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Body Fluid NMR Spectroscopy - Inborn Errors of Metabolism
Metabolomics – Metabolic diseases

^{1}H NMR-based metabolomics

Chemometric methods for the diagnosis of inborn errors of metabolism

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Introduction

^{1}H-NMR spectroscopy has successfully been applied to the field of inborn errors of metabolism (IEM). However, body fluid NMR spectra lead to a wealth of data and it can be a daunting task to find the relevant information.

Aim

To demonstrate the use of chemometric methods in NMR-based urine analyses to diagnose patients with IEM. Alkaptonuria (ALK) and methylmalonic aciduria (MMA) will be used as examples.

Materials and methods

Sample collection: Urine samples were obtained from 58 healthy volunteers, 5 patients with ALK and 6 patients with MMA.

NMR spectroscopy: Urine samples were measured at 500MHz. 1D ^{1}H-NMR spectra were acquired as 128 transients in 32K data points with a spectral width of 6002 Hz.

CHEMOMETRY: Spectra have been bucketed with a bucket size of 0.02 ppm, and scaled the creatinine peak at 3.13 ppm (Figure 1.) The region 4.6-4.9 ppm, which contains the residual water peak, was removed prior to statistical analysis.

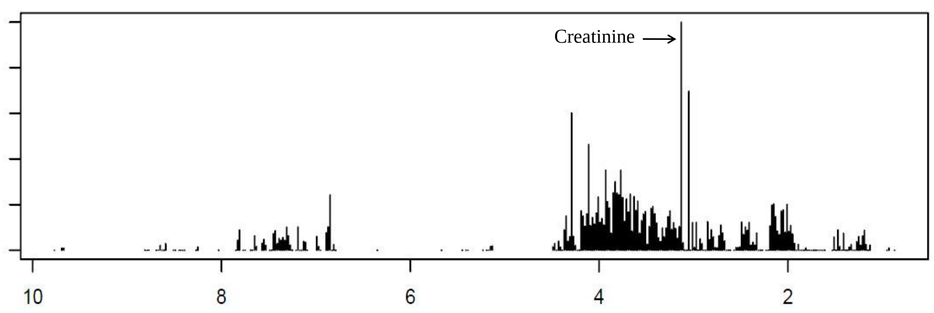

Figure 1: Randomly chosen urine NMR spectrum from a healthy volunteer. The spectrum is divided into equally sized regions (0.02 ppm).

Results

^{1}H-NMR spectra of urine may contain more than hundred resonances, deriving from endogenous or exogenous metabolites. Figure 2 shows examples of urine NMR spectra of a healthy volunteer (2A and B), a patient with ALK (2C) and a patient with MMA (2D).

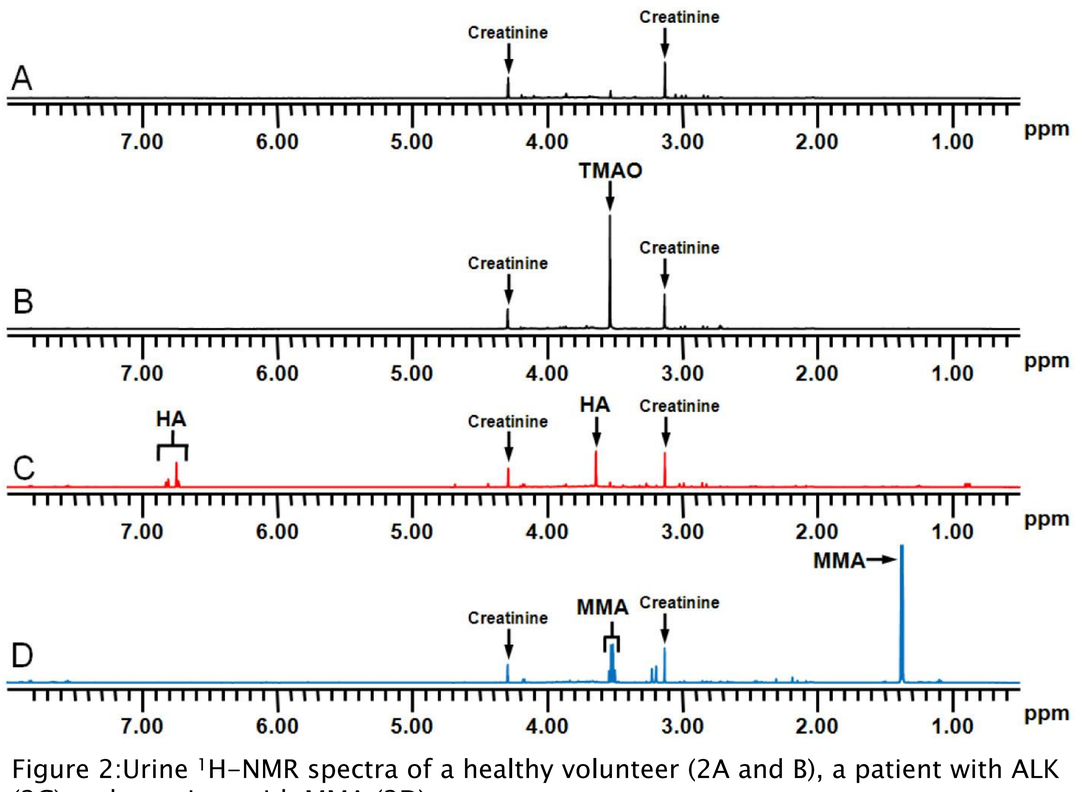

Figure 2:Urine ^{1}H-NMR spectra of a healthy volunteer (2A and B), a patient with ALK (2C) and a patient with MMA (2D).

Results

The urine NMR data were classified using Principal Component Analysis (PCA; Figure 3) and Partial Least Square Discriminant Analysis (PLS-DA; Figure 4).

PCA

PCA is a unsupervised since it does not take into account class labels. PCA can quickly provide insightful graphical overviews of the data, making it possible to identify gross outliers, group structure and relations between samples and variables.

Figure 3a shows the result of the PCA analysis of urine samples of healthy controls and the patients with MMA and ALK. The first and third PCs shown clearly discriminate between the three classes. The loading plots (Figure 3b) give an indication which variables are involved. On the horizontal axis, two variables jump out: ppm values 1.37 and 3.53. Both are signals from methylmalonic acid, the metabolite associated with MMA. On PC 3, we can clearly distinguish ppm values 3.63 and 6.75, coming from homogentisic acid, the metabolite associated with ALK.

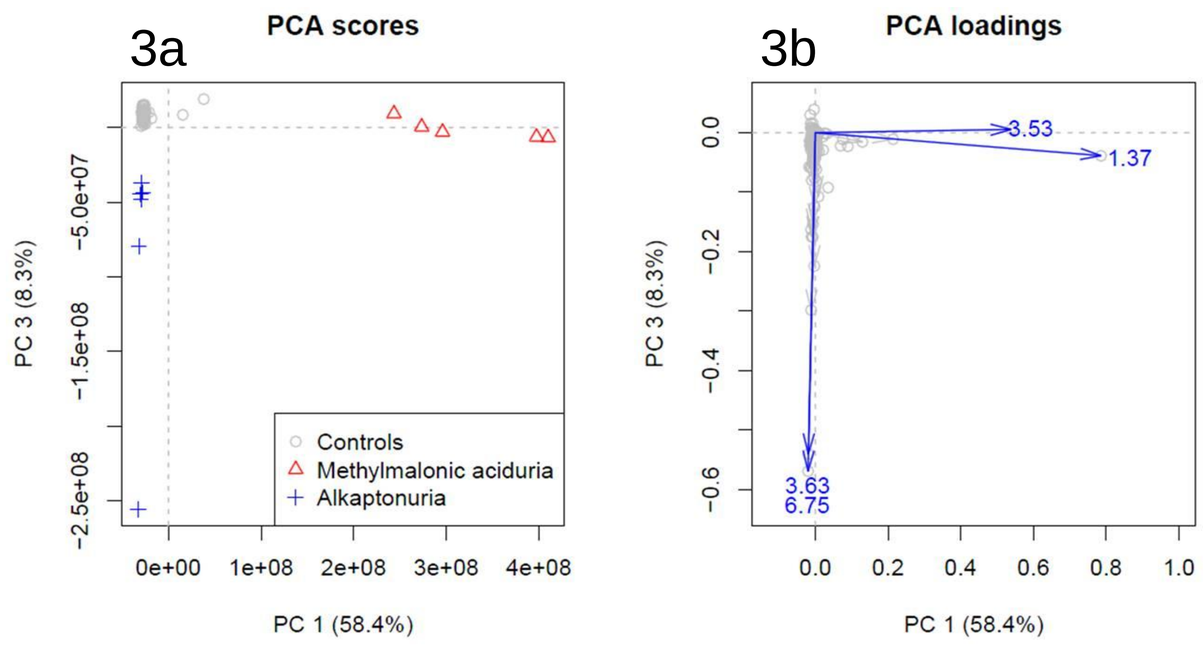

Figure 3: PCA analysis of urine. Scores (a) and loadings (b) plots

PLS-DA

This method is supervised, i.e. the class membership of the samples is included in the calculation. PLS-DA can be used to make models that discriminate between classes (diseased versus healthy).

Figure 4 shows PLS-DA scores of the three groups (log-scaled). Already the first component shows perfect separation between the three groups

Conclusion

Using PCA and PLS-DA analyses, discrimination of MMA and ALK urine samples from normal urine samples was possible by the different models produced.

Chemometric techniques can assist in the interpretation of complex urine NMR data and may be useful in the rapid screening of patients with IEM.

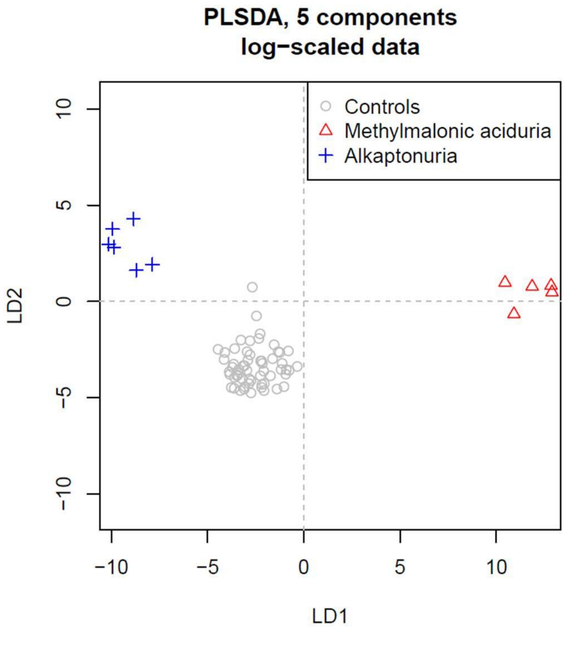

Figure 4: PLSDA scores