

Non-targeted dried urine filter paper (DUPF)-based metabolomics analysis applied to patients with Pompe disease

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For more than 50 years, filter paper has been used for screening different inherited metabolic disorders. Dried samples allow testing patient specimens for metabolic diseases using minimum sampling volume and low shipping costs to a specialized laboratory via traditional mail service. Since the 2000s, newborns from different countries have been screened by dried blood spot (DBS) for Pompe disease, a rare genetic disorder that causes progressive skeletal muscle and heart weakness. Another less utilized but very similar sampling approach is the analysis of dried urine on filter paper (DUPF). This approach has previously been used for monitoring many diseases as well as targeted metabolomics analysis for expanding bioanalytical demands and population health studies. Although the stability of urine metabolites on filter paper in short-term storage is already well established, to our knowledge, non-invasive methods have not been developed for identifying a specific biomarker to increase Pompe disease diagnosis accuracy using DUPF. In this study, we applied a non-targeted DUPF-based metabolomics method by Liquid Chromatography coupled to High-Resolution Mass Spectrometry (LC-HRMS) for patients with Pompe disease to search for complementary candidate biomarkers. For method development, 1.0 mL of urine samples from 12 Pompe patients (n = 25) and 25 urine samples from controls were added onto 2.5 x 5.0 cm filter papers (Whatman 903), which were dried for 24 h in plastic boxes (width 2.5 cm x length 5.0 cm) at room temperature (22 °C). The extraction was performed using 3 mL of MeCN:H₂O (1:1; % v/v) containing 0.1% of NH₄OH under agitation for 15 min. The extract was centrifuged and analyzed by LC-HRMS/MS in negative ionization mode using non-targeted experiments. Data obtained were normalized by LOWESS for intra-batch correction, followed by creatinine normalization for dilution effects. The unsupervised PCA analysis using the normalized data showed a trend of separation between the Pompe and control groups, with two PCs explaining 36.6% of the variability. For features selection, PLS-DA and Random Forest algorithm classification were used. Taken together, they revealed 32 metabolites showing a significant statistical difference (p < 0.05) from the control group and excellent area under the curve (AUC) values with ROC curve analysis (AUC > 0.7). These metabolites were classified as nucleosides (3), amino acids (9), fatty acyls (6), organic acids (3), purines (3), benzenoids (1), carbohydrates (4), pyridines (1), pyrimidines (1) and peptides (1). Interestingly, 6 out of the 32 biomarkers assessed have previously been found in the liquid urine of Pompe patients, including the traditional PD non-specific biomarker (Glc₄) as well as creatine, N-acetylaspartylglutamate, 2-aminobenzoic acid, Mannitol (or isomers), and 1-Methylguanine. Candidate biomarkers were identified using authentic standards (level 1) or putatively annotated (level 2 of identification). A distinct signature of these metabolites could significantly improve Pompe disease diagnosis. In conclusion, this study has demonstrated the potential of a non-invasive, easy-to-store and transport sampling method applied to Pompe disease investigation using filter paper. Moreover, this workflow might provide a useful approach for real-time health status monitoring, which is critical in the age of precision medicine.

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