Genetics in Medicine (2025) ■, 101493

ELSEVIER

Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ACMG STATEMENT

The emerging role of metabolomics analysis in genetic and genomic testing: A points to consider statement of the American College of Medical Genetics and Genomics (ACMG)

Marcus J. Miller[1], Maria Laura Duque Lasio[2], Adam D. Kennedy[3], Rebecca McClellan[4,5], Devin Oglesbee[6], Wendy E. Smith[7], V. Reid Sutton[8], Tina Cowan[9]; on behalf of the ACMG Laboratory Quality Assurance Committee[10,*]

Disclaimer: This Points to Consider document is designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to this Points to Consider document is voluntary and does not necessarily assure a successful medical outcome. These Points to Consider should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticists should apply their own professional judgment to the specific circumstances presented by the individual patient or specimen.
Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Points to Consider. They also are advised to take notice of the date this document was adopted, and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures. Where individual authors are listed, the views expressed may not reflect those of authors' employers or affiliated institutions.

ARTICLE INFO

Article history:
Received 2 June 2025
Accepted 9 June 2025
Available online xxxx

Keywords:
Global metabolite profiling
Metabolomic analyses for inherited disorders
Small molecule testing in inborn errors of metabolism

Introduction

Over the past decade, metabolomics testing has expanded from primarily research applications to a more prominent role in clinical testing, particularly for inherited disorders of metabolism. Metabolomics refers to the comprehensive analysis of the metabolome or the collection of all metabolites measured in a biological specimen.[1,2] The clinical analysis of the metabolome holds great promise for more efficient, sensitive, and specific testing that can be used to detect disease signatures, uncover new diseases and associated biomarkers, and provide functional support of results from other "omics" evaluations, such as genome sequencing. Nonetheless, the technical and bioinformatic complexities of metabolomics testing present challenges both among clinical laboratorians looking to incorporate metabolomics into their service, and clinicians who order these tests and utilize results to help guide patient care.

The Board of Directors of the American College of Medical Genetics and Genomics approved this statement on 28 April 2025.
*Correspondence: ACMG. *Email address:* documents@acmg.net
Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gim.2025.101493
1098-3600/© 2025 American College of Medical Genetics and Genomics. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

ARTICLE IN PRESS

The complexity of clinical metabolomics stems from the vast chemical diversity of analytes in a sample, with no single analytic method capable of achieving full coverage. Many technical approaches have been developed to address this, resulting in substantial differences between laboratories in both analytic methods and the scope of analytes detected. The situation is compounded by variation inherent to the metabolome itself, stemming from multiple factors including clinical and nutritional status, medications, specimen type, and many others. The postanalytic data processing is similarly complex and differs significantly from the well-established pipelines used for genomics analysis, again leading to interlaboratory variation in data analysis and reporting. This document presents 6 fundamental points to consider, summarized in Table 1, which were developed to guide laboratorians and clinicians involved in the clinical application of metabolomics testing for the detection of genetic disease.

Materials and Methods

This points to consider statement is based on expert opinion and informed by a literature review. A literature search was conducted in Medline (PubMed) on December 19, 2022 using the search strategy outlined in Table 2. The yield was deduplicated and limited to peer-reviewed articles published in the English language since 2005. The starting yield of 1180 articles was independently screened by 2 workgroup members serving as reviewers. During initial article screening, the reviewers included only original (randomized controlled trials, cohort studies, case-control studies, case reports, etc) and secondary research articles (eg, systematic reviews with or without meta-analyses and scoping reviews) and excluded commentaries, editorials, and narrative reviews. Each reviewer then read each article more thoroughly and scored articles using a simple classification of "definitely relevant" (score = 1), "possibly relevant" (score = 2), or "not relevant" (score = 3). This exercise identified 146 "high priority" articles flagged as relevant or possibly relevant by both reviewers (average score < 2). Workgroup members reviewed the high-priority articles and identified pertinent information to supplement workgroup discussions. The workgroup met 6 times to deliberate over the literature review findings and develop expert opinions for this Points to Consider document. Before finalizing the document, the workgroup added 5 relevant references published since December 2022.

Any conflicts of interests for workgroup members are listed at the end of the article. The American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee reviewed the document providing further input on the content, and a final draft was presented to the ACMG Board of Directors for review and approval to post on the ACMG website for member comment. Upon posting to the ACMG website, an email and link were sent to all ACMG members inviting participation in the 30-day open comment process. All members' comments and additional evidence received were assessed by the authors, and these recommendations were incorporated into the document as deemed appropriate. Member comments and author responses were reviewed by representatives of the ACMG Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved for publication by the ACMG Board of Directors.

Point 1: The human metabolome is large, diverse, and in constant flux

The metabolome encompasses a large, heterogeneous population of small molecule metabolites spanning a wide range of sizes (typically < 1500 Da) and physicochemical properties (eg, small polar amino acids, hydrophobic lipids, and bulky sterols).[1] The metabolome includes metabolites traditionally evaluated in clinical metabolic testing (eg, amino acids, acylcarnitines), as well as clinically relevant compounds not covered by current biochemical genetic laboratories (discussed in point 6) and many molecules derived from foods, medications, human microbiomes, and other environmental exposures. Collectively, more than 200,000 metabolites have been putatively identified in human biospecimens. However, only about 10% of these are currently associated with human disease, with many of the measured metabolites originating from exogenous compounds, chemical adducts, and derivatives.[3]

The metabolome is variable and highly influenced by both biological and technical factors. This is in stark contrast to the human genome, which remains relatively constant and has a defined reference set for comparison. Moreover, genomic results are typically discrete variables, whereas metabolomic results are continuous variables. Preanalytical considerations, such as sample type (eg, plasma or urine), collection tube type (presence and type of anticoagulant or

Table 1 Points to consider in the application of metabolomics testing to genetic disease

(1) The human metabolome is large, diverse, and in constant flux.
(2) Metabolomics is an emerging and heterogeneous field that lacks standard approaches to testing and quality assurance.
(3) The clinical application of metabolomics to individual specimens represents a fundamental change in testing.
(4) The yield of metabolomics testing is much lower than that of genomics testing in identifying genetic diseases.
(5) Metabolomics testing has limitations and does not replace standard biochemical genetics testing.
(6) Metabolomics has several useful clinical applications.

ARTICLE IN PRESS

Table 2 Literature search strategy performed on December 19, 2022

No.	Search	Yield
1	("Metabolism, Inborn Errors"[Mesh])	167,668
2	((inherited metabolic disease) OR (hereditary metabolic disorders)) OR (inborn errors of metabolism)	187,172
3	1 OR 2	187,172
4	("Metabolomics"[2][Mesh]) OR "Metabolome"[Mesh]	34,725
5	(((((metabolomics) OR (metabolomic testing)) OR (metabolite)) OR (metabolite testing)) OR (metabolomics analysis)) OR (clinical metabolomics)	360,852
6	4 OR 5	360,852
7	((((("Specimen Handling"[Mesh]) OR "Data Analysis"[Mesh]) OR "Incidental Findings"[Mesh]) OR "Disease Management"[Mesh]) OR "Practice Patterns, Physicians'"[Mesh]	537,342
8	((((((((((sample handling) OR (sample collection)) OR (untargeted metabolomics)) OR (targeted metabolomics)) OR (data analysis)) OR (data reporting)) OR (clinical utility)) OR (clinical use)) OR (testing indication*)) OR (limitation*)) OR (incidental findings)	9,595,523
9	7 OR 8	9,667,112
10	3 AND 6 AND 9	2022
11	10 [Filters: English, Human]	1632
12	11 [Filters: Years 2005 to present]	1180

Before finalizing the document, the workgroup added 5 relevant references published since December 2022.

other additive), and shipping and storage conditions can all affect the set of metabolites measured in a sample.[3-6] Clinical status (acute illness vs well) and nutritional status (including fasting, parenteral nutrition, ketogenic diet, and special formula) can also influence metabolomic results. Other variables include age, medications, biological sex, time of day, environmental exposures, and an individual's microbiome.[7-9] As a result, metabolomics studies provide only a snapshot of a portion of the metabolome at the time of sample collection and not a complete, static picture. This complexity presents challenges both for laboratories in determining which methods to employ and how to interpret and report results (point 2) and for clinicians in weighing the appropriateness of testing in a given clinical situation and the potential significance of diagnostic, indeterminate, and nondiagnostic results (points 3 and 4).

Point 2: Metabolomics is an emerging and heterogeneous field that lacks standard approaches to testing and quality assurance

There are many different approaches to metabolomics testing, each with inherent strengths and weaknesses. Metabolomic data acquisition can be targeted, with measurements restricted to a predetermined set of compounds, untargeted using unbiased detection parameters to more broadly interrogate the metabolome, or a hybrid of both approaches.

Targeted methods sacrifice the scope of compounds detected for improved sensitivity and specificity and can be designed to enable metabolite quantitation. Traditional biochemical genetics tests for amino acids, acylcarnitines, very-long-chain fatty acids, carnitine panels, and many others are examples of targeted metabolomics testing in that a discrete list of analytes is measured and reported.[10-12] In contrast, untargeted data acquisition methods are typically used in hypothesis-generating experiments to scan for all analytes within a particular mass range and detect a much broader list of metabolites. Results generated by untargeted acquisition are assessed for statistically significant increases or decreases, between populations or treatment groups (discussed further in point 3). Untargeted data are not quantitative, are typically less precise and reproducible than targeted data, and often require extensive curation to confidently identify metabolites. It is possible that a hybrid approach can be useful, incorporating stable isotopes to permit quantification of selected targets, while also performing untargeted discovery of deviations in analytes across the metabolome.[13,14]

It is important to note that data acquired through an untargeted acquisition method might subsequently be analyzed using a targeted approach in which only a subset of metabolites is evaluated. Thus, when referring to "targeted" and "untargeted" metabolomics, it is important to distinguish between the steps of data acquisition and analysis, with the most critical distinction lying in the analysis and data processing. Urine organic acid analysis, performed in biochemical genetics laboratories by gas chromatography mass spectrometry, often involves untargeted data acquisition followed by some form of targeted analysis to identify relevant compounds.[11]

Newly emerging metabolomics tools extend the scope of traditional clinical testing to reveal a much wider view of metabolism. However, gains in the amount of information gathered come with enormous technical challenges stemming from the chemical diversity of the metabolome, and no single analysis can comprehensively detect all major chemical classes. To achieve the broadest coverage, most metabolomics platforms use a composite of multiple orthogonal tests run in parallel utilizing multiple different approaches to sample preparation, chromatographic separation, and ionization. Numerous excellent reviews have been written describing effective metabolomics testing strategies and common pitfalls.[2,15-17]

ARTICLE IN PRESS

Approaches to metabolomics testing vary widely among laboratories.[18] Although details of individual testing strategies differ, all workflows include the basic steps: (1) sample collection, (2) analyte extraction, (3) analyte separation, (4) analyte detection (most commonly by mass spectrometry), (5) data curation to convert raw metabolomic data into meaningful metabolite identifications, (6) data analysis to glean biological or clinical significance from metabolite data, and (7) effective communication of the results to the ordering clinician. Methodological differences at every step can introduce biases that may skew results and their interpretation, which could range from minor differences in metabolic patterns to the failed detection of entire classes of compounds. Even when measuring the same commercial standards, results from different laboratories can vary substantially.[19] Similarly, assumptions made in applying statistical models to data analysis could inadvertently discard important compounds from the data set or amplify the importance of clinically insignificant ones.

The field is further complicated by the lack of practice resources and guidelines associated with clinical metabolomics testing. The continual technological advancements and relatively small number of laboratories offering such testing make it difficult to develop formal guidelines specific to clinical metabolomics testing, although guidelines do exist for clinical liquid chromatography-tandem mass spectrometry test validation that are relevant to metabolomics, particularly targeted applications.[20] Similarly, proficiency testing programs do not currently exist for metabolomics testing, and laboratories engaged in this work must therefore operate without an essential quality assurance mechanism. Many groups are working to address these deficiencies. For example, the Metabolomics Quality Assurance and Quality Control Consortium has developed several resources to improve quality assurance and quality control practices in the field of untargeted metabolomics testing.[21,22]

Because of the heterogeneity in metabolomics approaches and quality assurance practices, laboratories offering such testing should clearly describe their methodology and its limitations to help clinicians select proper testing and understand and apply the results. As with any clinical test, it is best when there is communication between the ordering clinician and laboratory regarding the indication for the test, limitations of the analysis, and significance of the results.

Point 3: The clinical application of metabolomics to individual specimens represents a fundamental change in testing

Historically, metabolomics studies have been confined to research settings and have relied on the statistical comparison of cohorts to identify biomarker abnormalities. This is largely due to the fact that a metabolomics profile of an individual participant can be influenced by numerous incidental biological or technical factors (see point 1) that can mask important biomarker findings or, worse, cause spurious elevations that can be misattributed to the test variable.

Cohort studies are not possible when performing diagnostic testing on a single individual ("N-of-1" studies), presenting a major challenge to the application of metabolomics in a clinical setting. N-of-1 studies lack the statistical power of cohort studies and therefore underperform in their ability to detect subtle biomarker abnormalities. In addition, metabolic profiles can be influenced by analytical factors such as suppression, enhancement, or interference that can occur in individual specimens from matrix effects (eg, medications and other exogenous factors). These factors are difficult, if not impossible, to recognize in individual data sets and can, in rare instances, lead to inaccuracies in analyte levels that would otherwise be statistically eliminated through large-scale cohort studies. Finally, individual metabolomics profiles must be compared with a reference sample set to make meaningful conclusions about a participant's metabolite levels. When comparing between samples, numerous variables can skew a specimen's metabolomics results, including deviations in collection protocols, storage intervals, dietary/fasted status, and/or interday instrument performance.

To minimize the imprecision that can result from these factors, research metabolomics studies are often designed to collect and store all test samples and reference samples concurrently following rigid protocols and to analyze all samples within the same instrument run. These workflows are not practical in a clinical setting and are therefore a potential source of inaccuracy in N-of-1 studies.

Despite these challenges, in recent years, multiple groups have reported successful approaches to diagnose inherited metabolic disease based on individual metabolomics profiles.[23-28] These methods use normalization strategies to bridge data between analytic batches and use outlier detection analyses, such as z-scoring, to prioritize metabolite findings and uncover abnormally increased/decreased or abnormally absent/present (rare) metabolites that characterize many inherited metabolic diseases. In all cases, outlier detection relies on comparisons of the participant's profile with a large reference data set comprising metabolomics data from unaffected individuals; reference data sets used in these studies typically exceed 100 samples.[24-26] The quality of outlier calling is directly related to the size and diversity of the reference set used in comparisons.

Point 4: The yield of metabolomics testing is much lower than that of genomics testing in identifying genetic diseases

Metabolomics provides biomarker coverage that is relevant to only a subset of genetic diseases and its diagnostic scope should not be conflated with other "omics" technologies, such as genomic sequencing. A recent study estimated that approximately 200 genetic diseases could be detected by

ARTICLE IN PRESS

metabolomics platforms when considering the list of known disease-associated biomarkers currently detectable by state-of-the-art untargeted metabolomics platforms.[23] Using a different metabolomics platform, a separate longitudinal study of 2000 participants detected disease-associated biomarkers in samples representing 70 different inherited metabolic diseases.[29] In both studies, metabolomics was informative almost exclusively for inborn errors of metabolism (IEM) as opposed to disorders not involving inherited disruptions in metabolic enzymes and transporters.[23,29] There are currently over 6800 Mendelian genetic diseases known in humans (https://omim.org/statistics/entry, accessed August 19, 2024) including approximately 1000 that are classified as IEM.[30] Therefore, unlike genomics testing, the majority of genetic diseases will not be identifiable by metabolomic testing. Even within the scope of metabolic diseases, current testing workflows are not informative for many classes of disorders, including lysosomal diseases, congenital disorders of glycosylation, steroid biosynthesis defects, porphyrias, lipoprotein metabolic defects, and copper metabolic defects.[23] To expand the clinical utility of metabolomics testing, labs must continue to discover and incorporate new disease biomarkers into their platforms. They must also acknowledge that many inherited metabolic diseases will never be detectable by traditional metabolomics studies and instead may lack a robust circulating biomarker or require the use of alternative methods, such as lipidomics or glycomics.

Point 5: Metabolomics testing has limitations and does not replace standard biochemical genetics testing

Although metabolomics testing widens the range of conditions detected, it does not replace the need for traditional evaluations, including amino acids, organic acids, and acylcarnitines. These tests provide rapid turnaround time, relatively low cost, and high sensitivity, specificity, and precision and can reliably detect many metabolic conditions in both their classic and variant forms. This is particularly important for the rapid diagnosis of an acutely ill patient, for which, in addition to concerns of turnaround time and test performance, metabolomic studies can be virtually uninterpretable because of variability introduced by acute clinical status (eg, metabolic decompensation and multiorgan dysfunction), medications, and other iatrogenic causes. Metabolomics testing can also miss metabolic conditions that could be detected by more precise traditional biochemical genetics tests, and false-negative clinical metabolomic findings have been reported due to missing analyte identifications (eg, failure to detect elevations of guanidinoacetate in individuals with guanidinoacetate methyltransferase deficiency, homogentisate in participants with alkaptonuria, and argininosuccinate in participants with argininosuccinic aciduria)[23,26,31] or an inability to detect subtle patterns of biomarker abnormalities (eg, failure to detect plasma lysine reductions in an individual with lysinuric protein intolerance or citrulline reductions in participants with ornithine transcarbamylase deficiency or a failure to resolve the isomeric biomarker alloisoleucine in participants with maple syrup urine disease, although, in this case, additional biomarkers could be used to narrow the differential diagnosis).[23,26,32] Standard tests (eg, amino acid analysis) also provide targeted, quantitative measurements needed for the ongoing monitoring of metabolic diseases; however, the time, cost, imprecision, and final reportable output of current metabolomics tests make them largely unsuitable for this purpose.

Beyond differences of test performance, the availability of metabolomics testing is much more limited compared with routine metabolic tests. At the time of this publication, a small number of US-based laboratories offer clinically validated metabolomics testing in urine and plasma samples. Metabolomic profiling is being done on a research basis at several institutions and has been performed by multiple groups as part of the Undiagnosed Diseases Network. Importantly, the scope and reliability of biomarker detections in metabolomics testing is highly platform specific and variable (point 2), and it can be difficult to draw conclusions about metabolism from a single analysis. For these reasons, nondiagnostic metabolomics results do not exclude the possibility of a metabolic disease and, as is standard practice for all biomarker testing in genetic disease, potentially diagnostic metabolomics results should be confirmed with additional testing, including DNA sequencing and other clinical laboratory studies.

Finally, false-positive results and incidental findings are more likely when using metabolomics testing compared with conventional targeted studies. In the largest retrospective clinical study to date, abnormalities were detected in over half (912 of 1807) of all participants receiving metabolomics testing and this included 655 cases with substantial findings considered suggestive of a specific category of IEM; ultimately only 128 cases (7.1% of all participants) detected by metabolomics testing received a confirmed diagnosis.[29] These findings highlight the substantial interpretative burden associated with metabolomics testing and demonstrate the importance of providing performing laboratories with pertinent patient information and the critical role of properly trained personnel in ongoing applications of metabolomics testing.

Point 6: Metabolomics has several useful clinical applications

Despite the limitations described above, the study of individual metabolomics profiles offers many useful clinical applications.

First, metabolomic testing provides access to a broad range of metabolite testing that is not otherwise available. There are numerous genetic diseases associated with biomarker abnormalities for which there are currently limited,

ARTICLE IN PRESS

or no, targeted clinical testing options. In these cases metabolomics might be the only option to confirm equivocal molecular findings or to monitor treatment; examples include the use of metabolomics to detect (1) outlier elevations of multiple plasma tryptophan pathway intermediates in individuals with biallelic *KYNU* variants of uncertain significance, (2) outlier accumulation of N-acetyl-D-mannosamine in biofluids of individuals with the recently discovered genetic disorder NANS deficiency, and (3) a diagnostic pattern of nicotinamide metabolite abnormalities and subsequent treatment-induced normalization in NAXE deficiency.[33-35] Metabolomics has also been shown to detect certain biomarkers in circulating biofluids that otherwise could only be identified in samples requiring invasive collection (eg, cerebrospinal fluid and muscle or liver biopsies), thus providing a less-invasive diagnostic approach and eliminating barriers to obtaining confirmatory testing.[36-38]

The holistic nature of metabolomic testing can also confer unique advantages. By simultaneously assaying the levels of large numbers of metabolites from diverse and interconnected metabolic pathways, investigators can broadly screen for a wide range of metabolic diseases. As a result, global metabolomic testing outperforms conventional targeted metabolic screening in terms of the diagnostic yield in individuals with an undifferentiated phenotype.[29] Metabolomics can also serve as a companion to genomic testing to functionally evaluate the pathogenicity of variants of uncertain significance in known disease genes or in candidate genes and to identify rare cases where genomics testing fails to detect the underlying genetic variant(s) in a true inherited metabolic disease.[39-41] Similarly, the rich data set provided by metabolomics testing can be used as a second-tier test to reduce false positives in newborn screening[27] and can also improve test specificity through the detection of treatment-related biomarkers that are necessary to distinguish medication induced metabolic abnormalities from those caused by inherited disease.[42]

Finally, perhaps the most exciting aspect of metabolomics testing lies in its ability to uncover novel findings that can not only support a genetic diagnosis but can expand our understanding of the pathophysiology of genetic disease. Already, metabolomics data sets have been mined to identify new biomarkers in multiple patient cohorts, including phenylketonuria, peroxisomal disorders, urea cycle disease, GLUT1 deficiency, and serine synthesis defects.[23,43-47] Continued growth of metabolomics data sets will enable further retrospective patient cohort analyses that will inform treatment options and guide development of targeted assays that can be used in future diagnosis and management.

Conflict of Interest

Adam D. Kennedy and V. Reid Sutton receive salary for providing clinical services that may be relevant to the content of this document in either the laboratory or patient care setting at their listed affiliations. All other authors declare no conflicts of interest.

Affiliations

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN; [2]Department of Pediatrics and Department of Pathology, Washington University School of Medicine, St. Louis, MO; [3]Metabolon Inc, Durham, NC; [4]Johns Hopkins Center for Inherited Heart Diseases, Baltimore, MD; [5]Neurogenetics, Kennedy Krieger Institute, Baltimore, MD; [6]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [7]Department of Pediatrics, Maine Medical Center, Portland, ME; [8]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; [9]Department of Pathology, Stanford University Medical Center, Stanford, CA; [10]American College of Medical Genetics and Genomics, Bethesda, MD

References

1. Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. *Physiol Rev*. 2019;99(4):1819-1875. http://doi.org/10.1152/physrev.00035.2018
2. Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harb Mol Case Stud*. 2015;1(1):a000588. http://doi.org/10.1101/mcs.a000588
3. Yin P, Peter A, Franken H, et al. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. *Clin Chem*. 2013;59(5):833-845. http://doi.org/10.1373/clinchem.2012.199257
4. Kamlage B, Maldonado SG, Bethan B, et al. Quality markers addressing preanalytical variations of blood and plasma processing identified by broad and targeted metabolite profiling. *Clin Chem*. 2014;60(2):399-412. http://doi.org/10.1373/clinchem.2013.211979
5. Roux A, Thévenot EA, Seguin F, Olivier MF, Junot C. Impact of collection conditions on the metabolite content of human urine samples as analyzed by liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectroscopy. *Metabolomics*. 2015;11(5):1095-1105. http://doi.org/10.1007/s11306-014-0764-5
6. Bi H, Guo Z, Jia X, Liu H, Ma L, Xue L. The key points in the pre-analytical procedures of blood and urine samples in metabolomics studies. *Metabolomics*. 2020;16(6):68. http://doi.org/10.1007/s11306-020-01666-2
7. Playdon MC, Tinker LF, Prentice RL, et al. Measuring diet by metabolomics: a 14-d controlled feeding study of weighed food intake. *Am J Clin Nutr*. 2024;119(2):511-526. http://doi.org/10.1016/j.ajcnut.2023.10.016
8. Hornburg D, Wu S, Moqri M, et al. Dynamic lipidome alterations associated with human health, disease and ageing. *Nat Metab*. 2023;5(9):1578-1594. http://doi.org/10.1038/s42255-023-00880-1
9. Davies SK, Ang JE, Revell VL, et al. Effect of sleep deprivation on the human metabolome. *Proc Natl Acad Sci U S A*. 2014;111(29):10761-10766. http://doi.org/10.1073/pnas.1402663111
10. Sharer JD, De Biase I, Matern D, et al. Laboratory analysis of amino acids, 2018 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2018;20(12):1499-1507. http://doi.org/10.1038/s41436-018-0328-6
11. Gallagher RC, Pollard L, Scott AI, et al. Laboratory analysis of organic acids, 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2018;20(7):683-691. http://doi.org/10.1038/gim.2018.45

ARTICLE IN PRESS

12. Miller MJ, Cusmano-Ozog K, Oglesbee D, Young S. Laboratory analysis of acylcarnitines, 2020 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(2):249-258. http://doi.org/10.1038/s41436-020-00990-1
13. Fiehn O. Metabolomics by gas chromatography-mass spectrometry: combined targeted and untargeted profiling. *Curr Protoc Mol Biol*. 2016;114:30.4.1-30.4.32. http://doi.org/10.1002/0471142727.mb3004s114
14. Gertsman I, Gangoiti JA, Barshop BA. Validation of a dual LC-HRMS platform for clinical metabolic diagnosis in serum, bridging quantitative analysis and untargeted metabolomics. *Metabolomics*. 2014;10(2):312-323. http://doi.org/10.1007/s11306-013-0582-1
15. Gertsman I, Barshop BA. Promises and pitfalls of untargeted metabolomics. *J Inherit Metab Dis*. 2018;41(3):355-366. http://doi.org/10.1007/s10545-017-0130-7
16. Alseekh S, Aharoni A, Brotman Y, et al. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. *Nat Methods*. 2021;18(7):747-756. http://doi.org/10.1038/s41592-021-01197-1
17. Ulmer CZ, Maus A, Hines J, Singh R. Challenges in translating clinical metabolomics data sets from the bench to the bedside. *Clin Chem*. 2021;67(12):1581-1583. http://doi.org/10.1093/clinchem/hvab210
18. Wurth R, Turgeon C, Stander Z, Oglesbee D. An evaluation of untargeted metabolomics methods to characterize inborn errors of metabolism. *Mol Genet Metab*. 2024;141(1):108115. http://doi.org/10.1016/j.ymgme.2023.108115
19. Thompson JW, Adams KJ, Adamski J, et al. International ring trial of a high resolution targeted metabolomics and lipidomics platform for serum and plasma analysis. *Anal Chem*. 2019;91(22):14407-14416. http://doi.org/10.1021/acs.analchem.9b02908
20. Bioanalytical method validation. Guidance for industry. United States Food and Drug Administration. Accessed September 4, 2024. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf
21. Kirwan JA, Gika H, Beger RD, et al. Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management. *Metabolomics*. 2022;18(9):70. http://doi.org/10.1007/s11306-022-01926-3
22. Lippa KA, Aristizabal-Henao JJ, Beger RD, et al. Reference materials for MS-based untargeted metabolomics and lipidomics: a review by the metabolomics quality assurance and quality control consortium (mQACC). *Metabolomics*. 2022;18(4):24. http://doi.org/10.1007/s11306-021-01848-6
23. Coene KLM, Kluijtmans LAJ, van der Heeft E, 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(3):337-353. http://doi.org/10.1007/s10545-017-0131-6
24. Webb-Robertson BM, Stratton KG, Kyle JE, et al. Statistically driven metabolite and lipid profiling of patients from the Undiagnosed Diseases Network. *Anal Chem*. 2020;92(2):1796-1803. http://doi.org/10.1021/acs.analchem.9b03522
25. Bonte R, Bongaerts M, Demirdas S, 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(12):289. http://doi.org/10.3390/metabo9120289
26. Miller MJ, Kennedy AD, Eckhart AD, et al. Untargeted metabolomic analysis for the clinical screening of inborn errors of metabolism. *J Inherit Metab Dis*. 2015;38(6):1029-1039. http://doi.org/10.1007/s10545-015-9843-7
27. Mak J, Peng G, Le A, et al. Validation of a targeted metabolomics panel for improved second-tier newborn screening. *J Inherit Metab Dis*. 2023;46(2):194-205. http://doi.org/10.1002/jimd.12591
28. Hou YCC, Yu HC, Martin R, et al. Precision medicine integrating whole-genome sequencing, comprehensive metabolomics, and advanced imaging. *Proc Natl Acad Sci U S A*. 2020;117(6):3053-3062. http://doi.org/10.1073/pnas.1909378117
29. Liu N, Xiao J, Gijavanekar C, et al. Comparison of untargeted metabolomic profiling vs traditional metabolic screening to identify inborn errors of metabolism. *JAMA Netw Open*. 2021;4(7):e2114155. http://doi.org/10.1001/jamanetworkopen.2021.14155
30. Ferreira CR, van Karnebeek CDM, Vockley J, Blau N. A proposed nosology of inborn errors of metabolism. *Genet Med*. 2019;21(1):102-106. http://doi.org/10.1038/s41436-018-0022-8
31. Almontashiri NAM, Zha L, Young K, et al. Clinical validation of targeted and untargeted metabolomics testing for genetic disorders: a 3 year comparative study. *Sci Rep*. 2020;10(1):9382. http://doi.org/10.1038/s41598-020-66401-2
32. Jacob M, Malkawi A, Albast N, et al. A targeted metabolomics approach for clinical diagnosis of inborn errors of metabolism. *Anal Chim Acta*. 2018;1025:141-153. http://doi.org/10.1016/j.aca.2018.03.058
33. Ehmke N, Cusmano-Ozog K, Koenig R, et al. Biallelic variants in KYNU cause a multisystemic syndrome with hand hyperphalangism. *Bone*. 2020;133:115219. http://doi.org/10.1016/j.bone.2019.115219
34. van Karnebeek CDM, Bonafé L, Wen XY, et al. NANS-mediated synthesis of sialic acid is required for brain and skeletal development. *Nat Genet*. 2016;48(7):777-784. http://doi.org/10.1038/ng.3578
35. Manor J, Calame D, Gijavanekar C, et al. NAXE deficiency: a neurometabolic disorder of NAD(P)HX repair amenable for metabolic correction. *Mol Genet Metab*. 2022;136(2):101-110. http://doi.org/10.1016/j.ymgme.2022.04.003
36. Donti TR, Cappuccio G, Hubert L, et al. Diagnosis of adenylosuccinate lyase deficiency by metabolomic profiling in plasma reveals a phenotypic spectrum. *Mol Genet Metab Rep*. 2016;8:61-66. http://doi.org/10.1016/j.ymgmr.2016.07.007
37. Kennedy AD, Pappan KL, Donti T, et al. 2-pyrrolidinone and succinimide as clinical screening biomarkers for GABA-transaminase deficiency: anti-seizure medications impact accurate diagnosis. *Front Neurosci*. 2019;13:394. http://doi.org/10.3389/fnins.2019.00394
38. Atwal PS, Donti TR, Cardon AL, et al. Aromatic L-amino acid decarboxylase deficiency diagnosed by clinical metabolomic profiling of plasma. *Mol Genet Metab*. 2015;115(2-3):91-94. http://doi.org/10.1016/j.ymgme.2015.04.008
39. Alaimo JT, Glinton KE, Liu N, et al. Integrated analysis of metabolomic profiling and exome data supplements sequence variant interpretation, classification, and diagnosis. *Genet Med*. 2020;22(9):1560-1566. http://doi.org/10.1038/s41436-020-0827-0
40. Graham E, Lee J, Price M, et al. Integration of genomics and metabolomics for prioritization of rare disease variants: a 2018 literature review. *J Inherit Metab Dis*. 2018;41(3):435-445. http://doi.org/10.1007/s10545-018-0139-6
41. Ferreira EA, Veenvliet ARJ, Engelke UFH, et al. Diagnosing, discarding, or de-VUSsing: a practical guide to (un)targeted metabolomics as variant-transcending functional tests. *Genet Med*. 2023;25(1):125-134. http://doi.org/10.1016/j.gim.2022.10.002
42. Pappan KL, Kennedy AD, Magoulas PL, Hanchard NA, Sun Q, Elsea SH. Clinical metabolomics to segregate aromatic amino acid decarboxylase deficiency from drug-induced metabolite elevations. *Pediatr Neurol*. 2017;75:66-72. http://doi.org/10.1016/j.pediatrneurol.2017.06.014
43. Wangler MF, Hubert L, Donti TR, et al. A metabolomic map of Zellweger spectrum disorders reveals novel disease biomarkers. *Genet Med*. 2018;20(10):1274-1283. http://doi.org/10.1038/gim.2017.262
44. Burrage LC, Thistlethwaite L, Stroup BM, et al. Untargeted metabolomic profiling reveals multiple pathway perturbations and new clinical biomarkers in urea cycle disorders. *Genet Med*. 2019;21(9):1977-1986. http://doi.org/10.1038/s41436-019-0442-0
45. Glinton KE, Benke PJ, Lines MA, et al. Disturbed phospholipid metabolism in serine biosynthesis defects revealed by metabolomic profiling. *Mol Genet Metab*. 2018;123(3):309-316. http://doi.org/10.1016/j.ymgme.2017.12.009
46. Václavík J, Coene KLM, Vrobel I, et al. Structural elucidation of novel biomarkers of known metabolic disorders based on multistage fragmentation mass spectra. *J Inherit Metab Dis*. 2018;41(3):407-414. http://doi.org/10.1007/s10545-017-0109-4
47. Cappuccio G, Pinelli M, Alagia M, et al. Biochemical phenotyping unravels novel metabolic abnormalities and potential biomarkers associated with treatment of GLUT1 deficiency with ketogenic diet. *PLoS One*. 2017;12(9):e0184022. http://doi.org/10.1371/journal.pone.0184022