Dissecting childhood asthma with nasal transcriptomics distinguishes subphenotypes of disease

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Background: Bronchial airway expression profiling has identified inflammatory subphenotypes of asthma, but the invasiveness of this technique has limited its application to childhood asthma.

Objectives: We sought to determine whether the nasal transcriptome can proxy expression changes in the lung airway transcriptome in asthmatic patients. We also sought to determine whether the nasal transcriptome can distinguish subphenotypes of asthma.

Methods: Whole-transcriptome RNA sequencing was performed on nasal airway brushings from 10 control subjects and 10 asthmatic subjects, which were compared with established bronchial and small-airway transcriptomes. Targeted RNA sequencing nasal expression analysis was used to profile 105 genes in 50 asthmatic subjects and 50 control subjects for differential expression and clustering analyses.

Results: We found 90.2% overlap in expressed genes and strong correlation in gene expression (ρ = .87) between the nasal and bronchial transcriptomes. Previously observed asthmatic bronchial differential expression was strongly correlated with asthmatic nasal differential expression (ρ = 0.77, $P = 5.6 \times 10^{-9}$). Clustering analysis identified T_H2-high and T_H2-low subjects differentiated by expression of 70 genes, including *IL13*, *IL5*, periostin *(POSTN)*, calcium-activated chloride channel regulator 1 *(CLCA1)*, and serpin peptidase inhibitor, clade B *(SERPINB2)*. T_H2-high subjects were more likely to have atopy (odds ratio, 10.3; $P = 3.5 \times 10^{-6}$), atopic asthma (odds ratio, 32.6; $P = 6.9 \times 10^{-7}$), high blood eosinophil counts (odds ratio, 9.1; $P = 2.6 \times 10^{-6}$), and rhinitis (odds ratio, 8.3; $P = 4.1 \times 10^{-6}$) compared with T_H2-low subjects. Nasal *IL13* expression levels were 3.9-fold higher in asthmatic participants who experienced an asthma exacerbation in the past year (P = .01). Several differentially expressed nasal genes were specific to asthma and independent of atopic status.

Conclusion: Nasal airway gene expression profiles largely recapitulate expression profiles in the lung airways. Nasal expression profiling can be used to identify subjects with *IL13*-driven asthma and a T_H2-skewed systemic immune response. (J Allergy Clin Immunol 2014;133:670-8.)

Key words: *Nasal airway epithelium, transcriptome, T_H2, asthma, bronchial airway epithelium*

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Asthma is the most common chronic childhood disease, affecting approximately 8.7 million children in the United States, and is characterized by chronic inflammation in the airways, leading to reversible airway obstruction.[1] Microarray-based expression profiling of bronchial airway epithelial brushings has revealed multiple genes with dysregulated expression in adult subjects with asthma.[2] These studies found a pattern of T_H2-driven inflammation that was characterized by expression of calcium-activated chloride channel regulator 1 *(CLCA1)*, periostin *(POSTN)*, and serpin peptidase inhibitor, clade B *(SERPINB2)*.[2,3] This so-called T_H2-high pattern was restricted to a subgroup (approximately 50%) of the asthmatic subjects screened, which is reflective of the known phenotypic heterogeneity of asthma.[3] The T_H2-high subphenotype appeared to have clinical significance because of its association with improved inhaled corticosteroid response, higher IgE levels, and higher peripheral blood eosinophil counts.[3] Given that there are multiple

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Abbreviations used

CLCA1: Calcium-activated chloride channel regulator 1
CPA3: Carboxypeptidase A3 gene
FPKM: Fragments per kilobase of transcript per million mapped reads
GALA II: Genes-environments and Admixture in Latino Americans
GWAS: Genome-wide association study
KRT5: Cytokeratin-5
MUC5B: Mucin 5b gene
OSM: Oncostatin M gene
POSTN: Periostin gene
RNA-seq: RNA sequencing
SERPINB2: Serpin peptidase inhibitor, clade B gene
ZPBP2: Zona pellucida binding protein 2 gene

novel biologic compounds targeting[4,5] components of the T_H2 inflammatory pathway, the ability to profile expression changes in the asthma-affected airway would be valuable not only in elucidating the pathogenesis of asthma but also for predicting and monitoring response to therapy and tailoring individual treatment regimens. However, carrying out bronchoscopy for evaluation of endotype and response to therapy would be invasive. An alternative to bronchial brushings would increase the practical utility of such findings, especially in children.

Similar to the bronchial airway epithelium, the nasal airway epithelium is populated by basal, ciliated, and secretory epithelial cells.[6] As such, the nasal airway presents an easily accessible alternative to the bronchial airway that might reflect much of the dysfunction present in the asthmatic bronchial airway. Supporting this, analysis of expression of approximately 2300 genes in nasal and bronchial airway brushings indicated a close relationship between these 2 airway sites.[7] Furthermore, a small study indicated that gene expression profiles were altered in the nasal brushings of asthmatic subjects versus those of healthy control subjects.[8] Finally, children experiencing asthma exacerbations exhibited altered gene expression in the nasal airway compared with children whose asthma was stable.[8]

In this study we used high-depth whole-transcriptome sequencing to comprehensively determine the degree to which the nasal airway serves as a biologic proxy for the bronchial airway. We also used novel targeted RNA sequencing (RNA-seq) technology to profile gene expression of candidate airway biomarkers in a larger group of well-characterized children with asthma and healthy control subjects. These data were used to determine the relationship between the nasal transcriptome and subphenotypes of asthma.

METHODS

Subject recruitment

Study subjects were a randomly selected subset of Puerto Rico islanders who were recruited as part of the ongoing Genes environments & Admixture in Latino Americans (GALA II) study described elsewhere.[9-11] Of the 100 study subjects who participated in this study, 92 were recontacted from their original GALA II study visit. Asthma was defined by a physician's diagnosis and the presence of 2 or more symptoms of coughing, wheezing, or shortness of breath in the 2 years before enrollment. Asthma exacerbations were defined by self-report of a subject having asthma symptoms requiring an emergency department visit. Atopy was defined by plasma testing with the Phadiatop Inhalant Multi-allergen ImmunoCAP (Phadia, Uppsala, Sweden)[12] as a dichotomous outcome. All study subjects had no history of smoking or recent nasal steroid use (within 4 weeks of recruitment). The study was approved by local institutional review boards, and written assent/consent was received from all subjects and their parents.

Nasal brushing collection and RNA extraction

Methods for nasal epithelial cell collection and processing were developed in collaboration with the National Institutes of Health/National Institute of Allergy and Infectious Diseases–sponsored Inner City Asthma Consortium, optimizing for collection and confirmation of columnar epithelial cell type, RNA yield, and specimen-collector training. Briefly, nasal epithelial cells were collected from behind the inferior turbinate with a cytology brush using a nasal illuminator (see Fig E1 in this article's Online Repository at www.jacionline.org). The collected brush was submerged in RLT Plus lysis buffer plus β-mercaptoethanol and frozen at −80°C until extraction. For more details, see the Methods section in this article's Online Repository at www.jacionline.org.

Whole-transcriptome gene expression and tissue comparison

RNA-seq libraries from 10 atopic asthmatic subjects and 10 nonatopic control subjects were constructed and barcoded with the Illumina Tru Seq RNA Sample Preparation version 2 protocol (Illumina, San Diego, Calif). Barcoded RNA-seq libraries from each of the 20 subjects were pooled and sequenced as 2×100-bp paired ends across 2 flow cells of an Illumina HiSeq 2000. Reads were mapped with TopHat (version 2.0.6) and quantified with Cufflinks (version 2.0.2), and differential expression was determined by using Cuffdiff.[13-15] Hierarchical clustering of samples was done with the cummeRbund package (version 2.0.0).[16] Bronchial epithelial and small-airway epithelium RNA-seq data sets were downloaded from the Sequence Read Archive (see the Methods section in this article's Online Repository).

RNA AmpliSeq analysis

RNA AmpliSeq libraries were prepared with a 105-gene multiplex design and sequenced with the Ion Torrent Proton on 3 P1 chips. Counts for reads mapping to target transcripts were tabulated using the torrent mapping alignment program and a Life Technologies in-house pipeline. Differential expression analysis was performed by using the nonparametric SAMseq[17] method available in the samr R package. High eosinophil count was tested as a dichotomous trait based on whether the subject's eosinophil percentage was greater than his or her predicted normal range. Hierarchical clustering and heat maps were generated with the Spearman rank correlation coefficient for genes and random forest proximity metrics for sample clustering (see the Methods section in this article's Online Repository).

Statistical methods

All statistical analyses outside of bioinformatics programs were performed in the R statistical package and are detailed in the Methods section in this article's Online Repository.

RESULTS

Whole-transcriptome gene expression signature of the nasal airway epithelium mirrors the bronchial airway epithelium

We performed whole-transcriptome sequencing of nasal airway epithelial brushings from 10 nonatopic control subjects and 10 atopic asthmatic subjects (Table I). Sequencing resulted in an average of 1.1×10^8 ($\pm 4 \times 10^7$) reads mapped per subject (see Table E1 in this article's Online Repository at www.jacionline.org). Mapped reads were used to generate fragments per kilobase of transcript per million mapped reads (FPKM) gene expression levels, which revealed 16,148 expressed genes in the healthy nasal transcriptome (see Table E1 and Fig E2 in this article's Online Repository at

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