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Gruzieva, Olena; Merid, Simon Kebede; Koppelman, Gerard H; Melén, Erik

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An update on the epigenetics of asthma

Olena Gruzieva[a,b,*], *Simon Kebede Merid*[c,*], *Gerard H. Koppelman*[d,e], *and Erik Melén*[c,f]

Purpose of review
Asthma is a common disease worldwide, however, its pathogenesis has not been fully elucidated. Emerging evidence suggests that epigenetic modifications may play a role in the development and natural history of asthma. The aim of this review is to highlight recent progress in research on epigenetic mechanisms in asthma.

Recent findings
Over the past years, epigenetic studies, in particular DNA methylation studies, have added to the growing body of evidence supporting a link between epigenetic regulation of gene expression and asthma. Recent studies demonstrate that epigenetic mechanisms also play a role in asthma remission. Although most existing studies in this field have been conducted on blood cells, recent evidence suggests that epigenetic signatures are also crucial for the regulation of airway epithelial cells. Studies conducted on nasal epithelium revealed highly replicable epigenetic patterns that could be used for diagnostic purposes.

Summary
Further research is needed to explore the diagnostic and therapeutic potential of epigenetic modifications in asthma. Multiomics studies on asthma will become increasingly important for a better understanding of etiology, heterogeneity, and severity of asthma, as well as establishing molecular biomarkers that could be combined with clinical information to improve the management of asthma patients.

Keywords
asthma, DNA methylation, epigenetics, histone modifications, microRNA

INTRODUCTION

Asthma is a chronic heterogeneous respiratory disease of the lower airways characterized by reversible airflow obstruction and airway inflammation. Despite that asthma has a hereditary component estimated between 50 and 60% [1], previous genetic studies have only been able to explain up to 20% of disease risk [2], leaving a significant portion of the heritability unexplained. Moreover, the remaining fraction is believed to originate from environmental exposures.

Analyses of epigenetic modifications have recently attracted substantial interest in the asthma research field. Unlike genotype, epigenetic regulation is receptive to environmental factors, and could thus bridge the gap between an individual's genetic susceptibility and the environment, providing mechanistic insights of asthma development, reflecting cell-type as well as cell activation, and facilitating identification of novel biomarkers and potentially new therapeutic targets. Further, epigenetics may also be implicated in age and sex differences in asthma occurrence and remission.

In contrast to the genome-wide association studies (GWAS), epigenome-wide association studies (EWAS) have recently started to emerge. To-date, most large-scale EWAS have been conducted on childhood populations. We found as many as 14 literature reviews summarizing existing evidence on epigenetic modifications associated with asthma published during the last 12 months [3–10],

[a]Institute of Environmental Medicine, Karolinska Institutet, [b]Centre for Occupational and Environmental Medicine, [c]Department of Clinical Sciences and Education, Karolinska Institutet, Södersjukhuset, Stockholm, Sweden, [d]University of Groningen, University Medical Center Groningen, Beatrix Children's Hospital, Department of Pediatric Pulmonology and Pediatric Allergology, [e]University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, The Netherlands and [f]Sachs Children's Hospital, Stockholm, Sweden

Correspondence to Erik Melén, MD, PhD, Department of Clinical Sciences and Education, Karolinska Institutet, Södersjukhuset, Stockholm, Sweden. E-mail: erik.melen@ki.se

*Olena Gruzieva and Simon Kebede Merid contributed equally.

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KEY POINTS

- Studies on methylation profiles in nasal epithelial cells have identified several new target genes for asthma, although most studies to date have focused on peripheral blood.
- Several of the implicated genes identified either in blood or nasal cells were enriched in pathways related to inflammation, immune response processes, transforming growth factor beta pathway.
- The epigenetic landscape is specific for a given cell type. Future studies on epigenetic marks measured in different cells within same individuals, along with single-cell omics analyses will likely shed light on the cell-specific molecular signatures related to asthma pathophysiology.
- The integration of information from multiple levels in omics studies on asthma is a promising tool in terms of understanding asthma pathogenesis and enhancing precision medicine care for asthma patients.

including miRNAs [11–16]. Overall conclusions were that, despite the huge technological advances that have enabled hypothesis-free genome-wide DNA methylation studies in the last few years, there is still lack of consistency between the findings from different studies, which might at least partly be due to differences in the studied populations, the definition of the asthma phenotype, cell compositions, as well as statistical methods used for analysis and interpretation of findings. This heterogeneity of the different analyzed populations, as well as unknown confounders, might limit the ability to replicate the findings of the discovery studies. In fact, as shown in a recent review on EWAS studies in asthma, among the thousands of cytosine-phosphate-guanine (CpGs) dinucleotide sites identified to be associated with asthma in recent years, only 41 of the associations were found in at least one other study [17]. Also noteworthy, most EWAS published so far are cross-sectional, thus not allowing to interpret if epigenetic changes are cause or consequence of asthma. The present review aims to highlight the latest advance on epigenetics of asthma and to offer future perspectives.

EPIGENETIC MECHANISMS

Epigenetics is the study of mitotically or meiotically heritable changes in gene activity without directly affecting the underlying DNA nucleotide sequence [18]. It has earlier been shown that epigenetic mechanisms regulate many genes including those implicated in inflammatory and immune responses [17]. The major epigenetic mechanisms that are currently being explored for asthma etiology and natural history include DNA methylation, histone modifications, and noncoding RNAs.

DNA methylation entails addition of a methyl group to cytosine residues in the CpG dinucleotide sequence. DNA methylation is often associated with suppression of gene transcription, even up to a complete gene inactivation. Compared to other epigenetic mechanisms, DNA methylation using both target-gene and genome-wide approaches and sampling different tissues has been most extensively investigated in human populations, largely due to the fact that it can be routinely studied using standardized methods and archived genomic DNA samples.

Histone modifications involve posttranslational modifications such as acetylation, phosphorylation, methylation, and ubiquitination of core histones, octameric proteins that build up chromatin [19]. Studies investigating histone modifications in context of asthma are less frequent compared to DNA methylation studies.

MicroRNAs (miRNAs) are a class of small noncoding RNAs that are involved in various biological processes such as cell signaling, biochemical pathways, tissue, and organ development [20]. They are known to regulate the innate functions of immune cells and their proliferation in response to pathogenic triggers [21].

LITERATURE SEARCH METHODOLOGY

To identify articles related to epigenetics of asthma, we conducted a systematic search of peer-reviewed articles on September 1st, 2020 using PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) applying the following search terms: (epigenetic*[Title/Abstract] OR methylation[Title/Abstract] OR histone[Title/Abstract] OR micro RNA[Title/Abstract]) AND (asthma*[Title/Abstract]), and limiting the results with '1 year' (publication dates), which led to the identification of 128 records. Those were subsequently subjected to a screening that aimed to filter out articles not reporting original data, that is, reviews, editorials, commentaries, textbook chapters ($n = 61$), or reporting epigenetic data obtained in animals or cell lines ($n = 18$), or reporting no epigenetic data on associations with asthma ($n = 35$). Further, we added one article that was not captured by the initial search [22▪▪]. The final selection comprised 15 original articles reporting associations between epigenetic data and asthma phenotypes. The descriptive information extracted from the eligible studies is presented in Supplementary Table S1, http://links.lww.com/COAI/A20.

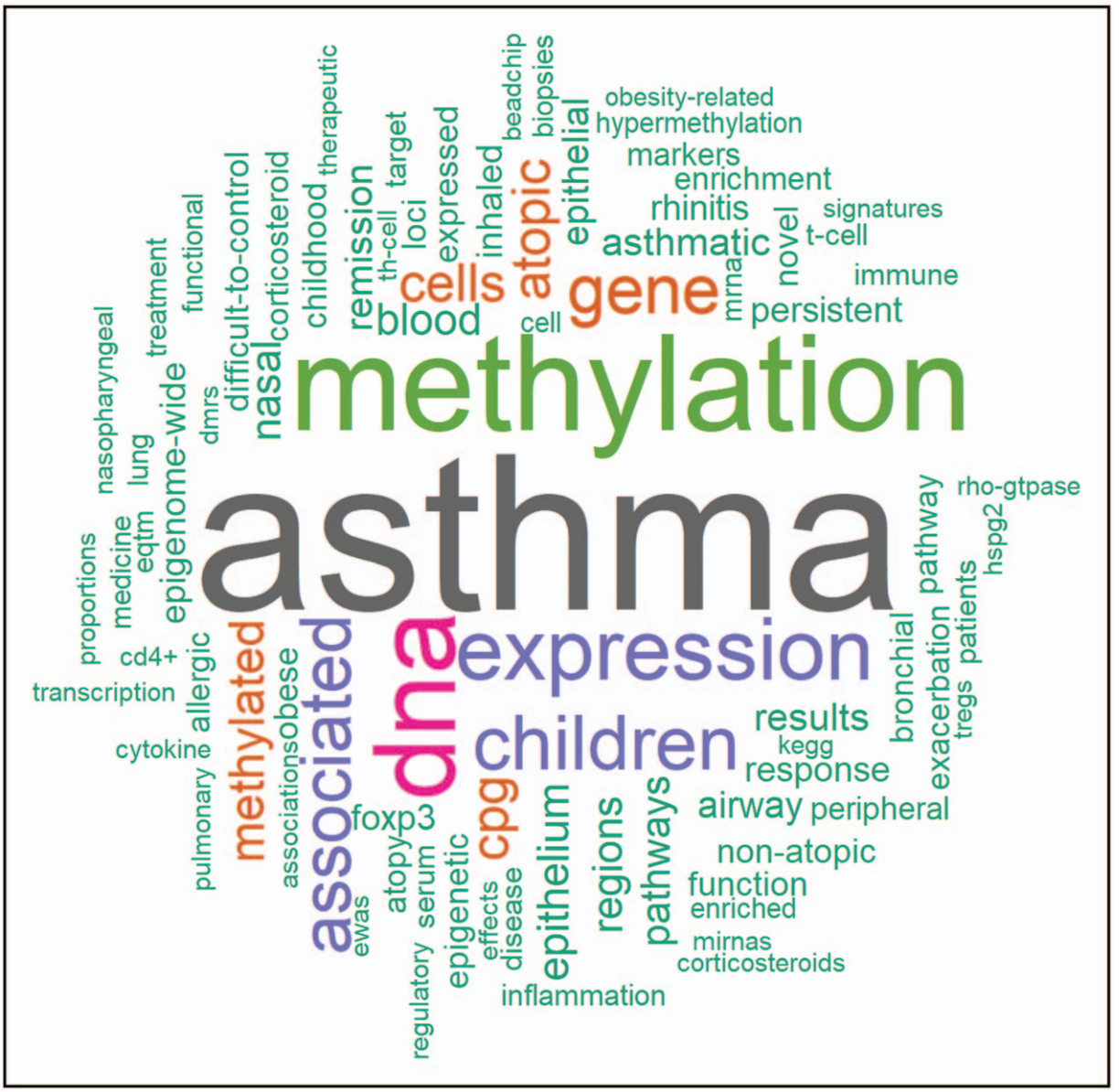

FIGURE 1. The word cloud of the weighted list of the words extracted from the abstracts of included articles.

Figure 1 demonstrates the word cloud of the weighted list of the words extracted from the abstracts of included articles.

REVIEW OF THE LATEST LITERATURE

Studies on children

Nine of the included studies were conducted on children and youth (up to 20 years). All but two studies [23,24▪▪] measured DNA methylation with Illumina Infinium HumanMethylation450K BeadChip array (Illumina, San Diego, CA), which is a platform investigating 1.6% of the total DNA methylome. Three studies focused on DNA methylation patterns in nasal brushed cells. Qi *et al.* found no epigenome-wide significant differentially methylated probe in a sample of 16 years old from the PIAMA cohort, however, using a less stringent cut-off ($P < 1 \times 10^{-4}$) the authors selected 95 CpG sites associated with asthma for replication in two independent studies, also using nasal epithelial cell data [25]. Of these 95 CpGs, cg08844313 (*PDE6A*) achieved statistical significance in meta-analyses of all 3 studies. Another study from Puerto Rico performed genome-wide expression quantitative trait methylation analysis in nasal epithelium of subjects aged 9–20 years [26▪]. In a mediation analysis, they identified 5,934 paths through which methylation markers could affect atopic asthma

through gene expression in nasal epithelium. In a study from China, the researchers screened critical genes and miRNAs involved in childhood atopic asthma utilizing Gene Expression Omnibus database of biosamples from atopic asthmatics and healthy controls [27]. They identified as many as 146 critical differentially expressed genes that were also differentially expressed in DNA methylome regions. Among these genes, *TNF* and *HLA-DPA1* were significantly related to the immune response process. Further, in the miRNA-target gene regulatory network, Hsa-miR-148b was the most significant node that regulated 21 target genes, including *TNF*. Taken together, these findings add further support to the previous work demonstrating biologically plausible specific methylation profiles in nasal epithelium in asthma [28▪▪].

Six studies investigated DNA methylation in blood cells. A meta-analysis of EWAS in white blood cells from Latino children identified one CpG (false discovery rate $P < 0.05$) located near the calcium/calmodulin dependent protein kinase (*CAMK1D*) [29▪], that has previously been associated with regulation of granulocyte function and shown to be upregulated in chronic obstructive pulmonary disease patients [30]. Further, the researchers found 25 differentially methylated regions (DMRs) associated with asthma (false discovery rate $P < 0.1$). The top annotated genes included *MUC6* that encodes for mucin protein linked to bronchial hypersecretion and moderate to severe asthma [31], as well as proline rich 5 gene (*PRR5*) that has been shown to affect asthma through ILC3-related pathways [32]. The top significant enriched pathway was transforming growth factor-beta signaling. Another study by Rastogi *et al*. employed a multiomics approach, including differential gene expression from $CD4^+$ Th cells, expression quantitative trait loci mapping, differential methylation, and methylation quantitative trait loci, to investigate cellular mechanisms underlying obesity-related asthma phenotype in Hispanic and African American children [24▪▪]. This integrative analysis revealed differential methylation and expression of genes implicated in the Rho-GTPase pathway, along with regulation of cellular metabolic processes and protein modification processes. In addition, differentially expressed and/or methylated genes, including ribosomal protein S27like (*RPS27L)*, recognized as a p53-inducible modulator of cell death [33], were associated with pulmonary function deficits estimated by FEV_1/FVC ratio and expiratory reserve volume. For the present review, we also included one candidate-gene study from China investigating expression and methylation levels of forkhead transcription factor P3 (*FOXP3*) in peripheral blood $CD4^+$ $CD25^+$ $CD25^+$ regulatory T cells (Tregs) in 30 children aged 5–14 years [23]. The average DNA methylation levels of 12 of the 16 available *FOXP3* CpG loci tended to be higher among asthmatic children compared to those without asthma, although only one CpG site 6 in exon 1 of sequence 1 reached statistical significance ($P < 0.05$). Summing up, the results of these studies provide evidence for the importance of integrative genomic approaches in blood, in particular CD4 Th cells, to further improve our understanding of the biologic mechanisms underlying pediatric asthma. The remaining three studies have focused on asthma medication and are further discussed under 'Asthma medication and epigenetic regulation' section below.

Studies on adult populations

A total of six studies included in this review examined associations of epigenetic marks with asthma in adults, of which two studies focused on miRNAs [22▪▪,34], whereas the rest on DNA methylation. One study assessed associations of various asthma exacerbation traits with selected serum miRNAs expression in 21 asthmatics aged 25–79 years with biosamples taken at the exacerbation and follow-up clinical visits [34]. The mean expression of serum miRNA-126a, miRNA-16, and miRNA-21 was significantly lower at the time of exacerbation visit. miRNA-29b correlated with FeNO, and most of the tested miRNA correlated with lung function tests. In another study based on subjects with complete asthma remission, persistent asthma, and healthy controls, the expression levels of 10 miRNAs (miR-320a, miR-193a-5p, miR-320c, miR-4532, miR-320d, miR-320b, miR-423–3p, miR-133b, miR-3960 and miR-126–3p) were found to differentiate complete remission from persistent asthma [22▪▪]. Moreover, by integrated miRNA, protein-coding RNA, and lncRNA expression through Bayesian network modeling, the authors identified a network characteristic of complete remission of asthma in which miRNAs and lncRNAs were abundantly present. A Dutch study investigated variation in DNA methylation in bronchial biopsies obtained from subjects with persistent asthma, remission subjects, and nonasthmatics aged 32–70 years [35▪▪]. The authors found four CpGs and 42 regions that were differentially methylated between persistent asthma and remission, as well as 1,163 CpG-sites and 328 regions between remission subjects and healthy controls. DNA methylation at two sites was correlated *in cis* with gene expression at atypical chemokine receptor 2 (*ACKR2)* and diacylglycerol kinase (*DGKQ)* genes that are involved in resolution of

inflammation by post-inflammatory clearance of chemokines and cellular signaling in inflammatory processes [36,37]. Further, in remission subjects, DNA methylation levels were correlated with expression of genes related to ciliated epithelium, indicating that DNA methylation patterns reflect cell type constitution in the airway biopsies.

In the largest EWAS of adult asthma conducted so far ($n = 2286$), 524 and 1086 CpGs appeared to be differentially methylated (false discovery rate $P < 0.05$) in blood from adults with nonatopic or atopic asthma (i.e., with coexistent IgE sensitization), respectively, compared to the control group, of which 104 CpG sites overlapped [38▪▪]. The look-up analyses in other studies using asthma-relevant tissues revealed as many as 68 of CpGs in nonatopic asthma and 288 in atopic asthma replicated in whole blood, 49 and 274, respectively, in eosinophils, 0 and 49 in airway epithelium, 151 and 875 in nasal epithelium. Substantial replication in nasal epithelium suggests that methylation in blood is a good proxy for asthma-relevant tissues in discovering novel differential methylation patterns of asthma. Further, identified genes were overrepresented in pathways related to the nervous system or inflammation. A study from China integrated genome-wide DNA methylation and metabolomic data obtained from 20 asthma patients aged 38–65 years [39]. The combination of epigenetic profiling with metabolic data revealed aldehyde dehydrogenase 3 family member A1 (*ALDH3A1)* as a potential marker for the diagnostic classification of difficult-to-control asthma. *ALDH3A1* has previously been implicated in oxidative stress induced by chronic obstructive pulmonary disease and air pollution [40]. Yet another study reported differential DNA methylation in airway epithelial cells (AECs) of asthma individuals 18–56 years of age [41]. Interestingly, the results appeared to differ depending on applied isolation technique for AECs. Thus, in pronase isolated AECs, 15 DNA regions were differentially methylated in asthmatics compared to subjects without asthma, whereas in bronchial brush isolated AECs, 849 differentially methylated DNA regions were discovered with no overlap to those found in pronase isolated cells. The top significant genes associated with the largest difference in DNA methylation (pronase, *DUSP22*; bronchial brush, *WNT7B*) have earlier been implicated in asthma pathogenesis [42]. In summary, the studies on adult asthma highlight miRNAs as promising prognostic markers of asthma and asthma exacerbations, but remain to be validated in the future studies. Cell type and cell isolation technics remain important aspects in epigenetic studies.

Asthma medication and epigenetic regulation

Although epigenetic mechanisms have been linked to both asthma susceptibility and severity, their role as a modifier of inhaled systemic corticosteroid (ICS) treatment response in asthma is poorly investigated and is often limited to very small samples restricted to the pediatric setting. Treatment with ICS is the standard for asthma care at present, still up to 30% of patients demonstrate a poor response to treatment [43]. Epigenetics can play a role in drug pharmacogenetics as suggested by *in vitro* studies reporting DNA methylation differences that affect dexamethasone sensitivity and resistance in human endothelial cells [44]. Earlier EWAS identified 130 differentially methylated CpGs in nasal epithelial cells related to treatment with sympathomimetic bronchodilators [45]. More recently, Kere *et al*. observed 20 CpGs to be associated with any or continuous ICS use in an EWAS based on 8-year-old children diagnosed with asthma, although none of them could be replicated [46]. The authors concluded therefore that ICS treatment does not affect peripheral blood cell DNA methylation levels to any major extent. An EWAS meta-analysis of three ethnically diverse cohorts of children with mild-to-moderate persistent asthma identified two differentially methylated CpGs in the upstream regions of IL12B and CORT genes associated with response to ICS treatment [47▪].

A recent study from Taiwan has demonstrated allergen-specific immunotherapy with the house dust mite cysteine protease Der p can affect PBMCs DNA methylome of children suffering from allergic asthma [48]. In this study, the authors reported 108 DMRs ($P < 0.05$) allied to genes such as major histocompatibility complex genes (i.e., *HLA-DRB6, HLA-DPB1, HLA-DRB1), GSTM1, HSPG2, BCL2L14*, and *BCL6*). The identified regions mapped to several pathways including antigen processing and presentation, extracellular matrix remodeling, and regulation of apoptosis. Several selected probes (i.e., *BCL6, HSPG2,* and *HSP90AA1*) were further validated by bisulfite pyrosequencing and qRT-PCR. Of these, B-cell CLL/lymphoma 6 (*BCL6*) was significantly hypomethylated, whereas heparan sulfate proteoglycan 2 (*HSPG2)* and heat shock protein 90 alpha family class A member 1 (*HSP90AA1*) showed hypermethylation in Der p immunotherapy group, compared to the allergic asthmatic without allergen-specific immunotherapy. Furthermore, significantly higher gene expression of *BCL6* and lower gene expression of *HSPG2* was observed in subjects receiving allergen-specific immunotherapy. Noteworthy, this study was based on a small sample (37 subjects split in 3 groups) and did not apply multiple testing correction.

None of the recent studies included in this review specifically examined histone modifications

or miRNAs in relation to asthma medication. The increasing use of biologics for severe asthma and allergic disease calls for precision medicine approaches to guide individual treatment, and in this context epigenetics and other omics data are promising tools [49]. However, we found no studies on epigenetics and biologics published in the last year. There is an obvious need of larger studies to determine robust epigenetic biomarkers predictive of response to asthma treatment, and to allow for precision medicine applications.

CONCLUSION AND FUTURE PERSPECTIVES

Recent research added promising results to the field of epigenetics of asthma. Several changes at DNA methylation or miRNA levels were observed between individuals with and without asthma. Some of these changes were linked to cell mediators or chemokines that might play a role in asthma disease process and hence can be potential therapeutic targets. Response to ICS treatment as well as allergen immunotherapy have also been associated with epigenetic changes in some studies. At the same time, limited power, the lack of reproducibility of the findings between studies, as well as limited understanding of their functional relevance, complicate the interpretation and clinical implementation of identified changes. The choice of most suitable tissue and cell type for analysis is still a debate, however, accumulating evidence points to the utility of blood and nasal cells for investigation of asthma-related epigenetics marks. The existing evidence regarding cell-specific DNA methylation signatures in asthma has recently been extensively reviewed, concluding that epigenetic changes that occur in certain cell type can not be directly translated into another one [50]. It is therefore, crucial with careful selection of the cell type of relevance for a given research question as well as taking care of potential confounding by differential cell proportions between cases and controls.

Recently, studies started to move from investigating genomics, epigenetics, or transcriptomics separately to a systems biology approach integrating multiple-omics data, thus enhancing our knowledge of cellular processes and gene regulatory mechanisms underlying asthma. Promising examples that also encoporate environmental exposures such as air pollutants have also been published recently [51▪]. Omics studies have large potential for causal modelling of relationships between cellular processes and asthma phenotypes, shared genetics/epigenetics between asthma and other allergic diseases [52], as well as identification of biomarkers that may allow development of individual risk prediction models to guide treatment, that is, precision medicine applications. Finally, prospective longitudinal studies with samples collected before the onset of asthma, are required to better investigate causality and the role of identified markers in the onset and course of asthma.

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Conflicts of interest

G.H.K. receives grant support of the Lung Foundation of the Netherlands, TEVA the Netherlands, GSK, Vertex, Ubbo Emmius Foundation, TETRI foundation, outside the submitted work. E.M. has received advisory board and lecture reimbursements from Chiesi, Novartis, AstraZeneca and Sanofi, outside the submitted work.

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