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This information is current as of January 4, 2011

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J Immunol; Prepublished online 27 December 2010;
doi:10.4049/jimmunol.1002568
<http://www.jimmunol.org/content/early/2010/12/24/jimmunol.1002568>

Supplementary Data	http://www.jimmunol.org/content/suppl/2010/12/27/jimmunol.1002568.DC1.html
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The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Gene Expression Patterns of Th2 Inflammation and Intercellular Communication in Asthmatic Airways

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Asthma is canonically thought of as a disorder of excessive Th2-driven inflammation in the airway, although recent studies have described heterogeneity with respect to asthma pathophysiology. We have previously described distinct phenotypes of asthma based on the presence or absence of a three-gene “Th2 signature” in bronchial epithelium, which differ in terms of eosinophilic inflammation, mucin composition, subepithelial fibrosis, and corticosteroid responsiveness. In the present analysis, we sought to describe Th2 inflammation in human asthmatic airways quantitatively with respect to known mediators of inflammation and intercellular communication. Using whole-genome microarray and quantitative real-time PCR analysis of endobronchial biopsies from 27 mild-to-moderate asthmatics and 13 healthy controls with associated clinical and demographic data, we found that asthmatic Th2 inflammation is expressed over a variable continuum, correlating significantly with local and systemic measures of allergy and eosinophilia. We evaluated a composite metric describing 79 coexpressed genes associated with Th2 inflammation against the biological space comprising cytokines, chemokines, and growth factors, identifying distinctive patterns of inflammatory mediators as well as Wnt, TGF- β , and platelet-derived growth factor family members. This integrated description of the factors regulating inflammation, cell migration, and tissue remodeling in asthmatic airways has important consequences for the pathophysiological and clinical impacts of emerging asthma therapeutics targeting Th2 inflammation. *The Journal of Immunology*, 2011, 186: 000–000.

Asthma has been described as an allergic disorder, with airway pathophysiology resulting from chronic Th2-driven eosinophilic inflammation (1). However, this description does not capture the complexity of clinical asthma, which exhibits heterogeneous pathophysiology and pharmacologic responsiveness related to the type of cellular inflammation in the

airway (2–4). In particular, the nature and intensity of granulocytic infiltrates (e.g., eosinophilic, neutrophilic, mixed, paucigranulocytic) may define distinct subtypes of asthma (5, 6). Molecular phenotyping of diseased tissues, using technologies such as gene expression microarrays, has the potential to provide insights into the phenotypic heterogeneity of disease and the identification of associated biomarkers (7) or strategies to select patients with an increased potential to respond to molecularly targeted therapies.

New investigational therapeutics for asthma targeting inflammatory cytokines are emerging examples of the potential advantage of patient selection. IL-5 is associated with the expansion, priming, recruitment, and prolonged tissue survival of eosinophils (8), and it has therefore been the subject of study as a potential drug target. Although early studies of IL-5 antagonism in asthmatics failed to show signs of efficacy (9–11), recent clinical studies in prespecified populations of eosinophilic asthmatics have demonstrated benefit (12, 13). Similarly, recent clinical trials of Abs targeting proinflammatory cytokines thought to contribute to asthma pathogenesis, such as TNF- α or IL-4 and IL-13 (1, 14, 15), failed to meet their primary endpoints in all comers, but in post hoc analyses there was evidence suggesting that subgroups of asthmatics experienced enhanced benefit (16, 17). However, the efficacy of molecularly targeted therapies in a clinical setting depends on both appropriate patient selection and appropriate outcome selection. These studies highlight the importance of understanding the underlying basis of heterogeneity in disease and the relationships between targeted pathways and in vivo pathophysiology for developing strategies to identify patient populations with maximal potential benefit from molecularly targeted therapies.

We have previously reported molecular signatures associated with clinical subphenotypes of asthma (18). We dichotomized a mild-to-moderate asthmatic population into “Th2-high” and

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Received for publication July 29, 2010. Accepted for publication November 23, 2010.

This work was supported by grants to J.V.F. and P.G.W. from Genentech; by National Institutes of Health Grants HL56385, HL080414, and HL66564 (to J.V.F.) and RR17002 and HL09572 (to P.G.W.); and by the Sandler Asthma Basic Research Center (to J.V.F.).

D.F.C., B.M., A.R.A., S.K., and H.F.C. designed and performed statistical data analyses; D.F.C., G.F., and Z.M. performed gene expression assessments; J.V.F. and P.G.W. designed and performed clinical assessments; L.C.W., J.V.F., P.G.W., and J.R.A. designed the study and experimental plan; and D.F.C. and J.R.A. wrote the manuscript.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BAL, bronchoalveolar lavage; CCGF, cytokines, chemokines, and growth factors; DE, differentially expressed; PCA, principal component analysis; PC1, principal component 1; PDGF, platelet-derived growth factor; qPCR, quantitative real-time PCR; SPCA, supervised principal component analysis; Th2 sig, Th2 signature metric.

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"Th2-low" subphenotypes on the basis of a signature of three IL-13 inducible genes in bronchial epithelial brushings. These subphenotypes exhibited distinct pathology and corticosteroid responsiveness. Molecular phenotyping of airway epithelium is therefore informative and relevant, as it describes a molecular basis for asthma pathophysiology (19). However, the pathophysiology of asthma is not limited to the epithelium. Endobronchial biopsies permit direct analysis of epithelial and subepithelial cellular and molecular mediators of asthma, including structural cells of the airway and inflammatory infiltrates. In the current study, our objective was to perform gene expression microarray analyses of endobronchial biopsies matched to previously characterized bronchial epithelial brushings, directly comparing Th2 subphenotypes. It is the premise of these analyses that complementary molecular phenotyping could elucidate a more granular description of the pathophysiological mediators of Th2 inflammation in asthma.

Materials and Methods

Subjects

Bronchial biopsy RNA from 27 mild-to-moderate nonsmoking asthma patients and healthy nonsmoking subjects was obtained from the University of California, San Francisco, Airway Tissue Bank, a specimen biorepository approved by the University of California, San Francisco, Committee on Human Research and managed by two of the authors (J.V.F. and P.G.W.). Endobronchial biopsies had been collected from a subset of patients in whom we have previously described gene expression profiles in bronchial epithelial brushings (as described in detail elsewhere; see Refs. 18, 20). Three to six endobronchial biopsies were collected from the carinae of second- to fourth-order bronchi, and the RNA was pooled after extraction using methods we have previously described. The clinical characteristics of the 13 healthy controls and 27 asthmatic subjects described in this study are shown in Table I. Informed consent was obtained from all human subjects after the nature and possible consequences of the study were explained.

Gene expression analyses

RNA was isolated from homogenized bronchial biopsies and quantitative real-time PCR (qPCR) for IL-5 and IL-13 was performed as described previously (18). TaqMan gene expression assays (Applied Biosystems, Foster City, CA) were purchased and conducted per the manufacturer's instructions for CST1 (i.d. Hs00606961_m1), MUC5B (i.d. Hs00861595_m1), CLCA1 (i.d. Hs00154490_m1), and CCL26 (i.d. Hs00171146_m1).

RNA was amplified (MessageAmp II; Ambion, Austin, TX) for Agilent (Santa Clara, CA) two-color Whole Human Genome 4 × 44K gene expression microarray analysis. Universal Human Reference RNA (Stratagene, La Jolla, CA) was used for the reference channel. Probe intensities were transformed as log₂ ratios of test and reference channels calculated by the Agilent Feature Extraction software, protocol GE2-v5_95 (Agilent). Flagged outliers were not included in any subsequent analyses. The microarray data are available in the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/projects/geo) under the accession number GSE23611.

Comparative analyses of differentially expressed genes in human disease data sets (asthma and eosinophilic esophagitis) was via Entrez Gene annotation for individual probes. Human to mouse comparisons were based on GenBank transcript annotation for each human or murine probe, followed by alignment through National Center for Biotechnology Information HomoloGene.

Statistical analyses

Analyses were performed using R for Windows version 2.9.2 (Ref. 21; also, refer to <http://www.R-project.org>), JMP 8.0.1 (SAS Institute, Cary, NC), and Partek Genomics Suite 6.5 (Partek, St. Louis, MO). Probes with features <50% present were removed prior to each statistical test. Multiple testing correction was addressed for indicated analyses by the calculation of the *q* value for determination of false discovery rate (22). Differential gene expression analysis between "Th2-high asthma" and "Th2-low asthma and healthy control" was conducted by a Welch *t* test, due to unequal sample sizes (14 and 26, respectively), and unequal variances were detected by a Bartlett test among array features. The *q* values were based on Welch *t* test *p* values and are reported in Supplemental Table I. Gene expression

similarity clustering was conducted using a Spearman correlation (dissimilarity metric) and Ward's agglomeration method.

A Th2 signature metric (Th2 sig) was derived from principal component 1 (PC1) of supervised PC analysis (SPCA) of the 93 differentially expressed (DE) probes between Th2-high asthma and Th2-low asthma and healthy control as described in *Results*. PCA was conducted on standard scores of expression values. Missing values in this computation (0.6%, 23 among 3720) was addressed by replacement with the response variable mean. Cytokines, chemokines, and growth factors (CCGf) genes were manually curated and are listed in Supplemental Table II. When comparing this approach to performing a simple *t* test of CCGf, using bronchial epithelial Th2 status as a factor, we find *t* test *t* and Spearman rho values to be nearly in perfect agreement (Supplemental Fig. 1A, $p < 1 \times 10^{-15}$, Spearman correlation). However, employing correlation analysis using the Th2 sig results in a much more sensitive detection of association with CCGf (Supplemental Fig. 1B).

Results

Differentially expressed bronchial biopsy genes are highly intercorrelated and relate directly to clinical measures of allergy and inflammation

To identify patterns of gene expression in bronchial mucosa corresponding to Th2 inflammation, we performed differential gene expression analysis in bronchial biopsies from 27 asthmatics and 13 healthy control subjects using discrete categorizations of bronchial epithelial Th2 signature status as prespecified stratification criteria. On the basis of a three-gene bronchial epithelial expression pattern, we have previously categorized these subjects into two clusters: one comprising Th2-high asthma and one comprising Th2-low asthma and healthy controls (18). A two-sample *t* test (see *Materials and Methods*) was employed to generate a DE probe list for Th2-high asthma versus Th2-low asthma and control, constituting 93 probes ($q < 0.05$) corresponding to 79 uniquely annotated genes (Supplemental Table I).

We verified a subset of the DE probe list by qPCR (Supplemental Table III). Among the verified genes were several that have been previously associated with asthma and/or Th2 inflammation: chloride channel, calcium-activated, family member 1 (CLCA1); mucin 5B, oligomeric mucus/gel-forming (MUC5B); and chemokine (C-C motif) ligand 26 (CCL26). CLCA1 is upregulated in airway epithelial cells by IL-13 stimulation *in vitro* and is expressed at elevated levels *in vivo* in asthmatic bronchial epithelium (20), which may have an indirect role in mediating calcium-activated chloride currents and mucus secretion (23). MUC5B is a member of a family of mucin glycoproteins produced by goblet cells in the lung that contribute to the viscoelastic and adhesive properties of airway mucus, and it has been shown to be downregulated in the airways of asthmatics, with concomitant upregulation of MUC5AC and MUC2 (18, 24).

CCL26 was the most highly differentially expressed gene between Th2-high asthma and Th2-low asthma and control by microarray (fold change = 4.06, $q = 1.3 \times 10^{-5}$) and confirmed by qPCR (Supplemental Table III). CCL26, also known as eotaxin-3, is a chemokine that binds to CCR3 and acts as a potent eosinophil chemoattractant. Stimulation by the Th2 cytokines IL-4 and IL-13 in *in vitro* systems strongly upregulates CCL26 expression in human bronchial epithelial cells (25) and PBMCs (26). CCL26 protein has been reported to be elevated in asthmatic bronchial mucosa (25). Furthermore, in a study of eosinophilic esophagitis, a Th2-associated disease of the esophageal mucosa, CCL26 was observed to be the mostly highly induced gene, whose expression strongly correlated with tissue eosinophilia and mastocytosis (27). We assessed the correlation between biopsy CCL26 gene expression and cytokine expression (quantified by qPCR) and observed strong positive correlations with the Th2 cytokines IL-5

($\rho = 0.48$, $p = 0.002$) and IL-13 ($\rho = 0.79$, $p < 0.0001$), but not with IL-4 (not shown), and a strong negative correlation with the Th1 cytokine IL-12A ($\rho = -0.53$, $p = 0.0006$) (Fig. 1A).

CCL26, IL-5, and IL-13 are widely held to be key effector molecules in the manifestation of Th2 inflammation in asthma (14, 15). Comparing quantitative local and systemic phenotypic assessments indicative of allergic inflammation (Fig. 1B), we found positive correlations between CCL26 gene expression and serum IgE

($\rho = 0.64$, $p < 0.0001$), peripheral blood eosinophil count ($\rho = 0.53$, $p = 0.0005$), and a trend for association with eosinophil percentage in bronchoalveolar lavage (BAL) fluid ($\rho = 0.30$, $p = 0.064$). Additionally, we noted several other genes among the DE probes known to be associated with Th2 inflammation: IgE; NO synthase 2A (NOS2A); histamine receptor H1; GPR44, also known as chemoattractant receptor-homologous molecule expressed on Th2 cells; and arachidonate 15-lipoxygenase (ALOX15) (18, 28–33). Taken together, the differential expression of these genes is consistent with the phenotypic descriptions of Th2-high and Th2-low asthma.

We observed a high degree of intercorrelation among the probes in the DE probe list described in Supplemental Table I. Two-way similarity clustering (Spearman correlation) of the 93 probes resulted in two major clusters of positively correlated probes (Fig. 1C). The clusters were anticorrelated with one another. Considering the high degree of intercorrelation and the direct relationship between CCL26 (probe i.d. A_24_P12573), qPCR assessments of IL-5, IL-13, and IL-12A, and local and systemic clinical measures of allergy and inflammation, it was not surprising to note a high prevalence of significant ($q < 0.05$) associations of these measures with individual expression of each DE probe. With the exception of eosinophil percentage in the BAL, each of these measures was significantly correlated ($q < 0.05$, Spearman correlation) with each individual DE probe, whereas BAL eosinophil percentage was correlated with 88 of 93 (95%) of the probes (Supplemental Table I).

Taken together, these data suggest that airway gene expression of Th2-associated molecules, such as CCL26, IL-5, IL-13, and other genes identified by differential gene expression analysis, have a direct and continuous relationship with local and systemic markers of Th2 inflammation in asthmatics. Due to the high degree of intercorrelation among the DE genes and direct correlation with clinical measures of Th2 inflammation, we hypothesized that a variable continuum of gene expression underlies discrete Th2-low and Th2-high phenotypes determined by bronchial epithelial gene expression (18) and that these DE genes described a Th2 inflammation signature gene set. To test this hypothesis, we: 1) developed a Th2 sig for the 93 DE probes and evaluated it against molecular and clinical markers of Th2 inflammation, and 2) assessed this summary metric against the biological space described by CCGf to more comprehensively describe the molecular processes associated with Th2 inflammation in asthmatic airways.

The biopsy Th2 sig describes a molecular and clinical continuum of Th2 inflammation

We summarized the expression of the 93 DE probes into a single continuous classifying metric by SPCA (34, 35). Scores from PC1, representing 49% of the variance of the highly intercorrelated Th2 signature gene set, are used as scalar values for the Th2 sig. As expected, Th2-high asthma is distinguished from control, exhibiting minimal overlap with Th2-low asthma along the PC1 axis (Supplemental Fig. 2). Organized by PCA factors, an intensity heat map of normalized DE probe intensity depicts an intuitive subject hierarchy on the basis of a coordinated continuum of Th2 signature gene expression (Fig. 2). Specifically, upregulated Th2-high genes, such as CCL26, CLCA1, and IgE, are coordinately expressed from low to high levels from Th2-low to Th2-high subjects, and conversely for downregulated Th2-low genes, such as MUC5B. As expected, this pattern corresponds well ($p < 0.0001$, ordinal logistic regression) with the dichotomous Th2-high and Th2-low phenotypes we have described in these subjects (18) on the basis of bronchial epithelial gene expression (Fig. 3A). The model performance is displayed by receiver operating

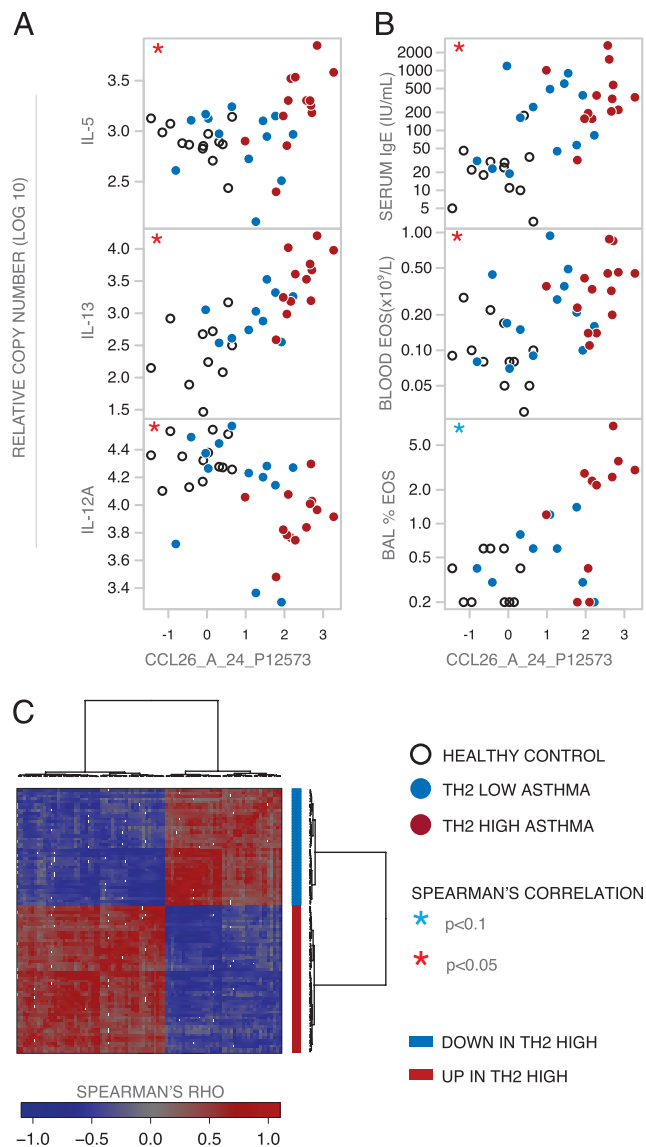


FIGURE 1. Relationship among differentially expressed bronchial biopsy genes and clinical measures of allergy and inflammation. CCL26 was the most highly differentially expressed gene between Th2-high asthma and Th2-low asthma and control (fold change = 4.06, $q = 1.3 \times 10^{-5}$) and is directly associated with Th2 inflammation. A, CCL26 (probe i.d.: A_24_P12573) positively correlates (Spearman rank order) with qPCR assessments of IL-5 ($\rho = 0.48$, $p = 0.002$) and IL-13 ($\rho = 0.79$, $p < 0.0001$) and negatively with IL-12A ($\rho = -0.53$, $p = 0.0006$). B, CCL26 (probe i.d.: A_24_P12573) correlates (Spearman rank order) with serum IgE ($\rho = 0.64$, $p < 0.0001$) and peripheral blood eosinophil count ($\rho = 0.53$, $p = 0.0005$) and weakly associates with BAL fluid eosinophil percentage ($\rho = 0.30$, $p = 0.064$). C, Similarity clustering analysis of DE probes (probe versus probe) illustrates a high degree of expression intercorrelation (Spearman rank order) among the DE probe list (Supplementary Table I). DE probes in Th2-high asthma are indicated by adjacent red (upregulated) and blue (downregulated) rectangles.

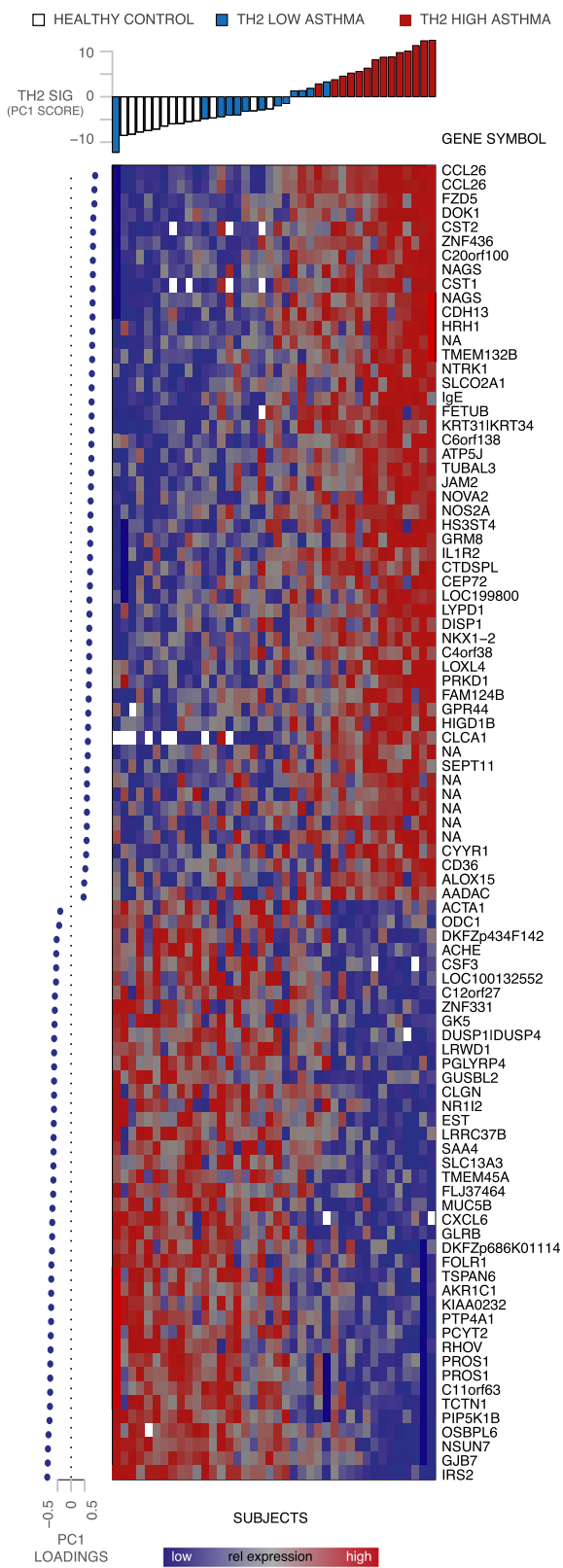


FIGURE 2. Th2 sig quantifies a continuum of Th2-associated gene expression. PCA is applied for data dimension reduction and derivation of the Th2 signature metric, which summarizes the expression of 93 signature gene set probes. PC1 retains 49% of the variance in the Th2 signature gene set and serves as a summarizing metric: Th2 sig. Normalized gene expression is represented by intensity heat map (missing probe values are represented in white). Subjects (columns) and probes (rows) are organized by PC1 score and PC1 loadings, respectively.

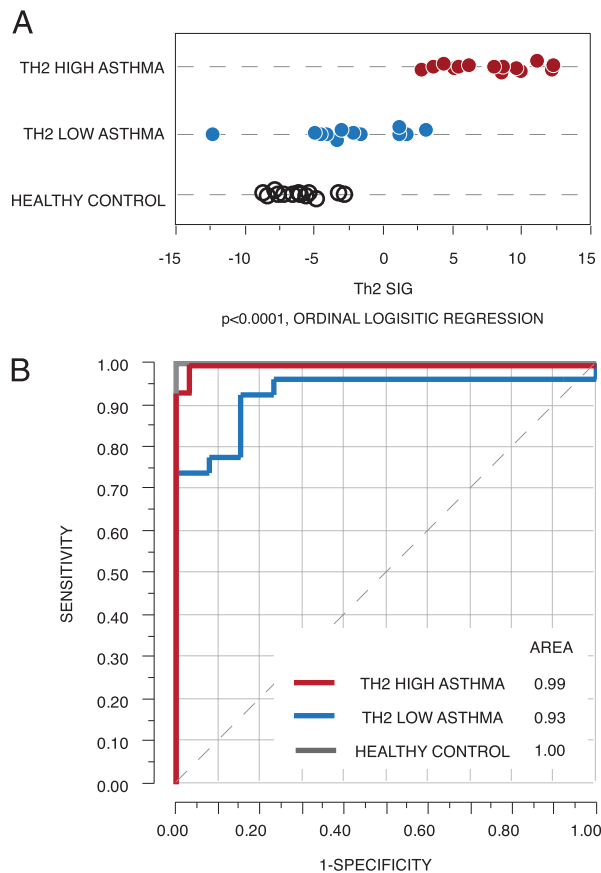


FIGURE 3. Logistic regression of bronchial epithelial Th2 phenotype by biopsy Th2 sig. Ordinal logistic regression was performed on bronchial epithelial Th2 phenotype, as defined previously (18), by biopsy Th2 sig. A high degree of association was observed ($p < 0.0001$) between the two metrics. A, Regression and (B) receiver operating characteristic plots from regression model.

characteristic analysis (Fig. 3B). Taken together, these analyses suggest that Th2 inflammation in underlying bronchial mucosa above a threshold level is necessary to trigger the dramatic phenotypic differences we observed between groups for bronchial epithelial gene expression, airway remodeling, and corticosteroid responsiveness (Table I).

Consistent with the observation that individual genes in the Th2 sig have a direct relationship with clinical measures of allergy and inflammation, Th2 sig, which summarizes the collective expression of 93 probes encoding 79 uniquely annotated genes, significantly correlates with qPCR assessments of IL-5 ($\rho = 0.45$, $p = 0.0038$), IL-13 ($\rho = 0.74$, $p < 0.0001$), and IL-12A ($\rho = -0.71$, $p < 0.0001$); serum IgE ($\rho = 0.62$, $p < 0.0001$); blood eosinophil count ($\rho = 0.53$, $p = 0.0005$); and BAL eosinophil percentage ($\rho = 0.39$, $p = 0.017$) (Fig. 4).

CCGf gene correlations with the biopsy Th2 sig

Asthma has been described as an allergic disorder, and the molecular effectors of Th2 inflammation have largely been described in terms of the biological space of CCGf due to their direct relevance as effector molecules in inflammation, cell migration, and tissue remodeling in addition to their relative tractability as therapeutic targets (1). We performed a focused correlation analysis of a manually curated gene set of known CCGf versus Th2 sig (Fig. 5, Supplemental Table II) within asthmatics to further elucidate the association between molecular and clinical components of Th2 inflammation in asthma and describe the Th2 inflammatory phe-

Table I. Clinical and demographic characteristics of the study population

	Healthy Control	Asthma
Sample size	13	27
Age, y	39 (31–58)	33 (20–55)
Gender, M/F	4:9	13:14
Ethnicity		
White	11	11
African-American	0	4
Hispanic	1	9
Asian/Pacific Islander	1	3
FEV ₁ , % predicted	102 (92–136)	87.7 (65–107)
Methacholine PC ₂₀	64 (21.5–64)	1.4 (0.05–7.27)
IgE (IU/ml)	23 (3–177)	221 (19–2627)
Blood eosinophils (×10 ⁹ /l)	0.085 (0.03–0.28)	0.27 (0.07–0.94)
BAL eosinophils (%)	0.2 (0–0.6)	0.5 (0–7.4)

Values are presented as median (range).
FEV₁, forced expiratory volume in 1 s; PC₂₀, provocative concentration required to cause a 20% decline in forced expiratory volume in 1 s.

notype more broadly within the biological space of intercellular mediators with generally well-characterized functions. Limiting our analysis to CCGf also serves as a useful analytical strategy to minimize multiple test correction penalties.

Among 212 genes encoding uniquely annotated CCGf (268 probes), 25 (27 probes) were positively or negatively correlated (Spearman correlation) with the Th2 sig below a *q* value threshold of 0.05, and 45 (49 probes) were correlated with a *q* value below a threshold of 0.10 (Fig. 5). Unsurprisingly, CCL26 and IL-13

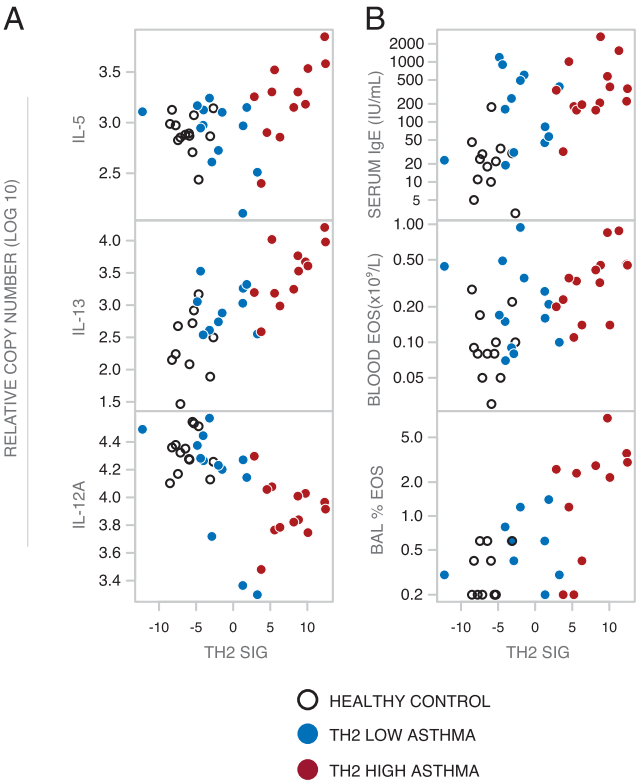


FIGURE 4. Characteristics of the Th2 sig. The Th2 sig relates directly to molecular and clinical measures of allergy, inflammation, and lung function. **A**, Correlation (Spearman) of the Th2 sig with qPCR assessments of IL-5 ($\rho = 0.45$, $p = 0.0038$), IL-13 ($\rho = 0.74$, $p < 0.0001$), and IL-12A ($\rho = -0.71$, $p < 0.0001$). **B**, Correlation (Spearman) of the Th2 sig with serum IgE ($\rho = 0.62$, $p < 0.0001$), blood eosinophil count ($\rho = 0.53$, $p = 0.0005$), and BAL eosinophil percentage ($\rho = 0.39$, $p = 0.017$).

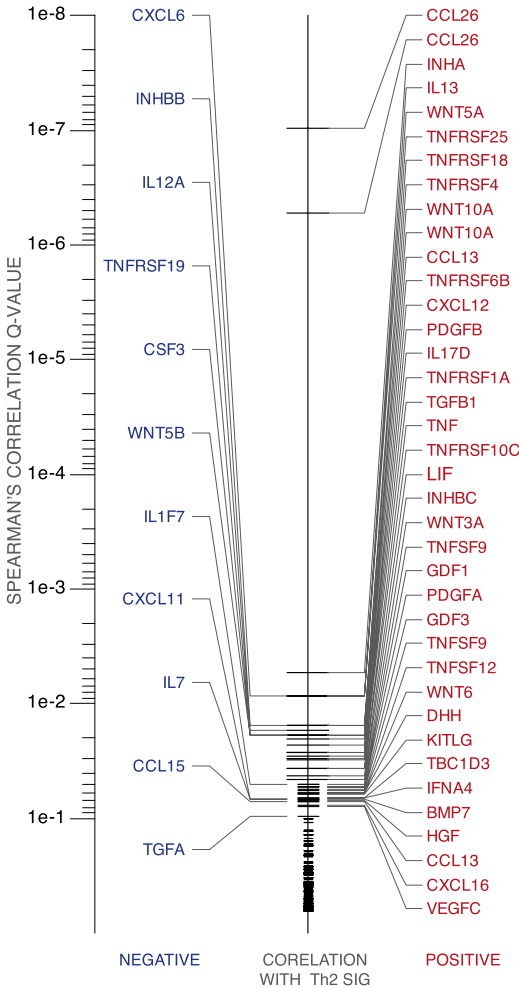


FIGURE 5. Correlation of asthma Th2 sig with CCGf. Correlation analysis (Spearman rank order) was performed among asthmatics ($n = 27$) with a manually curated list of CCGf. Correlation significance is plotted (all) and annotated ($q < 0.1$) by strip chart. Positive correlations are annotated in red to the right of the midline. Negative correlations are annotated in blue to the left of the midline.

were positively correlated with the Th2 sig, as was the CCR3-binding eosinophil attracting chemokine CCL13 (36). CCGf whose expression levels were strongly negatively associated with the Th2 sig included the Th1 cytokine IL-12A and the Th1 chemokine CXCL11 (37–40). Taken together, these findings suggest that Th2 inflammation may occur in the context of suppressed Th1 inflammation in asthma and further support the concept that the Th2 sig is a quantitative metric of Th2 burden in the airway. In addition to positive correlations between the Th2 sig and CCR3-binding eosinophil-attracting chemokines CCL13 and CCL26, we observe strong negative correlations between the Th2 sig and the CXCR1/2-binding neutrophil-attracting chemokine CXCL6, as well as the neutrophil hematopoietic factor CSF3 (G-CSF), which may underlie differences in airway granulocyte infiltration described for asthma subphenotypes (5, 6).

We observed positive correlations between diverse groups of inflammatory factors and the Th2 sig. TNF- α is produced along with Th2 cytokines from “inflammatory Th2” cells stimulated by OX40L-expressing dendritic cells (41), and we have previously shown that BAL macrophages from Th2 high asthmatics express elevated levels of TNF- α (18). Positive correlations between the Th2 sig and TNFRSF4 (OX40) and TNFSF9 (4-1BBL) suggest infiltration of activated helper T cells, which are likely sources of

many of the inflammatory cytokines observed (42). KITLG, also known as stem cell factor, is a mast cell growth factor and chemoattractant (43). LIF is a member of the IL-6 family of cytokines binding to the shared gp130 subunit of the IL-6R complex. Its expression is positively correlated with the Th2 sig and has been linked to airway remodeling in preclinical models (44). Taken together, these findings describe a complex interplay between mast cells, T cells, APCs, and airway stroma contributing to the Th2 inflammatory phenotype.

In addition to a large number of inflammatory cytokines and chemokines, several additional families of growth factors associated with epithelial-mesenchymal communication and tissue remodeling were correlated with the Th2 sig, including Wnt, TGF- β , and platelet-derived growth factor (PDGF) family members. Multiple Wnt genes were positively (Wnt3A, Wnt5A, Wnt6, and Wnt10A) or negatively (Wnt5B) correlated with the Th2 sig. Additionally, Fzd5, a Wnt receptor, is on the initial list of DE genes and is upregulated in Th2-high asthma (Supplementary Table I). Wnts have not previously been reported as being differentially expressed in human asthmatic airways, although Wnt5A can be induced by IL-13 in vitro in PBMCs (26) and by IL-6 family cytokines (45). Similarly intriguing patterns emerge with TGF- β family members, including TGF- β 1, BMP7, inhibins A and C, and GDFs 1 and 3, which are positively correlated with Th2 sig, whereas INHB is negatively correlated with the Th2 sig; as well as with PDGF family members, including PDGFs A and B, HGF, VEGFC, and the aforementioned KITLG, which are positively correlated with the Th2 sig, whereas TGFA is negatively correlated with the Th2 sig. Although many of these factors have been individually associated with allergic inflammation in vitro, in preclinical animal models, and, to a lesser extent, in vivo in human asthma, we present in this study a comprehensive overview of the relationships between key mediators of inflammation, cellular migration, and tissue remodeling as they relate to quantitative measures of allergic airway inflammation.

Discussion

Through gene expression profiling of asthmatic bronchial biopsies, directly comparing Th2 subphenotypes (18), we have found that highly differentially expressed asthma genes are part of a coordinated pattern of gene expression whose biological function and magnitude correspond to local and systemic measures of allergy and Th2 inflammation. We have summarized the expression of the Th2 inflammation gene set into a Th2 sig. The direct relationship that we observed between the Th2 sig and clinical manifestations of Th2 inflammation is consistent with a paradigm in which Th2 inflammation, both in molecular and cellular terms, exhibits a continuum of expression in asthma. Having established that the Th2 sig is a quantitative expression-based depiction of Th2 inflammation, we were able to comprehensively explore relationships between key intercellular mediators of inflammation, cell migration, and tissue remodeling directly in human asthmatic bronchial tissue.

There is a relative paucity of published whole genome expression studies of human asthmatic bronchial tissue, despite the potential insights that such studies may afford (46), thus precluding a comprehensive comparison of our findings with other datasets. Comprehensive expression analyses have been performed and published in experimental mouse models of asthma, and when comparing the report of upregulated pulmonary expression by IL-4, IL-13, and allergen challenges from one such study (47), we observe minimal overlap or consistency of direction of regulation for implicated asthma genes (Supplemental Fig. 3A). A small whole genome expression study of bronchial biopsies involving

four mild asthmatics identified 79 genes differentially expressed in asthma as compared with normal controls (48). Although only two genes (NOS2A and ALOX15) in that study are in common with our analyses above a nominal significance threshold of $q < 0.05$, we find a greater consistency in the direction of dysregulation than in comparison with mouse models (Supplemental Fig. 3B). The small number of subjects in that study and the degree of heterogeneity we have demonstrated in our larger cohort may contribute to the lack of overlap between studies. Interestingly, when comparing the genes reported to be differentially expressed in eosinophilic esophagitis, a Th2-associated disease of the esophageal mucosa (27), we observe a substantial intersection of genes with our DE list (Supplemental Fig. 3C), which suggests that common molecular and pathophysiological mechanisms underlie the mucosal inflammation observed in eosinophilic esophagitis and Th2-high asthma.

Biopsy Th2 sig

We addressed intrinsic heterogeneity in our asthma biopsy gene expression data set by using bronchial epithelial three-gene Th2 signature status (18) as a t test factor. We used this approach as a means to identify bronchial biopsy genes that are differentially expressed specifically in Th2-high asthmatics. As we had evidence that the differentially expressed genes represented a continuum of expression across the data set, we used SPCA (34, 35) to derive a biopsy Th2 signature as a continuous metric. An alternative classification technique to hierarchical clustering of microarray data, SPCA has been applied in situations when a continuous metric is desired (49). Through linear combinations of original variables, PCA projects the variance of correlated variables into a reduced number of dimensions without loss of information, that is, orthogonal principal components.

The genes that comprise the Th2 signature are highly coordinately regulated. The metric likely includes the effects of varying tissue cytology, where the unique transcriptional profiles of leukocytes, epithelial cells, and stromal cells of differing lineage, states of differentiation, and activation (50–52) contribute to the overall biopsy molecular profile. Therefore, individual genes within the Th2 sig may variably contribute to, or result from, components of asthma pathophysiology, but their coordinate regulation in asthmatic airways is consistent with the dramatic pathophysiological effects observed in asthma. In summary, we interpret the Th2 sig as a quantitative proxy for the net pathological state associated with airway Th2 inflammation. Note that this approach explicitly seeks to identify gene expression changes linked to Th2 inflammation. The possibility remains that orthogonal patterns of differential gene expression may underlie other pathophysiological features or subphenotypes of asthma.

Distinct subphenotypes of asthmatics have been described on the basis of the presence or absence of eosinophilic airway inflammation (6, 53, 54). A concept of discrete asthma subphenotypes driven by allergic inflammation or other factors may suggest disparate disease processes with a common final pathology of airway hyperreactivity and reversible obstruction. In a previous study of this cohort of asthmatics, we described distinct subphenotypes of asthma based on a limited three-gene signature in the airway epithelium. Dichotomizing asthmatics according to this signature into Th2-high and Th2-low subphenotypes is pathophysiologically and clinically relevant, as there are clear distinctions in terms of airway remodeling and mucus composition. Importantly, these subphenotypes have dramatic differences in their responsiveness to inhaled corticosteroid treatment, with improvements in airway function observed only in the Th2-high subset. However, allergic inflammation as measured by aeroallergen

sensitivity and serum IgE was still evident, if at lower levels, in the Th2-low subset (18). Taken together with our observations in bronchial mucosal biopsies in the current study, these findings suggest that a threshold level of underlying Th2 inflammation in the bronchial mucosa may be required to trigger the dichotomous epithelial gene expression and pathological and clinical phenotypes we have described. Although thresholds are essential for assigning subjects to nominal categories to assess clinical outcomes, our explicit objective in the current study was to comprehensively characterize gene expression related to Th2 inflammation in asthmatic bronchial tissue, and hence a continuous, quantitative measure such as the Th2 sig serves as a useful tool to accomplish this end.

Intercellular communication and asthma pathophysiology

We selectively examined the Th2 sig with respect to the expression of CCGf because this biological space comprises a broad but manageable number of well-characterized factors that mediate intercellular communication. Although many of the CCGf we identified as being coexpressed with the Th2 sig have been described as associated with Th2 airway inflammation via gene expression analyses using *in vitro* cell culture or preclinical animal models, this is to our knowledge the first attempt to characterize them globally in human asthmatic airway tissue. We found a consistent pattern of upregulated Th2 and eosinophil-attracting cytokines and chemokines (e.g., IL-13, CCL13, CCL26) with concomitant downregulation of Th1 and neutrophil-attracting cytokines and chemokines (e.g., IL-12A, CSF3, CXCL6, CXCL11). The finding that TNF- α and OX40 (TNFRSF4) expression are significantly coexpressed with Th2 inflammation in human asthma supports the notion that pathological Th2 inflammation may stem from a nonclassical inflammatory Th2 phenotype, which may be driven by OX40L–OX40 interactions (41). The respective positive and negative correlations between known Th2 and Th1 mediators and the Th2 sig illustrates a broad spectrum of mediators that actively play a role in bronchial mucosal inflammation and add confidence that other coexpressed genes are indeed relevant to Th2 inflammation in human asthma.

Airway remodeling, including reticular basement membrane thickening, smooth muscle hyperplasia, mucous metaplasia, and fibrosis, is thought to be an incompletely reversible long-term consequence of allergic airway inflammation, which may contribute to the progressive decline in pulmonary function observed over the course of many years in some asthmatics (55). We have previously shown that the epithelial Th2 signature is positively correlated with reticular basement membrane thickness, an index of bronchial fibrosis (18). Our findings of distinctive and coordinated expression patterns of Wnt, TGF, and PDGF family members coexpressed positively or negatively with the Th2 sig suggest an active process of tissue remodeling associated with Th2 inflammation. In particular, we noted that TGF- β 1 expression is positively correlated with the Th2 sig, consistent with other reports linking TGF- β expression with eosinophilic airway inflammation (56). Equally striking were the correlations between the noncanonical Wnt5A and the Th2 sig (Fig. 5), as well as between its cognate receptor Fzd5 (57) and Th2-high asthma (Fig. 2, Supplementary Table I). Wnt5A is highly expressed in fibroblasts isolated from subjects with idiopathic pulmonary fibrosis (58), which is intriguing in light of the fact that subepithelial fibrosis is significantly greater in Th2-high asthma as compared with Th2 low asthma (18). Given their established roles in tissue development, cellular differentiation, and patterning, it is tempting to speculate that Wnts may be involved in airway remodeling characteristic of allergic asthma. A recent study of therapeutic IL-5

blockade in severe eosinophilic asthmatics found a small but significant decrease in bronchial wall thickness over a 1-y period (12). With a large and growing number of novel therapeutic candidates targeting Th2 inflammation currently in development (15, 59, 60), it will be important to assess the ability of these molecules to modulate the expression of factors that regulate tissue remodeling and link those effects to pathophysiological and clinical outcomes.

We conducted this study in a cohort of mild-to-moderate asthmatics who were not currently taking steroids. An advantage of this approach is that, since our objective was to characterize patterns of gene expression associated with Th2 inflammation in human asthma, the potential confounding effects of steroids on gene expression were minimized. However, the most significant unmet medical need in asthma and the target population for emerging therapies directed against Th2 inflammation is more severe asthmatics whose disease is poorly controlled despite steroid treatment (15, 59, 60). In moderate asthmatics who require inhaled corticosteroids to control their disease, it appears that Th2 inflammation as assessed by airway IL-13 levels is largely suppressed, whereas in severe asthmatics uncontrolled despite steroid treatment, a subset with elevated airway IL-13 levels emerges (61). We have found that while Th2-high mild-to-moderate asthmatics respond well to inhaled corticosteroids, Th2-low subjects do not (18). Thus, it appears likely that severe asthmatics refractory to the effects of inhaled corticosteroids may have acquired resistance to the action of steroids, pathology driven by mechanisms not inherently susceptible to steroids, or a combination of the two. These mechanistic considerations will be important for understanding the clinical consequences of emerging therapies targeting Th2 inflammation in severe asthma. Now that we have laid the groundwork for understanding patterns of Th2 inflammation in asthmatic airways in the absence of steroids, future efforts should be directed at characterizing this pathway in the context of therapeutic interventions, including steroid treatment and new targeted therapeutics directed against components of Th2 inflammatory pathways.

In clinical studies, asthma therapies that specifically target components of Th2 inflammation, such as anti-IgE (omalizumab) or anti-IL-5 (mepolizumab), confer clinical benefits in the context of allergen challenge or exacerbation outcomes but not on airway obstruction outcomes, such as forced expiratory volume in 1 s (4, 9–13, 15, 29, 62–64). Our findings suggest that a predominant pattern of differential gene expression in asthma is related to Th2-driven airway inflammation; however, this pathway is linked to a large number of other factors associated with aspects of airway pathophysiology. Although Th2 inflammation is hypothesized to be a driver of disease in allergic asthma, it is as yet unclear whether Th2 inflammation is a cause or a consequence of the extended network of inflammatory and regulatory factors described in this study. Thus, it will be important to assess the clinical benefit of therapies targeting Th2 pathways on outcomes that are relevant to Th2 inflammation. This raises several important implications for future studies: 1) by defining components of asthma related to Th2 inflammation, we may now seek to identify components of asthma not related to Th2 inflammation that may underlie airway obstruction and hyperreactivity; 2) by relating distinct patterns of airway gene expression to specific domains of asthma pathophysiology, we may better interrogate the value of therapeutic interventions on clinical outcomes that are most relevant to the pathways being targeted; 3) by quantifying the degree to which Th2 inflammation is active in individual asthmatics, we may better identify patients most likely to benefit from therapeutics targeting Th2 inflammation; and 4) by developing a comprehensive, quantitative metric of Th2 airway inflammation, we are now equipped to interrogate the effectiveness of specific

molecular interventions on the large-scale pattern of gene expression associated with Th2 inflammation in asthma.

Acknowledgments

We thank Owen Solberg, Margaret Solon, Almut Ellwanger, the Genentech Sample Repository, Marco Sorani, Guiquan Jia, Sofia Mosesova, John Monroe, and Robert Gentleman for technical and statistical support, advice, and comments on the manuscript.

Disclosures

D.F.C., A.R.A., S.K., H.F.C., L.C.W., G.F., Z.M., and J.R.A. are current employees of Genentech and have equity interests in Roche Holding. B.M. is a former employee of Genentech.

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Supplementary Figure Legends

Figure Legends

Fig. S. 1. Th2 sig correlation with CCGf's versus bronchial epithelial Th2 status t-test with CCGf's

CCGf analyses using the biopsy Th2 sig or bronchial epithelial Th2 status as classifiers nearly identical rankings of association, though correlation analysis with Th2 sig is substantially more sensitive. A) Spearman's (rho) and Welch's t-test (t) test statistics are compared. A high degree of concordance ($p < 1 \times 10^{-15}$, Spearman's correlation) was observed among the test statistics. B) Q-values are compared between the two analytical approaches. Solid blue and dotted gray lines indicate $q < 0.05$ and $q < 0.1$ thresholds respectively.

Fig. S. 2. Principal Component Analysis of differentially expressed genes.

Principal Component scores and loadings plot illustrates that PC1 distinguishes “Th2 low” from “Th2 high” subjects.

Fig. S. 3. Comparative analyses of differential gene expression.

A volcano plot of {“Th2 high” asthma} versus {“Th2 low” asthma AND control}, t test statistics ($-\text{Log}_2$ p-value versus Log_2 Fold Change) are annotated by differentially expressed genes or orthologs in a comparative analysis of published studies: A) experimental asthma induced by recombinant IL4, IL13, allergen, or those in common

(47); B) human asthma biopsies (48); and C) eosinophilic esophagitis mucosa (27).

Horizontal dotted line indicates the $-\text{Log}_2$ p-value threshold that is equivalent to a 0.05 q-value.

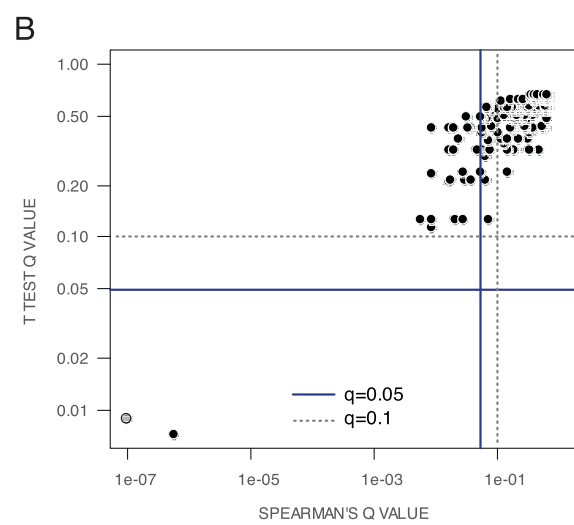
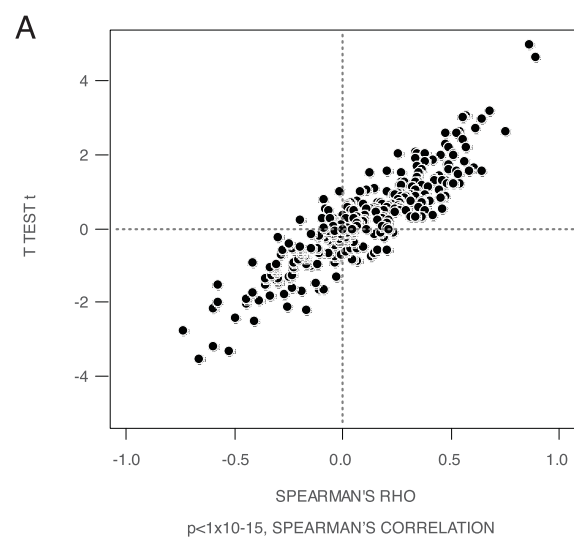


Fig. S. 1

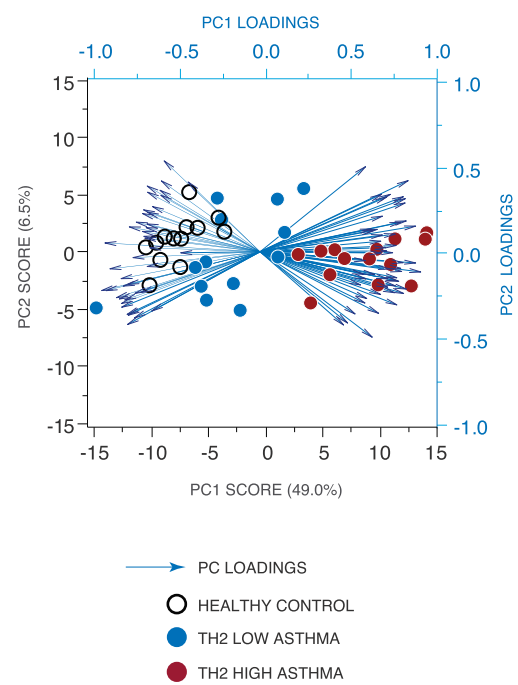


Fig. S. 2

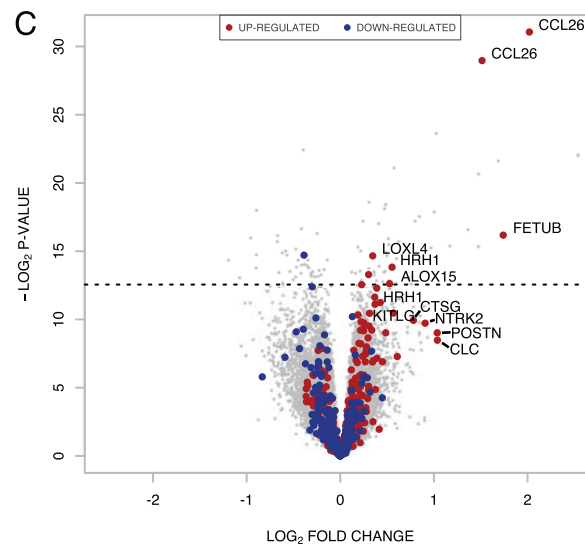
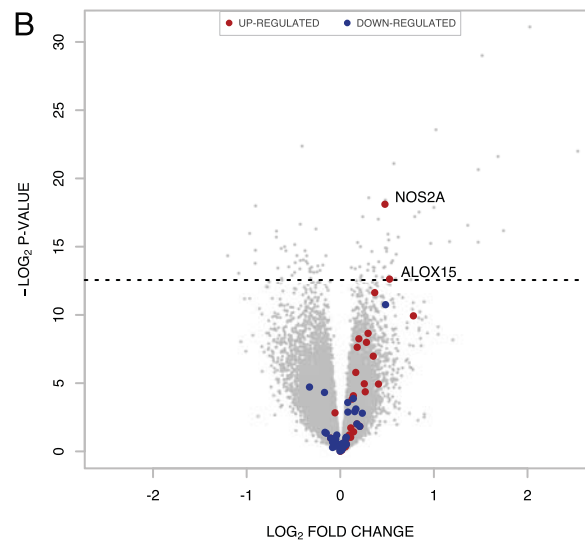
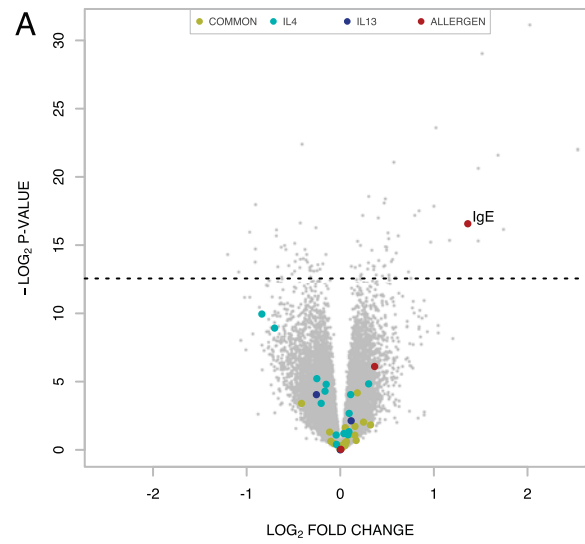


Fig. S. 3

Table SI: "Correlations between genes in the Th2 signature, cytokine gene expression, and clinical features of Th2 inflammation"

AGILENT PROBE ID	ENTREZ GENE ID	GENE NAME	GENE SYMBOL	MICROARRAY T TEST		CORRELATIONS					
				q value ↓	FC	QPCR			CLINICAL		
						IL5	IL13	IL12A	SERUM IgE	BLOOD EOS	BAL EOS%
A_24_P12573	10344	chemokine (C-C motif) ligand 26	CCL26	1.3E-05	4.06	4.36E-04	6.10E-10	1.82E-05	2.30E-06	4.00E-05	8.84E-03
A_23_P215484	10344	chemokine (C-C motif) ligand 26	CCL26	2.7E-05	2.86	4.45E-04	6.10E-10	1.02E-05	2.30E-06	4.00E-05	1.11E-02
A_24_P246825	442213	chromosome 6 open reading frame 138	C6orf138	7.7E-04	2.04	4.36E-04	4.27E-08	1.82E-05	1.80E-05	6.53E-08	9.49E-03
A_24_P154037	8660	insulin receptor substrate 2	IRS2	1.3E-03	-1.34	9.01E-04	3.98E-07	1.60E-06	9.49E-05	3.07E-04	6.67E-03
A_23_P51217	1179	chloride channel, calcium activated, family member 1	CLCA1	1.4E-03	5.76	5.79E-04	3.96E-07	1.43E-04	2.99E-06	1.57E-07	1.32E-02
A_23_P112859	1469	cystatin SN	CST1	1.5E-03	3.21	1.49E-03	1.70E-07	4.85E-06	2.30E-06	2.87E-06	1.25E-02
A_23_P95851	79861	tubulin, alpha-like 3	TUBAL3	1.8E-03	1.49	4.36E-04	1.83E-06	1.01E-05	3.77E-04	7.88E-04	9.28E-03
A_24_P237175	1470	cystatin SA	CST2	2.2E-03	2.78	1.77E-03	2.15E-07	1.35E-06	5.30E-06	7.02E-07	1.15E-02
A_32_P85999	1012	cadherin 13, H-cadherin (heart)	CDH13	8.1E-03	1.24	1.17E-03	6.36E-06	2.17E-06	1.23E-04	6.25E-05	5.17E-03
A_23_P120667	58494	junctional adhesion molecule 2	JAM2	8.2E-03	1.40	1.86E-03	5.86E-06	1.01E-05	1.80E-04	2.74E-03	9.49E-03
A_23_P502464	4843	nitric oxide synthase 2A (inducible, hepatocytes)	NOS2A	9.1E-03	1.39	1.01E-03	4.41E-06	3.20E-05	8.35E-06	4.30E-05	3.28E-02
A_23_P155755	6372	chemokine (C-X-C motif) ligand 6	CXCL6	9.1E-03	-1.88	1.17E-03	1.82E-06	3.09E-05	1.78E-03	3.23E-03	5.17E-03
A_23_P34804	4914	neurotrophic tyrosine kinase, receptor, type 1	NTRK1	9.1E-03	2.00	5.12E-04	1.70E-06	7.79E-05	3.87E-04	3.44E-04	3.70E-02
A_32_P101379	114795	transmembrane protein 132B	TMEM132B	1.1E-02	1.80	4.36E-04	1.07E-05	2.17E-06	1.10E-04	3.29E-05	3.51E-03
A_24_P115932	11251	G protein-coupled receptor 44	GPR44	1.2E-02	1.74	7.52E-04	7.60E-05	1.54E-04	4.68E-04	6.78E-04	4.30E-02
A_23_P51202	80818	zinc finger protein 436	ZNF436	1.2E-02	1.19	1.17E-03	5.23E-07	8.94E-07	1.08E-04	1.13E-03	1.85E-02
A_23_P79398	7850	interleukin 1 receptor, type II	IL1R2	1.3E-02	1.33	1.33E-03	8.52E-05	5.97E-06	6.26E-04	9.03E-03	4.78E-02
A_23_P53176	2348	folate receptor 1 (adult)	FOLR1	1.5E-02	-1.35	5.12E-04	1.94E-05	1.64E-05	2.58E-04	2.37E-04	3.25E-02
A_24_P109921	3497	Uncharacterized protein IGHE	IgE	1.5E-02	2.57	9.01E-04	5.28E-07	1.23E-04	2.30E-06	2.23E-04	6.81E-02
A_23_P134935	1843 1846	dual specificity phosphatase 1 dual specificity phosphatase 4	DUSP1 DUSP4	1.8E-02	-1.21	9.01E-04	2.00E-04	8.57E-05	1.66E-03	2.55E-03	3.19E-02
A_23_P144126	26998	fetuin B	FETUB	1.8E-02	3.34	2.63E-03	4.41E-06	1.35E-06	8.71E-05	6.57E-05	4.01E-02

A_24_P102650	727897	mucin 5B, oligomeric mucus/gel-forming	MUC5B	1.8E-02	-1.61	9.71E-04	1.70E-06	5.41E-06	1.30E-05	4.87E-06	8.35E-03
A_23_P87238	6291	serum amyloid A4, constitutive	SAA4	1.9E-02	-1.96	1.54E-03	4.66E-07	7.28E-07	4.87E-04	6.78E-04	3.44E-02
A_32_P155984	NA	expressed sequence tag	NA	1.9E-02	1.43	4.06E-03	1.41E-03	1.35E-04	2.16E-03	4.25E-03	2.87E-02
A_23_P207354	51751	HIG1 domain family, member 1B	HIGD1B	2.1E-02	1.54	1.96E-03	7.32E-06	4.76E-06	3.87E-04	2.46E-03	3.28E-02
A_24_P217489	2743	glycine receptor, beta	GLRB	2.1E-02	-1.61	1.94E-03	7.76E-06	5.19E-05	3.96E-04	2.07E-03	1.01E-03
A_23_P154566	84969	TOX high mobility group box family member 2	C20orf100	2.3E-02	1.44	5.29E-04	1.83E-06	2.45E-05	1.80E-04	5.30E-04	4.27E-03
A_32_P30600	NA	expressed sequence tag	NA	2.3E-02	2.25	5.29E-04	3.56E-07	5.04E-04	7.25E-05	6.53E-08	7.53E-03
A_23_P9711	9951	heparan sulfate (glucosamine) 3-O-sulfotransferase 4	HS3ST4	2.3E-02	2.78	9.01E-04	1.93E-05	1.95E-04	7.72E-04	9.37E-05	1.23E-02
A_32_P32739	162417	N-acetylglutamate synthase	NAGS	2.3E-02	1.14	4.49E-03	1.04E-05	2.12E-05	7.99E-05	1.14E-03	2.09E-03
A_32_P8221	2918	glutamate receptor, metabotropic 8	GRM8	2.4E-02	1.96	1.45E-03	1.83E-06	5.65E-04	1.71E-05	4.95E-05	5.17E-03
A_24_P119141	5627	protein S (alpha)	PROS1	2.4E-02	-1.41	1.12E-03	6.74E-06	7.28E-07	1.72E-04	2.28E-04	2.96E-03
A_23_P406187	162417	N-acetylglutamate synthase	NAGS	2.6E-02	1.17	2.79E-03	5.72E-06	7.44E-06	7.25E-05	7.81E-04	9.92E-04
A_23_P113212	55076	transmembrane protein 45A	TMEM45A	2.6E-02	-1.52	9.01E-04	4.41E-06	3.97E-05	1.95E-05	1.87E-06	2.96E-03
A_23_P107465	3881 3885	keratin 31 keratin 34	KRT31 KRT34	2.6E-02	1.41	5.09E-03	4.41E-06	3.38E-06	1.04E-05	7.54E-04	1.03E-02
A_32_P310335	522	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6	ATP5J	2.6E-02	1.42	1.54E-03	6.83E-06	2.38E-05	5.29E-05	2.71E-03	9.49E-03
A_23_P109785	8856	nuclear receptor subfamily 1, group I, member 2	NR1I2	2.6E-02	-1.56	5.29E-04	5.16E-05	7.48E-04	2.11E-04	4.38E-04	6.67E-03
A_23_P501754	1440	colony stimulating factor 3 (granulocyte)	CSF3	2.6E-02	-1.88	1.17E-03	2.94E-05	2.01E-06	1.10E-04	6.25E-05	4.30E-02
A_24_P220947	1645 648517	aldo-keto reductase family 1, member C1	AKR1C1	2.6E-02	-1.31	1.38E-03	6.34E-07	8.52E-05	2.11E-04	8.91E-04	1.01E-03
A_24_P60845	43	acetylcholinesterase (Yt blood group)	ACHE	2.6E-02	-1.50	1.96E-03	1.18E-04	3.97E-05	1.54E-04	4.87E-06	3.28E-02
A_23_P397999	7855	frizzled homolog 5 (Drosophila)	FZD5	2.6E-02	1.42	9.01E-04	2.84E-07	4.10E-06	2.47E-05	3.20E-05	3.51E-03
A_24_P406754	84171	lysyl oxidase-like 4	LOXL4	2.6E-02	1.27	4.45E-04	3.56E-07	5.27E-05	7.40E-04	3.00E-06	8.82E-03
A_32_P104334	390010	NK1 homeobox 2	NKX1-2	2.9E-02	1.52	1.10E-03	5.99E-05	5.42E-05	5.29E-05	1.01E-04	5.29E-03
A_23_P18684	1047	calmegin	CLGN	2.9E-02	-1.39	1.84E-03	1.16E-05	4.94E-06	2.62E-04	1.66E-04	6.36E-03
A_24_P332739	375513	glucuronidase, beta-like 2	GUSBL2	2.9E-02	-1.12	1.96E-03	4.91E-04	5.02E-06	2.28E-04	2.46E-03	2.05E-02
A_23_P30983	375519 728362 730838	gap junction protein, beta 7	GJB7	2.9E-02	-2.31	1.63E-03	5.28E-07	1.74E-06	1.79E-05	2.96E-04	5.04E-03

A_23_P250164	100132552	similar to homogentisate 1,2-dioxygenase	LOC100132552	2.9E-02	-1.29	9.92E-04	7.77E-05	1.82E-05	2.96E-03	1.31E-03	2.52E-02
A_32_P465742	8395	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	PIP5K1B	3.5E-02	-1.32	6.61E-04	1.70E-07	1.95E-05	7.76E-05	1.03E-04	2.71E-03
A_24_P269327	NA	cDNA, FLJ92257	DKFZp434F142	3.5E-02	-1.35	1.33E-03	8.40E-06	4.39E-05	2.11E-04	4.87E-06	2.55E-02
A_23_P80570	13	arylacetamide deacetylase (esterase)	AADAC	3.5E-02	1.48	1.38E-03	1.42E-04	6.32E-04	2.09E-03	2.27E-03	5.12E-02
A_24_P356916	64849	solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	SLC13A3	3.5E-02	-1.30	4.36E-04	4.79E-06	5.41E-06	4.49E-04	6.29E-04	5.12E-02
A_23_P5601	1796	docking protein 1, 62kDa (downstream of tyrosine kinase 1)	DOK1	3.6E-02	1.34	7.01E-04	1.05E-06	9.93E-06	1.40E-04	4.70E-04	3.51E-03
A_23_P433690	55422 730064 732358	zinc finger protein 331	ZNF331	3.6E-02	-1.41	2.63E-03	9.94E-05	6.16E-04	6.78E-04	2.07E-03	9.98E-03
A_24_P277211	3269	histamine receptor H1	HRH1	3.6E-02	1.47	5.29E-04	2.15E-07	1.35E-06	2.58E-04	1.90E-05	3.86E-03
A_23_P85503	57115	peptidoglycan recognition protein 4	PGLYRP4	3.6E-02	-1.89	9.74E-04	7.59E-05	5.70E-04	3.80E-04	6.57E-05	3.28E-02
A_23_P83751	79864	chromosome 11 open reading frame 63	C11orf63	3.6E-02	-1.28	5.29E-04	1.16E-05	1.35E-06	1.07E-03	1.13E-03	6.67E-03
A_24_P391230	116159	cysteine/tyrosine-rich 1	CYYR1	3.6E-02	1.24	2.23E-03	1.38E-04	2.55E-05	2.61E-04	2.09E-03	1.23E-02
A_23_P327069	9778	KIAA0232	KIAA0232	3.7E-02	-1.15	1.01E-03	1.19E-05	1.82E-05	1.31E-03	3.23E-03	3.86E-03
A_24_P294832	7803	protein tyrosine phosphatase type IVA, member 1	PTP4A1	3.7E-02	-1.19	2.09E-03	2.77E-06	1.13E-05	4.02E-04	8.00E-04	8.70E-03
A_24_P251534	10217	CTD small phosphatase-like	CTDSPL	3.7E-02	1.16	2.79E-03	5.33E-06	1.35E-06	7.06E-04	1.20E-03	2.91E-03
A_32_P61936	NA	expressed sequence tag	NA	3.7E-02	1.40	4.45E-04	1.34E-05	1.56E-03	1.10E-04	4.73E-03	2.05E-02
A_24_P7121	79730	NOL1/NOP2/Sun domain family, member 7	NSUN7	3.7E-02	-1.47	1.54E-03	2.81E-05	4.94E-06	4.92E-04	3.80E-03	5.43E-03
A_23_P171143	7105	tetraspanin 6	TSPAN6	3.7E-02	-1.25	9.39E-04	3.26E-06	2.17E-06	1.19E-03	2.36E-03	3.86E-03
A_23_P377717	4858	neuro-oncological ventral antigen 2	NOVA2	3.7E-02	1.28	2.26E-03	1.34E-05	2.57E-05	3.87E-04	5.54E-03	3.51E-03
A_24_P354496	152641	chromosome 4 open reading frame 38	C4orf38	3.7E-02	1.39	1.33E-02	9.24E-04	4.85E-06	2.79E-04	4.70E-03	1.03E-02
A_24_P341426	NA	expressed sequence tag	NA	3.7E-02	1.09	4.36E-04	1.49E-05	2.55E-04	4.02E-04	7.88E-04	5.43E-03
A_23_P106016	5587	protein kinase D1	PRKD1	3.7E-02	1.29	4.36E-04	7.64E-08	7.67E-06	1.35E-04	3.74E-06	5.52E-02
A_24_P314515	283460	chromosome 12 open reading frame 27	C12orf27	3.7E-02	-1.60	1.33E-03	3.22E-04	5.42E-05	2.39E-03	1.21E-03	1.03E-02

A_23_P117912	171177	ras homolog gene family, member V	RHOV	3.7E-02	-1.26	1.85E-03	5.28E-07	6.79E-06	2.30E-06	6.25E-05	5.17E-03
A_23_P113811	NA	expressed sequence tag	NA	3.7E-02	1.13	9.92E-04	1.30E-04	4.09E-04	2.30E-05	7.08E-04	1.75E-02
A_23_P73114	5627	protein S (alpha)	PROS1	3.7E-02	-1.36	8.11E-04	1.94E-05	2.01E-06	4.92E-04	1.31E-03	4.29E-03
A_23_P302654	55722	centrosomal protein 72kDa	CEP72	3.9E-02	1.23	1.77E-03	3.90E-05	2.62E-04	2.58E-04	7.00E-04	2.70E-02
A_23_P107295	114659	leucine rich repeat containing 37B	LRRC37B	3.9E-02	-1.17	5.29E-04	5.33E-06	9.84E-05	2.21E-05	7.48E-04	4.19E-02
A_23_P135990	6578	solute carrier organic anion transporter family, member 2A1	SLCO2A1	4.0E-02	1.29	2.23E-03	3.71E-05	2.45E-05	1.23E-04	1.31E-03	3.25E-02
A_24_P404245	5833	phosphate cytidyltransferase 2, ethanolamine	PCYT2	4.0E-02	-1.26	9.01E-04	3.20E-07	6.71E-06	2.58E-04	5.68E-04	3.51E-03
A_23_P360874	222229	leucine-rich repeats and WD repeat domain containing 1	LRWD1	4.0E-02	-1.25	1.59E-03	1.16E-05	1.38E-05	1.39E-03	1.87E-03	2.71E-02
A_23_P165840	4953	ornithine decarboxylase 1	ODC1	4.0E-02	-1.20	8.54E-03	3.02E-05	2.98E-04	1.10E-04	1.63E-04	5.43E-03
A_23_P258582	256356	glycerol kinase 5 (putative)	GK5	4.0E-02	-1.23	2.86E-03	7.82E-04	1.00E-04	7.25E-05	1.24E-03	6.67E-03
A_23_P108823	114880	oxysterol binding protein-like 6	OSBPL6	4.0E-02	-1.41	5.29E-04	1.83E-06	8.94E-07	2.35E-04	6.78E-04	5.17E-03
A_23_P111583	948	CD36 molecule (thrombospondin receptor)	CD36	4.1E-02	1.66	5.29E-04	2.05E-05	6.63E-03	1.10E-03	4.63E-04	4.83E-02
A_23_P1102	58	actin, alpha 1, skeletal muscle	ACTA1	4.1E-02	-2.13	2.15E-03	1.18E-03	3.93E-03	6.21E-04	6.04E-04	1.89E-02
A_23_P361544	283848	hypothetical protein FLJ37464	FLJ37464	4.5E-02	-1.44	2.53E-03	9.24E-04	2.01E-06	2.95E-03	3.23E-03	5.43E-03
A_23_P201319	84976	dispatched homolog 1 (Drosophila)	DISP1	4.5E-02	1.18	4.10E-03	4.84E-05	9.51E-06	7.40E-04	5.52E-03	2.53E-02
A_23_P212781	55752	septin 11	40432	4.5E-02	1.31	1.33E-03	2.33E-05	2.70E-04	1.80E-04	6.04E-04	1.11E-02
A_32_P101031	116372	LY6/PLAUR domain containing 1	LYPD1	4.5E-02	1.44	4.03E-03	3.67E-06	5.41E-06	3.77E-04	2.24E-04	2.52E-02
A_23_P27688	NA	hypothetical protein LOC199800	LOC199800	4.7E-02	1.37	1.74E-03	3.99E-06	1.77E-04	1.11E-04	2.71E-03	2.11E-02
A_32_P42989	NA	expressed sequence tag	NA	4.7E-02	1.17	9.81E-04	1.97E-04	7.94E-04	7.02E-04	3.37E-03	7.92E-03
A_23_P76402	79600	tectonic family member 1	TCTN1	4.8E-02	-1.25	4.36E-04	4.03E-07	6.79E-06	2.84E-04	6.04E-04	3.86E-03
A_32_P106807	NA	expressed sequence tag	EST	4.8E-02	-1.55	2.53E-03	1.18E-04	4.85E-06	4.86E-03	3.37E-03	9.49E-03
A_24_P349196	728621	hypothetical protein LOC728621	DKFZp686K01114	4.8E-02	-1.55	2.13E-03	2.02E-03	6.23E-05	1.52E-04	2.98E-03	1.36E-02
A_32_P224727	NA	expressed sequence tag	NA	4.8E-02	1.51	1.13E-03	2.32E-06	1.82E-05	2.30E-06	6.57E-05	2.09E-03
A_23_P55373	246	arachidonate 15-lipoxygenase	ALOX15	4.9E-02	1.44	6.61E-04	1.16E-05	7.65E-03	6.98E-05	2.28E-04	6.07E-02
A_23_P39525	79843	family with sequence similarity 124B	FAM124B	4.9E-02	1.69	9.71E-04	1.89E-06	2.55E-04	2.58E-04	2.71E-03	7.56E-03

Differential gene expression analysis was conducted by Welch's t test using bronchial epithelial Th2 signature status ({''Th2 high'' asthma} versus {''Th2 low'' asthma AND control}). Probes are ranked in ascending order of q value. Probes with $q < 0.05$ for t test are tabulated. Individual microarray probe expression values were tested for correlation (Spearman's) with qPCR assessments of IL5, IL13, IL12A, and clinical measures of allergy and inflammation (serum IgE, blood eosinophil count, and BAL eosinophil percentage). q value and fold change (FC) from these analyses are reported where appropriate. q values greater than 0.05 are indicated in red font.

Table SII: "Correlations between Th2 sig and cytokines, chemokines, and growth factors"

AGILENT PROBE ID	ENTREZ GENE ID	GENE NAME	GENE SYMBOL	rho	p value	q value	N
A_23_P215484	10344	chemokine (C-C motif) ligand 26	CCL26	0.89	5.52E-10	9.51E-08	27
A_24_P12573	10344	chemokine (C-C motif) ligand 26	CCL26	0.86	6.15E-09	5.30E-07	27
A_23_P51039	3623	inhibin, alpha	INHHA	0.68	9.32E-05	5.36E-03	27
A_23_P155755	6372	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	CXCL6	-0.67	2.27E-04	8.45E-03	25
A_23_P251031	3596	interleukin 13	IL13	0.75	3.16E-04	8.45E-03	18
A_23_P211926	7474	wingless-type MMTV integration site family, member 5A	WNT5A	0.64	3.20E-04	8.45E-03	27
A_23_P126844	8718	tumor necrosis factor receptor superfamily, member 25	TNFRSF25	0.64	3.43E-04	8.45E-03	27
A_24_P411121	8784	tumor necrosis factor receptor superfamily, member 18	TNFRSF18	0.61	8.28E-04	1.54E-02	27
A_32_P26092	7293	tumor necrosis factor receptor superfamily, member 4	TNFRSF4	0.60	8.41E-04	1.54E-02	27
A_23_P153964	3625	inhibin, beta B (activin AB beta polypeptide)	INHBB	-0.60	8.94E-04	1.54E-02	27
A_23_P91943	3592	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte	IL12A	-0.74	1.09E-03	1.71E-02	16
A_24_P56310	55504	tumor necrosis factor receptor superfamily, member 19	TNFRSF19	-0.58	1.37E-03	1.88E-02	27
A_23_P501754	1440	colony stimulating factor 3 (granulocyte)	CSF3	-0.60	1.50E-03	1.88E-02	25
A_23_P102117	80326	wingless-type MMTV integration site family, member 10A	WNT10A	0.58	1.54E-03	1.88E-02	27
A_23_P53588	81029	wingless-type MMTV integration site family, member 5B	WNT5B	-0.58	1.70E-03	1.88E-02	27
A_23_P102113	80326	wingless-type MMTV integration site family, member 10A	WNT10A	0.57	1.75E-03	1.88E-02	27
A_24_P125335	6357	chemokine (C-C motif) ligand 13	CCL13	0.57	1.98E-03	2.01E-02	27
A_23_P218646	51750 8771	tumor necrosis factor receptor superfamily, member 6b, decoy	TNFRSF6B	0.56	2.39E-03	2.29E-02	27
A_24_P412156	6387	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12	0.55	3.11E-03	2.68E-02	27
A_24_P339944	5155	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) on	PDGFB	0.55	3.11E-03	2.68E-02	27
A_23_P345692	53342	interleukin 17D	IL17D	0.54	3.53E-03	2.90E-02	27
A_23_P139722	7132	tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	0.54	3.81E-03	2.98E-02	27
A_24_P79054	7040	transforming growth factor, beta 1 (Camurati-Engelmann disease)	TGFB1	0.53	4.15E-03	3.11E-02	27
A_24_P50759	7124	tumor necrosis factor (TNF superfamily, member 2)	TNF	0.52	5.10E-03	3.65E-02	27

A_23_P256724	8794	tumor necrosis factor receptor superfamily, member 10c, decoy without an intrace	TNFRSF10C	0.52	5.29E-03	3.65E-02	27
A_24_P122137	3976	leukemia inhibitory factor (cholinergic differentiation factor)	LIF	0.51	6.38E-03	4.23E-02	27
A_23_P76051	3626	inhibin, beta C	INHBC	0.51	7.15E-03	4.56E-02	27
A_23_P5654	27178	interleukin 1 family, member 7 (zeta)	IL1F7	-0.50	8.36E-03	5.02E-02	27
A_23_P385690	89780	wingless-type MMTV integration site family, member 3A	WNT3A	0.50	8.46E-03	5.02E-02	27
A_24_P5856	8744	tumor necrosis factor (ligand) superfamily, member 9	TNFSF9	0.49	9.23E-03	5.30E-02	27
A_23_P209098	10715 2657	growth differentiation factor 1	GDF1	0.49	9.85E-03	5.36E-02	27
A_23_P113701	5154	platelet-derived growth factor alpha polypeptide	PDGFA	0.49	9.96E-03	5.36E-02	27
A_23_P72817	9573	growth differentiation factor 3	GDF3	0.48	1.06E-02	5.54E-02	27
A_23_P67224	8744	tumor necrosis factor (ligand) superfamily, member 9	TNFSF9	0.48	1.13E-02	5.73E-02	27
A_24_P245298	407977 8741 8742	tumor necrosis factor (ligand) superfamily, member 12	TNFSF12	0.47	1.26E-02	6.03E-02	27
A_23_P119916	7475	wingless-type MMTV integration site family, member 6	WNT6	0.47	1.27E-02	6.03E-02	27
A_23_P162314	50846	desert hedgehog homolog (Drosophila)	DHH	0.47	1.29E-02	6.03E-02	27
A_24_P133253	4254	KIT ligand	KITLG	0.47	1.35E-02	6.12E-02	27
A_23_P164210	414062 6349	TBC1 domain family, member 3	TBC1D3	0.46	1.51E-02	6.56E-02	27
A_24_P403459	3441 3442	interferon, alpha 4	IFNA4	0.46	1.52E-02	6.56E-02	27
A_23_P68487	655	bone morphogenetic protein 7 (osteogenic protein 1)	BMP7	0.46	1.60E-02	6.73E-02	27
A_23_P125278	6373	chemokine (C-X-C motif) ligand 11	CXCL11	-0.53	1.68E-02	6.87E-02	20
A_23_P8961	3574	interleukin 7	IL7	-0.45	1.71E-02	6.87E-02	27
A_23_P93780	3082	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	0.45	1.78E-02	6.98E-02	27
A_24_P301501	6358 6359	chemokine (C-C motif) ligand 15	CCL15	-0.45	1.87E-02	7.16E-02	27
A_23_P26965	6357	chemokine (C-C motif) ligand 13	CCL13	0.45	1.93E-02	7.21E-02	27
A_23_P38505	58191	chemokine (C-X-C motif) ligand 16	CXCL16	0.44	2.08E-02	7.62E-02	27
A_23_P167096	7424	vascular endothelial growth factor C	VEGFC	0.44	2.20E-02	7.89E-02	27
A_23_P377291	7039	transforming growth factor, alpha	TGFA	-0.42	2.74E-02	9.63E-02	27
A_23_P115190	4803	nerve growth factor, beta polypeptide	NGFB	-0.42	2.97E-02	1.01E-01	27
A_24_P944054	6387	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12	0.42	3.05E-02	1.01E-01	27
A_24_P228130	414062 6349	chemokine (C-C motif) ligand 3-like 3	CCL3L3	0.42	3.08E-02	1.01E-01	27
A_23_P45133	1435	colony stimulating factor 1 (macrophage)	CSF1	0.42	3.10E-02	1.01E-01	27

A_23_P49338	51330	tumor necrosis factor receptor superfamily, member 12A	TNFRSF12A	0.41	3.38E-02	1.08E-01	27
A_23_P10647	54360	cytokine-like 1	CYTL1	0.41	3.44E-02	1.08E-01	27
A_23_P90359	4902	neurturin	NRTN	0.40	4.11E-02	1.26E-01	27
A_23_P17065	6364	chemokine (C-C motif) ligand 20	CCL20	-0.39	4.25E-02	1.28E-01	27
A_24_P54174	7133	tumor necrosis factor receptor superfamily, member 1B	TNFRSF1B	0.39	4.32E-02	1.28E-01	27
A_24_P12401	7422	vascular endothelial growth factor	VEGF	0.39	4.43E-02	1.29E-01	27
A_23_P215491	6369	chemokine (C-C motif) ligand 24	CCL24	0.39	4.54E-02	1.29E-01	27
A_24_P364363	7132	tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	0.39	4.57E-02	1.29E-01	27
A_24_P313500	1489	cardiotrophin 1	CTF1	0.38	5.03E-02	1.38E-01	27
A_23_P76992	5228	placental growth factor, vascular endothelial growth factor-related protein	PGF	0.38	5.03E-02	1.38E-01	27
A_24_P20607	6373	chemokine (C-X-C motif) ligand 11	CXCL11	-0.41	5.26E-02	1.40E-01	23
A_23_P126908	8764	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediato	TNFRSF14	0.38	5.28E-02	1.40E-01	27
A_23_P136433	2246	fibroblast growth factor 1 (acidic)	FGF1	0.51	5.37E-02	1.40E-01	15
A_23_P321920	414062 6349	chemokine (C-C motif) ligand 3-like 3	CCL3L3	0.38	5.85E-02	1.51E-01	26
A_24_P319088	6368	chemokine (C-C motif) ligand 23	CCL23	-0.36	6.16E-02	1.56E-01	27
A_23_P135722	685	betacellulin	BTC	-0.36	6.35E-02	1.59E-01	27
A_23_P145669	2056	erythropoietin	EPO	0.36	6.54E-02	1.59E-01	27
A_24_P155502	3626	inhibin, beta C	INHBC	0.36	6.54E-02	1.59E-01	27
A_23_P67169	3589	interleukin 11	IL11	0.36	6.89E-02	1.65E-01	27
A_24_P38951	NA	tumor necrosis factor receptor superfamily, member 19-like	TNFRSF19L	0.35	7.31E-02	1.70E-01	27
A_23_P409438	282616	interleukin 28A (interferon, lambda 2)	IL28A	0.35	7.31E-02	1.70E-01	27
A_24_P35643	7481	wingless-type MMTV integration site family, member 11	WNT11	0.35	7.58E-02	1.74E-01	27
A_23_P334727	145957	neuregulin 4	NRG4	0.34	7.81E-02	1.75E-01	27
A_23_P255653	8797	tumor necrosis factor receptor superfamily, member 10a	TNFRSF10A	0.34	7.81E-02	1.75E-01	27
A_24_P79403	5196	platelet factor 4 (chemokine (C-X-C motif) ligand 4)	PF4	0.36	7.99E-02	1.76E-01	25
A_24_P257416	2920	chemokine (C-X-C motif) ligand 2	CXCL2	-0.34	8.15E-02	1.76E-01	27
A_23_P207194	2688	growth hormone 1	GH1	0.34	8.15E-02	1.76E-01	27
A_23_P213944	1839	heparin-binding EGF-like growth factor	HBEGF	0.33	9.06E-02	1.90E-01	27
A_24_P251040	9048	artemin	ARTN	0.33	9.06E-02	1.90E-01	27
A_24_P183150	2921	chemokine (C-X-C motif) ligand 3	CXCL3	-0.33	9.18E-02	1.91E-01	27
A_24_P253003	7481	wingless-type MMTV integration site family, member 11	WNT11	0.33	9.70E-02	1.99E-01	27

A_23_P218918	2247	fibroblast growth factor 2 (basic)	FGF2	0.32	1.02E-01	2.04E-01	27
A_24_P111106	2246	fibroblast growth factor 1 (acidic)	FGF1	0.33	1.02E-01	2.04E-01	26
A_23_P412389	8817	fibroblast growth factor 18	FGF18	-0.32	1.03E-01	2.04E-01	27
A_23_P94754	9966	tumor necrosis factor (ligand) superfamily, member 15	TNFSF15	-0.34	1.07E-01	2.09E-01	24
A_23_P76102	10220	growth differentiation factor 11	GDF11	0.31	1.10E-01	2.09E-01	27
A_23_P152620	407977 8741 8742	tumor necrosis factor (ligand) superfamily, member 13	TNFSF13	-0.31	1.12E-01	2.09E-01	27
A_23_P217319	2258 729045 730528	fibroblast growth factor 13	FGF13	0.31	1.12E-01	2.09E-01	27
A_23_P81805	7422	vascular endothelial growth factor	VEGF	0.31	1.13E-01	2.09E-01	27
A_23_P320054	647836 649279 7477	wingless-type MMTV integration site family, member 7B	WNT7B	-0.32	1.14E-01	2.09E-01	26
A_23_P382607	54361	wingless-type MMTV integration site family, member 4	WNT4	0.31	1.14E-01	2.09E-01	27
A_23_P104798	3606	interleukin 18 (interferon-gamma-inducing factor)	IL18	-0.31	1.20E-01	2.18E-01	27
A_23_P99386	8600	tumor necrosis factor (ligand) superfamily, member 11	TNFSF11	0.36	1.35E-01	2.39E-01	19
A_23_P501713	84639	interleukin 1 family, member 10 (theta)	IL1F10	0.30	1.35E-01	2.39E-01	27
A_23_P167882	112744	interleukin 17F	IL17F	0.33	1.41E-01	2.48E-01	21
A_23_P169030	8795 8797	tumor necrosis factor receptor superfamily, member 10b	TNFRSF10B	0.29	1.42E-01	2.48E-01	27
A_32_P865613	685	betacellulin	BTC	0.33	1.50E-01	2.59E-01	20
A_24_P63347	5197	platelet factor 4 variant 1	PF4V1	-0.29	1.53E-01	2.62E-01	26
A_32_P38125	3557	interleukin 1 receptor antagonist	IL1RN	-0.30	1.58E-01	2.66E-01	23
A_23_P410507	5623	persephin	PSPN	0.28	1.59E-01	2.66E-01	27
A_24_P133905	6368	chemokine (C-C motif) ligand 23	CCL23	0.28	1.62E-01	2.67E-01	27
A_23_P167585	2661	growth differentiation factor 9	GDF9	-0.28	1.63E-01	2.67E-01	26
A_24_P208513	7475	wingless-type MMTV integration site family, member 6	WNT6	0.27	1.65E-01	2.69E-01	27
A_23_P11787	54361	wingless-type MMTV integration site family, member 4	WNT4	0.27	1.69E-01	2.73E-01	27
A_24_P99244	2252	fibroblast growth factor 7 (keratinocyte growth factor)	FGF7	-0.31	1.75E-01	2.74E-01	21
A_23_P407012	1435	colony stimulating factor 1 (macrophage)	CSF1	0.27	1.78E-01	2.74E-01	27
A_23_P88404	7043	transforming growth factor, beta 3	TGFB3	0.27	1.78E-01	2.74E-01	27
A_24_P179400	7422	vascular endothelial growth factor	VEGF	0.27	1.78E-01	2.74E-01	27
A_23_P213745	9547	chemokine (C-X-C motif) ligand 14	CXCL14	0.27	1.79E-01	2.74E-01	27
A_23_P78742	2323	fms-related tyrosine kinase 3 ligand	FLT3LG	0.27	1.80E-01	2.74E-01	27
A_23_P111657	6469	sonic hedgehog homolog (Drosophila)	SHH	0.26	1.90E-01	2.87E-01	27

A_23_P29953	3600	interleukin 15	IL15	0.26	1.97E-01	2.95E-01	27
A_23_P46829	2253	fibroblast growth factor 8 (androgen-induced)	FGF8	0.33	2.02E-01	2.95E-01	17
A_23_P1594	7423	vascular endothelial growth factor B	VEGFB	0.25	2.02E-01	2.95E-01	27
A_23_P51951	11009	interleukin 24	IL24	0.29	2.03E-01	2.95E-01	21
A_23_P19723	653	bone morphogenetic protein 5	BMP5	-0.25	2.05E-01	2.95E-01	27
A_23_P55828	6370	chemokine (C-C motif) ligand 25	CCL25	-0.26	2.05E-01	2.95E-01	26
A_23_P337800	282618	interleukin 29 (interferon, lambda 1)	IL29	0.26	2.08E-01	2.96E-01	25
A_23_P315320	246778	interleukin 27	IL27	-0.25	2.10E-01	2.97E-01	27
A_23_P500614	943	tumor necrosis factor receptor superfamily, member 8	TNFRSF8	0.25	2.14E-01	2.99E-01	27
A_23_P122924	3624	inhibin, beta A (activin A, activin AB alpha polypeptide)	INHBA	0.25	2.17E-01	3.02E-01	27
A_23_P207564	6351	chemokine (C-C motif) ligand 4	CCL4	0.24	2.23E-01	3.04E-01	27
A_24_P141707	83729	inhibin, beta E	INHBE	0.24	2.23E-01	3.04E-01	27
A_23_P48088	NA	tumor necrosis factor receptor superfamily, member 7	TNFRSF7	0.24	2.26E-01	3.04E-01	27
A_23_P411157	7471	wingless-type MMTV integration site family, member 1	WNT1	-0.24	2.27E-01	3.04E-01	27
A_23_P18452	4283	chemokine (C-X-C motif) ligand 9	CXCL9	-0.24	2.28E-01	3.04E-01	27
A_23_P89587	7484	wingless-type MMTV integration site family, member 9B	WNT9B	-0.30	2.29E-01	3.04E-01	18
A_23_P121253	8743	tumor necrosis factor (ligand) superfamily, member 10	TNFSF10	-0.24	2.38E-01	3.13E-01	27
A_23_P136493	3084	neuregulin 1	NRG1	0.24	2.42E-01	3.17E-01	26
A_23_P166408	5008	oncostatin M	OSM	0.23	2.53E-01	3.26E-01	27
A_23_P17053	56300	interleukin 1 family, member 9	IL1F9	-0.27	2.54E-01	3.26E-01	20
A_23_P121596	5473	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	PPBP	0.25	2.56E-01	3.26E-01	23
A_23_P52714	9965	fibroblast growth factor 19	FGF19	-0.23	2.57E-01	3.26E-01	27
A_23_P213336	2246	fibroblast growth factor 1 (acidic)	FGF1	0.23	2.69E-01	3.38E-01	25
A_23_P94563	3442	interferon, alpha 5	IFNA5	-0.30	2.71E-01	3.39E-01	15
A_23_P93348	4050	lymphotoxin beta (TNF superfamily, member 3)	LTB	0.21	2.85E-01	3.52E-01	27
A_23_P259955	8200	growth differentiation factor 5 (cartilage-derived morphogenetic protein-1)	GDF5	0.21	2.87E-01	3.52E-01	27
A_24_P303091	3627	chemokine (C-X-C motif) ligand 10	CXCL10	-0.23	2.88E-01	3.52E-01	24
A_24_P167012	9966	tumor necrosis factor (ligand) superfamily, member 15	TNFSF15	0.26	2.99E-01	3.63E-01	18
A_23_P105803	2254	fibroblast growth factor 9 (glia-activating factor)	FGF9	0.20	3.08E-01	3.65E-01	27
A_23_P71530	4982	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	TNFRSF11B	-0.20	3.08E-01	3.65E-01	27
A_24_P91566	655	bone morphogenetic protein 7 (osteogenic protein 1)	BMP7	0.20	3.09E-01	3.65E-01	27
A_23_P49759	6346	chemokine (C-C motif) ligand 1	CCL1	0.22	3.10E-01	3.65E-01	24

A_24_P55971	7423	vascular endothelial growth factor B	VEGFB	0.20	3.21E-01	3.74E-01	27
A_23_P126735	3586	interleukin 10	IL10	0.20	3.21E-01	3.74E-01	27
A_24_P390495	6376	chemokine (C-X3-C motif) ligand 1	CX3CL1	-0.20	3.24E-01	3.75E-01	27
A_23_P45185	2277	c-fos induced growth factor (vascular endothelial growth factor D)	FIGF	-0.20	3.27E-01	3.75E-01	26
A_24_P370201	10148	Epstein-Barr virus induced gene 3	EBI3	0.20	3.29E-01	3.75E-01	27
A_24_P124349	80310	platelet derived growth factor D	PDGFD	-0.19	3.32E-01	3.76E-01	27
A_23_P209995	3557	interleukin 1 receptor antagonist	IL1RN	0.19	3.33E-01	3.76E-01	27
A_23_P332820	3605	interleukin 17A	IL17A	0.20	3.38E-01	3.78E-01	24
A_23_P259071	374	amphiregulin (schwannoma-derived growth factor)	AREG	-0.19	3.41E-01	3.79E-01	27
A_23_P14174	10673	tumor necrosis factor (ligand) superfamily, member 13b	TNFSF13B	-0.19	3.46E-01	3.82E-01	27
A_23_P151294	3458	interferon, gamma	IFNG	-0.21	3.52E-01	3.86E-01	22
A_23_P160336	10637	left-right determination factor 1	LEFTY1	0.19	3.54E-01	3.86E-01	27
A_23_P130158	7473	wingless-type MMTV integration site family, member 3	WNT3	0.18	3.57E-01	3.87E-01	27
A_23_P78037	6354	chemokine (C-C motif) ligand 7	CCL7	-0.25	3.62E-01	3.90E-01	15
A_23_P78944	268	anti-Mullerian hormone	AMH	0.18	3.69E-01	3.95E-01	27
A_23_P79518	3553	interleukin 1, beta	IL1B	-0.18	3.72E-01	3.96E-01	27
A_23_P91764	NA	tumor necrosis factor receptor superfamily, member 13C	TNFRSF13C	0.18	3.75E-01	3.97E-01	27
A_23_P135248	10850 730098 731532	chemokine (C-C motif) ligand 27	CCL27	0.17	3.84E-01	4.03E-01	27
A_32_P196029	2252	fibroblast growth factor 7 (keratinocyte growth factor)	FGF7	-0.23	3.87E-01	4.04E-01	16
A_23_P126836	7292	tumor necrosis factor (ligand) superfamily, member 4 (tax-transcriptionally acti	TNFSF4	-0.17	3.97E-01	4.12E-01	27
A_23_P46755	2658	growth differentiation factor 2	GDF2	0.20	4.08E-01	4.20E-01	19
A_23_P93787	3082	hepatocyte growth factor (hepapoietin A; scatter factor)	HGF	0.17	4.10E-01	4.20E-01	27
A_24_P233488	3976	leukemia inhibitory factor (cholinergic differentiation factor)	LIF	0.19	4.14E-01	4.22E-01	21
A_24_P911607	7476 7477	wingless-type MMTV integration site family, member 7B	WNT7B	0.21	4.25E-01	4.28E-01	16
A_24_P73599	3603	interleukin 16 (lymphocyte chemoattractant factor)	IL16	0.16	4.25E-01	4.28E-01	27
A_23_P70398	7422	vascular endothelial growth factor	VEGF	0.16	4.27E-01	4.28E-01	27
A_23_P360797	4908	neurotrophin 3	NTF3	0.16	4.34E-01	4.30E-01	27
A_23_P89431	6347 6357	chemokine (C-C motif) ligand 2	CCL2	0.16	4.34E-01	4.30E-01	27
A_23_P315364	2920	chemokine (C-X-C motif) ligand 2	CXCL2	-0.15	4.47E-01	4.40E-01	27
A_23_P46482	50604	interleukin 20	IL20	-0.17	4.53E-01	4.43E-01	22

A_23_P4899	4909	neurotrophin 5 (neurotrophin 4/5)	NTF5	0.15	4.70E-01	4.57E-01	27
A_23_P41344	2069	epiregulin	EREG	0.14	4.79E-01	4.63E-01	27
A_23_P37727	6376	chemokine (C-X3-C motif) ligand 1	CX3CL1	0.14	4.81E-01	4.63E-01	27
A_24_P377124	7066	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte	THPO	0.14	4.95E-01	4.74E-01	25
A_23_P207582	6360	chemokine (C-C motif) ligand 16	CCL16	0.13	5.08E-01	4.81E-01	27
A_23_P373017	6348 728830	chemokine (C-C motif) ligand 3	CCL3	0.13	5.08E-01	4.81E-01	27
A_23_P429363	8822	fibroblast growth factor 17	FGF17	-0.17	5.20E-01	4.90E-01	16
A_23_P138760	23529	cardiotrophin-like cytokine factor 1	CLCF1	-0.13	5.34E-01	5.00E-01	27
A_24_P355720	2249	fibroblast growth factor 4 (heparin secretory transforming protein 1, Kaposi sar	FGF4	-0.15	5.38E-01	5.01E-01	19
A_23_P121459	7066	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte	THPO	0.16	5.42E-01	5.01E-01	16
A_23_P121695	10563	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	CXCL13	-0.15	5.48E-01	5.01E-01	18
A_23_P7144	2919 2920	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	CXCL1	-0.12	5.50E-01	5.01E-01	27
A_23_P55270	6362	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	CCL18	-0.12	5.52E-01	5.01E-01	27
A_23_P258410	7476 7477	wingless-type MMTV integration site family, member 7A	WNT7A	0.14	5.52E-01	5.01E-01	20
A_23_P19624	654	bone morphogenetic protein 6	BMP6	0.12	5.60E-01	5.06E-01	27
A_23_P84705	23495	tumor necrosis factor receptor superfamily, member 13B	TNFRSF13B	-0.11	5.77E-01	5.17E-01	27
A_23_P133408	1437	colony stimulating factor 2 (granulocyte-macrophage)	CSF2	-0.12	5.80E-01	5.17E-01	22
A_23_P204654	4254	KIT ligand	KITLG	0.11	5.82E-01	5.17E-01	26
A_23_P212800	2250	fibroblast growth factor 5	FGF5	0.11	5.87E-01	5.19E-01	27
A_32_P87013	3576	interleukin 8	IL8	-0.11	5.98E-01	5.24E-01	26
A_23_P72096	3552	interleukin 1, alpha	IL1A	-0.12	5.99E-01	5.24E-01	23
A_24_P217520	2069	epiregulin	EREG	-0.11	6.03E-01	5.25E-01	25
A_24_P97342	60675	prokineticin 2	PROK2	-0.10	6.24E-01	5.40E-01	27
A_23_P315815	3084	neuregulin 1	NRG1	0.10	6.34E-01	5.46E-01	27
A_23_P202448	6387	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12	0.10	6.37E-01	5.46E-01	27
A_23_P58396	56034	platelet derived growth factor C	PDGFC	0.09	6.41E-01	5.47E-01	27
A_23_P26325	6361	chemokine (C-C motif) ligand 17	CCL17	-0.09	6.47E-01	5.47E-01	27
A_23_P54144	652	bone morphogenetic protein 4	BMP4	-0.09	6.47E-01	5.47E-01	27

A_24_P381901	6376	chemokine (C-X3-C motif) ligand 1	CX3CL1	0.09	6.56E-01	5.52E-01	27
A_23_P110531	10468	folliculin	FST	-0.09	6.65E-01	5.54E-01	27
A_23_P213706	3565	interleukin 4	IL4	0.09	6.65E-01	5.54E-01	27
A_24_P251969	2246	fibroblast growth factor 1 (acidic)	FGF1	0.11	6.80E-01	5.64E-01	16
A_23_P47735	84957	tumor necrosis factor receptor superfamily, member 19-like	TNFRSF19L	-0.08	6.89E-01	5.68E-01	27
A_23_P52227	2662	growth differentiation factor 10	GDF10	0.08	6.92E-01	5.68E-01	27
A_23_P76078	3593 51561	interleukin 23, alpha subunit p19	IL23A	0.08	7.05E-01	5.76E-01	27
A_24_P334300	2257	fibroblast growth factor 12	FGF12	-0.07	7.16E-01	5.80E-01	26
A_23_P37736	608	tumor necrosis factor receptor superfamily, member 17	TNFRSF17	0.07	7.16E-01	5.80E-01	27
A_23_P42065	27242	tumor necrosis factor receptor superfamily, member 21	TNFRSF21	-0.07	7.28E-01	5.81E-01	27
A_24_P192485	4982	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	TNFRSF11B	-0.07	7.28E-01	5.81E-01	27
A_23_P30122	3558	interleukin 2	IL2	-0.08	7.29E-01	5.81E-01	20
A_24_P401855	2250	fibroblast growth factor 5	FGF5	-0.09	7.43E-01	5.87E-01	17
A_23_P169257	944	tumor necrosis factor (ligand) superfamily, member 8	TNFSF8	0.09	7.45E-01	5.87E-01	16
A_23_P16523	9518	growth differentiation factor 15	GDF15	-0.07	7.46E-01	5.87E-01	27
A_23_P112470	6366	chemokine (C-C motif) ligand 21	CCL21	0.06	7.58E-01	5.92E-01	27
A_23_P49097	27189	interleukin 17C	IL17C	-0.08	7.59E-01	5.92E-01	19
A_23_P143331	650	bone morphogenetic protein 2	BMP2	-0.06	7.62E-01	5.92E-01	27
A_23_P378329	7483	wingless-type MMTV integration site family, member 9A	WNT9A	0.06	7.66E-01	5.92E-01	26
A_23_P211727	2257	fibroblast growth factor 12	FGF12	0.06	7.76E-01	5.93E-01	27
A_23_P66635	6356	chemokine (C-C motif) ligand 11	CCL11	0.06	7.81E-01	5.93E-01	27
A_23_P93027	8817	fibroblast growth factor 18	FGF18	0.06	7.81E-01	5.93E-01	27
A_23_P152838	6352	chemokine (C-C motif) ligand 5	CCL5	0.05	7.88E-01	5.93E-01	27
A_24_P218265	8795 8797	tumor necrosis factor receptor superfamily, member 10b	TNFRSF10B	0.05	7.90E-01	5.93E-01	27
A_23_P30666	27242	tumor necrosis factor receptor superfamily, member 21	TNFRSF21	-0.05	7.97E-01	5.93E-01	27
A_23_P380939	353500 656	bone morphogenetic protein 8b (osteogenic protein 2)	BMP8B	0.06	8.03E-01	5.93E-01	19
A_23_P138352	7482	wingless-type MMTV integration site family, member 2B	WNT2B	0.05	8.04E-01	5.93E-01	27
A_23_P427587	9965	fibroblast growth factor 19	FGF19	0.05	8.05E-01	5.93E-01	26
A_23_P14612	2252	fibroblast growth factor 7 (keratinocyte growth factor)	FGF7	-0.05	8.14E-01	5.93E-01	27
A_23_P127891	627	brain-derived neurotrophic factor	BDNF	0.05	8.16E-01	5.93E-01	27
A_24_P45476	6375 6846	chemokine (C motif) ligand 1	XCL1	-0.05	8.21E-01	5.93E-01	27

A_24_P140608	1839	heparin-binding EGF-like growth factor	HBEGF	-0.05	8.21E-01	5.93E-01	27
A_23_P119202	NA	tumor necrosis factor (ligand) superfamily, member 7	TNFSF7	0.04	8.25E-01	5.93E-01	27
A_23_P71037	3569	interleukin 6 (interferon, beta 2)	IL6	-0.04	8.25E-01	5.93E-01	27
A_23_P128503	55801	interleukin 26	IL26	0.04	8.28E-01	5.93E-01	27
A_23_P51936	3604	tumor necrosis factor receptor superfamily, member 9	TNFRSF9	-0.04	8.28E-01	5.93E-01	26
A_23_P155979	1950	epidermal growth factor (beta-urogastrone)	EGF	-0.04	8.31E-01	5.93E-01	26
A_23_P218369	6358 6359	chemokine (C-C motif) ligand 15	CCL15	-0.04	8.32E-01	5.93E-01	27
A_24_P355464	2662	growth differentiation factor 10	GDF10	0.04	8.51E-01	6.04E-01	26
A_24_P163168	56034	platelet derived growth factor C	PDGFC	0.04	8.65E-01	6.10E-01	23
A_23_P207456	6355	chemokine (C-C motif) ligand 8	CCL8	0.04	8.67E-01	6.10E-01	22
A_23_P113204	2248	fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2	FGF3	0.03	8.70E-01	6.10E-01	27
A_23_P100386	146433	interleukin 34	IL34	0.03	8.87E-01	6.19E-01	25
A_23_P156683	4049	lymphotoxin alpha (TNF superfamily, member 1)	LTA	-0.03	8.97E-01	6.22E-01	24
A_24_P402438	7042	transforming growth factor, beta 2	TGFB2	0.03	8.98E-01	6.22E-01	26
A_23_P137573	7044	left-right determination factor 2	LEFTY2	0.02	9.09E-01	6.24E-01	27
A_23_P140057	55504	tumor necrosis factor receptor superfamily, member 19	TNFRSF19	-0.02	9.17E-01	6.24E-01	26
A_23_P376488	7124	tumor necrosis factor (TNF superfamily, member 2)	TNF	-0.02	9.21E-01	6.24E-01	27
A_32_P7316	627	brain-derived neurotrophic factor	BDNF	0.02	9.22E-01	6.24E-01	26
A_24_P277367	6372 6374	chemokine (C-X-C motif) ligand 5	CXCL5	-0.02	9.23E-01	6.24E-01	27
A_23_P90925	27177	interleukin 1 family, member 8 (eta)	IL1F8	0.02	9.26E-01	6.24E-01	18
A_24_P251764	2921	chemokine (C-X-C motif) ligand 3	CXCL3	-0.02	9.35E-01	6.24E-01	27
A_23_P390518	8792	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	TNFRSF11A	0.02	9.37E-01	6.24E-01	27
A_23_P81058	651	bone morphogenetic protein 3 (osteogenic)	BMP3	0.02	9.40E-01	6.24E-01	16
A_23_P123853	6363	chemokine (C-C motif) ligand 19	CCL19	0.02	9.40E-01	6.24E-01	27
A_23_P119478	10148	Epstein-Barr virus induced gene 3	EBI3	0.01	9.42E-01	6.24E-01	27
A_23_P51534	6375 6846	chemokine (C motif) ligand 2	XCL2	-0.01	9.49E-01	6.24E-01	27
A_23_P73609	4693	Norrie disease (pseudoglioma)	NDP	0.01	9.54E-01	6.24E-01	27
A_23_P61057	3603	interleukin 16 (lymphocyte chemoattractant factor)	IL16	-0.01	9.54E-01	6.24E-01	27
A_23_P95417	8793	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated dea	TNFRSF10D	0.01	9.58E-01	6.24E-01	18
A_24_P237036	8740	tumor necrosis factor (ligand) superfamily, member 14	TNFSF14	-0.01	9.59E-01	6.24E-01	27

A_23_P35092	29949	interleukin 19	IL19	0.01	9.77E-01	6.33E-01	25
A_23_P31945	90865	interleukin 33	IL33	0.00	9.90E-01	6.39E-01	27
A_23_P101564	26291	fibroblast growth factor 21	FGF21	0.00	1	6.43E-01	19

Among asthmatics (N=27), the Th2 sig was correlated with a manually curated list of 212 Cytokines, Chemokines, and Growth factors (CCGf), encoded by 268 probes. Spearman's correlation rho, p-value, q-value, and N (number of subjects for whom reportable microarray data exists) are tabulated for each probe.

Table SIII: "qPCR verification of selected Th2 signature genes"

GENE SYMBOL	p value ↓	t	FC
CST1	7.94E-06	5.36	175.74
MUC5B	2.59E-03	-3.45	-20.01
CLCA1	3.03E-03	3.38	144.99
CCL26	0.038	2.15	9.40

Gene expression was verified by qPCR. Significance was assessed by Welch's *t* test, comparing subjects categorized by bronchial epithelial Th2 signature status ({"Th2 high" asthma} versus {"Th2 low" asthma AND control}).