Urine-Based Newborn Screening Project applying High Resolution NMR-Spectroscopy

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INFAI GmbH and Bruker BioSpin GmbH are leading a project to establish NMR-based Screening of Newborns in Turkey. 10 Hospitals all over Turkey have joined the project and provide support collecting newborn urine samples and relevant metadata.

The project is fully supported by ethics committee vote. NMR on urine has been chosen as it enables:

- non-invasive sample collection
- straightforward sample preparation without the need to derivatize
- highest reproducibility in the multiple detection of the smallest variations in concentration of all relevant compounds found in urine
- high throughput under full automation
- access to a much larger number of compounds in comparison with plasma analysis
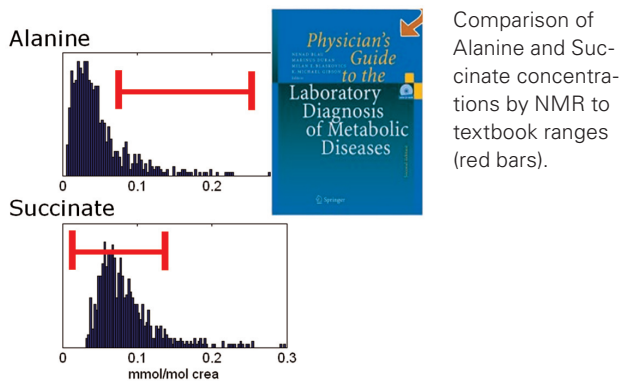- targeted and non-targeted screening in a single experiment

In the first part of the project, the task is to define a normal model for Turkish babies based on their urine NMR spectra. This model has to be extensively validated and then tested against further samples collected during the residual time of the project. Various statistical models will be developed

beyond the normal model, allowing the prediction of the overall status of the newborn in terms of growth, level of maturity, recognition of more frequent diseases and relation to parameters not directly visible by NMR, to mention a few.

In addition to the non-targeted approach using statistical methods, a substantial set of compounds will be quantified using the exact same experiment run for statistical evaluations. Compounds are safely identified using a rapid 2D experiment, implemented into the measurement sequence before quantification takes place. No internal standard is needed for quantification, with quantification accuracy not being dependent upon compound concentration. Based on modern digital NMR technology a dynamic range of compound concentrations of at least $2*10^5$ is possible within a single experiment.

Spectral databases of pure reference compounds and knowledge bases derived from thousands of urine samples support the project. More than 600 compounds occurring in urine are part of the reference compound spectral base, including compounds indicative for inborn errors of metabolism. The ability to quantify a large set of compounds in every sample enables the build up of valid concentration distributions, which is often different from the typical value sets available

Figure 1

Alanine

Succinate

Comparison of Alanine and Succinate concentrations by NMR to textbook ranges (red bars).

from textbooks (see Figure 1) and literature for inborn error analysis, where the number of samples is usually limited to small cohorts.

Figure 2

^{1}H NMR in urine

glutaric acid
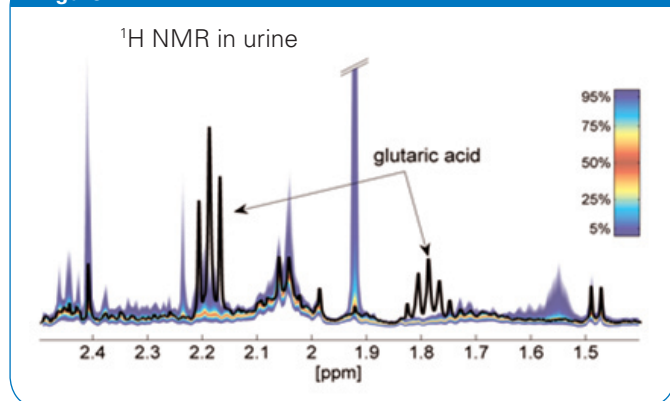
Non-targeted Screening

Non-targeted Screening enables the detection of all NMR visible deviations from normality, whether these are known or unknown deviations. Figure 2 shows the identification of deviations from normality. All NMR spectra forming the normal model are combined in a so-called quantile plot, shown as a color band over the NMR spectrum. For each point in the spectrum the intensity distribution can be visualized, where the color red shows the 50% average and blue indicates that only few samples in the model show such intensities. A new sample spectrum can be overlaid and investigated if all resonances fit into the envelope defined by the model. This testing can be done uni- or multivariate automatically.

The sample spectrum tested in Figure 2 represents a glutaric aciduria case and in the expansion of the overall NMR spectrum shown here, the signals of Glutaric Acid are clearly visible. The spectral section shown represents only about 15% of the overall spectrum. Since Glutaric Acid is part of the reference compound database, it can be identified automatically, however statistics would also reveal the existence of so far unknown deviations with the same certainty.

As can be seen, NMR has the potential to deliver statistical parameters and at the same time generate a set of quantitative parameters. The potential for NMR in the analysis of inborn errors is comprehensively described in the "Handbook of ^{1}H-NMR spectroscopy in inborn errors of metabolism" published by the group of R.A.Wevers in Nijmegen (SPS, ISBN-10: 3936145024), where around 80 different inborn errors detected by NMR are described.

This potential can be applied in a push button high through-put screening mode, once the normal model exists. Normal models will not be applicable on a worldwide basis, as there are differences in race, phenotype, nutrition and many more parameters. Therefore the applicability of a regional model has to be tested in surrounding regions, and eventually a different model has to be developed.

Contacts

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