Clinical Chemistry 67:12
1606–1617 (2021)

Review

Metabolomics in Clinical Practice: Improving Diagnosis and Informing Management

John D. Odom [a] and V. Reid Sutton[a,b,*]

BACKGROUND: Metabolomics is the study of small molecules to simultaneously identify multiple low molecular weight molecules in a system. Broadly speaking, metabolomics can be subdivided into targeted and untargeted types of analysis, each type having advantages and drawbacks. Targeted metabolomics can quantify analytes but only looks for known or expected analytes related to particular disease(s), whereas untargeted metabolomics is typically nonquantitative but can detect thousands of analytes from an agnostic or nonhypothesis driven perspective, allowing for novel discoveries.

CONTENT: One application of metabolomics is the study of inborn errors of metabolism (IEM). The biochemical hallmark of IEMs is decreased concentrations of analytes distal to the enzymatic defect and buildup of analytes proximal to the defect. Metabolomics can detect these changes with one test and is effective in screening for and diagnosis of IEMs. Metabolomics has also been used to study many nonmetabolic diseases such as autism spectrum disorder, various cancers, and multiple congenital anomalies syndromes. Metabolomics has led to the discovery of many novel biomarkers of disease. Recent publications demonstrate how metabolomics can be useful clinically in the diagnosis and management of patients, as well as for research and clinical discovery.

SUMMARY: Metabolomics has proved to be a useful tool clinically for screening and diagnostic purposes and from a research perspective for the detection of novel biomarkers. In the future, metabolomics will likely become a routine part of the evaluation for many diseases as either a supplementary test or it may simply replace historical analyses that require several individual tests and sample types.

[a]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; [b]Baylor Genetics Laboratory, Houston, TX.
*Address correspondence to this author at: 6701 Fannin St. Suite 1560, Houston, TX 77030, USA. Fax 832-825-4294; e-mail vrsutton@texaschildrens.org.
Received May 20, 2021; accepted August 17, 2021.
DOI: 10.1093/clinchem/hvab184

Introduction

The goal of metabolomics is to identify a broad range of low molecular weight molecules (<1000 Da) in a sample (1). These molecules can be endogenous to the organism or exogenous, reflecting environmental factors such as diet, an organism's microbiome, and medications. In recent years, metabolomics has proved to be a useful tool for the evaluation of diseases such as inborn errors of metabolism (IEM), cancer, and autism spectrum disorder (1–3). Most metabolomic platforms are based on the separation techniques of liquid chromatography (LC), gas chromatography (GC), nuclear magnetic resonance spectroscopy (NMR), capillary electrophoresis, or mass spectrometry (4). Combinations of techniques such as ultra-high-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) are used to detect an even wider range of small molecules (5). Metabolomics can be subdivided into either targeted or untargeted. Targeted metabolomics is widely used in clinical laboratories, and includes such testing as acylcarnitine, amino acids, and very-long-chain fatty acid analyses (6). Metabolomics is typically carried out on plasma, cerebrospinal fluid (CSF) or urine samples, but, as discussed later, can be used on a much wider variety of sample types. In targeted metabolomics, the chemicals to be analyzed are preselected. This allows for a highly sensitive test with a large dynamic range of measurements; however, with this test, only those molecules that have been selected for will be detected so it is not possible to discover novel analyte patterns (5). Untargeted metabolomics, on the other hand, can simultaneously identify a broad range and very large number of biochemical analytes in a sample. The challenges related to the analysis and interpretation of untargeted metabolomics include identification of analytes not included in the analysis library and in integrating the results of hundreds of biochemicals from diverse metabolic pathways to provide an interpretation and diagnosis (7). Metabolomic databases exist to help guide researchers but ultimately a reference standard is almost always needed for metabolite identification, and, even so, unidentifiable analytes are likely to be detected (8, 9). Additionally, as one is only looking at a snapshot of the metabolome, many factors that are difficult to control for can influence the results. Timing of sample

collection in relation to the last meal, exercise, medication administration, and even diurnal variation can all have profound effects on the results (10). Untargeted metabolomics is increasingly being used for both clinical and research applications. From a clinical perspective, both targeted and untargeted metabolomics offers the potential for screening tests that can be both broader and potentially cheaper than standard targeted analyses using traditional testing modalities. Many physicians will be familiar with urine organic acid analysis, a form of untargeted analysis typically using a GC–MS platform, which has been used for decades for the clinical diagnosis of IEM. Untargeted metabolomics expands on this type of analysis in both scope and diversity of analytes, being able to detect a much broader spectrum of biochemicals (such as acylcarnitines, amino acids, vitamins, phosphosphingolipids, neurotransmitters) and orders of magnitude more analytes (11). In addition, when used in conjunction with DNA sequencing techniques, metabolomics has been shown to improve interpretation of genomic variants (10, 12, 13). From a research perspective, the main advantage of metabolomics is its ability to show primary, secondary, and even tertiary biochemical effects of a condition and to discover novel biomarkers that may lead to an improved understanding of the pathophysiology of a disease and/or effects of treatment on the underlying biochemical perturbation(s) (12, 14). Here, we discuss the growing use of untargeted metabolomics and explore how both researchers and clinicians have been using metabolomics to investigate a wide range of disorders of inborn errors of metabolism such as aminoacidopathies, fatty acid oxidation disorders, and urea cycle disorders, as well as non-IEM conditions such as autism spectrum disorder, Rett syndrome, neuroinflammatory conditions, and cancer (2, 3, 15). We also discuss how untargeted metabolomics has led to the discovery of novel biomarkers for various diseases and how it has been used to assist in the interpretation of genomic data.

Disorders of Inborn Errors of Metabolism (IEM)

SCREENING AND DIAGNOSIS

Newborn screening (NBS) has come a long way since Dr. Robert Guthrie first introduced a bacterial inhibition assay for the detection of phenylketonuria (PKU; OMIM 261600) (16), and in the past decades, newborn screening programs have used targeted metabolomics to screen infants for various IEMs such as aminoacidopathies, organic acidemias, disorders of fatty acid oxidation, and urea cycle disorders. The first use of metabolomics for newborn screening purposes was implemented in 1998 at the New South Wales Newborn Screening Program (17). A larger scale targeted metabolomic analysis was conducted from 2007 to 2014 in the south of Italy, which has also shown reassuring results (10). The inherent ability of metabolomics to test for multiple conditions in a single sample, in this case a dried blood spot, has several advantages including rapid turnaround time with high-volume throughput and reduced amount of blood required from neonates. Additionally, cost-effectiveness studies have shown that the savings from NBS consistently outweigh the cost of implementation (18). Additionally, the diagnostic sensitivity and diagnostic specificity for most biochemical disorders screened is >99% (18, 19).

Untargeted metabolomics has shown promise in screening and diagnosis of individuals with IEMs (Table 1). It is worth noting that not all biochemical phenotypes included in Table 1 have associated clinical phenotypes. In one study, untargeted metabolomics was used to analyze plasma samples of 120 individuals with previously confirmed IEMs (1). The platform was based on GC–MS and LC–MS in both positive and negative ion mode. The researchers were able to detect multiple perturbations in the pathways of 20 out of the 21 different IEMs represented in their cohort, including fatty acid oxidation disorders, urea cycle disorders, cobalamin-related disorders, aminoacidopathies, and organic acidemias (1). A similar study was conducted by a different group (21) using untargeted metabolomics based on an LC–MS platform. This study analyzed the metabolomes of 260 controls vs 53 individuals with a confirmed IEM; 33 separate IEMs were represented in their cohort including aminoacidopathies, urea cycle disorders, organic acidurias, fatty acid oxidation defects, purine and pyrimidine disorders, and peroxisomal disorders. Two blinded laboratory specialists experienced in clinical biochemical genetics each independently reviewed the metabolic signatures of the 53 patients. The correct diagnosis was achieved in 90% of cases. In another study (20), researchers were able to correctly identify 42 of 46 different IEMs using their "next-generation metabolic screening" analysis based on an LC quadrupole time of flight platform. This platform was able to identify over 10 000 analytes in a sample, so to reduce data complexity researchers selected 340 metabolites previously associated with IEMs to use as a first filtering step. These analytes were then compared to control samples and *P* values were calculated for each. The types of disorders able to be detected in this way included organic acidemias, urea cycle, and fatty acid oxidation disorders, as well as disorders of nucleotide, creatine, folate, bile acid, ketone body, and amino acid metabolism.

In addition to screening purposes, metabolomics has also shown promise for diagnostic purposes. For example, adenylosuccinate lyase deficiency (OMIM 207900) is a disorder of purine synthesis and recycling. It is traditionally diagnosed based on the presence of

Table 1. Inborn errors of metabolism (IEM) that have been investigated using metabolomics, organized according to disease category and IEMbase nosology number. Citations are provided for reference to primary source.

Disorder category	IEMbase Nosology number	Condition	Reference paper(s)
Disorders of pyrimidine metabolism	1.03	Uridine monophosphate synthase deficiency	Coene, 2018 (20)
Disorders of purine metabolism	2.03	Adenylosuccinate lyase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Donti, 2016 (22)
	2.11	Hypoxanthine guanine phosphoribosyltransferase deficiency	Coene, 2018 (20)
Disorders of creatine metabolism	4.03	Guanidinoacetate methyltransferase deficiency	Coene, 2018 (20); Miller, 2015 (1)
Disorders of choline metabolism	5.01	Dimethylglycine dehydrogenase deficiency	Coene, 2018 (20)
Disorders of ammonia detoxification	7.02	Carbamoyl phosphate synthetase I deficiency	Bonte, 2019 (21)
	7.03	Ornithine transcarbamolyase deficiency	Bonte, 2019 (21); Burrage, 2019 (14); Miller, 2015 (1)
	7.04	Argininosuccinic acid synthetase deficiency	Bonte, 2019 (21); Burrage, 2019 (14); Coene, 2018 (20); Miller, 2015 (1)
	7.05	Argininosuccinic acid lyase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Miller, 2015 (1)
	7.06	Arginase deficiency	Bonte, 2019 (21); Burrage, 2019 (14); Miller, 2015 (1)
Disorders of amino acid transport	8.06	Lysinuric protein intolerance	Bonte, 2019 (21); Coene, 2018 (20); Miller, 2015 (1)
Aminoacylase deficiencies	9.01	Canavan disease	Coene, 2018 (20)
	9.02	Aminoacylase I deficiency	Bonte, 2019 (21); Coene, 2018 (20)
Disorders of monoamine metabolism	10.02	Aromatic L-amino acid decarboxylase deficiency	Atwal, 2015 (23)
Disorders of phenylalanine and tetrahydrobiopterin metabolism	11.01	Phenylketonuria	Bonte, 2019 (21); Blasco, 2016 (24); Coene, 2018 (20); Jacob, 2018 (6); Miller, 2015 (1); Xiong, 2015 (25)
Disorders of tyrosine metabolism	12.02	Tyrosinemia type II	Jacob, 2018 (6)
	12.05	Alkaptonuria	Bonte, 2019 (21); Coene, 2018 (20)
	12.07	Tyrosinemia type I	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6)

Continued

Disorder category	IEMbase Nosology number	Condition	Reference paper(s)
Disorders of sulfur amino acid and sulfide metabolism	13.01	Methionine adenosyltransferase I/III deficiency	Coene, 2018 (20)
	13.06	Cystathionine beta-synthase deficiency	Coene, 2018 (20); Bonte, 2019 (21); Miller, 2015 (1); Scolamiero, 2015 (10)
	13.1	Ethylmalonic encephalopathy	Coene, 2018 (20)
Disorders of branched-chain amino acid metabolism	14.02	Maple syrup urine disease	Bonte, 2019 (21); Coene, 2018 (20); Miller, 2015 (1); Jacob, 2018 (6)
	14.07	Isovaleric aciduria	Bonte, 2019 (21); Coene, 2018 (20); Miller, 2015 (1); Scolamiero, 2015 (10)
	14.08	Isobutyryl-CoA dehydrogenase deficiency	Scolamiero, 2015 (10)
	14.09	2-Methylbutyryl-CoA dehydrogenase deficiency	Coene, 2018 (20)
	14.1	3-Methylcrotonyl-CoA Carboxylase deficiency	Bonte, 2019 (21); Miller, 2015 (1); Scolamiero, 2015 (10)
	14.15	2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency	Bonte, 2019 (21)
	14.16	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency	Coene, 2018 (20)
	14.16	3-OH-3methylglutaryl-CoA lyase deficiency	Miller, 2015 (1)
	14.18	Propionic aciduria	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6); Miller, 2015 (1); Scolamiero, 2015 (10)
	14.2	Methylmalonyl-CoA epimerase deficiency	Coene, 2018 (20)
	14.21	Methylmalonic aciduria	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6); Miller, 2015 (1); Scolamiero, 2015 (10)
	14.22	Combined methylmalonic/malonic aciduria	Coene, 2018 (20)
	14.23	Malonyl-CoA decarboxylase deficiency	Bonte, 2019 (21)
Disorders of lysine metabolism	15.02	Hyperlysinemia type I and II	Coene, 2018 (20)

Continued

Disorder category	IEMbase Nosology number	Condition	Reference paper(s)
	15.02	Pyridoxine-dependent epilepsy	Coene, 2018 (20)
	15.05	Glutaric aciduria type I	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6); Miller, 2015 (1)
Disorders of proline and ornithine metabolism	16.1	Ornithine aminotransferase deficiency	Bonte, 2019 (21); Coene, 2018 (20)
Disorders of β- and γ-amino acids	17.03	3-Ureidopropionase deficiency	Coene, 2018 (20)
	17.05	GABA-transaminase deficiency	Kennedy, 2019 (26)
Disorders of histidine metabolism	18.01	Histidinemia	Coene, 2018 (20)
Disorders of serine metabolism	23.01	Phosphoglycerate dehydrogenase deficiency	Glinton, 2018 (27)
	23.02	Phosphoserine aminotransferase deficiency	Ferreira, 2018 (28); Glinton, 2018 (27)
	23.03	Phosphoserine phosphatase deficiency	Ferreira, 2018 (28); Glinton, 2018 (27)
Disorders of cobalamin metabolism	26	Cobalamin-related disorders	Miller, 2015 (1); Scolamiero, 2015 (10)
Disorders of folate metabolism	27.06	Formiminotransferase cyclodeaminase deficiency	Scolamiero, 2015 (10)
	27.06	Glutamate formiminotransferase deficiency	Bonte, 2019 (21); Coene, 2018 (20)
Disorders of biotin metabolism	28.02	Holocarboxylase synthetase deficiency	Miller, 2015 (1)
Disorders of riboflavin metabolism	30.08	Multiple acyl-CoA dehydrogenase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6)
Disorders of molybdenum metabolism	39.01	Molybdenum cofactor deficiency	Coene, 2018 (20)
	39.04	Xanthinuria, type II	Coene, 2018 (20)
Disorders of galactose metabolism	47.01	Galactosemia	Coene, 2018 (20)
Disorders of the pentose phosphate pathway and polyol metabolism	49.03	Transaldolase deficiency	Coene, 2018 (20)
Disorders of mitochondrial DNA depletion, multiple deletion, or	69.01	Progressive external ophthalmoplegia. Mitochondrial recessive ataxia syndrome (MIRAS)	Buzkova, 2018 (29)

Continued

Disorder category	IEMbase Nosology number	Condition	Reference paper(s)
intergenomic communication	69.08	Mitochondrial neurogastrointestinal encephalopathy	Bonte, 2019 (21); Miller, 2015 (1)
Disorders of mitochondrial tRNA	73.11	Mitochondrial tRNA(Leu) 1 deficiency aka. mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)	Buzkova, 2018 (29); Sharma, 2021 (30)
Disorders of carnitine metabolism	82.03	Carnitine palmitoyltransferase II deficiency	Bonte, 2019 (21)
	82.05	Trimethyllysine hydroxylase epsilon deficiency	Miller, 2015 (1)
Disorders of fatty acid oxidation and transport	83.02	Medium chain acyl-CoA dehydrogenase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Miller, 2015 (1); Scolamiero, 2015 (10)
	83.03	Very-long-chain acyl-CoA dehydrogenase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Jacob, 2018 (6); Miller, 2015 (1)
	83.05	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency	Bonte, 2019 (21); Coene, 2018 (20)
Disorders of ketone body metabolism	84.13	Beta-ketothiolase deficiency	Bonte, 2019 (21); Coene, 2018 (20); Scolamiero, 2015 (10)
Disorders of cholesterol biosynthesis	95.01	Mevalonic aciduria	Bonte, 2019 (21)
Disorders of steroid metabolism	96.04	3-Beta-hydroxysteroid dehydrogenase deficiency	Coene, 2018 (20)
Disorders of bile acid synthesis	97.04	Cerebrotendinous xanthomatosis	Coene, 2018 (20)
	97.05	Alpha-methylacyl-CoA racemase deficiency	Bonte, 2019 (21)
Oligosaccharidoses	103.04	Beta-mannosidosis	Bonte, 2019 (21)
Disorders of peroxisomal biogenesis	112.01	Zellweger spectrum disorders	Wangler, 2018 (31)
	112.01	Refsum disease	Coene, 2018 (20)
Disorders of monosaccharide synthesis and interconversion	125.03	*N*-acetylneuraminic acid phosphate synthase deficiency	Coene, 2018 (20)

elevated levels of succinylaminoimidazole carboxamide riboside and succinyladenosine in the urine, CSF, and to a lesser extent in plasma, but in 2016 untargeted metabolomics was used to diagnosis this condition. Researchers confirmed the diagnosis with traditional biochemical analysis confirming metabolomic results of increased succinyladenosine (22).

Urea cycle disorders arise from enzyme/transporter deficiencies in the metabolic pathway dedicated to detoxifying nitrogenous waste. The major risk to these patients is hyperammonemic encephalopathy and death if not treated properly. Diagnosis and monitoring of these disorders is traditionally based on targeted metabolomic platforms. However, untargeted metabolomics has also been used to study urea cycle disorders and was able to detect pathognomonic analytes traditionally used for diagnosis (1). These studies have also shed some light on novel biomarkers for urea cycle disorders and will be discussed later.

Ornithine transcarbamoylase deficiency (OTC; OMIM 311250) is the most common urea cycle disorder and is inherited in an X-linked manner (32). Untargeted metabolomics was performed using 3 separate platforms based on GC–MS, and LC–MS in both positive and then negative ion mode on samples taken from patients with OTC. Semiquantitative *Z*-scores for analytes were calculated by comparison to mean and standard deviation values of a reference population (1, 5, 14). Orotate was the only biomarker with a *Z*-score greater than 2 in patients with OTC deficiency (17 subjects). Since OTC is X-linked, females may be asymptomatic carriers or, due to skewed X inactivation, may have partial OTC deficiency and may be prone to hyperammonemia. The authors of one study (14) analyzed the metabolomes of 9 heterozygous females, 5 of which had a history of hyperammonemia. The group with a history of metabolic decompensation had increased orotate, uridine, and glutamine compared to the group without a history of decompensation. OTC diagnoses can be challenging, especially considering that up to 15% of patients with OTC may not have identifiable pathogenic variants on molecular testing (33); untargeted metabolomics is a promising tool to help with supplementing diagnostic work up for such patients.

Aromatic L-amino acid decarboxylase (AADC) (OMIM 608643) deficiency is a disorder of serotonin, dopamine, and catecholamine synthesis. It is characterized by developmental delay, hypotonia, abnormal movements, autonomic dysfunction, oculogyric crises, and seizures. AADC deficiency has traditionally been diagnosed by CSF neurotransmitter analysis, or by DNA testing. In 2015, untargeted plasma metabolomics analysis was reported to identify a diagnostic biochemical profile for AADC (23). Researchers found increased concentrations of 3-methoxytyrosine in the patient's plasma, which is notable since historically, biochemical testing has relied on CSF analysis of 5-hydroxyindoleacetic acid and homovanillic acid (making 3-methoxytyrosine a novel biomarker) and, in addition, a less invasive way of making a diagnosis (i.e., plasma sample, as compared to CSF analysis). This study shows the use of metabolomics offers less invasive testing options for clinicians, and it is not hard to imagine that eventually numerous targeted tests traditionally used to screen for and diagnose IEMs may be superseded by a single, comprehensive test.

NOVEL BIOMARKERS FOR IEMS

Metabolomics has begun to be used to identify novel biomarkers for diseases. This may be a single analyte that has not previously been reported or, it may be a collection of increased or depressed analytes that make up a biochemical fingerprint for the diseases.

PEROXISOMAL BIOGENESIS DISORDERS–ZELLWEGER SPECTRUM DISORDERS

Peroxisomal biogenesis disorders–Zellweger spectrum disorders (PBD–ZSD) are a group of neurodegenerative disorder traditionally characterized by biochemical defects in β-oxidation of very-long-chain fatty acids, plasmalogen synthesis, or peroxisomal enzyme localization. Analysis of the metabolomes of 19 patients with molecularly confirmed PBD–ZSD showed the expected increases in pipecolic acid and long-chain lysophosphatidylcholines, which are the analytes typically used for diagnostic purposes in PBD–ZSD. Additionally, the analysis also showed an unexpected yet consistent decrease in multiple sphingomyelin species (31). These results may provide some insight into the underlying pathophysiology of PBD–ZSD; hexadecanedioate has previously been implicated in blood pressure regulation, and it has been speculated (31) that this may explain the renal hypertension observed in older patients. The existence of a metabolomic signature for PBD–ZSD is especially promising when we consider that children with PBD–ZSD may present without classic biochemical findings on traditional testing (34).

UREA CYCLE DISORDERS

Arginase deficiency (also known as Argininemia; OMIM 207800) is a distal urea cycle disorder caused by bi-allelic, pathogenic variants in *ARG1*. In patients with arginase deficiency, multiple guanidino compounds are increased including: *N*-acetylarginine, guanidinoacetate, guanidinobutanoate, and 2-oxoraginine (14). Interestingly, the mean *Z*-score for some of these compounds is higher than that of arginine, which traditionally is the analyte used for newborn screening and diagnosis (14). These findings also provide some insight into the potential pathophysiology of arginase deficiency

because guanidinoacetate has been implicated in the pathogenesis of intellectual disability and seizures in guanidinoacetate methyltransferase deficiency. Its increase along with the other guanidino compounds in arginase deficiency may represent therapeutic targets or useful biomarkers for measuring responses to therapies (14).

PHENYLKETONURIA

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine breakdown due to deficiency of phenylalanine hydroxylase. High plasma concentrations of phenylalanine and low concentrations of tyrosine are the classic biochemical alterations of this disease. The neurotoxic effects of these alterations have been studied in detail (35). The secondary metabolites involved in this process have not been investigated until recently. In 2016, researchers using a targeted metabolomic approach based on GC–MS, amino acid analysis, and NMR found alterations in amino acid pathways of arginine, proline, alanine, aspartate, and glutamine metabolism (24).

NEUROMETABOLIC DISORDERS

γ-Aminobutyric acid (GABA)-transaminase deficiency (OMIM 613163) is a neurodevelopmental disorder characterized by GABA accumulation in the central nervous system. It is caused by pathogenic variants in *ABAT* and traditionally has been diagnosed via central nervous system (CNS) neurotransmitter studies. Metabolomic analysis for these patients was able to find increased concentrations of 2-pyrrolidinone in plasma. The same study also found increased succinimide and/or succinamic acid in plasma, urine, and CSF, and these appear to be reliable and novel biomarkers to allow this diagnosis to be made from a plasma sample (26).

Serine biogenesis is dependent on 3 enzymes: phosphoglycerate dehydrogenase (OMIM 601815), phosphoserine aminotransferase (OMIM 610992), and phosphoserine phosphatase (OMIM 614023). Alterations in any one of these enzymes results in a group of diseases aptly named the serine biogenesis disorders. These disorders can present with a range of phenotypic features from neonatal lethality associated with CNS, skin, and limb defects to more mild defects associated with childhood onset developmental delay and intellectual disability. These disorders are traditionally characterized by decreased concentrations of serine and glycine in the CSF and plasma. Metabolomic profiles of these patients showed the expected low glycine and serine as well as low glycerophosphocholine compounds, low glycerophosphoethanolamine compounds, and low sphingomyelin species (27). Another group found increases in deoxydihydroceramides of 18–22 carbon length in patients with serine biogenesis disorders (28). Of note, they also found similar increases in patients with mitochondrial disorders. One common phenotype between these 2 groups of disorders is peripheral neuropathy. The authors hypothesized that the neuropathy could be a result of the increases in deoxysphingolipids, providing potential therapeutic targets for future studies (28).

MITOCHONDRIAL DISORDERS

Mitochondrial disorders are a heterogeneous group of diseases characterized by mitochondrial dysfunction and a phenotype that typically involves progressive neurologic and muscular deterioration. Their overall prevalence is estimated around 1 in 4300, but individually these disorders are rare, and their diagnosis can be challenging (29). Several mitochondrial disorders have been investigated using targeted metabolomics based on a combination of ultra-performance LC coupled with triple-quadrupole MS or GC–MS analysis. Researchers found that patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS; OMIM 540000) had increases in carbohydrate derivatives such as sorbitol, glucuronate, myoinositol, and sucrose compared to age and gender-matched controls (29). Patients also had increases in alanine and decreases in arginine. There were also distinct analyte abnormalities in the metabolomes of patients with infantile-onset spinocerebellar ataxia (OMIM 271245), mitochondrial recessive ataxia syndrome (MIRAS) (OMIM 607459), and MIRAS carriers, as well as progressive external ophthalmoplegia (OMIM 157640). The researchers concluded that several changes in pathways were common to all mitochondrial disorders, such as the transsulfuration pathway (cysteine and methionine metabolism) and amino acid biosynthesis pathway (alanine, aspartate, and glutamate biosynthesis). They also noted dimethylglycine increases in carriers for MIRAS and their patients with inclusion body myositis (OMIM 147421), which is an inflammatory myopathy with secondary findings of mitochondrial dysfunction. In another study, researchers collected 102 patients with MELAS due to the common variant, m.3243A>G, and 32 health controls. Using LC–MS-based metabolomics, they found 23 metabolites that distinguished patients with MELAS from controls (30). Targeted metabolomic approaches identified analytes classically associated with mitochondrial disorders as well as some nominated markers including carnitine and creatinine. Lactate, pyruvate, and alanine are well known respiratory chain disease markers and were indeed increased in this cohort (30). Other analytes known to be associated with mitochondrial disorders such as tricarboxylic acid cycle intermediates and various acylcarnitines were also increased in MELAS metabolomes. Carnitine and creatine have been noted as potentially associated with mitochondrial

disorders previously and were confirmed by this study (30, 36). After targeted analysis, researchers performed untargeted metabolomics, and detected 5584 distinct analytes in the cohort, of which 237 analytes were able to be identified. They found 536 peaks that were able to significantly distinguish between patients with MELAS and controls. Of those 536, only 31 analytes were able to be identified. Six of those analytes overlapped with those found in the targeted metabolomics arm of the study and the rest could broadly be categorized into 3 metabolic families: *N*-lactoyl-amino acids, β-hydroxy acylcarnitines, and β-hydroxy fatty acids. Interestingly, this was only the third time that *N*-lactoyl-amino acids have been described in humans and they had not previously been linked with any mitochondrial disorder (30). The importance of these advances in our understanding of the biochemistry of mitochondrial disorders should not be understated. The existence of validated biomarkers for mitochondrial disorders could allow for empirical testing of therapeutics and improved clinical management for these patients.

Non-IEM Conditions

Metabolomics, by its nature, lends itself to the study of IEM. However, researchers have also used it to study other diseases and physiologies with promising results including autism spectrum disorder, Rett syndrome, neuro-inflammatory diseases, cancer, asthma, and multiple congenital anomalies syndromes. There are even studies looking at the metabolomics signatures associated with dietary intake of certain foods.

AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by repetitive patterns of restricted behavior with impaired social communication or interaction (37). Several studies have investigated the metabolomes of patients with ASD. In addition to the typical analysis on plasma and urine, metabolomics has also been used on fecal samples to explore the link between the gut microbiome and ASD (38). Researchers discovered increased concentrations of isopropanol in the feces of children with ASD as compared to their neurotypical peers (38). Over a dozen studies have in some way examined the metabolomes for children with ASD. Multiple pathways have been implicated, but consistent/conclusive results have not yet been shown (3).

RETT SYNDROME

Rett syndrome (OMIM 312750) is a neurodevelopmental disorder that occurs almost exclusively in females. It is characterized by a period of neurotypical development followed by developmental regression, seizures, and intellectual disability. Loss of function variants in the methyl-CpG-binding protein 2 gene (*MECP2*) lead to Rett syndrome. *MECP2* encodes a transcription factor that has previously been implicated in cholesterol metabolic pathways (39). In a study of 14 female subjects with Rett syndrome, metabolomics found increases in sphingolipid metabolism; these included sphinganine, sphinganine 1-phosphate, sphingosine, and sphingosine 1-phosphate (40). Sphingosine 1-phosphate is known to be an important regulator of mitochondrial function and gene expression, but more work is needed to determine its role in the pathology of Rett syndrome (40).

CSF INFECTIONS AND INFLAMMATION

When studying the CSF metabolomes of patients with neuroinflammatory conditions, targeted metabolomics in combination with more traditional markers of CNS infections and inflammation could accurately differentiate between neuroborreliosis, viral CNS infections, and autoimmune neuroinflammation. Researchers found that abnormalities in kynurenine and tryptophan were the most specific for this differentiation (15). This is significant because the clinical phenotypes for these diseases can be difficult to distinguish and diagnostic assays (pathogen-specific PCR vs antibody serologies) may not always be readily available.

CANCER

Metabolomics has also shown promise in the field of oncology. The metabolomes of cancer cells have been studied extensively for many years. Cancer cells are known to have a shift in cellular metabolism toward increased glycolysis and decreased oxidative phosphorylation; this is called the Warburg effect and was documented almost 100 years ago (41). More recently, untargeted metabolomic analysis of patients with cancer has been evaluated to understand tumor physiology and possibly develop new therapeutic strategies. In one study, researchers used mass spectrometry-based metabolomics on 395 paraganglioma samples to evaluate Krebs cycle metabolites. They found that they were able to accurately predict the underlying genotype of tumor cells based on metabolic profiles. These researchers noted increased succinate: fumarate or fumarate: malate ratios; they also noted high D/L ratios of 2-hydroxyglutarate in cancer cells with underling pathogenic variants in Krebs cycle genes (42). In another study (2), patients with lung cancer were found to have increased concentrations of maltose, palmitic acid, glycerol, ethanolamine, glutamic acid, and lactic acid. There were also decreased concentrations of tryptophan, lysine, and histidine. This report described 2 cohorts, one that exclusively contained patients with nonsmall cell lung cancer and the other with a variety of lung cancers. Most analytes were consistent across both groups,

suggesting a potential role for metabolomics in lung cancer screening and detection (2).

ASTHMA

Although metabolomics is typically used to analyze either plasma or urine samples, it can be applied to a wide variety of sample types. In a study of asthma, researchers performed NMR-based metabolomic analysis of exhaled breath condensate. Linear discriminant analysis found greater separability of asthma patients from controls based on NMR data as opposed to more traditional measures of lung functions such as exhaled nitric oxide and forced expiratory volume (43). These researchers were unable to identify the compounds used by their model but noted that they were primarily located between 1.7–2.2 ppm of the NMR spectrum and attributed them to acetylated compounds (43).

MULTIPLE CONGENITAL MALFORMATIONS

Metabolomics has also helped to shed light on the pathophysiology of a subset of multiple congenital malformation syndromes. Certain types of congenital malformation seem to cooccur in a nonrandom fashion, specifically congenital heart disease and vertebral anomalies. The genetic etiology for most of these cases remains unclear. However, using a targeted metabolomics platform based on UHPLC and GC–MS, researchers were able to confirm reduced concentrations of NAD^+ and NADH as well as a buildup of metabolites upstream of 3-hydroxyanthranilic acid 3,4-dioxygenase in select patients with heart and vertebral abnormalities (44). These were patients with suspected pathogenic variants in the gene encoding 3-hydroxyanthranilic acid 3,4-dioxygenase or the gene for kynureninase, which is also involved in the synthesis of NAD^+. This study not only helped elucidate the pathophysiology of some types of congenital malformations related to NAD^+ synthesis, but also demonstrated the ability of metabolomics to help decipher genomic variants, a concept discussed later in this review.

METABOLOMICS OF DIET

Until now, this review has covered analytes endogenous to the human body; however, untargeted metabolomics is equally capable of detecting exogenous analytes and their derivatives. The most obvious application of this is in pharmacologic research, but a growing amount of research has also been conducted on biomarkers of dietary intake. One benefit of this kind of research is its ability to offer objective measures of diet in population-based studies (45). Researchers conducted a year-long diet validation study to examine the correlation between food groups as measured by food frequency questionnaires or 24-hour diet recalls with metabolic profiles. The study consisted of 671 men and women of diverse backgrounds. Two separate metabolomic assessments were performed for each participant 6 months apart to help minimize the impact of short-term variations in diet. In addition to validating many putative biomarkers from prior studies, these researchers were also able to identify several novel biomarkers (45). Nutrimetabolomic profiles were described for a wide variety of foods ranging from general food groups such as whole grain products or cruciferous vegetables to individual food items such as avocados or poultry. This kind of work serves as an important example of the utility of metabolomics for nutritional epidemiological studies.

Functional Assessment of Genomic Variants

In the past, the typical workflow for obtaining a genetic diagnosis might involve testing a single gene or sending a panel for a select number of genes. Increasingly, laboratories are using expanded gene panels containing tens to hundreds of genes or simply using exome sequencing (ES), which sequences every gene (~20 000). Frequently, these identify novel variants, and the sheer number of variants detected in this way has complicated their interpretation. When performing ES, or any type of genetic test, the clinical significance of the results/variants exist on a spectrum from pathogenic to benign, with a host of categories in between. These "in between" results are deemed variants of uncertain significance (VUS) and are essentially genetic changes without enough evidence to be definitively determined as either benign or pathogenic. Guidelines exist to help standardize the interpretation of VUSs; however, variant interpretation remains one of the most challenging aspects of DNA analysis. In 2015, the American College of Medical Genetics and the Association for Molecular Pathology released updated joint consensus recommendations on variant classification (46). In their recommendations, the ultimate classification of a variant is based on the sum of positive and negative evidence for its pathogenicity. With enough supporting evidence, a VUS can be reclassified as either pathogenic or benign. Specifically, the American College of Medical Genetics addresses the role of established functional studies in variant interpretation and notes stronger evidence associated with functional studies that reflect broader biologic function (46). Metabolomics has consequently been assessed as a functional complement to ES results and has been shown to increase the diagnostic yield of ES (13). In one analysis, 170 patients were chosen whom both exome and metabolomic studies were ordered. The researchers found that supplemental metabolomics data aided the exome interpretation in 74 (43.5%) of patients. Using metabolomics data, they were able to reclassify 27 variants: 3 variants were reclassified as pathogenic, 15 as likely pathogenic, and 9 were

reclassified as benign (13). Another group was also able to use their metabolomics platform to reclassify a VUS in *ASPA* in a 1-year-old child with developmental delay, epilepsy, and hearing loss. Metabolomic analysis showed normal levels of *N*-acetylaspartate, effectively removing Canavan disease from the differential (20). These studies show the use of metabolomics in the interpretation of sequencing data and in the future, the most comprehensive diagnostic platforms will likely be those with an integrated multiomics-based approach.

Summary

Metabolomics is being increasingly used in the screening and diagnosis of a broad range of IEMs. It has also been used to study a number of non-IEM conditions including ASD, Rett syndrome, CNS inflammatory conditions, cancer, asthma, and multiple congenital anomaly syndromes. It should be noted that there are many more studies using metabolomics to study various diseases than could be discussed in this review including investigations into diabetes, preterm labor, and even death (47–49). Additionally, the field of metabolomics continues to advance with applications of machine learning and artificial intelligence. The sheer amount of data produced from untargeted metabolomics allows for training of powerful machine learning tools for classification and regression. While dimensional reduction techniques paired with support vector machine algorithms allows for classification models with only a small number of affected patients (12). Untargeted metabolomics has also contributed to our basic understandings of disease processes via identification of novel biomarkers for diseases such as PBD–ZSD, urea cycle disorders, PKU, GABA-transaminase deficiency, serine biogenesis disorders, and mitochondrial disorders. It has proven to be helpful in the interpretation of genomic variants which pose a substantial challenge for commercial laboratories and physicians alike. It is important to emphasize that substantial limitations exist, and more work is needed before untargeted metabolomics can be widely adopted. Given the relative newness of metabolomics, platforms are nonstandard, and in addition, untargeted metabolomic analysis is likely to detect analytes that may not be in the analysis software "library" and so unable to be identified. As the field advances we anticipate that analysis will become more standardized across laboratories overcoming some of these limitations; in addition, we anticipate that many standard biochemical assays may be replaced by singular, metabolomics-based analyses. We foresee this paralleling the changes in molecular genetic testing over the past decades with single, specialized tests being phased out by broader omics-based testing platforms. The broad applicability of metabolomics makes it a promising technology, and if recent years are any indication, there is still much to glean from both research and clinical applications of untargeted metabolomics.

Nonstandard Abbreviations: IEM, inborn errors of metabolism; LC, liquid chromatography; GC, gas chromatography; NMR, nuclear magnetic resonance spectroscopy; UHPLC, ultra-high-performance liquid chromatography; MS/MS, tandem mass spectrometry; NBS, newborn screening; PKU, phenylketonuria; CSF, cerebrospinal fluid; OTC, ornithine transcarbamoylase deficiency; AADC, aromatic L-amino acid decarboxylase; CNS, central nervous system; PBD–ZSD, peroxisomal biogenesis disorders–Zellweger spectrum disorders; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIRAS, mitochondrial recessive ataxia syndrome; ASD, autism spectrum disorder; ES, exome sequencing; VUS, variant of uncertain significance

Human Genes: *ARG1*, arginase 1; *ABAT*, 4-aminobutyrate aminotransferase; *MECP2*, methyl-CpG binding protein 2; *ASPA*, aspartoacylase.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.
Other Remuneration: V.R. Sutton, Baylor Genetics Laboratory.

Acknowledgments: J.D. Odom is supported by the National Institutes of General Medical Sciences Medical Genetics Research Fellowship Program award 5T32GM007526-44.

References

1. Miller MJ, Kennedy AD, Eckhart AD, Burrage LC, Wulff JE, Miller LAD, et al. Untargeted metabolomic analysis for the clinical screening of inborn errors of metabolism. J Inherit Metab Dis 2015;38:1029–39.
2. Miyamoto S, Taylor SL, Barupal DK, Taguchi A, Wohlgemuth G, Wikoff WR, et al. Systemic metabolomic changes in blood samples of lung cancer patients identified by gas chromatography time-of-flight mass spectrometry. Metabolites 2015;5:192–210.
3. Glinton KE, Elsea SH. Untargeted metabolomics for autism spectrum disorders: current status and future directions. Front Psychiatry 2019;10:647–15.
4. Lu W, Bennett BD, Rabinowitz JD. Analytical strategies for LC-MS-based targeted metabolomics. J Chromatogr B Analyt Technol Biomed Life Sci 2008;871:236–42.
5. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass

spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem 2009;81:6656–67.
6. Jacob M, Malkawi A, Albast N, Al Bougha S, Lopata A, Dasouki M, et al. A targeted metabolomics approach for clinical diagnosis of inborn errors of metabolism. Anal Chim Acta 2018;1025:141–53.
7. Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted metabolomics strategies–challenges and emerging directions. J Am Soc Mass Spectrom 2016;27:1897–905.
8. Pence HE, Williams A. ChemSpider: an online chemical information resource. J Chem Educ 2010;87:1123–4.
9. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0-The human metabolome database in 2013. Nucleic Acids Res 2013;41:D801–7.
10. Scolamiero E, Cozzolino C, Albano L, Ansalone A, Caterino M, Corbo G, et al. Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism. Mol BioSyst 2015;11:1525–35.
11. Gallagher RC, Pollard L, Scott AI, Huguenin S, Goodman S, Sun Q; ACMG Biochemical Genetics Subcommittee of the Laboratory Quality Assurance Committee. ACMG TECHNICAL STANDARD Laboratory analysis of organic acids, 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2018;20:683–91.
12. Mordaunt D, Cox D, Fuller M. Metabolomics to improve the diagnostic efficiency of inborn errors of metabolism. Int J Mol Sci 2020;21: 1195.
13. Alaimo JT, Glinton KE, Liu N, Xiao J, Yang Y, Reid Sutton V, Elsea SH. Integrated analysis of metabolomic profiling and exome data supplements sequence variant interpretation, classification, and diagnosis. Genet Med 2020; 22:1560–6.
14. Burrage LC, Thistlethwaite L, Stroup BM, Sun Q, Miller MJ, Nagamani SCS, et al. Untargeted metabolomic profiling reveals multiple pathway perturbations and new clinical biomarkers in urea cycle disorders. Genet Med 2019;21:1977–86.,.
15. Sühs KW, Novoselova N, Kuhn M, Seegers L, Kaever V, Müller-Vahl K, et al. Kynurenine is a cerebrospinal fluid biomarker for bacterial and viral central nervous system infections. J Infect Dis 2019;220:127–38.
16. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338–43.
17. Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. Acta Paediatr Suppl 1999;88:48–51.
18. Mak CM, Lee HCH, Chan AYW, Lam CW. Inborn errors of metabolism and expanded newborn screening: review and update. Crit Rev Clin Lab Sci 2013;50:142–62.
19. Pollitt RJ, Green A, McCabe CJ, Booth A, Cooper NJ, Leonard JV, et al. Neonatal screening for inborn errors of metabolism: cost, yield and outcome. Health Technol Assess 1997;1:i.
20. Coene KLM, Kluijtmans LAJ, van der Heeft E, Engelke UFH, de Boer S, Hoegen B, et al. Next-generation metabolic screening: targeted and untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients. J Inherit Metab Dis 2018;41:337–53.
21. Bonte R, Bongaerts M, Demirdas S, Langendonk JG, Huidekoper HH, Williams M, et al. Untargeted metabolomics-based screening method for inborn errors of metabolism using semi-automatic sample preparation with an UHPLC-orbitrap-MS platform. Metabolites 2019; 9:1–18.
22. Donti TR, Cappuccio G, Hubert L, Neira J, Atwal PS, Miller MJ, et al. Diagnosis of adenylosuccinate lyase deficiency by metabolomic profiling in plasma reveals a phenotypic spectrum. Mol Genet Metab Rep 2016;8:61–6.
23. Atwal PS, Donti TR, Cardon AL, Bacino CA, Sun Q, Emrick L, et al. Aromatic L-amino acid decarboxylase deficiency diagnosed by clinical metabolomic profiling of plasma. Mol Genet Metab 2015;115:91–4.
24. Blasco H, Veyrat-Durebex C, Bertrand M, Patin F, Labarthe F, Henique H, et al. A multiplatform metabolomics approach to characterize plasma levels of phenylalanine and tyrosine in phenylketonuria. JIMD Rep 2017; 32:69–79.
25. Xiong X, Sheng X, Liu D, Zeng T, Peng Y, Wang Y. A GC/MS-based metabolomic approach for reliable diagnosis of phenylketonuria. Anal Bioanal Chem 2015;407: 8825–33.
26. Kennedy AD, Pappan KL, Donti T, Delgado MR, Shinawi M, Pearson TS, et al. 2-Pyrrolidinone and succinimide as clinical screening biomarkers for GABA-transaminase deficiency: anti-seizure medications impact accurate diagnosis. Front Neurosci 2019;13:1–12.
27. Glinton KE, Benke PJ, Lines MA, Geraghty MT, Chakraborty P, Al-Dirbashi OY, et al. Disturbed phospholipid metabolism in serine biosynthesis defects revealed by metabolomic profiling. Mol Genet Metab 2018;123: 309–16.
28. Ferreira CR, Goorden SMI, Soldatos A, Byers HM, Ghauharali-van der Vlugt JMM, Beers-Stet FS, et al. Deoxysphingolipid precursors indicate abnormal sphingolipid metabolism in individuals with primary and secondary disturbances of serine availability. Mol Genet Metab 2018;124:204–9.
29. Buzkova J, Nikkanen J, Ahola S, Hakonen AH, Sevastianova K, Hovinen T, et al. Metabolomes of mitochondrial diseases and inclusion body myositis patients: treatment targets and biomarkers. EMBO Mol Med 2018;10:1–15.
30. Sharma R, Vivo DD, Mootha VK, Sharma R, Reinstadler B, Engelstad K, et al. Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity. J Clin Invest 2021;131:e136055.
31. Wangler MF, Hubert L, Donti TR, Ventura MJ, Miller MJ, Braverman N, et al. A metabolomic map of Zellweger spectrum disorders reveals novel disease biomarkers. Genet Med 2018;20:1274–83.
32. Summar ML, Koelker S, Freedenberg D, Le Mons C, Haberle J, Lee HS, et al.; Members of the Urea Cycle Disorders Consortium (UCDC). Electronic address: http://rarediseasesnetwork.epi.usf.edu/ucdc/. The incidence of urea cycle disorders. Mol Genet Metab 2013;110: 179–80.
33. Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M. Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. Hum Mutat 2006; 27:626–32.
34. Bacino CA, Chao YH, Seto E, Lotze T, Xia F, Jones RO, et al. A homozygous mutation in PEX16 identified by whole-exome sequencing ending a diagnostic odyssey. Mol Genet Metab Rep 2015;5:15–8.
35. Christ SE, Moffitt AJ, Peck D, White DA, Hilgard J. Decreased functional brain connectivity in individuals with early-treated phenylketonuria: evidence from resting state fMRI. J Inherit Metab Dis 2012;35:807–16.
36. Shaham O, Slate NG, Goldberger O, Xu Q, Ramanathan A, Souza AL, et al. A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci USA 2010;107:1571–5.
37. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed.). 10.1176/appi.books.9780890425596. 2013.
38. Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, et al. Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe 2018;49:121–31.
39. Segatto M, Trapani L, Di Tunno I, Sticozzi C, Valacchi G, Hayek J, et al. Cholesterol metabolism is altered in Rett syndrome: a study on plasma and primary cultured fibroblasts derived from patients. PLoS ONE 2014;9: e104834.
40. Cappuccio G, Donti T, Pinelli M, Bernardo P, Bravaccio C, Elsea SH, et al. Sphingolipid metabolism perturbations in Rett syndrome. Metabolites 2019;9: 221.
41. Warburg O. The metabolism of carcinoma cells. J Cancer Res 1925;9:148–63.
42. Richter S, Gieldon L, Pang Y, Peitzsch M, Huynh T, Leton R, et al. Metabolome-guided genomics to identify pathogenic variants in isocitrate dehydrogenase, fumarate hydratase, and succinate dehydrogenase genes in pheochromocytoma and paraganglioma. Genet Med 2019; 21:705–17.
43. Carraro S, Rezzi S, Reniero F, Héberger K, Giordano G, Zanconato S, et al. Metabolomics applied to exhaled breath condensate in childhood asthma. Am J Respir Crit Care Med 2007;175:986–90.
44. Shi H, Enriquez A, Rapadas M, Martin EMMA, Wang R, Moreau J, et al. NAD deficiency, congenital malformations, and niacin supplementation. N Engl J Med 2017; 377:544–52.
45. Wang Y, Hodge RA, Stevens VL, Hartman TJ, McCullough ML. Identification and reproducibility of plasma metabolomic biomarkers of habitual food intake in a US diet validation study. Metabolites 2020;10: 1–20.
46. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–23.
47. Filla LA, Edwards JL. Metabolomics in diabetic complications. Mol Biosyst 2016;12:1090–105.
48. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med 2010;23:1344–59.
49. Mora-Ortiz M, Trichard M, Oregioni A, Claus SP. Thanatometabolomics: introducing NMR-based metabolomics to identify metabolic biomarkers of the time of death. Metabolomics 2019;15:37.