Introduction

Monitoring of treatment in PKU patients is based on blood phenylalanine. Fluctuation of phenylalanine is often not well understood. Progress in NMR technology in analyzing the metabolome may offer a new horizon to monitor metabolic diseases. We used urinary analyses of treated patients with PKU as a model to define normality of profiles against healthy age matched controls.

Method and patient sample

60 patients (age 1-40 years) with dietary (43), sapropterin (12) and no treatment (5, supplemented with large neutral amino acids) (Table 1) were investigated using a Bruker Avance IVD System at 600 MHz. Statistical analyses against a reference of healthy children (n=43) using PCA-LDA were performed. Blood phenylalanine was measured according to local routine methods by tandem mass spectrometry in blood spots.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of samples</th>
<th>Mean Blood Phe µmol/l</th>
<th>Std Blood Phe</th>
<th>Mean Phe intake mg/day</th>
<th>Std Phe intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sapropterinhydrochloride</td>
<td>12</td>
<td>391</td>
<td>134</td>
<td>1663</td>
<td>600</td>
</tr>
<tr>
<td>Diet with Amino acids supplement</td>
<td>34</td>
<td>698</td>
<td>380</td>
<td>895</td>
<td>976</td>
</tr>
<tr>
<td>No Diet plus Large neutral amino acids supplement</td>
<td>5</td>
<td>1471</td>
<td>156</td>
<td>2430</td>
<td>1137</td>
</tr>
</tbody>
</table>

Table 1 Overview of patient characteristics with treatment group, blood phenylalanine and phenylalanine intake

Discussion

We present results of a preliminary pilot study showing the potential of investigating the metabolome in spontaneous urine samples of PKU patients with different treatment strategies. Separation of profiles from patients and controls is clearly possible and is not only due to phe control. Individual metabolic profiles can be displayed in this model and may predict quality of treatment based on high phe intake and phe control as can be seen by a significant difference in phenylalanine intake and blood phe concentration in dietary treated patients and sapropterin treated patients (Table). Further studies are in progress to elucidate the spectrum differences possibly caused by the amount of natural protein intake in the diet together with a phe control in the target range.

Conclusion

NMRs profiling in urine has a high potential to predict optimal dietary treatment in PKU patients.

Reference


Results

Since treatment strategies were very different we first build a model between the dietary treated group with amino acid supplement and the control group. As can be seen on the Fig 1, there is a clear separation between urinary profile of healthy controls (blue) and the patients on a phenylalanine restricted diet (red). Patients with a supplemental sapropterin treatment (10-20 mg/kg bodyweight) showed profiles between the healthy and PKU profiles (black points). Individual profiles of the non-treated group showed 3 of 5 as outliers (green). Differences in the spectrum profile are shown in Fig 2. There are several areas in the spectrum showing significant difference between the control and PKU patients on a phe restricted diet (Fig 2a). Differences of these spectra were not due to phenylalanine control as visible in urine (data not shown). However, in the non treated 5 patients on large neutral amino acids the main spectral differences were caused by high phenylalanine excretion in urine (Fig 2b).

Figure 2 shows the spectral differences coming from the different treatment. (on the top) Individual profiles of the 5 non treated (but supplemented with large neutral amino acids) show in Fig 2a a high phenylalanine excretion in urine. (on the bottom) The Fig 2b shows that the NMRs profiles from the patients treated with Kuvan are between the patients treated with Amino acids supplement and the controls.