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REVIEW

Omics for the future in asthma

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Abstract

Asthma is a common, complex, multifaceted disease. It comprises multiple phenotypes, which might benefit from treatment with different types of innovative targeted therapies. Refining these phenotypes and understanding their underlying biological structure would help to apply precision medicine approaches. Using different omics methods, such as (epi)genomics, transcriptomics, proteomics, metabolomics, microbiomics, and exposomics, allowed to view and investigate asthma from diverse angles. Technological advancement led to a large increase in the application of omics studies in the asthma field. Although the use of omics technologies has reduced the gap between bench to bedside, several design and methodological challenges still need to be tackled before omics can be applied in asthma patient care. Collaborating under a centralized harmonized work frame (such as in consortia, under consistent methodologies) could help worldwide research teams to tackle these challenges. In this review, we discuss the transition of single biomarker research to multi-omics studies. In addition, we deliberate challenges such as the lack of standardization of sampling and analytical methodologies and validation of findings, which comes in between omics and personalized patient care. The future of omics in asthma is encouraging but not completely clear with some unanswered questions, which have not been adequately addressed before. Therefore, we highlight these questions and emphasize on the importance of fulfilling them.

Keywords Omics · Biomarkers · Precision medicine · Integration · Phenotypes · Challenges

Introduction

Asthma is a chronic airway disease characterized by airway hyper-responsiveness, inflammation, and mucus secretion. It is one of the most prevalent chronic airway diseases, affecting approximately 339 million individuals worldwide, globally killing more than 1000 daily, and its incidence rises each year [1]. Asthma is a heterogeneous disease and comprises multiple clinical phenotypes that complicate appropriate diagnosis and therapeutic decisions.

Asthma is usually treated using a trial-and-error approach. In addition, it is mostly directed towards symptom control. Although this approach is traditionally used by many physicians worldwide, it may not be ideal in routine patient care. It does not achieve full asthma control in some patients, and therefore they can suffer from health-related complications and reduced quality of life. The healthcare community is now more aware of the need to tailor diagnostic and therapeutic decisions to the individual patient's need; this is also called "precision medicine."

The practice of precision medicine in asthma is currently far from optimal due to the lack of complete

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understanding of the complex nature of asthma. An example of a step towards the application of asthma precision medicine is the treatment of severe asthma patients with biologicals targeting underlying inflammatory pathways. Biologicals, such as omalizumab, mepolizumab, reslizumab, and dupilumab, have shown a great potential in terms of efficacy. However, the severe asthma group is composed of heterogeneous phenotypic subgroups, and different severe asthma patients might benefit from different types of phenotype-targeted treatment [2–4]. Therefore, efforts are being made to adequately characterize asthma patients to specific phenotypes, which will eventually improve patient outcome [3, 4].

In this review, we will discuss the transition of single biomarker asthma diagnosis/care to multiple omics approaches, current state, challenges and limitations, and the future of omics in asthma research and practice.

From single biomarker to multiple omics endotyping

Suppose we view asthma as a picture puzzle. A single puzzle piece (single biomarker) will not give enough information of the total picture. Multiple pieces (multiple biomarkers) are needed to get the fine details of the picture. Once all the pieces are brought together, one will get an idea of the picture size, shape, and colors and then decide on which wall this picture fits best (precise decision). A similar decision making strategy also applies to asthma, for which we aim to integrate the information of multiple omics to reveal the disease characteristics, biological pathways, and possible co-disease interactions (influence of other diseases/comorbidities on asthma) of a specific patient/group of patients. Hopefully, we can then identify new biomarkers leading to precisely target therapeutic options for each individual patient.

Historically, a single-feature approach has been used to classify asthma patients into certain phenotypes. For example, allergic versus nonallergic (extrinsic vs intrinsic) asthma, the former is being triggered by inhaled allergens and often occurs at a younger age [5]. Afterwards, classifications were based on certain clinical characteristics, for instance, smoking associated, exercise induced, early onset, and obesity related, or based on the inflammatory biomarkers, such as eosinophilic, neutrophilic, and paucigranulocytic asthma based on the presence of inflammatory cells in sputum [6]. Later, unsupervised approaches such as cluster analysis have been used to statistically identify asthma phenotypes with multiple associated clinical characteristics [7, 8].

Elucidation/refining the asthma phenotypes by revealing the underlying pathophysiological mechanistic pathways, "endotyping," is allowing us to get better insight in the complex nature of asthma. Sooner or later, those attempts to effectively categorize asthma patients into phenotypes and their corresponding endotypes will help to serve the greater purpose of precision medicine for the optimal patient outcomes. An example is the type 2 (T2)-high endotype, to which most developed biologicals are being directed to target immune cells (e.g., eosinophilia) involved with Th2 airway inflammation [9, 10]. Nevertheless, T2-low endotype, which lacks Th2 markers and response to corticosteroids but is neutrophilic (or paucigranulocytic), has fewer therapeutic options than the T2-high counterpart [9, 10]. The emerging fields of omics are bringing us one step closer to more refined endotyping [9, 11, 12] and to the ideal application of personalized medicine. Figure 1 shows the general interconnections of different omics technologies.

Emerging fields of omics

Terms	*Definition/explanation*
Omics	Depiction and quantification of a collection of biological molecules/biomarkers. It is added as a suffix (-ome/-omics) to address the subject of a specific study field
Genomics	Study of the genetic characterization (comprehensive information of DNA sequences) and its associated alterations of a specific tissue/cell
Epigenomics	Study of the DNA modifications (mainly chemical), which further influence gene activity regulation and expression of tissue/cell. It involves DNA methylations, histone modifications, and chromatin structure alterations
Transcriptomics	Study of the transcriptome, their structure, and function. A transcriptome includes set of RNA molecules within a tissue/cell such as messenger RNA (mRNA), noncoding RNA, transfer RNA (tRNA), microRNA (miRNA), ribosomal RNA (rRNA), and others
Proteomics	Study and identification of the sets of proteins/peptides within a tissue/cell, including abnormal alterations made to a particular set
Metabolomics	Study and identification of sets of low molecular weight metabolites. Metabolites are small molecules such as fatty acids, carbohydrates, organic compounds, and others, which are products of metabolic (enzymatic) reactions within a tissue/cell
Microbiomics	Study of the genetic composition of microorganisms (bacteria, viruses, and fungi) in samples/body compartments. This involves identification of both commensal (normal habitants of the human body) and pathogenic microorganisms and their cross talk
Exposomics	Study of the environmental exposure throughout a person's life and its influence on health and disease. This involves several exposures such as lifestyle factors (diet, smoking, etc.), air pollution, chemical exposures, and many others

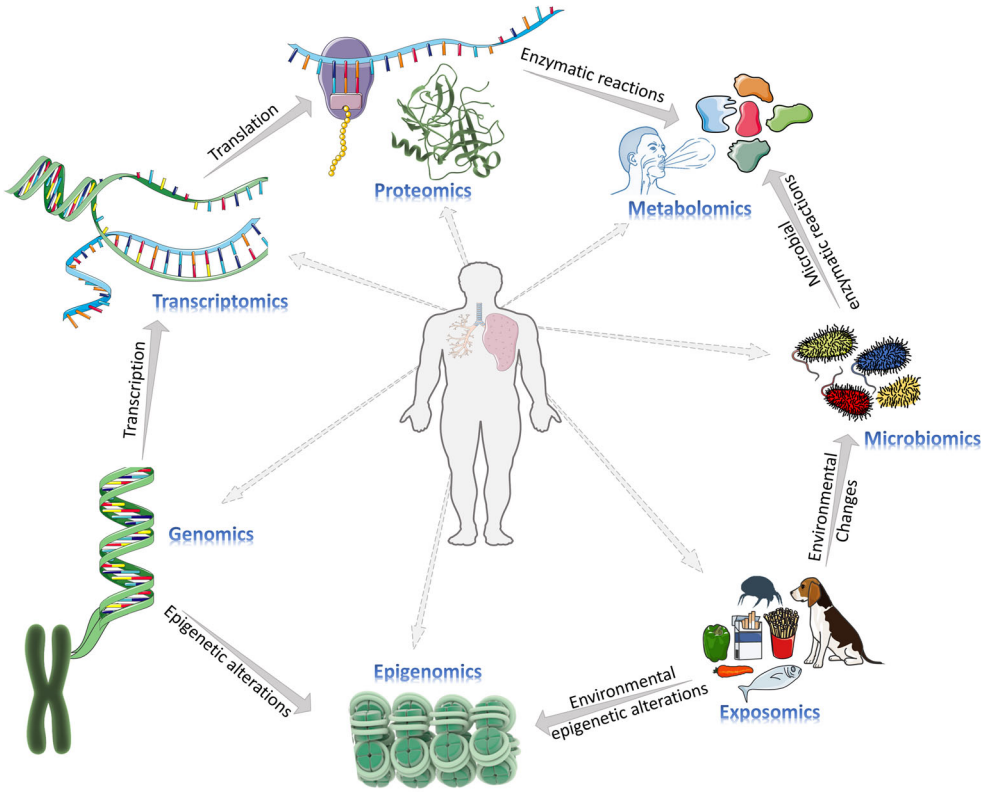

Fig. 1 Cross-connections and targets of omics investigations in the asthma patient. *Some of the drawn objects were adapted from Servier and Somersault18:24 corporations, which are used under creative commons license*

Multi-omics projects in asthma

Several wide-scale international collaborations have been initiated within the last two decades to better understand asthma. Multi-omics projects are usually the products of many years of intensive collaboration of multidisciplinary research teams. An example is the Severe Asthma Research Program (SARP), which is a multicenter US study, aiming to understand severe asthma in adults and children, by combining information at clinical and cellar level [13]. Another example is the Unbiased BIOmarkers in PREDiction of respiratory diseases outcomes (U-BIOPRED) project, which is a multicenter European study with the aim to investigate severe asthma in both adults and children, by integrating the information from multiple omics layers [14, 15]. A more general project is the Mechanisms of the Development of Allergy (MeDALL) study, which aimed to investigate the underlying mechanisms of allergic diseases including asthma through a multi-omics approach [16]. A recent example is the Systems Pharmacology Approach to Difficult-to-treat Pediatric Asthma (SysPharmPediA) study, which is an ongoing multicenter pan-European study, aiming to integrate multiple omics information to better understand the pathophysiology of pediatric asthma patients who are uncontrolled despite using inhaled corticosteroids (ICS).

Insight into omics layers investigated in asthma

Genomics

Genomic studies in asthma can be subdivided into different approaches: whole-genome sequencing (WGS), whole-exome sequencing (WES), genome-wide association studies (GWAS), or candidate gene association studies. GWAS studies focus mainly on detecting nucleotide polymorphisms (SNPs) across the whole genome (data-driven approach), while candidate gene approach focusses on detecting genetic alternations in (a) specific gene/s linked to an outcome of interest (hypothesis-driven). Some consider GWAS studies an unbiased approach to detect panels of SNPs associated with a certain disease or risk factors, which may further serve as a base for the candidate gene studies. Genetic studies, in general, require large number of patients to avoid false discoveries and non-reproducible findings. Within the last two decades, several asthma GWAS studies have been conducted to investigate genetic associations of different asthma-related characteristics in various populations and age groups. Among the largest investigations is an European GWAS study on allergic disease susceptibility conducted in 360,838 subjects, which identified 136 genetic variants to be associated with allergic disorders including asthma, implicating 132 nearby genes from 99 loci [17]. A more recent study identified 19 novel

significant gene-based associations on 11 risk loci shared between asthma, hay fever, and eczema allergic disorders out of 19,432 total genes tested [18]. With regard to childhood asthma risk in particular, variations in locus 17q21 were the most reproducible. The GABRIEL consortium genotyped 10,365 asthmatics and 16,110 healthy controls, which revealed that *ORMDL3* and *GSDMB* genes located on locus 17q21 were associated with childhood-onset asthma [19]. Other variations on locus 17q21 were also associated with increased risk of asthma exacerbations and response to ICS [20, 21].

Pharmacogenomics is another interesting field of genomics studies in asthma. It concerns with studying genetic alterations that are linked to patients' response to asthma medication and/or susceptibility to adverse effects. Although multiple genetic variants have been associated with the different responses to asthma medications, poor replication of findings stands against direct clinical applicability [22]. An exceptional finding is the genetic variation within the *ADRB2* gene, especially the Arg16 variant, which has been linked to long-acting β_2 adrenoceptor agonists (LABA) response in pediatric asthma patients across different studies [22–24]. Whether this will allow personalizing the use of LABA in pediatric asthma patients is currently investigated in the ongoing randomized PUFFIN control trial that aims to compare between ADRB2-guided treatment vs usual care in pediatric asthmatics [25]. Regarding genetically associated adverse effects of asthma medications, a recent study revealed that genetic variations within the *PDGFD* gene locus increase risk of corticosteroid-induced adrenal suppression in children and adults with asthma or COPD [26]. This might help to guide treatment in asthma patients by carefully tailoring corticosteroid-based regimens in sensitive patients.

In summary, GWAS studies revealed that some alterations in the genetic architecture could be linked to asthma or asthma-associated characteristics. Alterations at locus 17q21 were the most reproducible, while others could not be replicated. Likewise, findings within pharmacogenomics studies in asthma could not be adequately replicated in many instances, with the exception of few such as the variants of the *ADRB2* gene, which might help to direct therapeutic decisions.

Epigenomics

Epigenetics study the changes in genetic functions that are not related to genetic alterations. Epigenetic variations are dynamic and affected by environmental factors such as diet, chemical compounds (including the use of medication), air pollution, smoking, and others [27–29]. Many of these epigenetic changes in asthma occur during prenatal period and in early childhood [28–30]. Epigenetic studies in asthma often focus on changes in DNA methylation patterns, which can be examined by epigenome-wide association studies (EWAS). Other epigenetics studies may include the investigation of patterns of histone modifications or noncoding RNAs. Epigenetics caught the interest of asthma researchers because it is thought to mediate the link between environmental exposures and asthma disease. This also highlights why studying genetic alterations solely might not provide a complete picture, when environmental exposures are not considered [31].

Large-scale EWAS studies revealed that environmental exposures such as prenatal smoking or air pollution were associated with changes in DNA methylation patterns of several asthma-related genes [28, 29]. A meta-EWAS analysis of 8 newborn cohorts and 9 children cohorts showed differential blood DNA methylation profiles at several CpG sites, which were associated with the risk of school-age asthma [32]. DNA methylation profiles of nasal or airway epithelial cells were found to be different between asthmatics and healthy controls at levels surpassing those normally seen in blood [33–35]. Therefore, the selection of the specific tissues or cells for analysis is important for the appropriate interpretation and reproducibility of results [33].

Studies assessing histone modifications are less frequently conducted compared with DNA methylation studies. Asthmatic airway smooth muscle cells showed increased histone 3 acetylation and binding of histone acetyltransferase p300 compared to smooth muscles samples of non-asthmatics, which provides clarification of how CXCL8 is dysregulated in asthma [36]. In addition, histone deacetylase 9 (HDAC9) mRNA expression levels were associated with asthma disease severity [37]. In another study, asthmatic patients' airway smooth muscle cells showed abnormal histone methylation, which was linked to increased vascular endothelial growth factor (VEGF) secretion compared to non-asthmatics cells, and this abnormality was not observed at histone acetylation or DNA methylation levels [38].

In summary, epigenetics may mediate the link between the asthma disease process and different environmental exposures. Several epigenetic changes at DNA methylation or histone modification levels were observed between asthmatics and healthy controls. 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 targets for treatment.

Transcriptomics

Many transcriptomic asthma studies covering a wide range of tissue and cell types have been conducted within the last decade [39]. They are concerned with comparing transcripts of cells/tissues between asthmatics and healthy controls or between different asthma phenotypes to identify changes in genetic expressions that can be linked to functional hypothesis or biomarker discoveries. Transcriptomic approaches in asthma encompass two main techniques: gene expression microarrays and RNA-sequencing (RNA-seq). Microarrays were most

widely used in asthma due to their relatively low costs. Compared to microarray, RNA-seq was found to provide a wider dynamic range, more sensitivity, and more ability to detect biological isoforms [40]; however, it is costlier. Choosing a sample compartment for transcriptomic analysis is critical since different tissues and cells have distinctive transcriptome profiles [41].

The transcriptome profiles from different tissues/cells have provided significant insights on the role of gene expression in the asthma disease process. A study in healthy controls and mild, moderate, and severe asthmatics showed that $CD3^+$ T cells isolated from sputum and bronchoalveolar lavage fluid (BALF) had a distinct transcriptome profile from endobronchial epithelial brushings [42]. In the same study, sputum T cells in severe asthmatics showed most of the differentially expressed genes (DEGs) when compared to healthy controls [42]. Blood microarray analysis from 610 adults recruited in the U-BIOPRED study found a total of 1693 DEGs in patients with severe asthma compared to healthy controls [43]. In the same study, cluster analysis of DEGs revealed severe asthma subgroups with different response to oral corticosteroids [43]. In another U-BIOPRED study using sputum transcriptomic profiles of 418 adult asthmatics, cluster analysis revealed 4 clusters with distinct demographics and clinical characteristics such as asthma onset, severity, level of control, spirometry, smoking history, weight, and gender [44]. Unsupervised clustering of sputum DEGs from 104 adult moderate-to-severe asthmatics and 16 controls in the U-BIOPRED revealed 3 clusters that were associated with different inflammatory biomarkers and cytokines, with one cluster defined as Th2-high eosinophilic phenotype [11]. In addition, the SARP study analyzed the airway epithelial cell transcriptome from 155 asthmatic and healthy controls by weighted gene co-expression network to identify genetic networks and profiles associated with severe asthma [45]. Genes linked to neuronal function and epithelial growth and repair were prominently decreased in severe asthmatics [45]. A more recent U-BIOPRED study revealed that mRNA expression profiles of induced sputum, nasal brushings, and endobronchial brushings/biopsies significantly differentiated between adult-onset versus childhood-onset severe asthma, suggesting distinct molecular mechanisms underlying the two disease phenotypes [46].

Linking genomics to transcriptomics can provide more information on how genetic alterations (e.g., SNPs) can modulate genetic expression and, therefore, may uncover molecular mechanisms that underlie the complex asthma disease process. Genome-wide expression quantitative trait loci (eQTL) studies have emerged to investigate genomics–transcriptomics associations in asthma [47]. A large eQTL study conducted on 1111 human lung samples identified a strong association on locus 17q21 and mRNA expression levels of *GSDMA* [48]. *In the same study, a* suppressor of the cytokine signaling 3 (SOCS3) pathway was identified, through a genetic-gene expression network, as one of the important drivers of asthma [48]. *A combination of GWAS and lung eQTL analysis resulted in identification of some genes such as ABI3BP, NAF1, and MICA, which may play a role in the asthma disease process* [49]. *In a recent study, a cis-eQTL allele was found to reduce CHI3L1 mRNA expression, which was associated with late-onset adult asthma in a Japanese cohort* [50].

In summary, transcriptomics reflects the active status of functional genes in specific tissue/cells, which could be directly linked to asthma variability. In addition, it represents a mediating omics layer to study the associations between genetics and other omics; therefore, it can be considered as a principal bridging component in multi-omics-based asthma research. However, special focus should be given to the type of tissues or cells for which gene expression has been measured since the obtained signal can differ.

Proteomics

Proteins play a significant role in cellular processes, and their levels reflect the momentary state of tissues/cells at the time of investigation. Analytical proteomic techniques allow the characterization, identification, and quantification of proteins and their associated functions. Advances in proteomics research are improving the ability to take snapshots of the proteome while considering its static condition and dynamic response to space and time [51]. Different methods of proteomics are available, which can roughly be subdivided into immunoassay-based methods (e.g., ELISA, immunohistochemistry, and Western blot), mass spectrometry (MS)-based methods (e.g., tandem MS, electron capture, or electron-transfer dissociations), and protein microarrays methods. Generally, microarrays- and MS-based methods are more recent and allow identification of a larger portion of proteins at higher sensitivity, compared to immunoassay-based methods [52, 53].

Characterizing the proteome has provided essential information regarding the asthma research field. The SARP study could predict asthma phenotypes through the proteome profiles of a panel of 25 BALF cytokines from 84 asthmatics [54, 55]. A more recent SARP study conducted on 158 asthmatics found that protein profiles of BALF cytokines/chemokines were associated with asthma severity and with granulocytic inflammatory cells, especially neutrophils [56]. In U-BIOPRED, sputum proteomics showed that 10 out of 1129 proteins were significantly different between 4 previously established clinical asthma clusters [44]. Moreover, the sputum proteome profiles of adult U-BIOPRED asthmatics were distinguished between current, ex-smokers, and nonsmokers [57]. In the same study, RNA expression profiles of bronchial epithelial cells obtained from current and nonsmoking

asthmatics were significantly different according to pathway, gene set variant, and protein–protein interaction analyses [57], suggesting a link between transcriptomics and proteomics. A more recent U-BIOPRED study revealed that topological data analysis (TDA) of sputum proteomics from 246 subjects (206 asthmatics) identified 10 sub-phenotypic clusters, which were linked to granulocytic inflammation [58].

Like transcriptomics, it is important to note that proteome collected from a single body compartment/site or at certain moment (e.g., exacerbation, stable, etc.) will not provide the information on the complete dynamic proteome linked to the disease process [59]. Thus, comparative evaluation of the information collected from different sample sites and in different disease conditions and/or times may be needed to get more comprehensive information about the asthma–proteome link.

In summary, airway proteome profiles in asthma patients can be associated with different phenotypes. The importance of proteomics is that it can represent the active cellular state of different tissues/cells. However, adequate attention should be directed to tissue-associated proteome differences in comparative and possibly longitudinal studies.

Metabolomics

Metabolomics concerns with the study of low molecular weight organic compounds (50–1500 Da) that originate from the human-/microorganisms-related metabolism and are involved in biological processes. The state of metabolome profiles is a consequence of multiple factors such as genetic expression and associated changes in proteome, environmental exposures, and microbiome among others. Therefore, their characterization is important to reflect the combined effect of multiple factors in asthma.

The techniques used in metabolomics belong mainly to gas or liquid chromatography coupled with MS. Another method is nuclear magnetic resonance (NMR) spectroscopy, which has lower sensitivity and specificity compared to MS-based techniques and therefore requires higher analyte concentrations [60, 61]. Metabolomics research in pulmonary disorders has been conducted using different types of biological samples [62]. The advantages and disadvantages of most common sampling compartments in asthma metabolomics are summarized in Table S1.

Most conducted metabolomics studies in asthmatics focused on investigating metabolomics profiles in comparison to healthy controls, other respiratory disorders such as COPD, or to distinguish different asthma phenotypes. The main identified metabolites across different studies from various body compartments were related to immune reactions, inflammatory processes, tricarboxylic acid cycle, oxidative stress, hypoxia, and lipid metabolism pathways [63]. However, some limitations related to low sample sizes, different study populations, lack of standardization of analytical methodology, and absence of external validation make biomarker discovery and direct clinical applicability of metabolomics research challenging.

One of the emerging metabolomics techniques in asthma research is the measurement of volatile organic compounds (VOCs) in exhaled breath (breathomics). Techniques often used to measure VOCs are either gas chromatography/mass spectrometry (GC/MS) or electronic noses (eNoses). Using GC–MS-based methods, VOCs have been identified in asthmatics, which discriminated them from healthy controls and/or were linked to asthma disease characteristics [64, 65]. However, many of those identified VOCs were not replicated across different studies. In contrast, eNoses are not able to identify specific VOCs, but, rather, they are composed of cross-reactive sensor arrays that can detect signal patterns of VOCs mixtures without identification of single VOCs [66]. Interesting results have been reported for eNose studies in asthma. For instance, unsupervised clustering on eNose data of asthmatic and COPD patients revealed 5 distinct clusters which differ according to body mass index, ethnicity, atopy, fraction of exhaled nitric oxide (FeNO), exacerbation rate, and blood eosinophils and neutrophils counts [67]. In a U-BIOPRED study, unsupervised clustering on eNose profiles revealed 3 clusters, which differ in blood neutrophils and eosinophils percentages and oral corticosteroids intake [68]. Since eNoses are small, fast, easy to use, and can be connected to spirometry devices, they have a great potential to be used as point of care tools in physicians' offices [69, 70]. However, standardization of certain analytical devices and validation of findings are of utmost importance for implementation in clinical practice [65].

In summary, metabolomics research especially breathomics is expanding very rapidly in asthma. Using real-time breathomics techniques such as the eNoses provides opportunities for future applicability of omics in asthma diagnosis. However, analytical standardization and validation of findings are essential steps to move forward with applicability in direct patient care.

Microbiome

High-throughput sequencing studies investigating the microbiome–asthma associations started to emerge within the last decade. Microbiome investigations are divided into two main approaches: 16S ribosomal RNA sequencing or shotgun metagenomics. The earlier is less costly and less computational intensive compared to metagenomics and therefore used more frequently. However, it has lower potential to detect microbial taxa up to species level [71].

Microbiome research has disclosed some interesting associations between airway microbiome and asthma. For example, studies have shown that asthmatics had airway microbial dysbiosis compared to healthy controls, which was

characterized by enrichment in *Proteobacteria* phylum and deficiency in *Bacteroidetes* and *Actinobacteria* phyla [72–78]. Regarding asthma inflammatory phenotypes, neutrophilic asthma seems to be the most associated with microbial dysbiosis. Some studies reported that neutrophilic asthma patients exhibited higher airway abundances of *Proteobacteria* phylum, especially *Haemophilus* and *Moraxella* genera, and had lower bacterial richness and diversity compared with non-neutrophilic, eosinophilic, or paucigranulocytic asthma phenotypes [79–81].

In addition, the gut microbiome plays a very important role in the asthma disease process through the "gut-lung-axis." Gut microbiota ferment dietary carbohydrates to produce short-chain fatty acids (SCFAs) [82] that partly escape metabolism and exert effects on distal organs including the lungs. High vegetable fiber intake (source of SCFAs) was protective against allergic asthma in 476 children aged 11–14 years old [83]. In addition, children who had the highest levels of butyrate and propionate short-chain fatty acids (SCFAs) in their stool at 1 year of age were less likely to develop asthma at 3 and 6 years of age [84]. The levels of SCFAs were positively associated with the consumption of vegetables, fruits, yoghurt, and fish [84]. Moreover, SCFAs, especially butyrate, can promote epigenetic modulations [85, 86] which consequently may protect against airway inflammation [87, 88]. In addition, the microbiome was found to influence the production of different cytokines and chemokines that regulate the immunological and inflammatory processes within the lung [89, 90].

The microbiome has also been linked to additional omics layers such as host transcriptomics. An integrative study of nasal microbiome and nasal human transcriptomics conducted in young adults and children aged 6–20 years old revealed that *Moraxella catarrhalis* bacterial species was more abundant in asthmatics compared to controls, which was associated with a distinct RNA expression signature by the host [91]. In a similar study, the host RNA expression of the upstream regulator IL1A was significantly associated with nasal *Proteobacteria* abundance in asthmatic children [92]. This demonstrates the importance of studying the microbiome in relation to host genetics, epigenetics, and environmental exposures, especially diet intake.

We still do not completely know the "type" and "direction" of the link between microbiome and asthma, whether it is just an association or a causal relationship and whether it is a uni- or bidirectional effect. Elucidating more of the characteristics of this relationship will help to define diagnostic/therapeutic targets.

In summary, microbiomics is a fast-emerging research area, also in asthma, which is carried out to understand the long-lasting host microbiome–environment disease interaction. Microbial dysbiosis has been reported in the airways and intestines of asthmatic patients. In addition, distinct airway microbial profiles have been associated with inflammatory phenotypes, especially neutrophilic asthma, which highlights its importance in refining the classification of this phenotype. The association of microbiome profiles with host transcriptomics suggests that it has a genetic background, which may be later influenced by different environmental exposures.

Exposomics (environmental exposures)

Environmental factors, which might influence asthma risk, are numerous, ranging from lifestyle-related factors such as diet, smoking, exercise, and stress to environmental exposures related to pollution, allergens, occupational factors, as well as many others. Environmental exposures have always been of great interest to researchers of asthma. For instance, allergens-induced asthma was reported from prehistoric times and is considered as one of the very first asthma phenotypes studied [93]. To date, new findings related to environmental exposures with relation to asthma are reported [94–96].

Linking the exposome to other omics layers is now the focus of some asthma research groups. An example is the EXPOsOMICS consortium that investigates short- and long-term exposures to air and water contaminants and their biological effects on chronic diseases (including asthma) using a multi-omics approach [94, 97].

There are numerous studies, which investigated the associations between different environmental exposures and asthma. Providing a comprehensive summary of them is out of the scope of this review. For this review, we selected recent studies that investigated environmental exposures linked to asthma risk, development, or morbidity, taking omics into account. For example, prenatal exposure to nitrate air pollution was associated with reduced lung function in school-age children and increased asthma risk, especially in boys [98]. Prenatal nitrate exposure was found to be associated with blood differential DNA methylation and mRNA expression involved in antioxidant defense pathways [29]. Another study investigated genome-wide interaction of air pollution exposure and childhood asthma which found that nitrate exposure was associated with DNA methylation and expression of genes such as *B4GALT5*, *ADCY2*, and *DLG2* that could play a role in the pathogenesis of childhood asthma [99]. These studies highlight that environmental air pollution may produce its effects through an epigenome–transcriptome-mediated link. Others have shown that adult-onset asthma was associated with chronic air pollution (ultrafine particles) exposure, which was probably mediated by multiple metabolic pathways [94], and this suggests a strong exposome–metabolome link. Short-term exposure to particulate air pollution was found to be associated with nasal microbial dysbiosis in healthy subjects [100], which also suggest an exposome–microbiome relationship. Whether this relation can affect asthma development needs to be determined in further studies. In addition, the type of diet seems to influence asthma development. Western diet was suggested to promote a systemic pro-

inflammatory condition by lack of antioxidants and abundance of saturated fatty acid, which may cause innate immune activation, in contrast to a healthy diet, which was suggested to promote an anti-inflammatory state [101]. Reports have also shown that diet can affect risk of asthma at prenatal period. For example, a meta-analysis of 10 studies showed that vitamin D intake during pregnancy may reduce the risk of children on wheezing and on developing asthma later in life [102]. On the other hand, drug intake such as antibiotics during early childhood was associated with a greater risk of asthma later in life [103]. Moreover, the residential location has been shown to influence risk of asthma. Living and upbringing at farms and exposure to livestock were associated with a protective effect against asthma development [104, 105], which could not be explained by selective migration [106].

In summary, environmental exposures are connected directly or indirectly to other omics and with asthma. Studying environmental exposures in the context of other omics layers will be valuable to identify underlying pathological mechanisms associated with asthma.

Comprehensive omics insights, integrative omics, and single-cell omics

Integrative omics (multi-omics) in asthma is the process of combining the information of multiple omics layers, to get more insight in the asthma disease process. Most conducted attempts so far tried to pair the information of two omics layers, such as eQTL studies, and fewer attempts tried to integrate more layers. Figure 2 shows the sequence of integrating different omics layers. Integrating the information of more than two omics layers will provide better prospective on which combinations of omics layers will provide the appropriate harmony to understand underlying molecular mechanisms. It is preferred that integrating omics should be performed on layers with complementary information; otherwise, introducing more data noise might obscure important findings by less omics. Generally, it is believed that the more omics layers we integrate (more power), the less sample size will be needed. This is because complementary information obtained from multiple omics will overcome the need to sample more patients to reach significance levels of one layer's findings. An example is the study on omics integration which showed that combination of 5 to 7 omics layers leads to 100% correct classification of COPD patients with groups as small as 6 subjects [107]. In the same study, permutation and cross validation were implemented as methods to handle the risk of overfitting [107], which might be challenging in multi-omics studies when the number of predictors exceeds the sample size.

Several methods have been developed to help the integration of multiple omics layers together. Briefly, data analysis techniques are generally categorized into supervised and unsupervised approaches [108]. Supervised approaches mainly comprise several machine learning algorithms that classify or predict subjects based on previously defined subjects' labels (e.g., asthma versus healthy). On the other hand, unsupervised approaches, such as similarity network fusion [109], iClusterPlus [110], PINSPlus [111], and Spectrum [112], cluster the patients based on their similarity in the multi-omics profiles. One major drawback is that there is currently no gold standard "technique" that will provide the most robust clinical/statistical findings for the data under investigation. There is no single method, which always outperforms the

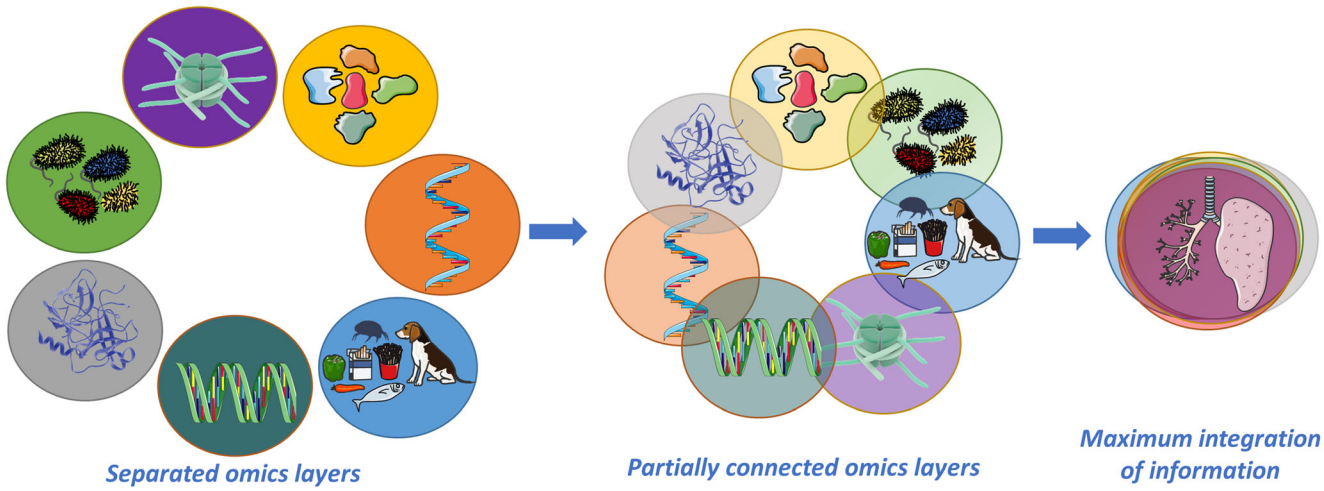

Fig. 2 Integrating multiple omics layers. Omics research in asthma started by investigations of separate omics layers (e.g., genetics, transcriptomics, microbiomics). Connecting two or more layers will likely reduce interdisciplinary gaps and allow better understanding of the underlying biological pathways. Maximizing the information obtained from different layers will eventually lead to more understanding of the multifactorial disease process of asthma. *Some of the drawn objects were adapted from Servier and Somersault18:24 corporations, which are used under creative commons license*

others, and different methods can study the data from different angles. Therefore, research groups should choose the ones that are suitable for the data features. Unsupervised techniques can be more valuable, especially in the exploratory phases of the analysis, because they do not assume any priori assumptions and therefore may eliminate the risk of bias. We suggest reading the article by Huang et al. [108] for a more comprehensive overview on the multi-omics data analysis techniques.

All previous discussed approaches characterize the omics in tissues or cells in bulk, while newer approaches try to investigate multiple molecular signals at a single-cell level (single-cell omics) [113]. This provides a more comprehensive understanding of molecular and pathophysiological processes at cellar level such as cellular state, dynamics (transition), and heterogeneity. Similar to the "bulk" omics approaches, it is possible to characterize the (epi)genome, transcriptome, proteome, and metabolome of a single cell after isolation and signal amplification and build a multi-omics profile for this cell (single-cell multi-omics) [114]. In asthma, very recent findings using single-cell transcriptomics revealed novel cell types and altered cellular states of asthmatic airways, which may provide new insights in the asthma pathogenesis [115, 116].

Challenges and limitations

Despite the growing importance of omics in the asthma research field, there are various challenges (Table 1) that may hinder appropriate analysis, interpretation, and clinical application of omics. Fulfilling them will help to quickly advance with omics in research and clinical applicability.

The future of omics in asthma: where should omics research in asthma go?

Asthma should be considered as a complex dynamic system consisting of several interacting units, rather than a single component structure. The transition of omics from being highly expensive to be relatively cheap and routinely performed in research labs [121] made it possible to be extensively investigated in asthma. The use of omics in asthma research has revealed an encouraging trail for the elucidation of the complex asthma disease process. An example is the AsthmaMap project, which focuses on comprehensive description of asthma mechanisms by showing links between several molecular

Table 1 Methodological, practical, and ethical challenges/limitations that might hinder knowledge transfer from bench to bedside

Challenge		Recommendation
Methodological	Risk of overfitting/data dimensionality when there are too many parameters for a relatively small sample	Techniques for dimension reduction or feature selection including cross validation should be used to overcome risk of overfitting, data noise, or highly correlated data
	Lack of power in omics studies	Multi-omics integration might lead to more power
	Lack of standardization of different sampling and analytical procedures	Implementation of centralized biobanks can help to increase the reproducibility of omics projects and integrity of scientific research by developing standardized protocols and/or operating procedures (SOPs) for sample collection, storage, and data sharing within different centers [117, 118]
	Lack of validation/replication of findings across different cohorts	The method of findings validation should be described in the analyses strategies of new or ongoing projects Centralized biobanks can help in the validation of findings by limiting variability associated with methodological conduct
	Commonly applied cross-sectional design or relatively short follow-up period might not capture complexity and dynamic nature of asthma	Longitudinal approach might reveal more about the biological dynamics that can occur overtime in asthmatics
Practical	High costs of omics studies may delay replication and validation of results in external cohorts	Combined efforts and resources of many international centers are needed for the success of multi-omics projects
	Lack of adequate supervision, frequent monitoring, objective feedback, and work management across different centers participating in joint omics projects may lead to work delays, methodological inconsistencies, and data or information loss	Working under a centralized harmonized work frame, such as under a consortium, with a principal coordinator may ensure appropriate monitoring and work harmonization within different centers
Ethical	Ownership and privacy of multi omics data sharing	Worldwide healthcare organizations and stakeholders should thoroughly discuss and unite (as possible) ethical legislation in a way that ensures progression and advancement of omics studies while securing patient data and keeping them integrate. An attempt is being done by the EU General Data Protection Regulation (GDPR) in order to harmonize data privacy across Europe [119]. However, this might influence biobanking and data sharing [120]

and cellular pathways [122]. Gathering the information on a multi-omics prospective will provide more insight into the role of different omics layers in the asthma disease process, especially if implemented in a longitudinal context. This can be further elaborated on by the application of systems biology. It may help to uncover interconnections of different omics layers in the context of different factors and disclose underlying molecular pathways and biological networks and possible new targets for treatment. Application of such complex approaches needs combined efforts from several multidisciplinary research teams working in close contact under harmonized structures. The transfer of knowledge from bench to bedside may become possible by using the omics information in a systems biology approach to characterize different asthma phenotypes for which different therapeutic targets can be directed. However, important criteria such as cost-effectiveness, feasibility, and rapid applicability in patient care are still to be determined in the future.

Several questions need to be answered to bring omics into the clinic. One of the questions to consider is "What are the optimum analysis techniques to analyze omics?". It is important to standardize techniques that can be used to best identify/predict patient phenotypes/endotypes by omics. Picking techniques randomly might obscure important signals, while conducting multiple techniques and only reporting ones with positive findings are considered "fishing" and are prone to bias. The analysis techniques should be suitable for the data under investigation and should be clearly described in the study protocol beforehand. "Do we need all of the omics/biomarkers together?" is another question that needs answering. Feature reduction and pinpointing the lowest number of biomarkers, preferably noninvasive and non-expensive, would help us to achieve the criteria needed for future omics clinical applicability. This will also help to characterize the gold standards needed in asthma-personalized care. Current possible omics examples that might have potential for future clinical application in asthma based on robust scientific evidence are exhaled metabolomics (e.g., eNoses), (pharmaco)genomics, and the microbiome that could be used as omics-guided care. eNoses (exhaled breath) have been shown in several studies to have high diagnostic accuracies in diagnosing asthma and/or asthma characteristics [65, 123]. With their real-time capability and feasibility to be integrated with conventional spirometry devices [69], they would be a feasible companion in the physician's office. However, as stated previously, appropriate validation is still required for direct implementation. In other fields, pharmacogenetic testing for the response and adverse reaction of various drugs has already made its way into some clinical settings. This has been achieved by using genetic chips that can identify hundreds of genetic variants (SNPs) related to drugs' response and/or adverse reactions [124, 125]. Designing chips that are customized for the asthma patient might be helpful to tailor therapeutic decisions in asthmatics. Likewise, recent advances in high-throughput technologies now allow sequencing and assembly of ultra-long human genetic reads using relatively cheap, pocket size, devices [126]. This may help in the future to facilitate identification of genetically linked treatable asthma traits in routine practice. In addition, identifying microorganisms in asthmatics that are up- or downregulated (abundant/deficient) and linked to different asthma characteristics might help to elucidate microbiome signatures that can be used in diagnostic and therapeutic asthma decisions [89]. Using multiple combined biomarkers identified by different omics may enhance applicability and precision of omics-guided care.

Although most scientists focus on reporting significant results, an important criterion that should be considered is the clinical relevancy of the omics findings: "Are they strong enough to be reflected clinically? Or are they mainly interesting for research purposes?" An example in genomics research is the low cutoff p values set to find significant SNPs. Although this is important from statistical point of view, a focus should be directed to the effect sizes of these significant SNPs [22]. This applies for other omics as well.

Once omics could be proven to be applicable in direct patient care, a question remains "Would they be cost effective?" and "When?". Studies for comparing cost-effectiveness of future omics-guided care to routine practice will be needed. The reduction of the costs of omics analysis with ongoing technological advances is encouraging in terms of future cost-effectiveness but should still be linked to clinical added value of implementing omics.

In summary, some of the omics have started to show potential to enhance the precision care for the asthma patient. Some unanswered questions remain which introduce knowledge gaps. Resolving them will define the optimum path to approach omics in asthma.

Concluding remarks

Omics studies in asthma are providing a promising tool in terms of understanding asthma. It is still in an early phase to provide direct clinical applicability for precision medicine, but in the future, there is a notable chance. The integration of information from multiple omics layers is what will help us to move forward in understanding the complex asthma disease features. However, this comes at the expense of tremendous costs, computational, and man powers required to run such projects. In addition, high expertise and multiple disciplinary teams are needed for adequate application of mathematical modeling and computational tools and for the appropriate interpretation of the results.

What is still not known?/future directions of omics research in asthma

1. Single-cell multi-omics to investigate the heterogeneity of omics signals of single cells and whether there are specific cells that may enhance complementation of other omics or better represent the asthma disease process. What this new knowledge will bring us in regard to more precise insights in the pathophysiology/phenotyping, target finding, and clinical application for diagnostics and precision treatment? "To what extent cells differ?"
2. Lowest number of omics needed to adequately refine asthma phenotypes. "Do we need all omics together?"
3. What would be the most optimum analysis techniques in multi-omics research? "Should we just pick one by random?"
4. How strong are effect sizes for omics in asthma? Could this be pathophysiologically or clinically relevant? Or mainly interesting for research purposes? "How much could they explain?"
5. Future cost-effectiveness of omics techniques in direct patient care. "Is it worth the trouble?"
6. The direction of the link between certain omics such as genetic associations or microbial dysbiosis and asthma characteristics. "Is it just an association or causation?"

Compliance with ethical standards

Conflict of interest AHM has been reimbursed for visiting the ATS by Chiesi, received a fee for participating in advisory boards for Boehringer Ingelheim and Astra Zeneca, and received an unrestricted research grant from GSK. ADK received grants/research support from several companies of Janssen, GSK, Nutricia Research, Friesland Campina, and NTRC.

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Table S1: Advantages and disadvantages of some biological samples used in metabolomics research of asthma.

Biological sample	Advantages	Disadvantages/precautions	References
Blood/plasma/serum	• Minimally invasive • It is a central compartment which connects tissues and fluids together, so measurement of metabolites provides a comprehensive representation of the whole body metabolome	• High protein and salt content require samples preprocessing. • Sample collection method, tubes, clotting time and temperature can affect the metabolome • Not directly linked to lung metabolome	[1-3]
Urine	• Non-invasive. • Relatively low concentrations of cells and proteins • Suitable for all age groups • Minimal pre-treatment for required for nuclear magnetic resonance (NMR) analysis	• High salt concentration requires samples preprocessing • Metabolome can change with diurnal variations or timing of samples collection. • Special patient instructions such as “midstream fast void urine sample” should be given to subjects • Not directly linked to lung metabolome	[1, 2, 4]
Bronchoalveolar lavage fluid (BALF)	Good representation of the airway metabolome	• Invasive • Exogenous fluid is introduced to the alveolar space, thus requires concentration • Samples preprocessing is required • Potential plasma leakage to airways • Risk of contamination during aspiration	[2, 3, 5]
Induced sputum	• Can be representative of the lung metabolome • Standardized protocols for samples collection are available	• Can be invasive or not suitable for very severe asthma patients • Viscosity and different layers have to be dealt with • Samples preprocessing is required • Salivary contamination can occur	[5-7]
Saliva	• Non-invasive	• Some confounding factors,	[3, 8]

	• Easy to collect • Relatively low concentrations of cells and proteins	such as gender differences and smoking status should be accounted for	
Exhaled Breath/ Exhaled Breath Condensate (EBC)	• Non-invasive • Low protein and cellular content • Can detect volatile organic compounds	• Very dilute samples which requires concentration • Highly subject to variability and confounders such as smoking, alcohol and diet consumption, temperature, humidity, equipment, and others	[2, 3, 5, 9, 10]

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