Phenylketonuria: is blood phenylalanine the only and best biomarker?

Introduction
Phenylketonuria (PKU) is an inborn error of phenylalanine metabolism, caused by a deficiency of phenylalanine hydroxylase. Management of PKU primarily consists of a protein-restricted diet to keep phenylalanine levels within an acceptable range to prevent severe brain damage.

Phenylalanine is a well-known biomarker of phenylketonuria (PKU). However, despite intensive treatment monitoring clinical outcome remains sub-optimal. Some of the cerebral processes are linked to pathways involving lipids. Therefore, we aimed to perform lipidomics in a well described/studied cohort of PKU patients.

Study design
A Case-Control study

- treated PKU\(^1\) (n=22)
- control (n=22)
  Age & gender matched

B Correlation studies

- treated PKU\(^1\) (n=35)

Figure 1 Study design

JC van der Weerd\(^2\), AMJ van Wegberg\(^1\),
TS Boer\(^2\), K van Vliet\(^1\), P de Blaauw\(^2\),
SC Huijbregts\(^3\), FJ van Spronsen\(^1\),
MR Heiner-Fokkema\(^2\)

1Division of Metabolic Diseases, Beatrix Children’s Hospital, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands.
2Laboratory of Metabolic Diseases, Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, PO Box 30.001, 9700 RB, The Netherlands.
3Department of Clinical Child and Adolescent Studies-Neurodevelopmental Disorders, Faculty of Social Sciences, Leiden University, Leiden, The Netherlands.

*Van Wegberg and van der Weerd equally contributed

Correspondence:
j.c.van.der.weerd@umcg.nl
Experimental

Matrix
- EDTA-plasma

Lipid extraction
- One-phase extraction (MeOH/MTBE/CHCl₃)
- Untargeted
- Acquity UPLC CSH C18 column (2.1 x 100 mm, 1.7 µm) (Waters, Manchester, UK)
- ESI +/- mode
- QTOF mass spectrometry (Waters, Manchester, UK)

LC MS/MS
- Acquity UPLC CSH C18 column (2.1 x 100 mm, 1.7 µm) (Waters, Manchester, UK)
- ESI +/- mode
- QTOF mass spectrometry (Waters, Manchester, UK)
- Untargeted

Preprocessing
- MassLynx

Processing
- Progenesis QI (Nonlinear Dynamics, Newcastle, UK)
  - Alignment, peak picking, annotation (<5 ppm, LipidBlast, HMDB)
- Biological outlier removal
- SERRF normalization
- Remove noise and inconsistent data (CV ≥20% in QC samples)

Data analysis
- Univariate analysis (Mann-Whitney U test)
- Multivariate analysis (PCA, OPLS-DA)
- Correlation analysis (Spearman’s rank-order correlation test)

Results

Figure 2 Summary of the discriminating features selection criteria. 216 of 2490 features were significantly different. Orthogonal partial least squares analysis resulted in the identification of 16 potential lipid biomarkers for PKU disease.

Figure 3 PCA with 216 features for PKU patients (Type 1) and control group (Type 2). PKU patients are represented by the red circles (n=19) and controls by the blue triangles (n=22)
Results

**Figure 4 Biomarker extraction.** S-plot derived from the OPLS-DA model. In the plot the covariance \( p[1] \) is plotted against the correlation \( p(\text{corr}) \) of variables. Features higher in PKU are located in the top right corner, while features that are lower are in the lower left corner of the graph. 16 features; and their preliminary annotation that satisfy the selection criteria \( p(\text{corr}) \leq 0.5 \), \( p[1] \geq 0.5 \) and VIP≥1) are described in figure 4 and are located in the blue and pink squares respectively. In total, 7 features could not be identified with the LipidBlast, HMDB or Lipid Maps database within a mass error range of 5 ppm and therefore require further investigation.

**Figure 5 Correlations with phenylalanine levels.** Biomarker unknown 1 (retention time 16,62 min. & 1542,6450 m/z) was found to be significantly correlated with phenylalanine levels.)
Conclusions and future work

216 out of 2490 features from our lipidomics pipeline were significantly different between treated PKU patients and healthy controls.

16 features were highly discriminating, annotated features have been found to relate to increased oxidative stress and dietary intake.

Only 1 feature correlated with plasma phenylalanine, the identity remains to be investigated.

Plasma lipidomics in treated PKU seems valuable for investigation of the diet.

- Future work will focus on identification of the found markers and on correlations with the diet and with neurocognitive functioning.

For questions please contact: j.c.van.der.weerd@umcg.nl

References