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Future clinical implications emerging from recent genome-wide expression studies in asthma

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Host susceptibility to environmental triggers is the most likely explanation for the development of asthma. Quantifying gene expression levels in disease-relevant tissues and cell types using fast evolving genomic technologies have generated new hypotheses about the pathogenesis of asthma and identified new therapeutic targets to treat asthma and asthma-exacerbations. New biomarkers and distinct transcriptomic phenotypes in blood, sputum and other tissues were also identified and proved effective to refine asthma classification and guide targeted therapies. The wealth of information provided by transcriptomic studies in asthma is yet to be fully exploited, but discoveries in this field may soon be implemented in clinical settings to improve diagnosis and treatment of patients afflicted with this common disease.

KEYWORDS: asthma • biomarkers • gene expression profiling • predictive testing • transcriptomics

Asthma is defined as a chronic airway disorder along with recurrent symptoms including cough, shortness of breath, chest tightness and wheezing provoked by airway smooth-muscle constriction and inflammation of the bronchi [1]. According to the Global Initiative for Asthma, the number of patients with asthma worldwide is about 300 million [2]. It affects both children and adults of all ethnicities with variable prevalence by country [3,4]. Asthma is a complex trait influenced by a combination of genetic factors, environmental factors and gene–environment interactions. Substantial efforts were deployed during the last decades to understand the genetic and environmental causes of asthma. So far, hundreds of genes have been related to asthma [5], yet, they explain only a small percentage of its total heritability [6].

Asthma is a heterogeneous disease and its pathogenesis is not fully understood. Recent insights from genetic studies and measurements of new markers are redefining asthma [7]. However, some dominating features are observed in asthma patients. Chronic airway inflammation is present in the majority of asthmatic individuals and causes different functional changes in the airways [8]. The

inflammation of the airways is typically caused by activated T-helper (Th) lymphocytes, eosinophils and mast cells. Th2 cells generate the main cytokines responsible for inflammation, especially in allergic asthma, and the release of cytokines is one of the most investigated mechanisms causing asthma [8,9]. Asthma is also characterized by airway hyperresponsiveness (AHR). AHR occurs in patients following exposure to a specific bronchoconstrictive stimulus that normally has no effect on airways narrowing [10]. Airway remodeling is also an important feature of asthma pathogenesis. Remodeling consists of structural changes in the lung parenchyma and the airways, characterized by increased thickness of the airway walls and reduction of the airway lumen. Remodeling fosters inflammation and accumulation of mucus in airways and further increases airway resistance [11–13].

Airway remodeling, chronic inflammation and AHR are very broad pathobiological processes to describe asthma. A much finer scale understanding is needed to identify disease-causing genes and molecular pathways. Advances in genomic technologies are progressively being used to study asthma and a number of approaches are now commonly used including

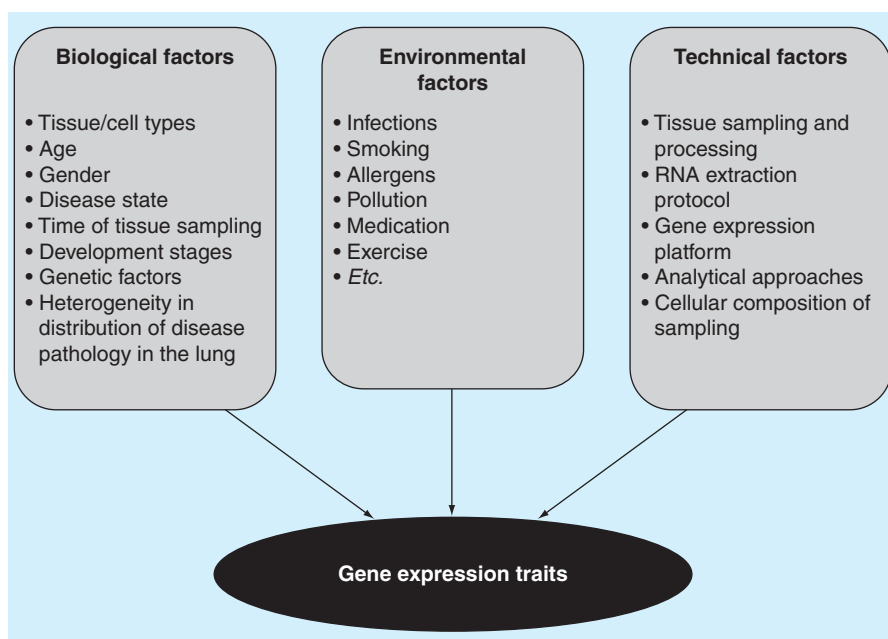


Figure 1. Gene expression traits are sensitive to biological, environmental and technical factors.

genome-wide association study (GWAS) and genome-wide gene expression measurements in relevant tissues and cell types. Many recent reviews describe the results from GWAS [14,15]. In contrast, transcriptomic efforts are accumulating in the field of asthma and reviewing the literature is timely. In this article, we attempt to fill this gap and provide an overview of recent transcriptomic studies in asthma. We start by describing the dynamic nature of gene expression and how it is context-dependent. We address the heterogeneity of methodological approaches used to study gene expression profiles in asthma including the study design, experimental models and source of RNA. Moreover, we highlight the findings from published studies and provide recommendations for future studies in the field. Finally, we outline emerging clinical implications/applications derived from transcriptomic studies in asthma.

Gene expression traits

Different methods are used to measure gene expression and the most common are quantitative real-time PCR (qPCR) [16], microarrays [17] and RNA sequencing (RNA-Seq) [18]. So far, most studies measuring gene expression of thousands of transcripts have used microarrays. Microarray studies have limitations including a lower and upper limit of gene expression quantification. One of the main concerns of microarrays is that it contains only probes that interrogate known transcripts. It does rely on the existing knowledge of the genome sequence and cannot interrogate unknown transcripts or sequence variants. Genetic polymorphisms can also alter the hybridization of RNAs to specific probes [19]. Despite these limitations, microarray in combination with qPCR remains a very useful strategy to explore gene expression patterns of complex diseases at a reasonable cost. Advances in genomic technologies now

allow sequencing of RNA, which is becoming the state-of-the-art method for whole-genome gene expression, or transcriptome, analysis. RNA-Seq provides several advantages over microarrays since all transcripts, including non-coding RNAs, can be sequenced and quantified. RNA-Seq is more sensitive than microarrays and can thus detect expression events below the level of detection of standard microarrays [20,21]. In addition, RNA-Seq has no upper limit of quantification, allowing for a much larger dynamic range of expression levels. RNA-Seq provides single-base resolution of transcription boundaries and detection of sequence variants in the transcribed portion of the genome, providing opportunities to study allele-specific expression. One drawback of RNA-Seq is that it is still relatively expensive compared with microarrays, and sequencing several samples is not affordable for most research teams. So

far, only few gene profiling studies in asthma have used RNA-Seq [22,23]. However, RNA-Seq is likely to become the standard method to perform whole-genome gene expression studies as the cost of sequencing is continuously dropping.

It is important to understand that gene expression traits are highly sensitive to environment cues, technical issues (e.g., gene expression platforms) and are spatiotemporal dependent. A study conducted on blood-cell-derived RNA from healthy volunteers demonstrated that age, gender and time of day at which the sample was taken influence interindividual variations in gene expression [24]. Extra care must thus be taken to collect tissues or cell types in order to minimize technical and interindividual variations in gene expression. In addition, for the same cell type measured with the same platform, interindividual variations differed widely among transcripts [25]. In fact, the transcript levels of some genes are tightly controlled from one individual to the other, while some genes are highly variable. There are also temporal variations of gene expression observed within an individual [26]. It has also been shown that gene expression levels are less tightly controlled and more heterogeneous with age [27]. Time and age of research participants are thus important to consider in longitudinal studies. In addition, gene expression patterns differ by tissues and cell types [28–31]. The source of tissue to extract RNA is thus critical for a given disease. The interindividual response in gene expression to environmental conditions is also known to be under strong genetic influence [25,32]. FIGURE 1 summarizes the factors influencing gene expression traits.

Although gene expression traits are highly sensitive, disease-relevant gene expression signatures can be detected over the background noise. The study mentioned above on blood-cell-derived RNA from healthy volunteers showed that the

alterations in global gene expression observed in the blood of healthy subjects were strikingly smaller than the variations observed in samples from diseased subjects [24]. Modifications of gene expression are thus significantly greater in the diseased state compared with background variation in normal condition. Transcriptomic studies can thus yield novel findings that can lead to a better understanding of disease mechanisms.

Study design & biological material

Starting in late 90s, genome-wide expression studies have been increasingly used to study human biology and understand diseases, and the field of asthma is no exception. Several human tissues and cell types as well as experimental models relevant for asthma were studied in concert with evolving technologies to profile gene expression. As indicated above, the source of RNA deeply influenced the gene expression measurements and ultimately the information that can be derived from the experiment (TABLE 1). Studying the transcriptome of the whole lung is certainly relevant for asthma research, but cellular heterogeneity of the lung is an inherent limitation. Similarly, bronchial biopsies as well as airway and nasal epithelial brushings are relevant to study asthma, but cellular composition of specimens is a concern. In addition, the distribution of disease pathology varies within the same patient, so the location of sampled material is also a concern. Transcriptomic results must thus be interpreted with caution. Gene expression patterns of each tissue reflect their respective role in the pathogenesis of asthma. A typical study design is to detect genes differentially expressed between two conditions, for example, gene differentially expressed in a diseased compared with healthy tissue or changes in gene expression before and after a given treatment. However, the study design can take many forms including time-series analysis and comparisons among many experimental groups. In humans, gene expression studies have been used to observe changes occurring in a given disease. In most cases, genome-wide expression studies have provided only a snapshot of expression levels in a given time point and tissue or cell type. The regulation factors leading to gene expression differences cannot be directly inferred from gene expression studies alone. However, identifying genes differentially expressed in particular disease states is an important step to unveil potential causal genes implicated in the disease pathogenic processes. FIGURE 2 shows the main decisional stages to design a genome-wide gene expression study in asthma.

Asthma transcriptomics

A large number of transcriptomic studies in asthma have been performed [33–37]. We first describe studies in animal models of asthma and then on human cells and tissues. For ease of discussion, the human section is divided into three broad categories based on the main objective of each study: identify genes and/or pathways underlying molecular mechanisms of asthma; understand responses to environmental triggers and asthma therapies; find new biomarkers to improve asthma classification and targeted treatment. We have made an attempt to

comprehensively review the transcriptomic studies published in the field of asthma, but our main goal was to capture the clinical implications of these studies. In addition, more emphasis was directed to studies published during the last 5 years (2009–2013). The main clinical findings and/or implications of each study described below are summarized in TABLES 2–5.

Transcriptomic studies in animal models of asthma

Animal models have been developed to study the pathogenesis of asthma [38–40] and used to identify transcriptomic signatures. Not exhaustively, we describe some of the key findings identified in mice, rats, monkeys and horses published during the last 5 years. Earlier transcriptomic studies in animals were reviewed previously [36]. The main clinical implications are summarized in TABLE 2.

Mouse

The whole lung gene expression response to allergen was evaluated in mouse models. Tolgyesi *et al.* [41] evaluated changes occurring in lung gene expression levels at different time points in the murine ovalbumin asthma model (BALB/c). The lung gene expression pattern at 4 h after the first allergen challenge was characterized by an acute inflammatory response and corresponded to a marked increase in neutrophil cell count observed in bronchoalveolar lavage. Twenty-four hours after the third and last allergen challenge, early neutrophilic infiltration was replaced by Th2-driven eosinophilic inflammation. Compared with controls, 1134 transcripts were differentially expressed at that time with a characteristic signature of Th2 inflammation. Paraoxonase-1 (*Pon1*), an anti-oxidant enzyme, was found among the top downregulated genes. The downregulation of *Pon1* was confirmed at the protein level. The serum PON1 activity was then evaluated in eight patients during asthma exacerbation. Serum PON1 activity was lower during the exacerbation phase and increased with the relief of exacerbation symptoms following treatment with methylprednisolone. Individual changes in serum PON1 activity may thus be used as a biomarker to evaluate the effectiveness of asthma therapy.

Dharajiya *et al.* [42] performed a gene expression profiling study in a second model of allergic asthma in BALB/c mice sensitized and challenged with ragweed. Lung RNA was extracted to compare gene expression in mice challenged with PBS or ragweed. Analyses were performed 4 h post-challenge, where peaked *Th2* gene expression was observed. The ragweed challenge modulated the expression of 352 genes in the lungs. Although a dominant induction of *Th2* genes was observed during the early stages of the ragweed challenge, *Th1* genes were found simultaneously upregulated in the lungs. Studying the kinetics of key genes by qPCR revealed that *Th1* genes remain upregulated longer than *Th2* genes. This study suggests that allergen-induced airway inflammation is concurrently regulated by both *Th1* and *Th2* genes and that persistent upregulation of *Th1* genes counterbalances the effects of Th2 cytokines and eventually resolves allergic inflammation.

Table 1. Human tissue types used for genome-wide expression profiling to study asthma.

Tissue type	Study (year)	Ref.
Blood		
Whole blood	Orsmark-Pietras <i>et al.</i> (2013)	[60]
	Kam <i>et al.</i> (2012)	[64]
	Baines <i>et al.</i> (2011)	[87]
Peripheral blood mononuclear cells	Subrata <i>et al.</i> (2009)	[67]
	Aoki <i>et al.</i> (2009)	[68]
	Bjornsdottir <i>et al.</i> (2011)	[69]
	Shin <i>et al.</i> (2011)	[85]
	Brutsche <i>et al.</i> (2001)	[102]
	Brutsche <i>et al.</i> (2001)	[103]
	Brutsche <i>et al.</i> (2002)	[104]
CD2 ⁺	Hakonarson <i>et al.</i> (2005)	[105]
	Chen <i>et al.</i> (2009)	[75]
CD4 ⁺	Tsitsiou <i>et al.</i> (2012)	[62]
	Hunninghake <i>et al.</i> (2011)	[63]
	Murphy <i>et al.</i> (2010)	[106]
	Wohlfahrt <i>et al.</i> (2003)	[107]
	Syed <i>et al.</i> (1999)	[108]
	Kapitein <i>et al.</i> (2008)	[109]
	Hansel <i>et al.</i> (2005)	[110]
CD8 ⁺	Tsitsiou <i>et al.</i> (2012)	[62]
Neutrophils	Radom-Aizik <i>et al.</i> (2008)	[73]
	Baines <i>et al.</i> (2010)	[88]
Basophils	Youssef <i>et al.</i> (2007)	[111]
Sputum/lavage		
Induced sputum	Esnault <i>et al.</i> (2013)	[66]
	Bosco <i>et al.</i> (2010)	[70]
	Hallstrand <i>et al.</i> (2010)	[74]
	Fu <i>et al.</i> (2013)	[84]
	Baines <i>et al.</i> (2011)	[82]
	Baines <i>et al.</i> (2014)	[83]
Bronchoalveolar lavage cells	Yang <i>et al.</i> (2012)	[65]
	Esnault <i>et al.</i> (2013)	[66]
Nasal lavage	Bosco <i>et al.</i> (2012)	[71]
Alveolar macrophages	Madore <i>et al.</i> (2010)	[58]
Cells		
Primary bronchial epithelial cells	Pennino <i>et al.</i> (2013)	[78]
	Yuyama <i>et al.</i> (2002)	[112]
	Lee <i>et al.</i> (2001)	[113]
Primary airway smooth muscle cells	Hakonarson <i>et al.</i> (2001)	[114]
	Sutcliffe <i>et al.</i> (2012)	[57]
	Masuno <i>et al.</i> (2011)	[77]
	Lee <i>et al.</i> (2001)	[113]
	Jarai <i>et al.</i> (2004)	[115]
	Syed <i>et al.</i> (2005)	[116]
Lung fibroblasts	Lee <i>et al.</i> (2001)	[113]
Macrophages	Yao <i>et al.</i> (2005)	[117]

Table 1. Human tissue types used for genome-wide expression profiling to study asthma (cont.).

Tissue type	Study (year)	Ref.
Cells (cont.)		
Lymphoblastoid cell line	Moffatt <i>et al.</i> (2007)	[118]
	Cusanovich <i>et al.</i> (2012)	[119]
	Liang <i>et al.</i> (2013)	[120]
Lung and airway tissues		
Endobronchial biopsies	Laprise <i>et al.</i> (2004)	[52]
	Chamberland <i>et al.</i> (2009)	[53]
	Yick <i>et al.</i> (2013)	[23]
	Choy <i>et al.</i> (2011)	[80]
Bronchial brushing	Kicic <i>et al.</i> (2010)	[56]
	Dougherty <i>et al.</i> (2010)	[79]
	Woodruff <i>et al.</i> (2009)	[55]
	Woodruff <i>et al.</i> (2007)	[54]
	Yang <i>et al.</i> (2012)	[65]
	Bochkov <i>et al.</i> (2010)	[72]
	Lilly <i>et al.</i> (2005)	[121]
Nasal brushing	Giovannini-Chami <i>et al.</i> (2012)	[59]
	Guajardo <i>et al.</i> (2005)	[122]
Whole lung from surgical resection	Hao <i>et al.</i> (2012)	[93]
Fetal lung tissue	Sharma <i>et al.</i> (2010)	[90]
	Melen <i>et al.</i> (2011)	[91]
Airway smooth muscles	Yick <i>et al.</i> (2013)	[22]

The whole lung gene expression response to allergen was also evaluated in different strains of mouse. Tumes *et al.* [43] performed a microarray experiment in asthma-susceptible (BALB/c and C57BL/6) and asthma-resistant strains (CBA/Ca). Immunized and ovalbumin challenged mice compared with unchallenged mice revealed a unique signature of 64 differentially expressed genes in the asthma-susceptible strains including many enzymes involved in inflammation. Survivin, an anti-apoptotic protein, was found biologically connected to many of these differentially expressed genes using gene network analysis and upregulated in asthma-susceptible strains after allergen exposure. Survivin was thus suspected as a candidate to explain the high levels of eosinophils observed in asthma-susceptible strains. mRNA and protein levels of survivin was then explored in the lung eosinophils obtained from bronchoalveolar lavage and found more expressed in asthma-susceptible strains compared with the asthma-resistant strains, suggesting that low expression of survivin favors apoptosis in these cells. This study suggested that reduction of survivin in eosinophils is a new therapeutic target to resolve airway inflammation in asthma, most particularly allergic and eosinophilic asthma.

The whole lung is a relevant tissue to study asthma. However, cellular heterogeneity of the lung may impair the interpretation of results and justify the need to conduct transcriptomic studies in specific cell types. Siddiqui *et al.* [44] used the murine ovalbumin asthma model (BALB/c) to study the transcriptome of macrophages purified from bronchoalveolar lavages.

Bronchoalveolar macrophages, most particularly the subpopulation M2, are known to secrete chemokines that play a role in eosinophil migration into the lung. The transcriptome of macrophages from mice sensitized and challenged with ovalbumin compared with naïve mice revealed a distinct chemokine expression pattern. A total of 15 chemokine/chemokine receptor genes were highly upregulated and three were downregulated. Notably, *Ccl-8*, *Ccl-17* and *Ccl-24* (eotaxin-2) were highly upregulated. Genes associated with alternatively activated M2 macrophages were also upregulated. This study supports the role of M2 bronchoalveolar macrophages in leukocyte migration in asthma and reveals new chemokines involved in this process. More cell-specific transcriptomic studies will be needed in mouse models.

Two transcriptomic studies in mouse models have investigated the molecular actions of drugs. First, Camateros *et al.* [45] conducted a microarray experiment in mice to clarify the molecular mechanisms by which resiquimod treatment prevents the development of allergen-induced experimental allergic asthma. Treatment with resiquimod 1 day prior to each of the three ovalbumin challenges was showed to modulate the expression levels of 565 and 697 genes in the lung of A/J and C57BL/6 mice collected 3 h after the last challenge. Genes involved in airway remodeling and chemokine signaling were normalized by the treatment. Genes involved in NK cell-mediated cytotoxicity were also altered in treated mice. This study also demonstrated that NK cells were recruited in the

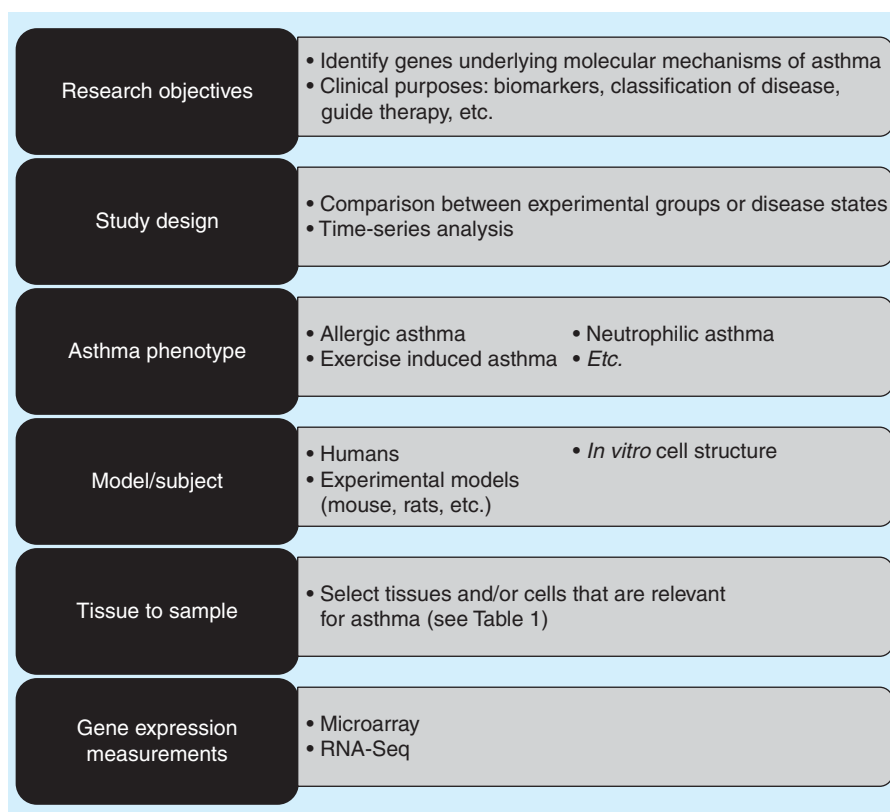


Figure 2. Decisional stages to perform a genome-wide expression study in asthma.

lung of mice treated with resiquimod, suggesting that it may be a promising approach for the treatment of infection-induced asthma exacerbations. Second, Lopez *et al.* [46] demonstrated that galectin-3 delivered by gene therapy was a potential therapeutic approach to improve Th2 inflammation in a chronic asthma mouse model (inhalation of ovalbumin 3 days per week for 12 weeks). The transcriptomic effects of galectin-3 gene treatment were evaluated in lungs of A/J mice collected 24 h after the last antigen administration. A ‘Gal-3 treated signature’ was identified and characterized by reduced levels of inflammatory and regulatory genes. Processes implicated in negative regulation of inflammation were particularly enriched, notably by *Socs* genes that are known to play a role in Th1/Th2 balance. Lower *Socs1* and *Socs3* at the mRNA and protein levels were confirmed in treated mice. The downregulation of *Socs1* and *3* in lungs of mice treated with Gal-3 gene therapy is thus a promising therapeutic approach in asthma.

Rat

The transcriptome of the developing lung has also been investigated in asthma-susceptible rat models. Carpe *et al.* [47] studied the transcriptomic signatures in the lung of Lewis (normoresponsive), Fisher (airway hyperresponsive) and Brown Norway (atopic) rat pups. Whole-genome gene expression was evaluated at postnatal days 1, 7 and 14 in the three rat models. Overall, 1376 genes were found variably expressed during that period.

The very diverse transcriptomic signatures observed among strains correlated with their respective and distinct respiratory phenotypes. Genes temporally expressed during postnatal life in asthma rat models revealed early molecular factors contributing to asthma pathogenesis and provided solid experiment data to investigate how the environmental triggers early in life can influence these molecular factors, and subsequently asthma susceptibility.

Carpe *et al.* [48] also investigated the transcriptomic differences observed in rat pups exposed to allergen *in utero*. Again, the lung of Lewis and Brown Norway pups were interrogated by microarrays at postnatal days 1, 7 and 14. In this study, pups were from dams exposed or not to ovalbumin in order to evaluate the transcriptome response to maternal allergen exposure, but also to compare this response between strains. The results revealed important strain-specific differences in gene expression response to maternal allergen exposure. In fact, only 14% of the top 799 allergen-induced probes were common in both strains. This suggests that gene expression patterns in response to maternal allergen exposure

are genetically encoded and, transposed to human development, may explain part of the interindividual susceptibility to asthma.

Monkey

Abbas *et al.* [49] studied the gene expression in lung airways of a rhesus house dust mite (HDM) allergic asthma model. Lung airway biopsies of 12 sensitized monkeys and 10 controls were obtained. The transcriptome of monkeys was compared with the transcriptome of humans with and without asthma obtained from bronchial epithelial brushings and the lung transcriptome of BALB/c mice exposed to ovalbumin-induced asthma. A 35-gene signature was shared across the three species characterized by Th2 inflammation. However, more globally, most genes differentially expressed in asthma were species-specific. Only 11 genes were commonly upregulated in asthma in the three species. Greater concordance in genes and pathways was observed between humans and monkeys compared with humans and mice, suggesting that the HDM-induced asthma model in rhesus monkeys better mirrored gene expression profile observed in human asthma.

Cheng *et al.* [50] used a second model of monkey, namely the cynomolgus monkey model of asthma, to profile gene expression in bronchoalveolar lavage cells before and after allergen challenge (*Ascaris suum*) to evaluate the role of thymic stromal lymphopoietin (*TSLP*) in allergic inflammation. Studying eight monkeys, hundreds of genes were upregulated

Table 2. Clinical implications of transcriptomic studies in animal models of asthma.

Study (year)	PubMed ID	Data access (GEO or EBI)	Main clinical implication for asthma	Ref.
Mouse				
Tolgyesi <i>et al.</i> (2009)	19556304	GSE11911	Serum paraxonase-1 (PON1) activity is a biomarker to evaluate the effectiveness of asthma therapy	[41]
Dharajiya <i>et al.</i> (2009)	20027288	GSE18083	Persistent expression of Th1-associated genes in the lung resolves allergic airway inflammation	[42]
Tumes <i>et al.</i> (2009)	19395375	GSE15429	Decreasing survivin level in eosinophils is a potential target to develop anti-inflammatory drugs	[43]
Siddiqui <i>et al.</i> (2013)	23685352	NA	Chemokines secreted by bronchoalveolar macrophages are new potential targets to prevent eosinophilic infiltration in the lung	[44]
Camateros <i>et al.</i> (2009)	19491150	GSE13032	Resiquimod treatment represents a promising drug to prevent and treat virus-induced asthma exacerbations	[45]
Lopez <i>et al.</i> (2011)	21547260	NA	Galectin-3 gene therapy is a negative regulator of inflammation and a therapeutic approach to treat asthma	[46]
Rat				
Carpe <i>et al.</i> (2010)	20118217	GSE19666	Identification of early postnatal molecular factors contributing to asthma pathogenesis	[47]
Carpe <i>et al.</i> (2012)	22983352	GSE32622	Maternal allergen exposure during pregnancy contributes to asthma susceptibility later in life	[48]
Monkey				
Abbas <i>et al.</i> (2011)	21819959	GSE23327 GSE23611	House dust mite antigen-induced asthma in rhesus monkeys is a valuable preclinical asthma model	[49]
Cheng <i>et al.</i> (2013)	23810153	GSE41329	TSLPR blockade represents a promising approach to reduce allergic lung inflammation and improve lung function	[50]
Horse				
Lavoie <i>et al.</i> (2012)	22235296	NA	RhoA, PPP3CB/NFAT and LTB4/GPR44 are therapeutic targets to develop anti-inflammatory and anti-remodeling agents	[51]

EBI: European Bioinformatics Institute; GEO: Gene expression omnibus; NA: Not available.

by the allergen challenge from which 11 overlapped with a *TSLP* gene expression signature observed in dendritic cells isolated from human blood. Seven monkeys went through the same protocol, but were treated with a monoclonal antibody against the TSLP receptor. After 6 weeks, monkeys treated with the anti-TSLPR had fewer genes differentially expressed in response to the allergen challenge and the *TSLP* gene signature was also significantly reduced, leading to an improvement of lung function. This experiment further demonstrated the role of *TSLP* in allergic inflammation.

Horse

One study by Lavoie *et al.* [51] identified genes differentially expressed in an equine chronic asthma model using the suppression subtractive hybridization technique. Peripheral lung biopsies of six heaves-affected horses and five controls were studied before and after antigen challenge (hay and barn dust). Differentially expressed genes confirmed by qPCR were associated with asthmatic airway inflammation and remodeling.

Differentially expressed genes are related to signaling pathways such as *PPP3CB/NFAT*, *RHOA* and *LTB4/GPR44*. These pathways are potential targets for future therapies.

Transcriptomic studies in human asthma Identify genes and/or pathways underlying molecular mechanisms of asthma

The main clinical implications of this section are in TABLE 3 for studies published between 2009 and 2013. In human subjects, the classical case-control design has been applied repeatedly to detect genes differentially expressed in asthma. These studies are important to highlight the transcriptomic signature of asthma and subsequently develop compounds or other treatment strategies that reverse the disease signature. The first study to do so was performed by Laprise *et al.* [52] in human bronchial biopsies from four controls and four patients with asthma. A total of 79 genes were found to be differently expressed between subjects with and without asthma. The same team did a similar experiment using a second set of bronchial biopsies

Table 3. Clinical implications of transcriptomic studies identifying genes and/or pathways underlying molecular mechanisms of asthma.

Study (year)	PubMed ID	Data access (GEO or EBI)	Main clinical implication for asthma	Ref.
Chamberland <i>et al.</i> (2009)	19842841	GSE41649	Microarray results in human bronchial biopsies are partly reproducible	[53]
Yick <i>et al.</i> (2013)	23314903	GSE38994	RNA-Seq in human bronchial biopsies identified transcriptional profiles that differ between asthma and controls	[23]
Kicic <i>et al.</i> (2010)	20110557	GSE18965	Fibronectin can restore the dysregulated epithelial response to injury observed in asthmatic patients	[56]
Sutcliffe <i>et al.</i> (2012)	22108207	NA	Knockdown of <i>NOX4</i> inhibits the hypercontractility in smooth muscle cells observed in asthma patients and is a new therapeutic target	[57]
Madore <i>et al.</i> (2010)	19913588	GSE22528	HSP60 protein level is a possible new biomarker for alveolar macrophages activities in the development of allergic asthma	[58]
Giovannini-Chami <i>et al.</i> (2012)	22005912	GSE19190 GSE22147	Restoring proper IFN response in epithelial cells is a therapeutic avenue for patients with uncontrolled asthma	[59]
Orsmark-Pietras <i>et al.</i> (2013)	23222870	GSE27011	Bitter taste receptors have anti-inflammatory and bronchodilator actions and represent new therapeutic targets for asthma	[60]
Tsitsiou <i>et al.</i> (2012)	21917308	GSE31773	Severe asthma is associated with a large transcriptional activation of circulating CD8 ⁺ T cells, but little changes in CD4 ⁺ T cells	[62]
Hunninghake <i>et al.</i> (2011)	21473777	GSE22324	Expression of IL17RB in peripheral blood CD4 ⁺ T cells correlated with serum IgE level in asthmatic men	[63]

EBI: European Bioinformatics Institute; GEO: Gene expression omnibus; HSP60: Heat shock protein 60; NA: Not available; RNA-Seq: RNA sequencing.

tested on a new version of the Affymetrix microarray chip (Affymetrix U133A vs U95Av2) and found 40 genes differentially expressed [53]. Fifty-five percent of them were found in the first set suggesting that microarray results are at least partly consistent. These early microarray studies in human bronchial biopsies demonstrated the feasibility of this approach in order to identify genes and molecular pathways deregulated in diseased airways.

Feasibility was also recently demonstrated to perform asthma transcriptomic studies by RNA-Seq using human endobronchial biopsies [23]. The transcriptome of four atopic asthmatic patients was compared with five healthy non-atopic controls. A total of 46 genes were identified as differentially expressed. Interestingly, two genes differentially expressed (*SLC26A4* and *POSTN*) had already been identified by previous transcriptomic studies [54,55]. More RNA-Seq studies will be needed in asthma.

A number of transcriptomic studies have elucidated new molecular mechanisms of asthma and thus novel therapeutic targets. Kicic *et al.* [56] investigated the transcriptome of airway epithelium specimens obtained by bronchial brushing in children. Gene expression profiling was performed in seven healthy non-atopic and nine atopic asthmatic subjects with a particular attention on genes implicated in epithelial repair. A total of 1612 genes were found differentially expressed. A gene set involved in response to wounding was identified as

downregulated in atopic asthmatic subjects. Fibronectin was the only gene encoding an extracellular matrix protein that was downregulated in this gene set. Knockdown and overexpression of fibronectin in cultured epithelial cells were shown to impair and restore wound healing, respectively, suggesting that reduced fibronectin levels explains the abnormal epithelial repair observed in asthmatic airways.

Sutcliffe *et al.* [57] performed gene expression profiling to investigate the greater oxidative stress burden observed in airway smooth muscle of patients with asthma compared with controls. Airway smooth muscle cells were isolated from bronchial biopsies taken from six control subjects and six individuals with asthma. Cells were cultured and evaluated by microarrays. A total of 37 genes were found differentially expressed between cases and controls. Focusing on genes involved in oxidative stress, higher expression of *NOX4* in asthma was confirmed by qPCR. Silencing of this gene by siRNA eliminated the hypercontractility induced by spasmogens in airway smooth muscle cells from individuals with asthma. Inhibitors of *NOX4* are thus potential treatment for asthma.

Madore *et al.* [58] aimed to detect genes differentially expressed in alveolar macrophages isolated from bronchoalveolar lavage. A total of five allergic asthmatics and five controls were compared and 50 genes were found differentially expressed. Nine of these genes underexpressed in asthma were

Table 4. Clinical implications of transcriptomic studies studying responses to environmental triggers and asthma therapy.

Study (year)	PubMed ID	Data access (GEO or EBI)	Main clinical implication for asthma	Ref.
Antigen				
Kam <i>et al.</i> (2012)	22316092	GSE34172	Allergen inhalation challenge induces changes in the blood transcriptome of asthma patients. Technical considerations to perform transcriptomic study in blood were addressed	[64]
Yang <i>et al.</i> (2012)	22246175	GSE8190 GSE5272	Host transcriptomic response to specific asthma environmental exposures can be used to develop targeted therapies	[65]
Esnault <i>et al.</i> (2013)	23844029	GSE46159 GSE46238	Specific eosinophil genes induced by allergen provide new candidates to treat eosinophilic asthma	[66]
Infections and exacerbations				
Subrata <i>et al.</i> (2009)	19620293	GSE16032	Most children hospitalized for asthma exacerbations are virally infected owing to interactions between innate antiviral and atopic immunoinflammatory pathways in susceptible individuals	[67]
Aoki <i>et al.</i> (2009)	19187333	NA	Genes induced by asthma exacerbation in PBMC can be used as biomarkers to monitor disease state	[68]
Bjornsdottir <i>et al.</i> (2011)	21779351	GSE19301	Identify gene expression signatures in blood that delineate three distinct subgroups of patients with asthma exacerbation	[69]
Bosco <i>et al.</i> (2010)	20336062	GSE19903	Identification of new candidate genes to prevent the development of chronic airflow obstruction in children with recurrent asthma exacerbations	[70]
Bosco <i>et al.</i> (2012)	22112518	GSE30326	<i>IRF7</i> represents a novel therapeutic target for asthma exacerbations	[71]
Bochkov <i>et al.</i> (2010)	19710636	GSE13396	The transcriptomic response of epithelial cells to infection differs in asthma patients mostly because of existing differences at baseline	[72]
Exercise				
Hallstrand <i>et al.</i> (2010)	20052409	GSE13785	<i>TGM2</i> is a new target to treat exercise-induced bronchoconstriction	[74]
Socioeconomic status				
Chen <i>et al.</i> (2009)	19001005	NA	Socioeconomic status influences the transcriptome of circulating lymphocytes in children with asthma and may explain part of the variability in treatment response to asthma medications	[75]
Corticosteroids				
Masuno <i>et al.</i> (2011)	21257922	GSE34313	<i>Klf15</i> expressed in airway smooth muscle cells is a new therapeutic target to alleviate airway hyperreactivity in asthma	[77]
Yick <i>et al.</i> (2013)	23491407	GSE40996	Oral glucocorticoids modulate gene expression in airway smooth muscle and this knowledge may help to develop new asthma therapy	[22]
IL-22 and IFN-γ				
Pennino <i>et al.</i> (2013)	23174657	NA	IL-22 inhibits the proinflammatory effects of IFN- γ in bronchial epithelial cells	[78]

EBI: European Bioinformatics Institute; GEO: Gene expression omnibus; NA: Not available.

part of the heat shock protein (*HSP*) family. One member of this family, *HSP60* was found upregulated at the protein level (2.28-fold) in alveolar macrophages from allergic asthmatics. The exact role of *HSP60* and other *HSP* members in allergic asthma is still unclear from this study, but the results suggested an implication in alveolar macrophage functions.

Giovannini-Chami *et al.* [59] performed a genome-wide expression study to determine the transcriptional signatures in epithelial cells sampled from upper airways (nasal brushings). Four groups of children were investigated: allergic rhinitis with controlled asthma ($n = 7$), allergic rhinitis with uncontrolled asthma ($n = 6$), allergic rhinitis without asthma ($n = 14$) and

Table 5. Clinical implications of transcriptomic studies to find new biomarkers to improve asthma classification and targeted treatment.

Study (year)	PubMed ID	Data access (GEO or EBI)	Main clinical implication for asthma	Ref.
Woodruff <i>et al.</i> (2009)	19483109	GSE4302	Asthma can be divided into distinct molecular phenotypes, Th2 high and Th2 low, based on airway epithelial cell gene signatures	[55]
Dougherty <i>et al.</i> (2010)	20451039	GSE4302	Expression levels of mast cell genes in airway epithelial brushings are biomarkers for inhaled corticosteroid responsiveness	[79]
Choy <i>et al.</i> (2011)	21187436	GSE23611	A Th2 gene expression signature in bronchial biopsy is used to classify asthma patients in two groups that are likely to respond differently to new asthma therapies targeting Th2 inflammation	[80]
Baines <i>et al.</i> (2011)	21211650	NA	Transcriptional phenotypes in sputum delineate three distinct groups of asthma patients that can be used to guide treatment strategies	[82]
Baines <i>et al.</i> (2014)	24582314	GSE45111	Gene expression of 6 biomarkers in sputum can delineate inflammatory phenotypes of asthma and predict response to inhaled corticosteroids	[83]
Fu <i>et al.</i> (2013)	23438328	GSE43397	Targeting systemic inflammation is a possible strategy to treat neutrophilic asthma	[84]
Shin <i>et al.</i> (2011)	21966607	NA	The mRNA expression levels of three genes (<i>MEPE</i> , <i>MLSTD1</i> , <i>TRIM37</i>) in PBMC can be used to predict subjects with and without asthma	[85]
Baines <i>et al.</i> (2011)	21785157	NA	α -defensins and neutrophil proteases in whole blood can be used as biomarkers to identify neutrophilic asthma	[87]
Baines <i>et al.</i> (2010)	19797135	NA	Identification of molecular targets that distinguish non-eosinophilic and eosinophilic asthma from circulating neutrophils	[88]

EBI: European Bioinformatics Institute; GEO: Gene expression omnibus; NA: Not available.

healthy controls (n = 11). No gene was found differentially expressed between allergic rhinitis patients with (n = 13) and without (n = 14) asthma. However, the expression levels of 160 genes were different in subjects with uncontrolled compared with controlled asthma. A subset of 41 of these genes was found to discriminate children with controlled and uncontrolled asthma with good prediction accuracy. Nine out of 41 were downregulated in uncontrolled asthma and linked to the interferon response. Altered interferon production in epithelial cells may thus be targeted to develop new therapies in children with difficult-to-treat asthma.

Recently, Orsmark-Pietras *et al.* [60] conducted a microarray profiling study in white blood cells of three groups of children including 17 patients with severe therapy-resistant asthma, 19 with medically controlled asthma and 18 healthy controls. One thousand three hundred and seventy-eight genes were found differentially expressed in the three pairwise comparisons. Pathway analyses identified bitter taste transduction receptors (*TAS2Rs*) upregulated in severe asthma. Further experiments demonstrated that *TAS2R* agonists inhibit lipopolysaccharide (LPS)-induced cytokine release in a second set of blood leukocyte obtained from asthmatic adults, which suggests that *TAS2Rs* have anti-inflammatory actions. These results, coupled with the known bronchodilator property of *TAS2Rs* in the lung [61], suggested a protective role of these receptors in asthma and represent new therapeutic

targets that are urgently needed for patients with severe therapy-resistant asthma.

Tsitsiou *et al.* [62] also investigated the transcriptomic differences in inflammatory cells between cases and controls. Gene expression levels were examined in circulating CD4⁺ and CD8⁺ T cells isolated from healthy controls (n = 8) and patients with non-severe (n = 4) and severe asthma (n = 12). No change in gene expression was observed between healthy controls and non-severe asthma. In contrast, significant differences were observed between healthy controls and severe asthma including a relatively small number of transcripts (n = 40) in CD4⁺ T cells and a large number of transcripts (n = 1566) in CD8⁺ T cells. Changes were also observed in the expression profile of miRNA and long non-coding RNAs in CD8⁺ T cells from severe asthma. Although the impact on cell functions is still unclear, results suggested major changes in circulating CD8⁺ but not CD4⁺ T cells in severe asthma.

Also in circulating inflammatory cells, Hunninghake *et al.* [63] had for objective to evaluate the correlation between gene expression in peripheral blood CD4⁺ T cells and serum IgE levels. Blood samples were obtained from 223 young adults with asthma. Only the mRNA expression levels of *IL17RB* was significantly associated with IgE levels. Sex-specific analyses indicated that this relationship was restricted to men, supporting additional evidences that the genetic determinants of serum IgE levels are different between men and women.

Understand responses to environmental triggers & asthma therapy

The main clinical implications of this section are described in TABLE 4.

Antigen

Kam *et al.* [64] performed a transcriptomic study in patients with asthma to understand response to an allergen inhalation challenge in peripheral blood. Nine subjects with mild atopic asthma were recruited and blood samples were collected before and 2 h after allergen inhalation. A total of 1126 up- and 469 downregulated genes were detected after the allergen challenge. Transcriptomic changes observed in blood may help us to elucidate the molecular mechanisms causing forced expiratory volume in 1 s to drop by more than 20% following the allergen inhalation challenge. More sampling at different time points will be required to study temporal change in blood gene expression. Of note, critical technical considerations were addressed in this article and should be reviewed before planning a transcriptomic study in blood.

Yang *et al.* [65] analyzed the transcriptional response to antigens in airway epithelial cells and inflammatory cells obtained by bronchial brushing and bronchoalveolar lavage, respectively. 12 atopic subjects, 14 atopic asthmatics and 13 non-atopic controls had three subsegmental airways instilled with saline, LPS and HDM antigen. Brushing of each site and bronchoalveolar lavage was obtained 4 h after the challenges. This design is particularly suited to reveal responsive genes and molecular pathways to environmental triggers that may differ between cases and controls. The main results demonstrated a pronounced transcriptional response to LPS in both epithelial and inflammatory cells that was largely independent of the asthma and atopic status. In contrast, HDM stimulation resulted in more subtle changes in gene expression of both cell types, but some changes in inflammatory cells were different between patients with or without asthma. Individual response to environmental triggers in immune or pulmonary cells may eventually help us to personalize treatment.

Esnault *et al.* [66] performed another study using an *in vivo* allergen challenge to identify transcriptional response specific to eosinophils. Three eosinophilic airway models were compared in cells derived from BAL and sputum. First, 299 transcripts were found upregulated in total BAL cells collected before and 48 h after segmental bronchoprovocation with an allergen (SBP-Ag). Second, the same challenge was performed but 1 month after a single dose of mepolizumab (anti-IL-5 monoclonal antibody). This treatment reduced eosinophils counts by 54.5% and suppressed 99 of the 299 transcripts induced by the allergen challenge. Third, 365 genes were induced in sputum collected before and after 48 h of whole lung allergen challenge, which included 168 genes that overlapped with the 299 responsive genes observed in BAL cells. Intersection of these 168 genes with the ones suppressed by mepolizumab revealed 57 genes specifically expressed by airway eosinophils. The majority of these 57 genes have never been

studied in eosinophils and represent new therapeutic targets for eosinophilic asthma.

Infections & exacerbations

Infection is the most common exacerbating factor in asthma. A number of studies have been conducted to evaluate transcriptomic alterations associated with infections. Tissue sampling is a concern for asthma patients with exacerbation. Accordingly, most of these studies were conducted in cell/tissue readily available. Subrata *et al.* [67] investigated the transcriptional profile of circulating cells in children hospitalized for acute asthma. Peripheral blood mononuclear cells (PBMC) were collected during exacerbation and at convalescence. Five RNA pools at both stages were obtained from 25 children. Pathway analyses were conducted on the list of genes differentially expressed during exacerbation and pathways related to inflammation and immunity were prioritized. Fifty-two genes were found upregulated during acute exacerbation. These genes were then followed-up by qPCR in subpopulation of PBMC sorted by flow cytometry in an independent set of 10 subjects. Monocytes and dendritic cells were largely responsible for the upregulation of these genes. The expansion of monocytes during exacerbation was associated with higher expression of the high-affinity IgE receptor. The mRNA expression of *CCR2* was particularly elevated in purified CD14⁺ monocytes during exacerbation, which was confirmed in cultured monocytes exposed to Th2 cytokines. More globally, the exacerbation signature was characterized by type 1 *IFN*-sensitive and Th2 cytokine-sensitive genes. This work suggested that viral infections in susceptible atopic children activate atopic-associated pathways that promote and maintain airway inflammation.

Aoki *et al.* [68] also investigated the transcriptional response to PBMC during asthma exacerbation in children. Gene expression was compared between 12 asthma patients with exacerbation and 11 stable asthma patients. The experiment revealed upregulation and downregulation of 137 and 16 genes, respectively. 62 genes were found non-specific to asthma exacerbation as they were differentially expressed in 6 infected controls compared with five non-infected controls. These results suggested that the transcriptional response of PBMC substantially overlap between asthma exacerbation and respiratory infections. Asthma-exacerbation related genes can serve as new biomarkers to monitor acute and convalescence phases of exacerbations.

Bjornsdottir *et al.* [69] identified gene expression signatures of asthma exacerbation in PBMC. Cells were obtained during stable asthma, exacerbation and 2 weeks after exacerbation in 118 patients with a range of asthma severity that experienced at least one exacerbation. Gene expression in exacerbation samples was compared with the average of their corresponding samples obtained during quiescent asthma. This analysis identified 1079 probe sets nominally associated with exacerbation. More revealing in this study is the heterogeneity of the gene expression response among the samples. Samples were then partitioned into three subgroups based on the expression of

exacerbation-induced genes. The first subgroup included 18% of samples and was characterized by 1081 exacerbation-induced genes, many of which were involved in innate immunity pathways. The second subgroup consisted of 38% of samples with 574 genes altered by exacerbation that implies the activation of lymphocytes typical of adaptive immunity. Finally, the third subgroup included 43% of samples with only 286 genes induced by exacerbation. The majority of the latter overlapped with the other two subgroups and was less statistically significant. This study highlighted host differences in transcriptional response to exacerbation and provided biomarkers to personalize the management of asthma patients experiencing exacerbation attacks.

Bosco *et al.* [70] investigated the transcriptional response to exacerbations in induced sputum cells collected from two groups of 10 asthmatic children at the extreme tails of the forced expiratory volume in 1 s/forced vital capacity distribution. Sputum was collected during and after acute exacerbation. The aim of the study was to identify gene expression patterns during acute asthma exacerbations that differed between children with or without chronic airflow obstruction. During exacerbations, children with airflow obstruction at baseline demonstrated a decrease in the activation of Th1-like/cytotoxic and interferon signaling pathways, which was independent of the presence of atopy, picornaviruses infections and medications used during exacerbations. Impairment of these pathways in response to viruses or other environmental insults may lead to chronic deficits in lung function. Targeting genes in these pathways may provide new solutions to prevent the development of airway obstruction in children with recurrent asthma attacks.

Bosco *et al.* [71] also studied the transcriptional response of virus-induced exacerbation in nasal lavages of 16 children obtained during and after moderate exacerbations. A total of 1121 genes were found upregulated and 77 genes downregulated during the acute illness compared with 7 and 14 days later. Coexpression network analysis was performed and eight modules (i.e., gene sets) were identified. Module 2 contained 197 genes and 45 of them interact with the interferon regulatory factor 7. Interferon regulatory factor 7 is thus a major hub in virus-induced asthma exacerbations and represents a novel treatment target for asthma attacks.

As indicated above, experiments focusing on asthma exacerbations are limited by tissue sampling. To cope with this limitation, Bochkov *et al.* [72] developed an *in vitro* model to evaluate the transcriptional response of primary bronchial epithelial cells to rhinovirus infection. Cells were obtained from six mild asthmatic subjects and six controls. The goal of this study was to determine whether the epithelial cell response to infection differs in asthma. The infection modulated the expression of 1044 genes in both groups combined for which many overlapped ($n = 423$). Interestingly, the majority of genes induced by infection that were specific to cells from asthma patients were differentially expressed in this group prior to infection. This study suggested that asthmatic epithelial cells

respond differently to rhinovirus infections mostly because of gene expression pattern that is different at baseline.

Exercise

Exercise is a well-described asthma trigger for a subset of patients. The mechanisms that trigger exercise-induced asthma in certain individuals are still not apparent. A single bout of heavy aerobic exercise (i.e., 30 min at 80% peak O_2 uptake) in healthy individuals is known to affect gene expression in PBMCs and isolated neutrophils [73], including genes known to be involved in asthma. To identify molecular pathways underlying exercise-induced bronchoconstriction, Hallstrand *et al.* [74] investigated the transcriptional response of airway cells obtained from induced sputum in 12 asthma patients with or without exercise-induced bronchoconstriction. Gene expression was compared before and after exercise challenge within and between the two groups. The exercise challenge was shown to induce the expression of nine genes in patients with exercise-induced bronchoconstriction while no change was observed in patients without exercise-induced bronchoconstriction. At baseline only one gene, transglutaminase 2 (*TGM2*), was found differentially expressed between asthma patients with or without exercise-induced bronchoconstriction. At post-exercise, 19 genes were differentially expressed between the two groups. Further functional *in vitro* analysis revealed that *TGM2* increased the enzyme activity of sPLA₂-X, which is known to catalyze the first limiting step of eicosanoid production. Thus, higher expression of *TGM2* in airway cells may be responsible for increased concentration of inflammatory eicosanoids observed in asthma patients with exercise-induced bronchoconstriction.

Socioeconomic status

Chen *et al.* [75] linked gene expression patterns in CD2⁺ T cells to social class in asthmatic children. Fifty-six genes were found differentially expressed comparing gene expression of children from low ($n = 16$) and high ($n = 15$) socioeconomic status. Genes upregulated in patients with low socioeconomic status environment are known for their roles in inflammation and chemokine activity as well as stress and wound responses. These results provide empirical data that social environment modulated gene expression levels in circulating T lymphocytes.

Corticosteroids

Gene expression changes due to asthma medications were also investigated [76]. In two studies, the transcriptional response of asthmatics to corticosteroids treatment has been evaluated in bronchial epithelium [52,54]. Several genes were identified as modulated by inhaled fluticasone and one particular gene, *SERPINE2*, was revealed as highly modulated in both studies. A third study conducted by Masuno *et al.* [77] evaluated the transcriptomic effect of dexamethasone in airway smooth muscle cell lines. Overall, 1594 and 7547 transcripts were found differentially expressed in cells treated with dexamethasone for 4 and 24 h, respectively. With only two time points (4 and 24 h), this study highlights substantial temporal changes in

gene expression of smooth muscle cells following treatment with a single dose of dexamethasone. One strong candidate gene potentially involved in airway smooth muscle function was followed-up in a mouse KO model sensitized and challenged with ovalbumin. KO mice for the *Klf15* gene were characterized by typical airway inflammation observed in the murine asthma model, but also by reduced acetylcholine-induced airway contraction, suggesting a role of *Klf15* in airway contractility.

Recently, Yick *et al.* [22] evaluated gene expression changes induced by glucocorticoids in airway smooth muscle obtained by laser-dissected endobronchial biopsies. Global gene expression of airway smooth muscle from patients with asthma receiving either oral prednisolone ($n = 6$) or placebo ($n = 6$) was measured by RNA-Seq. Biopsies were obtained before and after 14 days of treatment. Fifteen genes were found differentially expressed in subjects treated with prednisolone including two genes that were associated with airway hyperresponsiveness: *FAM129A* and *SYNPO2*. This study demonstrated that airway smooth muscle directly responded to glucocorticoids and this knowledge may help to develop new asthma therapy.

IL-22 & IFN- γ

Pennino *et al.* [78] evaluated the gene expression profile of primary human bronchial epithelial cells following exposure to either IL-22 or IFN- γ , or in combination. Compared with untreated cells, IL-22 and IFN- γ induced the expression of 118 and 2920 genes, respectively. Interestingly, 75% of the IFN- γ induced genes were inhibited by co-treatment with IL-22. These results suggested that recombinant IL-22 represents a potential therapy to inhibit the proinflammatory effects of IFN- γ in human primary epithelial cells.

Find new biomarkers to improve asthma classification & targeted treatment

Gene expression can also be used to refine asthma phenotype, and eventually, identify the most appropriate treatment. The main clinical implications derived from these studies during the last 5 years are listed in TABLE 5. Woodruff *et al.* [54] conducted a transcriptomic analysis of airway epithelial brushings and revealed 22 genes differentially expressed between subjects with asthma ($n = 42$) and healthy controls ($n = 28$). Three upregulated genes in asthma, namely *CLCA1*, periostin and serpinB2, were found downregulated in asthmatics treated with inhaled fluticasone compared with placebo. These corticosteroid responsive genes were induced by IL-13 in airway epithelial cells in culture, an effect that was suppressed by corticosteroids. A subsequent analyses of the same airway epithelial brushing gene expression dataset suggested that asthma is divided in two distinct molecular phenotypes, namely Th2 high and Th2 low [55]. The two subgroups were significantly different on several aspects such as airway responsiveness, subepithelial fibrosis and serum IgE. This Th2 high and low clustering was based on the gene expression levels of *CLCA1*, periostin and serpinB2 as well as the higher levels of IL-13 and IL-5 expression

observed in bronchial biopsies of subjects with Th2 high asthma. Interestingly, lung function improvement following inhaled fluticasone treatment was limited to the Th2 high group, suggesting that Th2 high group is steroid responsive. The molecular phenotypes defined by the expression levels of these three genes in airway epithelial brushing can thus be used as biomarkers to delineate responders and non-responders to corticosteroids.

These distinct Th2 phenotypes defined by the expression of three genes in bronchial epithelium were followed-up in two additional studies. First, Dougherty *et al.* [79] found the three-gene signature correlated with a larger mast cell genes cluster in the same airway epithelial brushing microarray dataset. Similar to the three gene signature, the mast cell gene expression was correlated with lung function response to corticosteroids. The number of intraepithelial mast cells was then evaluated and found significantly higher in the Th2 high asthma subphenotype. This study pointed out a new mechanism implying mast cells accumulation in the epithelium of asthmatics with a Th2 high phenotype. Second, Choy *et al.* [80] studied the transcriptome of bronchial biopsies from 27 mild-to-moderate asthmatics and 13 controls. Seventy-nine genes were found differentially expressed in 'Th2 high' asthma compared with 'Th2 low' asthma and controls. A composite metric representing the Th2 signature was derived from the principal component one of expression values of these 79 genes. The Th2 signature demonstrated a direct relation with markers of inflammation and allergy used in clinic, such as serum IgE and blood eosinophil count. These studies on Th2 inflammation patterns ultimately resulted in the development of a gene expression signature to treat and diagnose asthma [81].

Baines *et al.* [82] also identified transcriptional phenotypes of asthma in induced sputum. Unsupervised hierarchical clustering analysis of 7436 genes called present in sputum of 59 asthma patients, identified three transcriptional phenotypes of asthma. Nearly one-third of asthma patients was grouped in each phenotype and was clinically different in terms of lung function values, asthma control and sputum inflammatory cell profile. More recently, the same team investigated gene expression biomarkers in induced sputum to delineate asthma inflammatory phenotypes (eosinophilic, neutrophilic and paucigranulocytic asthma) and predict response to inhaled corticosteroids [83]. Genes differentially expressed between inflammatory phenotypes were identified in 47 patients with asthma. Thirty-five genes were selected as potential biomarkers and validated by qPCR. Twenty-three genes were validated as differentially expressed between inflammatory phenotypes in a second set of 59 asthma patients. A six-gene signature with optimal discriminatory performance was then identified in the combined dataset. The six-gene panel (*CLC*, *CPA3*, *DNASE1L3*, *IL1B*, *ALPL* and *CXCR2*) was able to discriminate eosinophilic asthma from other phenotypes as well as to discriminate asthma patients from healthy controls ($n = 20$). The six-gene was also found to predict inhaled corticosteroid responsiveness to a greater extent than sputum eosinophil percentage in a group of 71 asthma patients. Fu *et al.* [84] also used sputum cells to identify

transcriptomic differences in asthma patients with and without systemic inflammation. Systemic inflammation status was determined according to the plasma levels of IL-6 and C-reactive protein. Microarray results of 18 and 16 subjects with and without systemic inflammation, respectively, revealed 449 genes differentially expressed. Genes mapped to different pathways related to innate immune response, defense and inflammatory response and neutrophilic inflammation. This study shows that neutrophilic airway inflammation is related to systemic inflammation. Treating the latter might prove beneficial in patients with neutrophilic asthma.

Shin *et al.* [85] were able to classify subjects with or without asthma based on the gene expression profile in PBMC. This classification was elaborated in a Korean population including 42 patients with asthma and 10 healthy controls. Microarray measurement of gene expression levels showed 170 genes differentially expressed between the two groups. More stringent criteria to define differentially expressed genes ($p \leq 0.001$ and a fold change ≥ 5) led to the identification of eight genes. All possible combinations of these eight biomarkers were tested and a three-gene model with optimal discriminatory performance was identified consisting of *MEPE*, *MLSTD1* and *TRIM37*. The performance of this gene panel to differentiate subjects with and without asthma needs to be validated in larger sample sets as well as in populations of diverse ethnicities.

Other investigators used circulating cells to find transcriptional phenotypes of asthma. Different inflammatory phenotypes of asthma have been described based on the predominant cell type present in induced sputum including eosinophilic, neutrophilic, paucigranulocytic and mixture of eosinophilic and neutrophilic asthma [86]. Baines *et al.* [87] compared whole blood gene expression levels in 36 samples with the four different inflammatory phenotypes. Results showed six genes differentially expressed between the four phenotypes of asthma including α -defensins (*DEFA1*, *DEFA1B*, *DEFA3* and *DEFA4*), cathepsin G (*CTSG*) and elastase (*ELA2*). All six genes were overexpressed for neutrophilic asthma. Thus, α -defensins and serine proteases may prove to be good biomarkers to identify neutrophilic asthma.

Baines *et al.* [88] also used inflammatory phenotypes to characterize the transcriptome of circulating neutrophils. In this case, only two asthma inflammatory phenotypes were considered: eosinophilic asthma ($n = 5$) and non-eosinophilic asthma ($n = 4$). Genome-wide expression profiling was conducted in neutrophils isolated from peripheral blood cultured or not with LPS. Three pairwise gene expression comparisons were performed: resting and LPS-stimulated neutrophils, resting neutrophils in non-eosinophilic and eosinophilic asthma and LPS-stimulated neutrophils in non-eosinophilic and eosinophilic asthma. As expected, LPS stimulation provoked an important transcriptomic response in neutrophils (1080 genes) that is characteristic of the proinflammatory state of neutrophil activation. Comparing the resting neutrophil transcriptome between the two asthma phenotypes revealed 317 significantly

modulated genes. About half of these genes (54%) were also modulated following LPS stimulation. For the comparison of LPS-stimulated neutrophils of eosinophilic and non-eosinophilic asthmatics, 221 genes were differentially expressed. This study indicated that the type of airway inflammation (non-eosinophilic vs eosinophilic asthma) is associated with distinct circulating neutrophil gene expression profiles, and thus, implied different disease pathogenesis and the need for different therapy.

As described above, other transcriptomic classifications were described including the identification of children with controlled and uncontrolled asthma in nasal brushings [59] and distinct subgroups based on the expression of exacerbation-induced genes in PBMC [69]. The latter led to the development of a method [89] assisting in the assessment, diagnosis or prognosis of asthma exacerbations, by assessing the level of expression of specific biomarkers such as *IFIT1*, *MX1* and *CCL2*. Together, these studies demonstrated the potential of genome-wide expression measurement to refine asthma phenotype and guide treatment strategies.

Integrative genomic approaches

Efforts to understand asthma from lists of genes that are differentially expressed are incomplete. Gene expression studies needed to be integrated with additional genome-wide molecular phenotypes such as genetic variants and epigenetic marks. Few studies have combined transcriptomic information with genetic variants. First, Sharma *et al.* [90] compared gene expression profiling in human fetal lung tissues at two normal developmental stages: pseudoglandular ($n = 27$) versus canalicular ($n = 9$). One thousand seven hundred and seventy-six differentially expressed genes were found between the two stages including five genes implicated in the Wnt signaling pathway. Follow-up genotyping data of nearly 1000 children from two distinct asthma cohorts revealed polymorphisms in two of these five genes associated with lung function in children, namely *WISP1* and *WIF1*. This study showed that transcriptomic studies of developing lung can be used to discover asthma susceptibility genes involved in developmental pathways influencing lung function later in life. Second, Melen *et al.* [91] investigated the transcriptomic profile of developing human and murine lung in relation with 96 well-established asthma susceptibility genes. This study took advantage of four published genome-wide expression datasets of developing lungs, one in human and three in different mouse strains. The goal was to test whether known candidate genes of asthma were differentially expressed during lung development. Thirty-two percent of the 96 asthma candidate genes were differentially expressed during human lung development. However, this figure was not different from non-asthma genes. Interestingly, some asthma genes were constantly differentially expressed in the four datasets including *NOD1*, *EDN1*, *CCL5*, *RORA* and *HLA-G*. This study showed that a number of asthma susceptibility genes are likely to exert their effect during lung development.

More studies combining genome-wide expression values with additional molecular phenotypes in the same individuals are needed. Recently, expression quantitative trait loci (eQTL) mapping studies were performed in the field of asthma [92,93]. This approach identifies single nucleotide polymorphisms associated with gene expression levels in a specific tissue or cell type. eQTL mapping studies will be highly complementary to transcriptomic studies and have already been particularly useful to aid in the interpretation of GWAS findings, which were described in detail elsewhere [92].

Expert commentary & five-year view

During the last decade, we have observed an increasing number of transcriptomic studies being reported in the field of asthma. In this review, we attempted to summarize the clinical implications of these studies. This evolving field of transcriptomics has certainly highlighted many new genes and pathways potentially implicated in the pathobiology of asthma as well as new biomarkers to improve clinical management of asthma patients. However, we must recognize that clinical interpretation of these studies is challenged by many factors. First, gene expression traits have been found highly sensitive and spatiotemporal dependent. Second, the nature of gene expression values has changed with evolving genomic technologies. Third, transcriptomic studies have been performed in a large variety of tissues and cell types (TABLE 1). Finally, the variety of study designs and the heterogeneity of asthma also add layers of complexity to distinguish common trends in asthma gene expression. Together, all possible variations of transcriptomic studies in asthma (FIGURE 2) leave most studies performed to date as unique. It is thus not surprising that at this stage, the results and clinical outcomes of all these studies only partially overlap. Furthermore, the methodologies used to study asthma transcriptomics can be extended on many levels. So far, our knowledge on asthma transcriptomics is derived from studies with relatively small sample sizes. In addition, no longitudinal studies were reported, only a few RNA-Seq studies were performed, and single cell studies are just emerging [94]. Accordingly, major progresses in the field of asthma are needed in all these areas.

A large number of transcriptomic studies in asthma have been performed in animal models (TABLE 2). Akin to human studies, the wide variety of experiments and methods makes the comparison between studies difficult. Despite this challenge, important lessons were derived from experimental models. We now know that the transcriptomic signature of asthma is species specific [49]. The baseline lung transcriptome differs in asthma-susceptible strains of mice [43] and the transcriptional changes during lung development are strain-specific in rats [47]. It is also well established that the transcriptomic response to allergens is strain-specific in mice [43] and rats [48]. This clearly demonstrated that the genetic background largely shapes the transcriptome. Further studies will be needed to identify the genetic factors involved. Animal models have also been useful to study the mechanisms of action of new asthma drugs including resiquimod [45], galectin-3 gene therapy [46] and TSLP

receptor blockade [50]. Animal studies also identified new therapeutic targets or strategies to prevent eosinophilic infiltration in the lung [44] and resolve airway inflammation [42,43,51]. Finally, paraxonase-1 activity in serum was found as a new biomarker to monitor the effectiveness of asthma therapy [41]. Additional development is needed in animal models to delineate the contribution of specific cell types in asthma pathogenesis. The transcriptional effects of more therapeutic agents studied at many time points and cell types will need to be investigated using experimental models.

As observed in animal models, the human transcriptome is genetically determined. Heterogeneity of gene expression response to infection is observed [69] and may largely be explained by existing differences at baseline [72]. The specific genetic factors are still largely unknown, but progresses are imminent as polymorphisms in genes differentially expressed during lung development have been found associated with asthma [90,91]. Studying gene regulation will be critical to understand genetic predispositions to asthma as demonstrated by GWAS-nominated variants residing largely in regulatory regions [95]. Accordingly, transcriptomic studies along with eQTL mapping studies [92,96] as well as more integrative genomic approaches in large sample size are likely to reveal soon the genetic factors that account for variation in gene expression levels.

Stimulating discoveries were made during the last few years using transcriptomic approaches in human asthma. A number of new therapeutic targets for asthma were found including fibronectin [56], *NOX4* [57], *Klf15* [77] and the bitter taste receptors [60]. Novel targets were also identified for asthma-exacerbations [71], severe uncontrolled asthma [59,61], chronic airflow obstruction in children with recurrent exacerbations [70], exercise-induced asthma [74] and eosinophilic asthma [66]. A number of biomarkers to improve clinical management of asthma patients were also elucidated. Biomarkers were found to predict patients with or without asthma [85], to monitor disease state [68] and the activity of specific cell type [58] as well as to identify patients that will respond to inhaled corticosteroids [79,83] and patients with recognized asthma phenotypes, for example, neutrophilic asthma [83,87].

Although promising, we need to emphasize that most discoveries derived from transcriptomic studies in asthma have not been translated into clinical applications. However, this is likely to occur in a not too distant future. Distinct transcriptional phenotypes of asthma were found in induced sputum [82,83], airway epithelial cells [54,55], circulating neutrophils [88], PBMC [69] and nasal brushings [59]. These asthma subphenotypes respond differently to conventional asthma therapy, that is, corticosteroid treatment, and may also pinpoint subsets of individuals that will respond to new therapeutic agents, for example, asthma therapies targeting Th2 inflammation [66,80]. Targeting medication on particular subgroups of asthma patients will eventually lead to more personalized treatments. The host transcriptomic response to environmental triggers may soon be used to guide targeted therapies [65]. With our

improving understanding of the transcriptomic signatures of asthma agents [22,77,78], it may be possible to find compounds or drugs that reverse the specific disease-related transcriptomic signature [97]. Accordingly, fine asthma phenotyping derived from transcriptomic data has the potential to predict responsiveness to asthma drugs akin to genotyping data predicting responsiveness to bronchodilators and glucocorticoid therapies [98,99]. More transcriptomic studies in asthma relevant tissues and cell types testing the impact of conventional and emerging drugs will be needed to achieve this goal. In the short term, it will be important to develop robust biomarkers in readily available non-invasive samples such as nasal brushings [59], buccal mucosa [100], blood [69,88] or sputum [82] to ensure point-of-care implementations of new transcriptomic knowledge.

In conclusion, the wealth of information provided by transcriptomic studies in asthma is yet to be fully exploited to improve diagnosis and treatment of asthma. During the last decade or so, we have certainly learn about the feasibility [23,52] and proper methodologies [64] to carry out whole-genome gene expression studies in asthma relevant tissues and cell types. In short term, sequencing of RNA will likely revolutionize our understanding of the transcriptome [18]. Coupled with new functional annotations of the human genome [101] and evolving bioinformatics tools, new genes and regulatory mechanisms implicated in asthma will be discovered. The clinical applications of the new knowledge derived from transcriptomic studies of asthma are

starting to emerge to improve asthma classification and guide treatment options. Transcriptomic studies are not yet ready to revolutionize the management of patients with asthma, but it is hoped that the major investments in time and resources as well as promising discoveries in this field will soon refine and personalize the clinical management of patients with asthma.

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Key issues

- Gene expression traits are highly sensitive, spatiotemporal dependent and different in nature based on rapidly evolving measurement platforms.
- Methodologies vary widely to perform genome-wide expression studies in asthma in terms of source of tissues/cells, species studied, asthma phenotypes, gene expression platforms, treatment groups and time points, which make these studies difficult to compare and summarize at this point.
- Animal models of asthma clearly demonstrated that the baseline transcriptome as well as changes that occur during development stages and following exposure to environmental triggers are genetically determined.
- Transcriptomic studies in human identified a number of new therapeutic targets that required further investigation.
- The first clinical applications of transcriptomic studies in asthma are starting to be developed and may soon improve asthma classification and guide the use of conventional and emerging drugs.

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