

Molecular Endotypes Contribute to the Heterogeneity of Asthma



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KEYWORDS

- Asthma • Endotypes • Biomarker • Transcriptomics • Sputum • Epithelium
- Human rhinovirus infection • Steroid-resistant asthma

KEY POINTS

- Molecular endotyping in sputum, airway epithelial cells, and blood increases our understanding of the clinical heterogeneity of asthma.
- Varying genes and pathways contribute to the development and progression of asthma depending on the tissue, trigger, and disease status.
- Endotyping can be a screening tool to identify useful biomarkers of disease, risk assessment, and treatment response.
- Well-characterized, longitudinal cohorts are necessary to define critical endotypes to advance the diagnosis, classification, and treatment of asthmatic patients.

Asthma is one of the most common chronic diseases. Commonly, clinical measurements have been used to diagnose and manage the disease. Depending on symptom severity, guideline-supported approaches recommend inhaled corticosteroids (ICS) for disease control and β_2 -agonists for disease rescue. However, this one-size-fits-all approach to disease management has been called into question, particularly in light of the increasing recognition that asthma is heterogeneous, one that is not stable over time but responsive to ever-changing environmental stimuli, and corticosteroids (CS) may not always be effective. Defining subgroups of asthmatic patients based on clinical parameters and cluster analyses incorporating lung function, medication usage, and other clinical variables has been challenging. Identification of biomarkers that associate with these subgroups have centered around measurements of airway inflammation,¹ levels of exhaled nitric oxide (FeNO),² and serum periostin levels.^{3,4} Although useful, these nonmolecular biomarkers provide limited resolution of disease

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subgroups and little information regarding the underlying pathobiology of the disease in individual subjects. In contrast, molecular data generated from asthmatic patients hold great promise in the delineation of pathobiological subgroups, bioactive pathways, and true disease endotypes. In particular, the ability to query the entire transcriptome in a single assay (microarray technology and/or RNA-sequencing [RNA-seq]) has provided a powerful tool to comprehensively define the molecular dysfunction of a tissue at a patient level and then relate these molecular patterns to clinical traits. The complex cause of asthma defined by endotyping of a single tissue will only provide a partial picture of the disease. Reflecting this, expression studies of sputum, airway, and blood have all contributed significant information to our understanding of asthma pathobiology; but results have been discordant. Additionally, the dynamic nature of the transcriptome requires studies examining tissues at different developmental and disease stages, environmental exposures or infection status which will contribute unique information about asthma. Collectively, these studies have and will continue to provide a more comprehensive picture of asthma endotypes, associated biomarkers, and potential pharmacologic targets, as discussed later.

MOLECULAR ENDOTYPES CORRELATE WITH ASTHMA PHENOTYPES USING SPUTUM TRANSCRIPTOMICS

Most asthmatic patients have some degree of chronic inflammation of the airways. Analyses of induced sputum cells offer a noninvasive technique to collect and quantify inflammatory cell populations in the airways of asthmatic patients. To a large extent the composition of these samples includes both innate (macrophages, neutrophils, eosinophils, innate lymphocytes) and adaptive immune cells (CD4⁺ and CD8⁺ lymphocytes). Although sputum eosinophilic airway inflammation in asthmatic patients is the most prevalent, asthmatic patients can be classified into 4 subgroups based on the cell composition at a particular point in time: eosinophilic, neutrophilic, mixed granulocytic (eosinophilic and neutrophilic), and pauci-granulocytic (normal or near normal eosinophil/neutrophil numbers).^{5,6} Compared with the pauci-granulocytic phenotype, eosinophilic, neutrophilic, and mixed granulocytic asthmatic patients exhibit poorer lung function.⁵ Severe asthma has tended to associate with increased neutrophil numbers rather than eosinophils in their airways (neutrophilic asthma), a phenomenon typically related to nonallergic asthma and thought to be triggered by particulates in the air, pollution, viruses, or bacteria.⁷ Important in understanding this inflammatory cell heterogeneity is the impact of drugs. Whereas eosinophils seem to be CS-sensitive and readily undergoing apoptosis, neutrophils seem to be insensitive to CS.⁸ Although attempts have been made to track these airway inflammatory subgroups by monitoring blood granulocyte levels, this has only been partially helpful for eosinophilic disease and poorly for neutrophilic asthma.

Studies have been extended to the molecular level through generation of gene expression profiles from sputum cells and peripheral blood to determine whether the clinical heterogeneity of asthma is also reflected in underlying molecular pathways. Sputum microarray transcriptomic data from 100 asthmatic subjects were recently used to identify transcriptomic endotypes of asthma (TEA) clusters.⁹ The expression of genes within predefined pathways was used in unsupervised clustering analyses of TEA groups based on the pathway distance between samples and resulted in 3 distinct clusters. To determine the phenotypic correlates of these TEA subgroups, clinical variables were compared: TEA cluster 1 was associated with lower prebronchodilator forced expiratory volume in the first second of inspiration (FEV1), a higher bronchodilator response, higher FeNO levels, and ICS usage; TEA cluster 2 contained

individuals with prior hospitalization for asthma and no atopy history: TEA cluster 3 was enriched for patients with mild asthma, associated with better lung function, decreased FeNO, and lower ICS requirements. A similar approach was taken in asthmatic patients where both sputum and peripheral blood expression data were available. An expression signature encompassing 53 genes in the blood separated the population into clusters that closely resembled the TEA subgroups observed in the sputum. The identification and clinical characteristics of TEA clusters represented by this gene signature were largely replicated in the Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE) cohort. Importantly, the investigators did not find these TEA clusters to be associated with sputum inflammatory cell numbers per se, supporting the importance of molecular sputum analyses for clinical associations. Moreover, these studies suggested that blood gene expression patterns can be informative in clinically defined clustering of asthmatic patients.

Comprehensive approaches have been taken by the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) consortia to test if molecular differences in the sputum and other tissues, for example, blood, exist between clinically assigned groups but also to discover distinct asthma phenotypes based on endo-typic signatures. This European cohort included nonsmoking patients with severe asthma (SA), smoking/ex-smoking patients with SA, nonsmoking patients with mild/moderate asthma (MMA), and healthy nonsmoking controls.¹⁰ One focus of this cohort study was to perform molecular phenotyping of sputum cells to define endotypes in patients with severe asthma that relate to sputum granulocytic inflammation.¹¹ Inflammatory cell counts revealed the highest sputum eosinophils in smoking and nonsmoking SA, whereas neutrophilic inflammation did not vary between groups. When transcriptomic profiles were compared between the 4 different subgroups, 801 transcripts were differentially expressed in nonsmoking SA versus healthy controls and 172 genes comparing nonsmoking SA with MMA, respectively. Key genes that were upregulated in the sputum of nonsmoking SA included members of the interleukin 1 (IL-1) receptor (*IL-1R*) family and the inflammasome pathway (eg, *IL1RL1*, *IL18R1*, *NLRP3*, *ILRL1*, *IL1RL2*, *IRAK3*, *IL18RAP*). When *IL-1R* and inflammasome-related genes were correlated with specific sputum granulocyte subtypes, a predominance of eosinophilic inflammation, enhanced T helper 2 (Th2) cytokine protein expression, and *IL1RL1* messenger RNA (mRNA) levels was detected. In contrast, elevated expression of *IL-1* and *IL-18R* family members, *NLRP1*, *NLRP3*, *NLRP4*, and other members of the inflammasome were associated with neutrophilic asthma. Of note, these changes were validated in the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) cohort¹² but seemed specific for sputum, as similar effects were not seen in bronchial brushings or biopsies from SA in the U-BIOPRED cohort.¹¹ Further validation of the relevance of the *NLRP3* inflammasome was provided in a model of experimental asthma. In vivo administration of the inflammasome inhibitor CRID3 prevented the development of lung allergic responses and reduced the expression of IL-1 β , Th2 cytokines, and neutrophil- and eosinophil-associated chemokines. Such studies underscore the need to incorporate both human and animal studies to validate biologically relevant mechanisms and translate the clinical heterogeneity of human asthma through mechanistic understanding of the pathobiology.

Additional molecular endotyping studies of sputum have focused on defining the presence or absence of type 2 cytokine-mediated airway inflammation. For example, Peters and colleagues¹³ found gene expression changes of the type 2 cytokines *IL-4*, *IL-5*, and *IL-13* in sputum cell pellets. Moreover, they developed an algorithm to classify asthmatic patients as having type 2-high or type 2-low disease. Subjects with type 2-high disease had more severe disease and increased sputum and blood eosinophil

numbers. A transcriptome-wide study was conducted to define molecular mechanisms of type 2-high and type 2-low asthma in the sputum of patients with severe disease (U-BIOPRED¹⁰) and identified involvement of 10 relevant pathways. These pathways included immune activation and cytokine production, regulation of cysteine-type endopeptidase activity, pattern recognition receptor signaling, response to interferon- γ , ice protease-activating factor inflammasome complex, and Nucleotide-binding, oligomerization domain like receptor signaling.¹⁴ Moreover, this study identified interferon-, tumor necrosis factor- α -, and inflammasome-associated signatures that were associated with type 2-low, neutrophilic asthma. Pauci-granulocytic patients exhibited a molecular profile characteristic of increased mitochondrial energy metabolic pathways, with the highest expression scores for mitochondrial oxidative stress (oxidative phosphorylation) and aging.

Taken together, these studies confirm the value of molecular analyses of sputum cells to increase our understanding of the clinical heterogeneity of asthma, to delineate the varying airway inflammatory pathways in asthma, and to define useful biomarkers that associate with disease. One limitation of these studies is that they are single-point-in-time analyses and do not reflect the dynamic nature of asthma.

TRANSCRIPTOMIC PROFILES IN EPITHELIAL CELLS OF ASTHMATIC PATIENTS ARE DISTINCT FROM HEALTHY INDIVIDUALS

One potential mechanism contributing to asthma pathobiology is through disrupted epithelial barrier function of airway epithelial cells due to transcriptional reprogramming.^{15,16} However, dysregulated genes and pathways involved in this signaling process are poorly understood. One of the first mechanistic studies to characterize molecular endotypes in airway epithelial brushings from asthmatic patients identified a gene signature consisting of *PERIOSTIN*, *CLCA1*, and *SERPINB2* as potential markers of the IL-13–driven, CS-responsive asthma phenotype.¹⁷ Based on the initial data, asthmatic patients with a Th2-high or a Th2-low profile were defined.¹⁸ Key features within the Th2-high group were increased IL-5 and IL-13 levels, bronchial hyper-responsiveness, elevated serum immunoglobulin E levels, and blood and airway eosinophilia. The Th2-high group was more likely to be atopic and responsive to ICS, although this has been less consistent in other studies.¹⁹

A similar pattern distinguishing Th2-high versus Th2-low asthmatic patients was demonstrated in nasal epithelial cell brushings.²⁰ Overall, expression patterns in nasal brushings largely recapitulated changes in the airways (bronchial brushings) with an overlap of more than 90%. Hierarchic clustering analyses of nasal epithelial cells separated the Th2-high versus Th2-low group by global expression changes in 70 genes, including *PERIOSTIN*, *CLCA1*, *SERPINB*, *IL-13*, and *IL-5*. An IL-13–induced/Th2-high signature was also found in the sputum of asthmatic patients, although with a lower magnitude,¹¹ suggesting that Th2 signatures in the sputum and epithelial cells may be driven by different pathways. Identification of patients as Th2-high may predict those who would benefit from treatment specifically targeting type 2 pathways, for example, dupilumab, yet these asthmatic patients were shown to be well controlled with standard ICS treatment.

Studies analyzing paired bronchial biopsies and epithelial brushings from the same patient have shown the relationship between gene expression clusters and unique clinical signatures in patients with moderate to severe asthma (U-BIOPRED).¹⁹ Surprisingly, none of the gene sets was significantly different in epithelial brushings from SA compared with patients with moderate asthma. In contrast, unsupervised and hierarchical clustering of bronchial biopsy transcriptomes identified 2 clusters

(cluster A and cluster non-A). Cluster A was characterized by high submucosal eosinophilia and higher FeNO and oral CS use with 26 out of 42 gene sets differentially expressed. These gene sets were associated with asthma, immune, and/or inflammatory pathways. In addition, an overlapping 9-gene set signature between bronchial biopsies and epithelial brushings distinguished patients exhibiting high Th2 activation (groups 1 and 3) versus a group of non-Th2-mediated asthma subtypes (groups 2 and 4). In this study, one interesting finding was the identification of a subgroup of patients (group 1) with a unique molecular endotype associated with a Th2 signature, increased CD3⁺ and CD8⁺ T cells, and a CS insensitivity, which is in contrast to previous findings.¹⁸ This group of SA who appear refractory (insensitive) to CS are a clinical challenge. One possible mechanism, as suggested here and in the authors' previous work, is through the involvement of increased numbers of CD8⁺ T cells that are insensitive to CS^{21,22} and can undergo transcriptional reprogramming and conversion to IL-13 production (Tc2) in an IL-4-dependent manner through an orchestrated cascade of molecular and cellular events.^{23,24}

Transcriptional changes across multiple airway tissues has been investigated by combining data from epithelial brushings and airway T cells from the sputum and bronchoalveolar lavage (BAL) fluid from the same patient.²⁵ In sorted CD3⁺ T cells, a distinct transcriptomic signature was observed when compared to epithelial brushings, while no differences were seen between T cells isolated from the BAL and sputum. The changes primarily reflected cell type differences rather than disease-related effects. As previously shown for the epithelium,^{17,20,26} IL-13-response genes including *PERIOSTIN*, *CLCA1*, and *SERPINB2* were differentially expressed in patients with mild asthma compared with controls.²⁵ In addition, mast cell mediators (*CPA3*, *TPSAB1*), inducible nitric oxide synthase, and cystatin (*CST1*, *CST2*, *CST4*) genes were upregulated in asthmatic patients. After CS treatment of patients with moderate asthma, mRNA levels were downregulated with the exception of cystatin genes, indicating asthma control in these patients. Interestingly, in BAL T cells, none of the genes were altered in patients with mild asthma versus controls; *SCGB1A1* was the only gene with changes in patients with moderate asthma, suggesting that sputum may be more reflective of ongoing inflammation. The largest number of gene expression changes was observed in the sputum of SA, a predominantly neutrophilic phenotype, compared with controls. As predicted, some gene signatures were associated with IL-17-inducible chemokines (*CXCL1*, *CXCL2*, *CXCL3*, *IL-8*, *CSF3*) and neutrophil chemoattractants (*IL-8*, *CCL3*, *LGALS3*), whereas others were related to T cells and monocytes. The investigators concluded that an *IL-13* signature was detected primarily in patients with mild asthma, whereas patients with more severe asthma were prone to neutrophilic inflammation with steroid-insensitive *IL-17* transcriptomic patterns.

As suggested earlier, a major challenge is comparing molecular patterns across different studies and linking them to disease severity or phenotype. In a study mining public microarray data sets from 8 independent studies of asthmatic bronchial epithelial cells, consistent expression differences involving 159 genes in at least 5 studies that were tightly linked to lung and epithelial biology were shown.²⁶ Subsequent network analyses classified hubs of 12 genes involved in more than one functional pathway supporting biological relevance. In this study, *PERIOSTIN* was among the 12 marker genes. Surprisingly, an underlying suppression of epithelial differentiation, including insulin and Notch signaling pathways (*EFNB2*, *FGFR1*, *FGFR2*, *INSR*, *IRS2*, *NOTCH2*, *TLE1*, and *NTRK2*), was most prominent. Epithelial dedifferentiation programs were shared across the cohorts of mild, moderate, and severe asthmatic patients, which were more pronounced with increasing disease severity. Further

functional validation of these findings was provided after performing RNA-seq on cultured human bronchial epithelial cells. In healthy controls versus asthmatic patients, the number of differentially expressed genes increased from 1912 to 2721 genes comparing cells at baseline in submerged growth conditions to fully differentiated air-liquid interface cells. Of note, insulin deprivation of bronchial epithelial cells from healthy controls resulted in a similar loss of epithelial differentiation gene expression signatures as seen in asthmatic cells, including the down-regulation of *MUC5AC*, *EPCAM*, *FGFR2*, *NOTCH2* and paralleled by upregulation of *PERIOSTIN* and *SERPINB2*. This comprehensive study emphasizes the importance of the biological state of the epithelium in determining the transcriptome, a factor often ignored. On the other hand, despite the caveats, epithelial gene expression signatures (eg, *PERIOSTIN* and *SERPINB2*) across different studies^{17,18,20,25,26} did associate with asthma and could serve as disease biomarkers.

GENOMIC DYSREGULATION IN ASTHMATIC PATIENTS IN RESPONSE TO HUMAN RHINOVIRUS INFECTION

Endotyping and biomarkers identification in non-type 2 asthma has received less attention than type 2-mediated asthma. Based on Th2-high versus Th2-low molecular signatures,^{18,27} the incidence of adult non-type 2 asthma may approximate 30%; but this is deceiving because both types may be involved at different stages of the disease.²⁸ One of the major triggers of severe asthma exacerbations typically inducing a non-type 2 neutrophilic inflammatory response in the upper airways is human rhinovirus (HRV) infection.²⁹ Genome-wide transcriptome analyses in airway epithelial cells from a small cohort of asthmatic patients and controls have begun to uncover endotypes associated with HRV infection (rhinovirus [RV]-A16) contributing to asthma.³⁰ Overall, 10 functional pathways were attributable to HRV infection in vitro independent of disease status, including epithelial cilia movement and morphogenesis, type I and type II interferon responses, and innate immune responses. In addition, an overlap of genes enriched for a previously reported Th2 signature^{17,18} was shown.³⁰ For some genes, differential expression in the vehicle-treated cells from asthmatic patients and controls was already present at baseline, indicating an intrinsic molecular difference between asthmatic and nonasthmatic donors before viral infection. In cells from asthmatic patients, sets of genes associated with viral responses were detected, which were related to inflammatory pathways, epithelial structure and remodeling, and cilia assembly and function (eg, *CCL5*, *CXCL10*, *CX3CL1*, *MUC5AC*, and *CDHR3*). The investigators concluded that airway epithelial cells derived from asthmatic patients have fundamental structural differences at baseline in the absence of infection, which are further amplified in response to HRV infection. However, cytokine data were not consistent.

To more directly assess the effects on global gene expression patterns in responses to HRV in the host, nasal epithelial cells from adult asthmatic patients and healthy controls were obtained before in vivo inoculation with HRV (RV-A16) as well as at different time points after viral inoculation.³¹ Overall changes in the transcriptome were apparent at baseline, during infection, and during the onset of convalescence/repair between both groups. At baseline (7 days before inoculation), 57 genes were differentially expressed between asthmatic patients and controls with lower levels of gene expression of viral replication inhibitors in individuals with asthma together with increased mRNA levels for genes involved in inflammation. In asthmatic patients, a much larger set of genes ($n = 1329$) was significantly altered from baseline to 36 hours after inoculation. These large gene expression changes were not seen in the control

group. An increase in *IL-10* signaling was restricted to healthy individuals without a history of asthma, which may have contributed to a protective effect in these individuals. The investigators suggested that the upregulation of *SPINK5* only in the control group during the convalescence/repair phase identified the potential relevance of this protease inhibitor in the recovery phase.

The effectiveness of whole transcriptome analyses to classify differential responsiveness to clinically relevant stimuli, such as HRV, was used to predict the exacerbation risk in asthmatic children.³² Biological process pathways were built using a training set based on global expression in peripheral blood mononuclear cells (PBMC) from healthy controls subjected to in vitro exposure to HRV. These classifications were then used to predict exacerbations in asthmatic children^{33,34} dependent on gene expression changes in PBMCs before and after virus exposure.³² Although the classifier was based on healthy controls, asthmatic patients were categorized with a 70% accuracy at low (no exacerbation) or high risk (recurrent exacerbation) to experience an exacerbation. It remains to be confirmed if gene expression in PBMCs reflect mechanistic changes in the lung. However, this minimally invasive procedure showed that developing unbiased genomic profiles combined with a clinically relevant environmental risk assessment can provide a tool to predict exacerbations in asthmatic children.

WHOLE TRANSCRIPTOME ANALYSES AS A MEANS TO PREDICT THE RESPONSE TO CORTICOSTEROID IN ASTHMATIC PATIENTS

The ability to analyze expression levels of thousands of genes at the same time has advanced the identification for molecular mechanisms involved in disease pathobiology, enabled the prediction of asthma exacerbation risk, and aided in defining biomarkers for drug responses in asthmatic patients. To delineate key molecules as predictors, the transcriptome of primary airway smooth muscle cells was studied in response to dexamethasone in a controlled in vitro environment.³⁵ Overall, 316 genes were altered after dexamethasone exposure, including well-known (*DUSP1*, *KLF15*, *PER1*, *TSC22D3*) and less obvious glucocorticoid-responsive genes (*C7*, *CCDC69*, *CRISPLD2*). *CRISPLD2* was selected for further functional analyses, as single nucleotide polymorphisms in *CRISPLD2* were nominally associated in a genome-wide study for ICS resistance and bronchodilator response. Its role as a potential modulator in the antiinflammatory response to glucocorticoid was further validated after *CRISPLD2* knock-down, which led to an increase in the production of the proinflammatory cytokines IL-6 and IL-8 after stimulation with IL-1 β .

Analyzing PBMCs in 2 independent cohorts of steroid-sensitive and steroid-resistant asthmatics, 15 genes separated the response to ICS treatment with a suggested 84% accuracy.³⁶ Among the identified candidates predicting the clinical response to ICS were NF- κ B, *STAT4*, and *IL-4R*, genes associated with asthma pathogenesis.^{37–41} A more recent study following whole transcriptome analyses in induced sputum from asthmatic patients distinguished the 4 clinical asthma phenotypes (eosinophilic, non-eosinophilic, pauci-granulocytic or neutrophilic) from healthy controls based on differential expression of 6 key genes (*CLC*, *CPA3*, *DNASE1L3*, *IL-1B*, *ALPL*, and *CXCR2*) with an area under the curve ranging from 85.7 to 92.6.⁴² When changes in expression levels were compared before and after ICS treatment, each of these candidate genes was affected in patients with eosinophilic asthma, strengthening their potential as a useful diagnostic and predictive tool in asthma management.

A similar approach was taken in a small cohort of atopic asthmatic subjects versus atopic and nonatopic controls.^{43,44} Endobronchial biopsies were taken before and after 14 days of oral prednisolone or placebo. Each set of subjects was distinguishable

through specific gene signatures in bronchial biopsies. In the asthmatic subjects, significant changes in global expression levels were found for 15 genes enriched for 3 pathways in contrast to the placebo group which were associated with cellular growth, proliferation, and development. After the 14-day treatment of oral prednisolone, increased *FAM129A* and *SYNPO2* gene expression was observed which correlated with reduced airway hyperresponsiveness.

Such studies lend support to the relevance of unbiased transcriptional approaches to delineate gene expression profiles that are related to treatment responses. As the number of studies and subjects enrolled are relatively small, further work will be necessary to validate these early findings and explore mechanistic differences in larger cohorts.

SUMMARY

This review selectively summarizes some of the latest developments linking transcriptional biomarkers, endotyping, and clinically relevant asthma outcomes. Distinct transcriptomic profiles have defined complex molecular tissue-specific pathways involved in disease pathogenesis, which may have potential as therapeutic targets. To date, expression of *PERIOSTIN* and *SERPINB2* in epithelial cells appears as the most consistent genetic markers identified across multiple studies.^{17,18,20,25,26} Classification of asthmatic patients based on molecular endotypes in addition to traditional clinical parameters will be needed to better assess the heterogeneity of asthma as well as guide personalized interventions to improve outcomes. Although perhaps less vital in the group of patients with steroid-responsive mild-moderate asthma, a molecular approach will be essential in asthmatic patients who fail to respond to CS if we are to intervene in appropriate ways. An important caveat in most of these studies is that they were focused on patients with type 2-high immune responses and were not performed in a longitudinal manner. The best way to study specific interventions with the rapidly increasing number of new biologicals or supplements^{45–48} will require more rigid selection criteria for patient entry, in part driven by the dysregulation of distinct pathophysiologic mechanisms, and analyses at more than a single time point.

To improve the effectiveness and applicability of molecular endotyping and biomarker identification, investigations will need to be developed in well-characterized populations with sufficient sample power. As asthma is not a single disease and pathobiology may vary over time, sampling of different immune/inflammatory-relevant cells and tissues from the same patient at multiple time points to monitor longitudinal contributions to disease pathogenesis is needed to address the multifactorial subtypes of asthma. Adding to the complexity are findings that regulatory regions of genes may not be actively transcribed until an appropriate environmental signal is received. It is essential to couple transcriptomic analyses at a steady state as well as after activation to identify genes that truly control expression and to track their stability and/or plasticity over time. Finally, identification of transcriptomic changes mediated through specific genes and/or pathways will only become relevant when the findings are directly coupled to functional outcomes of asthma. This endeavor can only be accomplished by forming consortia using comparable definitions of asthma and disease control, selected target tissues, and uniform analyses of molecular events across studied populations.

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