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EDITORIAL

Metabolomics in the prevention and management of asthma

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1. Overview of metabolomics

Asthma is a complex syndrome that is influenced by both genetic and environmental factors. Determination of its pathobiology requires investigations on the outcome of transcriptional and translational processes, such as a comprehensive analysis of metabolites. Metabolites are a function of the one's genetic make-up and environmental influences which provide approximate information of a biosystem's functional state [1]. The recent advent of several analytical techniques has enabled us to simultaneously examine the global collection of small molecules, including sugars, amino acids, organic acids, and lipids (generally <1,800 daltons), in an organism, tissue, or cell – i.e. the metabolome [2]. Metabolomics is the systematic analysis of the group of functional metabolites that are present in a biological system [2]. There are three major analytical techniques used in metabolomics: nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS) [3]. Metabolomics is generally applied through either a targeted or global (or untargeted) measurement approach [3]. Targeted metabolomics is a quantitative approach that allows the measurement of specific metabolite concentrations. In contrast, global metabolomics undertakes a simultaneous assessment of metabolites without *a priori* sample knowledge for hypothesis generation [3]. Global metabolomics is a comprehensive strategy for identifying changes in different pathophysiological states. However, a stringent significance threshold (e.g. Bonferroni or false discovery rate) needs to be applied to account for multiple testing and the discovered metabolites need to be validated in an independent cohort to avoid potential false-positive findings. Additionally, metabolomics studies generate highly collinear and sparse data which may not be handled well by most conventional statistical models. This constitutes statistical and methodological challenges, particularly when combining with another omics data. Thus, choosing appropriate statistical methods for analyzing metabolomics data – such as partial least squares-discriminant analysis (PLS-DA), sparse PLS-DA, and dimension reduction approaches (e.g. principal component analysis) – is essential [4]. Please see Table 1 for a glossary of common terms used in metabolomics.

2. Evidence on metabolomics and asthma etiology

Despite the clinical and public health importance of asthma [5], the molecular determinants of asthma pathogenesis are not yet fully understood [6]. One of the most important genomic regions that are associated with asthma development is chromosome 17q21. This region contains the genetic variants that regulate *ORMDL3* gene expression, which plays an important role in sphingolipid metabolism. Indeed, Zhang et al. showed, by using a human lung epithelial cell model, that siRNA knockdown of *ORMDL3* modulates the activity of serine palmitoyltransferase which is involved in the rate-limiting step of the production of sphingolipids and increases sphingolipid metabolism, such as increased ceramides and sphingosine-1-phosphate (S1P) level [7]. This finding is consistent with prior findings of *ORMDL3* and sphingolipid metabolism in mouse airway epithelium [8] and *ORMDL3* transgenic mice models [9]. Also a recent study by Kelly et al. suggested that prenatal vitamin D supplementation may lower the risk of recurrent wheeze by age 3 years through alterations of sphingolipid metabolite levels depending on the *ORMDL3* genotype [10]. Additionally, our recent studies demonstrated that increased levels of sphingolipid metabolites in infants' nasopharyngeal airway and serum are associated with higher bronchiolitis severity – one of the most important risk factors for asthma development [11,12]. Furthermore, a cross-sectional study of 325 Costa Rican children with asthma used transcriptomic and metabolomic data, and discovered associations of *ORMDL3* and dysregulated sphingolipid metabolism in blood with impaired lung function [13]. In addition to the host-derived metabolites, the microbe-derived metabolites may also play a role in the asthma pathobiology. For example, Levan et al. reported that gut microbes produce 12,13-diHOME – a metabolite that decreases the number of regulatory T cells in the lungs and promotes airway inflammation in asthma [14]. Together, these studies suggest an integrated role of host genetics (e.g. *ORMDL3* gene), microbiome and their related metabolites in the development of childhood asthma.

3. Evidence on metabolomics and asthma phenotypes

Asthma research increasingly emphasizes the heterogeneity of asthma. As metabolites are inherently sensitive to subtle alterations in biological pathways, metabolomics can provide insights

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Table 1. Metabolomics glossary.

Collinearity	A statistical condition that two variables (e.g. metabolites) linearly correlated with each other.
Dalton	A mass unit to measure weight of small molecules.
Gas chromatography mass spectrometry (GC-MS)	An analytical chemistry technique that combines gas chromatography and mass spectrometry to separate and analyze volatile and semi-volatile compounds.
Global metabolomics	The comprehensive screening of large number of metabolites without a specific hypothesis.
Liquid chromatography mass spectrometry (LC-MS)	An analytical technique that combines liquid chromatography and mass spectrometry to separate molecules according to hydrophobicity or hydrophilicity.
Metabolome	The collection of metabolites in a biological system.
Metabolomics	The systematic analysis of small functional molecules – metabolites (e.g. sugars, amino acids, organic acids, and lipids, excluding nucleic acids and proteins) – within cells, biofluids, tissues, or organisms.
Multiple testing	Any instance that involves the simultaneous testing of more than one hypothesis.
Nuclear magnetic resonance (NMR) spectroscopy	An analytical technique that uses the magnetic properties of atomic nuclei to determine the physical and chemical properties of atoms or molecules.
Partial least squares discriminant analysis (PLS-DA)	A statistical method that identifies the differential metabolite concentrations between two groups of patients, with superior handling of highly collinear and noisy data; PLS-DA is commonly used in metabolomics analyses.
Sparsity	A condition that data contain relatively high proportion of null or zero values.
Targeted metabolomics	The quantification and identification of one metabolite or a small number of metabolites with a certain hypothesis.

into biomarker discovery and pathophysiological mechanisms that underlie different phenotypes. Indeed, several small studies – by applying metabolomics approaches to plasma, urine, and exhaled breath condensate – have identified distinct metabolome profiles in different asthma phenotypes (e.g. high fractional exhaled nitric oxide (FE_{NO}) and corticosteroid-refractory asthma) [1]. For example, linoleic acid – an ω-6 polyunsaturated fatty acid – was found to play roles in corticosteroid resistance in patients with asthma. Panda et al. reported, in allergic asthma mouse and human bronchial epithelial cell models, that 13-S-hydroxyoctadecadienoic acid (HODE) – 12/15–lipoxygenase metabolite of linoleic acid – increases pNF-κB levels and reduces GR-α transcripts, thereby inducing corticosteroid resistance [15]. Similarly, arachidonate (another ω-6 polyunsaturated fatty acid) is a precursor of the arachidonic cascade that produces lipid mediators (e.g. prostaglandins and leukotrienes), thereby contributing to airway inflammation [16]. Thus, targeting these metabolites may be beneficial to successful resolution of airway inflammation in asthma [17]. Furthermore, a large cohort study also reported that ω-6 polyunsaturated fatty acids levels in the nasopharyngeal airway of infants are associated with a specific immune response profile (e.g. low 25-hydroxyvitamin D levels) and higher severity of bronchiolitis – both of which are potential risk factors for the development of wheezing illness and possibly asthma in childhood [18]. While the current asthma phenotyping relies heavily on conventional clinical and laboratory data (e.g. presence of atopic diseases, IgE sensitization), metabolomics – along with other molecular biomarkers – are likely to provide further insights into the identification of asthma phenotypes and their underlying mechanisms.

4. Potential interventions based on metabolomics

4.1. Primary prevention of asthma

The primary prevention of asthma depends on early (e.g. infancy) identification of modifiable risk factors (e.g. metabolism pathways) that precede asthma inception. Previous studies have indicated a potential role of sphingolipid metabolism in the development of childhood asthma [13]. Sphingolipids are not only major components of the cell membrane but also serve as signaling molecules involved in immune response, inflammation, cell proliferation, and apoptosis [19]. Therapeutic drug and dietary interventions are starting to target the sphingolipid metabolism pathway. For example, in an asthma mouse model, the use of sphingosine kinase inhibitor attenuated airway inflammation and hyperresponsiveness [20]. Similarly, in another allergic asthma mouse model, inhibitions of sphingosine kinase reduced proinflammatory NF-κB and suppressed airway inflammation [21]. Additionally, enhanced fruit and vegetable intake in young adults led to decreased levels of 24:0 ceramide (a sphingolipid pathway metabolite) and inflammatory cytokines, such as monocyte chemoattractant protein-4 and thymus- and activation-regulated chemokines [22]. While these studies provide tantalizing leads, an application of metabolomics to asthma prevention is at its developmental stage. Many confounding factors – such as demographics, diet, physical activity, and genetics – contribute to both metabolic variations and asthma pathogenesis, which have made omics-based investigations challenging in asthma. To disentangle these confounding effects and delineate the mechanisms of asthma development, large prospective cohort studies – with high-quality biospecimens – are essential.

4.2. Asthma treatment and secondary prevention

Development of effective asthma treatment and secondary prevention strategies involves several major challenges, such as identification of the responsible mechanisms (e.g. upregulation of lipid mediators), accurate risk stratification, and the heterogeneity of asthma itself. For example, a subset of patients with asthma requires high-dose-inhaled corticosteroids and sometimes chronic use of systemic corticosteroids. As these patients with corticosteroid-refractory asthma have large morbidity burden and healthcare use, the identification of such patients and underlying

mechanisms is essential. Several metabolites' pathways – e.g. tyrosine, glutathione, and linoleic acid metabolism – were known to be involved in corticosteroid responsiveness and severity [15,23]. For example, in an aforementioned study showing the role of HODE (a linoleic acid metabolite), its inhibition by pyrrolidine dithiocarbamate increased sensitivity to corticosteroids and reduced airway inflammation [15]. While these early observations require further investigations, they offer new avenues for risk stratification (e.g. early identification of patients at high risk for corticosteroids resistance) and treatment strategies (e.g. modulation of ω-6 polyunsaturated fatty acids and dietary interventions).

5. Next steps and future directions

To advance the metabolome science and clarify the role of metabolomics in the prevention and management of asthma, rigorous transdisciplinary research efforts – involving, for example, allergy/immunology, pulmonology, epidemiology, genomics, metabolomics, and systems biology – are critical. In addition to the Wayne County Health, Environment,

Allergy and Asthma Longitudinal Study (WHEALS) [14], Vitamin D Antenatal Asthma Reduction Trial (VDAART) [10], and Infant Susceptibility to Pulmonary Infections and Asthma Following RSV Exposure Study (INSPIRE) [24] cohort studies that are adept at generating high-quality science, through our cooperative agreement with the National Institute of Allergy and Infectious Diseases (U01AI87881 and R01AI127,507) and membership in the National Institutes of Health Environmental Influences on Child Health Outcomes (ECHO) Consortium (UG3/UH3 OD-023253) [25], our research team – the Emergency Medicine Network [26] – is currently conducting the 35th and 43th Multicenter Airway Collaboration (MARC-35/43) Cohort studies. MARC-35 is a prospective cohort comprised of 1016 racially/ethnically diverse infants who were hospitalized with bronchiolitis at 17 U.S. sites. MARC-43 is parallel prospective cohort comprised of 720 healthy infants at five U.S. sites. These studies are poised to address the role of many novel factors – e.g. respiratory viruses (including rhinovirus species), genome, epigenome, microbiome, proteome, and metabolome. Both studies collect high-quality biospecimens – e.g. nasopharyngeal aspirates, nasal swabs, blood, and stool – at multiple time points. Because quality assurance of sampling and processing is essential in metabolomics (and other omics) investigations, the cohorts have used a standardized collection protocol and applied thorough quality control [11]. Investigators follow these children with telephone interviews, review of medical records, and in-person exams at ages 3 and 6 years, with the latter focused on asthma diagnosis and its phenotypic characterization. Our team has already applied global metabolomics (with complex lipid profiling – lipidomics) to the nasopharyngeal airway and serum samples in the MARC-35 participants [11,12]. We aim to define the role of airway metabolome profiles during infancy – an important period of lung development and critical window for primary intervention – in the development of childhood asthma. Additionally, by integrating the comprehensive metabolome data with extensive clinical and multi-level omics data, we also aim to disentangle the interrelated contributions of genome (e.g. *ORMDL3* polymorphism), microbiome, and metabolome (e.g. altered metabolism of sphingolipids and ω-6 polyunsaturated fatty acids) to the different asthma phenotypes of these children. We will also validate these novel findings in independent cohorts, such as MARC-43 cohort and ECHO Consortium. These efforts will enable us to uncover the complex web of the genome, environmental factors, confounders, and mediators in the pathogenesis of asthma and its phenotypes, which will, in turn, guide the development of primary prevention and targeted treatment strategies for asthma.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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