Endotyping Asthma: Profiling the Metabolic Dimension?

Asthma has long been recognized as a heterogeneous disease, and the notion of discrete endotypes of asthma has been prevalent for more than a decade (1). However, despite the success in identifying and targeting type 2 inflammation in asthma, the identification of tractable endotypes has remained elusive, with not a single endotype defined with certainty.

In this issue of the *Journal*, Kelly and colleagues (pp. 288–299) have reported putative novel asthma endotypes, defined using metabolomics (metaboendotypes), in childhood asthma (2). The metabolomics included unbiased multiplatform metabolic profiling, using liquid chromatography and tandem mass spectrometry. The metabolic endotypes generated were replicated against clinical outcomes in an independent cohort, underscoring both the validity and the potential importance of the approach. The work is novel and important owing to the comprehensive and "agnostic" metabolomics approach, enabling the quantification of metabolic fingerprints that may reflect underpinning gene–environment interactions in asthma.

The study population included two independent (discovery/replication) childhood asthma cohorts. The Genetics of Asthma in Costa Rica Study (GACRS) ($n = 1{,}165$) included children aged 6–14 years. CAMP (Childhood Asthma Management Program) included children aged 5–12 years, with mild to moderate asthma (3). The two cohorts were well matched for both age and sex, which is important because of the phenotypic and life course changes associated with asthma and lung function, as children enter more advanced school age and puberty (4, 5). However, the cohorts differed significantly in terms of asthma treatment use; whereas ∼30% of the CAMP cohort were on inhaled budesonide, none of the GACRS cohort were on inhaled steroids. In addition, all of the GACRS cohort were Hispanic in ethnicity, whereas just over two-thirds of the CAMP cohort were of White ethnicity.

Despite the apparent differences in the two cohorts, five discrete metaboendotypes are reported in both cohorts, with the most consistent associations observed with both pre- and post-bronchodilator FEV_1% and FEV_1/FVC. Metaboendotype 3 demonstrated the best lung function, whereas metaboendotype 2 demonstrated the lowest lung function, although the overall numerical differences in lung function across the endotypes were very small indeed. Notable metabolic differences between these two endotypes identified by metabolite set enrichment/depletion analyses were depletion of hydroxy and unsaturated fatty acids, carnitines, and cholesterol esters, with enrichment of triglycerides in endotype 3. Endotype 2 was characterized by depletion of triglycerides, unsaturated phosphatidylcholines, lysophosphatidylcholines, and unsaturated fatty acids.

Overall, cholesterol esters, phospholipids, triglycerides, and long chain polyunsaturated fatty acids were among the most important drivers of metaboendotype membership. Intriguingly, although differences in the prevalence of a blood eosinophilia across endotypes were observed, no differences were seen in other relevant phenotypic traits, such as hospital admissions, emergency department visits, IgE level, or the presence of atopic dermatitis, across any of the five metaboendotypes.

Previous studies have demonstrated depletion of relevant airway/systemic phospholipids in association with reduced lung function in asthma (6, 7); these observations are concordant with the associations observed with lung function in both endotypes 2 and 3. In addition, patients within endotype 2 demonstrated depletion of polyunsaturated fatty acids, which have an important role in the resolution of inflammation via proresolvin pathways (8). Triglyceride metabolism differed among the two endotypes, with enrichment in endotype 3 and depletion in endotype 2. Alterations in fasting serum triglyceride have previously been reported in children with asthma (9), associated with asthma severity (10), and may indicate differences in dietary fat intake between the endotypes or alterations in lipid metabolism due to inflammation. Indeed, a previous study in an allergic mouse model of asthma has demonstrated significant increases in phosphatidylcholines, diglycerides, triglycerides, and cholesterol that were reversed on exposure to dexamethasone (11). The metabolic profiles reported by Kelly and colleagues provide reinforcing evidence that the lipid, purine, and energy metabolism pathways are key mechanistic targets for understanding asthma pathogenesis (12). Perhaps more significantly, these findings provide new evidence for the translational potential metabolomics holds as a tool, not only for supporting the classification of clinical subphenotypes but for deriving them (*viz.*, metabolomic-led endotypes).

Strengths of the study by Kelly and colleagues include the use of robust replication and a consistent association of metabolite profiles with lung function and blood eosinophilia. Profiling of a broad range of metabolites ($n = 589$, with approximately two-thirds confirmed using authentic standards) across three different analytical workflows is another strength. Finally, the use of an unbiased and metabolic biomarker–driven "bottom up" endotyping approach—specifically, a combination of data analytic techniques including *1*) similarity network fusion, *2*) spectral clustering, and *3*) chemical metabolite set enrichment—enabled the identification of putative multimetabolite-driven endotypes.

Potential limitations of the study and areas for future development include the cross-sectional design of the two cohorts, which rendered causal inference challenging. Samples in the CAMP cohort were acquired at the end of the study period—consequently,

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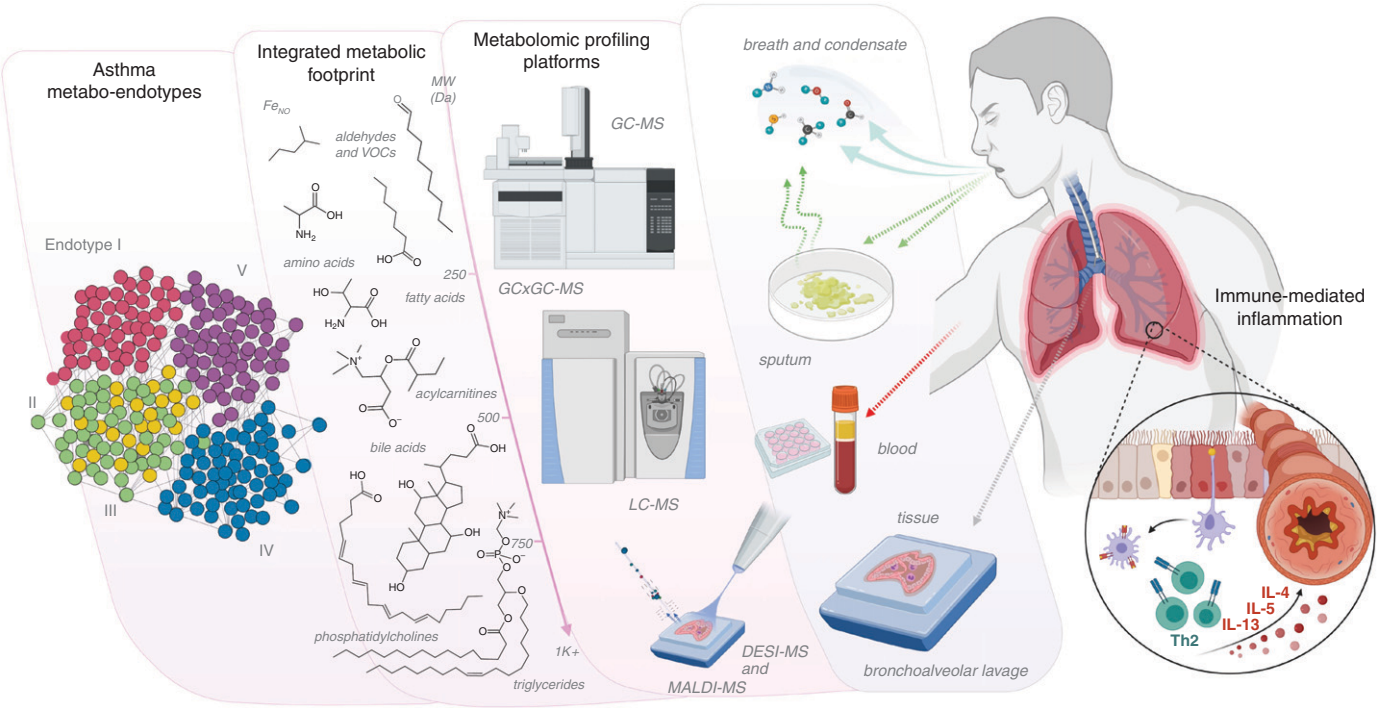

Figure 1. A collective representation of complementary approaches for the comprehensive capture of the respiratory metabolome and metabolic dysregulation caused by immune-mediated inflammation. Future adoption of multimodal designs, coupling data from tissue scale and liquid and gas phase analyses from across analytical platforms, will provide a more integrated, better annotated understanding of the metabolome, its associated metabolic endotypes, and its potential for therapeutic development in respiratory disease. DESI-MS = desorption electrospray ionization–mass spectrometry; Fe_{NO} = fractional exhaled nitric oxide; GC-MS = gas chromatography–mass spectrometry; GCxGC-MS = two-dimensional gas chromatography–mass spectrometry; LC-MS = liquid chromatography–mass spectrometry; MALDI-MS = matrix-assisted laser desorption/ionization-time of flight–mass spectrometry; MW = molecular weight; Th2 = T-helper cell type 2; VOCs = volatile organic compounds.

although CAMP was designed to measure lung growth over a 5- to 6-year period (3), it was not possible to assess the impact of endotypes membership on this outcome or indeed of inhaled steroid exposure. Furthermore, although an (extensive) untargeted approach was adopted to leverage the wealth of information of the global plasma metabolome, removal of unnamed metabolites after data acquisition constrains the findings to metabolites most studied, perpetuating their occurrence as key mediators. Tools for processing high-dimensional metabolomic data sets, such as the chemical similarity enrichment analysis (ChemRICH) tool used herein (13), are now including ways of incorporating unknowns, deriving subclass structure for assigning chemically similar metabolite sets from mass spectra.

In addition, although broad metabolic insights could be derived in this study, the precise tissue-cellular scale events driving metabolic dysregulation in childhood asthma warrant further detailed study. Future studies should integrate the full spectrum of mass spectrometry approaches available across tissue-organ scales (Figure 1), to build a more complete picture of the metabolome and provide insight into the mechanisms of metabolic dysregulation in asthma.

In summary, the study by Kelly and colleagues provides an important and comprehensive snapshot of the plasma metabolome in childhood asthma. The methodology deployed in this study will ultimately enable a more comprehensive understanding of the hitherto elusive asthma endotype(s), once embedded within a broader framework contextualizing the outputs to precise cellular and tissue mechanisms and in the context of broader multiomic profiling. ■

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An Alarmin Role for $P2Y_{13}$ Receptor during Viral-driven Asthma Exacerbations

An array of distinct, nonspecific stimuli may evoke a prototypic type 2 immune response in the airways. This is achieved through their shared capacity to induce the release of stress signals or "alarmins" from airway sentinels such as airway epithelial cells (AECs), which, in turn, function to initiate and amplify type 2 inflammation by acting primarily upon type 2 innate lymphoid cells and $CD4^+$ (cluster of differentiation 4–positive) T-helper type 2 (Th2) cells (1). IL-33 and HMGB1 (high-mobility group box 1 protein) are two alarmins released from AECs fundamental to establishing a type 2 inflammatory response. Levels of IL-33 and HMGB1 are elevated in patients with asthma, correlating with Th2 inflammation and disease severity, whereas targeting these mediators in animal models ameliorates inflammation and pathology (1–5). Intriguingly, targeting HMGB1 diminishes IL-33–induced type 2 inflammation, suggesting that these mediators coordinate in a feed-forward circuit to amplify Th2 responses (6).

Although the importance of IL-33 and HMGB1 in driving type 2 inflammation is irrefutable, the mechanisms by which diverse stimuli converge to drive their release from AECs is less well defined. Both IL-33 and HMGB1 are constitutively expressed in the nuclei of AECs, and it is proposed that their extracellular availability results from passive release from necrotic or damaged cells or active cell death–independent secretion. Allergen-induced activation of TLR4 or protease-activated receptors and viral-induced necroptosis are all purported mechanisms driving the secretion of these alarmins (7–10). Increasing evidence suggests a signaling cascade whereby AEC exposure to these environmental stimuli elicits extracellular ATP accumulation and autocrine purinergic P2 receptor activation as an intermediary step in driving alarmin release (8, 11, 12); however, the precise receptor subtype responsible remained ambiguous. Genome-wide association studies have highlighted $P2Y_{13}$-R, a purinergic receptor that is highly sensitive to ADP, as a risk factor for asthma (13). $P2Y_{13}$-R is upregulated on AECs of allergen-exposed mice, and ADP administration was sufficient to induce IL-33 release and airway eosinophilia (13).

In this issue of the *Journal*, Werder and colleagues (pp. 300–312) provide compelling evidence implicating $P2Y_{13}$-R as a key regulator of IL-33 and HMGB1 release (14). An analysis of human lung biopsies showed that the majority of AECs coexpress $P2Y_{13}$-R, IL-33, and HMGB1. Exposure of AECs to HDM allergen induced the release of the $P2Y_{13}$-R agonists, ADP and ATP, whereas subsequent pharmacological inhibition of $P2Y_{13}$-R abrogated cytoplasmic translocation and release of IL-33 and HMGB1 to multiple aeroallergens or rhinovirus. Importantly, the authors showed that these effects were specific to $P2Y_{13}$-R, as other purinergic receptor antagonists had no effect, whereas AECs from $P2Y_{13}$-$R^{-/-}$ mice showed reduced allergen-induced alarmin release. Consistent with *in vitro* findings, IL-33 and HMGB1 release into the airways of acute allergen–exposed mice was preceded by a rapid increase in ADP and/or ATP. Genetic or pharmacological inhibition of $P2Y_{13}$-R inhibited this allergen-induced alarmin release and downstream inflammation. In an HDM-induced experimental asthma model, $P2Y_{13}$-R antagonist administration again ameliorated Th2 inflammation and aspects of airway remodeling, but the effect on airway hyperresponsiveness was not assessed, and the benefit was only apparent when antagonist administration preceded allergen exposure, questioning the potential effectiveness of $P2Y_{13}$-R antagonists in patients with established asthma. However, the authors subsequently demonstrated that therapeutic $P2Y_{13}$-R antagonism ameliorated airway inflammation and promoted viral clearance in a mouse rhinovirus-induced asthma exacerbation model.

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