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 2017;139:1478-88.)

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

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Abbreviations used
ALOX15: Arachidonate 15-lipoxygenase
CAPN14: Calpain 14
DMP: Differentially methylated probe
DMR: Differentially methylated region
POSTN: Periostin

The increase in the prevalence, incidence, and severity of asthma over the last 20 years[1] 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-Mendelian[3] and parent-of-origin[4] patterns of inheritance that are characteristic of asthma. Additionally, epigenetic marks can be influenced by the environment[5]; 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 asthma[10] 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 allergens[12] 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 genome[19]; (2) it increases power to detect associations[20]; 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 PBMCs[10] 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 effects[24]; (2) fit linear models with limma[25]; and (3) use comb-p[26] 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 limma[25] 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