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Asthma Metabolomics and the Potential for Integrative Omics in Research and the Clinic

Rachel S. Kelly, PhD; Amber Dahlin, PhD, MMSc; Michael J. McGeachie, PhD; Weiliang Qiu, PhD; Joanne Sordillo, PhD; Emily S. Wan, MD; Ann Chen Wu, MD; and Jessica Lasky-Su, ScD

Asthma is a complex disease well-suited to metabolomic profiling, both for the development of novel biomarkers and for the improved understanding of pathophysiology. In this review, we summarize the 21 existing metabolomic studies of asthma in humans, all of which reported significant findings and concluded that individual metabolites and metabolomic profiles measured in exhaled breath condensate, urine, plasma, and serum could identify people with asthma and asthma phenotypes with high discriminatory ability. There was considerable consistency across the studies in terms of the reported biomarkers, regardless of biospecimen, profiling technology, and population age. In particular, acetate, adenosine, alanine, hippurate, succinate, threonine, and trans-aconitate, and pathways relating to hypoxia response, oxidative stress, immunity, inflammation, lipid metabolism and the tricarboxylic acid cycle were all identified as significant in at least two studies. There were also a number of nonreplicated results; however, the literature is not yet sufficiently developed to determine whether these represent spurious findings or reflect the substantial heterogeneity and limited statistical power in the studies and their methods to date. This review highlights the need for additional asthma metabolomic studies to explore these issues, and, further, the need for standardized methods in the way these studies are conducted. We conclude by discussing the potential of translation of these metabolomic findings into clinically useful biomarkers and the crucial role that integrated omics is likely to play in this endeavor. CHEST 2017; 151(2):262-277

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Asthma is a complex disease with both environmental and genetic influences; however, the role of molecular determinants as mediators of asthma is not yet fully understood.[1] Metabolomics, the systematic analysis of small molecules, including carbohydrates, amino acids, organic acids, nucleotides, and lipids, has identified new biomarkers and novel pathogenic pathways for a number of complex chronic diseases.[2] Metabolomics is well-suited to the study of diseases with an environmental etiological

ABBREVIATIONS: AUC = area under the curve; EBC = exhaled breath condensate; MS = mass spectrometry; NMR = nuclear magnetic resonance spectroscopy; PLS-DA = partial least squares- discriminant analysis; SNP = single nucleotide polymorphism; VOC = volatile organic compounds

AFFILIATIONS: From the Channing Division of Network Medicine (Drs Kelly, Dahlin, McGeachie, Qiu, Wan, and Lasky-Su), Brigham Women's Hospital and Harvard Medical School, Boston, MA; VA Boston Healthcare System (Dr Wan), Department of Veterans Affairs, Boston, MA; and the Department of Population Medicine (Drs Sordillo and Wu), Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA.

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CORRESPONDENCE TO: Jessica Lasky-Su, ScD, Channing Division of Network Medicine, Brigham Women's Hospital and Harvard Medical School, 181 Longwood Ave, Boston, MA 02115; e-mail: rejas@channing.harvard.edu

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component because it has the potential to capture the history of the cellular response to past exposures. Metabolite fluctuations represent an integrated pathophysiologic profile encompassing genetic and environmental interactions; therefore, metabolic profiles can be instrumental in elucidating the understanding of the biologic mechanisms of asthma. Although the application of metabolomics to study asthma is recent, the body of literature is rapidly growing. Critical analysis of this literature will afford an improved understanding of the status of asthma metabolomics and help to inform future studies.

Methods

The National Center for Biotechnology Information PubMed database was searched to identify studies of asthma in humans using mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) to identify and quantify metabolites associated with asthma or asthma-related outcomes. The references of each identified study were evaluated to identify additional qualifying manuscripts. Twenty-one studies using metabolomic profiling of exhaled breathe condensate (EBC) (n = 11),[3-13] urine (n = 4),[14-17] serum (n = 3),[18-20] and plasma (n = 3)[21-23] were identified (Table 1). Twelve studies evaluated children,[3-10,14,15,21,22] eight evaluated adults,[12,13,16-20,23] and one included both.[11] The majority used MS-based methods; six used NMR.[10-13,15,20] All but four[16,17,21,22] were case-control in design, and the total number of people with asthma ranged from 10[16] to 343.[9] The primary aim of most studies was to examine the differences between asthma cases and healthy control patients, with a smaller number of studies examining disease severity or phenotypes. One study of recurrent wheeze was also included.[7]

Results

All 21 studies reported significant findings and concluded that metabolomic profiles in EBC, urine, and blood could distinguish asthma and asthma phenotypes (Table 2). The utility of such profiles is twofold: (1) the identification of metabolite biomarkers for asthma and (2) the improved understanding of the pathophysiology of asthma. The majority of the studies focused on the former by building metabolomic signatures that were subsequently assessed for discriminative ability. These signatures were created by identifying associated metabolites from the total number measured, which ranged by study from two[4] to almost 9,000.[14] However, the interrogation of the metabolites and pathways composing these signatures also provided important insights into asthma pathophysiology. In this review, we compare the metabolomic signatures and the biological information they impart. In particular, we focus on how different methods and techniques may affect metabolomic signatures, and the implications thereof, as the metabolomics field begins to shift toward clinical translation.

Metabolomic Biomarkers of Asthma

Predictive Indices: The indices of prediction reported by the included studies suggest extremely good classification accuracy (≥ 85%), particularly when differentiating asthma cases from healthy control patients. Discriminatory power was lower for mild vs severe asthma, but would still be ranked as good to excellent (≥ 80%) in most cases. There was no evidence that discriminatory ability differed in adults as opposed to children, with comparable values reported in both groups. The most commonly used model was partial least squares-discriminant analysis (PLS-DA), which is appropriate for analysis of datasets with many correlated predictors, as is common in metabolomics. The R^2 and Q^2, which are used to assess PLS-DA models, had high values in all studies; however, PLS-DA is known to overestimate predictive ability and only a few studies addressed this.[24,25] Four studies reported area under the receiver operator characteristic (AUC) curves, demonstrating strong predictive ability, with the highest AUC of 0.977 for a 10-biomarker profile.[20] The highest AUC for a single metabolite was 0.976 for succinate in serum.[19] On the basis of these studies, there was no evidence that including larger numbers of metabolites in the profile increased discriminatory ability.

Regardless of the reported predictive indices, the true test of discriminatory ability is validation. In the search for biomarkers several possible validation strategies can be used, including: (1) testing on a separately recruited and ascertained validation cohort; (2) using a hold-out data set; and (3) performing permutation testing by label shuffling.[24,25] Eight[5,7,8,14,15,17,19,21] and four[9,12,13,20] studies used permutation and a hold-out dataset, respectively. Both approaches provided support for accuracy of the reported biomarkers. However, the most robust measure of validation is replication in an entirely independent cohort; this was not performed by any included study. In this review, we highlight the metabolites and metabolomic pathways that are replicated between the included studies to inform the development of comprehensive and effective metabolomic asthma biomarkers.

TABLE 1] Characteristics of the 21 Asthma Metabolomic Studies Conducted in Humans

Biological Sample	Age Group	Method	Authors	No. of Cases	No. of Control Patients	Diagnostic Criteria	Main Aim	Population	Metabolomic Profiling
EBC	Children	LC-MS	Esther et al (2009)[3]	11	28	PD	Metabolomic profile of asthma vs healthy	United States	Targeted: adenosine, AMP, and purine biomarkers
			Montuschi (2009)[4]	20 atopic patients without asthma, 25 steroid-naïve atopic mild patients with asthma, 22 atopic mild-to-moderate patients with asthma	15	PD; skin-prick testing	Leukotriene profile of asthma vs healthy	Italy	Targeted: leukotrienes
			Carraro et al (2012)[5]	31 patients with nonsevere asthma, 11 patients with severe asthma	15	PD; GINA guidelines	Discrimination of different asthma phenotypes	Italy	Untargeted
		GC-MS	Caldeira et al (2012)[6]	32 atopic patients with asthma	27	PD	Metabolomic profile of asthma vs healthy	Portugal	Targeted: alkanes, alkenes, aldehydes, and ketones
			van de Kant et al (2013)[7]	202 recurrent wheezers	50	≥ 2 parental-reported episodes of wheeze during life	Metabolomic profile of recurrent wheeze vs no recurrent wheeze	ADEM study, Netherlands	Targeted: VOCs
			Gahleitner et al (2013)[8]	11	12	Health questionnaire; respiratory examination	Metabolomic profile of asthma vs healthy	United Kingdom	Targeted: VOCs
EBC	Children	GC-MS	Smolinska et al (2014)[9]	343	185 healthy and 546 transient wheezers	PD	Metabolomic profile of asthma vs transient wheeze	ADEM study, Netherlands	Targeted: VOCs

(Continued)

TABLE 1] *(Continued)*

Biological Sample	Age Group	Method	Authors	No. of Cases	No. of Control Patients	Diagnostic Criteria	Main Aim	Population	Metabolomic Profiling
		NMR	Carraro et al (2007)[10]	17 patients with persistent asthma treated with inhaled corticosteroids, 8 corticosteroid-naïve intermittent patients with asthma	11	PD; GINA guidelines	Metabolomic profile of asthma vs healthy	Italy	Untargeted
	All ages	NMR	Sinha et al (2012)[11]	7 adults with asthma, 58 children with asthma	10	PD	Metabolomic profile of asthma vs healthy	India	Untargeted
	Adults	NMR	Ibrahim et al (2013)[12]	82	35	Reported symptoms; treatment	Metabolomic profile of asthma vs healthy	ASMAL study, United Kingdom	Untargeted
			Motta et al (2014)[13]	35 patients with mild asthma	35	PD; GINA guidelines; DSS	Metabolomic profile of asthma vs healthy	Italy	Targeted and untargeted
Urine	Children	LC-MS	Mattarucchi et al (2012)[14]	41	12	PD; GINA guidelines	Metabolomic profile of asthma vs healthy	Italy	Untargeted
		NMR	Saude et al (2011)[15]	73 patients with stable asthma, 20 patients with unstable asthma	42	PD	Metabolomic profile of asthma vs healthy, and of different asthma endotypes	Canada	Targeted
	Adults	GC-MS	Loureiro et al (2014)[16]	7 patients with allergic asthma, 3 patients with nonallergic asthma	NA	PD	Metabolomic changes with asthma exacerbation	Portugal	Targeted: aldehydes and alkanes and central metabolites
			Loureiro et al (2016)[17]	57	NA	PD	Metabolomic profile of asthma severity	Portugal	Targeted: aliphatic aldehydes and alkanes

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TABLE 1] *(Continued)*

Biological Sample	Age Group	Method	Authors	No. of Cases	No. of Control Patients	Diagnostic Criteria	Main Aim	Population	Metabolomic Profiling
Serum	Adults	GC-MS	Ried et al (2013)[18]	147	2,778	Self-report and medical examination	Metabolomic profile of asthma vs healthy	KORA Study, Germany	Targeted
			Chang et al (2015)[19]	17 patients with mild persistent asthma	17	PD; GINA guidelines	Metabolomic profile of asthma vs healthy	China	Untargeted
Plasma	Children	LC-MS	McGeachie et al (2015)[21]	20	NA	PD	Identification of predictors of asthma control	CARE Network cohort, United States	Targeted lipidomics
			Fitzpatrick et al (2014)[22]	22 patients with mild/moderate asthma, 35 patients with severe asthma	NA	Spirometry	Metabolomic profile of mild-moderate vs severe asthma	United States	Untargeted
Plasma	Adults	NMR	Jung et al (2013)[20]	39	26	PD	Metabolomic profile of asthma vs healthy	South Korea	Untargeted and targeted
		MS	Comhair et al (2015)[23]	20	10	ATS Workshop on Refractory Asthma Guidelines	Metabolomic profile of asthma vs healthy, and of different asthma endotypes	United States	Untargeted and targeted

ADEM = Asthma Detection and Monitoring Study; AMP = adenosine monophosphate; ASMAL = Assessment of Manchester Asthmatics Longitudinally Study; ATS = American Thoracic Society; CARE = Childhood Asthma Research and Education Study; DSS = disease severity score; EBC = exhaled breath condensate; GC-MS = gas chromatography–mass spectrometry; GINA = Global Initiative for Asthma; KORA = Cooperative Health Research in the Region Augsburg Study; LC-MS = liquid chromatography–mass spectrometry; NA = not applicable; NMR = nuclear magnetic resonance spectroscopy; PD = physician diagnosed; VOC = volatile organic compounds.

TABLE 2] Summary of Results for the 21 Asthma Metabolomic Studies in Humans

Authors	No. of Metabolites	Results	Significant Metabolites	Implicated Pathways	Conclusions	Validation
Esther et al (2009)[3]	6	Adenosine-to-urea ratio elevated in asthma (median, 1.5) vs control (median, 0.4) ($P < .05$)	Adenosine	Neutrophilic airway inflammation	EBC adenosine-to-urea ratio is a potential noninvasive biomarker of airways disease	No
Montuschi et al (2009)[4]	2	Exhaled leukotrienes were increased in children with asthma children and were highest in steroid-naive children	Leukotrienes	Leukotriene-related pathways, inflammatory pathways	EBC leukotriene B4 and eicosanoids represent potential noninvasive biomarkers of airway inflammation and therapy monitoring	No
Carraro et al (2012)[5]	NR	PLS-DA models could distinguish severe asthma cases from healthy control patients ($R^2 = 0.93$; $Q^2 = 0.75$); and severe from nonsevere asthma cases ($R^2 = 0.84$; $Q^2 = 0.47$).	Retinoic acid, adenosine, and vitamin D	NR	Metabolomic profiling of EBC could clearly distinguish asthmatic children	Internal cross-validation
Caldeira et al (2012)[6]	134	PLS-DA model had a classification rate of 98% and showed 96% sensitivity and 95% specificity for distinguishing patients with asthma from healthy control patients	Nonane, 2,2,4,6, 6-pentamethylheptane, decane, 3, 6-dimethyldecane, dodecane, and tetradecane	Oxidative stress and inflammatory processes	EBC metabolome is able to accurately distinguish healthy children from children with asthma	No
van de Kant et al (2013)[7]	913	Sparse logistic regression model on the basis of 28 VOCs correctly classified 73% of recurrent wheezers (79% sensitivity, 50% specificity)	28 VOCs	NR	VOC profiles in EBC are able to distinguish children with and without recurrent wheeze	Internal cross-validation
Gahleitner et al (2013)[8]	NR	PLS-DA model on the basis of 8 metabolites distinguished patients with asthma from healthy children with 100% accuracy	1-(methylsulfanyl)propane, ethylbenzene, 1,4-dichlorobenzene, 4-isopropenyl-1-methylcyclohexene, 2-octenal, octadecyne, 1-isopropyl-3-methylbenzene, and 1, 7-dimethylnaphtalene	NR	VOC profiles in EBC are able to distinguish children with and without asthma	Internal cross-validation

(Continued)

TABLE 2] *(Continued)*

Authors	No. of Metabolites	Results	Significant Metabolites	Implicated Pathways	Conclusions	Validation
Smolinska et al (2014)[9]	NR	PLS-DA model on the basis of 17 VOCs distinguished children with asthma from transient wheezers with a prediction rate of 80%	Alkanes, acetone, 2, 4-dimethylpentane, 2, 4-dimethylheptane, 2,2, 4-trimethylheptane, 1-methyl-4-(1-methylethenyl) Cyclohexen, 2,3, 6-trimethyloctane, 2-undecenal, Biphenyl, 2-ethenylnaphtalene, 2,6, 10-trimethyldodecane, Octane, 2-methylpentane, 2,4-dimethylheptane, and 2-methylhexane	Oxidative stress and lipid peroxidation	VOCs in EBC predict development of asthma	Split into a training and test set (80:20)
Carraro et al (2007)[10]	101 spectral regions	NMR-based PLS-DA model distinguished patients with asthma from healthy children with a classification rate of 95%	Oxidized and acetylated compounds.	Oxidative stress	Metabolomic profiling of EBC affords potential for noninvasive biomarker development	No
Sinha et al (2012)[11]	NR	Trident peak at 7 ppm reliably distinguishes EBC samples from patients with and without asthma	Ammonium ions	Glutamate-glutamine cycle	Distinct metabolomic profiles of asthmatics and healthy control patients can be identified in NMR-based metabolomic profiling of EBC	No
Ibrahim et al (2013)[12]	367 spectral bins	13 spectral regions discriminated patients with asthma from healthy control patients; AUC, 0.91; overall accuracy, 82.3%; PPV, 83.1%; NPV 78.6%	Reported spectral regions	NR	Distinct metabolomic profiles of patients with asthma and healthy control patients can be identified in NMR-based metabolomic profiling of EBC	Split into a training and test set (70:30)
Motta et al (2014)[13]	NR	PLS-DA model distinguished patients with mild asthma from healthy subjects ($R^2 = 0.90$, $Q^2 = 0.84$)	Saturated fatty acids, valine, adenosine, hippurate, alanine, formate, urocanic acid, proline, acetate, ethanol, methanol, isoleucine, propionate,	Histidine conversion pathways	EBC metabolome is determined by asthma status	External validation models (n = 40 drawn from

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TABLE 2] *(Continued)*

Authors	No. of Metabolites	Results	Significant Metabolites	Implicated Pathways	Conclusions	Validation
			4OH-phenylacetate, tyrosine, arginine, trans-aconitate, and phenylalanine			same population)
Mattarucchi et al (2012)[14]	6,744 features	PLS-DA models distinguished patients with well-controlled symptoms (resulting from drugs), well-controlled symptoms (not from drugs), and poorly controlled symptoms (despite using drugs). Prediction rate > 90% for all models	Urocanic acid and methyl-imidazoleacetic acid	Modulation of immunity	LC-MS urinary metabolic profiles can characterize asthma in children	Internal cross-validation
Saude et al (2011)[15]	70	PLS-DA model on the basis of 23 metabolites could distinguish patients with asthma from healthy children; sensitivity, 94%; specificity, 95%; $R^2 = 0.84$; $Q^2 = 0.74$	2-oxaloglutarate, succinate, fuma- rate, 3-hydroxy-3-methylglutarate, threonine, and cis-aconitate and trans-aconitate	Hypoxia, TCA cycle	NMR urinary metabolomic profiles can characterize asthma in children	Internal cross-validation
Loureiro et al (2014)[16]	32	During exacerbations, urine revealed increased levels of aldehydes and alkanes and alterations in a number of nonvolatile metabolites	Threonine, lactate, alanine, carnitine, acetylcarnitine, trimethylamine-N-oxide, acetate, citrate, malonate, hippurate, dimethylglycine, and phenylacetylglutamine	Oxidative stress, tricarboxylic acid cycle	Urinary metabolic composition in asthmatics is highly altered during exacerbations	No
Loureiro et al (2016)[17]	34	Metabolites related to lipid peroxidation levels could predict clinical and laboratory parameters including disease severity, lung function, FeNO, and blood eosinophils in nonobese patients (R^2 0.53-0.90)	Aliphatic aldehydes and alkanes	Lipid peroxidation	Metabolomics can provide vital insights into asthma mechanisms	Internal cross-validation

(Continued)

TABLE 2] *(Continued)*

Authors	No. of Metabolites	Results	Significant Metabolites	Implicated Pathways	Conclusions	Validation
Ried et al (2013)[18]	151	Identified 4 metabolites associated with asthma risk loci and asthma status	Phosphatidylcholines, lyso-phosphatidylcholines, PC.aa.C42:2 and PC.aa.C42:4	Lipid metabolism	GC-MS serum-based metabolomics affords potential for asthma biomarker development	No
Chang et al (2015)[19]	272	14 discriminatory metabolites were identified. Top metabolite AUCs: 2-ketovaleric acid (0.874), 3, 4-dihydroxybenzoic acid (0.965), 5-aminovaleric acid (0.948), ascorbate (0.917), dehydroascorbic acid (0.896), inosine (0.962), phenylalanine (0.927), and succinic acid (0.976)	2-ketova-leric acid, 3, 4-dihydroxybenzoic acid, 5-aminovaleric acid, ascorbate, dehydroascorbic acid, inosine, phenylalanine, and succinic acid (succinate), β-glycerophosphoric acid, maleamate, maleic acid, monoolein, ribose, and trans-4-hydroxy-L-proline	TCA cycle, nitrogen metabolism, glutamine and glutamate metabolism, ribose metabolism, and phenylalanine metabolism, alterations in amino acid metabolism, and hypoxia	Distinct metabolomic profiles of asthmatics and healthy control patients can be identified in GC-MS based metabolomic profiling of serum	Internal cross-validation
McGeachie et al (2015)[21]	25	Integrated genomic-metabolomic model could predict asthma control (AUC, 95%)	monoHETE0863, and sphingosine-1-phosphate, arachidonic acid, PGE2 and S1P	Cellular immune response, interferon signaling, and cytokine-related signaling	Metabolomic profiling of plasma provides insight into the pathophysiology of asthma control	Bootstrapping and cross-validation
Fitzpatrick et al (2014)[22]	8,953 features	Identified 164 Discriminatory metabolites	Glycine, serine, and threonine	Oxidative stress: the glycine, serine, and threonine metabolism pathway and the N-acylethanolamine, and N-acyltransferase pathway	Severe, corticosteroid refractory asthma in children is associated with metabolic derangements	No
Jung et al (2013)[20]	64	PLS-DA model distinguished patients with asthma from healthy adults; training set AUC, 1 ($P < .001$); validation set: 0.9771 ($P < .001$). Prediction in validation set: 90.9% for asthma and 100% for control subjects	Formate, methanol, acetate, choline, O-phosphocholine, arginine, and glucose	Asthma status: hypermethylation, response to hypoxia, and immune reaction; severity: lipid metabolism	(1)H-NMR–based metabolite profiling of serum may be useful for the effective diagnosis of asthma and a further understanding of its pathogenesis	External validation models (n = 10 drawn from same population)

(Continued)

TABLE 2] (Continued)

Authors	No. of Metabolites	Results	Significant Metabolites	Implicated Pathways	Conclusions	Validation
Comhair et al (2015)[23]	293	25 compounds were significantly different between cases and control patients, 18 differed by asthma severity levels	Taurine, lathosterol, bile acids (taurocholate and glycodeoxycholate), nicotinamide, and adenosine-5-phosphate	Asthma status: steroid and amino acid/protein metabolism, inflammatory and immune pathways. Severity: bile acid metabolism and taurine transport	The plasma metabolome differs between patients with asthma and healthy control patients and by asthma severity	No

AUC = area under the curve; FeNO = fractional exhaled nitric oxide; NPV = negative predictive value; NR = not reported; PGE2 = prostaglandin E_2; PLS-DA = partial least squares discriminant analysis; PPV = positive predictive value; TCA = tricarboxylic acid; VOC = volatile organic compound. See Table 1 legend for expansion of other abbreviations.

Outcomes: A large number of common metabolites were associated with asthma case status, severity, exacerbations, and phenotype discrimination, suggesting metabolites contributing to disease onset may also contribute to its severity (Table 3). Even where individual metabolites were not concordant across similar studies, there was consistency in the enriched metabolic pathways (Table 4). Similarly, many were common to both the studies of adults and children. This finding of common metabolomic signatures in children and adults with asthma may support a shared etiology and pathophysiology for these two entities, in contrast to the prevailing belief that childhood asthma is influenced more by genetic predisposition, whereas adult asthma is more affected by environmental factors and obesity.[26] In fact, it may be age at asthma onset that is most important in this regard. This was not reported in the studies and, given that 95% of asthma is postulated to start in childhood,[27] it can be assumed the included studies are not representative of adult-onset asthma. Further metabolomic studies with rigorously characterized adult-onset asthma are required to determine if and how the metabolomic profiles of such cases differ.

Biological Samples: Unlike the genetic sequence, metabolite profiles can vary depending upon the biomaterial being assessed. Throughout the variety of biospecimens used in these studies, there was considerable consistency in the metabolites and metabolomic pathways identified as significant (Tables 3 and 4); however, larger numbers were not replicated between biospecimens. The lack of replication between studies using the same biospecimen should also be noted. This may be in part attributable to specimen collection conditions and processing procedures, which can affect the metabolome; however, there was no evidence of systematic bias relating to such variables for plasma or urine in these studies.

A number of variables should be considered for EBC, including whole breath vs end-tidal gases, collection device used as well as whether inhaled medications, spirometry, exercise, or other procedures have occurred before sample collection. Motta et al[13] investigated the impact of different condensation temperatures on the EBC metabolome (−27.3 and −4.8°C). They reported that although the samples collected at both temperatures resulted in metabolomics profiles that could distinguish asthma cases from control patients, the constituent metabolites of the profiles varied. Their work and that of others highlights that susceptibility to such external

TABLE 3] Metabolites Identified as Significant in More Than 1 Study

Group	Metabolite	Biospecimen	Population	Method	Outcome
Acid salt	Formate	Plasma,[20] EBC[13]	Adults[13,20]	NMR[13,20]	AvH[13,20]
	Hippurate	Urine,[16] EBC[13]	Adults[13,16]	GC-MS[16]; NMR[13]	AvH,[13] exacerbation[16]
	Succinate	Serum,[19] urine[15]	Children,[15] adults[19]	GC-MS[19]; NMR[15]	AvH,[15,19] phenotype[15]
Alcohol	Methanol	Plasma,[20] EBC[13]	Adults[13,20]	NMR[13,20]	AvH[13,20]
Amino acid	Alanine	Urine,[16] EBC[13]	Adults[13,16]	GC-MS,[16] NMR[13]	AvH,[13] exacerbation[16]
	Arginine	Plasma,[20] EBC[13]	Adults[13,20]	NMR[13,20]	AvH[13,20]
	Phenylalanine	Serum,[19] EBC[13]	Adults[13,19]	GC-MS,[19] NMR[13]	AvH[13,19]
	Threonine	Plasma,[22] urine[15,16]	Children,[15,22] adults[16]	LC-MS,[22] GC-MS,[16] NMR[15]	AvH,[15] phenotype,[15,22] exacerbation[16]
Intermediate in the catabolism of histidine	Urocanic acid	Urine,[14] EBC[13]	Children,[14] adults[13]	LC-MS,[14] NMR[13]	AvH[13,14]
Organic acid	Trans-aconitate	Urine,[15] EBC[13]	Children,[15] adults[13]	NMR[13,15]	AvH,[13,15], phenotype[15]
Purine nucleoside	Adenosine	EBC,[3,5,13] plasma[23]	Children[3,5]; Adults[13,23]	MS[3,5,23]; NMR[13]	AvH[3,13]; Phenotype[5,23]
Salt	Acetate	Plasma,[20] urine,[16] EBC[9,13]	Children,[9,20] adults[13,16]	LC-MS,[20] GC-MS,[9,16] NMR[13]	AvH,[13,20] AvW,[9] exacerbations[16]
VOC	1,4-dichloro-benzene	EBC[7,8]	Children[7,8]	GC-MS[7,8]	AvH,[8] WvH[7]
	2,4-dimethyl-1-heptene	EBC[7,9]	Children[7,9]	GC-MS[7,9]	WvH,[7] AvW[9]

AvH = asthma cases vs healthy control patients; AvW = asthma cases vs wheeze cases; phenotype = measures of asthma phenotypes and severity; WvH = heeze cases vs healthy control patients. See Table 1 legend for expansion of other abbreviations.

influences tends to be metabolite dependent. Further, it underlines the importance of standardizing collection and metabolite assays for biospecimens—a goal that has yet to be achieved in this field. Last, none of the studies profiled metabolites in more than one biospecimen type; therefore, it is not possible to determine the relationship between metabolites across biospecimens from the same individual, nor whether discriminatory metabolites could be detected in different biospecimens from the same subject.

Metabolomic Profiling: Metabolomic profiling technique may also account for differences in study findings. NMR uses the magnetic properties of atomic nuclei to generate information on structure and thereby identify metabolites in the biofluid under investigation by their unique pattern of chemical shifts and peak intensities.[28] Liquid or gas chromatography tandem mass spectrometry combines chromatography, a technique that separates metabolites, with MS, which measures their abundance. The complement of metabolites measured by NMR and MS may not always be comparable. Nevertheless, multiple metabolites and metabolomic pathways were defined as significant under both methods (Tables 3 and 4). Others were only identified as significant in the studies using NMR profiling such as arginine, formate, methanol,[13,20] and trans-aconitate.[13,15] However whether this is a function of the profiling method or other sources of heterogeneity between the studies cannot be discerned.

One fundamental source of heterogeneity is the use of a global untargeted metabolomic profiling approach, which aims to capture all metabolites in a biological system as opposed to a hypothesis-driven approach targeting specific metabolites, or metabolite classes. Fifteen of the included studies were targeted or semitargeted in nature: three focused on volatile organic compounds (VOCs)[7,9,13]; three on a combination of alkanes, alkenes, aldehydes, and ketones[6,16,17]; one on

TABLE 4] Metabolomic Pathways Identified as Significant in More Than 1 Study

Pathway	Biospecimen	Population	Method	Outcome
Amino acid metabolism	Serum,[19] plasma[23]	Adults[19,23]	GC-MS,[19] MS[23]	AvH,[19,23] phenotype[23]
Glutamate-glutamine cycle; glutamine and glutamate metabolism	EBC,[11] serum[19]	Children,[11] adults[11,19]	NMR,[11] GC-MS[19]	AvH[11,19]
Hypoxia response pathways	Serum,[19] plasma,[20] urine[15]	Children,[15] adults[19,20]	GC-MS,[19] NMR[15,20]	AvH,[15,19,20] phenotype[15]
Immune pathways	Plasma,[20,21,23] urine[14]	Children,[14,21] adults[20,23]	MS,[23] LC-MS,[14,2] NMR[20]	AvH,[14,20,23] phenotype,[23] asthma control[21]
Inflammatory pathways	EBC,[4,6] plasma[23]	Children,[4,6] adult[23]	GC-MS,[6] LC-MS,[4] MS[23]	AvH,[4,6,23] phenotype[23]
Lipid metabolism	Plasma,[20] serum,[18] EBC,[9] urine[17]	Children,[9] adults[17,18,20]	NMR,[20] GC-MS[9,17,18]	AvH,[18,20] AvW,[9] phenotype[17]
Oxidative stress	EBC,[6,9,10] plasma,[22] urine[16]	Children,[6,9,10,22] adults[16]	GC-MS,[6,9,16] LC-MS,[22] NMR[10]	AvH,[6,9,10] exacerbations,[16] phenotype[22]
Tricarboxylic acid cycle	Urine,[16] serum,[19] urine[15]	Children,[15] adults[16,19]	GC-MS,[16,19] NMR[15]	AvH,[15,19] phenotype[15,16]

See Table 1 and 3 legends for expansion of abbreviations.

leukotrienes[4]; and the rest on a variety of metabolites from specific panels. A targeted approach allows for optimal sensitivity in the measurement of these metabolites because it uses tailored and calibrated methods; however, it lacks the broad range of an untargeted approach and may miss novel but important metabolites without an a priori biological hypothesis. Crucially, it also hampers the replication and validation of findings between studies.

Other Variables: Replication may also have been affected by heterogeneity in the diagnostic criteria for asthma, with variable use of physician diagnosis, spirometric criteria, and/or subject self-report. Even where a definitive diagnosis can be made, asthma reflects a broad spectrum of disorders of varying severity. The concordance between the studies across a range of asthma outcomes suggests a similar underlying pathogenesis, however.

The metabolome is known to fluctuate with factors such as BMI and to be highly sensitive to external influences including diet, smoking status, and treatment regime. Three[7,12,20] studies withheld treatment for a set period before sample collection. Others reported on medication usage, some used treatment as a measure of asthma severity, and five[3,8,9,11,19] did not report on treatment at all. Currently, the data are too limited to draw conclusions regarding the effect of treatment on the asthma metabolome. Similarly, the data on potential confounders are not yet comprehensive enough for analysis, but will benefit from the efforts of the wider metabolomics community to identify the metabolomic shifts induced by various environmental factors and physiological characteristics, and the "healthy" metabolome.

Biological Insights Into Asthma Pathophysiology and Treatment

An expanding list of human metabolites has been annotated and comprehensively mapped to specific biological pathways. In the reviewed studies, a large number of pathways were reported to be associated with asthma outcomes in a variety of biospecimens (Table 2). Although diverse, these pathways can be broadly categorized on the basis of general physiological or molecular roles: (1) immune response, signaling, and inflammation; (2) metabolism of amino acids, sugars, bile acids, steroids, and lipids; (3) oxidative stress and hypoxia; (4) cellular energy homeostasis; and (5) DNA hypermethylation. Among studies investigating asthma status, all were represented. In analyses of asthma phenotypes, immune responses, oxidative stress, energy metabolism, and metabolism of amino acids and lipids were enriched, whereas asthma control[21] was associated primarily with immune response pathways.

Aberrant immune responses and acute inflammation are hallmark features of all asthmatic phenotypes, and the predominance of inflammatory and immunological

response pathways is not surprising. An enrichment of pathways reflecting the increased metabolism of amino acids, lipids, steroids, and bile acids that are fundamental to asthma pathogenesis is also anticipated. Amino acids are mediators of immunological activities in asthma and have antioxidant functions; in particular, taurine, glycine, glutamine, and glutamate may have potentially protective effects, whereas phenylalanine may have adverse effects. Lipid mediators are key drivers of inflammatory responses in asthma and have well-characterized roles in T-cell recruitment and energy metabolism; therefore, the enrichment of lipid metabolism pathways in asthma metabolomic studies is consistent with the biological importance of these molecules in asthma pathogenesis. The role of oxidative stress in asthma has also been well-studied, and evidence suggests that an imbalance between oxidation and reducing systems, in the favor of oxidative states, contributes to asthma severity. Both endogenous and exogenous reactive oxygen species including superoxide and reactive nitrogen and hydrogen species, increase airway inflammation, and are key determinants of asthma severity. Activated inflammatory cells in the airway produce reactive oxygen species that contribute to poor asthma control by reducing the ability of the airway epithelium to repair damage resulting from oxidative stress.

Pathways related to hypoxia were also significantly enriched. Increased hypoxic responses by the inflamed airway have been observed in asthma and were reported to lead to exacerbations in acute and chronic experimental allergic models of asthma, but not in healthy, noninflamed lung tissue.[29] The increase in oxidative and hypoxic stress responses in asthma coincides with considerable alterations in cellular energy metabolism. Levels of metabolites participating in the tricarboxylic acid cycle were altered in asthma, and fluctuations of metabolites in pathways involved in cellular energy metabolism in the lungs have been observed in mouse models of experimental asthma.[30] Potentially, alterations in these pathways may reflect the reduced ability of the damaged lung to meet the substantial energy demands of activated inflammatory cells in the allergic airway. Finally, epigenetic effects have a strong impact on asthma severity, and metabolites related to the methyl transfer pathway were also reported.[13,20] DNA methylation may increase airway inflammation by predisposing immune responses towards a Th2 phenotype; increased hypermethylation may therefore represent a novel epigenetic mechanism underlying asthma pathogenesis.[20]

Discussion: The Future of Asthma Omics

Asthma metabolomics studies to date are limited but encouraging and report a number of replicated biologically plausible metabolites and metabolomic pathways associated with the development and manifestation of asthma. Whether the nonreplicated results represent spurious findings or heterogeneity between the studies cannot be assessed with the literature available to date. Much of this heterogeneity stems from lack of standardization in the field, and highlights the need for the development of a rigorous set of criteria for conducting and reporting metabolomic studies.

If clinical translation is the end goal, several factors must be considered. First, the determination of specificity: the biomarkers must be specific to the asthma phenotype rather than representing a general profile of a biological system in a dysregulated physiological state. In these studies, the VOC profile of wheeze[7] was similar to many of the asthma profiles. This is perhaps not unexpected; however, Esther et al[3] also reported similarities with cystic fibrosis profiles. In the wider literature, more distinct respiratory disorders such as ARDS as well as exposure to environmental pollutants that may affect lung function[31] were also associated with a number of the metabolites identified in this review. Perhaps most importantly, many "asthma metabolites," particularly the amino acids and those involved in choline metabolism, have been associated with other chronic diseases including multiple malignancies.[32] Although this does not negate their possible involvement in the pathogenesis of asthma, it does call into question their utility as stand-alone biomarkers.

A further question involves the role of the biomarkers. Most studies focused on distinguishing asthma cases from healthy control patients; however, established clinical markers and criteria for the diagnosis of asthma already exist. A more useful role for metabolomic biomarkers may be in the discrimination of different subtypes, which are currently not well-defined. Prediction is arguably of the greatest clinical use. No studies used a prospective design to identify predictive biomarkers, although one focused on wheezing in preschoolers, which could be considered an early asthma phenotype. In terms of the most optimal biospecimen, EBC is an attractive, noninvasive method approach for collecting samples with more direct relevance to the end organ of interest. However, in the included studies, there was no evidence that EBC-based biomarkers outperformed blood or urine.

Clearly, further refinement of biomarkers is required before clinical translation is a viable option. Metabolomic data alone may be insufficient to fully characterize complex pathologies.[33] The integration of metabolomics with other omic data to identify the interactions and synergisms between the different hierarchical components of the "central biological dogma" represents a potential strategy that will allow the visualization of a biological system on a global level.

Ried et al[18] integrated metabolomic profiles with asthma-associated single nucleotide polymorphisms (SNPs) and observed that several SNPs at the asthma susceptibility locus 17q21 influenced asthma-associated metabolites, particularly phosphatidylcholines, and concluded that the simultaneous analysis of metabolite and genetic data provide an improved understanding of diseases mechanisms on a molecular and functional level. McGeachie et al[21] expanded on this approach by additionally incorporating gene expression and methylation data into their analysis. This led to both an increased understanding of physiology and an increased predictive accuracy, relative to the use of a single omic technology, again supporting the integration of multiple data types.

To date, no studies have integrated metabolomics and proteomic data, although this may in fact be the most informative integrative strategy. A single gene can generate multiple different proteins through alternative splicing, and posttranslational modifications and proteolytic processes. These proteins form the main structural components of all cells and control the majority of their biological functions.[34] One crucial role is as enzymes to catalyze metabolic and signaling pathways; however, it can be difficult to ascertain the endogenous physiological function of these different enzymes because they often exist as part of large networks and are regulated by posttranslational events. Metabolomic profiling of the substrates involved in these reactions can help assign biochemical functions to these enzymes providing access to "a portion of biomolecular space that is inaccessible to genomics and proteomics"[35] and have the potential to identify functionally relevant biological targets.

The field of proteomics has developed almost in parallel with metabolomics, although the terminology was coined slightly earlier in 1994.[36] Analogous to metabolomics, some of the most commonly used technologies for protein separation and identification include liquid chromatography and mass spectrometry.[37] The proteome is also similarly dynamic and sensitive to exogenous exposures and intracellular stimuli.[38] Together, these omes provide a downstream read out of the genome and its direct interaction with the environment.

As with metabolomics, proteomics studies remain limited in respiratory medicine, specifically asthma.[34] Studies are hindered by many of the same issues, including sample size, lack of standards for sample collection, handling and storage, multiple incompatible profiling technologies, and underdeveloped analytical methods.[38] Similarly, quantification and identification of proteins is challenging; as with the metabolome, the entire proteome is yet to be characterized. Unidentified proteins may account for up to 60% of the total proteomic database and it is unclear exactly how large it is.[37,39] Mapping of the proteome is more advanced than the metabolome, however, with drafts of the human and lung proteomes available.[34] Crucially, as with metabolomics, the majority of reported proteomic findings have yet to be validated.[40]

One notable difference between metabolomics and proteomics is in the biospecimens used. EBC, which has a low protein content, forms only a minority of the literature, whereas sputum, lung epithelial lining, or BAL fluid, which more directly reflect the lungs activity, are much more commonly used.[39] The abundance of proteins in plasma and serum can be both an advantage and a disadvantage, particularly for the measurement of the less abundant, lower molecular weight proteins.[41] The choice of biospecimen may therefore affect the findings[34] and is an important consideration for integrated omics moving forward.

The proteomics asthma literature to date has been summarized in several comprehensive reviews.[38,40] In plasma proteins involved in iron metabolism, coagulation cascade, acute-phase response, responses to stress and pathogens, and in complement cascades have all been reported. Complement cascades were also identified among the sputum and BAL fluid literature, together with signaling; calcium-binding and lung remodeling proteins; proteins involved in cellular movement, immune cell trafficking, collagen fibrillogenesis and chemotaxis; cytokines; chemokines; matrix metalloproteinases; signaling; and, crucially for integration, metabolic enzymes. Yet no proteomic biomarkers with clinical applications in asthma have emerged thus far.[38] These findings broadly support those of the existing metabolomics studies, which is, in

particular, an important role for the immune system and for identifying novel potentially important processes. Going forward, however, it may be anticipated that the exact points(s) of dysregulation, within a pathway, and therefore more clinically relevant information, can be identified by actually combining these two data sets.

Conclusion

Omics technologies remain in the early stages. Although increasingly promising results are being reported, metabolomics and proteomics in particular are limited by a lack of standards in the field and uncertainty in the optimal analytical methods. Additionally it is becoming increasingly clear that integrated omic analyses are necessary to maximally leverage these data. As more large population-based studies begin to generate multiomic data, it is likely to represent the newest frontier in asthma research. However, clinical utility has yet to be demonstrated, and whether the future of asthma metabolomics and integrative omics lie in the development of biomarkers, or whether it is better suited to increasing the understanding of its underlying biology remains to be determined.

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