Metabolomic approaches to mitochondrial disease: correlation of urine organic acids

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Abstract

In order to examine correlations which might be useful in ascertaining or confirming the diagnosis of mitochondrial disease, a retrospective analysis of urine organic acids was performed. Among 3646 analyses from randomly selected samples referred to our laboratory, there were 258 specimens from 67 patients with various known disorders of mitochondrial oxidative function, most of whom were known to have chronic and persistent elevations of blood lactic acid, and 176 samples from 21 patients with diagnosed organic acidemia. Urine lactate was not a useful discriminator; only 7.6% of results from infants with mitochondrial disease fell the 95th percentile for patients without mitochondrial disease. Most of the Krebs cycle intermediates were also not useful in discriminating patients with mitochondrial disorders. Interestingly, there was strikingly poor correlation among most of those analytes in all patient groups, but fumarate and malate were uniquely well correlated ($r^2=0.840$). Fumarate and malate were also the most useful in distinguishing patients with mitochondrial disease and organic acidemia from the pool of unselected or undiagnosed patients, although the utility was somewhat limited. Using a cutoff value of approximately 90 mmol/mol creatinine for fumarate or malate at age < 1 year, or a cutoff of approximately 25 for older patients, 25–30% of mitochondrial disease patients can be distinguished with a 5% false positive rate. Further refinements to this approach may better characterize the metabolomic profile and may improve the diagnostic utility of quantitative organic acid analysis in mitochondrial disease.

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1. Introduction

The diagnosis of mitochondrial electron transport chain defects and energy metabolism disorders has been difficult, due to the heterogeneity and variability of mitochondrial disease. There have been a few notable attempts to establish consensus guidelines for diagnostic criteria (Walker et al., 1996; Bernier et al., 2002; Wolf and Smeitink, 2002), and metabolic laboratory test abnormalities have been included as minor criteria. However, there has been no clear specification of which metabolites should be considered nor

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the exact values which should be considered diagnostic. This study is a retrospective analysis of urine organic acids to examine correlations which might be useful in ascertaining or confirming the diagnosis of mitochondrial disease.

2. Methods

Urine organic acids were analyzed by gas chromatography–mass spectrometry following formation of the pentafluorobenzyl oximes of oxoacids, aldehydes, and ketones, batch-wise liquid partition chromatography on silicic acid and formation of trimethylsilyl adducts of carboxylate and alcohol groups, according to the method of Hoffmann et al. (1989). The computer records of all recent organic acid analyses performed at the UCSD Biochemical Genetics Laboratory over a period of approximately two years were compiled. There were a total of 3646 organic acid panel results in the database at the time it was queried.

The UCSD Biochemical Genetics Laboratory is a referral center, and many of the patients and their diagnoses are not known in detail. However, included in the dataset were the results of a set of known patients with mitochondrial disease, and those were considered separately. Those data included 258 specimens from 67 patients with mitochondrial diagnoses. The diagnoses were Complex I deficiency ($N=11$), Complexes I and IV deficiency ($N=4$), Complex III deficiency ($N=1$), Complex IV deficiency ($N=6$), Kearns–Sayre syndrome associated with mtDNA deletion ($N=3$), Pearson syndrome associated with mtDNA deletion ($N=3$), Leigh syndrome with no detected electron transport defect or mitochondrial DNA abnormality after muscle biopsy ($N=11$), MELAS A3243G ($N=19$), MELAS T3271C ($N=1$), NARP T8993G ($N=4$), PDH E1α defect ($N=2$), and mtDNA tRNA$_{Lys}$ G8363A ($N=2$). The patients’ ages ranged from 4
Fig. 2. Correlations among urinary organic acids. Data are shown for citrate (C), aconitate (A), isocitrate (I), 2-oxoglutarate (O), succinate (S), fumarate (F), and malate (M).
months to 59 years. Most of these patients were enrolled in a study which was predicated on documented consistent elevation of blood and/or CSF lactate concentrations (≥3 mM). The samples were collected at times of baseline health in most cases; in only 3 patients (total of 10 samples) were these analyses performed at the time of acute illness. There were also 176 specimens from 21 patients known or found to have organic acidemias, and these were tabulated separately. The diagnoses included propionic acidemia (10 patients), maple syrup urine disease (3 patients), methylmalonic acidemia (2 patients), isovaleric acidemia (2 patients), hydroxyisobutyric acidemia, 3-oxothiolase deficiency, pyruvate carboxylase deficiency, and glutaric acidemia type I (1 patient each). It is unknown how many other samples from other patients with mitochondrial disease referred from other centers (or possibly with unrecognized organic acidemias) were included in the dataset.

3. Results

Urine lactate was not a strong discriminator in this population of mitochondrial patients (Fig. 1). Among the samples from patients with mitochondrial disease (of all ages), 27.1% had lactate values above the upper limit of normal (197 mmol/mol creatinine), but 13.5% of samples from unselected patients also had values above the normal range. It is interesting that urine lactate was not elevated in the majority of samples from these mitochondrial patients, particularly since most of those had blood lactate levels above 3 mmol/L. There were >50 samples from patients who were not identified as having mitochondrial disease or organic acidemia, with urine lactate concentrations among the highest values in the dataset. The clinical details of those cases without specific diagnosis are not known; presumably many of those patients were extremely ill and perhaps in clinical shock at the time metabolic tests were ordered. Based on available information, the statistical specificity of identifying patients with mitochondrial disease or organic acidemia based on urinary lactate was not good. For example, by choosing a cutoff value for lactate which distinguished 5.0% of samples from patients ≤1 year old without known mitochondrial disease or organic acidemia (1108 mmol/mol creatinine), only 7.6% of samples from patients with mitochondrial disease would have been flagged.

The correlation among derivatives of Krebs cycle intermediates detected in the urine organic acid analyses of all 3646 samples is shown in Fig. 2. In general, there is a strikingly poor correlation among the analytes, but the correlation between fumarate and malate is relatively high, with a correlation coefficient ($r^2$) of 0.840. The poor correlation of the other Krebs cycle metabolites is evident on inspection of the results of individual patients’ organic acid results, with isolated elevations of individual analytes may be inconsistent from time to time. The distributions of results for the Krebs cycle intermediates in each diagnostic category are tabulated (Table 1).

By successive approximation, cutoff values were determined which would flag 5.0% of samples from patients without specified diagnosis (i.e. a statistical specificity of 0.95, calculated as the ‘true negative’ rate

Table 1
Dispersion of urine Krebs cycle organic acid results

<table>
<thead>
<tr>
<th>Category</th>
<th>Age</th>
<th>N</th>
<th>Citrate</th>
<th>Aconitate</th>
<th>Isocitrate</th>
<th>Oxoglutarate</th>
<th>Succinate</th>
<th>Fumarate</th>
<th>Malate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected</td>
<td></td>
<td></td>
<td>685±684</td>
<td>119±95</td>
<td>105±75</td>
<td>172±184</td>
<td>52±77</td>
<td>25±36</td>
<td>29±39</td>
</tr>
<tr>
<td>Mito</td>
<td></td>
<td></td>
<td>478±440</td>
<td>108±91</td>
<td>93±66</td>
<td>60±86</td>
<td>25±41</td>
<td>9±30</td>
<td>9±23</td>
</tr>
<tr>
<td>OA</td>
<td></td>
<td></td>
<td>663±359</td>
<td>222±162</td>
<td>146±54</td>
<td>250±229</td>
<td>46±39</td>
<td>108±143</td>
<td>87±100</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>393±341</td>
<td>183±158</td>
<td>120±72</td>
<td>114±166</td>
<td>31±51</td>
<td>44±114</td>
<td>32±67</td>
</tr>
</tbody>
</table>

Values are means±SD expressed in mmol/mol creatinine. The categories of patients relate to diagnosis: mito, mitochondrial/oxidative phosphorylation defect; OA, organic acidemia; unselected, all other patients.
divided by the sum of the ‘false positive’ and ‘true negative’ rates). Statistical sensitivity for identification of the known mitochondrial disease patients at those cutoff values were calculated as the ‘true positive’ rate over the sum of the ‘false negative’ and ‘true positive’ rates. The results are tabulated (Table 2).

Samples from patients with mitochondrial disease tended to have more frequent elevations of fumarate
and malate (Figs. 3 and 4), and that was also true for patients with organic acidemias. Aconitate was elevated more often in the mitochondrial disease patients than in those with organic acidemias, but the sensitivity of fumarate and malate was greater overall.

4. Discussion

Abnormalities in organic acid patterns have long been known to be a feature of mitochondrial diseases that affect energy metabolism and the electron transport chain. Of course, most organic acidemias are in a broader sense mitochondrial diseases, and the enzymes may be affected by intramitochondrial chemical alterations, or perhaps by direct interactions with the electron transport chain. Although the mechanisms are generally not known exactly, there are numerous examples where specific metabolites, even those considered pathognomonic for specific organic acidemias, are elevated in mitochondrial disease. Few such cases have been reported; for example, two of the patients in this group were siblings with Complex I deficiency who consistently excreted very high amounts of tiglylglycine and were initially misassessed to have 3-oxothiolase deficiency. A patient with MELAS and the A3243G mutation was reported to present with excretion of 3-methylglutaconic acid and clinical features of Barth syndrome (De Kremer et al., 2001). Abnormal fatty acid oxidation (Venizelos et al., 1998) and acylcarnitine profiles (Sim et al., 2002) have been documented in cells of patients with electron transport chain defects. Mitochondrial respiratory chain defects may give rise to clinical...
features of fatty acid oxidation disorders (Enns et al., 2000), and conversely, there may be secondary respiratory chain abnormalities in patients with \( \beta \)-oxidation defects (Das et al., 2000).

Consensus diagnostic criteria for mitochondrial disease were published in 1996 by Walker et al. (1996), and an attempt was made in 2002 to refine them for application to pediatric cases by Bernier et al. (2002). In those schemes, a minor diagnostic criterion was ‘one or more metabolic indicators of impaired respiratory chain function,’ but the indicators were no more specifically defined. The Nijmegen Mitochondrial Disease Criteria (Wolf and Smeitink, 2002) included urine organic acid abnormalities as minor criteria, specifically as ‘elevated excretion of lactate or TCA cycle intermediates,’ and ‘elevated excretion of ethylmalonic acid or 3-methylglutaconic acid or dicarboxic acids (adipic, suberic and sebacic acid)’. The present work is an initial attempt to determine specific parameters, and the predictive value and limitations of using organic acid results in the diagnosis of mitochondrial disease.

There is a certain amount of uncertainty in estimating the statistical specificity (without knowing the true diagnostic status of all patients studied); incorrectly attributed patients with mitochondrial disease who were not known would have been included among the ‘false positives,’ but that would make the calculated specificities may be minimum estimates. Owing to the variability of the values and the wide dispersion of results in the unselected population, there is limited statistical power. The relatively high specificity of fumarate and malate may relate to the fact that their normal concentrations are the lowest among the Krebs cycle intermediates in urine organic acids. The singular correlation between those two Krebs cycle intermediates may arise from their extramitochondrial equilibration through the fumarase reaction.

The present study shows that there is a limited ability to distinguish samples from patients with oxidative phosphorylation defects against the background of the general samples (often sick or stressed patients) sent to a Biochemical Genetics laboratory for organic acid analysis. At best, it appears that using a cutoff value of about 90 mmol/mol creatinine for fumarate or malate in infants, or a value of 25 for older individuals, 25–30% of mitochondrial disease patients can be distinguished with a 5% false positive rate. Further refinement of this approach, such as applying predictive criteria using combinations of organic acid concentrations, may lead to greater utility.

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**References**


