UNTERTAGED MASS SPECTROMETRY-BASED METABOLOMICS IN THE DIAGNOSIS OF INBORN ERRORS OF METABOLISM IN AN INDIVIDUAL PATIENT

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Background and Goal
In a proof-of-concept study, untargeted liquid chromatography - mass spectrometry (LC-MS) based metabolomics was applied to the diseases phenylketonuria (PKU), 3-methylcrotonyl-CoA carboxylase (3MCC) deficiency and medium chain acyl-Co-A dehydrogenase (MCAD) deficiency. Goal of the study was to find and identify, in an untargeted approach, the biomarkers in plasma from individual patients with PKU, 3MCC and MCAD.

Methods

Blood plasma
- 20 controls, 1 patient
- Samples in duplicate

Agilent-UHPLC-QTOF-MS
- Reversed phase liquid chromatography
- Electrospray ionization - positive ion mode

XCMS (www.xcms.org)
- Peak comparison

Statistic pipeline
- PCA
- t-test
- PPI

Metabolite identification
- HMDB (www.hmdb.ca)
- Retention time of pure compound

Diagnosis

Results

Table 1: Diagnostic metabolites, neutral masses (MW) and retention times (RT) identified in plasma of patients with PKU, MCAD and 3MCC deficiency. Intensity plots are provided for three metabolites as examples (Fig 1, 2 and 3).

PKU

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MW</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>165.07898</td>
<td>3.67</td>
</tr>
<tr>
<td>Glutamylphenylalanine</td>
<td>294.12157</td>
<td>5.88</td>
</tr>
<tr>
<td>Unknown</td>
<td>327.13180</td>
<td>4.01</td>
</tr>
<tr>
<td>N-Acetylglutamylala</td>
<td>207.08954</td>
<td>7.65</td>
</tr>
</tbody>
</table>

MCAD deficiency

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MW</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanoylcarnitine</td>
<td>287.20966</td>
<td>9.54</td>
</tr>
<tr>
<td>9-Decenoylcarnitine</td>
<td>313.22531</td>
<td>10.73</td>
</tr>
<tr>
<td>Hexanoylcarnitine</td>
<td>259.17836</td>
<td>6.84</td>
</tr>
<tr>
<td>Capryloylglycine</td>
<td>201.13649</td>
<td>10.86</td>
</tr>
<tr>
<td>2-trans,4-cis-Dodecenoylcarnitine</td>
<td>311.20966</td>
<td>9.83</td>
</tr>
<tr>
<td>Nonanoylcarnitine</td>
<td>301.22531</td>
<td>10.32</td>
</tr>
<tr>
<td>Suberylglycine</td>
<td>231.11067</td>
<td>6.36</td>
</tr>
<tr>
<td>Hexanoylglycerol</td>
<td>173.10519</td>
<td>7.63</td>
</tr>
<tr>
<td>Heptanoylcarnitine</td>
<td>273.19401</td>
<td>8.29</td>
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</table>

3MCC deficiency

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MW</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxyisovalerylcarnitine</td>
<td>261.15760</td>
<td>3.17</td>
</tr>
<tr>
<td>3-Hydroxyisovaleric acid</td>
<td>118.06299</td>
<td>3.62</td>
</tr>
<tr>
<td>3-Methylcrotonylglycine</td>
<td>157.07389</td>
<td>5.08</td>
</tr>
</tbody>
</table>

Conclusion

Untargeted MS-based metabolomics in plasma can be used to find abnormal metabolites in patients with an inborn error of metabolism. This approach can be used to examine patients for possible new metabolic disorders. The method works in individual patients. Untargeted metabolomics can be applied in parallel with whole exome/genome sequencing. This may indicate metabolic perturbations and may help prioritize candidate genes in whole exome sequencing interpretation.

**Additional Information**
- Table 1 provides diagnostic metabolites for PKU, MCAD, and 3MCC deficiencies.
- Figures 1, 2, and 3 show intensity plots for specific metabolites.
- The method is applied to find biomarkers in individual patients with PKU, 3MCC, and MCAD.
- Metabolomics is a powerful tool for identifying metabolic disorders.