Rapid and Effective Screening for Lysosomal Storage Disease: How Close Are We?

The introduction of tandem mass spectrometry (MS/MS)\(^1\) into newborn screening programs during the past 15 years has been revolutionary, and it has resulted in a marked expansion of the number of detectable metabolic disorders, from a handful to more than 30 (1). The majority of these are defects of amino acid and fatty acid transport and catabolism, and most are responsive to treatment. The success of this development has prompted much interest in further expansion of newborn screening programs, particularly for lysosomal storage disorders (LSDs) that cause significant morbidity and mortality. Until recently, these disorders received low priority (2) because of the lack of effective treatment and practicable screening methodologies. Enzyme replacement and bone marrow transplant therapies have become available for several LSDs, however, and other therapies are under development (3), giving new hope for affected individuals. Furthermore, the development of viable screening methods based on measurements of lysosomal enzyme activities in dried blood spots (DBS) has paved the way for newborn screening for these disorders (4).

Enzyme activities of LSDs in DBS have been measured successfully both spectrofluorometrically, using substrates that release the fluorophore 4-methylumbelliferone (5, 6), and by tandem mass spectrometry, using substrates that are, for some of these assays, more closely related to or homologous with the natural enzyme precursors (7). The MS/MS method has the advantage that it can detect multiple enzyme products simultaneously because each product has a different \(m/z\). In this issue of Clinical Chemistry, 2 papers (8, 9) address developments with MS/MS-based enzyme assays that bring the newborn screening option for LSDs a step closer to reality.

Zhang et al. (8) describe substantial methodological improvements to the MS/MS-based method developed by Li et al. (7) for multiplex assays of up to 5 lysosomal enzyme activities. This assay in principle requires dividing the extract from one 3-mm DBS punch into separate incubations for the enzymes that target Pompe, Fabry, Gaucher, and Nieman-Pick diseases. Each aliquot of DBS extract is exposed to a substrate targeted for a particular enzyme using optimized buffers. Internal standards, also included in the incubation mixture, are either isotope-labeled analogs or closely related homologs of the enzyme reaction product. The activity for the enzyme that is defective in Krabbe disease is assayed by directly incubating a second 3-mm DBS punch with a specific substrate cocktail. The products of all the enzyme reactions and their internal standards have different \(m/z\) values, enabling their simultaneous analysis by MS/MS after recombining the reaction products into a single 96-well plate before analysis. Before the final analysis, liquid-liquid extraction with ethyl acetate and solid-phase chromatography are required to remove salts and other reagents incompatible with the MS/MS assay. The most notable methodological development is that reagent cocktails containing premixed substrates and internal standards have been optimized for each assay and made commercially available. According to the authors, these and other improvements have resulted in higher assay precision and reproducibility, and hence at least a 2-fold greater discriminating power between affected newborns and normal controls compared with the original method. These conclusions are based on DBS results obtained from 149 adult controls, 100 newborn controls, and cohorts of 10–14 patients affected with each of the 5 targeted diseases.

The commercial availability of assay-specific reagents is an important step toward the deployment of MS/MS-based newborn screening for LSDs. Screening programs will now have the option to select any or all of the 5 LSDs targeted by the new reagents. In a proof-of-concept report, Dajnoki et al. (9) describe the application of MS/MS to determine the activity of acid \(\alpha\)-glucosidase (GAA, the enzyme deficient in Pompe disease) in more than 10 000 anonymized DBS from the newborn screening program in Austria. Results were compared with 29 patient samples, 14 of which were confirmed with infantile Pompe disease and the remainder with later-onset Pompe disease. Only 4 of the 10279 newborns (0.039%) would have been recalled for diagnostic testing based on their established lower limit of normal GAA activity in DBS of 2 \(\mu\)mol/h/L. The authors also tested intra- and interassay imprecision and the effect of environmental conditions and sampling point within the DBS, concluding that

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1 Nonstandard abbreviations: MS/MS, tandem mass spectrometry; LSD, lysosomal storage disorder; DBS, dried blood spots.
the assay was robust under typical conditions encountered in their newborn screening program. It is noteworthy that the MS/MS-based DBS assay does not discriminate infantile from later-onset Pompe disease, as reported for the fluorometric enzyme assay (6). Results from a pilot newborn screening program for Pompe disease in Taiwan that applied the fluorometric assay have recently been published (10). Of 132,538 newborns screened, 1093 (0.82%) were recalled for a repeat newborn screen, and 121 (0.09%) were recalled for additional diagnostic evaluation. Pompe disease was confirmed in 4 patients, and enzyme therapy was initiated in 3 of them based on clinical evaluation. Although the 2 assay methods have not been compared with the same specimens, the salient difference between the fluorometric and MS/MS-based assay for Pompe disease would appear to be a substantially lower expected recall rate for MS/MS because of increased discriminating power between affected and unaffected patients. However, the MS/MS method has yet to be prospectively used in a newborn screening program.

The reports discussed here provide compelling evidence that newborn screening for Pompe disease and other lysosomal storage diseases using MS/MS-based enzyme assays is practicable. The newborn screening quality control program administered by the Centers for Disease Control and Prevention (CDC) is developing quality assurance materials for these assays, thus providing screening programs with an invaluable tool to assess the effectiveness of their assays as they are introduced.

Newborn screening programs should be aware of several key issues before screening for LSDs by MS/MS. These issues should be debated or discussed at least at the program level, by the advisory committee, and perhaps also at the national level. The first is that the multiplex method refers only to the final analytical step, and requires several additional steps that are not familiar to current practice in newborn screening. This may present a challenge for newborn screening laboratories, especially the need to incubate several 96-well plates in parallel and then recombine enzyme reaction products and perform liquid–liquid extraction and solid-phase purification. Second, although the analyses can in principle be accomplished using the MS/MS systems already in place for the acylcarnitine and amino acid multiplex assay, these assays must be performed consecutively. In high-throughput laboratories that currently run in excess of 500 specimens per day, there is unlikely to be enough capacity on the existing instrumentation for the increased workload, and additional instruments and personnel will likely be required to deploy these assays. Third, the results from LSD assays will not be available for at least 24 h after the specimens are prepared because of the incubation period required by the enzyme reaction, and this may delay the reporting of newborn screening results. Another cogent issue is the fact that some of patients targeted by LSD newborn screening may not benefit from early detection. The Taiwan study (10) was performed as a research program requiring informed consent, thus enabling at least some of the ethical issues raised by newborn screening for LSDs (11, 12) to be addressed. These include (a) the detection of disorders with later-onset forms for which it is not clear at what point therapy should begin, (b) the detection of disorders in which affected organs (such as the brain in Gaucher disease) are not targeted by current therapies, or (c) the detection of disorders in which some patients may not respond to therapy or develop resistance due to an immune response. In Fabry disease, enzyme assay will fail to detect about 30% of the female heterozygotes who will develop symptoms in later life (13). In a mandated setting, it must be recognized that these and other ethical aspects are not adequately addressed.

Despite the challenges inherent in newborn screening for LSDs, the advent of the improved reagents and MS/MS screening methods described here will result in their deployment in at least some programs. As more data are gathered regarding the outcomes of patients detected by these methods, we will be in a better position to assess the benefits and risks to affected patients.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: David S. Millington, TMS Biosciences Honoraria: None declared.
Research Funding: David S. Millington, Genzyme Corporation.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played a direct role in the preparation of the manuscript and final approval of the manuscript.

— David S. Millington
Pediatrics, Medical Genetics Division
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DOI: 10.1373/clinchem.2008.112110