Propionic (PA) and methylmalonic (MMA) acidemias are inborn errors of the metabolism of several important amino acids, the side chain of the cholesterol molecule, and dietary odd chain-length fatty acids. PA results from a deficiency of mitochondrial propionyl-CoA carboxylase, an enzyme that requires biotin as a cofactor and converts propionyl-CoA to D-methylmalonyl-CoA. Classical MMA results from deficiency of methylmalonyl-CoA mutase, a cobalamin- (vitamin B₁₂) dependent enzyme, which converts L-methylmalonyl-CoA to succinyl-CoA. The 2 enzymes are almost adjacent in this important enzyme, which converts L-methylmalonyl-CoA to succinyl-CoA. The 2 defects are very similar from the perspective of clinical presentation and metabolic pathway, and the 2 defects are very similar from the perspective of clinical presentation and metabolic biomarkers (1). For example, in expanded screening programs for metabolic diseases in newborns, observation of increased propionyl-carnitine (C₃-carnitine) by tandem mass spectrometric analysis is predictive of the occurrence of both defects (2). Currently, the 2 defects are differentiated biochemically with targeted analysis by gas chromatography/mass spectrometry of characteristic urinary organic acid. Specifically, large amounts of MMA acid and several propionate metabolites are found in the urine from patients with MMA, whereas increased MMA acid has not been found in tests of urine, and more recently dried blood spots, from patients with PA (3).

Although both of these disorders have been studied for more than 4 decades, several features and metabolic issues remain unresolved. Before the introduction of diagnostic methods to measure urinary organic acids, these disorders were defined as a syndrome called ketotic hyperglycinemia (4). The mechanism that leads to increased blood glycine has never been determined, and most patients remain hyperglycinemic throughout life. It is not known if hyperglycinemia contributes at all to the phenotype. It has been established, however, that several alternate pathways of propionyl-CoA metabolism that exist as minor pathways in healthy individuals are used extensively in patients with these diseases. The extent to which the use of these alternative pathways leads to the clinical phenotype has not been determined. One of these alternate pathways involves the condensation of propionyl-CoA with oxaloacetate to form methylcitrate. This pathway uses enzymes of the Krebs cycle (5). Whether this additional input into a vital metabolic pathway is beneficial or harmful is not known.

Our understanding of the pathogenesis of metabolic diseases is maturing. Initially, the concept was held that a single gene defect results only in a 1-step involvement in a single well-defined metabolic pathway. Now we have come to the realization that abnormalities in 1 step in a single pathway are very likely to impact multiple other pathways. Furthermore, we are recognizing that involvement of alternate pathways may additionally impact the disease process. The evolving metabolomic tools that allow us to simultaneous study multiple metabolic pathways have been used mainly in targeted studies of candidate alternate pathways such as studies of the involvement of the Krebs cycle in PA and MMA. Targeted analysis is likely to provide incomplete knowledge of all metabolic interactions that may result from a single gene defect.

In this issue of Clinical Chemistry, Wikoff et al. (6) provide proof of principle that untargeted metabolomic analysis may generate additional information regarding the complexity of the metabolic ramifications of a metabolic disease. In this study, methanol-extracted plasma samples from patients with PA or mutase-deficient MMA were subjected to an approximately 1.25-h long capillary C-18 reversed-phase gradient liquid chromatography separation process. Peaks eluting from the column were detected by electrospray ionization time-of-flight mass spectrometry, and spectral data accumulation was across a mass range of m/z 75–1000. A total of more than 4000 features (peaks) were detected with the equivalent of just 8 μL of plasma. Some of these features had characteristic and known spectra for which positive identification was possible and some were not known but were putatively identified. Other unidentified compounds were not subjected to further studies to help identify them. The authors focused their major efforts on identification of features that can be used to distinguish samples from patients with PA or MMA from control samples and from each other.

As proof of the principle that untargeted analysis can hit the right note, the most characteristic and statistically significant feature that distinguished both types of disease samples from healthy samples was a compound subsequently confirmed to be propionyl-carnitine. This compound is used for identification of both conditions in newborn screening programs and is a routine targeted metabolic marker for monitoring disease status (2). All patients with these 2 diseases have consistently increased concentrations of propionyl-carnitine, which may fluctuate according to metabolic status.

A 2nd probable acyl-carnitine species, which may be the 6:1 or a branched-chain methyl carnitine, was also identified as being significantly increased in both groups of patients. Less consistently, γ-butyrobetaine, an intermediate on the carnitine biosynthetic pathway, was also increased. The contribution of exogenous carnitine therapy, which is routinely provided for PA and MMA patients, may partly explain these findings of carnitine metabolic changes. PA and MMA patients are on chronic long-term high-dose therapy, whereas the control patients that took carnitine in this study did so for a short time only. The relative effects of carnitine medication, long-term or short-term, on metabolic processes are unknown. It also remains to be seen if the basic metabolic defect leads to alteration of carnitine metabolism itself, as was indicated by the increased concentrations of γ-butyrobetaine.
A total of 71 features differentiated PA from MMA, a finding that is quite remarkable given the very close proximity of the 2 metabolic defects. The most notable feature that differed between the 2 was the presence of isovaleryl carnitine (IV-carnitine), which was increased by a factor of 5.1 in MMA but did not differ from normal in PA. No other features that differed between PA and MMA were positively identified in this study, but identification remains possible on the basis of chromatographic retention time and the mass spectral information that was obtained. We should anticipate further experimentation to reveal features that uncover important metabolomic interactions in PA and MMA.

Most intramitochondrial diseases with primary accumulation of coenzyme A intermediates, such as PA and MMA, eventually lead to the accumulation of acylcarnitine species, presumably owing to the interaction of otherwise minor carnitine esterification pathways. This study clearly identifies involvement of carnitine metabolism in the complex metabolic perturbations seen in PA and MMA and appears to have identified a considerable number of different features between PA and MMA. The observation of increased IV-carnitine in MMA but not PA was clearly unanticipated and at the moment not easily explained. Increased accumulation of IV-carnitine presumably results from increased accumulation of isovaleryl-CoA, which is the major targeted marker for isovaleric acidemia, a disorder of leucine metabolism, and also of multiple acyl-CoA dehydrogenation defects resulting from impaired electron transport (7, 8). Leucine, however, is not a precursor of propionyl-CoA nor is it known to be a precursor or downstream metabolic product of L-methylmalonyl-CoA. Patients with MMA do not have all of the features of isovaleric acidemia, including the characteristic odor of sweaty feet, which is related to isovaleryl-CoA accumulation. The finding of increased IV-carnitine requires additional investigation to discern whether or not it is an important observation that will lead to better understanding of the complexity of MMA and perhaps provide an insight into a different disease mechanism for PA.

At the end of this study, there remained many unidentified features. The identification of useful components among this large list of potentially useful biomarkers presents a major technological challenge, and whether technology can answer those important clinical and metabolic questions remains unknown until these additional features are identified. In other emerging areas in which untargeted study of proteomic biomarkers seeks primarily to identify new disease biomarkers to aid diagnosis, the need for absolute analyte identification is controversial. To some investigators, it matters less what component X might be as long as it is measurable and shown to be the best biomarker for that particular disease state [Refs. (9, 10) show some of the many recent examples in which positive identification was not deemed necessary]. Other investigators express the view that molecular identification is important for clinical application of these predominantly proteomic biomarkers (11–14). Further advances in untargeted metabolomic analysis of metabolic diseases requires absolute identification of important analytes so that we can unravel the metabolic complexity of these diseases and perhaps design better therapeutic regimens. We already have targeted biomarkers for most of the metabolic diseases, and identification of markers for diagnosis is less of an issue. This field requires identification of the novel features that can help us explain the complex clinical phenotypes. The present study, although providing few new insights, is the first to use untargeted analysis. That predictable features were found is good. It is now time to use this novel and potentially useful technique to dig deeper in the search for unidentified but clinically different features.

References
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