Metabolomics of Respiratory Diseases

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Abstract

Metabolomics is an expanding field of systems biology that is gaining significant attention in respiratory research. As a unique approach to understanding and diagnosing diseases, metabolomics provides a snapshot of all metabolites present in biological samples such as exhaled breath condensate, bronchoalveolar lavage, plasma, serum, urine, and other specimens that may be obtained from patients with respiratory diseases. In this article, we review the rapidly expanding field of metabolomics in its application to respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), pneumonia, and acute lung injury, along with its more severe form, adult respiratory disease syndrome. We also discuss

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V. Ghini et al. (eds.), *Metabolomics and Its Impact on Health and Diseases*,
Handbook of Experimental Pharmacology 277, https://doi.org/10.1007/164_2022_614

the potential applications of metabolomics for monitoring exposure to aerosolized occupational and environmental materials. With the latest advances in our understanding of the microbiome, we discuss microbiome-derived metabolites that arise from the gut and lung in asthma and COPD that have mechanistic implications for these diseases. Recent literature has suggested that metabolomics analysis using nuclear magnetic resonance (NMR) and mass spectrometry (MS) approaches may provide clinicians with the opportunity to identify new biomarkers that may predict progression to more severe diseases which may be fatal for many patients each year.

Keywords

Acute lung injury · Acute respiratory distress syndrome (ARDS) · Asthma · Chronic obstructive pulmonary disease (COPD) · Pneumonia

1 Introduction

Metabolomics is an established field of systems biology that has generated substantial new findings in respiratory research. The ability of metabolomics to produce a "snapshot" of small molecules within a given sample from the body provides a powerful tool for temporal analyses to follow the distribution and concentration of these molecules (Patti et al. 2012). Small molecules of interest include chemicals (such as drugs) and metabolites (including waste products of metabolism). These small molecules are a distinct group of compounds from the larger proteins and nucleic acids (RNA, DNA), and their measurement provides a valuable complement to other fields of systems biology (transcriptomics, genomics, proteomics, and others). Further, metabolomics informs other areas of systems biology as it lies downstream of proteins, RNA, and genes. Because of its ability to detect small molecules, metabolomics has the potential to discover novel biomarkers of disease as well as environmental and occupational exposure (Madsen et al. 2010; Robertson et al. 2011). To understand the relevance of metabolomics in respiratory diseases, it is important to establish how the metabolome is defined and how this aligns with other approaches in systems biology.

The metabolome of an organism reflects events that occur in the proteome, transcriptome, and genome. Changes in proteins, RNA, and genes result in alterations of metabolite concentrations in biological fluids and tissues. Perhaps unsurprisingly, measurement of metabolites in human samples is not a new procedure, since metabolites have been used for millennia to aid in the diagnosis of disease. For example, diabetes mellitus has been diagnosed since ancient times based on the taste of glucose, a small molecule metabolite, in urine from patients with type I diabetes. While this may be an unappetizing and perhaps unsafe practice these days, this was essentially the first diagnostic test for a metabolite in urine samples. The recognition that urine contains important biomarkers of disease led to the development of analytical tools to measure these in a variety of samples from the

body today. Today, the measurement of small molecules in human samples forms the basis of clinical chemistry, established to assist health professionals in diagnosis of illnesses.

Most clinical chemistry tests rely on the measurement of handful of metabolites, and often these are only qualitative (positive or negative) rather than quantitative tests. Because most metabolites measured using clinical tests are abundant and not specific to any one disease, their detection must always be taken into consideration with other clinical descriptors. Thus, the focus of clinical chemistry on such a small group of metabolites is a significant limitation that prevents the applicability of metabolite detection in the specific diagnosis of many diseases.

The limitations of traditional clinical chemistry highlight the advantages of metabolomics. Recent improvements in the sensitivity and specificity of metabolite detection using metabolomics have allowed the characterization and quantification of complex metabolic profiles resulting in concurrent analysis of hundreds of metabolites in a single sample. Metabolomics seeks to quantitatively assess complex metabolic patterns in patient samples and is coupled with computational technologies to allow the interpretation of data in the context of known metabolic pathways. The complexity of the metabolome in a patient sample is further augmented by the presence of metabolites that derive from the microbiome, which is present in almost all samples obtained from the human body. The microbiome generates metabolites that are unique to prokaryotic organisms and may be distinguished from the host's own metabolome, thus providing another possible approach for enhancing the diagnosis and prognosis of disease.

Despite substantial investments in genome analysis in diseases, genetic mutations that result in the manifestation of disease are rare. Only 1–2% of disease risk for a spectrum of conditions including asthma, chronic obstructive pulmonary disease (COPD), and acute respiratory distress syndrome (ARDS) can be explained by genetic mutations. Transcriptomic and proteomic analysis has generated more insight into their potential as biomarkers, but these too have not developed into standard disease indicators. In contrast, metabolomics and clinical chemistry reproducibly demonstrate that metabolites are highly predictive for a large proportion of complex diseases (Xia et al. 2013). Samples may be used from a broad range of sources including saliva, nasal lavage, exhaled breath condensate (EBC), bronchial washings, sweat, blood (plasma and serum), urine, feces, among others. Examples of established metabolic biomarkers include glucose for diabetes, as mentioned above, creatinine to detect kidney disease, cholesterol and triglycerides to evaluate the risk of developing cardiovascular disease, uric acid for gout detection, and thyroxine for hypo/hyperthyroidism. There are undoubtedly other metabolites that may be used to serve as biomarkers in a range of diseases.

These findings indicate that the metabolome is a much more dynamic group of analytes than the proteome, transcriptome, or genome, as it can change immediately in response to environmental or physiological changes (Fig. 1) (Wishart 2005). To appreciate the contribution that metabolomics may make to diagnosis of disease, it is useful to compare the impact of environmental and physiological impact on proteins, RNA, and genes. Environmental and physiological changes have negligible impact

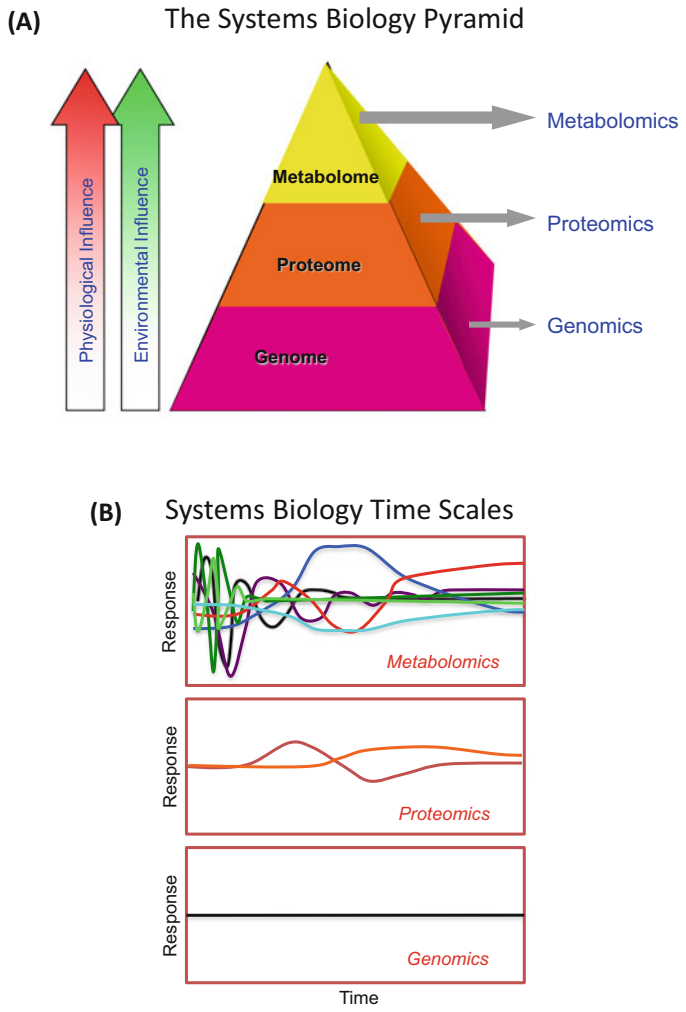

Fig. 1 The systems biology pyramid and time scales of responses to environmental influences. (**a**) Over 25,000 genes have been identified in human genomics, compared with a smaller number of enzymes and even smaller number of metabolites. The responsiveness to physiological and environmental insults of each of these components increases as we go from genomics to metabolomics. (**b**) While metabolomics shows rapid changes in multiple metabolites in a short period of time, proteomics shows smaller changes in abundance while genomics shows negligible changes over the same period

on somatic gene expression, while some transcriptomic and proteomic changes have been detected. In contrast, metabolomic changes in response to environmental and physiological factors closely correlate with these events and can be altered within seconds of exposure (Fig. 1b). Therefore, significant changes in metabolites may be measurable in samples over far shorter time scales than by other systems biology

approaches. This allows for a powerful approach for detection of changes in biomarkers in real time and provides an opportunity to use metabolomics as a biomonitoring tool in health and disease. Historically we have adhered to the concept of a single biomarker for each disease, but this limits the accuracy, precision, and sensitivity of the assay. New and developing metabolomics approaches suggest that we may use a pattern of metabolites to describe a given disease. However, by using multiple biomarkers for each disease, techniques become more sophisticated, and the computing power used to analyze the data becomes much more complicated.

In this article, we review the expanding field of metabolomics in its application to respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), pneumonia, acute lung injury/acute respiratory distress syndrome, and occupational and environmental lung diseases. We also discuss the metabolomics associated with the lung microbiome in asthma and COPD. These findings show that there remains a considerable amount of experimental work to be done to understand the role of the metabolome in respiratory diseases, and how this may be applied to the diagnosis and/or prognosis of illness. Recent findings have shone some light onto the relationship between the gut and lung microbiome metabolites in generating metabolic signatures that may provide mechanistic insights into various lung diseases, as well as deliver potential biomarkers associated with specific lung conditions.

2 Respiratory Diseases with Metabolomic Signatures

2.1 Asthma

Asthma is an inflammatory disease of the airways that is often triggered by exogenous perturbations. The recent Global Burden of Disease report stated that an estimated 262 million people were affected by asthma in 2019, and 461,000 people died from this disease in that year (Vos et al. 2020). Asthma is a highly heterogeneous disease with different phenotypic variations, as well as multiple causative agents, etiology, and complex inflammatory and pathophysiological features. Thus, it is proposed that significant metabolic changes are associated with different phenotypes of disease.

Metabolic profiling has demonstrated significant variations in serological and urinary metabolomic pathways that are distinct in various phenotypes of asthma and provides valuable information about the accuracy and precision of asthma diagnosis, disease progression, and response to treatment (Fig. 2) (Kelly, "Pharmacometabolomics of Asthma as a road map to Precision Medicine"). Using technologies such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), several metabolomic studies have demonstrated comprehensive evidence of metabolic alterations in asthma. In Table 1, we have summarized some of the recent reports of metabolomic studies in asthma.

Studies conducted on asthma patients with varying degrees of disease severity, ages, or obesity have been reported. Interestingly, all these studies showed a high

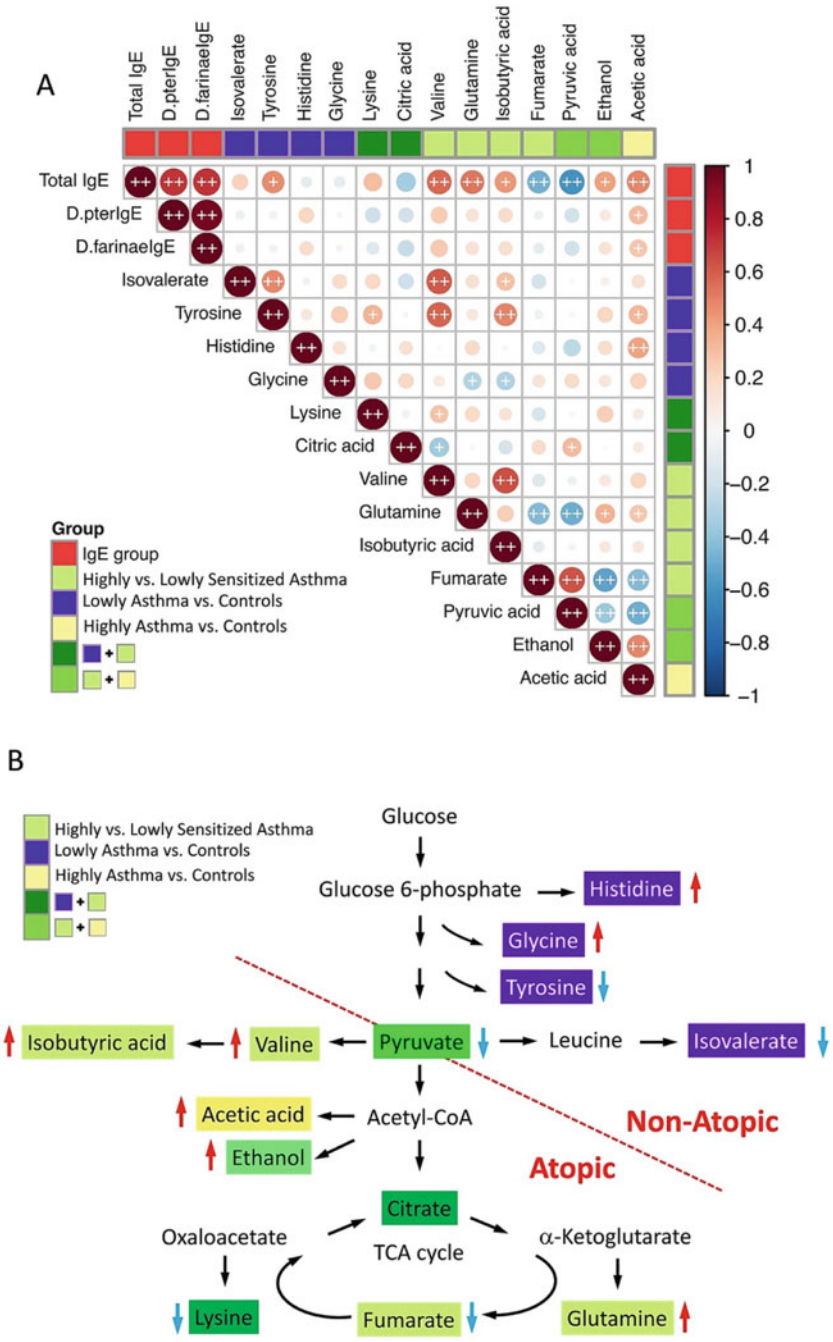

Fig. 2 Metabolites and metabolic pathways in childhood asthma. Heatmap of Spearman's rank correlation coefficients between metabolites associated with lowly and highly sensitized asthma (**a**) and metabolic pathways of metabolites associated with atopic and non-atopic asthma (**b**). Red color

correlation among specific metabolites with disease. Park et al. (2017) showed that in severe asthma in children (≤15 years of age) exhibiting corticosteroid resistance, tyrosine metabolism, degradation of aromatic compounds, and glutathione metabolism were suggested to be significant pathways related to corticosteroid resistance based on urine metabolites. A targeted LC-MS-based study for the presence of a unique biomarker in asthmatic children showed a combination of 2-isopropylmalic acid and betaine can classify children with asthma and controls. It was also shown in the same study that asthmatics had lower relative concentrations of serum ascorbic acid, 2-isopropylmalic acid, shikimate-3-phosphate, 6-phospho-D-gluconate, and reduced glutathione. In the case of overweight children, niacin concentrations were elevated in serum samples (Checkley et al. 2016). Loureiro et al. showed that lipid peroxidation-related metabolites in urine samples are associated with asthma severity and lung function, along with eosinophilic inflammation in nonobese asthmatic patients (Loureiro et al. 2016). In other studies, it was shown that metabolic pathways and pathway components like arginine, proline, taurine, hypotaurine, glyoxylate, and dicarboxylate in serum and urine samples were closely related to acute exacerbations of asthma as well as the choice of corticosteroid treatment (Quan-Jun et al. 2017). It was also found that a set of 15 volatile carbon compounds may discriminate between controlled and uncontrolled asthma and that 7 of these compounds detected in exhaled breath samples could predict exacerbation within the next 14 days with 88% sensitivity and 75% specificity (van Vliet et al. 2017).

Lipids have also been correlated with the diagnosis of asthma. Kang et al. showed that certain metabolites, primarily lipid biomolecules in bronchoalveolar lavage (BAL) fluid, could be markedly elevated in asthma compared to non-asthmatic healthy individuals (Kang et al. 2014). This observation, supported by other studies (Loureiro et al. 2016; Ghosh et al. 2020), indicates that lipid metabolism is altered in asthma, potentially as a result of increased oxidative stress. Such altered lipid metabolism was also associated with asthma severity, reduced lung function, and higher eosinophilic inflammation in asthmatic individuals (Loureiro et al. 2016). While asthma and obesity are known to share common systemic manifestations, Maniscalco et al. showed that methane, pyruvate, and glyoxylate and dicarboxylate metabolic pathways in EBC also greatly vary between obese and nonobese asthma patients (Maniscalco et al. 2017), which indicates more complex crosstalk between asthma and obesity than previously recognized.

Other recent studies have reported intriguing results of altered profiles of structural lipid molecules in asthma compared to healthy individuals (Kang et al. 2014; Ghosh et al. 2020; Reinke et al. 2017; Pang et al. 2018; Jiang et al. 2021). Bian et al. reported that some derivatives of serum arachidonic acid that serve as potential mediators for allergic responses were significantly elevated in asthma (Bian et al.

Fig. 2 (continued) represents positive correlations; blue color represents negative correlations; red arrow represents increase; blue arrow represents decrease. + symbol means a P-value < 0.05; + + symbol means a P-value < 0.01. [Reproduced from Chiu et al. (2021)]

Table 1 Metabolomics studies – asthma

Study	Study population (adult/children)	Sample/method	Summary of results
Kang et al. (2014)	Adults (38 asthmatics and 13 healthy)	Quadrupole time-of-flight (QTOF) MS of bronchoalveolar lavage fluid (BALF)	• ↑ Lysophosphatidylcholine (LPC), triglyceride (TG), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylserine (PS), and sphingomyelin (SM) in non-steroidal bronchial asthma NSBA) compared to healthy subjects • No difference was observed between steroid-treated bronchial asthma (SBA) and healthy subjects
Loureiro et al. (2016)	Adults (57 asthmatics)	Targeted solid phase microextraction (SPME) with two-dimensional gas chromatography and time-of-flight MS (GCxGC-TOF/MS) of urine	Metabolites related to lipid peroxidation were associated with ↑ asthma severity, ↓ lung function, and ↑ eosinophilic inflammation in nonobese patients with asthma
Ghosh et al. (2020)	Adults (34 asthmatics, 30 COPD, 35 ACO, 33 healthy)	GC-MS of serum	↑ 2-Palmitoylglycerol, cholesterol, serine, threonine, ethanolamine, glucose, stearic acid, linoleic acid, D-mannose, succinic acid in asthma than healthy ↓ Lactic acid, 2-palmitoylglycerol in asthma than healthy
Maniscalco et al. (2017)	Adults (25 obese patients with asthma, 30 obese patients without asthma, 30 lean patients with asthma and 72 adults in the external validation set)	Untargeted LC-MS of EBC	Participants with asthma, obesity, and obesity + asthma showed distinct variations in respiratory metabolic fingerprint
Reinke et al. (2017)	Adults (54 asthmatics, 22 healthy)	Untargeted LC-MS of serum	↑ Ceramide (C16:0, C18:0, C20:0, C22:0, C24:0, C24:1), sphingomyelin (C18:0, C18:1), hexosylceramide (C18:0, C24:1), and cysteinyl leukotriene E_4 (LTE_4) in asthma than healthy ↓ 14,15-Dihydroxyeicosatetraenoic acid (DiHETE), 19,20-Dihydroxydocosapentaenoic acid (DiHDPA) in asthmatics than healthy

(continued)

Table 1 (continued)

Study	Study population (adult/children)	Sample/method	Summary of results
Pang et al. (2018)	Adults (29 asthmatics, 15 healthy)	Ultra-performance liquid chromatography-tandem MS (UPLC-MS) of serum	↑ Monosaccharides, LysoPC(o-18:0, 18:1), Retinyl ester, PC(18:1/2:0), PC(16:0/18:1), arachidonic acid, PE(18:3/14:0) in asthma ↓ Glycerophosphocholine, PS(18:0/22:5), cholesterol glucuronide, Phytosphingosine, Sphinganine, LysoPC(p-18:1), retinols, PC(20:4/16:1)
Jiang et al. (2021)	Adults (33 asthmatics, 28 healthy)	LC-MS/MS of plasma	↑ Phosphatidylethanolamine (PE) (18:1p/22:6), PE (20:0/18:1), PE (38:1), sphingomyelin (SM) (d18:1/18:1), triglyceride (TG) (16:0/16:0/18:1) in asthmatics than healthy ↓ Phosphatidylinositol (PI) (16:0/20:4), TG (17:0/18:1/18:1), phosphatidylglycerol (PG) (44:0), ceramide (d16:0/27:2), lysoPC (22:4) in asthma
Chiu et al. (2020, 2018)	Adults (30 asthmatics, 30 healthy)	NMR of urine	↑ Guanidoacetate ↓ 1-Methylnicotinamide, allantoin
Chiu et al. (2021)	Children (28 asthmatics, 25 healthy)	NMR of plasma	↑ Lysine, isovalerate, histidine, tyrosine, glycine, citric acid, ethanol, acetic acid, pyruvic acid in asthma
Chang-Chien et al. (2021)	Adults (92 asthmatics, 73 healthy)	NMR of EBC	↑ Lactate, formate, butyric acid, isobutyrate in asthma
Bian et al. (2017)	Adults (15 asthmatics and 15 healthy)	Ultra-high performance liquid chromatography quadrupole time-of-flight (UHPLC)-Q-TOF- MS of serum	↑ 5(S)-Hydroxyeicosatetraenoic acid (HETE), 8(S)-HETE, 11(S)-HETE, 12(S)-HETE, 15(S)-HETE, 15(S)-Hydroxyeicosapentaenoic acid (HEPE), prostaglandin (PG)A2, PGB2, PGF1a, PGF2a, PGJ2, 15-keto-PGF2a in asthma compared to healthy ↓ Palmitic acid, Lauric acid in asthma than healthy
Checkley et al. (2016)	Children (50 asthmatics and 49 healthy between 9 and 19 years)	Targeted liquid chromatography-MS (LC-MS) of serum	↓ Relative concentrations of serum ascorbic acid, 2-isopropylmalic acid, shikimate-3-phosphate, 6-phospho-D-gluconate, and reduced glutathione in asthmatics than healthy

(continued)

Table 1 (continued)

Study	Study population (adult/children)	Sample/method	Summary of results
Kelly et al. (2017)	Children (380 asthmatics)	Targeted LC-MS (complementary methods) of plasma	• Metabolites (primarily glycerophospholipid, linoleic acid, and pyrimidine) were associated with airway hyperreactivity, and pre- and postbronchodilator FEV_1/FVC • Distinct metabolites showed moderate but important signatures between disease severity
Tao et al. (2019)	Children (80 asthmatics, 29 healthy)	GC-MS of urine	↑ Aspartic acid, Xanthosine, hypoxanthine, N-acetylgalactosamine ↓ Stearic acid, Heptadecanoic acid, uric acid, D-threitol
Li et al. (2020)	Children (30 asthmatics, 30 healthy)	GC-MS of urine	↑ Azelaic acid, citraconic acid 4, D-altrose 1, D-erythro-sphingosine 1, gentiobiose 2, 2-hydroxybutanoic acid, L-allothreonine 1, leucine, stearic acid, succinic acid, tyramine in asthmatics than healthy ↓ 3,4-dihydroxycinnamic acid, methionine 1, purine riboside, malonic acid 1, cysteine, erythrose 1, lactamide 1, uric acid, valine in asthma
Matysiak et al. (2020)	Children (13 asthmatics, 17 healthy)	LC-MS/MS of blood	↑ L-arginine, B-alanine, Y-amino-N-butyric acid, L-histidine, Hydroxy-L-proline in asthma ↓ D,L-B-Aminoisobutyric acid, taurine, L-tryptophan, L-valine in asthma
Ferraro et al. (2020)	Children (26 asthmatics, 16 healthy)	UPLC-MS of EBC	↑ 9-amino-nonanoic acid, 12-amino-dodecanoic acid, lactone of PGF-MUM, N-linoleoyl taurine, 17-phenoxy trinor PGF2α ethyl amide, lysoPC (18:2(9Z,12Z)) in asthma
Van Vliet et al. (2017, 2016)	Children (96 asthmatics)	Targeted GC-TOF/MS for VOCs in EBC	• 7 VOCs (3 aldehydes, 1 hydrocarbon, 1 ketone, 1 aromatic compound, and 1 unidentified VOC) in exhaled breath could predict asthma exacerbations

2017). They described that some saturated fatty acids such as palmitic acid and lauric acid levels were decreased in asthma. In addition, metabolites derived from protein or carbohydrate metabolisms were found altered in asthma compared to non-asthmatic healthy individuals in EBC, plasma, and urine (Chang-Chien et al. 2021; Chiu et al. 2018, 2020). However, in adults, the asthmatic response can be caused, triggered, or aggravated by different risk factors such as allergy, environmental exposures, active or passive smoking, and workplace conditions. Therefore, more studies on different adult asthma phenotypes are required to better understand those metabolic alterations.

On the other hand, asthma in children is mostly caused by allergic conditions or genetic predisposition (such as parental atopy or asthma) and, to some extent, pregnancy-related issues such as gestational smoking. In a group of asthmatic and non-asthmatic children, Checkley et al. showed lowered relative concentrations of serum ascorbic acid, reduced glutathione (GSH), and some carbohydrate derivatives in asthma (Checkley et al. 2016). Kelly et al. further showed association between certain plasma metabolites (glycerophospholipid, linoleic acid, and pyrimidine) and airway hyperreactivity in asthmatic children (Kelly et al. 2017). They were able to demonstrate moderate but clinically important signatures of distinct metabolites in accordance with the disease severity (Kelly et al. 2017). Several other reports have demonstrated distinct metabolomic profiles in asthmatic children compared to non-asthmatic healthy individuals (Tao et al. 2019; Li et al. 2020; Matysiak et al. 2020; Chiu et al. 2021).

Recently, breath analysis has suggested some intriguing metabolic alterations in asthma, particularly related to volatile organic compounds (VOCs) in the EBC that could predict asthma exacerbations in children (van Vliet et al. 2017; Ferraro et al. 2020; Van Vliet et al. 2016). However, most of those analyses did not consider potential risk factors or confounding factors as mentioned earlier. Therefore, clinical correlations between metabolites and symptoms/severity are important to consider while inferring those results into clinical practice.

2.2 Chronic Obstructive Pulmonary Disease (COPD)

COPD is a major lung disease worldwide that causes significant morbidity and mortality and is among the top causes of death in many countries (Keogh and Mark 2021). COPD is a chronic inflammatory disease of the lungs that is progressive and irreversible in nature (Devine 2008). Although cigarette smoking is the most common major risk factor for COPD, occupational or environmental insults are also known to be prominent triggers for the onset and progression of this debilitating lung condition.

Recent studies have demonstrated that COPD is a variable condition with multimodal phenotypic variants, particularly because of the differences in causal agents, course, and progression of the disease. Although many metabolic alterations of COPD were unknown until the beginning of the twenty-first century, these have

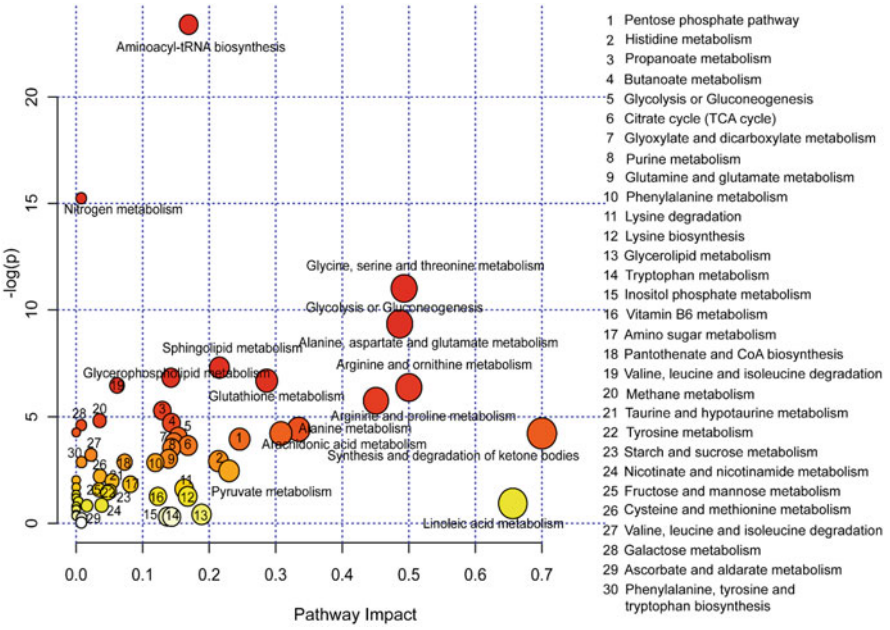

Fig. 3 Distinct metabolites identified in COPD-associated metabolomics studies. Metabolic pathways analysis based on distinct metabolites published in chronic obstructive pulmonary disease (COPD)-associated metabolomics studies performed by applying the Metabo-Analyst 4.0 platform. The names of 44 disturbed metabolic pathways were marked in the pathway figure, which mainly involved dysfunctions of amino acid metabolism, lipid metabolism, energy production pathways, and imbalance of oxidation and antioxidation. [Reproduced from Ran et al. (2019)]

so far exhibited an intriguing panorama based on what has been discovered to date (Fig. 3). In this section, we briefly describe some of the important findings from metabolomic research in COPD.

Several studies have identified metabolites that are distinctive in COPD (Turano, "NMR-based metabolomics to evaluate individual response to treatments"). Novotna et al. (2018) examined 10 COPD patients and 10 healthy individuals and observed that two amino acids, alanine and phenylalanine, were significantly lower in the peripheral blood of COPD patients than healthy individuals, while pyroglutamate level was higher in COPD patients. They also observed that the free carnitine to acylcarnitine ratio was significantly lower in COPD patients than the healthy individuals. Another report by Diao et al. (2019) further demonstrated that COPD patients had reduced serum levels of creatine, glycine, histidine, and threonine compared to non-COPD smokers. Although these findings indicate a possible subclinical malnutrition in the context of respiratory disease, results are still inconclusive regarding the association of these specific metabolites with COPD.

Body composition is greatly affected in COPD as the disease progresses (Schols et al. 2005). Chronic bronchitis and emphysema are the two distinct phenotype

variations of COPD and patients with these diseases have different body silhouettes, presumably due to difference in lipid metabolic pathways. Some reports have suggested that perturbation of lipid metabolism occurs in COPD (Chen et al. 2019; Rafie et al. 2018). In the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS) cohort, Halper-Stromberg et al. (2019) observed that phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, leucine, and lysine from BAL fluid in COPD patients were associated with higher odds of developing emphysema. Liang et al. (2019) identified that serum metabolites such as glutamine, glycine, histidine, hypoxanthine, α-N-phenylacetyl-L-glutamine, L-pipecolic acid, P-chlorophenylalanine, pseudouridine, and L-citrulline levels were markedly different between asthma and COPD.

There is an increasing body of evidence suggesting that sphingolipids, which that play crucial roles in the structure and function of plasma membranes and signal transduction, also have roles in the pathogenesis of COPD, asthma, and other lung conditions (Vlahos 2020). Lipidomic studies have shown that COPD patients have higher plasma concentrations of very low density lipoprotein (VLDL) compared to healthy individuals, which strongly correlates with higher central and peripheral airway resistance (Rafie et al. 2018). Nambiar et al. (2021) found that blood palmitoleic acid, linoleic acid, and dihydrotestosterone were lower in COPD patients than healthy controls. Similarly, another study showed that the levels of serum lysophosphatidylcholine (LPC) 18:3, lysophosphatidylethanolamine (LPE) 16:1, and phosphatidylinositol (PI) 32:1 were markedly reduced in acute exacerbations in COPD, thus highlighting the role of glycerophospholipids in the pathophysiology of COPD (Gai et al. 2021).

Another recent report reiterated these findings in the context of disease onset and stages in COPD where the authors observed that phosphatidylcholine and LPC were key indicators of COPD onset and that phosphatidylserine and diacylglycerol could potentially indicate the various COPD stages (Zhou et al. 2020). In line with these observations, polyunsaturated acid metabolites were found to be associated with reduced lung function and disease severity in COPD (Ran et al. 2019; Yu et al. 2019; Xue et al. 2020). Pinto-Plata et al. identified plasma lipid metabolites that may predict survival differences in COPD patients (Pinto-Plata et al. 2019). Using the Karolinska COSMIC cohort, Naz et al. (2017) found that the autotaxin-lysophosphatidic acid axis may be dysregulated due to oxidative stress in COPD and that sex-regulated phenotypes are influential in the pathophysiology of disease. However, despite several reports demonstrating associations between metabolites with disease progression and severity in COPD, it is still not clear whether these metabolites may influence pathophysiological mechanisms. Furthermore, there may be several residual confounders that influence the dysregulation of metabolic pathways in disease conditions. Therefore, any interpretation and conclusions made from these metabolic outcomes should be made cautiously (Kilk et al. 2018).

2.3 Pneumonia

Community-acquired pneumonia remains a major cause of morbidity and mortality around the world, with over a million hospitalizations each year in the USA prior to COVID-19 (Griffin et al. 2013). Among the most common bacterial strains involved in community-acquired pneumonia are *Streptococcus pneumoniae* and *Staphylococcus aureus*, which are also found as commensal bacteria in healthy humans. The challenge in controlling the incidence of pneumonia is to determine the etiological process by which it occurs in individual patients. Using systems biology approaches, it is hoped that diagnosis and monitoring of disease may be enhanced to allow for more accurate prescription of drugs in pneumonia and similar inflammatory lung diseases (Wheelock et al. 2013).

Application of NMR analysis of pneumonia patient urine suggests that definitive metabolic profiles could be applied to infection with *S. pneumoniae* (Fig. 4). The pattern of urinary metabolites detected in pneumococcal pneumonia could be distinguished from pneumonia associated with viruses and other bacterial strains (Slupsky et al. 2009a). An animal model of pneumonia also demonstrated that distinct metabolic profiles could be detected in the urine of mice infected with *S. pneumoniae* or methicillin-resistant *S. aureus*, a major cause of antibiotic-resistant pneumonia (Fig. 5) (Slupsky et al. 2009b). These studies indicate that metabolomics has potential for the diagnosis, monitoring, and clinical management of pneumococcal diseases.

2.4 Acute Lung Injury/Acute Respiratory Distress Syndrome (ARDS)

Acute lung injury and its more severe form, ARDS, is characterized by infiltration of an inflammatory, fibrin-rich exudate into the pulmonary interstitium and alveolar spaces (Gattinoni et al. 2014; Martin and Matute-Bello 2011; Ware and Matthay

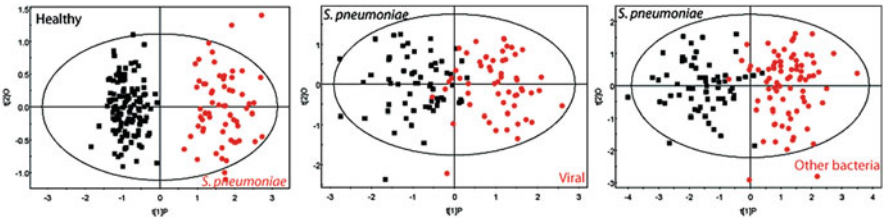

Fig. 4 Differentiating between different types of pneumonia in human patients. Urinary metabolites were found to be distinct in pneumonia caused by *S. pneumoniae* and other pathogens. These graphs show OPLS-DA models based on 61 measured metabolites found in the urine from *S. pneumoniae* patients compared with those found in viral pneumonia and other bacteria (including *Mycoplasma tuberculosis*, *Legionella pneumophila*, *S. aureus*, and others). Reprinted with permission from Slupsky et al. (2009b) J. Proteome Res. 8:5550–5558. Copyright 2009 American Chemical Society

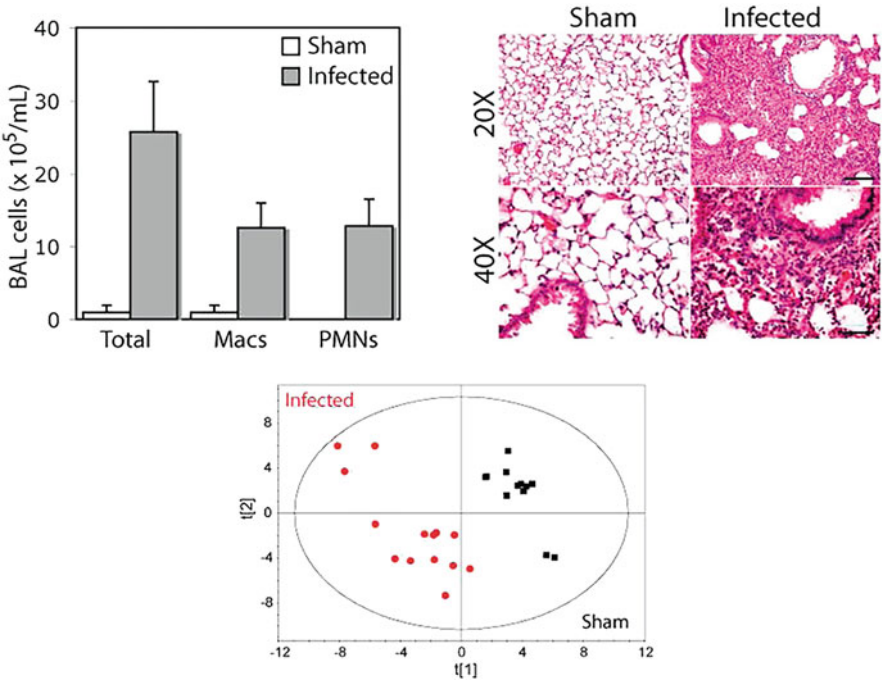

Fig. 5 Metabolic profiles in mice infected with *S. pneumoniae*. An inbred strain of mice (C57BL/6), maintained in specific virus antigen-free housing with autoclaved bedding and identical dietary supplies, was infected intratracheally with a clinical isolate of *S. pneumoniae*, serotype 14. After 24 h of infection, bronchoalveolar lavage (BAL) samples were analyzed for cell counts (**a**) and histology was carried out on lung sections (**b**) to confirm inflammation arising from infection. At the same time, urine samples were collected from animals that were subjected to NMR analysis and a PCA model of urinary metabolite concentrations was generated (**c**). Reprinted with permission from Slupsky et al. (2009a) J. Proteome Res. 8(6):3029–3036. Copyright 2009 American Chemical Society

2000; Li et al. 2011). This influx leads to impaired lung function and diminished gas exchange (Ware and Matthay 2000). First described in 1967 by Ashbaugh et al. (1967), ARDS is precipitated by many different causes, with the most common being sepsis, pneumonia, severe trauma, and more recently, severe COVID-19 (Huang et al. 2020). ARDS is accompanied by an extraordinarily high mortality rate (approximately 30% of patients die upon diagnosis of ARDS), and to date there have been few effective pharmacotherapies for its treatment that mainly serve to shorten the duration of illness rather than reverse it entirely. In addition, no effective predictive or prognostic biomarkers are available to indicate the likelihood of a patient developing ARDS. This has prompted a search for biomarkers of ARDS, which has been led by genomics and proteomics, although neither field has yielded suitable markers, and no candidate has progressed beyond the initial discovery phase (Serkova et al. 2011; Rogers and Matthay 2014; Meyer 2013, 2014). This is likely

due to the heterogeneity of disease, and much of the variation could lie beyond the proteome or genome, possibly in the metabolome (Serkova et al. 2011; Rogers and Matthay 2014). Thus, metabolomics presents itself as a potentially valuable tool for analysis in ARDS.

A challenge with understanding mechanisms associated with ARDS is that there are no translational animal models that accurately mimic human disease (Martin and Matute-Bello 2011; Matute-Bello et al. 2011; Matute-Bello and Downey 2013). Despite this limitation, there have been several metabolomic studies carried out in rodent models that demonstrate changes in metabolites (Stringer et al. 2016). In early experimental models, mechanical ventilation-induced ARDS in rodents generated metabolic profiles in serum, lung tissue, and BAL samples (Izquierdo-Garcia et al. 2014). Putative metabolites of ARDS were reported to be increased lactate and decreased glucose and glycine in lung tissues, together with increased glucose, lactate, acetate, 3-hydroxybutyrate, and creatine in BAL samples. NMR-detected metabolites in lung samples were associated with markers of ARDS phenotype (peak inspiratory pressure, PaO_2, and lung histology), but there was no association between these ARDS indices and serum metabolites. In one of the first studies examining the metabolomics of experimental ARDS, a cytokine-induced lung injury model was tested to determine the temporal association between inflammation in the lungs and changes in lung metabolome (Serkova et al. 2008). Cytokine-induced lung injury resulted in decreased ATP, energy balance, and energy charge levels, suggesting a decreased energy state. Together with this there was a significant increase in glycolytic activity, measured as elevated lactate-to-glucose levels that normalized 24 h after the induction of injury. Collectively these findings indicate that a shift in cell energy metabolism occurs in lung tissues in ARDS. The benefit of this study was that it demonstrated an association between phenotypic and metabolic changes, an important first step in biomarker discovery. To date, biomarkers have not been found that can differentiate between the two extremes of mild interstitial edema and extensive cellular injury in the spectrum of acute lung injury. However, continued analysis by magnetic resonance imaging and metabolic NMR spectroscopy may enhance the development of more robust and predictive longitudinal processes of experimental lung injury. Other animal models have shown significant metabolic shifts in ARDS induced by a variety of stimuli, reviewed in detail in Stringer et al. (2016).

Few clinical studies have reported metabolomics analysis of patients with ARDS. Several studies suggest that the use of BAL samples could provide insight into the metabolomic profile associated with ARDS. In one study, at least 26 and 18 endogenous metabolites, respectively, could be used to differentiate ARDS from healthy BAL samples using liquid chromatography-MS analysis (Evans et al. 2014). These included lactate and other energy metabolism-associated metabolites such as citrate, creatine, and creatinine which are increased in the plasma of patients with ARDS (Stringer et al. 2011). These findings demonstrate the utility of BAL as a biofluid for metabolomics analysis.

In addition, some reports have demonstrated the utility of exhaled breath as a vehicle for metabolomics analysis (Schubert et al. 1998; Bos 2018). For example, Schubert et al. demonstrated the utility of exhaled breath as a sample for metabolomics analysis (Schubert et al. 1998). This was furthered in a study by Bos et al. (2014) which found that three metabolites, octane, acetaldehyde, and 3-methylheptane, were able to discriminate ARDS from non-ARDS patients. Octane is an end-product of lipid peroxidation, one of the degenerative processes caused by oxidative stress (Riely et al. 1974; Horvat et al. 1964).

Interestingly, a recent study examining EBC from patients on mechanical ventilation due to severe COVID-19 or non-COVID-19 ARDS showed a characteristic "breathprint" for COVID-19 (Grassin-Delyle et al. 2021) that could be distinguished from non-COVID-19 ARDS. In this study, the four most prominent volatile compounds in COVID-19 patients were methylpent-2-enal, 2,4-octadiene, 1-chlorohelptane, and nonanal, suggesting that real-time metabolomics analysis of exhaled breath may identify patients with COVID-19. Nonanal is a sub-product of oxidative stress-mediated destruction of the cell membrane (Rahman 2003).

In summary, the metabolomics data generated from experimental and clinical studies of ARDS demonstrate that a disturbance in oxidative stress metabolism and energy levels occur in this disease, which is consistent with what has been described for the pathology of ARDS. To date, there appears to be no multi-center prospective studies done for metabolomics analysis of ARDS. Our understanding of ARDS metabolomics has been based on small studies that demonstrate feasibility for evaluation of ARDS phenotypes and for determining lung injury severity. Going forward, we will need to establish clinical trials aimed at testing prevention and treatment strategies in ARDS patients by applying metabolomics analysis to the spectrum of disease that presents in this population.

2.5 Occupational and Environmental Lung Diseases

Occupational exposure is one of the major risk factors associated with respiratory illnesses, and the incidence of occupational lung diseases is increasing due to expanding populations and consumer needs (Moitra et al. 2015). According to the report of the International Labour Organization, nearly two million people die each year due to workplace accidents, of which over 30% die due to lung cancer or other lung diseases as a result of workplace exposure (Cullinan et al. 2017). In many cases, occupational lung diseases are improperly recorded or detected, often due to a lack of causal evidence, all of which contributes to a significant underestimation of the true burden of these diseases. Although several biomarker-related reports have been published in the context of occupational exposure, metabolomic studies have been very limited to date. We discuss some of the few studies below on occupational and environmental lung diseases.

Among a group of workers employed in carbon-coating friction systems, Maniscalco et al. (2018) found that the concentrations of VOCs, including

1,2-propanediol, phenylalanine, 3-hydroxybutyrate, and isopropanol, were significantly elevated in the EBC of the workers who did not wear a mask at the work, compared to those who routinely wore masks.

Other markers such as polycyclic aromatic hydrocarbons (PAH) have been found to be associated with occupational exposure. Wei et al. studied the joint effects of arsenic exposure, smoking, and physical exercise on lung function changes among a group of coke-oven workers and found that urinary concentrations of PAH were significantly higher in coke-oven workers than office workers in the same industry who were not directly exposed to the ovens (Wei et al. 2021). Using a nationwide biomonitoring survey of the Korean National Environmental Health Survey, Koh et al. collected measurements of urinary 1-hydroxypyrene (1-OHP) as a metabolite of interest for PAH exposure at workplace. They found that the level of urinary 1-OHP was highest among people engaged in construction and mine-related occupations. Although that study did not explicitly study associations between urinary metabolites and respiratory health, the effect of PAH on respiratory health is already well known and therefore, urinary 1-OHP could potentially be a marker of PAH-associated respiratory dysfunctions.

The collapse of the World Trade Center (WTC) on 9/11 introduced a novel and unprecedented exposure scenario in which hundreds of thousands of New Yorkers were affected in the ensuing years. Firefighters and all first responders were exposed to huge amounts of dust containing numerous fibrous, chemical, and hazardous substances. To date, several reports have been published on the respiratory health of the workers who were exposed to WTC dust, resulting in a condition known as WTC lung disease (also known as WTC sarcoid-like granulomatous pulmonary disease). For example, a recent study provided novel insights into metabolic syndrome as a risk factor for lung function decline in a cohort of firefighters exposed to materials arising from the collapse of the WTC (Kwon et al. 2021). They also proposed that regulating metabolic syndrome, particularly dyslipidemia, could also help to decrease the risk of developing WTC lung disease. This group also showed previously that the serum metabolome, particularly the sphingolipid cluster containing sphingosine-1-phosphate, a pleiotropic inflammatory mediator, was low in WTC lung diseases, suggesting decreased bioavailability and increased risk of compromised vascular integrity in WTC lung disease (Crowley et al. 2018). A mouse model of WTC particulate matter exposure was also investigated and showed that several prominent metabolic pathways were affected, including advanced glycation end-products and lipids (including sphingolipids), that correlated with inflammatory changes and attenuation of antioxidant potential (Veerappan et al. 2020). However, despite these interesting outcomes, correlations between metabolomics and clinical evaluation in occupational lung diseases remain limited, and therefore more studies are required to elucidate the crosstalk between these two aspects.

3 Metabolomics of Lung Microbiome in Respiratory Diseases

3.1 Asthma

The lung microbiota and metabolome are likely to play a pivotal role in the onset of disease in the case of asthma (Barcik et al. 2020). It is now emerging that metabolically active microbiota that reside in the lung under normal conditions maintain a complex network of crosstalk with the host in a symbiotic manner. In disease conditions, however, this symbiosis is transformed into dysbiosis that can alter the host immune response, which influences the overall lung health (Loverdos et al. 2019). The composition of normal lung microbiota consists of *Bacteroidetes* and *Firmicutes* (the most abundant two genera), and apart from these two, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria* have also been found by 16S rRNA sequencing in endobronchial brushing samples (Charlson et al. 2011; Bassis et al. 2015). Although normal lung microbiota consists of a relatively small bacterial population, estimated to be around 10^3 to $10^5/cm^2$, their intensely intricate crosstalk is thought to be primarily responsible for the conduct of most of the host-microbiome interplay (Charlson et al. 2011; Bassis et al. 2015; Hilty et al. 2010; Mathieu et al. 2018; Denner et al. 2016; Goleva et al. 2013).

In asthma, the bacterial pattern of the pulmonary microbiome has been characterized in several studies. It is evident that some bacterial species become elevated in nasopharyngeal swabs from asthmatics, such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Moraxella catarrhalis*, compared to healthy controls. These bacteria are well-known pathogens that can cause infectious exacerbations (Dickson et al. 2016). Interestingly, Huang et al. showed in patients with severe asthma, *Actinobacteria* is present at high abundance in correlation with elevated sputum leukocytes and eosinophils in bronchial biopsies (Huang et al. 2015). It has been also shown that elevated eosinophil numbers in lavage, along with reduced FEV_1, correlate with bacterial α-diversity (based on comparison of different species present in same sample) in endobronchial brushings of asthmatic subjects. Bacterial species associated with lower airway obstruction show distinctive features associated with FEV_1 levels. For example, patients with asthma exhibiting $FEV_1 < 60\%$ had low α-diversity but high β-diversity compared to asthma patients with $FEV_1 > 80\%$ (Denner et al. 2016).

Interestingly, the gut microbiome is an important component of asthma pathophysiology which has not been explored in detail. The human gut possesses a surface area of 150–200 m^2, which harbors 100,000 to 100 billion bacteria per mL of sample, depending on the region of sample collection (Sender et al. 2016). A relationship between the gut and lung was discovered upon the observation that different lung diseases may be influenced by changes in the gut microflora and vice versa. The microbiota in these two sites is therefore connected by a gut-lung axis that is important in relation to asthma (Marsland et al. 2015). Among many different metabolites produced by the gut microbiome, short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, have been found to regulate physiological and immunological responses in humans. It is well known that not only do SCFAs

provide a source of energy, but they also function as signaling molecules. SCFAs have been shown to have multiple signaling effects: they inhibit histone deacetylases (HDACs) that increase cytokine gene expression by promoting an anti-inflammatory cell phenotype to maintain homeostasis, suppress transcription factors (nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB)), and reduce tumor necrosis factor-α (TNF-α) production (Durack et al. 2017; Chambers et al. 2018; Tan et al. 2014). Depletion of SCFA-producing bacteria as a mechanistic link between the microbiome and asthma susceptibility or severity has been suggested by Cait et al. (2018). Hence, SCFAs derived from the metabolic activity of gut microbiota inhibit proinflammatory responses in the lungs. Although the mechanism underlying this pathway is unclear, the most likely mechanistic explanation is that the hepatic system may weaken innate immune responses by SCFAs binding to G protein-coupled receptors and/or inhibition of the mevalonate/isoprenoid pathway through HMGCoA reductase (Young et al. 2016). The specific mediators that make up the communication between gut and lung is still unclear, but it has been speculated that gut epithelial cells and immune cells absorb signals from the endothelium to form local cytokine microenvironments, and eventually this alters the immune response in distal sites such as the lung (Budden et al. 2017).

Overall, these studies demonstrate that the gut and lung microbiome, and its associated metabolome, have an enormous impact on patient outcomes in asthma. Findings from these reports could contribute to the discovery of mechanisms and novel biomarkers for asthma and its associated exacerbations.

3.2 Chronic Obstructive Pulmonary Disease (COPD)

Recent evidence suggests an association between the lung microbiome and COPD, suggesting a contribution of the lung bacterial community to disease progression in the form of dysbiosis (Hilty et al. 2010; Erb-Downward et al. 2011; Zakharkina et al. 2013; Pragman et al. 2012). Phylogenetic analysis of microbial populations in samples collected from the oropharynx and bronchial brushings from COPD patients and healthy controls showed increased populations of pathogenic *Proteobacteria* (*Haemophilus* spp.) over *Bacteroidetes* (*Prevotella* spp.), with the latter being especially reduced (Hilty et al. 2010). Other studies also demonstrated that healthy individuals commonly exhibit higher populations of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria*, in contrast to pathogenic *Haemophilus*, *Streptococcus*, *Klebsiella*, *Pseudomonas*, and *Moraxella* in COPD patients (Wu et al. 2014; Murphy et al. 2005). In addition, several reports have described that exposure to tobacco smoke can modify bacterial populations in the mouth and lungs. Though studies are limited in the context of COPD, numerous reports indicate an alteration of the oral and respiratory bacterial microbiome as an effect of tobacco smoking (Morris et al. 2013; Zhang et al. 2018; Huang and Shi 2019). In COPD patients, commensal colonization of *H. influenzae*, *S. pneumoniae*,

Pseudomonas, and *Moraxella* are frequently observed in the lungs (Simpson et al. 2016).

The gut-lung axis also features prominently in COPD (Young et al. 2016). Fecal microbiota derived from COPD patients have been demonstrated to contribute to the development of COPD in a mouse model (Li et al. 2021). The fecal microbiota of COPD patients were found to contain lower levels of SCFAs, which could contribute to the manifestation of COPD.

In another recent study comparing the metabolomic profiles of COPD patients with healthy humans (Bowerman et al. 2020), it was found that COPD patients and healthy individuals manifest significantly different sets of microbial and metabolic signatures in fecal samples. As many as 146 bacterial species differ in between these two groups, along with a group of the top 50 indicator metabolites that distinguished between healthy and COPD individuals, consisting of mostly lipids (46%), amino acids (20%), and xenobiotic compounds (20%). Hence, it can be deduced that the intricate mechanisms associated with the gut-lung axis and the host's microbial community play a crucial role in the manifestation and progression of COPD.

4 Conclusive Remarks

Taken together, we have reviewed some of the literature associated with metabolomics analysis of biological fluids obtained from patients and experimental animal models with a range of respiratory diseases. Metabolomics is a fundamental part of systems biology analysis that has enormous clinical potential in discovering novel biomarkers as well as understanding disease pathophysiology. Because of its rapidly changing properties in health as well as disease, metabolomics has the power to generate snapshots of metabolites from a given sample that can be followed over time with repeated sampling. Several high-throughput systems have the ability to capture the identities and qualities of metabolites in a rapid manner using NMR or MS-based techniques. Challenges remain with the application of NMR in complex biological samples, which is less sensitive to small amounts of metabolites in many cases than MS. An important distinction to make is that analysis of metabolites in lung-specific samples is predicted to provide greater sensitivity to the tissue-specific metabolome over that of blood-derived (plasma, serum, or whole blood) or urinary metabolites. This is especially evident in the case of analysis of the metabolomics of the lung microbiome. Variability of NMR-measured metabolites is also an issue, with differing results found within a single facility as well as multiple locations (Lacy et al. 2014). In addition, a substantial number of unknown metabolites have been detected by MS that await more detailed identification in biological samples. We look forward to a future where we can implement increasingly sophisticated analyses of biological samples using systems biology approaches in respiratory diseases.

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