E-07	0.00395	CAPN14	0	exon+utr5	CpG: 79	-16391	
chr2	31440450	31440451	-0.129	6.29E-08	0.00065	CAPN14	-39	intergenic	CpG: 79	-16289	
chr2	43986105	43986106	-0.133	1.51E-06	0.00627	PLEKHH2	0	cds	CpG: 39	-72659	
chr2	67516851	67516852	-0.104	1.63E-07	0.00126	LOC10280044	475	intergenic	CpG: 50	-107520	
chr2	70322658	70322659	-0.055	8.53E-08	0.00078	PCBP1	6324	intergenic	CpG: 186	7168	
chr2	75283270	75283271	-0.095	2.42E-07	0.00170	TACR1	0	intron	CpG: 74	97093	
chr2	98552471	98552472	-0.127	4.68E-09	0.00008	TMEM131	0	intron	CpG: 146	-59261	
chr2	1.03E+08	1.03E+08	-0.154	4.42E-09	0.00008	SLC9A4	0	exon+utr3	CpG: 111	-85881	
chr2	1.22E+08	1.22E+08	-0.122	1.55E-06	0.00627	CLASP1	0	intron	CpG: 51	-824	shore
chr2	1.39E+08	1.39E+08	-0.142	2.44E-08	0.00033	HNMT	109409	intergenic	CpG: 84	-375689	
chr2	1.81E+08	1.81E+08	-0.189	7.35E-07	0.00384	CWC22	-172551	intergenic	CpG: 27	172457	
chr2	2.35E+08	2.35E+08	-0.204	3.57E-11	0.00000	SPP2	174674	intergenic	CpG: 184	-244051	
chr2	2.35E+08	2.35E+08	-0.080	2.79E-07	0.00188	SPP2	181154	intergenic	CpG: 184	-237571	
chr3	1.02E+08	1.02E+08	-0.232	1.51E-11	0.00000	LOC152225	177435	intergenic	CpG: 118	325159	
chr3	1.12E+08	1.12E+08	-0.104	1.27E-06	0.00589	TAGLN3	0;0	exon+utr5;intron+utr5	CpG: 40	19918	
chr3	1.29E+08	1.29E+08	-0.102	1.04E-07	0.00089	RAB7A	0	intron+utr5	CpG: 30	22468	
chr3	1.51E+08	1.51E+08	-0.123	1.17E-07	0.00098	MED12L	0	intron	CpG: 227	58404	
chr3	1.52E+08	1.52E+08	-0.212	1.24E-09	0.00003	RP11-454C18.1	0	nc_intron	CpG: 101	-366404	
chr3	1.57E+08	1.57E+08	-0.154	7.97E-09	0.00013	VEPH1	0	cds	CpG: 67	22307	

chr3	1.7E+08	1.7E+08	-0.134	8.92E-07	0.00438	LRRC31	0	cds	CpG: 58	57018	
chr4	3640480	3640481	-0.108	1.04E-07	0.00089	RP3-513G18.2	34838	intergenic	CpG: 66	-3288	
chr4	11531390	11531391	-0.207	1.04E-08	0.00016	HS3ST1	-100853	intergenic	CpG: 86	100134	
chr4	1.46E+08	1.46E+08	-0.101	1.54E-06	0.00627	ANAPC10	0;0;0	intron+utr5;nc_intron;intr	CpG: 59	-581	shore
chr4	1.54E+08	1.54E+08	0.115	1.93E-10	0.00001	KIAA0922	0	intron	CpG: 23	669	shore
chr5	15927183	15927184	-0.193	1.50E-13	0.00000	FBXL7	0;0	intron;intron+utr5	CpG: 34	-904	shore
chr5	1.07E+08	1.07E+08	-0.185	2.06E-09	0.00004	EFNA5	0	intron	CpG: 256	-145265	
chr5	1.32E+08	1.32E+08	-0.121	1.50E-06	0.00627	C5orf56	0	nc_intron	CpG: 72	8051	
chr5	1.39E+08	1.39E+08	-0.118	3.16E-07	0.00210	PAIP2;PAIP2	0;0	nc_intron;intron	CpG: 43	-11541	
chr5	1.49E+08	1.49E+08	-0.191	2.07E-08	0.00029	PDE6A	0	cds	CpG: 102	-99402	
chr5	1.71E+08	1.71E+08	-0.078	3.57E-07	0.00227	FBXW11	0	intron	CpG: 90	-60309	
chr6	2977292	2977293	-0.241	4.27E-08	0.00050	SERPINB6	-4893	intergenic	CpG: 106	5330	
chr6	41168910	41168911	-0.127	2.22E-07	0.00159	TREML2	0	exon+utr5	CpG: 70	99567	
chr6	41168940	41168941	-0.118	4.32E-08	0.00050	TREML2	-15	intergenic	CpG: 70	99597	
chr6	1.06E+08	1.06E+08	-0.066	1.39E-06	0.00614	PRDM1	-94404	intergenic	CpG: 91	-2196	shore
chr6	1.17E+08	1.17E+08	-0.214	4.11E-12	0.00000	FAM26D	0	exon+utr3	CpG: 62	-12743	
chr6	1.65E+08	1.65E+08	-0.111	1.46E-06	0.00627	QKI	531947	intergenic	CpG: 52	137751	
chr7	857980	857981	-0.099	4.95E-08	0.00055	SUN1	0;0	intron;intron+utr5	CpG: 99	1233	shore
chr7	2851990	2851991	-0.156	4.95E-07	0.00290	GNA12	0	intron	CpG: 94	-31447	
chr7	22882511	22882512	-0.158	6.38E-09	0.00011	SNORD93	-13719	intergenic	CpG: 70	-11282	
chr7	29233475	29233476	-0.053	1.36E-06	0.00613	CHN2	-644	intergenic	CpG: 112	-228	shore
chr7	1.02E+08	1.02E+08	-0.094	1.51E-07	0.00120	CUX1	0	intron	CpG: 20	129022	
chr7	1.06E+08	1.06E+08	-0.273	1.69E-14	0.00000	ATXN7L1	-4084	intergenic	CpG: 88	3152	
chr7	1.07E+08	1.07E+08	-0.197	1.19E-09	0.00003	DUS4L	0;0	intron+utr5;nc_intron	CpG: 59	594	shore
chr7	1.08E+08	1.08E+08	-0.197	3.68E-08	0.00045	NRCAM	0	intron+utr5	CpG: 177	-125564	
chr7	1.5E+08	1.5E+08	-0.118	1.50E-06	0.00627	ZNF862	0	intron	CpG: 91	7098	
chr7	1.5E+08	1.5E+08	-0.150	1.55E-06	0.00627	ZNF862	0	intron	CpG: 91	7127	
chr7	1.5E+08	1.5E+08	-0.166	1.02E-07	0.00089	ZNF862	0	intron	CpG: 91	7139	
chr8	59105651	59105652	-0.095	5.90E-07	0.00322	FAM110B	43374	intergenic	CpG: 90	46237	
chr8	68022261	68022262	-0.121	1.08E-06	0.00514	CSPP1	0	intron	CpG: 85	44813	
chr8	90794646	90794647	0.071	5.49E-07	0.00306	RIPK2	0	intron	CpG: 92	24189	
chr8	1.45E+08	1.45E+08	-0.215	2.30E-10	0.00001	EPPK1	-17562	intergenic	CpG: 20	3314	
chr9	97545513	97545514	-0.072	8.53E-07	0.00423	C9orf3	0	intron	CpG: 40	56341	
chr10	4386801	4386802	-0.261	5.18E-13	0.00000	LINC00703	-39635	intergenic	CpG: 75	-481323	

chr10	95228649	95228650	-0.111	1.00E-06	0.00480	MYOF	0	intron	CpG: 54	-27483	
chr10	1.34E+08	1.34E+08	-0.108	2.53E-07	0.00175	JAKMIP3	-39224	intergenic	CpG: 23	0	island
chr10	1.35E+08	1.35E+08	-0.168	3.57E-08	0.00044	CYP2E1	-421	intergenic	CpG: 119	-810	shore
chr11	3815588	3815589	-0.116	7.16E-08	0.00071	NUP98	0	intron+utr5	CpG: 88	-2858	shore
chr11	36030085	36030086	-0.288	1.75E-10	0.00001	LDLRAD3	0	intron	CpG: 73	63631	
chr11	46534979	46534980	-0.153	9.92E-10	0.00002	AMBRA1	0	intron	CpG: 45	-80217	
chr11	57267100	57267101	-0.072	4.73E-07	0.00280	SLC43A1	0	intron	CpG: 27	0	island
chr11	72411399	72411400	-0.101	2.20E-07	0.00159	ARAP1	0	intron	CpG: 39	2886	shore
chr11	1.18E+08	1.18E+08	-0.127	4.72E-07	0.00280	SCN2B	-874	intergenic	CpG: 90	24017	
chr11	1.18E+08	1.18E+08	-0.156	1.59E-08	0.00024	SCN2B	-878	intergenic	CpG: 90	24021	
chr11	1.21E+08	1.21E+08	-0.120	3.71E-07	0.00231	SORL1	0	intron	CpG: 73	97903	
chr11	1.21E+08	1.21E+08	-0.189	9.48E-10	0.00002	SORL1	0	cds	CpG: 73	97995	
chr12	28124664	28124665	-0.080	1.53E-06	0.00627	PTHLH	0	intron+utr5	CpG: 95	891	shore
chr12	58162286	58162287	-0.270	4.74E-13	0.00000	METTL1	63	intergenic	CpG: 91	2286	shore
chr12	71587793	71587794	-0.123	3.33E-07	0.00215	TSPAN8	-36014	intergenic	CpG: 104	-245398	
chr12	1.23E+08	1.23E+08	-0.054	1.15E-06	0.00541	CLIP1	0;0	nc_exon;intron+utr5	CpG: 72	-22050	
chr13	38172802	38172803	-0.116	7.47E-08	0.00073	POSTN	0	cds	CpG: 45	-270879	
chr14	23769998	23769999	-0.069	6.87E-07	0.00362	PPP1R3E	0	exon+utr3	CpG: 155	-513	shore
chr14	53418878	53418879	-0.092	4.23E-07	0.00257	FERMT2	-1063	intergenic	CpG: 137	539	shore
chr14	78446339	78446340	-0.136	5.64E-07	0.00311	ADCK1	46042	intergenic	CpG: 37	-999	shore
chr14	81610811	81610812	-0.141	4.55E-09	0.00008	TSHR	0	exon+utr3	CpG: 132	-75573	
chr14	91017835	91017836	-0.077	2.68E-08	0.00035	TTC7B	0	intron	CpG: 18	9926	
chr14	1.04E+08	1.04E+08	-0.083	1.39E-07	0.00115	LINC00637	22204	intergenic	CpG: 197	31600	
chr15	22891953	22891954	-0.094	8.52E-08	0.00078	CYFIP1	-694	intergenic	CpG: 184	0	island
chr15	27784587	27784588	-0.115	1.58E-07	0.00124	GABRG3	6214	intergenic	CpG: 40	-2130	shore
chr15	39544143	39544144	-0.229	1.13E-09	0.00003	C15orf54	0	intron+utr5	CpG: 109	-328383	
chr15	45449436	45449437	-0.227	7.62E-15	0.00000	DUOX1	0	intron	CpG: 18	4731	
chr15	90455798	90455799	-0.092	8.27E-07	0.00414	C15orf38	0;0;0	intron;intron;exon+utr5	CpG: 71	0	island
chr15	1.02E+08	1.02E+08	-0.251	2.95E-12	0.00000	PCSK6;PCSK6	0;0	intron;exon+utr3	CpG: 100	51207	
chr15	1.02E+08	1.02E+08	-0.143	1.83E-07	0.00138	PCSK6	0	intron	CpG: 176	-223	shore
chr16	67051004	67051005	-0.094	1.85E-10	0.00001	CES4A	7345	intergenic	CpG: 79	2534	shore
chr16	85655006	85655007	-0.115	1.26E-06	0.00589	GSE1;GSE1	0;0	intron;intron+utr5	CpG: 23	-3735	
chr16	86653214	86653215	-0.156	6.19E-07	0.00334	FOX1	37910	intergenic	CpG: 154	39393	

chr16	88558222	88558223	-0.295	5.29E-10	0.00001	ZFPM1	0	intron	CpG: 22	0	island
chr17	4541333	4541334	-0.101	8.54E-08	0.00078	ALOX15	0	intron	CpG: 146	-1381	shore
chr17	15394552	15394553	-0.088	1.74E-08	0.00025	TVP23C-CDRT4	0;0	nc_intron;intron	CpG: 42	-71806	
chr17	17088576	17088577	0.069	1.33E-06	0.00604	MPRIP	0	exon+utr3	CpG: 95	-20194	
chr17	29667325	29667326	-0.142	5.49E-08	0.00059	NF1	0	intron	CpG: 29	-5009	
chr17	38599365	38599366	-0.076	3.60E-07	0.00227	IGFBP4	-309	intergenic	CpG: 20	0	island
chr17	62307786	62307787	-0.098	3.81E-07	0.00234	TEX2	0	intron+utr5	CpG: 99	-32016	
chr17	62309292	62309293	-0.159	3.31E-07	0.00215	TEX2	0	intron+utr5	CpG: 99	-30510	
chr17	67557526	67557527	-0.080	7.01E-08	0.00071	MAP2K6	19056	intergenic	CpG: 116	233689	
chr17	75181835	75181836	-0.142	6.86E-07	0.00362	SEC14L1	0;0	intron;intron+utr5	CpG: 18	-27623	
chr18	33529996	33529997	-0.052	7.50E-07	0.00387	C18orf21	-22590	intergenic	CpG: 53	0	island
chr18	43652436	43652437	-0.084	1.40E-06	0.00614	PSTPIP2	-186	intergenic	CpG: 80	0	island
chr19	6459299	6459300	-0.070	5.13E-07	0.00296	SLC25A23	0	intron	CpG: 117	0	island
chr20	11898477	11898478	-0.086	1.48E-07	0.00120	BTBD3	0;0	intron;intron+utr5	CpG: 104	26270	
chr20	17680944	17680945	-0.139	1.71E-08	0.00025	BANF2	0;0	intron+utr5;intron	CpG: 177	17488	
chr20	48998833	48998834	0.102	9.28E-08	0.00083	RP11-290F20.2	-60954	intergenic	CpG: 15	38460	
chr21	16340624	16340625	-0.212	1.46E-11	0.00000	NRIP1	0	exon+utr5	CpG: 148	-95871	
chr21	16340721	16340722	-0.162	2.71E-07	0.00185	NRIP1	0	exon+utr5	CpG: 148	-95774	
chr22	19471092	19471093	-0.244	1.68E-11	0.00000	CDC45	0	intron	CpG: 109	3281	
chr22	29458675	29458676	-0.076	5.21E-07	0.00297	C22orf31	-768	intergenic	CpG: 54	-8615	
chr22	29458722	29458723	-0.205	3.44E-11	0.00000	C22orf31	-815	intergenic	CpG: 54	-8568	
chr22	38819701	38819702	0.085	1.46E-06	0.00627	KCNJ4	2630	intergenic	CpG: 98	-3177	

Supplemental Table S6. Methylation status in nasal epithelia of 81 asthma-associated DMRs from analysis of the pBMC epigenome in the same study population.

Chr	Start	End	# Probes	PBMC SLK p value	PBMC SLK-Sidak p value	Gene	Location	Nasal epithelial cell SLK p value
chr1	6341139	6341327	4	3.79E-08	9.79E-05	ACOT7;BACH	exon;intron;nc_exon;nc_intron	4.14E-08
chr6	31846768	31847028	10	6.90E-06	0.01281	SLC44A4	utr5	1.65E-06
chr16	88558064	88558237	3	1.59E-06	0.004442	ZFPM1	intron	7.55E-06
chr17	41278134	41278429	12	1.59E-08	2.62E-05	NBR2	nc_intron	9.53E-05
chr1	21900986	21901260	3	3.79E-06	0.006684	ALPL	intron	0.0001325
chr10	126330844	126331180	4	2.03E-07	0.0002926	FAM53B	intron	0.000309
chr1	110306506	110306642	3	5.01E-07	0.001787	EPS8L3;EPS8R3	utr5	0.001151
chr1	6355007	6355060	2	1.41E-07	0.001295	ACOT7;BACH	exon;intron;nc_exon;nc_intron	0.003273
chr8	127569580	127569677	4	8.24E-07	0.004117	FAM84B	exon;utr5	0.004076
chr1	154300116	154300241	2	5.46E-06	0.021	ATP8B2	utr5_intron	0.01168
chr16	85676291	85676526	4	1.86E-05	0.03767	KIAA0182	intron;utr5_intron	0.01661
chr16	85551477	85551748	3	1.59E-06	0.002845	KIAA0182	93281	0.0221
chr8	125462981	125463310	6	1.73E-06	0.002554	TRMT12	exon;utr5	0.02409
chr10	101824919	101825185	5	3.92E-06	0.00713	CPN1	exon;intron	0.02932
chr1	2230600	2230923	4	4.52E-07	0.0006791	SKI	intron	0.0431
chr17	79615651	79615861	4	4.00E-06	0.009209	OCSP;TSPAN10	nc_exon;utr3	0.04494
chr10	134222217	134222507	4	2.63E-06	0.004397	PWWP2B	intron;utr3_intron	0.05256
chr11	34460297	34460557	7	7.88E-07	0.00147	CAT	utr5	0.05552
chr1	19011015	19011172	2	3.83E-06	0.01177	PAX7	intron	0.05623
chr16	86714535	86714834	4	1.63E-06	0.002635	BC041439	39457	0.0566
chr10	3823757	3824016	5	8.39E-09	1.57E-05	KLF6	exon;intron	0.06753
chr21	47845609	47845863	4	1.79E-09	3.42E-06	PCNT	exon;intron	0.09023
chr1	229163551	229163577	2	2.00E-06	0.03665	AX748369	-110597	0.0933
chr1	21877523	21877781	5	3.59E-06	0.006726	ALPL	utr5_intron	0.1125
chr19	55314878	55315083	6	3.44E-06	0.008115	KIR2DL4;KIR2DS2;KIR3DL1	intron;utr5	0.1297
chr3	11651535	11651881	3	1.25E-07	0.000175	VGLL4	intron	0.1426
chr20	35504370	35504553	4	1.33E-06	0.003526	C20orf118	17	0.1469
chr19	1256998	1257217	3	1.38E-05	0.03013	MIDN	exon	0.1564

chr15	91369956	91370233	2	2.72E-05	0.0465	BLM	-11270	0.1609
chr3	114012658	114012912	4	1.67E-05	0.03144	TIGIT	exon;utr5	0.1812
chr6	31867697	31868025	20	2.89E-08	4.28E-05	DKFZp779M0311;ZBTB12	exon;utr3;utr5_intron	0.1815
chr13	114172889	114173024	3	1.19E-05	0.04192	TMCO3	intron	0.2276
chr2	232393063	232393256	3	1.15E-08	2.90E-05	NMUR1	exon	0.2452
chr1	25240864	25240938	3	1.63E-07	0.001069	RUNX3	intron	0.309
chr2	242867554	242867611	2	5.56E-07	0.004724	AK097934	45223	0.3133
chr2	8423121	8423155	2	1.02E-06	0.01438	AK127578;BC043563	nc_intron	0.3202
chr7	73645722	73645966	4	9.19E-08	0.0001827	RFC2	utr3	0.3204
chr2	8422235	8422332	3	9.66E-08	0.0004833	AK127578;BC043563	nc_intron	0.3321
chr1	27683138	27683501	5	1.96E-07	0.0002621	MAP3K6	exon;intron	0.3472
chr7	38356728	38357047	4	2.71E-06	0.004121	TCRg;TRGV9	exon;intron;nc_exon	0.3815
chr11	36421229	36421301	2	7.07E-07	0.004753	FLJ14213	intron;utr5_intron	0.4064
chr7	2089599	2089880	3	7.89E-07	0.001363	MAD1;MAD1L1	intron	0.4194
chr17	80880758	80880952	3	1.15E-06	0.002882	KIAA0988;TBCD	intron	0.4279
chr5	131993613	131994061	6	5.34E-08	5.79E-05	IL13	exon;intron;utr5	0.4356
chr2	27799206	27799411	3	7.47E-06	0.01754	C2orf16	utr5	0.438
chr10	858816	858923	3	8.24E-06	0.0367	LARP5	exon;utr3	0.4393
chr1	215740423	215740701	3	2.66E-05	0.0453	KCTD3	34	0.4554
chr1	45273494	45273680	3	1.74E-05	0.04438	TCTEX1D4	-537	0.4701
chr5	125695686	125695848	3	3.32E-10	9.96E-07	GRAMD3	63253	0.474
chr12	123596154	123596413	2	3.43E-08	6.43E-05	PITPNM2	utr5_intron	0.4744
chr6	28911758	28912166	10	8.39E-07	0.0009973	TRIM27	-19990	0.4764
chr11	62622172	62622234	3	1.62E-06	0.01262	SNHG1	nc_intron	0.4818
chr7	75053026	75053196	3	1.05E-05	0.0296	POM121C	intron	0.4982
chr3	52812366	52812520	4	5.39E-06	0.01686	ITIH1	exon;nc_exon	0.5327
chr16	88547578	88547861	3	1.39E-05	0.02358	ZFPM1	intron	0.5663
chr1	1101301	1101462	4	5.59E-07	0.001684	CS444397	1075	0.5685
chr20	55982702	55982978	3	3.23E-06	0.005656	RBM38	exon;utr3	0.5812
chr7	2728775	2728912	3	8.55E-06	0.02983	AMZ1;KIAA1950	utr5_intron	0.5893
chr2	66664534	66664798	3	4.82E-07	0.0008866	MEIS1	intron	0.6073
chr4	1243848	1244086	7	5.29E-07	0.001079	AK125775	nc_exon	0.615
chr19	18260329	18260515	4	3.31E-08	8.64E-05	MAST3	exon	0.6401
chr6	109774737	109775161	3	1.42E-08	1.62E-05	MICAL1	exon;intron	0.705

chr17	40125607	40125781	3	5.59E-08	0.0001559	CNP	exon	0.7071
chr19	51220097	51220537	9	1.02E-07	0.000113	SHANK1	exon;utr5	0.7141
chr12	131568440	131568647	4	1.33E-05	0.03062	DKFZp434B1272;GPR133	intron;utr5_intron	0.7275
chr22	46685471	46685728	4	3.11E-09	5.88E-06	TTC38	exon;intron	0.7287
chr17	72757292	72757302	2	9.75E-07	0.04621	NHERF;SLC9A3R1	intron;nc_intron	0.7297
chr17	80393503	80393666	4	1.35E-05	0.03938	HEXDC	exon;intron	0.7378
chr7	922050	922235	3	3.47E-08	9.11E-05	C7orf20;UNC84A	intron;nc_intron	0.7674
chr11	129980477	129980529	3	2.10E-06	0.01937	APLP2	exon;utr5_intron	0.7682
chr17	47297267	47297512	2	1.18E-05	0.02301	ABI3	exon;intron	0.7826
chr6	513210	513297	2	4.91E-06	0.027	EXOC2	intron	0.7922
chr13	114806954	114807180	3	1.34E-05	0.02828	RASA3	intron;utr5_intron	0.8069
chr6	26226202	26226256	2	1.41E-07	0.001263	HIST1H3F	utr3	0.8089
chr14	99787362	99787620	3	3.11E-06	0.00583	BCL11B	-49540	0.8129
chr19	10662581	10662782	5	1.46E-05	0.03466	APG4-D;ATG4D	exon;intron;nc_exon;nc_intron;utr5	0.8322
chr14	105184056	105184176	3	2.80E-06	0.01125	INF2	intron;nc_intron;utr3_intron	0.8372
chr10	3824386	3824687	4	3.08E-08	4.96E-05	KLF6	exon;intron	0.8462
chr10	72360347	72360448	3	1.27E-07	0.0006084	PRF1	exon	0.8717
chr1	15392307	15392433	2	4.38E-10	1.69E-06	HRIHFB2003;KIAA1026	intron;utr3	0.9432
chr11	64533346	64533623	3	3.71E-06	0.006473	SF1	exon;intron	0.9681

Supplemental Table S7. Differentially methylated regions (DMRs) associated with environmental tobacco smoke exposure (ETS) in nasal epithelia.

Chr	Start	End	# Probes	SLK-Sidak p	% Methylati on Difference	refGene Name	refGene Distance	refGene Feature	CpG Island	CpG Island Distance	CpG Island Feature
chr21	47604051	47604654	5	0.000597	-0.216	SPATC1L	0	TSS+intron+ exon+utr5	CpG: 28	1311	shore
chr13	1.14E+08	1.14E+08	2	0.05	0.073	MCF2L	0	intron	CpG: 18	-4526	NA
chr10	22765644	22766320	5	0.00289	0.031	LOC100499489	-38786	intergenic	CpG: 217	0	island
chr10	90966523	90967233	10	0.01553	0.059	CH25H	0	TSS+cds	CpG: 33	-322	shore
chr10	1.04E+08	1.04E+08	4	0.000939	0.078	FGF8	0	utr3+cds	CpG: 102	-5381	NA
chr10	1.35E+08	1.35E+08	6	0.01215	-0.033	LINC01168	48037	intergenic	CpG: 88	2139	shore
chr12	6166873	6167147	4	0.0377	0.079	VWF	0	intron+cds	CpG: 30	0	island
chr22	30476088	30476525	11	0.000662	0.032	HORMAD2- AS1;HORMAD2	0;0	nc_intron+n c_exon;TSS+ intron+exon +utr5	CpG: 50	0	island
chr6	31275147	31276669	26	2.19E-06	-0.075	HLA-C	-35234	intergenic	CpG: 38	0	island
chr6	32063773	32064613	26	0.000302	-0.066	TNXB	0	cds	CpG: 150	0	island
chr6	33033175	33033484	3	4.29E-05	0.359	HLA-DPA1	0	intron+utr3	CpG: 35	-14932	NA
chr6	1.18E+08	1.18E+08	6	0.02246	0.037	DCBLD1	-1033	intergenic	CpG: 112	-711	shore
chr8	1049166	1049477	5	0.01604	-0.051	ERICH1-AS1	0	nc_intron	CpG: 48	293	shore
chr8	19796298	19796844	7	0.000829	-0.045	LPL	0	TSS+exon+u tr5	CpG: 112	0	island
chr8	21899861	21900438	7	0.0171	0.019	FGF17	0	TSS+exon+u tr5	CpG: 28	-5023	NA
chr8	41167801	41168481	10	0.005745	0.034	SFRP1	-811	intergenic	CpG: 147	661	shore
chr8	1.05E+08	1.05E+08	11	0.02097	0.035	DPYS	0	TSS+intron+ utr5+cds	CpG: 74	0	island

chr5	1093579	1094035	5	0.004668	0.059	SLC12A7	0	intron+cds	CpG: 23	0	island
chr5	72510845	72511061	3	0.000522	0.063	TMEM174	39875	intergenic	CpG: 62	-659	shore
chr4	53588359	53588850	6	0.006775	-0.103	DANCR	8054	intergenic	CpG: 46	0	island
chr7	4652670	4652842	4	0.02486	0.045	FO XK1	-69087	intergenic	CpG: 91	-28591	NA
chr3	1.81E+08	1.81E+08	4	0.008704	0.05	SOX2-OT	0	nc_intron	CpG: 100	7680	NA
chr16	8806358	8807043	13	1.24E-05	0.04	ABAT	0;0	TSS+exon+u tr5;intron+u tr5	CpG: 45	37739	NA
chr15	68120756	68121177	3	0.02818	-0.042	SKOR1	0	intron	CpG: 542	0	island
chr20	43935221	43935551	10	0.004638	0.051	MATN4;RBPJL	0;0	intron+utr5; TSS+exon+u tr5	CpG: 39	0	island
chr14	37123677	37124434	7	0.009666	0.042	PAX9	-2338	intergenic	CpG: 39	-785	shore
chr14	37132489	37132774	4	0.001391	-0.063	PAX9	0	intron+cds	CpG: 121	0	island
chr14	1.05E+08	1.05E+08	6	0.0168	0.037	C14orf180	-105081	intergenic	CpG: 23	0	island
chrX	75004942	75005360	8	4.79E-08	0.063	MAGEE2	0	TSS+exon	CpG: 26	-243968	NA
chrX	1.18E+08	1.18E+08	10	0.02946	0.038	KIAA1210	0	TSS+cds	CpG: 39	0	island
chrX	1.54E+08	1.54E+08	10	0.007501	0.046	DNASE1L1	0;0;0;0	intron+utr5; TSS+exon+u tr5;intron+e xon+utr5;TS S+intron+ex on+utr5	CpG: 93	-1907	shore
chr2	1.09E+08	1.09E+08	6	0.003016	0.036	SULT1C4	0	TSS+exon+u tr5	CpG: 47	-70981	NA
chr2	1.6E+08	1.6E+08	4	0.008665	-0.038	DAPL1	0	TSS+intron+ utr5+cds	CpG: 127	-172835	NA
chr2	2.23E+08	2.23E+08	14	0.000134	0.071	CCDC140;PAX3	0;0	TSS+intron+ exon+utr5;T SS+intron+u tr5+cds	CpG: 75	0	island
chr2	2.23E+08	2.23E+08	6	0.006335	-0.068	CCDC140	0	intron+utr5+ cds	CpG: 26	0	island

chr2	2.23E+08	2.23E+08	8	0.000258	-0.057	CCDC140	6230	intergenic	CpG: 72	0	island
chr1	25257504	25258332	21	1.14E-05	0.044	RUNX3	0	intron	CpG: 311	0	island
chr1	75198210	75199117	11	0.000686	-0.065	TYW3;TYW3;CRYZ	0;0;0	TSS+intron+ utr5+cds;nc _exon;TSS+i ntron+exon +utr5	CpG: 40	-391700	NA
chr1	1.1E+08	1.1E+08	10	0.03667	0.062	GSTM5	0	TSS+intron+ utr5+cds	CpG: 32	24047	NA
chr1	2.06E+08	2.06E+08	8	0.00041	0.111	PM20D1	0	TSS+intron+ utr5+cds	CpG: 23	0	island
chr1	2.13E+08	2.13E+08	4	1.00E-05	0.072	LOC101929565	-30038	intergenic	CpG: 42	313	shore
chr19	2041904	2042088	5	0.02795	-0.054	MKNK2	0	intron+cds	CpG: 77	0	island
chr19	2607725	2607903	4	0.005284	-0.043	GNG7	0	intron+utr5	CpG: 18	0	island
chr19	4153363	4153713	7	0.01746	0.083	CREB3L3	0	TSS+exon+u tr5	CpG: 29	-19440	NA
chr19	10736005	10736448	8	0.008151	-0.026	SLC44A2	0;0	TSS+intron+ utr5+cds;int ron	CpG: 46	0	island
chr19	37825008	37825679	8	0.03561	-0.046	HKR1	0	TSS+exon+u tr5	CpG: 45	0	island
chr19	38684236	38684328	4	0.04134	0.083	SIPA1L3	0	cds	CpG: 26	0	island
chr19	44306564	44307056	5	0.01252	0.006	LYPD5	0;0	intron+utr5; TSS+exon+u tr5	CpG: 56	3388	NA

Supplemental Table S8. Differentially expressed genes ($q < 0.05$) in asthmatic compared to control nasal epithelia.**(A)** Statistics for Differential Expression

Probe ID	Gene	SystematicName	Average Expression	p value	q value	log (Fold Change)
A_19_P00804124	A_19_P00804124	lincRNA:chr14:23018	9.514	1.91E-05	0.0187	-0.999
A_23_P55373	ALOX15	NM_001140	8.652	1.55E-05	0.0171	1.018
A_33_P3361348	ALOX15	NM_001140	7.485	1.47E-06	3.75E-03	1.087
A_24_P87036	ANO1	NM_018043	5.205	1.24E-06	3.75E-03	0.724
A_33_P3263614	ASAP3	NM_001143778	5.031	7.27E-06	0.0115	0.323
A_23_P128744	BDKRB1	NM_000710	6.875	9.42E-05	0.0420	0.373
A_32_P167076	CAPN14	NM_001145122	5.712	1.34E-04	0.0475	1.072
A_23_P35912	CASP4	NM_033306	11.190	5.17E-05	0.0329	-0.214
A_23_P425681	CCK	NM_000729	6.059	7.83E-09	1.45E-04	1.190
A_23_P152838	CCL5	NM_002985	7.954	6.53E-05	0.0344	-0.846
A_23_P98410	CD3G	NM_000073	7.147	1.33E-04	0.0475	-0.580
A_24_P188377	CD55	NM_000574	10.569	1.56E-05	0.0171	0.542
A_23_P311875	CD6	NM_006725	6.695	1.07E-04	0.0432	-0.582
A_23_P70095	CD74	NM_001025158	8.739	1.27E-04	0.0470	-0.599
A_23_P502957	CDH26	NM_021810	9.273	3.14E-05	0.0268	1.024
A_23_P153301	CEACAM5	NM_004363	10.511	2.78E-05	0.0248	0.566
A_23_P18017	CPA3	NM_001870	5.861	1.38E-07	5.65E-04	1.674
A_23_P112859	CST1	NM_001898	6.111	1.41E-08	1.45E-04	3.434
A_33_P3268472	CTSC	NM_001114173	7.039	4.63E-05	0.0322	0.503
A_23_P1552	CTSC	NM_001814	8.377	5.15E-08	3.52E-04	0.759
A_23_P140384	CTSG	NM_001911	5.042	7.01E-08	3.60E-04	1.254
A_23_P109913	CXCR6	NM_006564	6.669	8.63E-06	0.0127	-0.812
A_23_P129014	EFCAB11	NM_145231	6.712	9.61E-05	0.0420	-0.350
A_23_P156497	ELOVL5	NM_021814	7.594	8.37E-05	0.0391	0.441
A_23_P108501	EPHA4	NM_004438	6.895	6.31E-05	0.0344	0.564
A_33_P3388391	GJB4	NM_153212	7.039	3.46E-05	0.0284	0.522
A_33_P3423365	GSN	NM_001127663	9.104	4.41E-06	8.24E-03	0.611
A_32_P351968	HLA-DMB	NM_002118	10.056	4.70E-05	0.0322	-0.649
A_32_P356316	HLA-DOA	NM_002119	9.075	1.04E-04	0.0432	-0.972
A_32_P42197	HNRNPA1	NM_031157	11.356	1.07E-04	0.0432	-0.236
A_23_P99906	HOMER2	NM_199330	6.965	4.49E-05	0.0322	0.464
A_23_P95790	ITLN1	NM_017625	6.236	1.52E-05	0.0171	2.260

A_23_P148475	KIF4A	NM_012310	4.729	5.10E-05	0.0329	-0.431
A_23_P99275	KLRB1	NM_002258	7.296	5.41E-05	0.0329	-0.856
A_33_P3242543	MAOA	NM_000240	8.690	8.79E-05	0.0401	0.475
A_33_P3245922	MAP3K4	NM_006724	8.232	2.16E-05	0.0201	0.352
A_23_P321511	MARCH3	NM_178450	6.116	8.32E-05	0.0391	0.375
A_23_P50108	NDC80	NM_006101	6.188	8.27E-05	0.0391	-0.345
A_24_P394533	NEU1	NM_000434	8.379	6.47E-05	0.0344	0.190
A_23_P217280	NOX1	NM_007052	5.015	5.92E-05	0.0337	-0.549
A_24_P95822	NPTN	NM_012428	8.731	1.50E-06	3.75E-03	0.387
A_33_P3322804	NTRK2	NM_001018064	5.203	1.64E-06	3.75E-03	1.046
A_33_P3401284	RMRP	NR_003051	10.385	5.60E-05	0.0329	0.812
A_33_P3363082	SCARNA5	NR_003008	6.732	5.52E-05	0.0329	0.939
A_24_P245379	SERPINB2	NM_002575	6.042	8.27E-05	0.0391	0.754
A_23_P115726	SLC16A9	NM_194298	7.596	3.94E-05	0.0311	0.472
A_23_P571	SLC2A1	NM_006516	8.832	1.14E-04	0.0442	0.299
A_33_P3274696	SLC5A2	NM_024531	10.911	1.28E-04	0.0470	0.238
A_24_P388528	ST6GAL1	NM_173216	10.332	4.11E-05	0.0313	0.438
A_33_P3237674	TCRA,TRA@,TRAC	ENST00000478163	6.281	1.14E-04	0.0442	-0.732
A_33_P3415698	TMIGD2	NM_144615	6.348	1.05E-05	0.0144	-0.843
A_23_P37702	TPSAB1	NM_003294	5.870	5.24E-06	8.97E-03	1.413
A_33_P3382324	TPSD1	NM_012217	5.831	3.23E-06	6.64E-03	1.418
A_23_P105562	VWF	NM_000552	6.569	1.92E-05	0.0187	0.765
A_32_P206479	ZNF831	NM_178457	4.726	1.03E-04	0.0432	-0.488

(B) Function and cell/tissue expression patterns from literature. Red indicates genes with strong evidence for immune cell specific expression.

Gene	Function	General Lung-Relevant Expression (http://biogps.org/#goto=genereport &id=153811)	Human Immune Cells (Garvan; www.immgen.org)
A_19_P00804124	lincRNA:chr14:23018310-23025460	no data	no data
ALOX15	arachidonate lipoxxygenase family; genetic associations with asthma; differentially expressed in human asthma; expression in non-epithelial cells leads to bronchial epithelial injury	high expression in the trachea, ubiquitous lower expression in other cells/tissues	highest in DCs, medium in eosinophils but generally low in immune cells
ALOX15	epithelial injury		

Sheet1

ANO1	calcium activated chloride channel (TMEM16 family); decreased activity and expression associated with abnormal cystic fibrosis airway epithelial repair; functional expression in human airway smooth muscle	expressed in the trachea but not in immune cells	no data
ASAP3	subfamily of ADP-ribosylation factor(Arf) GTPase-activating proteins; promotes cell differentiation and migration and has been implicated in cancer cell invasion	ubiquitous expression	no data
BDKRB1	bradykinin receptor; bradykinin, a 9 aa peptide, is generated in pathophysiologic conditions such as inflammation, trauma, burns, shock, and allergy; associations with asthma and allergy	very high in smooth muscle, ubiquitous in other cell/tissues	highest in effector memory T cells and NK cells but expressed in all immune cells
CAPN14	calpain family of cytosolic calcium-activated cysteine proteases involved in a variety of cellular processes including apoptosis, cell division, modulation of integrin-cytoskeletal interactions; gene family regulates mucus secretion	ubiquitous expression	no data
CASP4	inflammatory caspase; part fo the inflammasome complex; crucial for cell response to LPS	moderate/high expression in all immune cells (highest in monocytes); some expression in the bronchial epithelium; low expression in other cells/tissues	highest expression in neutrophils
CCK	not expressed in the lung at baseline but induced by ozone, LPS, and other innate immune stimuli	high expression in the brain and gut; low in all other cells/tissues	expressed in low levels in mast cells, NK cells, activated eosinophils and activated neutrophils

Sheet1

CCL5	chemoattractant for blood monocytes, memory T helper cells and eosinophils; causes the release of histamine from basophils and activates eosinophils	high expression in T cells, NK cells, whole blood; medium expression in whole lung tissue; some expression in trachea and very low in bronchial epithelium	high expression in NK cells, activated DCs and macrophages, and effector memory T cells; medium expression in basophils and Th1 cells
CD3G	one of the polypeptides of the TCR; part of the T-cell receptor-CD3 complex	high in the thymus and all T cells; low in whole lung, bronchial epithelium and trachea	high in all T cells, some expression in NK cells and basophils
CD55	glycoprotein involved in the regulation of the complement cascade; antigens present on this protein constitute the Cromer blood group system	high in whole blood, monocytes, B cells; medium expression in whole lung tissue; some expression in trachea and very low in bronchial epithelium	no data
CD6	found on the outer membrane of T-lymphocytes as well as some other immune cells	expressed at low/med levels similar to other cells in the bronchial epithelium; high in CD4+ and CD8+ cells	high in all T cells, some expression in NK cells
CD74	invariant polypeptide of MHCII; macrophage migration inhibitory factor receptor	medium expression in trachea; high in B cells, monocytes, and DCs and whole lung tissue	high expression in DCs, and B cells; medium expression in eosinophils and basophils
CDH26	cadherin domain-containing protein; cell-	ubiquitous expression	no data
CEACAM5	cell adhesion	high expression in the trachea, medium expression in whole lung, no expression in immune cells	highest in memory T cells, neutrophils and eosinophils but overall low expression
CPA3	mast cell carboxypeptidase; differentially expressed in epithelial brushing of Th2-high vs low; sputum biomarker of asthma immune phenotypes	high in HSCs (CD34+)	high in mast cells and basophils
CST1	cystatin superfamily, locus on chr 20; upregulated in patients with allergy	high expression in salivary gland	high in DCs, neutrophils and basophils, medium in eosinophils, mast cells, NK cells and T cells
CTSC	also DPPI, a mast cell peptidase; central		

Sheet1

CTSC	coordinator for activation of many serine proteinases in immune/inflammatory cells; IL-4 and IL-13 induce an alternative splice form in the bronchial epithelium; KO mice protected from SV-induced mucous cell metaplasia	moderate expression in the bronchial epithelium and lung in general; also high in CD34+ cells, DCs, NK cells, monocytes, and whole blood	highest in immature DCs but expressed in all immune cells (very low in B cells and eosinophils)
CTSG	neutrophil serine protease; processes IL-33 into mature form; higher expression in NA sputum and PB; released by neutrophils to detach epithelial cells from basement membrane	high in bone marrow and HSCs (CD34+); low in whole lung, trachea and bronchial epithelium	expressed highly and specifically on mast cell progenitors and terminally differentiated mast cells
CXCR6	CXCL16, the only ligand for CXCR6, highly expressed by human bronchial epithelial cells; important in homeostasis and activation of CD1d-restricted NKT cells	ubiquitous expression	high in Th2 cells
EFCAB11	EF-hand calcium binding domain 11	ubiquitous expression	no data
ELOVL5	plays a role in elongation of long-chain polyunsaturated fatty acids	low/med in bronchial epithelia, trachea and whole lung; high in whole blood	high in neutrophils and eosinophils
EPHA4	ephrin receptor family; implicated in mediating developmental events, particularly in the nervous system but also in the lung epithelium	low in bronchial epithelia, trachea, and whole lung; low in all immune cells	expressed in central memory T cells, otherwise very low
GJB4	transmembrane connexin protein that is a component of gap junctions; cx-based channels play several central roles in promoting a regulated inflammatory response and facilitating lung repair, enabling the pulmonary epithelium and vasculature to behave as integrated systems	ubiquitous expression	ubiquitous expression

Sheet1

GSN	actin-binding protein that is a key regulator of actin filament assembly and disassembly; released by epithelial cells into the airways, its secretion is increased by IL-4 in vitro; the concentration of both IL-4 and gelsolin were raised in the bronchoalveolar lavage of patients with asthma	medium expression in trachea and whole lung, low in bronchial epithelia; low in immune cells	expressed at low levels in DCs, macrophages, and neutrophils
HLA-DMB	MHC II	very low in bronchial epithelia and trachea, medium in whole lung; high in monocytes, macrophages, DCs, and B cells	high in B cells
HLA-DOA	found in lysosomes in B cells and regulates HLA-DM-mediated peptide loading on MHC class II molecules; very little sequence variation compared to other HLA genes	low in bronchial epithelia, trachea, and whole lung; high and specific expression in B cells	high in B cells
HNRNPA1	involved in the packaging of pre-mRNA into hnRNP particles, transport of poly A+ mRNA from the nucleus to the cytoplasm, and may modulate splice site selection	ubiquitous expression; high in immune cells, medium in bronchial epithelia	no data
HOMER2	regulates group 1 metabotropic glutamate receptor function	medium/high in bronchial epithelia; low in immune cells	highest in Th2 T cells and immature DCs but low in all immune cells
ITLN1	omentin; adipokine; plays an anti-inflammatory role through inhibition of TNF- α -induced superoxide production in vascular smooth muscle cells.	very high expression in whole lung tissue; low in immune cells	no data
KIF4A	kinesin 4 subfamily of kinesin related proteins; component of cilia; KIF3A SNPs and expression associated with asthma	low in all cells relevant to lung	highest in Th1 and Th2 cells

Sheet1

KLRB1	also CD161; killer cell lectin-like receptor; also a marker of Th17 T cells; LLT1, ligand for KLRB1, is expressed by respiratory epithelia	high in NK cells, also expressed in T cells; no expression in whole lung, trachea and bronchial epithelia	highest in NK and effector memory T cells
MAOA	mitochondrial enzyme which catalyzes the oxidative deamination of amines	medium level of expression in bronchial epithelia and whole lung; low in immune cells	expressed in DCs, no expression in other immune cells
MAP3K4	activated by innate immune stimuli such as LPS	ubiquitous expression; high in immune cells	expressed in all immune cells except for neutrophils; highest in activated macrophages
MARCH3	E3 ubiquitin-protein ligase that may be involved in regulation of the endosomal transport pathway	low expression in all lung-relevant cells except for B-cells	no data
NDC80	functions to organize and stabilize microtubule-kinetochore interactions and is required for proper chromosome segregation	some expression in whole lung, trachea and bronchial epithelia; low in immune cells except for CD105+ endothelial	no data
NEU1	cleaves terminal sialic acid residues from substrates such as glycoproteins and glycolipids	low/med in bronchial epithelia and whole lung; med/high on monocyte-derived macs and DCs, alveolar macs	high in activated eosinophils, medium in activated neutrophils and immature DCs
NOX1	a member of the NADPH oxidase family of enzymes responsible for the catalytic one-electron transfer of oxygen to generate superoxide or hydrogen peroxide; oxidative	ubiquitous low level of expression	high in activated eosinophils, low in all other immune cells
NPTN	Ig superfamily; cell-cell interactions or cell-substrate interactions	medium level of expression in trachea, bronchial epithelia and whole lung; expression in monocytes and whole blood	no data
NTRK2	also TRKB; neurotrophic tyrosine receptor kinase; upon neurotrophin binding, phosphorylates itself and members of the MAPK pathway; necessary for normal lung development; important in airway hyperreactivity in asthma	ubiquitous expression	low ubiquitous expression in all immune cells

Sheet1

RMRP	RNA component of mitochondrial RNA processing endoribonuclease	no data	no data
SCARNA5	also U87; small Cajal body-specific RNA 5	no data	no data
SERPINB2	also PAI2; one of Th2 high/low epithelial cell classifier genes	high and specific expression by bronchial epithelia; no expression in immune cells	only expressed in activated macrophages
SLC16A9	solute carrier family member; unknown function	expressed in the tracheal and CD105+ endothelial cells	no data
SLC2A1	glucose transporter	ubiquitous low level of expression	low level in all immune cells
SLC52A2	riboflavin transporter	ubiquitous expression; high in bronchial epithelia and NK cells	no data
ST6GAL1	catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates; is involved in the generation of the cell-surface carbohydrate determinants and differentiation antigens HB-6, CD75, and CD76	medium expression in trachea and whole lung, low but expressed in bronchial epithelia; also high in immature B cells and generally expressed by immune cells except for neutrophils	high in B cells, NK cells, basophils and memory T cells
TCRA,TRA@,TRAC	T cell receptor alpha constant	high only in T cells, thymus, and medium in lymph nodes; very low to none in trachea, bronchial epithelium, and whole lung	high in all T cells
TMIGD2	also IGPR1; cell adhesion receptor that is	ubiquitous expression	no data
TPSAB1	tryptase beta; specific for mast cells	very high and specific expression in whole lung tissue	expressed highly and specifically in mast cells
TPSD1	tryptase delta	ubiquitous expression	expressed on mast cells and neutrophils at low levels
VWF	von Willebrand factor; the glycoprotein encoded by this gene functions as both an antihemophilic factor carrier and a platelet-vessel wall mediator in the blood coagulation system	very high expression in whole lung tissue; low in immune cells	highest in activated macrophages but generally low in all immune cells
ZNF831	transcription factor with unknown function	ubiquitous expression	no data

Supplemental Table S9. Significant methyl-eQTRs ($q < 0.05$) for differentially methylated **(A)** and expressed **(B)** genes in asthmatic nasal epithelia.

(A) me-eQTR analysis focused on differentially methylated genes in asthma

Chr	Start	End	Methylation-Expression correlation, r	p value	q value	# Probes	Expression Probe	Method	refGene Name	refGene Strand	refGene Distance
chr17	4541333	4541334	-0.043363	3.72E-06	0.000223	1	A_23_P55373	lm	ALOX15	-	0
chr17	4541333	4541334	-0.045305	5.17E-06	0.000237	1	A_33_P3361348	lm	ALOX15	-	0
chr9	97545513	97545514	-0.14079	0.00044	0.010378	1	A_23_P73012	lm	C9orf3	+	0
chr7	1.07E+08	1.07E+08	-0.21178	0.00246	0.034793	4	A_23_P215778	liptak	DUS4L	+	0
chr5	15927183	15927184	-0.14042	0.003302	0.041009	1	A_23_P144827	lm	FBXL7	+	0
chr11	63973845	63974229	-0.17409	4.00E-05	0.001179	7	A_23_P64044	liptak	FERMT3	+	0
chr17	37893637	37894636	-0.12268	7.70E-07	0.000182	11	A_23_P163992	liptak	GRB7	+	0
chr16	69143314	69143815	-0.10379	0.002663	0.034911	5	A_23_P393034	liptak	HAS3	+	0
chr6	33041217	33041697	-0.10138	0.000572	0.011933	8	A_33_P3234277	liptak	HLA-DPA1	-	0
chr6	33041217	33041697	-0.086185	0.002506	0.034793	8	A_23_P30913	liptak	HLA-DPA1	-	0
chr6	33041217	33041697	-0.09153	0.001037	0.01883	8	A_33_P3271635	liptak	HLA-DPB1	+	-2005
chr6	33041217	33041697	-0.095205	0.001194	0.020121	8	A_33_P3271651	liptak	HLA-DPB1	+	-2005
chr6	33041217	33041697	-0.098032	0.001512	0.023794	8	A_24_P166443	liptak	HLA-DPB1	+	-2005
chr12	6881302	6881303	0.073226	9.96E-05	0.002611	1	A_23_P116942	lm	LAG3	+	-366
chr1	1.84E+08	1.84E+08	-0.13024	3.78E-06	0.000223	1	A_23_P138194	lm	NCF2	-	0
chr10	5488224	5488628	-0.138	2.98E-06	0.000223	8	A_23_P83931	liptak	NET1	+	0
chr10	5488224	5488628	-0.13423	6.03E-06	0.000237	8	A_33_P3210085	liptak	NET1	+	0
chr15	1.02E+08	1.02E+08	-0.2094	1.29E-05	0.000434	1	A_23_P151907	lm	PCSK6	-	0
chr8	90794646	90794647	-0.10586	0.004004	0.047249	1	A_23_P252106	lm	RIPK2	+	0
chr7	857980	857981	-0.13893	0.000607	0.011933	1	A_23_P169838	lm	SUN1	+	0
chr14	91017835	91017836	-0.074467	0.00424	0.047644	1	A_23_P25974	lm	TTC7B	-	0

(B) me-eQTR analysis focused on differentially expressed genes in asthma

Chr	Start	End	Methylation- Expression correlation, r	p value	q value	# Probes	Expression Probe	Method	refGene Name	refGene Strand	refGene Distance
chr17	4536236	4536237	-0.077278	1.02E-09	6.86E-07	1	A_33_P3361348	lm	ALOX15	-	0
chr17	4536236	4536237	-0.072262	1.85E-09	1.03E-06	1	A_23_P55373	lm	ALOX15	-	0
chr17	4534891	4534892	-0.060045	1.96E-06	0.000173	1	A_33_P3361348	lm	ALOX15	-	0
chr17	4534891	4534892	-0.056084	2.77E-06	0.000226	1	A_23_P55373	lm	ALOX15	-	0
chr17	4541333	4541334	-0.043363	3.72E-06	0.000277	1	A_23_P55373	lm	ALOX15	-	0
chr17	4541333	4541334	-0.045305	5.17E-06	0.000369	1	A_33_P3361348	lm	ALOX15	-	0
chr17	4546449	4546450	-0.11317	7.82E-06	0.000476	1	A_33_P3361348	lm	ALOX15	-	-1489
chr17	4546449	4546450	-0.1037	1.74E-05	0.000938	1	A_23_P55373	lm	ALOX15	-	-1489
chr17	4547473	4547474	-0.10692	3.39E-05	0.001514	1	A_23_P55373	lm	ALOX15	-	-2513
chr17	4547473	4547474	-0.11197	4.09E-05	0.001693	1	A_33_P3361348	lm	ALOX15	-	-2513
chr17	4545720	4545721	-0.061395	0.000108	0.003555	1	A_23_P55373	lm	ALOX15	-	-760
chr17	4545720	4545721	-0.06457	0.00012	0.003819	1	A_33_P3361348	lm	ALOX15	-	-760
chr17	4544924	4545157	-0.033504	0.001737	0.026093	5	A_33_P3361348	liptak	ALOX15	-	0
chr17	4544924	4545157	-0.032568	0.00207	0.029256	5	A_23_P55373	liptak	ALOX15	-	0
chr11	69925135	69925268	-0.065652	2.58E-05	0.001272	2	A_24_P87036	liptak	ANO1	+	0
chr11	69962357	69962465	0.031523	0.001278	0.021196	3	A_24_P87036	liptak	ANO1	+	0
chr11	70002830	70002831	-0.10022	0.002873	0.036911	1	A_24_P87036	lm	ANO1	+	0
chr2	31397282	31397283	-0.086778	0.001337	0.021849	1	A_32_P167076	lm	CAPN14	-	0
chr17	34207331	34207663	-0.041022	0.000898	0.016619	4	A_23_P152838	liptak	CCL5	-	0
chr17	34202460	34202461	-0.058995	0.00272	0.035315	1	A_23_P152838	lm	CCL5	-	0
chr11	1.18E+08	1.18E+08	-0.089057	4.62E-05	0.001889	3	A_23_P98410	liptak	CD3G	+	-1644
chr11	1.18E+08	1.18E+08	0.049564	0.000508	0.010908	1	A_23_P98410	lm	CD3G	+	0
chr11	1.18E+08	1.18E+08	-0.06475	0.000579	0.011889	5	A_23_P98410	liptak	CD3G	+	0
chr11	60775232	60775233	0.11827	8.65E-11	9.65E-08	1	A_23_P311875	lm	CD6	+	0
chr11	60775544	60775545	0.14806	5.43E-08	1.40E-05	1	A_23_P311875	lm	CD6	+	0
chr11	60774951	60774952	0.082191	9.11E-05	0.003213	1	A_23_P311875	lm	CD6	+	0
chr5	1.5E+08	1.5E+08	-0.051878	0.000111	0.003626	1	A_23_P70095	lm	CD74	-	2235
chr5	1.5E+08	1.5E+08	-0.036566	0.00145	0.023137	1	A_23_P70095	lm	CD74	-	0
chr5	1.5E+08	1.5E+08	-0.046245	0.003495	0.04152	1	A_23_P70095	lm	CD74	-	-936

chr19	42213762	42213763	-0.061478	7.90E-05	0.002847	1	A_23_P153301	lm	CEACAM5	+	0
chr19	42234061	42234062	-0.060997	0.000251	0.006241	1	A_23_P153301	lm	CEACAM5	+	0
chr20	23728813	23728814	-0.0081078	0.003005	0.037702	1	A_23_P112859	lm	CST1	-	0
chr20	23728431	23728432	-0.018356	0.003459	0.041327	1	A_23_P112859	lm	CST1	-	0
chr11	88069168	88069169	-0.087032	3.43E-05	0.001514	1	A_23_P1552	lm	CTSC	-	0
chr11	88069168	88069169	-0.096344	3.92E-05	0.001664	1	A_33_P3268472	lm	CTSC	-	0
chr11	88059526	88059527	-0.17441	0.002692	0.03523	1	A_33_P3283480	lm	CTSC	-	0
chr3	45984742	45985168	-0.10823	1.24E-05	0.00073	4	A_23_P109913	liptak	CXCR6	+	0
chr3	45983791	45983792	0.10054	0.000147	0.004403	1	A_23_P109913	lm	CXCR6	+	-1180
chr6	53147640	53147641	-0.15441	5.51E-05	0.002164	1	A_23_P156497	lm	ELOVL5	-	0
chr6	53191789	53191790	-0.17768	0.000143	0.004333	1	A_23_P156497	lm	ELOVL5	-	0
chr6	53152717	53152718	-0.12109	0.000794	0.015371	1	A_23_P156497	lm	ELOVL5	-	0
chr6	53187373	53187374	-0.14042	0.000838	0.015783	1	A_23_P156497	lm	ELOVL5	-	0
chr6	53208622	53208623	-0.20475	0.000841	0.015783	1	A_23_P156497	lm	ELOVL5	-	0
chr6	53200696	53200697	0.14014	0.001771	0.026475	1	A_23_P156497	lm	ELOVL5	-	0
chr2	2.22E+08	2.22E+08	0.069609	0.000645	0.012946	1	A_23_P108501	lm	EPHA4	-	0
chr1	35223246	35223247	0.050123	0.000286	0.006983	1	A_33_P3388391	lm	GJB4	+	-2094
chr1	35223054	35223055	0.038377	0.000421	0.009409	1	A_33_P3388391	lm	GJB4	+	-2286
chr9	1.24E+08	1.24E+08	-0.053909	0.000158	0.004524	4	A_23_P255884	liptak	GSN	+	-331
chr9	1.24E+08	1.24E+08	-0.06284	0.00023	0.005831	4	A_33_P3423365	liptak	GSN	+	-331
chr9	1.24E+08	1.24E+08	-0.046617	0.000415	0.00932	1	A_33_P3423365	lm	GSN	+	0
chr9	1.24E+08	1.24E+08	-0.040872	0.000475	0.010343	1	A_23_P255884	lm	GSN	+	0
chr9	1.24E+08	1.24E+08	-0.039675	0.003331	0.040872	1	A_23_P255884	lm	GSN	+	0
chr9	1.24E+08	1.24E+08	-0.044516	0.003361	0.041093	1	A_33_P3423365	lm	GSN	+	0
chr15	50534286	50534287	0.043024	0.001778	0.026475	1	A_23_P117662	lm	HDC	-	0
chr6	32901535	32901642	0.057866	1.41E-05	0.0008	3	A_32_P351968	liptak	HLA-DMB	-	763
chr6	32908238	32908239	-0.11096	6.46E-05	0.002486	1	A_32_P351968	lm	HLA-DMB	-	0
chr6	32913124	32913125	-0.088048	0.001359	0.021987	1	A_32_P351968	lm	HLA-DMB	-	-4277
chr6	32976483	32976484	-0.053641	8.32E-07	9.61E-05	1	A_32_P356316	lm	HLA-DOA	-	0
chr6	32976048	32976049	-0.017913	0.000325	0.007787	1	A_32_P356316	lm	HLA-DOA	-	0

chr6	32977579	32977580	-0.026154	0.001869	0.027064	1	A_32_P356316	lm	HLA-DOA	-	-190
chr6	32974121	32974122	0.021009	0.002901	0.03709	1	A_32_P356316	lm	HLA-DOA	-	0
chr6	32974478	32974479	0.025647	0.003463	0.041327	1	A_32_P356316	lm	HLA-DOA	-	0
chr15	83544283	83544284	-0.07804	0.000184	0.004929	1	A_23_P99906	lm	HOMER2	-	0
chr15	83620320	83620321	-0.09893	0.003281	0.040554	1	A_23_P99906	lm	HOMER2	-	0
chr1	1.61E+08	1.61E+08	-0.033275	0.001209	0.020348	3	A_23_P95790	liptak	ITLN1	-	0
chr1	1.61E+08	1.61E+08	-0.01794	0.002061	0.029253	1	A_23_P95790	lm	ITLN1	-	0
chr12	9760325	9760326	-0.062347	3.42E-06	0.00026	1	A_23_P99275	lm	KLRB1	-	0
chr12	9757160	9757161	-0.033739	0.000134	0.004133	1	A_23_P99275	lm	KLRB1	-	0
chrX	43517028	43517029	-0.1256	0.000397	0.009054	1	A_33_P3242543	lm	MAOA	+	0
chr5	1.26E+08	1.26E+08	0.078068	0.003848	0.044912	1	A_23_P321511	lm	MARCH3	-	0
chr6	31827899	31827900	-0.12695	0.000924	0.016907	1	A_24_P394533	lm	NEU1	-	0
chr9	87285692	87285693	-0.04446	0.000173	0.004739	1	A_33_P3322804	lm	NTRK2	+	0
chr9	87433863	87433864	-0.054708	0.000678	0.013435	1	A_33_P3322804	lm	NTRK2	+	0
chr10	61434210	61434211	-0.16065	0.001354	0.021987	1	A_23_P115726	lm	SLC16A9	-	0
chr10	61412026	61412027	0.061463	0.002413	0.032866	1	A_23_P115726	lm	SLC16A9	-	0
chr10	61468157	61468158	-0.16088	0.004359	0.049534	1	A_23_P115726	lm	SLC16A9	-	0
chr1	43399326	43399327	-0.11789	0.000276	0.006796	1	A_23_P571	lm	SLC2A1	-	0
chr3	1.87E+08	1.87E+08	-0.15076	3.63E-05	0.001559	1	A_24_P388528	lm	ST6GAL1	+	0
chr3	1.87E+08	1.87E+08	-0.088453	0.000504	0.010897	1	A_24_P388528	lm	ST6GAL1	+	0
chr3	1.87E+08	1.87E+08	-0.084581	0.000707	0.013847	1	A_24_P388528	lm	ST6GAL1	+	0
chr14	22993035	22993190	-0.12978	1.88E-11	3.15E-08	2	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr14	22981234	22981235	-0.07131	1.22E-10	1.02E-07	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22986465	22986466	-0.087763	6.26E-08	1.50E-05	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22974143	22974144	-0.070781	1.37E-07	2.70E-05	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0

chr14	22984400	22984401	-0.078184	2.74E-07	4.83E-05	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22964912	22964913	-0.064425	4.00E-07	6.37E-05	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22953112	22953113	-0.079308	7.50E-07	8.98E-05	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22978072	22978195	-0.093246	1.84E-06	0.000166	2	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr14	22974950	22975521	-0.095348	5.08E-06	0.000369	5	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr14	22945180	22945457	-0.070813	1.90E-05	0.000993	2	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr14	22958398	22958402	-0.047488	6.90E-05	0.002625	2	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr14	22969694	22969695	-0.0399	0.000175	0.004739	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22982776	22982777	-0.034644	0.000299	0.007266	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22465675	22465676	0.045632	0.000519	0.011065	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22987688	22987689	-0.047232	0.000833	0.015783	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0

chr14	22978728	22978729	-0.081547	0.001495	0.023344	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22941182	22941183	-0.021065	0.002485	0.033572	1	A_33_P3237674	lm	TCRA,TRA @,TRAC	+	0
chr14	22314957	22315066	0.032473	0.00295	0.037435	2	A_33_P3237674	liptak	TCRA,TRA @,TRAC	+	0
chr19	4302994	4303012	-0.045129	3.06E-06	0.000241	2	A_33_P3415698	liptak	TMIGD2	-	-566
chr12	6178605	6178606	-0.034197	0.000213	0.005491	1	A_23_P105562	lm	VWF	-	0
chr12	6054361	6054362	-0.096809	0.000224	0.005717	1	A_23_P105562	lm	VWF	-	3677
chr12	6232978	6232979	0.069844	0.000306	0.007372	1	A_23_P105562	lm	VWF	-	0
chr12	6056641	6056642	0.027111	0.002479	0.033572	1	A_23_P105562	lm	VWF	-	1397
chr12	6197836	6197837	-0.039153	0.002876	0.036911	1	A_23_P105562	lm	VWF	-	0
chr20	57765814	57766332	0.059602	7.06E-05	0.002659	5	A_32_P206479	liptak	ZNF831	+	0
chr20	57796490	57796491	0.10228	0.000159	0.004524	1	A_32_P206479	lm	ZNF831	+	0
chr20	57798095	57798303	0.058712	0.000441	0.009789	2	A_32_P206479	liptak	ZNF831	+	0
chr20	57800724	57800725	0.072577	0.001081	0.018755	1	A_32_P206479	lm	ZNF831	+	0
chr20	57769798	57769799	0.033632	0.001448	0.023137	1	A_32_P206479	lm	ZNF831	+	0
chr20	57797318	57797377	0.052089	0.003151	0.039095	2	A_32_P206479	liptak	ZNF831	+	0

Supplemental Table S10. Upstream regulator analysis of the 50 genes from the methyl-eQTR results.

Upstream Regulator	Molecule Type	Activation z-score	p-value of overlap	Target molecules in dataset
IL13	cytokine	-2.695	8.09E-08	ALOX15,CCL5,CD36,CTSC,GSN,HDC,HOMER2,MAOA,RIPK2
TGFB1	growth factor	-1.307	1.31E-07	ALOX15,C9orf3,CCL5,CD36,CEACAM5,CTSC,CXCR6,GSN,HAS3,KLRB1,MAOA,NCF2,NET1,SLC16A9,SLC2A1,VWF
IFNG	cytokine	-0.705	2.22E-07	ALOX15,CCL5,CD36,CD74,CEACAM5,CTSC,HLA-DMB,HLA-DOA,LAG3,NCF2,NTRK2,RIPK2,SLC16A9,SLC2A1
IL27	cytokine	-0.577	2.93E-07	CCL5,CD74,HLA-DMB,HLA-DOA,HLA-DPA1,LAG3
CIITA	transcription regulator		8.63E-07	CD74,HLA-DMB,HLA-DOA,HLA-DPA1
IL4	cytokine	-2.405	2.38E-05	ALOX15,CCL5,CD36,CD6,CTSC,HOMER2,KLRB1,MAOA,SLC2A1
IL6	cytokine	-0.152	8.93E-05	CCL5,CD36,CD74,CTSC,ITLN1,KLRB1,LAG3,NCF2
HCK	kinase		2.05E-04	CD36,MAOA
STAR	transporter		3.00E-04	CCL5,CD36
TNF	cytokine	-1.026	3.57E-04	CCL5,CD36,CTSC,HDC,NCF2,NET1,PCSK6,RIPK2,SLC2A1,ST6GAL1,STMN1
ERBB2	kinase		3.61E-04	CCL5,CD36,HOMER2,MAOA,NET1,SLC2A1,VWF
CSF2	cytokine	0.532	3.68E-04	CD74,CTSC,HDC,NET1,RIPK2,STMN1
RFX5	transcription regulator		4.75E-04	CD74,HLA-DMB
CXCL12	cytokine	-2	5.86E-04	CCL5,CD36,CD3G,CXCR6
IL2	cytokine	-2.186	6.54E-04	CCL5,CD3G,CTSC,CXCR6,EPHA4,KLRB1
ITK	kinase		7.01E-04	CCL5,KLRB1,SLC2A1
HTT	transcription regulator		7.19E-04	CCL5,CD74,GSN,NTRK2,RIPK2,SLC2A1,STMN1
NRAS	enzyme		8.94E-04	CCL5,NCF2,SLC2A1

Supplemental Table S11. Pyrosequencing validation of selected DNA methylation marks in the same asthma cases and controls (A) and an independent population of asthmatics (B). Green denotes hypomethylation and red hypermethylation in asthma cases compared to controls.

(A) Internal Validation

DMR/DMP Coordinates	Gene	Illumina Adjusted p Value	Illumina Methylation Change	Coordinates of Measured Cs	Pyrosequencing Asthma Mean (SD)	Pyrosequencing Control Mean (SD)	Pyrosequencing Methylation Change	One-tailed t-test
chr17:4541333-4541334	ALOX15	7.82x10 ⁻⁴	-10.1	4541333	76.6 (8.7)	85.3 (3.0)	-8.7	1.55x10 ⁻⁷
chr6:33041220-33041697	HLA-DPA1	1.04x10 ⁻⁶	9.8	33041528	24.0 (10.4)	15.0 (10.1)	9	2.10x10 ⁻⁴
chr1:35258778-35258933	GJA4	6.00x10 ⁻³	-9.8	35258850, 35258855	9.2 (3.8), 29.7 (11.3)	12.6 (3.0), 37.5 (10.4)	-3.4, -7.8	6.0x10 ⁻⁵ , 1.74x10 ⁻³
chr13:38172802-38172803	POSTN	7.25 x10 ⁻⁴	-11.6	38172802	30.9 (10.3)	39.8 (9.8)	-8.9	1.70x10 ⁻⁴
chr11:36030085-36030086	LDLRAD3	6.23x10 ⁻⁶	-28.8	36030086	72.2 (19.3)	93.1 (8.2)	-20.9	2.18x10 ⁻⁷
chr7:105521115-105521116	ATXN7L1	4.09x10 ⁻⁹	-27.3	105521115	48.8 (14.8)	73.6 (5.8)	-24.8	2.60x10 ⁻¹⁴
chr12:58162286-58162287	METTL1	5.03x10 ⁻⁸	-27	58162286	63.3 (16.9)	86.1 (6.3)	-22.8	8.25x10 ⁻¹¹
chr3:45984742-45985168	CXCR6	7.30x10 ⁻⁴	5.9	45984839, 45984841	78.4 (7.0), 78.6 (7.1)	72.7 (8.1), 72.6 (9.3)	5.7, 6.0	9.89x10 ⁻⁴ , 1.48x10 ⁻³
chr17:34202460-34202461	CCL5	0.017	1.9	34202460	60.4 (11.0)	56.0 (11.2)	4.4	0.048
chr11:88059526-88059527	CTSC	1.51x10 ⁻³	-1.1	88059526	41.4 (16.5)	46.1 (16.8)	-4.7	0.12

(B) External Validation

Coordinates of Measured Cs	Pyrosequencing Asthma Mean (SD)	Pyrosequencing Control Mean (SD)	Pyrosequencing Methylation Change	One-tailed t-test
4541333	74.7 (8.3)	85.3 (3.0)	-10.6	7.50x10 ⁻¹⁰
33041528	23.1 (10.7)	15.0 (10.1)	8.1	1.27x10 ⁻³
35258850, 35258855	7.9 (3.0), 23.8 (6.8)	12.6 (3.0), 37.5 (10.4)	-4.7, -13.7	2.03x10 ⁻⁸ , 2.26x10 ⁻⁸
38172802	26.2 (6.4)	39.8 (9.8)	-13.6	6.5x10 ⁻⁹
36030086	69.3 (18.4)	93.1 (8.2)	-23.8	8.80x10 ⁻⁹
105521115	42.5 (12.8)	73.6 (5.8)	-31.1	4.91x10 ⁻²⁰
58162286	59.0 (15.6)	86.1 (6.3)	-27.1	6.02x10 ⁻¹⁴
45984839, 45984841	76.9 (8.6), 77.3 (8.5)	72.7 (8.1), 72.6 (9.3)	4.2, 4.7	0.0227, 0.0188
34202460	63.8 (8.3)	56.0 (11.2)	7.8	1.6x10 ⁻³

88059526	31. 6 (11.4)	46.1 (16.8)	-14.5	8.2×10^{-5}
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Supplemental Table 12. Clinical characteristics of the NJH adult asthmatic cohort.

	Asthma Subjects (N=12)	Control Subjects (N=12)	p-value †
Age at recruitment (yr)	37 [24,74]	29 [24,45]	0.16
Gender (Male)	4 (33.3%)	6 (50.0%)	0.59
Total serum IgE (kU/l)	115 [7,1085]	25.5 [7, 71]	0.0027
Baseline - FEV1(% predicted) ‡	74.7 ± 15.7	98.7 ± 12.0	**
Baseline - FEV1/FVC §	66.4 ± 13.0	79.7 ± 5.4	**
Inhaled corticosteroids	12 (100%)	0 (0%)	**
LABA	6 (50%)	0 (0%)	**

* Data are presented as mean ± standard deviations, medians [range] or numbers (%).

† P-values were calculated with the use of the Chi-Square¹ test for categorical variables and the Mann-Whitney² test for continuous variables.

‡ FEV₁ denotes forced expiratory volume in one second

§ FVC denotes forced vital capacity

** By protocol design

Supplemental Table S13. Significant (genome-wide $p < 0.05$) methylation changes in nasal epithelia of adult Caucasian asthmatics compared to controls.

(A) DMPs

geneSymbol	probe	chr	pos	p value	q value	methy change
ABO	cg11879188	chr9	136149908	0.001143076	0.0133	-0.116
ABO	cg21160290	chr9	136149941	8.54E-05	0.0133	-0.130
ABO	cg22535403	chr9	136150032	0.002053806	0.0133	-0.114
ABO	cg13506600	chr9	136150361	0.002482881	0.0133	-0.123
AMBRA1	cg24079972	chr11	46486318	0.000780103	0.0133	-0.057
ARID3A	cg01950129	chr19	941919	0.006577278	0.0133	-0.057
ARID3A	cg08553572	chr19	947765	0.003777537	0.0133	0.065
ARID3A	cg23048215	chr19	947936	0.00526776	0.0133	0.066
ARID3A	cg02466798	chr19	941571	0.007315283	0.0133	-0.083
ARID3A	cg23966363	chr19	937630	0.001245351	0.0133	0.103
ARID3A	cg01774027	chr19	947712	0.002906991	0.0133	0.079
ARID3A	cg02695969	chr19	932813	0.004974175	0.0133	0.061
ARID3A	cg26305504	chr19	947612	0.009943272	0.0139	0.051
ARID3A	cg26857315	chr19	932759	0.004115812	0.0133	0.086
C15orf38	cg01256846	chr15	90456541	0.006884349	0.0133	-0.035
C15orf38	cg05173987	chr15	90455332	0.009058066	0.0136	-0.053
C15orf54	cg15648896	chr15	39542877	0.008418634	0.0135	0.054
C15orf54	cg10099207	chr15	39544144	8.41E-05	0.0133	-0.130
C22orf31	cg13586696	chr22	29458723	0.001772149	0.0133	-0.122
C22orf31	cg03665785	chr22	29458676	0.000264166	0.0133	-0.135
C5orf56	cg05452391	chr5	131781406	0.003006595	0.0133	-0.099
C5orf56	cg21171858	chr5	131792825	0.002847782	0.0133	0.088
C5orf56	cg00594167	chr5	131772039	0.003611808	0.0133	0.031
C9orf3	cg13972491	chr9	97848736	0.00782505	0.0134	-0.061
C9orf3	cg14372245	chr9	97683653	0.006943569	0.0133	-0.091
C9orf3	cg01993208	chr9	97808906	0.006645547	0.0133	-0.123
C9orf3	cg14601621	chr9	97848457	0.002277549	0.0133	-0.044
C9orf3	cg17406248	chr9	97807693	0.004849372	0.0133	-0.096
C9orf3	cg21189611	chr9	97691844	0.003164101	0.0133	-0.107
C9orf3	cg13761321	chr9	97768771	0.007915222	0.0134	-0.111

CAPN14	cg16504661	chr2	31441554	0.002119488	0.0133	-0.044
CAPN14	cg15741855	chr2	31440451	0.000543626	0.0133	-0.225
CAPN14	cg15941014	chr2	31439687	0.008787912	0.0135	0.061
CHN2	cg22747501	chr7	29233476	0.004961766	0.0133	-0.093
CHN2	cg21472642	chr7	29233468	0.004584035	0.0133	-0.102
COL16A1	cg02989257	chr1	32169274	0.000266514	0.0133	-0.059
COL5A2	cg02420724	chr2	190044983	0.007416453	0.0133	-0.076
CYP2E1	cg00436603	chr10	135340740	0.003721872	0.0133	-0.107
CYP2E1	cg09208540	chr10	135340467	0.000465951	0.0133	-0.133
CYP2E1	cg10986462	chr10	135340539	1.38E-05	0.0133	-0.147
CYP2E1	cg07381788	chr10	135340445	7.26E-05	0.0133	-0.165
CYP2E1	cg14250048	chr10	135340785	0.000737926	0.0133	-0.123
DAB2IP	cg07949217	chr9	124497981	0.007605343	0.0133	-0.070
DAB2IP	cg14594111	chr9	124463457	0.001621046	0.0133	-0.073
DEGS2	cg26002632	chr14	100625216	0.005329227	0.0133	-0.054
DEGS2	cg01974091	chr14	100625027	0.003290293	0.0133	-0.108
EFNA5	cg08942527	chr5	106801945	0.004170889	0.0133	-0.118
EFNA5	cg03718773	chr5	106941252	0.008546015	0.0135	0.057
EFNA5	cg07575204	chr5	107002135	0.008552733	0.0135	-0.108
FAM110B	cg00889709	chr8	59043471	0.008511859	0.0135	-0.067
FAM110B	cg05237114	chr8	58905672	0.003242303	0.0133	-0.067
FAM26D	cg15314855	chr6	116879209	0.003949988	0.0133	-0.110
FAM26D	cg25351588	chr6	116879492	0.009786352	0.0138	-0.132
FAM65A	cg01872947	chr16	67580610	0.006537064	0.0133	0.044
FBXL7	cg25623727	chr5	15932416	0.007015989	0.0133	-0.073
FBXL7	cg23041295	chr5	15502456	0.007533013	0.0133	-0.067
FBXL7	cg18373623	chr5	15694721	0.002717818	0.0133	0.064
FBXL7	cg00664723	chr5	15927184	2.29E-05	0.0133	-0.268
FBXL7	cg26233084	chr5	15510258	0.000735024	0.0133	-0.030
FBXL7	cg01572460	chr5	15865364	0.008444282	0.0135	0.049
FOXL1	cg08645670	chr16	86612451	0.004178858	0.0133	0.095
GJA4	cg02902318	chr1	35258061	0.005550898	0.0133	-0.092
GJA4	cg02480666	chr1	35259746	0.002852418	0.0133	-0.072
GJA4	cg15006973	chr1	35258933	7.79E-05	0.0133	-0.150

GNA12	cg01670430	chr7	2773010	0.00480659	0.0133	-0.072
GNA12	cg13493071	chr7	2842687	0.004596868	0.0133	-0.081
GNA12	cg19524238	chr7	2802976	0.002389959	0.0133	-0.051
GNA12	cg06895747	chr7	2842593	0.002607676	0.0133	-0.117
GNA12	cg05793240	chr7	2802953	0.000277259	0.0133	-0.056
GNA12	cg16765393	chr7	2842746	0.002746296	0.0133	-0.109
GRAMD1B	cg01699740	chr11	123396486	0.001951043	0.0133	-0.055
GRAMD1B	cg18041960	chr11	123415568	0.000892836	0.0133	-0.031
GRAMD1B	cg13247967	chr11	123487358	0.008497382	0.0135	-0.096
GRAMD1B	cg03627771	chr11	123396039	0.009839759	0.0138	-0.049
HLA-DPA1	cg10921592	chr6	33039414	0.008947039	0.0136	0.065
HLA-DPA1	cg05366160	chr6	33039481	0.007545592	0.0133	0.080
HLA-DPA1	cg15253861	chr6	33040743	0.007655736	0.0134	0.117
HOXB3	cg21474786	chr17	46631948	0.004887749	0.0133	-0.066
HOXB3	cg17616537	chr17	46628663	0.008747498	0.0135	-0.094
HOXB3	cg16787431	chr17	46629350	0.007318158	0.0133	-0.072
HOXB3	cg12570134	chr17	46640347	0.006924508	0.0133	-0.061
HOXB3	cg02836478	chr17	46652501	0.003571445	0.0133	-0.093
HOXB3	cg02873421	chr17	46629306	0.003200792	0.0133	-0.065
HOXB3	cg19236493	chr17	46629596	0.007051487	0.0133	-0.081
HOXB3	cg20152430	chr17	46641504	0.005935831	0.0133	-0.105
HOXB3	cg00842549	chr17	46651920	0.006234692	0.0133	-0.042
HOXB3	cg05323879	chr17	46642104	0.006952758	0.0133	-0.058
HOXB3	cg03019986	chr17	46628850	0.009844959	0.0138	-0.084
HOXB3	cg06186155	chr17	46648582	0.009484163	0.0137	-0.055
HOXB3	cg13293524	chr17	46651822	0.002849748	0.0133	-0.058
HOXB3	cg21399057	chr17	46631910	0.008825264	0.0135	-0.063
HOXB4	cg07015911	chr17	46655580	0.004156607	0.0133	0.062
HOXB4	cg11971423	chr17	46653711	0.004034321	0.0133	-0.078
HOXB4	cg12806763	chr17	46655836	0.005135863	0.0133	0.061
HOXB4	cg02422694	chr17	46655790	0.006145626	0.0133	0.052
HOXB4	cg21460081	chr17	46656012	0.000622603	0.0133	0.093
HOXB4	cg24114154	chr17	46655829	0.004235016	0.0133	0.073
HS3ST1	cg14047130	chr4	11427700	0.001273056	0.0133	-0.123

IGFBP4	cg13385210	chr17	38599271	0.004765413	0.0133	-0.084
JAKMIP3	cg09955413	chr10	133995286	0.004656377	0.0133	-0.109
JAKMIP3	cg27636330	chr10	133976763	0.00431117	0.0133	-0.077
LAG3	cg16352928	chr12	6881590	0.009449025	0.0137	0.023
LCE3D	cg18116418	chr1	152553244	0.001001312	0.0133	-0.179
LDLRAD3	cg02984184	chr11	35966322	0.002295896	0.0133	-0.088
LDLRAD3	cg24656207	chr11	36030086	0.00073453	0.0133	-0.134
LDLRAD3	cg15948836	chr11	36114126	0.003534542	0.0133	0.048
LRRC31	cg12183875	chr3	169587454	0.003877628	0.0133	-0.126
MAP2K6	cg18326562	chr17	67413885	0.003578262	0.0133	-0.080
MAP2K6	cg17583957	chr17	67536299	0.006704792	0.0133	-0.100
MED12L	cg16946840	chr3	150888616	0.006119202	0.0133	0.013
MEGF6	cg08425229	chr1	3470175	0.00445629	0.0133	-0.088
MMP14	cg10418289	chr14	23308294	0.00128283	0.0133	-0.095
NFIL3	cg07084627	chr9	94183408	0.009875654	0.0138	-0.060
NRIP1	cg00712106	chr21	16340625	1.31E-05	0.0133	-0.206
NTRK1	cg11177059	chr1	156808680	0.006737065	0.0133	-0.029
PCBP1	cg26487157	chr2	70313295	0.002083163	0.0133	0.066
PDE6A	cg19910679	chr5	149240728	0.008578497	0.0135	0.059
PIK3CD	cg23251761	chr1	9750061	0.008539475	0.0135	-0.098
PIK3CD	cg24859236	chr1	9750213	0.003352907	0.0133	-0.057
PIK3CD	cg04287259	chr1	9730401	0.001956012	0.0133	0.093
PLEKHH2	cg18856652	chr2	43864913	0.003951643	0.0133	-0.055
PLEKHH2	cg05939149	chr2	43986106	0.000413291	0.0133	-0.121
PPFIBP2	cg05736186	chr11	7633488	0.004921602	0.0133	-0.031
PPFIBP2	cg11333968	chr11	7569641	0.007856874	0.0134	0.039
PSTPIP2	cg26436583	chr18	43649176	0.007243767	0.0133	-0.077
RAB7A	cg10143911	chr3	128506519	0.006911152	0.0133	-0.085
RIPK2	cg23632791	chr8	90794647	0.009641252	0.0138	0.107
SCN2B	cg13348944	chr11	118047121	0.009267978	0.0136	-0.079
SERPINB6	cg05082466	chr6	2953123	0.001874408	0.0133	-0.107
SLC25A23	cg17100943	chr19	6459300	0.000477324	0.0133	-0.141
SLC43A1	cg17559156	chr11	57269634	0.002117509	0.0133	-0.095
SLC9A4	cg20061812	chr2	103149495	0.004592655	0.0133	-0.106

SLC9A4	cg02552255	chr2	103104330	0.001334819	0.0133	-0.117
SORL1	cg11586591	chr11	121466463	0.002519502	0.0133	-0.078
SORL1	cg20069407	chr11	121460973	0.004183726	0.0133	0.078
SORL1	cg05075579	chr11	121421206	0.0007696	0.0133	-0.122
SORL1	cg03055520	chr11	121460793	0.006171245	0.0133	0.086
SORL1	cg25418363	chr11	121421298	0.001804004	0.0133	-0.157
TEX2	cg26007445	chr17	62287566	0.000310879	0.0133	-0.103
TMEM131	cg04223924	chr2	98552472	0.003778509	0.0133	-0.081
TMEM131	cg02995664	chr2	98613076	0.001202544	0.0133	0.082
TNS3	cg26559707	chr7	47367816	0.002334377	0.0133	-0.091
TNS3	cg10158598	chr7	47344579	0.001379009	0.0133	0.027
TNS3	cg07395763	chr7	47574050	0.000119839	0.0133	-0.077
TNS3	cg09905635	chr7	47525243	0.002541394	0.0133	-0.095
TNS3	cg23895495	chr7	47539837	0.007479538	0.0133	0.047
TREML2	cg12045237	chr6	41169171	0.006568255	0.0133	0.054
TSHR	cg22855021	chr14	81610812	0.001328763	0.0133	-0.152
TSPAN8	cg15684563	chr12	71552188	0.006584455	0.0133	-0.108
TSPAN8	cg02965411	chr12	71551926	0.008233806	0.0134	-0.084
TSPAN8	cg12965512	chr12	71552336	0.003218566	0.0133	-0.095
TTC7B	cg15959205	chr14	91281552	0.000664984	0.0133	-0.096
TTC7B	cg12720152	chr14	91087476	0.004623057	0.0133	-0.057
TTC7B	cg24036116	chr14	91281636	0.008202439	0.0134	-0.133
TTC7B	cg01955025	chr14	91091782	0.000158518	0.0133	-0.070
TTC7B	cg18986218	chr14	91225366	0.007857906	0.0134	0.055
ZFPM1	cg05183538	chr16	88536950	0.004037839	0.0133	0.022
ZFPM1	cg01062937	chr16	88537260	0.003107957	0.0133	0.062
ZFPM1	cg09502865	chr16	88600155	0.004017257	0.0133	-0.070
ZFPM1	cg07099810	chr16	88575557	0.005856174	0.0133	-0.080
ZFPM1	cg04387347	chr16	88537187	0.006106756	0.0133	0.026
ZFPM1	cg09247061	chr16	88571844	0.007059881	0.0133	-0.058
ZNF862	cg19507256	chr7	149552478	0.008281194	0.0134	-0.061

(B) DMRs

genes	probes	chr	start	stop	n_probes	min p value	slk p value	slk-sidak p value	methylation change
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ABO;ABO;ABO cg11879188;cg chr9	136149908	136150033	3	0.0121	2.00E-06	0.00772	-0.120
CYP2E1;CYP2E1 cg07381788;cg chr10	135340445	135340851	7	0.0064	2.09E-08	0.00003	-0.124
FERMT3;FERMT3 cg25338707;cg chr11	63973846	63974230	7	0.0097	2.14E-06	0.00270	0.075
FERMT3;FERMT3 cg01647936;cg chr11	63974772	63975027	4	0.0271	3.36E-06	0.00637	0.079
HOXB4;HOXB4; cg08089301;cg chr17	46655561	46656013	12	0.0074	1.92E-06	0.00206	0.052
LFNG;LFNG;LFN cg24939194;cg chr7	2563611	2563910	4	0.0375	9.14E-06	0.01471	-0.111
NCOR2;NCOR2; cg07800907;cg chr12	124950669	124950991	5	0.0194	1.01E-05	0.01506	0.065
NRIP1;NRIP1 cg00712106;cg chr21	16340625	16340723	2	0.0143	5.45E-06	0.02663	-0.130

Supplemental Table S14. Correlation of clinical/demographic and laboratory data with top 10 principal components in methylation data.

PC #	Variance Explained	Variable	Variable Type	# Samples	Correlation	ANOVA Groups	p	Adjusted p
1	0.296	albuterol	categorical	72	na	2	0.0875	1
1	0.296	any_ind_skin	categorical	72	na	2	0.0905	1
1	0.296	asthma	categorical	72	na	2	0.0905	1
1	0.296	cat_wheal	numeric	72	-0.254	na	0.0315	1
1	0.296	concentration	numeric	72	-0.278	na	0.0181	1
1	0.296	eastern_pos	categorical	72	na	2	0.00758	1
1	0.296	eastern_wheal	numeric	72	-0.21	na	0.0769	1
1	0.296	ethnic_mexican	categorical	10	na	2	0.0171	1
1	0.296	juniper_pos	categorical	15	na	2	0.0832	1
1	0.296	meds	categorical	72	na	5	0.0584	1
1	0.296	ragweed_pos	categorical	72	na	2	0.0507	1
1	0.296	ragweed_wheal	numeric	72	-0.25	na	0.0339	1
1	0.296	Sample.TC	numeric	72	0.234	na	0.0475	1
1	0.296	SentrixBarcode_A	numeric	72	0.299	na	0.0106	1
1	0.296	sol_4_FEV1	numeric	6	0.971	na	0.00121	1
1	0.296	sol_5_FEV1	numeric	5	0.955	na	0.0115	1
2	0.145	alt_pos	categorical	72	na	2	0.0054	1
2	0.145	alt_wheal	numeric	72	-0.246	na	0.0376	1
2	0.145	americanroach_pos	categorical	72	na	2	0.0195	1
2	0.145	americanroach_wheal	numeric	72	-0.207	na	0.0817	1
2	0.145	bermuda_pos	categorical	35	na	2	0.0568	1
2	0.145	cat_6mo	categorical	72	na	2	0.0824	1
2	0.145	derfderp_pos	categorical	72	na	2	0.0502	1
2	0.145	gender	categorical	72	na	2	6.36E-80	1.00E-76
2	0.145	nasal_steroid	categorical	58	na	2	0.0352	1
2	0.145	post_FEF2575pred	numeric	31	-0.402	na	0.025	1
2	0.145	post_FEV1	numeric	31	-0.308	na	0.0921	1
2	0.145	post_FEV1pred	numeric	31	-0.356	na	0.0496	1
2	0.145	race_indian_alaska	categorical	72	na	2	0.0838	1
2	0.145	ragweed_pos	categorical	72	na	2	0.0698	1
3	0.075	albuterol	categorical	72	na	2	0.00466	1

3	0.075	alt_pos	categorical	72	na	2	0.00236	1
3	0.075	alt_wheal	numeric	72	0.408	na	0.000375	0.593
3	0.075	americanroach_pos	categorical	72	na	2	0.0314	1
3	0.075	antihist	categorical	72	na	2	0.0714	1
3	0.075	any_ind_skin	categorical	72	na	2	0.00637	1
3	0.075	aspergillus_pos	categorical	72	na	2	0.0289	1
3	0.075	aspergillus_wheal	numeric	72	0.347	na	0.00281	1
3	0.075	asthma	categorical	72	na	2	0.00637	1
3	0.075	atopy_count	categorical	72	na	12	0.075	1
3	0.075	bermuda_pos	categorical	35	na	2	0.000813	1
3	0.075	bermuda_wheal	numeric	35	0.325	na	0.0571	1
3	0.075	cat_wheal	numeric	72	0.205	na	0.0846	1
3	0.075	Collection.Site	categorical	72	na	6	0.00195	1
3	0.075	derfderp_pos	categorical	72	na	2	0.00623	1
3	0.075	derfderp_wheal	numeric	72	0.335	na	0.00398	1
3	0.075	eastern_pos	categorical	72	na	2	0.0131	1
3	0.075	eastern_wheal	numeric	72	0.244	na	0.0385	1
3	0.075	ethnic_puertorican	categorical	10	na	2	0.0946	1
3	0.075	FEF2575	numeric	72	-0.22	na	0.0631	1
3	0.075	FEV1FVC	numeric	72	-0.285	na	0.0152	1
3	0.075	FEVpred	numeric	72	-0.205	na	0.0835	1
3	0.075	germanroach_pos	categorical	72	na	2	0.0646	1
3	0.075	grassmix_pos	categorical	72	na	2	0.0401	1
3	0.075	grassmix_wheal	numeric	72	0.288	na	0.014	1
3	0.075	inh_steroid	categorical	72	na	2	0.0146	1
3	0.075	juniper_pos	categorical	15	na	2	0.0114	1
3	0.075	lnige	numeric	72	0.36	na	0.00189	1
3	0.075	meds	categorical	72	na	5	0.0445	1
3	0.075	montelukast	categorical	72	na	2	0.00655	1
3	0.075	nasal_steroid	categorical	58	na	2	0.0328	1
3	0.075	pos_ctrl	numeric	72	0.33	na	0.00461	1
3	0.075	post_FEV1pred	numeric	31	-0.335	na	0.0651	1
3	0.075	race_a_carrib	categorical	72	na	2	0.0966	1
3	0.075	race_aa	categorical	72	na	2	0.0415	1

3	0.075	ragweed_pos	categorical	72	na	2	0.00409	1
3	0.075	ragweed_wheal	numeric	72	0.274	na	0.0198	1
3	0.075	rat_pos	categorical	72	na	2	0.0128	1
3	0.075	rat_wheal	numeric	72	0.243	na	0.0397	1
3	0.075	Sample.TC	numeric	72	0.294	na	0.0122	1
3	0.075	SentrixBarcode_A	numeric	72	0.202	na	0.0893	1
3	0.075	SentrixPosition_A	categorical	72	na	12	0.00371	1
3	0.075	Site	categorical	72	na	6	0.00195	1
3	0.075	skintest_source	categorical	72	na	2	0.0475	1
3	0.075	sol_4_FEV1	numeric	6	-0.841	na	0.036	1
3	0.075	sol_4_FVC	numeric	6	-0.937	na	0.00584	1
3	0.075	sol_5_FEV1	numeric	5	-0.805	na	0.1	1
3	0.075	sol_5_FVC	numeric	5	-0.956	na	0.011	1
3	0.075	sol_7_FVC	numeric	3	-0.989	na	0.0954	1
3	0.075	subtype	categorical	72	na	4	0.0155	1
3	0.075	total_IgE	numeric	72	0.207	na	0.0806	1
4	0.04	age	numeric	72	-0.325	na	0.00541	1
4	0.04	aspergillus_pos	categorical	72	na	2	0.0896	1
4	0.04	barcode	numeric	72	0.199	na	0.0934	1
4	0.04	BR_use3	categorical	72	na	2	0.0208	1
4	0.04	concentration	numeric	72	-0.282	na	0.0163	1
4	0.04	DNA.conc..1st.elution	numeric	68	-0.233	na	0.0562	1
4	0.04	DNA.conc.2nd.elution	numeric	68	-0.259	na	0.0331	1
4	0.04	Hyb.Date	categorical	72	na	9	0.0782	1
4	0.04	inh_steroid	categorical	72	na	2	0.0312	1
4	0.04	juniper_pos	categorical	15	na	2	0.0869	1
4	0.04	juniper_wheal	numeric	15	-0.478	na	0.0713	1
4	0.04	labelling	categorical	72	na	3	0.0553	1
4	0.04	neg_ctrl	numeric	72	0.215	na	0.0694	1
4	0.04	SentrixBarcode_A	numeric	72	-0.399	na	0.000521	0.823
4	0.04	signal_strength	numeric	72	0.277	na	0.0185	1
4	0.04	skintest_source	categorical	72	na	2	0.0668	1
4	0.04	sol_5_FEV1	numeric	5	-0.904	na	0.0353	1
4	0.04	ug.DNA	numeric	68	-0.233	na	0.0562	1

5	0.023	alt_pos	categorical	72	na	2	0.0597	1
5	0.023	alt_wheal	numeric	72	0.203	na	0.0867	1
5	0.023	americanroach_pos	categorical	72	na	2	0.0264	1
5	0.023	americanroach_wheal	numeric	72	0.265	na	0.0245	1
5	0.023	bermuda_pos	categorical	35	na	2	0.00903	1
5	0.023	bermuda_wheal	numeric	35	0.34	na	0.0458	1
5	0.023	Collection.Site	categorical	72	na	6	0.00153	1
5	0.023	derfderp_pos	categorical	72	na	2	0.0335	1
5	0.023	derfderp_wheal	numeric	72	0.2	na	0.092	1
5	0.023	dog_6mo	categorical	72	na	2	0.0273	1
5	0.023	eastern_pos	categorical	72	na	2	0.0965	1
5	0.023	juniper_pos	categorical	15	na	2	0.0301	1
5	0.023	race_hispanic	categorical	72	na	2	0.00508	1
5	0.023	race_white	categorical	72	na	2	0.0768	1
5	0.023	ragweed_pos	categorical	72	na	2	0.0128	1
5	0.023	rat_pos	categorical	72	na	2	0.0792	1
5	0.023	roach_12mo	categorical	72	na	2	0.0371	1
5	0.023	SentrixBarcode_A	numeric	72	0.325	na	0.00537	1
5	0.023	SentrixPosition_A	categorical	72	na	12	0.0624	1
5	0.023	signal_strength	numeric	72	-0.251	na	0.0337	1
5	0.023	Site	categorical	72	na	6	0.00153	1
6	0.017	FEV1	numeric	72	-0.232	na	0.0497	1
6	0.017	FVC	numeric	72	-0.216	na	0.0681	1
6	0.017	juniper_pos	categorical	15	na	2	0.0204	1
6	0.017	meds	categorical	72	na	5	0.0973	1
6	0.017	montelukast	categorical	72	na	2	0.0831	1
6	0.017	postFEV1_80per	numeric	7	-0.742	na	0.0563	1
6	0.017	race_white	categorical	72	na	2	0.0174	1
6	0.017	Sample.TC	numeric	72	-0.198	na	0.0948	1
6	0.017	SentrixPosition_A	categorical	72	na	12	6.73E-05	0.106
6	0.017	sol_0_postFEV1	numeric	7	-0.742	na	0.0559	1
6	0.017	sol_1_FVC	numeric	7	-0.681	na	0.092	1
6	0.017	sol_2_FEV1	numeric	7	-0.705	na	0.0767	1
6	0.017	sol_2_FVC	numeric	7	-0.712	na	0.0727	1

6	0.017	sol_3_FEV1	numeric	7	-0.763	na	0.0459	1
6	0.017	sol_3_FVC	numeric	7	-0.791	na	0.0342	1
6	0.017	subtype	categorical	72	na	4	0.0648	1
7	0.013	albuterol	categorical	72	na	2	0.00815	1
7	0.013	americanroach_pos	categorical	72	na	2	0.0125	1
7	0.013	americanroach_wheal	numeric	72	0.226	na	0.0563	1
7	0.013	any_ind_skin	categorical	72	na	2	0.00884	1
7	0.013	aspergillus_pos	categorical	72	na	2	0.0231	1
7	0.013	aspergillus_wheal	numeric	72	0.282	na	0.0164	1
7	0.013	asthma	categorical	72	na	2	0.00884	1
7	0.013	bermuda_wheal	numeric	35	0.327	na	0.0554	1
7	0.013	cat_pos	categorical	72	na	2	0.0727	1
7	0.013	cat_wheal	numeric	72	0.265	na	0.0244	1
7	0.013	derfderp_pos	categorical	72	na	2	0.00232	1
7	0.013	derfderp_wheal	numeric	72	0.388	na	0.000761	1
7	0.013	dog_6mo	categorical	72	na	2	0.0407	1
7	0.013	dog_pos	categorical	72	na	2	0.0911	1
7	0.013	eastern_pos	categorical	72	na	2	0.00509	1
7	0.013	eastern_wheal	numeric	72	0.266	na	0.0241	1
7	0.013	ethnic_otherhisp	categorical	10	na	2	0.0421	1
7	0.013	FEV1FVC	numeric	72	-0.212	na	0.0739	1
7	0.013	FEV2575pred	numeric	72	-0.319	na	0.00627	1
7	0.013	FEVpred	numeric	72	-0.216	na	0.0691	1
7	0.013	germanroach_pos	categorical	72	na	2	0.00244	1
7	0.013	germanroach_wheal	numeric	72	0.281	na	0.0169	1
7	0.013	grassmix_pos	categorical	72	na	2	0.0255	1
7	0.013	grassmix_wheal	numeric	72	0.246	na	0.0376	1
7	0.013	inh_steroid	categorical	72	na	2	0.0309	1
7	0.013	lnige	numeric	72	0.241	na	0.0413	1
7	0.013	meds	categorical	72	na	5	0.0542	1
7	0.013	mouse_pos	categorical	72	na	2	0.0439	1
7	0.013	ragweed_pos	categorical	72	na	2	0.0973	1
7	0.013	rat_pos	categorical	72	na	2	0.00415	1
7	0.013	roach_IgE	numeric	71	0.201	na	0.0935	1

7	0.013	skintest_source	categorical	72	na	2	0.0046	1
7	0.013	subtype	categorical	72	na	4	0.0415	1
7	0.013	total_igE	numeric	72	0.284	na	0.0156	1
8	0.012	albuterol	categorical	72	na	2	0.0189	1
8	0.012	any_ind_skin	categorical	72	na	2	0.00569	1
8	0.012	asthma	categorical	72	na	2	0.00569	1
8	0.012	BR_use1	categorical	72	na	2	0.0497	1
8	0.012	cat_pos	categorical	72	na	2	0.0134	1
8	0.012	cat_wheal	numeric	72	-0.286	na	0.015	1
8	0.012	Collection.Site	categorical	72	na	6	0.00262	1
8	0.012	concentration	numeric	72	0.332	na	0.0044	1
8	0.012	elution.volume	categorical	72	na	2	0.0593	1
8	0.012	ethnic_mexican	categorical	10	na	2	0.0108	1
8	0.012	ethnic_puertorican	categorical	10	na	2	0.0471	1
8	0.012	Hyb.Date	categorical	72	na	9	0.0977	1
8	0.012	inh_steroid	categorical	72	na	2	0.0323	1
8	0.012	meds	categorical	72	na	5	0.00239	1
8	0.012	race_white	categorical	72	na	2	0.0365	1
8	0.012	rodent_6mo	categorical	72	na	2	0.0721	1
8	0.012	SentrixBarcode_A	numeric	72	-0.376	na	0.00114	1
8	0.012	Site	categorical	72	na	6	0.00262	1
8	0.012	subtype	categorical	72	na	4	0.00268	1
9	0.012	alt_wheal	numeric	72	-0.327	na	0.00501	1
9	0.012	americanroach_pos	categorical	72	na	2	0.0985	1
9	0.012	any_ind_skin	categorical	72	na	2	0.091	1
9	0.012	aspergillus_pos	categorical	72	na	2	0.0222	1
9	0.012	aspergillus_wheal	numeric	72	-0.315	na	0.00697	1
9	0.012	asthma	categorical	72	na	2	0.091	1
9	0.012	bermuda_wheal	numeric	35	-0.338	na	0.0471	1
9	0.012	BR_use1	categorical	72	na	2	0.0391	1
9	0.012	BR_use2	categorical	72	na	2	0.0627	1
9	0.012	caretaker_smokes	categorical	72	na	2	0.0952	1
9	0.012	Collection.Site	categorical	72	na	6	0.0842	1
9	0.012	eastern_pos	categorical	72	na	2	0.0195	1

9	0.012	eastern_wheal	numeric	72	-0.268	na	0.0228	1
9	0.012	FEV1FVC	numeric	72	0.214	na	0.0717	1
9	0.012	grassmix_pos	categorical	72	na	2	0.0368	1
9	0.012	grassmix_wheal	numeric	72	-0.29	na	0.0133	1
9	0.012	Hyb.Date	categorical	72	na	9	0.0102	1
9	0.012	juniper_pos	categorical	15	na	2	0.00921	1
9	0.012	juniper_wheal	numeric	15	-0.535	na	0.0398	1
9	0.012	neg_ctrl	numeric	72	0.239	na	0.043	1
9	0.012	post_FEF2575	numeric	31	0.335	na	0.0656	1
9	0.012	post_FEV1FVC	numeric	31	0.353	na	0.0516	1
9	0.012	race_indian_alaska	categorical	72	na	2	0.0753	1
9	0.012	ragweed_pos	categorical	72	na	2	0.00822	1
9	0.012	ragweed_wheal	numeric	72	-0.393	na	0.00063	0.995
9	0.012	Site	categorical	72	na	6	0.0842	1
9	0.012	skintest_source	categorical	72	na	2	0.0177	1
9	0.012	sol_2_FVC	numeric	7	-0.701	na	0.0794	1
9	0.012	sol_3_FVC	numeric	7	-0.755	na	0.05	1
9	0.012	sol_5_FVC	numeric	5	-0.809	na	0.0974	1
9	0.012	sol_7_FVC	numeric	3	-1	na	3.83E-05	0.0606
10	0.009	around_smokers	categorical	72	na	4	0.0855	1
10	0.009	aspergillus_pos	categorical	72	na	2	0.0654	1
10	0.009	barcode	numeric	72	0.219	na	0.0642	1
10	0.009	post_FEV1change	numeric	31	-0.328	na	0.0718	1
10	0.009	postFEV1_80per	numeric	7	-0.68	na	0.0928	1
10	0.009	sol_0_postFEV1	numeric	7	-0.678	na	0.0943	1
10	0.009	sol_1_FEV1	numeric	7	-0.747	na	0.0539	1
10	0.009	sol_1_FVC	numeric	7	-0.741	na	0.0567	1
10	0.009	sol_2_FVC	numeric	7	-0.711	na	0.0734	1
10	0.009	sol_3_FVC	numeric	7	-0.724	na	0.066	1

