

# Gene Expression Profiling in Human Asthma

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Asthma is a chronic inflammatory disease of the lungs, characterized by airway hyperreactivity, mucus hypersecretion, and airflow obstruction. Despite recent advances, the genetic regulation of asthma pathogenesis is still largely unknown. Gene expression profiling techniques are well suited to study complex diseases and hold substantial promise for identifying novel genes and pathways in asthma; however, relatively few studies have been completed in human asthma. The few studies that have been done have identified many novel candidate genes and pathways in asthma pathogenesis, including ALOX15 and serine proteinase inhibitors cathepsin C and G. The interpretation of results of these studies should be cautious, as limitations include small sample sizes and heterogeneity of study populations and tissues sampled. In the future, the promise of gene expression studies would be enhanced by the use of larger sample sizes and attempts to standardize phenotype, sample collection techniques, and analysis. As the field of expression profiling in asthma advances, we hope it will improve our understanding of critical questions about mechanisms involved in susceptibility to the disease, as well as help to personalize care by improving appropriate selection of patients for prevention and treatment strategies.

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Gene expression profiling techniques are often used as high-throughput tools allowing for the simultaneous analysis of a large quantity of transcripts. These tools hold promise in identifying novel genes and pathways involved in disease pathogenesis and have increasingly been used to study complex diseases such as asthma (1). Expression profiling tools include cDNA and oligonucleotide microarrays (2–4), differential display (5), and subtractive hybridization (6–8). Several expression profiling studies have been done in mouse models and purified cell cultures relevant to asthma (9–13); however, despite the potential for identifying novel genes and gene expression patterns, relatively few studies have been done in human asthmatic tissue samples. The purpose of this review is to highlight expression profiling studies that have been performed in human asthma.

## BRONCHIAL BIOPSY

Asthma is considered to be an inflammatory disease principally affecting the lungs (14). Thus, the use of lung tissue is ideal for the investigation of asthma pathogenesis, and several recent studies have analyzed global gene expression in bronchial biopsy specimens. Laprise and colleagues (15) used oligonucleotide microarrays to compare the gene expression of bronchial biopsy

specimens taken from subjects with mild asthma ( $n = 4$ ) who were not using inhaled corticosteroid therapy with biopsy specimens from normal control subjects. Of the genes that were either up-regulated (20 genes) or down-regulated (54 genes) in patients with asthma compared with control subjects, 13 were related to immune signaling molecules, 8 to extracellular proteins, 9 to immune response, 9 to intracellular signaling component pathways, 8 to proteolytic enzymes, and 5 to transmembrane proteins. Many of the identified genes were known to be involved in asthma pathogenesis, including nitric oxide synthase 2A (NOS2A), glutathione peroxidase 3 (GPX3), and T-cell receptor  $\alpha$  locus (TCR $\alpha$ ). However, several genes were newly implicated in asthma, including arachidonate 15-lipoxygenase (ALOX15) and fractalkine receptor (CX3CR1). The 15-lipoxygenase pathway generates both 15S-hydroxyeicosatetraenoic acid (15S-HETE) and the lipoxins. 15S-HETE may be involved in airway remodeling (16), whereas lipoxins are increasingly appreciated for their antiinflammatory properties (17). Likewise, detection of CX3CR1 is a novel finding, but was found previously to be elevated in peripheral CD4<sup>+</sup> lymphocytes of subjects with asthma compared with normal control subjects (18). Genes included in the serine proteinase inhibitor (serpin) family of protease inhibitors, such as cathepsin C and cathepsin G, were also identified as differentially regulated in subjects with asthma compared with control subjects, and their potential role in asthma has been supported by other expression profiling studies (19, 20). Genes in the serpin family were among 12 differentially expressed genes in interleukin (IL)-4- and IL-13-stimulated human bronchial epithelial cell lines (19). SERPINB3 (SCCA1) and SERPINB4 (SCCA2) were subsequently elevated in cDNA libraries constructed from bronchial tissues of subjects with asthma compared with control subjects. Furthermore, serum levels of SCCA were significantly higher in subjects with asthma compared with control subjects and higher during an asthma attack compared with the convalescent stage of an asthma exacerbation. The precise role of these genes in asthma pathogenesis is unknown; however, they may exert a protective role by inhibiting endogenous proteases associated with the inflammatory response (19, 21). The results of the expression studies suggest that the role of serpin proteases in asthma pathogenesis merits further study.

In another study using bronchial biopsy specimens, Dolganov and colleagues (20) used a real-time polymerase chain reaction (PCR)-based method specifically designed for quantification of multiple low-abundance transcripts to analyze gene expression in 11 patients with asthma and 8 healthy control subjects. This study identified 19 up-regulated genes. Several of these genes had been identified in the study by Laprise and coworkers (15), including cathepsin C (CTSC), cathepsin G (CTSG), and RANTES (regulated upon activation, normal T-cell expressed and secreted), all of which have previously been implicated in asthma pathogenesis (22–25). Several other genes in the Dolganov study were previously known to be involved in the pathogenesis of asthma, including cytokines (i.e., IL-5, IL-13), cytokine receptors (i.e., IL-13RA2, IL-13RA1, IL-4R $\alpha$ , IFN- $\gamma$ R2), transcription factors (i.e., STAT4, STAT6), and chemokines. When the authors examined gene expression by disease severity, as measured by lung function (FEV<sub>1</sub>% predicted), in subjects with

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asthma, only IL-13 expression was associated with severity, and it was inversely related to lung function. The authors also identified an eightfold increased expression of NKCC1, a  $\text{Na}^+\text{K}^+\text{Cl}^-$  cotransporter not previously identified in the pathogenesis of asthma. Furthermore, increased protein expression of NKCC1 in airway epithelium goblet cells was confirmed by immunohistochemical methods. The role of NKCC1 in asthma pathogenesis is not clear; however, it may have a role in mucus hypersecretion and, if so, it may represent a novel therapeutic target.

Gene expression profiles obtained from bronchial biopsy specimens must be interpreted with caution and may be difficult to compare across studies. Differing biopsy techniques and heterogeneity of distribution of disease pathology can affect the proportion of epithelial cells, fibroblasts, smooth muscle cells, and inflammatory cells that are obtained in each specimen. Therefore, although these findings come from a highly relevant tissue, reproducibility of gene expression differences across studies may be impacted by differences in cellular composition of the biopsy specimen.

### AIRWAY EPITHELIAL CELLS

Results of several other studies using microarray techniques have emphasized the active role that airway epithelial cells play in the asthmatic response. Lilly and colleagues (26) investigated the response of the airway epithelium to segmental allergen challenge in five individuals with mild asthma and identified 149 differentially (141 increased, 8 decreased) expressed genes pre- and post-allergen challenge in airway epithelial cells obtained by bronchoscopic brushings. Differentially regulated genes included those involved in tissue repair, proliferation, and survival response genes. Up-regulated genes included those previously implicated in asthma pathogenesis, such as IL-1 $\beta$ , IL-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced protein 6, lipocortin-1, as well as novel genes including plasminogen activator inhibitor 2. In another study, nasal epithelial brushings were used as a surrogate for lower airway epithelium to determine gene expression profiles using microarrays in children with stable asthma ( $n = 10$ ), children experiencing an acute exacerbation ( $n = 10$ ), and nonatopic control children ( $n = 10$ ) (27). In this study, discrete gene expression profiles were found in acute versus stable asthma, with several immune-regulated genes being up-regulated and cilia-related genes being down-regulated. These results suggest that distinct genes are induced during asthma exacerbations as compared with stable asthma (27). Although these results are interesting, it is not yet clear the extent to which gene expression changes in nasal epithelial brushings may provide an early signal for impending exacerbations, or the extent to which they may be confounded by rhinitis, atopy, or other factors.

In comparing the results of the above two studies with *in vitro* studies of IL-4- and IL-13-stimulated human airway epithelial cells (19, 28), there is little apparent overlap with differentially expressed genes. The lack of overlap of findings is not surprising, as one may expect differences in the response to allergen exposure compared with cytokine exposure, different cell types in the brushings compared with cell lines studied, or differences in the responses of airway epithelial cells in culture as compared with those *in vivo*. Bronchial epithelial brushings have been used to obtain cytokeratin-positive airway epithelial cells with approximately 95% purity; however, gene expression results can be confounded by nonepithelial cells, such as inflammatory cells (neutrophils and eosinophils), which may be present in varying proportions (26, 27). Therefore, epithelial brushings may be subject to similar limitations as bronchial biopsy specimens.

### PERIPHERAL BLOOD MONONUCLEAR CELLS

Although the lung is the main site of inflammation, there is substantial evidence that asthma is a systemic disease with activated circulating cells (29, 30). Brutsche and colleagues, using peripheral blood mononuclear cells (PBMCs) of atopic individuals with and without asthma compared with nonatopic control subjects, showed that gene array techniques can be used to detect alterations in the expression of several genes associated with B-cell isotype control, IgE production (31), and cell survival and/or apoptosis (32). In this study, the proto-oncogene MYC, as well as MADH1, CD86, TNF receptor-associated factor (TRAF-3), extracellular signaling regulated kinase 1 (ERK1), and mitogen-activated protein kinase-activated protein kinase were up-regulated in subjects with severe asthma compared with subjects with milder disease. The ataxia telangiectasia gene ATM and the TNF- $\alpha$  inositol hexaphosphate gene showed reduced expression (31, 32). Furthermore, Brutsche and colleagues developed a gene expression-based score (composite atopy gene expression [CAGE] score) for the assessment of asthma disease activity and atopic status (33). The score was based on the expression of 10 genes, including IL-1 receptor type 1, IL-6, and IFN- $\alpha\beta$  receptor  $\beta$ -chain. The CAGE score was able to identify individuals with atopy with 96% sensitivity and 92% specificity. In addition, the CAGE score was significantly correlated with total IgE in atopic individuals ( $r^2 = 0.75$ ,  $p < 0.001$ ) and asthma severity ( $p = 0.051$ ), suggesting that gene expression profiles of PBMCs may be used to assess atopic disease activity.

Fukuda and colleagues compared the gene expression of stimulated and nonstimulated PBMCs in subjects with asthma (34). When stimulated with mite antigens, PBMCs obtained from patients with mite sensitization and asthma showed increased expression of 13 genes by subtractive hybridization. Six of these genes were mitochondrial genes, suggesting that activation of T cells by mite antigen stimulation may require an increase in energy production. There was no overlap of these genes with those identified from Brutsche and colleagues. This difference in findings may be related to the characteristics of the patients studied, the use of stimulated versus nonstimulated PBMCs for analysis, or different methods used (custom DNA microarray vs. subtractive hybridization).

### PERIPHERAL BLOOD LYMPHOCYTES

Studies that concentrate on a specific cell type might reduce the complexity of interpretation of gene expression studies. CD4<sup>+</sup> lymphocytes play a central role in the pathogenesis of asthma and peripheral CD4<sup>+</sup> lymphocytes have shown evidence for increased activation in patients with acute, severe asthma (35, 36). Given the key role of this cell type, several studies have investigated the global gene expression of CD4<sup>+</sup> lymphocytes in asthma.

In one such study, Hansel and colleagues (37) compared the gene expression of CD4<sup>+</sup> lymphocytes of subjects with severe ( $n = 5$ ) versus mild asthma ( $n = 5$ ) using oligonucleotide microarray techniques. A total of 40 differentially regulated genes with at least a twofold up- or down-regulation (37 up-regulated and 3 down-regulated) were identified. Up-regulated genes included transforming growth factor- $\beta$  (TGF- $\beta$ ) and others involved in T-cell activation, proliferation, and cytoskeletal changes. Novel genes with plausible biological links to asthma were also identified, including urotensin and leukocyte-derived arginine aminopeptidase (LRAP). Chromosome 19p13-13.3 has previously been implicated to harbor susceptibility loci associated with atopic phenotypes and inflammatory conditions (38-40). Seven of the 37 up-regulated genes localized to this region,

including B4GALT1, GTF2F1, JunD, PRKCSH, PTBP1, POLR2E, and WDR18. These results highlight the potential to identify candidate genes responsible for disease severity that might not have been otherwise targeted for investigation.

Wohlfahrt and colleagues (18), in another study of peripheral CD4<sup>+</sup> lymphocytes, comparing subjects with asthma with normal control subjects using cDNA microarray techniques, also found up-regulation of TGF- $\beta$ 1 and other members of the TGF- $\beta$  family. Furthermore, they found evidence for up-regulation of several other gene families in asthma, including those for chemokines, chemokine receptors, fibroblast growth factors, and matrix metalloproteinases and their inhibitors.

Another small study evaluated the gene expression of peripheral CD4<sup>+</sup> lymphocytes, using custom cDNA arrays, in three subjects with asthma (one subject with steroid-sensitive asthma [SSA] and two subjects with steroid-resistant asthma [SRA]) compared with six nonasthmatic control subjects (41). HLA-DR, c-Jun, and c-Fos were up-regulated in the subjects with SSA compared with control subjects, and TNF receptor-2 was up-regulated in both SSA and SRA. CCR7 was down-regulated in both SSA and SRA, and laminin receptor (LAMR1) and integrin  $\beta$ 5 (ITGB5) were down-regulated only in SRA. Although this study identified several genes potentially involved in asthma pathogenesis, and previous evidence suggests that some patients respond to certain medications better than others (42), it is difficult to reach firm conclusions from the findings of differences between SRA and SSA subjects, due to the small number of subjects studied. A larger study may help to elucidate novel molecular targets for therapeutic intervention and may help identify subjects who will respond better to steroids in order to individualize asthma treatment strategies.

In a study by Katsunuma and colleagues, eight atopic children with asthma had peripheral blood drawn and isolation of peripheral T lymphocytes (CD3<sup>+</sup> cells) during the time of an exacerbation, and again after improvement of symptoms and lung function (43). Differential display reverse transcriptase (RT)-PCR was used to identify 49 bands that were differentially expressed during the asthma exacerbation compared with after recovery; however, only one gene, lipocortin (annexin) II, was confirmed by quantitative RT-PCR to be elevated during exacerbations. Although other expression studies have not identified lipocortin II, another expression study did identify a different member of the lipocortin family. Lilly and colleagues showed increased lipocortin-1 in airway epithelial cells in asthmatic after allergen challenge (26). Lipocortin I is believed to play an antiinflammatory role and has previously been implicated as a marker in T cells in children with exacerbated asthma (44, 45). Lipocortin II is believed to have antifibrinolytic properties (45), but its precise role in asthma or asthma exacerbations is unknown. The particular role of the lipocortins in asthma pathogenesis likely merits further investigation.

## PERIPHERAL EOSINOPHILS

Increased numbers of eosinophils in the peripheral blood and inflammatory tissue are characteristic features of asthma, and eosinophils are associated with disease severity (46). Eosinophils are activated by several inflammatory mediators, and migrate from the circulation to the airway (47). In a comparison of gene expression in peripheral eosinophils of subjects with asthma compared with nonasthmatic subjects by differential display RT-PCR, only 10 bands of over 500 bp in length were found to be consistently up-regulated (48). Only four of these bands were confirmed by reverse dot blots and none of them represented an open reading frame. Although evidence suggests that stimulated eosinophils *in vitro* (49, 50) and peripheral eosinophils of subjects

with asthma (51) may have increased cytokine expression, these results suggest that there are relatively few genes significantly differentially expressed in resting peripheral eosinophils of individuals with asthma and nonasthmatic individuals.

## CONCLUSIONS

Advancement in gene expression profiling techniques, such as microarrays, has provided powerful tools for studying complex diseases such as asthma. To date, there have been relatively few studies, many of which have relied on small sample sizes. Reassuringly, despite the small size of the studies, the use of these techniques in human asthma has confirmed many genes already known to be relevant to the disease. Many novel candidate genes and pathways in asthma pathogenesis also have been identified, although the relevance of most of these genes and pathways remains to be confirmed. It should also be noted there has been little overlap in the novel genes identified across studies. The lack of consistency of findings across studies to date is likely due to several limitations, which should be considered when interpreting the results of the body of gene expression studies. First, the lack of a standard definition of asthma raises the risk of studying a heterogeneous disease. Small sample sizes and heterogeneity of study populations increase the chance that findings are confounded by patient characteristics (e.g., sex or age) and important exposures, such as medications that may affect gene expression (52, 53). Furthermore, the use of different sampling strategies, laboratory techniques, and approaches to data analysis can all contribute to disparate results. Finally, the study conditions vary considerably (e.g., stable vs. unstable, stimulated vs. unstimulated), which also has made it difficult to expect consistency across studies. In the future, the promise of gene expression studies would be enhanced by the use of larger sample sizes and as much standardization as possible. Ideally, studies could use consistent definitions of asthma, standardized techniques to obtain and process samples, and similar statistical approaches to the analyses. It is hoped, as the field of expression profiling in asthma advances, that this will improve our understanding of critical questions about mechanisms involved in susceptibility to the disease, as well as help with key clinical challenges such as the appropriate selection of patients for specific prevention and treatment strategies.

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