Analytical Chemistry in Newborn Screening

Essay for the Humanity in Science Award

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Professors Gelb and Turecek have developed new methods for the effective and accurate screening of genetic diseases causing debilitating errors of metabolism in children. The new procedures have been tested in pilot projects in the U. S. and Europe and are being applied for large-scale diagnostics of newborns. Timely and accurate diagnosis is prerequisite for the application of modern therapeutic procedures to save and improve lives of affected children.

Diseases caused by inborn errors of metabolism represent a diverse group of several thousand known syndromes. Some affect specific organs, some are systemic, and their effects range from mild that appear in adolescence or adulthood, to severe that, if untreated, result in an early death of affected children. Inborn errors of metabolism are rare on an individual basis, but their combined prevalence can be as high as one in a few thousand newborns. In the past decade, a breakthrough progress has been made by U.S. pharmaceutical companies in developing sophisticated therapies, such as those based on enzyme replacement or erythropoietic stem cell transplantation, to treat metabolic diseases and save lives of affected children. The new therapies are expensive and carry inherent risks, which places extremely stringent criteria on diagnostic accuracy. Detection of inborn errors poses specific problems, as the disorder phenotype may not be obvious in the early stages of the disease to apply the suitable therapy. Therefore, most disorders are detected by chemical or bioanalytical methods using blood samples from newborns. The premier method of detection relies on enzyme activity analysis as a powerful chemical approach in which specific enzymes are targeted and the lack of their activity signals a metabolic error.

The principle of enzyme activity measurements is quite simple. A biological sample from the patient is incubated in vitro with a synthetic substrate which is recognized and chemically modified by the target enzyme. The product of the enzyme-catalyzed reaction is then quantitatively analyzed and used to calculate the enzyme activity which is compared to the mean activity measured for samples from many thousands of other individuals. If the activity is below a statistically determined cutoff value, the sample is flagged for follow up genotype analysis to determine the DNA mutation. The analytical procedure must have figures of merit showing good reproducibility, high specificity, and a very low rate of false positives. In addition, in order to be applied in a large scale format of newborn screening the enzyme activity measurements must be fast and inexpensive. We have developed new methods for newborn screening that combine high performance liquid chromatography or just simple flow injection with tandem mass spectrometry for multiplex detection of lysosomal storage disorders (LSD).

LSD are disorders affecting activities of ca. 50 hydrolytic enzymes that catalyze degradation of biopolymers in the lysosome. The affected newborns appear normal after birth and for the first few months of life, but their development and overall health decline as the metabolic products keep accumulating. Those affected with severe forms die in the first decade. The course of the disease, if untreated, extols substantial emotional hardship on parents. This is

further aggravated by the diagnostic "odyssey" the parents often undergo because of lack of pediatricians trained in recognizing these diseases and the general difficulty of assigning the patient phenotype to the disease.

Lysosomal enzymes retain latent activity in dried blood spots (DBS) and can be assayed after rehydration in a suitable buffer. Since DBS are used as a common biological sample in newborn screening, our efforts have been focused on developing the chemistry of substrates and internal standards, bioanalytical work up procedures, and mass spectrometric analysis. Particular emphasis has been placed on multiplexing all these steps so that multiple enzyme activities could be determined simultaneously in one or two parallel DBS incubations. Our efforts were focused on both high performance liquid chromatography and flow injection of samples to provide newborn screening centers with alternative technologies suitable for their workflow.

Our new generation substrates were designed to comprise three major parts: (i) a structure moiety that is recognized by the enzyme, (ii) a group allowing facile introduction of stable isotope label, and (iii) a functional group directing the ion fragmentation in the mass spectrometer into one dominant channel producing the reporter ion. At the same time, the compounds were designed such that the enzyme reaction products were readily separated from the incubation medium by a single step procedure, e.g., extraction or ultrafast liquid chromatography keeping the analysis time per injection below 2 minutes.

As of now, we have developed substrates and assay procedures for 15 lysosomal enzymes. Six of these assays have been included in a six-plex procedure that utilizes liquid chromatography-MS/MS in a pilot program of newborn screening in Illinois state. A pilot study of a triplex assay (GLA, GAA and IDUA) has been successfully carried out with >100,000 samples and a six-plex pilot study is underway in Washington state. A diagnostic newborn screening of GALC deficiency (Krabbe disease) that is based on our technology has been running in New York state and has so far included over 1,500,000 samples. In 2014, our technology has been licensed to Perkin-Elmer and is being further developed into a kit for FDA approval and general use by newborn screening centers worldwide.