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Endotyping Asthma - Profiling the Metabolic Dimension?

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Asthma has long been recognised as a heterogeneous disease, and the notion of discrete endotypes of asthma has been prevalent for over 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.

Here, Kelly et al, have reported putative novel asthma endotypes, defined using metabolomics (metabo-endotypes), in childhood asthma [2]. The metabolomics included unbiased multi-platform, metabolic profiling, using liquid chromatography and tandem mass spectrometry (LC-MS). 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 due 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 Rico Cohort (n=1,165), included children aged 6-14 years. The Childhood Asthma Management Program (CAMP), included children aged 5-12 years, with mild-moderate asthma [3]. The two cohorts, were well matched for both age and gender which is important due to the phenotypic and life course changes associated with asthma and lung function, as children enter more advance school age and puberty [4-5]. However, the cohorts differed significantly in terms of asthma treatment utilisation, whilst approximately 30% of the CAMP cohort were on inhaled Budesonide - none of the GARCS cohort were on inhaled steroids. Additionally, all of the GARCS cohort were Hispanic in ethnicity, whilst just over two thirds of the CAMP cohort were of Caucasian ethnicity.

Despite the apparent differences in the two cohorts, five discrete metabo-endotypes are reported in both cohorts; with the most consistent associations observed with both pre and post bronchodilator FEV₁% and FEV₁/FVC. Metabo-endotype-3 demonstrated the best lung
function, whilst metabo-endotype-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. Whilst endotype-2 was characterised 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 metabo-endotype membership. Intriguingly, whilst 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 metabo-endotypes.

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 pro-resolvin pathways [8]. Triglyceride metabolism differed amongst 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 upon exposure to dexamethasone [11]. The metabolic profiles reported by Kelly et al 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 sub-phenotypes but for deriving them (viz. metabolomic-led endotypes).

Strengths of the study by Kelly et al, 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 unbiased and metabolic biomarker driven ‘bottom up’ endotyping approach - specifically, a combination of data analytic techniques (I) similarity network fusion, (II) spectral clustering and (III) chemical metabolite set enrichment, enabled the identification of putative multi-metabolite driven endotypes.

Potential limitations of the study and areas for future development include the cross-sectional design of the two cohorts, rendering causal inference challenging. Samples in the CAMP cohort were acquired at the end of the study period - consequently, whilst CAMP was designed to measure lung growth over a 5–6-year period [3], it was not possible to assess the impact of endotypes membership on this outcome or indeed of inhaled steroid exposure. Furthermore, whilst an (extensive) untargeted approach was adopted to leverage the wealth of information of the global plasma metabolome, removal of unnamed metabolites during post data acquisition constrains the findings to metabolites most studied, perpetuating their occurrence as key mediators. Tools for processing high dimensional metabolomic datasets, such as the ChemRICH tool utilised herein [13], are now including ways of incorporating unknowns, deriving sub-class structure for assigning chemically similar metabolite sets from MS/MS spectra.

In addition, whilst 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 (Fig. 1), to build a more complete picture of the metabolome and shed insight into the mechanisms of metabolic dysregulation in asthma.

In summary the study by Kelly et al, 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 contextualising the outputs to precise cellular and tissue mechanisms and in the context of broader multi-omic profiling.
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FIGURE LEGEND

**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, liquid and gas phase analyses from across analytical platforms will provide a more integrated, better annotated understanding of the metabolome, associated metabolic endotypes and its potential for therapeutic development in respiratory disease.
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, liquid and gas phase analyses from across analytical platforms will provide a more integrated, better annotated understanding of the metabolome, associated metabolic endotypes and it's potential for therapeutic development in respiratory disease.