# A narrative review of metabolomics in the era of "-omics": integration into clinical practice for inborn errors of metabolism

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**Background and Objective:** Traditional targeted metabolomic investigations identify a pre-defined list of analytes in samples and have been widely used for decades in the diagnosis and monitoring of inborn errors of metabolism (IEMs). Recent technological advances have resulted in the development and maturation of untargeted metabolomics: a holistic, unbiased, analytical approach to detecting metabolic disturbances in human disease. We aim to provide a summary of untargeted metabolomics [focusing on tandem mass spectrometry (MS-MS)] and its application in the field of IEMs.

**Methods:** Data for this review was identified through a literature search using PubMed, Google Scholar, and personal repositories of articles collected by the authors. Findings are presented within several sections describing the metabolome, the current use of targeted metabolomics in the diagnostic pathway of patients with IEMs, the more recent integration of untargeted metabolomics into clinical care, and the limitations of this newly employed analytical technique.

**Key Content and Findings:** Untargeted metabolomic investigations are increasingly utilized in screening for rare disorders, improving understanding of cellular and subcellular physiology, discovering novel biomarkers, monitoring therapy, and functionally validating genomic variants. Although the untargeted metabolomic approach has some limitations, this "next generation metabolic screening" platform is becoming increasingly affordable and accessible.

**Conclusions:** When used in conjunction with genomics and the other promising "-omic" technologies, untargeted metabolomics has the potential to revolutionize the diagnostics of IEMs (and other rare disorders), improving both clinical and health economic outcomes.

**Keywords:** Inborn errors of metabolism (IEMs); untargeted metabolomics; "-omics"; diagnosis; biomarker

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## Introduction

Metabolites are small organic molecules (≤1,000 m/z) that are the intermediate or end products of enzymatic processes. Their levels in cells, tissues, or biological fluids may vary due to gene function, disease processes, diet, environment, medications, and other factors (1,2).

Inborn errors of metabolism (IEMs), although individually rare, are a key group of over 1,000 inherited disorders that result in altered levels of metabolites (3). Some of these disorders affect the processing of carbohydrates, lipids, or proteins, leading to intoxication or energy deficiency in the case of small molecules, and storage or deficiency in the case of complex molecules (4). In many of these conditions, early diagnosis allows for timely medical intervention, reducing mortality and significantly improving quality of life. Such prompt diagnoses of IEMs can be achieved through newborn screening (NBS) programs, which involve dried blood spot (DBS) analysis using mass spectrometry techniques. Depending on the country and region, these programs can detect around 30 disorders in the pre-symptomatic phase, facilitating early intervention and improving clinical outcomes (5-8).

Diagnosis of many IEMs, either through NBS or diagnostic testing, requires metabolomic analysis. This traditional approach to the investigation of metabolites is referred to as "targeted metabolomics". Different techniques (such as gas or liquid chromatography-mass spectrometry) are used to analyze a predefined, small list of metabolites so that they can be separated, detected, annotated, and quantified. For decades, these approaches have been widely and successfully used for the diagnosis and monitoring of patients with IEMs.

More recently, a powerful "next-generation metabolic screening" technology has become available to assess the metabolome in an unbiased fashion. This has been facilitated by the development of high-resolution accurate mass spectrometers, as well as progress in the understanding of biochemical pathways and bioinformatics. As a result, untargeted metabolomics has significantly matured in the last decade and offers an increased diagnostic yield when compared to traditional approaches (9-14). The applications of both targeted and untargeted metabolomics may be varied; however, one crucial application is in the diagnosis and therapeutic monitoring of patients with IEMs (15,16).

Similar to what has occurred in the field of genomic testing with next generation sequencing (NGS), untargeted metabolomics is beginning to alter the diagnostic approach to the investigation of IEMs due to its broader coverage and scope. Over the past two decades untargeted metabolomic approaches are increasingly being utilized in the investigation and diagnosis of patients with IEMs (15).

Not only does untargeted metabolomics assist with diagnosis, it also allows for increased understanding of disease mechanisms (17-19), customization of drug treatments (20), and monitoring of therapeutic response (21-24). As genomic studies unravel new disorders, understanding their effects on cellular and subcellular function becomes essential to confirm causation of disease and develop effective therapies (25). In addition to the use of traditional sample types (urine, plasma and cerebrospinal fluid), other tissue and cell types are also amenable to metabolomic studies, which will advance the understanding of intracellular and organ-specific metabolic pathways (26). Concurrently, the other rapidly expanding "-omic" fields (including transcriptomics, epigenomics, proteomics, lipidomics and glycomics) will also be increasingly contributing to these improvements (25,27).

This review aims to highlight the clinical utility of untargeted metabolomics, and its complementary nature to currently utilized "-omic" approaches, for the diagnosis and monitoring of IEMs. Current methods and limitations of untargeted metabolomics will be reviewed, in comparison to targeted metabolomics. Future applications of untargeted metabolomics in the field of IEMs, including identifying 'metabolomic signatures', monitoring therapeutic responses and integrating genomic and metabolomic data, will also be discussed. Over time, widespread availability of this technology will improve understanding of molecular mechanisms of disease and increase diagnostic yield, resulting in positive impacts on patient outcomes. We present the following article in accordance with the Narrative Review reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-105/rc).

## Methods

In this narrative review, authors performed a literature search using PubMed and Google Scholar using the MeSH terms: "Metabolomic", "Diagnosis", "Untargeted Metabolomics", "Inborn Errors of Metabolism". Article inclusion and exclusion criteria are outlined in *Table 1*. Additionally, where applicable, authors also utilized their own repositories of articles.

## Evaluating the metabolome

For targeted metabolomics, gas chromatography-mass

**Table 1** The search strategy summary

| Items | Specification |
|---|---|
| Date of search | July 25th, 2022 |
| Databases and other sources searched | PubMed; Google Scholar |
| Search terms used | "Metabolomic", "Diagnosis", "Untargeted Metabolomics", "Inborn Errors of Metabolism" |
| | Please refer to Table S1 for detailed search strategy |
| Timeframe | January 1st, 2002 to July 25th, 2022 |
| Inclusion and exclusion criteria | Inclusion criteria: articles in English pertaining to the use of "Metabolomic", "Diagnosis", "Inborn Errors of Metabolism" published from 2002 onwards, and "Untargeted Metabolomics" in "Inborn Errors of Metabolism" published from 2013 onwards |
| | Exclusion criteria: articles not focusing on "untargeted metabolomics" or "inborn errors of metabolism" |
| Selection process | AH, AS and AAT selected candidate articles for inclusion. Articles were not included unless 50% of the authorship agreed |

spectrometry and liquid chromatography tandem mass spectrometry (LC/MS-MS) are both heavily utilized in the clinical setting, as sample preparation is often simple and analytical results can be available rapidly. With these techniques, a finite number of compounds are analyzed, allowing optimization of sensitivity and specificity (28,29). There are now universal databases that provide reference spectra (30).

In untargeted metabolomics, the sheer number and complexity of metabolites detected is far greater (even up to 40,000 metabolites has been reported) (14) and provides an unbiased view of the entire metabolome similar to whole genome sequencing (WGS) in genomic evaluation. Untargeted metabolomics can be performed by ultra-high performance mass spectrometry based approaches in tandem with liquid chromatography (31,32). Additionally, there are other modern technologies and methodologies that are being employed to detect large numbers of metabolites, including the ultra-high resolution of Orbitrap-mass spectrometry which leads to accurate detection of molecular ions (<1 ppm mass deviations) with extremely high specificity (33). Matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry carries the benefits of short sample analysis time, wide mass range of detectable metabolites and low sample consumption (<1 μL) (34). Other platforms, such as $^1$H-nuclear magnetic resonance (NMR) spectral analysis, are now employed in both targeted and untargeted approaches (35,36). However, this mini-review will focus primarily on

mass spectrometry-based techniques.

Automated bioinformatic curation, based on population z-scores, reduces the number of compounds requiring manual review downstream (37,38). Similar to genomic pipelines assisting in interpretation of large volumes of genomic data, biochemical pipelines that help streamline and prioritize metabolite analysis have also been developed (10).

## Current diagnostic pathways: targeted metabolomics

Traditionally, IEMs have been diagnosed in a targeted manner (driven by phenotypic or molecular findings) (29,39). Such biomarker assays, as outlined by Saudubray and colleagues, are developed and validated with a high level of sensitivity and specificity for disease and are suitable for use in clinical practice (4). This targeted approach streamlines the diagnostic pathway, allowing for timely implementation of treatment in patients presenting during an acute metabolic crisis. However, a targeted approach comes with limitations in terms of the quantity of metabolites analyzed, as well as an inability to easily assess interactions between metabolic pathways.

With the targeted approach, patients presenting with clinical features of an IEM (acute metabolic encephalopathy, with basic biochemistry suggestive of small molecule intoxication), are rapidly diagnosed through tests such as urine metabolic screening (including organic acids), plasma acylcarnitine and/or amino acid analyses (29).

Other patients presenting with a more chronic process due to accumulation of a complex molecule, such as glycosaminoglycans, sphingolipids or very long chain fatty acids, have a separate set of metabolomic biomarkers that are evaluated to arrive at a diagnosis. The benefits of a targeted metabolomic approach include comprehensive understanding of the relevant enzyme pathways, optimized sample preparation (with reduction in high-abundance molecules) and the ability to more effectively filter out analytical artefacts (40,41). Newer technologies, such as flow injection mass spectrometry, can reduce run times, enabling faster analysis that could be suitable for high-throughput testing (38).

With the increasing utility of NGS technologies, targeted metabolomic studies are also playing a role in assisting with molecular diagnostics. Detection of a specific metabolic derangement can facilitate a simpler bioinformatic gene panel analysis, rather than an open whole exome approach with a non-specific phenotype (42). However, it is important to consider that the absence of a metabolomic marker does not preclude a diagnosis, as these may only be elevated during times of metabolic stress.

Whilst the clinical utility of metabolomics currently lies in targeted approaches, advances in technology and bioinformatics have facilitated untargeted metabolic analysis in both the clinical and research spheres, allowing for subsequent integration into the clinical workflow.

## Future trends and integration of untargeted metabolomics to the bedside

Untargeted metabolomic technologies allow for analysis of a comprehensive array of metabolites that would otherwise require individualized assays and sample preparations (43). This technology is also likely to offer substantial clinical benefit not achieved by targeted metabolomic testing, and may be further enhanced by developments in machine learning (44) and statistical modelling (45,46). The clinical potential of untargeted metabolomics in diagnosis of IEM has been investigated extensively (11,13,15,18,22,33,47-53), and complements other "-omic" approaches in the diagnosis of IEM and other genetic disorders (16,42,54-58).

### Biomarker discovery and increased understanding of disease pathophysiology

A key application of untargeted metabolomics is in identification of novel, or more sensitive biomarkers

for disease. To be considered an ideal biomarker (for diagnostic and prognostic features) of disease, the candidate analytes derived in a research setting need to be validated extensively before translating into clinical practice (59). There are multiple considerations when identifying an ideal disease biomarker. These analytes must be sensitive, in that patients with the disease should have a derangement in the biomarker (such as allo-isoleucine in maple syrup urine disease) (60). They should be specific; for instance, lactate is a poor biomarker for IEMs because its elevation can occur due to multiple other aetiologies (including sepsis, seizures or cardiac dysfunction) (61). The extent of biomarker derangement should correlate with disease severity, as well as clinical response to therapy. There are also analytical factors that should be considered, such as sample robustness (during handling, storage and freeze-thaw cycles) and reproducibility (62,63). It is unlikely that new candidate biomarkers would be found using targeted, or even semi-targeted approaches, given the limited number of metabolites and pathways interrogated. However, with untargeted analytical approaches, the unbiased screening of the metabolome facilitates biomarker discovery.

For instance, Burrage and colleagues (17) performed untargeted metabolomics using mass spectrometry on plasma samples of 48 patients with urea cycle disorders. This involved the detection of over 900 different metabolites and normalization of subject samples to an invariant anchor specimen, which could then be compared to population reference ranges to generate z-scores. Metabolites of sodium benzoate and sodium phenylbutyrate were detected, allowing for monitoring of therapeutic compliance. In addition, marked increases in multiple guanidino compounds were observed in patients with arginase deficiency, which were elevated over-and-above the plasma arginine level. Wangler and colleagues studied individuals with confirmed peroxisome biogenesis disorders in the Zellweger spectrum (PBD-ZSD). The untargeted metabolomic profiling results from plasma detected over 650 compounds, with increases observed in pipecolic acid and long-chain lysophosphatidylcholines, along with unexpected decreases in multiple sphingomyelin species (64). These findings could serve as more sensitive biomarkers for the initial diagnosis, act as early signs of impending clinical deterioration, determine prognosis or prove beneficial in monitoring response to novel therapies. Once identified, these biomarkers, or patterns of biomarkers can be incorporated into targeted metabolomic analysis.

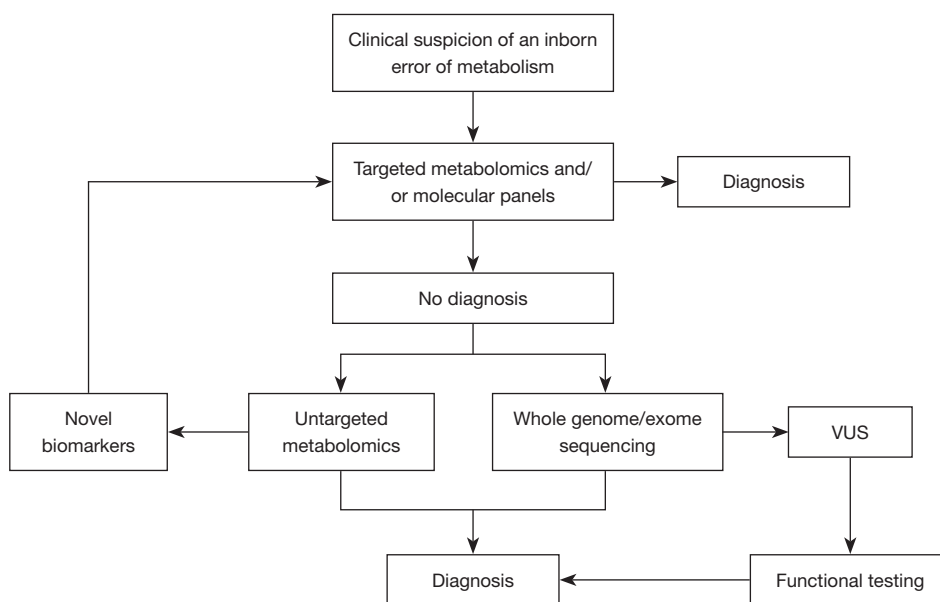Identification of novel markers of disease also provides

**Figure 1** The integration of untargeted metabolomics into the diagnostic pipeline of IEMs. Patients who have no diagnosis despite targeted metabolomic and molecular testing are candidates for an untargeted approach, including metabolomics and WGS. Novel biomarkers discovered during this process can then be re-incorporated into targeted metabolomic analysis. Any VUSs unresolved by the combined genomic-metabolomic approach can be further investigated through functional characterization studies in the research setting. VUS, variant of uncertain significance; IEMs, inborn errors of metabolism; WGS, whole genome sequencing.

additional insight into disease mechanisms and pathogenesis. Glinton and colleagues, utilizing untargeted metabolomic profiling for four patients with serine biosynthetic defects, demonstrated low levels of glycerophosphocholine, glycerophosphoethanolamine and sphingomyelin, as well as the well-known deficiencies of serine and glycine (18). Most of these phospholipid compounds normalized following treatment with serine and glycine. The authors suggested that deficiency of phospholipids (secondary to serine deficiency) may be contributing to the neurological manifestations of the condition, and this could lend itself to additional targeted therapies in the future. As exemplified by this study, and others, untargeted methods allow for a better understanding of disease pathogenesis, as whole pathways can be analyzed, rather than individual metabolites (32,56,57,65-69).

The use of untargeted approaches can also help generate a 'metabolomic signature' similar to the increasing utility of methylation episignatures for detection of BAFopathies (70). Venter and colleagues performed untargeted metabolomic testing on urine samples for patients with suspected respiratory chain disorders, identifying a 12-compound signature that distinguished patients with biopsy-proven

respiratory chain abnormalities in muscle, from those with a suspicion of a mitochondrial myopathy who did not have these changes (with a sensitivity of 98%, and specificity of 80%) (71). There are also several other reports of utilization of untargeted metabolomics to generate disease specific 'fingerprints' (58,72-74).

Additionally, diagnostic evaluation of samples from 16 patients with pyruvate kinase deficiency (compared to 32 controls) generated a highly specific and accurate metabolomic signature that included glycolytic intermediates, polyamines and acylcarnitine species (75). Such comparison of patient samples to a library of known metabolomic signatures could be beneficial when a more targeted approach has not achieved a diagnosis (*Figure 1*). Similar to the 100,000 Genomes Pilot Study (76), cohort analysis of large numbers of such patients may also help identify and refine candidate biomarkers and metabolomic signatures.

### Using 'metabolomic signatures' to identify candidate biomarkers for treatment response

With increased throughput and understanding of individual metabolites in disease pathogenesis, metabolomic signatures

could be built upon to incorporate response to treatment. There are already examples in the literature of untargeted metabolomic analysis assisting in treatment monitoring for IEMs (77,78). Pillai and colleagues reported a patient with riboflavin transporter deficiency with bi-allelic variants one pathogenic and one a variant of uncertain significance (VUS) in *SLC52A2* (77). Untargeted metabolomic analysis identified multiple compounds associated with abnormal flavin adenine nucleotide function, which normalized after riboflavin therapy. This both assisted in confirmation of the molecular diagnosis and provided a mechanism for identification of candidate biomarkers that can potentially be validated to be used for monitoring therapeutic response through targeted assays.

These cases demonstrate that, aside from its diagnostic role, untargeted metabolomics may also identify biomarkers that assist in therapeutic decision-making. This not only applies to patients with IEMs, but also in other diseases with larger health economic considerations, including cancer, cardiovascular disease and infectious diseases (79). Untargeted metabolomics may also facilitate a personalized medical approach that is not only patient-specific, but also incorporates contemporaneous external factors such as diet, treatment, and environment. Although outside the scope of this review, the recent advances in single-cell (or organelle) metabolomics also have the potential to revolutionize the treatment approaches in cancer as well as assist in understanding of cell senescence (80).

### Incorporation of untargeted metabolomics into NBS

Given the comprehensive analysis provided by untargeted metabolomics, it may play a significant role in the future of NBS. Untargeted metabolomics can be successfully performed on DBS (81-83). To our knowledge, results of a large-scale study involving untargeted metabolomics in an asymptomatic population are not available. Liu and colleagues reviewed 4,464 clinical samples analyzed via targeted metabolomics, compared with 2,000 samples (from a separate cohort) analyzed using untargeted techniques (12). The former had a 1.3% diagnostic rate [14 conditions identified, three of which were not in the Recommended Uniform Screening Panel (RUSP) (84)]. The latter had a diagnostic rate of 7.1%, but this included 49 conditions that were not included in the RUSP (19 of which do not have any known treatment). This study demonstrates that untargeted metabolomics can identify conditions detected by NBS; however, the risks of identifying additional

disorders not routinely included in the RUSP are also high. Typically, the RUSP scoring system considers diagnostic and clinical evidence for a disease (and its treatment) prior to recommendation: identifying non-RUSP conditions, some of which are less defined clinical entities, may create more uncertainty.

Over a decade ago, the National Institutes of Health (NIH)-funded Newborn Sequencing In Genomic medicine and public HealTh (NSIGHT) Consortium commenced pilot programs for whole exome sequencing (WES)- and WGS-based NBS in the United States of America (85). Many lessons have been learned from these initiatives including those associated with technical issues (data analysis and handling) and those associated with ethical and societal challenges (secondary findings of adult-onset disorders for which no therapies are currently available) (86). The latter pose a significant ethical dilemma with the possibility of interfering with the neonate's right to self-determination (85). A NBS pilot study of a combined genomic-untargeted metabolomic approach, which only analyzes genes and pathways associated with treatable childhood-onset conditions, may help to reduce this risk of secondary findings.

### Untargeted metabolomics as a diagnostic companion to genomics

With more rapid analytical techniques (87) now available, untargeted metabolomic testing is complementing genomic approaches for the diagnosis of patients suspected of having an IEM. For example, in a patient with intellectual disability and skeletal dysplasia, untargeted metabolomics identified the unusual metabolite N-acetylated mannosamine. As a result, two missense variants in *NANS* detected by WGS were prioritized for further work-up (88). This case was combined with eight other similar patients ascertained through WGS, and further functional work confirmed impairment in NANS enzyme activity as the basis for these patients' clinical condition.

In other instances where molecular testing has already identified VUSs, untargeted metabolomic analysis can contribute significantly to variant interpretation (42,55,89-92). In their "cross-omics" study, Kerkhofs and colleagues showed that for accurate prioritization of disease-causing genes in IEMs, it is essential to take into account the primary pathway of the affected protein as well as the broader network of metabolites (55). Alaimo and colleagues reviewed data on 170 patients who had both WES and

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untargeted metabolomics (89). The metabolomic data contributed to variant interpretation in 74 individuals (43.5%) and confirmed a clinical diagnosis in 21 of these cases (12.3% diagnostic rate). The American College of Medical Genetics and Genomics (ACMG) criteria give specific provision for the incorporation of results generated by such high-quality functional validation studies into variant classification (93,94). Increasingly, the combination of "-omic" technologies will provide the best approach for re-evaluating previously unsolved cases.

## Limitations of untargeted metabolomics

As with all analytical techniques, there are significant limitations and challenges with untargeted metabolomics (95). Similar to targeted metabolomics, the instrumentation required for untargeted metabolomics is expensive to both purchase and maintain (96). The specialized methodology requires a high level of expertise across both wet lab sample preparation through to dry lab results interpretation (97). Additionally, when compared with other "-omic" technologies (such as genomics), metabolomic results are heavily influenced by physiological factors such as gender, diet, and drug treatment. Age is also a particularly important confounder, as metabolomic signatures are influenced by both gestational age and chronological age (98,99). However, newer regression models are being developed to control for these factors (100). Additionally, ambient temperature and humidity can affect sample preparation (101) and assay implementation steps which in turn can influence the data produced and associated interpretation of metabolic profiling.

The bioinformatic pipelines that are required for untargeted metabolomic analysis also have specific challenges. For instance, bioinformatic pre-processing methods can impact results under different settings (102). Metabolite identification (including consideration of the associated pathways) is both complex and time consuming (2). Also, the specialized expertise required for analysis and interpretation needs to be integrated into the laboratory workflow for fast and accurate clinical outcomes (103).

Drawing parallels with genomics, early allele frequency databases lacked ethnic diversity, which negatively impacted variant curation in minority groups (104,105). Similarly, more work is required to compile the "metabolomes" of different populations and ethnic groups around the world for metabolomics to address ethnic specificity of a given disease (106). One key additional challenge will be "filtering out" metabolite variation seen in normal populations

during catabolic stress (107), to avoid misattribution of normal physiology to an IEM. As point of difference from genomic investigations, reference libraries for metabolite identification are not as well established as genomic reference sequences. Additionally, fragmentation and isotope patterns, m/z ratios, and retention time data are available for many yet unidentified metabolites; however, this is changing with increased testing. For instance, the most recent update to the Human Metabolome Database (HMDB) includes a nearly two-fold increase in the number of annotated metabolites (from 114,000 to 217,920), as well as improved spectral and pathway visualization tools (108).

Many IEMs have organ-specific manifestations, such as encephalopathy and seizures, or isolated cardiomyopathy (109,110). These conditions are likely to have distinct metabolomic profiles in different tissues and sample types, which also need to be accounted for in the analysis.

Given the above limitations, the sensitivity of untargeted metabolomics for diagnosing established IEMs is lower than that of targeted metabolomics. Almontashiri and colleagues performed untargeted metabolomic analysis in 87 patients with confirmed IEMs, with a sensitivity of 86% (111). This is analogous to the challenges with coverage observed in WES and WGS, as compared to targeted testing (112). Steinbusch and colleagues reported similar sensitivity (87%), with seven out of the 10 'missed' cases being patients already on therapy (113).

Many of the other difficulties are likely to be overcome with increasing use of untargeted metabolomics. Testing will become more affordable over time, resulting in increased accessibility. Normal population databases (including ethnically diverse minority populations) and bioinformatic pipelines will become more robust, facilitating identification of pathological states. Considering the incredible potential of untargeted metabolomics in revolutionizing the diagnosis, monitoring and treatment of IEMs, the challenges are outweighed by the benefits.

## Conclusions

Metabolomics originally developed as a targeted testing methodology, quantifying a panel of key metabolites to diagnose a limited number of clinically relevant IEMs. However, with the recent advances in technology, untargeted metabolomics is increasingly being applied in screening for a broad range of rare diseases, as well as for functional validation of genomic variants, identification of drug targets, and monitoring response to therapy. Decreasing

costs over time will allow for increased use of untargeted metabolomics, in the field of IEMs but also in others such as oncology, cardiology and infectious diseases. The more holistic analytical approach will considerably improve understanding of the pathophysiology of many IEMs, as well as identifying additional secondary metabolites to create disease-specific metabolic signatures. Tissue-specific, single cell, and even single organelle, metabolomic investigations also offer the opportunity to diagnose disorders with selective expression and can help improve treatment options for many diseases. The potential applications in screening of asymptomatic populations (as in NBS) may have significant health economic benefits: however, it would be essential to reduce the risk of secondary findings by using appropriate bioinformatic curation tools. Integration of untargeted metabolomics, along with genomics, into routine clinical care is likely to improve diagnostic workflows and patient management, resulting in both improved clinical and health economic outcomes.

## Footnote

*Reporting Checklist:* The authors have completed the Narrative Review reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-105/rc

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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