

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 ($\rho = .87$) between the nasal and bronchial transcriptomes. Previously observed asthmatic bronchial differential expression was strongly correlated with asthmatic nasal differential expression ($\rho = 0.77$, $P = 5.6 \times 10^{-9}$). 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;■■■:■■■-■■■.)

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 novel biologic compounds targeting^{4,5} components of the T_H2 inflammatory pathway, the ability to profile expression changes

Abbreviations used

<i>CLCA1</i> :	Calcium-activated chloride channel regulator 1
<i>CPA3</i> :	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
<i>KRT5</i> :	Cytokeratin-5
<i>MUC5B</i> :	Mucin 5b gene
<i>OSM</i> :	Oncostatin M gene
<i>POSTN</i> :	Periostin gene
RNA-seq:	RNA sequencing
<i>SERPINB2</i> :	Serpin peptidase inhibitor, clade B gene
<i>ZBP2</i> :	Zona pellucida binding protein 2 gene

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 SAMseq¹⁷ 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

TABLE I. Clinical data for subjects included in the nasal whole-transcriptome and AmpliSeq expression studies

	WTS			AmpliSeq		
	Control subjects	Asthmatic subjects	P value	Control subjects	Asthmatic subjects	P value
Sample size	10	10		50	50	
Age (y)	15.2 ± 2.8	15.2 ± 2.5	.991	15.3 ± 2.8	15.0 ± 3.1	.682
Sex (M/F)	5/5	7/3		22/28	30/20	
FEV ₁ (% predicted)	83.4-112.2 (92.1)	68.8-92.7 (79.0)	.0003	83.2-126.9 (98.8)	83.4 ± 16.9	2.62e-11
Eosinophils*	L: 0.9-8.2 (2.7) H: 1.5-8 (5.8)	L: 1.3-13.4 (4.7) H: 0.7-0.7 (0.7) (n = 8)	L: .159 H: .500	L: 0.2-11.6 (2.8) H: 0.5-8.6 (2.4)	L: 0.7-15.6 (6.2) H: 0.7-14.7 (3.6) (n = 45)	L: .010 H: .226
IgE	23.4-219.0 (38.2)	17.4-2034.5 (158.7) (n = 9)	.095	4.9-2999.6 (114.6) (n = 47)	8.6-2992.9 (182.5) (n = 45)	.154
Phadiatop (+/-)	0/10	10/0		30/20 (n = 39)	37/12 (n = 45)	
Ancestry						
African	0.12-0.51 (0.29)	0.07-0.19 (0.15)	.001	0.08-0.51 (0.22)	0.07-0.49 (0.19)	.693
European	0.43-0.74 (0.59)	0.66-0.83 (0.73)	.001	0.43-0.84 (0.68)	0.43-0.83 (0.69)	.534
North American	0.06-0.14 (0.10)	0.08-0.16 (0.11)	.247	0.06-0.22 (0.11)	0.07-0.25 (0.11)	.917

All values are presented as either means ± SDs with *P* values calculated by using the 2-sided *t* test or when nonnormal as minimum-maximum (median) values with *P* values calculated by using the 2-sided Wilcoxon Mann-Whitney rank test. Sample sizes differing from the "Sample size" row are noted.

WTS, Whole-transcriptome sequencing.

*Data categories are divided into low (*L*) and high (*H*), indicating normal ranges of 0.0 to 4.0 and 0.0 to 7.0, respectively.

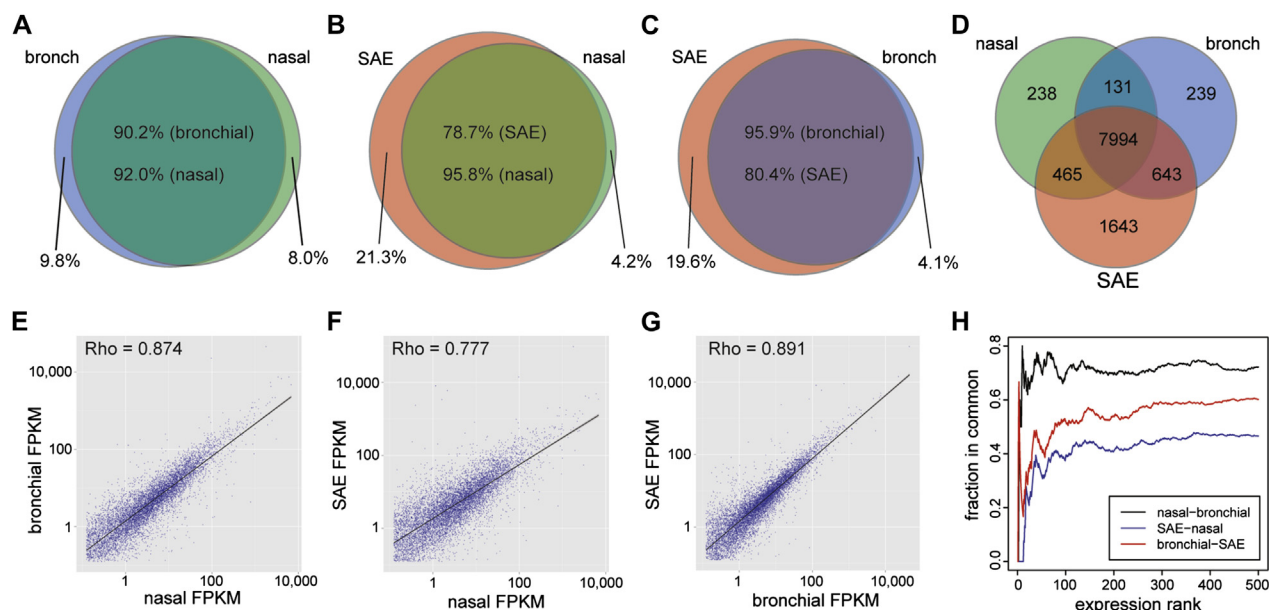


FIG 1. Comparison of nonubiquitous gene expression between airway tissues. **A-D**, Overlap of expressed genes between nasal-bronchial (Fig 1, A), nasal-SAE (Fig 1, B), and bronchial-SAE (Fig 1, C) tissues and between all tissues (Fig 1, D). **E-G**, Scatter plot of mean expression levels for genes commonly expressed between nasal-bronchial (Fig 1, E), nasal-SAE (Fig 1, F), and bronchial-SAE (Fig 1, G) tissues. **H**, Correspondence-at-top plot for the top 500 genes ranked by expression level from highest to lowest for each tissue.

www.jacionline.org). We accessed publically available transcriptome sequencing data to generate transcriptomes for the healthy bronchial and lung small airways (sixth-generation airways) for comparison with our healthy nasal transcriptome.^{18,19} We removed 7,331 ubiquitously expressed genes from each airway data set, which were defined by expression in a diverse panel of 23 human and murine cell types and tissues.²⁰ This resulted in 8,828, 9,007, and 10,745 nonubiquitously expressed genes in the nasal, bronchial, and small-airway transcriptomes, respectively. Examining the overlap of the nonubiquitously expressed nasal and bronchial genes, we found that 90.2%

of the genes expressed in the bronchial samples were also present in the nasal airway transcriptome (Fig 1, A). This was only slightly lower than the overlap with small-airway epithelium, which expressed 95.9% of the nonubiquitous bronchial transcriptome (Fig 1, B). In fact, the nasal transcriptome contained 78.7% of the genes expressed in the more distal small-airway transcriptome (Fig 1, C and D).

We next examined whether expression levels of these genes were correlated between the different airway sites. We found a high correlation ($\rho = 0.87$) between the nasal and bronchial transcriptomes, which was similar to the correlation between the bronchial and small-airway transcriptomes ($\rho = 0.89$;

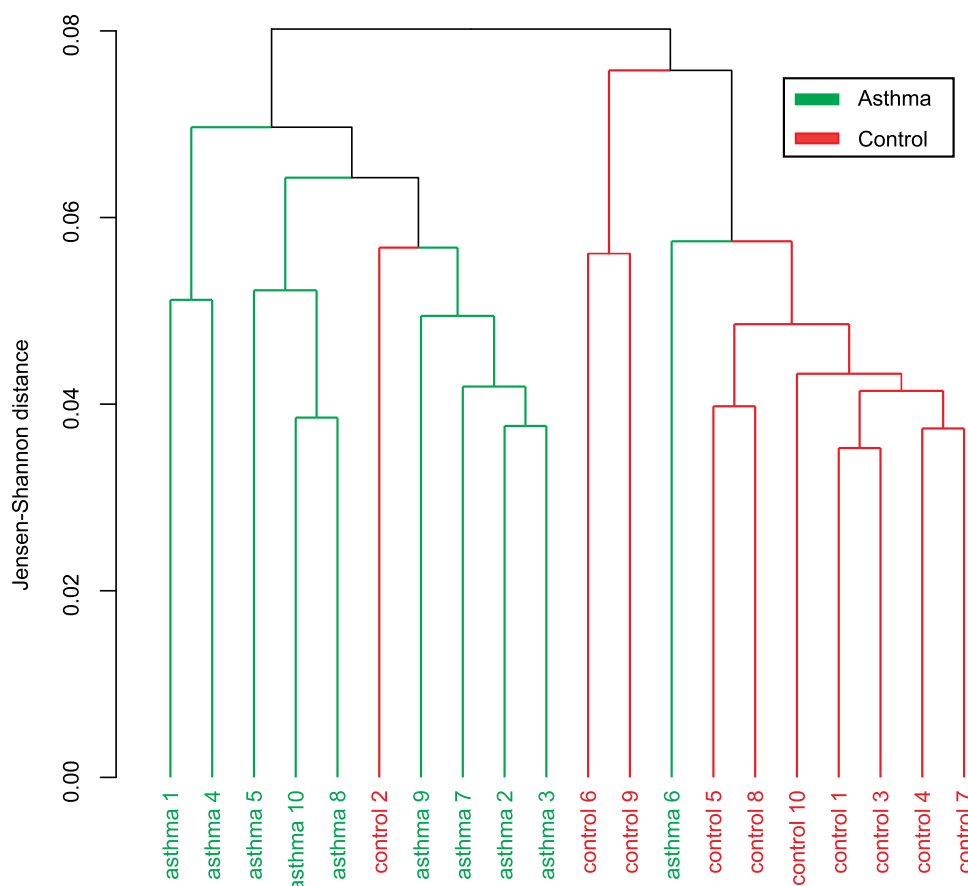


FIG 2. Unsupervised clustering of subjects with atopic asthma and healthy control subjects by using nasal transcriptome expression levels. FPKM expression levels for all genes in the nasal whole-transcriptome sequencing data were used for clustering.

Fig 1, E and G). Strong correlation was also observed between the nasal and small-airway transcriptomes ($\rho = 0.78$; Fig 1, F). Lastly, we examined concordance among the 3 data sets among the 500 most highly expressed genes. We found 72% overlap of these genes between nasal/bronchial airways, 60% overlap between bronchial/small airways, and 47% overlap between nasal/small airways (Fig 1, H). Taken together, these results demonstrate that the composition and structure of the bronchial and lung small-airway transcriptome is closely mirrored by that of the nasal airway.

The nasal airway transcriptome is altered in subjects with atopic asthma, and expression changes reflect asthmatic differential expression in the bronchial airway

We performed an unsupervised cluster analysis of the entire nasal transcriptome data set to determine whether nasal expression could be used to segregate the 10 atopic asthmatic subjects from the 10 nonatopic healthy control subjects. Clustering by using Jensen-Shannon distance separated asthmatic from control subjects, with only 1 outlier sample in each of 2 top-level clusters (Fig 2). We then performed an uncorrected analysis of differential expression for all gene transcripts to identify individual gene transcripts that might be driving the separate clustering of asthmatic subjects for later confirmation in a larger set of subjects. The 50

genes with the largest differential expression statistic are listed in Table E2 in this article's Online Repository at www.jacionline.org. The gene with the lowest P value for differential expression was carboxypeptidase A3 (*CPA3*), a mast cell gene product. Interestingly, *CPA3* was previously identified as the second most differentially expressed gene in the bronchial airway of asthmatic subjects.² Therefore we examined the ability of nasal airway expression to recapitulate the expression pattern in the bronchial airways of asthmatic subjects. This was done by comparing the fold changes of the top 20 overexpressed and underexpressed genes in the bronchial airways of asthmatic subjects to the fold changes of these genes in the nasal airways of asthmatic subjects.² Despite differences in expression platforms (microarray vs RNA-seq), the direction and magnitude of fold changes for these 40 bronchial genes were strongly correlated with the asthmatic fold changes for these genes in our nasal data ($\rho = 0.77$, $P = 5.6 \times 10^{-9}$, Fig 3). These results suggest that the nasal airway gene expression profile is altered in asthmatic subjects and that these changes are reflective of those observed in the bronchial airways of asthmatic subjects.

Targeted RNA-seq of the nasal airway epithelium reveals a T_H2 pattern associated with atopic asthma

We used targeted RNA-seq (AmpliSeq) technology to quantitate expression of 105 genes (see Table E3 in this article's Online

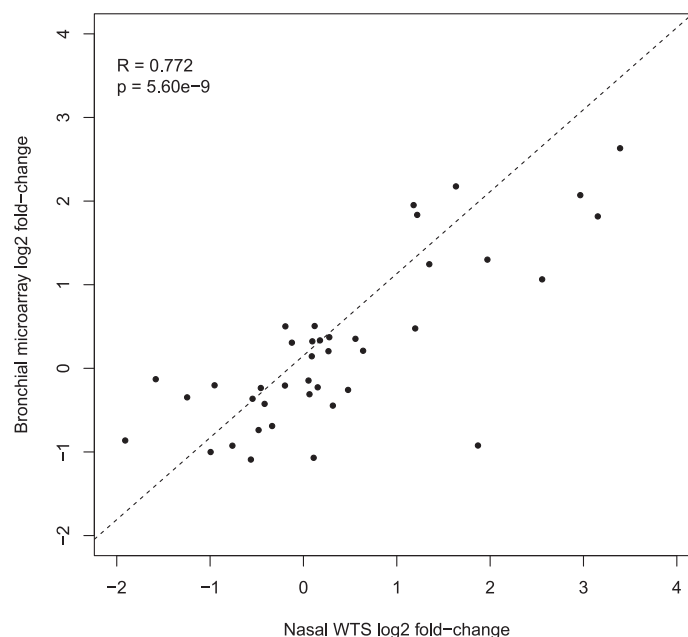


FIG 3. Comparison of gene expression fold changes in asthmatic subjects between bronchial and nasal airway expression data for bronchial airway biomarker genes. Scatter plot of previously reported bronchial airway gene expression log₂ fold changes in asthmatic subjects for the top 20 upregulated and downregulated genes versus fold changes in asthma for these genes in the nasal airway transcriptome data. The linear regression best-fit line is shown. WTS, Whole-transcriptome sequencing.

Repository at www.jacionline.org). The AmpliSeq assay included 3 gene groups: (1) we targeted the top 50 differentially expressed nasal genes in asthmatic subjects for confirmation of our whole-transcriptome analysis; (2) we targeted the top 20 overexpressed and top 10 underexpressed genes in the asthmatic bronchial epithelium according to the study by Woodruff et al² to validate the ability of nasal airway expression to proxy bronchial airway expression biomarkers of asthma; and (3) to provide genetic and biological context, we targeted a select set of 29 “asthma candidate genes,” which were defined as such by being either implicated in genome-wide association studies (GWASs) of asthma, other asthma genetic studies, or implicated in mechanistic studies of asthma.

We performed the AmpliSeq assay in a larger group of Puerto Rican children with asthma ($n = 50$) and control subjects ($n = 50$, Table I). To validate both gene expression methods, the 20 whole-transcriptome subjects were a subset of these 100 subjects (see Fig E3 in this article’s Online Repository at www.jacionline.org). These subjects were characterized by high rates of atopy in both case (75.5%) and control subjects (60%). Additionally, we found that 62% of subjects with asthma and 14% of nonasthmatic subjects self-reported rhinitis. The heterogeneity among both subjects with and without asthma in terms of these allergic phenotypes allowed us to investigate their relationship with nasal gene expression.

At our sequencing depth (1.3×10^6 reads per sample), we were able to detect expression in the nasal airways for 103 of the 105 genes screened. The AmpliSeq assay had the sensitivity to measure rare cytokine transcripts, such as *IL13*, *IL4*, and *IL5*, which have been below the detection level of prior microarray-based studies of bronchial airway gene expression.² We found that 48 of the 105 genes assayed were differentially expressed in asthmatic subjects after correction for multiple testing, including 26 overexpressed and 22 underexpressed genes (see Table E3). We note that 17 genes, or 57% of the bronchial

differential expression set screened, were also significantly differentially expressed in the nasal airways, strongly suggesting that gene expression among children with asthma is altered similarly in the bronchial and nasal airways (see Table E4 in this article’s Online Repository at www.jacionline.org). Among the differentially expressed genes, we noted epithelial genes (eg, *POSTN*, *CLCA1*, *SERPINB2*, dipeptidyl peptidase-4 [*DPP4*], cystatin SN [*CST1*], and cystatin-S [*CST4*]) and mast cell genes (eg, tryptase alpha/beta 1 [*TPSAB1*], IgE Fc receptor subunit beta [*MSA42*], and *CPA3*). The cardinal T_H2 cytokine, *IL13*, was upregulated in children with asthma. IL-13 was shown *in vitro* to drive upregulation of several genes differentially expressed in the bronchial epithelium of asthmatic subjects. Therefore we leveraged our ability to measure *IL13* transcripts in the airway to examine the correlation between *IL13* transcription levels and the other 47 differentially expressed genes *in situ*. We found that 18 of the 26 upregulated genes exhibited a strong positive correlation ($p > 0.5$) with *IL13* levels, whereas 14 of the 22 downregulated genes exhibited a strong negative correlation ($p < -0.5$) with *IL13* levels (Fig 4). Moreover, examining asthma severity, we found nasal *IL13* levels were 3.9-fold higher ($P = .01$) in subjects who experienced an asthma exacerbation in the past year versus those who did not (see Fig E4 in this article’s Online Repository at www.jacionline.org). These data suggest that differential expression at both epithelial sites (nasal and bronchial) is orchestrated in common by an underlying T_H2/IL-13 skew in the systemic immune system.

To investigate this idea, we tested the genes for differential expression by atopic status, a measure of systemic T_H2 immune system skew. We found 70 of the 103 expressed genes were differentially expressed by atopic status. We used hierarchical clustering of these 70 genes in all subjects to determine gene relationships and examine clustering of subjects with varying asthma and allergic status. The first branching point separated

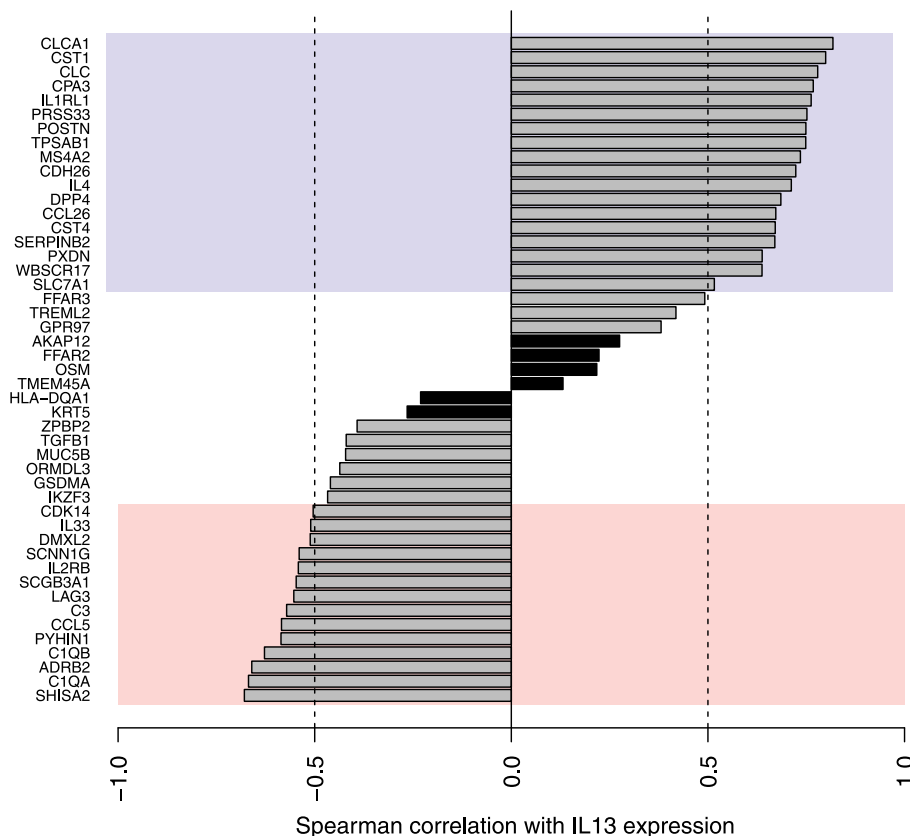


FIG 4. Correlation between AmpliSeq nasal gene expression of *IL13* and the other 47 genes differentially expressed in asthmatic subjects. Genes are ranked from top to bottom by decreasing Spearman correlation coefficient (p) values. Purple and pink regions correspond to levels of high positive ($p > 0.5$) and negative ($p < -0.5$) correlation, respectively. Open bars, Significant *IL13* correlations; solid bars, nonsignificant *IL13* correlations.

T_H2 -high (*IL13*-high) from T_H2 -low (*IL13*-low) subjects (Fig 5). The odds ratio for atopy, regardless of asthma status, was 10.33 among subjects with a T_H2 -high expression pattern ($P = 3.5 \times 10^{-6}$). We also found 9.1-fold increased odds for high blood eosinophil counts among subjects with a T_H2 -high pattern, another measure of systemic T_H2 skew ($P = 2.6 \times 10^{-6}$). Self-reported rhinitis also clustered tightly with the T_H2 -high pattern (odds ratio, 8.3; $P = 4.1 \times 10^{-6}$). Overall, 42 of the 48 differentially expressed genes in asthmatic subjects were among the 70 differentially expressed genes in atopic subjects, suggesting that the atopic asthmatic subgroup was driving the majority of the differentially expressed genes by asthma status. The odds ratio for atopic asthma among subjects with a T_H2 -high pattern was 32.6 compared with that seen in nonatopic healthy control subjects ($P = 6.9 \times 10^{-7}$).

Asthma-specific and T_H2 -independent gene expression in the nasal transcriptome

In contrast, 6 genes (cytokeratin-5 [*KRT5*], mucin 5b [*MUC5B*], zona pellucida binding protein 2 [*ZBP2*], triggering receptor expressed on myeloid cells-like 2 [*TREML2*], oncostatin M [*OSM*], and free fatty acid receptor 2 [*FFAR2*]) were associated with asthma and not atopy. None of these 6 genes were strongly correlated with *IL13* levels ($0.5 > r > -0.5$, Fig 4). Differential expression in these genes was driven by both atopic and nonatopic subjects with asthma (Fig 6).

Asthma GWAS genes differentially expressed in the nasal airway epithelium

We were able to detect gene expression in the nasal airway brushings of all 16 asthma GWAS genes screened with the AmpliSeq assay. We found that nasal expression of 9 (56.3%) and 11 (68.8%) of these candidate genes were associated with asthma and atopy, respectively. The genes and their fold changes in expression are listed in Table E5 in this article's Online Repository at www.jacionline.org. Among the differentially expressed genes verified through large GWAS meta-analyses were the cytokine *IL33* and its receptor, *IL1RL1* (ST2). Expression of *IL1RL1* was strongly upregulated in both atopy and asthma, whereas *IL33* was downregulated in both of these groups.

The 17q21 asthma GWAS risk locus contains 5 genes. In the nasal AmpliSeq assay we found *ORMDL3* and *GSDMB* were highly expressed, *IKZF3* was moderately expressed, and *GSDMA* and *ZBP2* were expressed at a low level. We found *ORMDL3*, *IKZF3*, and *GSDMA* were all downregulated in both atopic and asthmatic subjects. *ZBP2* and *GSDMB* were downregulated in just asthmatic and atopic subjects, respectively.

DISCUSSION

Transcriptional profiling of the bronchial airways has shown that T_H2 inflammation is present in only approximately 50% of asthmatic subjects, revealing the phenotypic heterogeneity of

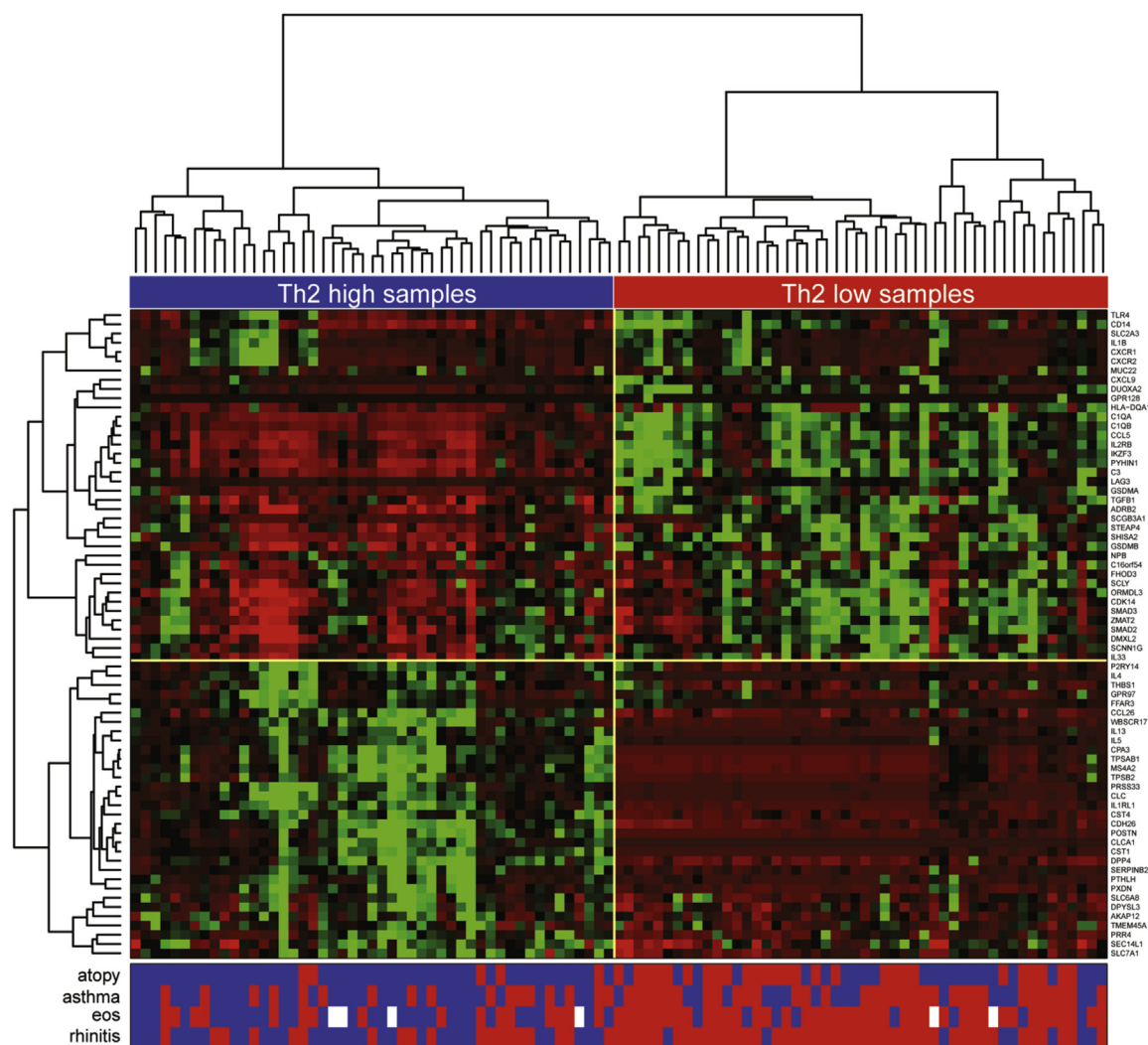


FIG 5. Clustering of AmpliSeq nasal gene expression levels in study subjects. Clustering was generated by using relative nasal expression levels for the 70 genes differentially expressed in the setting of atopy ($n = 99$). The heat map represents normalized expression counts (red, low; green, high) for each gene. The presence (blue) or absence (red) of atopy, asthma, eosinophil levels, and rhinitis is displayed directly below the heat map. Open squares, Missing data.

asthma.³ Bronchial airway gene expression changes are associated with inhaled corticosteroid response² and clinical characteristics, such as eosinophil levels,²¹ and have identified candidate genes (eg, *CLCA1*)²² that, on further study, have increased understanding of asthma pathogenesis.

However, the widespread application of these methods and findings to childhood asthma in large research studies and eventually clinical practice is impeded by the safety, expense, and time limitations presented by obtaining bronchoscopic brushings in children. In this study we used high-depth whole-transcriptome sequencing to prove nasal airway brushings can perform as an excellent proxy for bronchial airway brushings in asthmatic children. We found that more than 90% of non-uniquitous transcripts expressed in the bronchial airways are also expressed in the nasal airways, with strong correlation between expression of the 2 transcriptomes. The correlation in gene expression between the nasal and lung small airways was also strong, indicating that the nasal airways can serve as a good surrogate for both the bronchial and small airways. Our results

confirm and extend to a whole-transcriptome level the observations of prior studies, which reported large similarities in bronchial and nasal expression of surface receptor genes²³ and a subset of bronchial genes examined by microarray.⁷ Strikingly, we found unsupervised clustering of the nasal whole-transcriptome data resulted in near-complete separation of atopic asthmatic from healthy control subjects, an exciting result that will need to be confirmed in additional populations.

We demonstrate IL-13-centric T_H2 inflammation in the nasal airways of asthmatic subjects that is analogous to the T_H2 inflammation previously observed in the bronchial airways. The largest study of differential gene expression in the asthmatic bronchial airways identified a clear pattern of T_H2 -driven inflammation marked by the IL-13-responsive genes *POSTN*, *CLCA1*, and *SERPINE2*. We found both *IL13* and these IL-13-responsive genes were all differentially expressed among asthmatic subjects in our large targeted RNA-seq screen of the nasal airways. Moreover, 68% of the genes we associated with asthma in the targeted RNA-seq screen were strongly correlated with *IL13* levels. Finally,

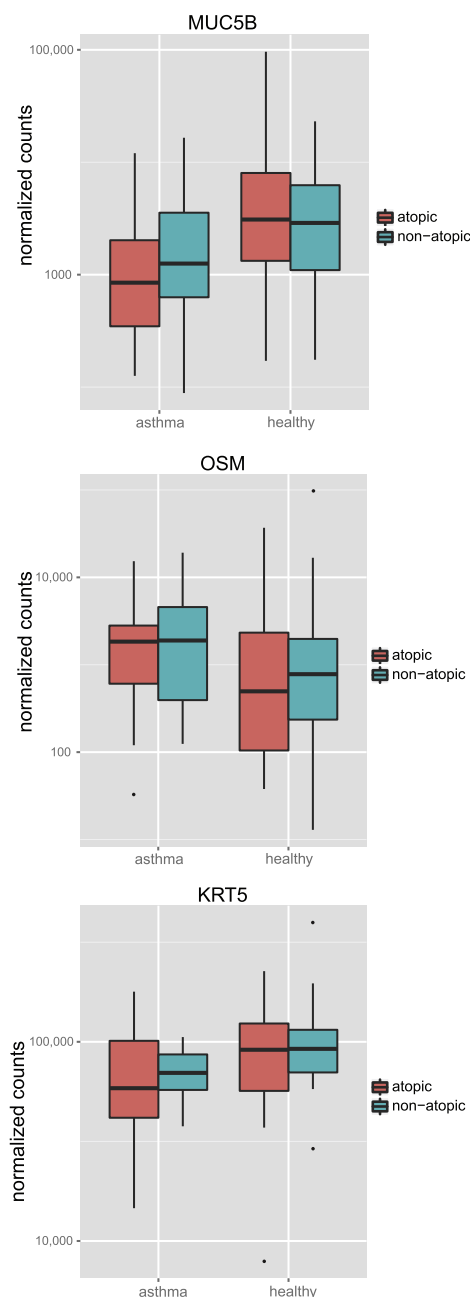


FIG 6. Box plots of genes differentially expressed in asthmatic but not atopic subjects in the nasal airway. AmpliSeq-normalized expression counts for 3 of the 6 genes (*MUC5B*, *OSM*, and *KRT5*) differentially expressed in the setting of asthma but not atopy (+1 pseudocount and log₁₀ scale) are plotted according to the subject's asthma and atopy status.

we found the direction and fold change of the top 40 differentially expressed genes in the bronchial airways was mirrored by our nasal transcriptome differential expression results.

As expected, nasal T_H2 inflammation was associated with rhinitis, but we found 17 T_H2-high subjects had atopy but not rhinitis, showing the nasal signature is not just a manifestation of rhinitis but rather a risk factor for rhinitis. We speculate that the common pattern of T_H2 inflammation in both the bronchial and nasal airways is driven by an underlying T_H2-skewed systemic immune system. Supporting this, both atopy status and blood eosinophil levels were strongly associated with the T_H2-high

pattern of nasal gene expression, regardless of asthma status. Moreover, the T_H2-high nasal expression pattern was present in 64% of asthmatic subjects, and we found 29 of these 32 subjects were atopic. Likewise, the bronchial airway profiling study found a T_H2-high expression pattern in 53% of asthmatic subjects, who were characterized by higher IgE levels and both higher blood and bronchoalveolar lavage eosinophil counts. These results suggest that T_H2 airway inflammation is a part of the mechanistic basis of asthma in atopic or more systemically allergic subjects.

Identification of T_H2-high subjects is feasible based on nasal brushings and could have a large impact on both biomedical and clinical research. For example, the ability to stratify subjects with asthma by the presence of T_H2 airway inflammation should create a more homogeneous group of subjects with regard to asthma pathogenesis and increase the power of future biomedical and clinical research studies. Moreover, this stratification would allow the search for genetic determinants of non-T_H2-driven asthma, for which little is known regarding disease pathogenesis. The fact that 9 of the 16 asthma GWAS genes tested were differentially expressed in the nasal epithelium suggest there might be a genetic basis to these disease subphenotypes. A prior study revealing inhaled corticosteroid response was almost entirely restricted to bronchial T_H2-high subjects, suggests the T_H2 designation has great clinical utility.² Moreover, it is straightforward to imagine T_H2-high airway status would be a highly relevant biomarker in T_H2-targeted therapeutic trials. Supporting this, serum levels of periostin have recently been used as a biomarker in a clinical trial of an IL-13 inhibitor.⁴ Finally, our association of nasal *IL13* levels with asthma exacerbations suggests nasal airway expression levels might be predictive of loss in asthma control and useful for clinical management. Although encouraging, these retrospective results will need to be further evaluated in prospective studies to determine their true clinical utility.

Interestingly, we identified 6 genes that were differentially expressed in asthmatic but not atopic subjects. This observation suggests that there exists dysregulation in nasal airway expression beyond T_H2 inflammation that is relevant to both atopic and nonatopic asthma. Nasal expression of both *KRT5*, a marker of basal airway epithelial cells, and *MUC5B*, a marker of secretory cells, was downregulated in asthmatic subjects. Changes in the expression of these genes might reflect airway remodeling of subjects with chronic asthma, as characterized by changes in the cellular and mucosal composition of the airway. Nasal overexpression of the *OSM* gene supports this idea because *OSM* gene and protein expression (an IL-6 family member) has previously been shown to be upregulated in the sputum of asthmatic subjects with irreversible airway obstruction.²⁴

In conclusion, we found that the nasal airways are an excellent and less-invasive proxy for the bronchial airways in transcriptional profiling studies. The pattern of T_H2 inflammation in the nasal airways is highly similar to the T_H2 pattern observed in the bronchial airways of asthmatic subjects. We find this T_H2 airway inflammation signature is highly enriched in atopic asthmatic subjects. These data clearly show the utility of nasal airway brushings for subphenotyping of asthmatic children in both research and clinical settings.

We thank the families and patients for their participation and thank the numerous health care providers and community clinics and schools for their support and participation in GALA II. In particular, we thank study coordinator Sandra Salazar and the recruiters who obtained the data: Jaime Colon and Vivian Medina.

Clinical implications: Nasal airway gene expression profiling can be used to easily identify the T_H2-high subphenotype of asthma in children and also other genes dysregulated in the asthmatic airway but independent of atopic status.

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METHODS

Nasal brushing collection and RNA extraction

Nasal epithelial cells were collected from behind the inferior turbinate with a sterile cytology brush (Cyto-Soft Cytology Brush CYB-1, #S7766-1A; Cardinal Health, Dublin, Ohio) by using a nasal illuminator. The collected brush was submerged in RLT Plus lysis buffer plus β -mercaptoethanol and frozen at -80°C until extraction. For some subjects ($n = 20$), the nasal brush was lightly touched to a glass slide to transfer some cells for staining before submerging the brush in lysis buffer. Wright staining of the slides revealed sheets of airway epithelial cells on all 20 slides (Fig E1). RNA quality was determined with the Agilent Bioanalyzer (Agilent, Santa Clara, Calif) for 50 of the 100 samples, and these samples had an RNA integrity number of greater than 8.2.

Whole-transcriptome gene expression

Barcoded Illumina RNA-seq libraries were prepared for each subject with the Illumina Tru Seq RNA Sample Preparation Kit (version 2). Libraries were pooled and sequenced across 2 full flow cells of an Illumina HiSeq 2000 at the University of Colorado Anschutz Medical Campus sequencing core. Raw sequencing quality was assessed with the fastx software package (version 0.0.13.2).^{E1} Reads from each sample were consolidated and mapped to the hg19 genome by using TopHat (version 2.0.6)^{E2,E3} with Bowtie (version 2.1.0).^{E4} After mapping, fragments were further filtered to remove sequences in which mates were unmapped or mapped to another chromosome or either mate had an alignment quality score of less than 20. Genes and transcripts were identified from mapped reads and quantified with Cufflinks (version 2.0.2).^{E5} Transcript assembly was restricted to annotated isoforms by using the “-G” option. Differential expression between asthmatic and control subjects was performed with the Cuffdiff tool^{E6} in the Cufflinks package with default settings. The iGenomes transcript annotation file (UCSC hg19) was downloaded (<http://tophat.cbcb.umd.edu/igenomes.shtml>) and used in both mapping and transcript assembly, as instructed by the TopHat/Cufflinks documentation.

Tissue comparison

Public RNA-seq data sets were downloaded from the sequence read archive.^{E7} Specifically, bronchial epithelial RNA-seq data were obtained from bronchial brushings of a pooled sample of 3 healthy (race not reported) nonsmoking subjects (SRR192333).^{E8} Small-airway epithelial data came from 5 black nonsmokers (SRR094862, SRR094863, SRR094864, SRR094906, and SRR094907) in a study by Hackett et al.^{E9} For each tissue, FPKM expression levels from healthy subjects were averaged across all genes. Genes obtaining a FPKM value of 0.125 or greater were considered expressed for all 3 data sets because we considered this biologically significant expression because an FPKM of 0.125 corresponds to approximately 1 transcript expressed per 8 cells (Fig 1).^{E9} Genes ubiquitously expressed across multiple tissues, as described by Ramskold,^{E10} were removed. Bronchial airway gene expression fold-changes in asthmatic subjects were obtained from the Woodruff et al^{E11} bronchial brushing microarray study at the following Web site: http://woodrufflab.ucsf.edu/genomics_pub/EPITH_top1000.html.

AmpliSeq expression analysis

Selection criteria for a subset of 29 asthma candidate genes screened in the AmpliSeq analysis were as follows: we selected 23 asthma genetic candidates selected from GWASs (16 genes) and other smaller genetic studies of asthma or related traits (7 genes). We selected an additional 7 biologic candidates implicated in mechanistic studies of asthma. (Note: One gene was selected as both a genetic and biologic candidate, resulting in 29 total genes.) Table E3 provides a reference for each candidate gene selected. Targeted RNA-seq expression analysis was performed with Ion RNA AmpliSeq (Life Technologies, Carlsbad, Calif). RNA AmpliSeq libraries were designed and generated by using Life Technologies design

software, a standard protocol, and reagents from the Ion AmpliSeq RNA Library Kit (Life Technologies). Namely, Life Technologies multiplexing software was used to design primers to amplify approximately 100-bp amplicons for 105 genes in a multiplex reaction (primer designs available on request). According to a standard library generation protocol, 10 ng of RNA was reverse transcribed, followed by multiplex PCR amplification of cDNA with the multiplex primer panel, adapter and barcode ligation to generate sequencing library, and library amplification. RNA AmpliSeq libraries were sequenced by using the Ion Torrent Proton on three P1 chips (Life Technologies). Reads mapping to target transcript amplicons were tabulated as expression count data with the torrent mapping alignment program and a Life Technologies in-house pipeline. Raw AmpliSeq expression values for each gene were determined by counting the number of reads mapping to the corresponding amplicon target. Differential expression analysis was performed on raw count data by using the nonparametric SAMseq^{E12} method available in the samr R package with default settings, except for increasing the number of permutations to 10,000. Differential expression testing in the SAMseq package uses a modified Wilcoxon statistic to test for differential expression with a multiple resampling strategy to correct for differences in total read counts between samples. Significance of results is determined by generating a null distribution of the modified Wilcoxon statistic by means of permutation. Significant genes after correcting for multiple testing are determined by using the false discovery rate method, directly computing the q value. The q value represents the expected proportion of false-positive results incurred when calling that gene significant. We considered genes with q values of less than .05 to be differentially expressed.

Statistical methods

The correlation between gene expression of commonly expressed nonubiquitous genes from different airway sites was calculated by using the Spearman rank correlation coefficient (ρ ; Fig 1). Venn diagrams were produced with the VennDiagram package (version 1.5.1) in R software.

All plots and calculations were performed by using the R statistical package, and correspondence-at-top plot values were calculated, as previously described.^{E13}

Hierarchical clustering of samples in Fig 2 was done with the csDendro function in the cummeRbund package (Version 2.0.0),^{E14} with default parameters. The method uses the Jensen-Shannon distance and complete linkage for dendrogram construction.

The correlation between $\log_2$ -transformed nasal and bronchial asthma fold changes in gene expression was determined by using the Pearson correlation coefficient (Fig 3).

Raw count values were normalized to correct for unequal sample mixing by using DESeq^{E15} for display of expression data and clustering analyses. Hierarchical clustering of genes (Fig 5) used the Spearman rank correlation coefficient ρ (as dissimilarity metric $[1 - \rho]$) and complete linkage. A heat map of expression values (Fig 5) was generated by transforming normalized counts for each gene into z scores, binning, and assigning bin colors from red to green, representing low and high expression levels, respectively, with black representing the mean expression level. Samples were clustered with the proximity score from a random forest classifier^{E16} trained to segregate samples into atopic or nonatopic status (positive or negative Phadiatop test result) by using the randomForest R package^{E17} (version 4.6-7) implementation. The randomForest classifier was run by using the default settings for number of trees grown (500) and gene sampling size (square root of the total number of genes). Subjects were sampled, with an equal number of atopic and nonatopic subjects (20 of each) used to construct each tree. The subject sampling strategy was determined empirically by maximizing the classifier accuracy on samples withheld from the training set. To form clusters, the distance between any 2 samples was equal to 1 minus the proximity score, and clusters were aggregated by using complete linkage. The Fisher exact test was used to calculate odds ratio P values for association of clinical phenotypes with T_H2-high and T_H2-low sample clusters.

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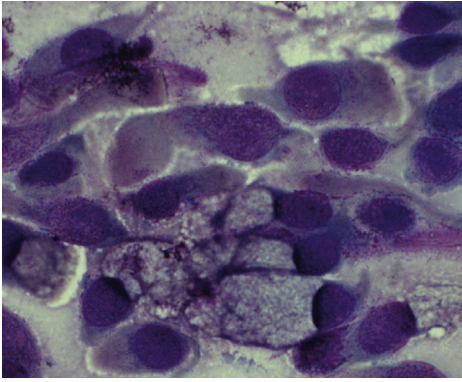


FIG E1. Representative nasal brush smear staining. A Wright-stained nasal brush cell smear showing nasal airway epithelial cells.

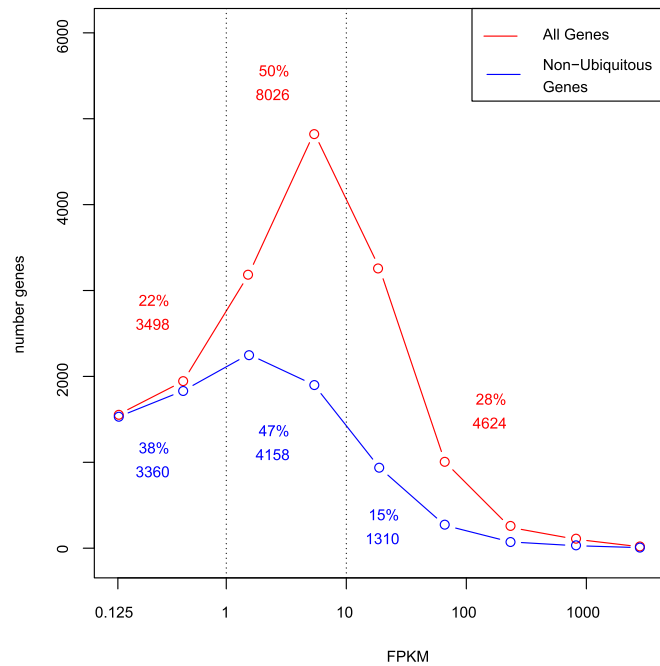


FIG E2. Nasal airway epithelial whole-transcriptome gene expression in healthy subjects. Expressed genes are categorized as having low (0.125-1 FPKM), medium (1-10 FPKM), and high (>10 FPKM) expression. The expression distribution for all genes is shown in *red* and the non-ubiquitously expressed genes is shown in *blue* (determined per Ramskold et al method).^{E10}

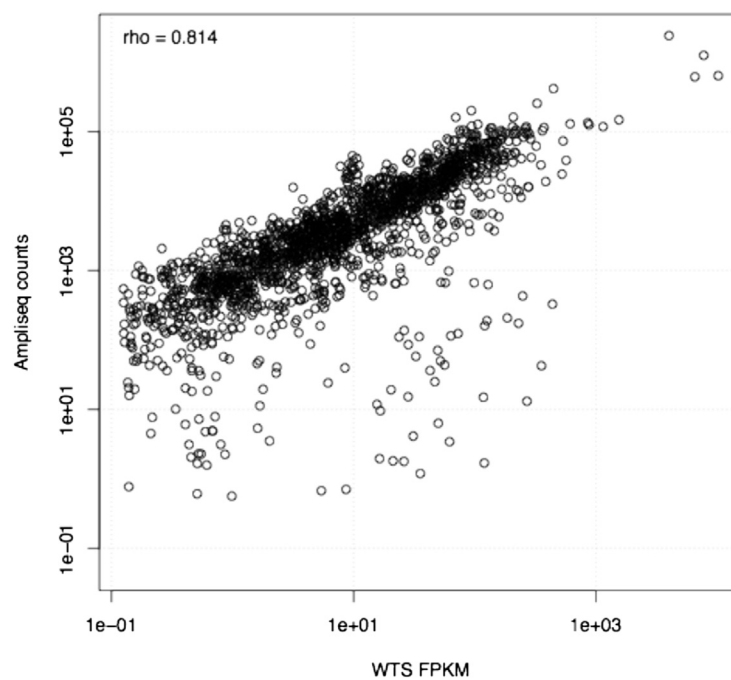


FIG E3. Scatter plot of AmpliSeq gene expression versus nasal whole-transcriptome gene expression data. Data are shown for the 20 overlapping subjects and genes between the 2 expression methods. The FPKM cutoff was 0.125. Spearman correlation coefficient is shown. *WTS*, Whole-transcriptome sequencing.

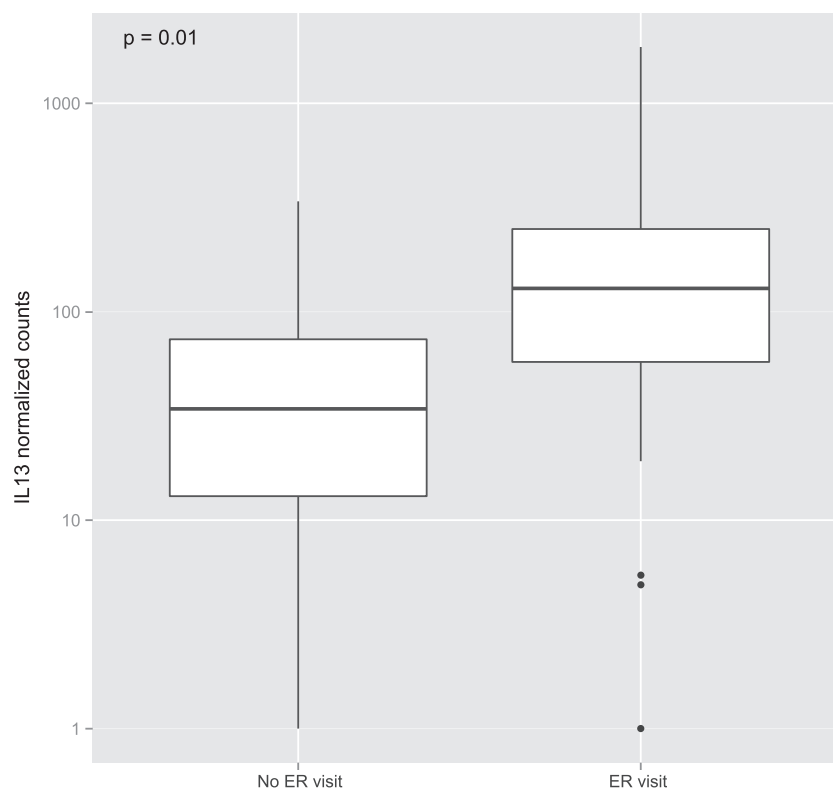


FIG E4. *IL13* AmpliSeq nasal gene expression levels categorized by asthmatic exacerbations. *Box plots* display normalized *IL13* expression count data for subjects requiring an asthma-related emergency department (*ER*) visit in the past year ($n = 30$) compared with those who did not ($n = 19$). A 1-read pseudocount was added to each sample to allow log transformation of genes with expression values of zero. The *P* value for differential expression between the groups was calculated by using a nonparametric Wilcoxon Mann-Whitney test.

TABLE E1. Whole-transcriptome sequencing and mapping metrics

	Control subjects	Asthmatic subjects	Total
Sample size	10	10	20
Read length	2 × 100 bp	2 × 100 bp	2 × 100 bp
Average pairs sequenced	69,534,854.0 ± 29,853,578	54,273,218.5 ± 5,888,143	61,904,036 ± 22,358,026
Average read ends sequenced	139,069,708 ± 59,707,157	108,546,437 ± 11,776,285	123,808,073 ± 44,716,051
Reads mapped	92.56% ± 4.1%	91.22% ± 4.98%	91.89% ± 4.49%
Mapped reads in genes	90.49% ± 2.09%	88.77% ± 2.09%	89.63% ± 2.22%
Mapped reads in exons	85.25% ± 3.18%	83.19% ± 2.80%	84.22% ± 3.10%
Mapped reads in introns	5.24% ± 1.38%	5.58% ± 1.00%	5.41% ± 1.19%
Mapped intergenic reads	9.45% ± 2.10%	11.17% ± 2.10%	10.31% ± 2.22%
Mapped aligning to rRNA	2.77% ± 3.94%	2.33% ± 1.28%	2.55% ± 2.86%

Values are means and SDs calculated on a subject-by-subject basis (not pooled). Reads were mapped with TopHat (version 2.0.6; using Bowtie2 [version 2.0.5]), and mapping summary statistics were obtained with RNA-SeQC (version 1.1.7).

TABLE E2. Cufflinks/Cuffdiff top 50 differentially expressed genes in nasal whole-transcriptome data

Gene	hg19 coordinates	FPKM, healthy subjects	FPKM, asthmatic subjects	Fold change	P value
<i>CPA3</i>	chr3:148583042-148614872	6.25	55.65	8.897	.003
<i>CLC</i>	chr19:40221892-40228669	0.34	27.44	80.103	.004
<i>RPPH1</i>	chr14:20811229-20811570	112.84	5.35	0.047	.004
<i>FOS</i>	chr14:75745480-75748937	9.81	33.22	3.386	.005
<i>WBSCR17</i>	chr7:70597788-71178584	0.15	2.13	14.538	.007
<i>PXDN</i>	chr2:1635658-1748291	0.42	2.62	6.177	.009
<i>CD69</i>	chr12:9905081-9913497	3.67	20.3	5.53	.01
<i>CLCA1</i>	chr1:86934525-86965974	2.42	25.44	10.499	.011
<i>OSM</i>	chr22:30658818-30662829	2.31	13.18	5.696	.011
<i>CCL5</i>	chr17:34198495-34207377	55.16	18.19	0.33	.012
<i>GPRI28</i>	chr3:100328432-100414323	1.81	0.01	0.005	.012
<i>HLA-DQA1</i>	chr6:32605182-32611429	110.78	32.61	0.294	.015
<i>TPSAB1</i>	chr16:1290677-1292555	17.8	104.78	5.885	.016
<i>CXCR1</i>	chr2:219027567-219031716	2.21	10.93	4.935	.02
<i>CSF2RB</i>	chr22:37309674-37336479	2.62	9.28	3.539	.021
<i>MUC5B</i>	chr11:1244294-1283406	2.03	0.54	0.266	.022
<i>AKAP12</i>	chr6:151561133-151679694	1.42	5.11	3.605	.023
<i>IL1B</i>	chr2:113587336-113594356	11.15	49.25	4.416	.026
<i>SAMSN1</i>	chr21:15857548-15955723	3.7	13.69	3.697	.026
<i>SLC2A3</i>	chr12:8071823-8088892	2.92	9.11	3.119	.027
<i>KRT16</i>	chr17:39766030-39769079	1.79	9.2	5.139	.03
<i>MS4A2</i>	chr11:59856136-59865940	0.35	2.72	7.762	.03
<i>PRSS33</i>	chr16:2833953-2836708	0.03	1.01	28.994	.032
<i>GPR97</i>	chr16:57702156-57723290	0.96	4.57	4.77	.032
<i>SOCS3</i>	chr17:76352858-76356158	8.5	25.86	3.043	.033
<i>TPSB2</i>	chr16:1278335-1280185	16.63	69.55	4.183	.034
<i>FFAR3</i>	chr19:35849487-35851389	0.13	2.65	20.367	.034
<i>G0S2</i>	chr1:209848669-209849735	11.79	40.09	3.401	.035
<i>TREML2</i>	chr6:41157551-41168925	0.34	1.98	5.84	.035
<i>CXCL9</i>	chr4:76922622-76928641	21.8	5.39	0.247	.035
<i>PTHLH</i>	chr12:28111016-28124916	1.64	17.46	10.663	.036
<i>RMRP</i>	chr9:35657747-35658015	128.94	18.99	0.147	.036
<i>SRGN</i>	chr10:70847827-70864567	43.71	133.91	3.064	.038
<i>FFAR2</i>	chr19:35940616-35942669	2.69	10.65	3.956	.039
<i>EGR1</i>	chr5:137801180-137805004	4.08	10.9	2.671	.04
<i>THBS1</i>	chr15:39873279-39889668	0.99	3.31	3.352	.042
<i>RGS2</i>	chr1:192778168-192781407	14.62	36.36	2.488	.048
<i>LAG3</i>	chr12:6881669-6887621	5.21	1.19	0.227	.049
<i>SLC6A8</i>	chrX:152953751-152962048	25.79	66.89	2.594	.051
<i>NDRG1</i>	chr8:134249413-134309547	23.28	57.7	2.479	.051
<i>CXCR2</i>	chr2:218990012-219001976	2.67	10.97	4.103	.052
<i>KRT6A</i>	chr12:52880957-52887181	23.2	71.76	3.093	.054
<i>DPYSL3</i>	chr5:146770370-146889619	4.29	10.51	2.448	.056
<i>DPP4</i>	chr2:162848754-162931052	1.68	5.25	3.127	.056
<i>MMP12</i>	chr11:102733463-102745764	1.46	7.71	5.283	.058
<i>HCAR3</i>	chr12:123199302-123201439	7.14	24.95	3.493	.058
<i>DUOXA2</i>	chr15:45406522-45422057	52.3	19.24	0.368	.059
<i>C1QA</i>	chr1:22963117-22966175	35.62	13.51	0.379	.059
<i>CCL26</i>	chr7:75398841-75419064	5.68	31.76	5.589	.06
<i>LGALS7B</i>	chr19:39279849-39282394	49.25	123.57	2.509	.06

TABLE E3. SAMseq differential expression for genes in the targeted nasal AmpliSeq analysis

Gene	Source*	Fold change in asthmatic patients	Asthmatic patient, q value	Fold change in atopic subjects	Atopic subjects, q value	Candidate reason/ reference
<i>ADRB2</i>	Asthma	0.89	0.034	0.706	0	Genetics ^{E18-E20}
<i>AKAP12</i>	WTS	1.229	0.048	1.277	0	
<i>BCL2A1</i>	WTS	1.575	0.137	0.658	0.092	
<i>C16orf54</i>	Bronch	0.871	0.241	0.655	0	
<i>C1QA</i>	WTS	0.606	0	0.486	0	
<i>C1QB</i>	WTS	0.564	0	0.436	0	
<i>C3</i>	Bronch	0.534	0.034	0.475	0.007	
<i>CCL26</i>	WTS	1.585	0	1.922	0	
<i>CCL5</i>	WTS	0.569	0	0.636	0	
<i>CD14</i>	Asthma	0.946	0.148	0.657	0	Genetics ^{E21}
<i>CD69</i>	WTS	1.105	0.137	1.097	0.236	
<i>CDH26</i>	Bronch	1.553	0	2.28	0	
<i>CDK14</i>	Bronch	0.705	0	0.721	0.007	
<i>CLC</i>	WTS	6.116	0	33.342	0	
<i>CLCA1</i>	Bronch, WTS	85.818	0	579.677	0	
<i>CPA3</i>	Bronch, WTS	2.755	0	31.045	0	
<i>CSF2RB</i>	WTS	1.28	0.101	0.891	0.221	
<i>CST1</i>	Bronch	23.664	0	619.824	0	
<i>CST4</i>	Bronch	2.346	0	4.42	0	
<i>CXCL9</i>	WTS	0.661	0.118	0.595	0.007	
<i>CXCR1</i>	WTS	0.816	0.369	0.247	0.012	
<i>CXCR2</i>	WTS	0.814	0.364	0.374	0.007	
<i>DHX35</i>	Bronch	1.021	0.369	1.039	0.306	
<i>DMXL2</i>	Bronch	0.943	0.046	0.938	0.017	
<i>DPP4</i>	WTS	2.1	0	2.651	0	
<i>DPYSL3</i>	WTS	1.006	0.339	1.388	0	
<i>DUOXA2</i>	WTS	0.889	0.158	0.302	0	
<i>EGR1</i>	WTS	1.159	0.272	0.871	0.258	
<i>FFAR2</i>	WTS	2.052	0.048	0.709	0.306	
<i>FFAR3</i>	WTS	2.752	0	2.51	0.022	
<i>FHOD3</i>	Bronch	0.865	0.227	0.755	0.017	
<i>FOS</i>	WTS	1.224	0.137	0.942	0.19	
<i>G0S2</i>	WTS	0.966	0.213	0.614	0.076	
<i>GPR128</i>	WTS	0.479	0.158	0.24	0.012	
<i>GPR97</i>	WTS	1.27	0.048	1.502	0.036	
<i>GSDMA</i>	Asthma	0.523	0.034	0.45	0	Genetics ^{E22,E23}
<i>GSDMB</i>	Asthma	0.993	0.369	0.816	0.007	Genetics ^{E22,E23}
<i>HCAR3</i>	WTS	1	0.369	1	0.325	
<i>HLA-DQA1</i>	WTS	0.318	0.046	0.362	0	
<i>IKZF3</i>	Asthma	0.796	0.014	0.669	0	Genetics ^{E22,E23}
<i>IL13</i>	Asthma	3.581	0.014	19.729	0	Bio/genetics ^{E22,E24,E25}
<i>IL18R1</i>	Asthma	0.917	0.294	1.046	0.136	Genetics ^{E22,E23,E26}
<i>IL1B</i>	WTS	1.36	0.28	0.445	0.027	
<i>IL1RL1</i>	Asthma	3.59	0	6.406	0	Genetics ^{E22,E23,E26}
<i>IL2RB</i>	Asthma	0.588	0	0.574	0	Genetics ^{E22}
<i>IL33</i>	Asthma	0.792	0	0.796	0	Genetics ^{E22,E23}
<i>IL4</i>	Asthma	8.675	0.048	8.58E+08	0	Bio ^{E27}
<i>IL5</i>	Asthma	2.495	0.13	9.497	0	Bio ^{E28}
<i>KCNIP4</i>	Asthma	1	0.06	1	0.306	Genetics ^{E29}
<i>KLK3</i>	Asthma	1	0.369	1	0.325	Genetics ^{E30}
<i>KRT14</i>	Asthma	1.195	0.272	0.953	0.306	Bio ^{E31}
<i>KRT16</i>	WTS	1.605	0.08	1.339	0.135	
<i>KRT5</i>	Asthma	0.683	0	1.079	0.092	Bio ^{E31}
<i>KRT6A</i>	WTS	1.425	0.178	1.18	0.314	
<i>LAG3</i>	WTS	0.637	0	0.67	0.007	
<i>LGALS7B</i>	WTS	0.697	0.193	1.324	0.147	
<i>MFGE8</i>	Asthma	1.126	0.329	1.028	0.216	Bio ^{E32}
<i>MMP12</i>	WTS	1.385	0.054	1.217	0.236	
<i>MS4A2</i>	WTS	3.122	0	21.388	0	
<i>MUC21</i>	Asthma	1.159	0.272	0.856	0.092	Genetics ^{E33}
<i>MUC22</i>	Asthma	1.05	0.329	0.756	0.007	Genetics ^{E33}
<i>MUC5B</i>	Bronch, WTS	0.287	0	0.716	0.1	

(Continued)

TABLE E3. (Continued)

Gene	Source*	Fold change in asthmatic patients	Asthmatic patient, q value	Fold change in atopic subjects	Atopic subjects, q value	Candidate reason/ reference
<i>MUC7</i>	Asthma	0.614	0.066	0.776	0.183	Genetics ^{E34}
<i>MXRA7</i>	Bronch	0.889	0.305	1.08	0.2	
<i>NDRG1</i>	WTS	0.914	0.339	1.148	0.136	
<i>NPB</i>	Bronch	0.954	0.148	0.608	0.012	
<i>ORMDL3</i>	Asthma	0.839	0.034	0.849	0.007	Genetics ^{E22,E23}
<i>OSM</i>	WTS	3.133	0.032	0.905	0.306	
<i>P2RY14</i>	Bronch	1.159	0.089	1.39	0	
<i>POSTN</i>	Bronch	1.853	0.048	11.237	0	
<i>PRR4</i>	Bronch	1.149	0.28	1.705	0	
<i>PRSS33</i>	WTS	4.859	0	38.281	0	
<i>PTHLH</i>	WTS	1.481	0.089	2.815	0	
<i>PXDN</i>	WTS	1.691	0.032	2.609	0	
<i>PYHIN1</i>	Asthma	0.668	0	0.472	0	Genetics ^{E23}
<i>RG2</i>	WTS	1.01	0.28	0.886	0.092	
<i>SAMSN1</i>	WTS	1.184	0.089	1.168	0.204	
<i>SCGB3A1</i>	Bronch	0.56	0.027	0.468	0	
<i>SCLY</i>	Bronch	0.828	0.066	0.811	0.007	
<i>SCNN1G</i>	Bronch	0.68	0	0.796	0.007	
<i>SDK2</i>	Asthma	0.899	0.148	1.079	0.202	Genetics ^{E34}
<i>SEC14L1</i>	Bronch	0.997	0.329	1.198	0	
<i>SERPINB2</i>	Bronch	1.491	0	1.871	0	
<i>SHISA2</i>	Bronch	0.684	0.034	0.559	0.007	
<i>SLC2A3</i>	WTS	1.098	0.213	0.728	0.027	
<i>SLC6A8</i>	WTS	1.13	0.095	1.306	0	
<i>SLC7A1</i>	Bronch	1.201	0	1.365	0	
<i>SMAD2</i>	Asthma	0.868	0.148	0.831	0	Genetics ^{E35}
<i>SMAD3</i>	Asthma	0.969	0.294	0.923	0.007	Genetics ^{E22}
<i>SOCS3</i>	WTS	1.167	0.339	1.023	0.183	
<i>SOX2</i>	Bronch	0.998	0.339	1.037	0.282	
<i>SRGN</i>	WTS	1.415	0.106	0.945	0.216	
<i>STEAP4</i>	Bronch	0.801	0.06	0.75	0	
<i>STOM</i>	Bronch	1.05	0.213	1.041	0.325	
<i>TGFB1</i>	Asthma	0.836	0	0.801	0	Bio ^{E36}
<i>THBS1</i>	WTS	0.96	0.369	1.544	0.036	
<i>TLR4</i>	Asthma	1.117	0.339	0.612	0	Genetics ^{E37}
<i>TMEM45A</i>	Bronch	1.439	0	1.484	0	
<i>TPSAB1</i>	Bronch, WTS	2.522	0	21.858	0	
<i>TPSB2</i>	WTS	1.378	0.08	486599925	0	
<i>TREML2</i>	WTS	1.814	0.026	1.177	0.135	
<i>TSLP</i>	Asthma	0.872	0.073	1.082	0.287	Genetics ^{E23,E38}
<i>WBSCR17</i>	WTS	2.302	0	4.497	0	
<i>ZMAT2</i>	Bronch	1.011	0.369	0.912	0.007	
<i>ZPBP2</i>	Asthma	0.073	0	0.725	0.258	Genetics ^{E22,E23}

*Reason for inclusion: bronchial asthma biomarker (*Bronch*) in top 50 differentially expressed genes from nasal whole-transcriptome sequencing data (*WTS*) and asthma candidate genes (*Asthma*) subclassified as (*Genetics*) or biological (*Bio*) candidates.

TABLE E4. Bronchial biomarkers found to be differentially expressed in the nasal airways of subjects with asthma by using the AmpliSeq assay

Gene	Log ₂ fold change, nasal*	Log ₂ fold change, bronchial†
<i>POSTN</i>	0.89	2.071
<i>CPA3</i>	1.462	1.817
<i>TPSAB1</i>	1.335	1.064
<i>SERPINB2</i>	0.576	1.835
<i>CLCA1</i>	6.423	2.632
<i>CST4</i>	1.23	1.245
<i>CST1</i>	4.565	2.176
<i>SLC7A1</i>	0.264	0.21
<i>CDH26</i>	0.635	0.477
<i>TMEM45A</i>	0.525	−0.923
<i>SCNN1G</i>	−0.556	−0.925
<i>DMXL2</i>	−0.085	−0.31
<i>MUC5B</i>	−1.801	−0.863
<i>SCGB3A1</i>	−0.837	−1.091
<i>CDK14</i>	−0.504	−0.235
<i>C3</i>	−0.905	−1.001
<i>SHISA2</i>	−0.548	−1.07

*Values derived from SAMseq analysis of AmpliSeq data.

†From microarray data from the study by Woodruff et al.^{E11}

TABLE E5. SAMseq differential expression in asthma GWAS genes

Gene	Fold change, asthmatic subjects	Q value, asthmatic subjects	Fold change, atopic subjects	Q value, atopic subjects	Reference
<i>GSDMA</i>	0.523	0.034	0.45	0	E22,E23
<i>GSDMB</i>	0.993	0.193	0.816	0.007	E22,E23
<i>IKZF3</i>	0.796	0.014	0.669	0	E22,E23
<i>IL13</i>	3.581	0.014	19.729	0	E22,E24
<i>IL18R1</i>	0.917	0.294	1.046	0.136	E22,E23,E26
<i>IL1RL1</i>	3.59	0	6.406	0	E22,E23,E26
<i>IL2RB</i>	0.588	0	0.574	0	E22
<i>IL33</i>	0.792	0	0.796	0	E22,E23
<i>KCNIP4</i>	1	0.060	1	0.306	E29
<i>KLK3</i>	1	0.369	1	0.325	E30
<i>ORMDL3</i>	0.839	0.034	0.849	0.007	E22,E23
<i>PYHIN1</i>	0.668	0	0.472	0	E23
<i>SMAD2</i>	0.868	0.148	0.831	0	E35
<i>SMAD3</i>	0.969	0.294	0.923	0.007	E22
<i>TSLP</i>	0.872	0.073	1.082	0.287	E23,E38
<i>ZPBP2</i>	0.073	0	0.725	0.306	E22,E23