

EDITORIAL

Utilizing metabolomics to distinguish asthma phenotypes: strategies and clinical implications

DOI:10.1111/all.12238

Asthma is a multifaceted disease that can present with a variety of clinical manifestations. In spite of considerable efforts, diagnostic tools that can be used to classify asthma or predict responsiveness to asthma therapy are still lacking. As several phenotypes of asthma exist (1–8) (many with potentially overlapping mechanisms), it is unlikely that a single biomarker can be used to distinguish between phenotypes or predict responsiveness to medication. In addition, studies targeting single molecules are not able to capture the dynamics of multiple networks, many of which are likely unknown at present.

Metabolomics has been shown to be a valuable tool for the discovery of biomarkers and for the elucidation of complex mechanisms in cardiovascular disease, nutrition, cancer, and other diseases, including asthma (9–14) (Table 1). Mattarucchi and colleagues specifically used liquid chromatography–mass spectrometry (LC-MS)-based urinary metabolomics to differentiate pediatric phenotypes and to identify possible metabolite sources of inflammation, also demonstrating that molecules relevant to lung disease can be detected in urine (11). Ho and colleagues used LC-MS metabolomics of sensitized and challenged mice to evaluate changes in metabolism and found reversible changes in carbohydrate, lipid, and sterol metabolism upon treatment with dexamethasone (9). Additional applications of metabolomics to asthma include nuclear magnetic resonance (NMR) of bronchiolar lavage fluid (BALF) in mouse models (15) and exhaled breath condensate (EBC) in humans (13, 16).

In the current issue of *Allergy*, the work by Ibrahim and colleagues expands on this growing body of knowledge by using metabolomics in an important first step toward the development of a noninvasive diagnostic tool (17). Specifically, the authors used EBC samples to determine whether metabolomics profiles could be used to discriminate asthmatics from nonasthmatics and whether these profiles could be used to distinguish asthmatic phenotypes. Importantly, the study includes the use of a relatively large adult cohort, training and test data sets and a methodology that could potentially be translated into the clinical laboratory. Using nuclear magnetic resonance spectroscopy (NMR), the authors were able to develop a classification model that could be used to distinguish asthmatics from nonasthmatics. Five spectral regions, of variable reproducibility, were used for the model and provided a robust area under the receiver operating curve (AUROC) of 0.84. Additional regions were used to distinguish between sputum neutrophilia and use of inhaled corticosteroids, but were not able to discriminate between eosinophilia and asthma control.

Overall, the work by Ibrahim and others can be used to highlight progress, strategies, and issues in the field (17). While prior metabolomics studies have utilized only a limited number of subjects or sample types, questions remain regarding how best to preclassify subjects. Exploratory 'omics studies are often ancillary to larger clinical trials that focus on specific phenotypic or mechanistic questions or on response to medication (e.g. determining the role of Th2 pathways in obesity-related asthma or the response to corticosteroids in a pediatric cohort). This necessarily biases an after-the-fact metabolomics study toward the original question being asked and, because sample numbers are often limited, does not enable the exploration of broader questions. For example, while Ibrahim was able to examine neutrophilic and eosinophilic phenotypes, the authors were not able to explore questions regarding the role of T-helper pathways, atopy, or paucigranulocytic phenotypes (17). Inclusion of metabolomics researchers and basic immunologists at study inception would greatly expand the potential for knowledge gained by large-scale clinical studies.

In spite of these challenges, progress continues to be made, and Ibrahim and others have developed tools that can be potentially used to differentiate between asthmatics and nonasthmatics. To date, no tool exists that can distinguish between the myriad of asthmatic phenotypes, highlighting the multifactorial nature and overall complexity of asthma and emphasizing the need for not only multiplex tests but also multiple platforms of discovery. While NMR has relatively poor sensitivity and specificity compared with LC-MS, it is nondestructive and high throughput. NMR can be used to quantitate a limited number of molecules (<200), whereas LC-MS can be used to analyze several hundreds, with close to 4000 small molecules in plasma alone (18). However, positive identification of metabolites remains a challenge in LC-MS-based metabolomics, and statistical analysis of multidimensional data is still evolving for both fields. LC-MS has already been shown to be a powerful clinical laboratory tool, and there is great potential for NMR in the clinical laboratory; however, the power of these technologies may lie not only in the potential for biomarker discovery, but also in the fact that they are complementary. Following the use of NMR to determine differences, LC-MS can be used to specifically identify many of the compounds detected, adding important mechanistic information.

In addition to technical challenges, the dynamic interplay between genes, proteins, and metabolites in the context of environmental stimuli cannot be understood using a single platform. The state of the asthmatic lung is also variable,

Table 1. Summary of recent studies utilizing NMR or LC-MS metabolomics in the field of clinical asthma

Author and year	Study purpose	Study population	Technological platform used	Sample source	Knowledge gained	Issues
Carraro 2013 (13)	Discrimination based on asthma severity	Pediatric cohort: nonsevere asthma ($n = 31$), severe asthma ($n = 11$), and nonasthmatic (15)	LC-MS	EBC	Groups could be distinguished using bidirectional orthogonal projections to latent structures discriminant analysis (O2PLS-DA) with a naïve Bayes classification technique	Use of ICS in a portion of the participants complicated analysis. Limited number of metabolites used to distinguish between groups and functional significance is unknown.
Ho 2013 (9)	Biomarker discovery in challenged mice	Mouse model	LC-MS and GC-MS	BALF	Found differences in key energy pathways that were reversed with dexamethasone	Application of mouse models to human disease is still not clear
Ibriham 2013 (17)	Differentiate phenotypes	82 asthmatics split into 4 phenotypes with number of subjects between 18 and 43 in each group. 35 nonasthmatics	NMR	EBC	Model could distinguish between asthmatics and nonasthmatics and between sputum neutrophilia and use of inhaled corticosteroids, but could not discriminate between eosinophilia and asthma control.	Limited ability to phenotype, in part due to small sample sizes of individual phenotypes
Jung 2013 (10)	Asthma diagnosis	Asthma ($n = 30$) vs control ($n = 26$)	NMR	Serum	Several identified metabolites could be used to distinguish asthmatics from nonasthmatics	Small study with limited clinical utility unless validated by others or in a large cohort
Mattarucchi 2011 (11)	Differentiate phenotypes	Pediatric cohort of atopic asthmatics ($n = 41$) vs nonasthmatics ($n = 12$).	LC-MS	Urine	Model could distinguish between well controlled with SABA ($n = 14$), well controlled with daily controller ($n = 16$), and poor control with daily controller ($n = 11$). Demonstrates utility of urine	Small study size, final dataset consisted of only 27–72 metabolites used for modeling due to background noise.
Saude 2011 (26)	Asthma exacerbation in children	Pediatric cohort of stable asthma ($n = 72$), unstable asthma ($n = 20$), or nonasthmatic ($n = 42$)	NMR	Urine	Model could distinguish between groups and several relevant metabolites with physiological significance were identified	Confounding variables include atopy and medication use.
Izquierdo-Garcia 2011 (27) and Sinha 2012 (16)	Utility of NMR for EBC analysis	Not applicable	NMR	EBC	Conflicting studies illustrating technical differences in EBC analysis using NMR	

due to differential medication use or factors such as pollution, allergens, and the presence of bacteria or viruses. It is unclear whether a single sampling is sufficient to capture the overall disease state or whether repeated measures will be required. Therefore, well-designed biomarker discovery studies will require longitudinal samples, preferably with data before and after treatment. Unfortunately, the design and implementation of a study with sufficient subjects with specific phenotypes, with longitudinal samples, is presently beyond reach. Therefore, for the time being, those interested in the role of small molecules in asthma will necessarily rely on ancillary trials, where access to suitable samples is an additional challenge.

Although central dogma states that disease-relevant molecules are most likely to be found in tissues nearest to the sites of damage/disease, the use of airway fluids in research has proven challenging for several reasons (19, 20). These include a lack of normalization schemes, inability to include internal standards, and contamination between fluids, especially saliva. However, the noninvasive nature of sputum and breath collection remains appealing, and potential markers with relevance to asthma such as proteins, prostaglandin D₂, and leukotrienes (LTEs) have been found in airway and other sources (21, 22). Although experimental design, including the randomization of contaminants among controls and asthmatics, minimizes effects, concerns remain, and there have been conflicting conclusions from NMR (largely claiming no contamination) and LC-MS laboratories (showing contamination and poor coefficients of variation) (23). Preliminary work in our laboratory has shown that urinary LTE₄ can be used to distinguish between asthmatics and nonasthmatics and that there is a strong correlation between EBC and salivary levels of LTEs and PGs. However, salivary contamination may result in artificially increased signal and therefore greater differences between asthmatics and nonasthmatics. Therefore, efforts to quantitate salivary contamination should be

included in any study, and analysis of the overlap between fluids is warranted. Overall, the clinical implications for a breath-based diagnostic are significant, with potential for fast, in-office diagnostic tests, and the use of research tools such as NMR and LC-MS to study breath is an important first step toward this goal.

In spite of considerable challenges, there is immense potential for metabolomics to discover molecules that can be used to improve diagnosis and treatment of asthma through accurate phenotyping of patients. In addition, our own studies have shown potential for small molecules to be used to predict response to leukotriene receptor agonist medication and to predict risk of severe exacerbations in children exposed to tobacco smoke (24, 25). These studies asked specific questions and suggest potential for metabolomics to discover new biomarkers; however, the need for comprehensive, large-scale clinical metabolomics studies remains. With the advancement of metabolomics techniques, including instrumentation, software, and evolving databases, these studies have high potential for success, and in the near future, we expect that metabolomics studies will result in novel diagnostic approaches for asthma.

Acknowledgments

The authors are supported by Reisdorph P20 HL113445-01 and R01 DK081166.

Conflict of interest

The authors have no conflict of interests relevant to this article.

N. Reisdorph and M. E. Wechsler
National Jewish Health, Denver, CO, USA
E-mail: wechslerm@njhealth.org

References

1. Lötvald J, Akdis CA, Bacharier LB, Björner L, Casale TB, Custovic A et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;**127**:355–360.
2. Kowalski ML, Cieslak M, Pérez-Novo CA, Makowska JS, Bachert C. Clinical and immunological determinants of severe/refractory asthma (SRA): association with Staphylococcal superantigen-specific IgE antibodies. *Allergy* 2011;**66**:32–38.
3. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 cells: new players in asthma pathogenesis. *Allergy* 2011;**66**:989–998.
4. Isidoro-Garcia M, Sanz C, Garcia-Solaesa V, Pascual M, Pescador DB, Lorente F et al. PTGDR gene in asthma: a functional, genetic, and epigenetic study. *Allergy* 2011;**66**:1553–1562.
5. Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. *Allergy* 2012;**67**:835–846.
6. Papadopoulos NG, Arakawa H, Carlsen KH, Custovic A, Gern J, Lemanske R et al. International consensus on (ICON) pediatric asthma. *Allergy* 2012;**67**:976–997.
7. Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH et al. Three phenotypes of adult-onset asthma. *Allergy* 2013;**68**:674–680.
8. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop CM, Winn J et al. Multiple atopy phenotypes and their association with asthma: similar findings from two birth cohorts. *Allergy* 2013;**68**:764–770.
9. Ho WE, Xu YJ, Xu F, Cheng C, Peh HY, Tannenbaum SR et al. Metabolomics reveals altered metabolic pathways in experimental asthma. *Am J Respir Cell Mol Biol* 2013;**48**:204–211.
10. Jung J, Kim SH, Lee HS, Choi GS, Jung YS, Ryu DH et al. Serum metabolomics reveals pathways and biomarkers associated with asthma pathogenesis. *Clin Exp Allergy* 2013;**43**:425–433.
11. Mattarucci E, Baraldi E, Guillou C. Metabolomics applied to urine samples in childhood asthma: differentiation between asthma phenotypes and identification of relevant metabolites. *Biomed Chromatogr* 2012;**26**:89–94.
12. Ried JS, Baurecht H, Stücker F, Krumsiek J, Gieger C, Heinrich J et al. Integrative genetic and metabolite profiling analysis sug-

- gests altered phosphatidylcholine metabolism in asthma. *Allergy* 2013;**68**:629–636.
13. Carraro S, Giordano G, Reniero F, Carpi D, Stocchero M, Sterk PJ et al. Asthma severity in childhood and metabolomic profiling of breath condensate. *Allergy* 2013;**68**:110–117.
14. Ponsonby AL, Kemp A. Investigation of the hygiene hypothesis: current issues and future directions. *Allergy* 2008;**63**:506–508.
15. Saude EJ, Obiefuna IP, Somorjai RL, Ajamian F, Skappak C, Ahmad T et al. Metabolomic biomarkers in a model of asthma exacerbation: urine nuclear magnetic resonance. *Am J Respir Crit Care Med* 2009;**179**:25–34.
16. Sinha A, Krishnan V, Sethi T, Roy S, Ghosh B, Lodha R et al. Metabolomic signatures in nuclear magnetic resonance spectra of exhaled breath condensate identify asthma. *Eur Respir J* 2012;**39**:500–502.
17. Ibrahim B, Marsden P, Smith JA, Custovic A, Nilsson M, Fowler SJ. Breath metabolomic profiling by nuclear magnetic resonance spectroscopy in asthma. *Allergy* 2013;**68**:1050–1056.
18. Yang Y, Cruickshank C, Armstrong M, Mahaffey S, Reisdorph R, Reisdorph N. New sample preparation approach for mass spectrometry-based profiling of plasma results in improved coverage of metabolome. *J Chromatogr A* 2013;**1300**:217–226.
19. Effros RM, Dunning MB III, Biller J, Shaker R. The promise and perils of exhaled breath condensates. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L1073–L1080.
20. Kim CK, Koh YY, Callaway Z. The validity of induced sputum and bronchoalveolar lavage in childhood asthma. *J Asthma* 2009;**46**:105–112.
21. Torregrosa Paredes P, Esser J, Admyre C, Nord M, Rahman QK, Lukic A et al. Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma. *Allergy* 2012;**67**:911–919.
22. Hallstrand TS, Henderson WR Jr. An update on the role of leukotrienes in asthma. *Curr Opin Allergy Clin Immunol* 2010;**10**:60–66.
23. Liu J, Conrad DH, Chow S, Tran VH, Yates DH, Thomas PS. Collection devices influence the constituents of exhaled breath condensate. *Eur Respir J* 2007;**30**:807–808.
24. Armstrong M, Liu AH, Harbeck R, Reisdorph R, Rabinovitch N, Reisdorph N. Leukotriene-E4 in human urine: comparison of on-line purification and liquid chromatography-tandem mass spectrometry to affinity purification followed by enzyme immunoassay. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;**877**:3169–3174.
25. Rabinovitch N, Reisdorph N, Silveira L, Gelfand EW. Urinary leukotriene E(4) levels identify children with tobacco smoke exposure at risk for asthma exacerbation. *J Allergy Clin Immunol* 2011;**128**:323–327.
26. Saude EJ, Skappak CD, Regush S, Cook K, Ben-Zvi A, Becker A et al. Metabolomic profiling of asthma: diagnostic utility of urine nuclear magnetic resonance spectroscopy. *J Allergy Clin Immunol* 2011;**127**:757–764.
27. Izquierdo-Garcia JL, Peces-Barba G, Heili S, Diaz R, Want E, Ruiz-Cabello J. Is NMR-based metabolomic analysis of exhaled breath condensate accurate? *Eur Respir J* 2011;**37**:468–470.