Newborn Screening for Lysosomal Storage Disorders

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Introduction

With the advent of enzyme replacement therapy (ERT) and bone marrow transplantation (BMT) for selected lysosomal disorders (LSDs), are there disorders that should be considered for newborn screening?

Enzyme replacement therapy for Gaucher disease[1] and Fabry disease[2] has been demonstrated to be effective in alleviating the major clinical symptoms and anatomic alterations leading to morbidity and mortality. Although there is no convincing evidence that ERT can alter the neurologic components in Gaucher disease types II and III, it can certainly improve the somatic alterations caused by the disease. This is especially true for Gaucher disease type I, where there is reduction in liver and spleen volume and prevention of bony degeneration once ERT has been initiated. Similarly for Fabry disease, there is good evidence for resolution of peripheral pain, improvement of cardiac function, and removal of the lipid storage in the skin and kidney.[2,3] Although Gaucher and Fabry diseases typically do not present in early childhood with irreversible changes, an argument can be made that their early detection would allow for appropriate timing of ERT.

Other disorders for which ERT is currently available or being evaluated are mucopolysaccharidosis (MPS)-I (Hurler syndrome), MPS-II (Hunter syndrome), MPS-VI (Maroteaux-Lamy syndrome), and glycogenosis, type II (Pompe disease). Early evidence would suggest that patients with MPS-I benefit from ERT by improvement in the somatic features of the disorder. There is some evidence that early intervention for some patients with Pompe disease improves muscle strength and allows for improved developmental landmarks.[4]

Another therapeutic modality, which may alter the natural history of Krabbe disease, metachromatic leukodystrophy (MLD) and Niemann-Pick B, is BMT. For these disorders BMT prior to the onset of neurologic symptoms has been reported to dramatically alter the slowly progressive neurologic involvement.[5] Because of the observations from ERT and BMT, there have been initial efforts to devise technologies for their early recognition that would allow for presymptomatic treatment (**Table 1**).

Table 1. Potential candidates for newborn screening of LSDs.

Disorder	Effective Tx	Meets Criteria for NB Screening
Gaucher	ERT	possibly
Fabry	ERT	possibly
MPS-I	ERT	yes
Pompe	ERT	yes
Krabbe	BMT	yes
Metachromatic leukodystrophy	BMT	yes
Niemann-Pick B	BMT	possibly
MPS-VI	ERT	possibly

ERT=enzyme replacement therapy; BMT=bone marrow transplantation; MPS=mucopolysaccharidosis; NB=newborn; Tx=treatment

Screening Newborns for Lysosomal Storage Disorders

Interest in screening newborns for LSDs has been prevalent for the last decade.[6] The interest was stimulated by the results of clinical studies on the effectiveness of ERT or BMT in modifying the natural history of the disorders. A leader in this approach has been the LSD unit in North Adelaide, Australia, under the direction of Dr. John Hopwood.

Protein Analytes: The Adelaide program has focused on identifying analytes that are increased in individuals affected with these disorders. They have identified 2 proteins that are associated with the lysosomal membrane and have developed techniques for quantitating the proteins in blood. These proteins are called lysosome-associated membrane protein-1 (LAMP-1) and lysosome-associated membrane protein-2 (LAMP-2). The method involves a sensitive fluorescence immunoassay that can be performed on extracts obtained from blood spots. They have obtained quantitative measurements from individuals affected with LSDs and have compared the results with those of normal newborns.[7,8] Their published data indicate that for LAMP-1, if

Table 2. Detection of LSDs by increased concentration of LAMP-1 or LAMP-2 in plasma.

Disorder	% > 95th Percentile of Normal Newborns LAMP-1	LAMP-2
Galactosialidosis	100	100
I-cell	100	100
MPS-I	100	100
MPS-II & III	100	100
Sandoff	100	0
Tay-Sachs	100	0
Gaucher	92	92
Fabry	5	0
Niemann-Pick A/B	33	20
Krabbe	17	8
Pompe	25	0
MLD	19	16

Adapted from Meikle et al., 1997; Hua et al., 1998.

MLD=metachromatic leukodystrophy; MPS=mucopolysaccharidosis; LAMP=lysosome-associated membrane protein

they use a cutoff that is above the 95th percentile for the normal population, they are able to achieve a sensitivity of 72% for patients with known LSDs. This included 320 affected individuals representing 25 different LSDs. A selection of disorders for which LAMP-1 was greater than the 95th percentile as compared to controls, is listed in **Table 2**.

A second protein, LAMP-2, was separately evaluated using an immunoquantitation assay.[9] A wider quantitative range was observed in patients affected with LSDs as compared with controls from normal infants. LAMP-2, however, turned out to be less sensitive than LAMP-1 in discriminating between affected and unaffected individuals, and was elevated beyond the 95th percentile in only 66% of patients with LSDs.

The measurement of LAMP-1 and -2, however, shows promise as an initial screen for newborns unsuspected of having an LSD. The Adelaide program continues to work on technology that would select samples above the 95–99 percentile and follow the procedure with tandem mass spectroscopy that can identify specific abnormal lipids or oligosaccharides present in the newborn blood spot to more accurately identify affected patients. This approach depends on a 2-tiered system of evaluation, similar to what is used by some programs that are screening for cystic fibrosis.

Glucose Tetrasaccharide: Another analyte used for detection of an LSD has been the evaluation of a glucose tetrasaccharide as a putative biomarker for the diagnosis of Pompe disease. It was noted by the group at Duke University that patients with Pompe disease excreted in urine a tetraglucoseoligimer (Glc-Glc-Glc-Glc, or Glc_4) in increased quantities.[9] A high pressure liquid chromatography (HPLC) method was developed for identification and quantitation of this Glc_4 molecule. It was confirmed that all patients affected with Pompe disease, irrespective of age, excreted an increased quantity of Glc_4 compared with normal controls.

The method was improved by adaptation to electrospray ionization tandem mass spectroscopy.[10] The technique was validated on urine samples from affected patients and extended to the documentation that the Glc_4 was also elevated in plasma. There was good agreement between the urine samples, with an r^2=0.94, but less agreement between the HPLC method and the tandem mass spectrometry method for plasma samples, r^2=0.53. This raised a question regarding the immediate application of the method to newborn blood spots for the detection of Pompe disease.

In evaluating this method in Adelaide, the Australian program confirmed that an increase in Glc_4 levels could be detected in urine from infantile onset Pompe disease, when measured by electrospray ionization tandem mass spectroscopy, and that Glc_4 levels could be monitored in plasma samples.[11] Although the method was sensitive enough to determine Glc_4 levels in a single 3-mm blood spot, it could not confirm the difference between individuals affected with Pompe disease and normal controls.[12] Thus, this particular method appears limited in its application for the detection of Pompe disease from blood spots routinely submitted for newborn screening (**Table 3**).

Enzyme Analysis: A more direct method for evaluating newborns at risk for an LSD would be to measure the gene product responsible for each of the disorders. This involves the assay of lysosomal enzymes from blood samples submitted on newborn screening cards. Chamoles et al have documented that in retrospective sampling of newborn blood spots from individuals later diagnosed with LSDs, the enzyme deficiency can be detected using conventional artificial substrates, the 4-methylum-belliferyl substrates that are traditionally used in fluorescent enzyme assays of white cells or fibroblasts.[13-16] These disorders included Gaucher, Fabry, Hurler, Niemann-Pick, and the GM-2 gangliosidoses.

Table 3. Data on the assay of Glc_4 in urine (A), plasma (B) and blood spots (C) in normal persons and those with Pompe.

A

Age (yr)	Affected with Pompe (mmole/molCr)	Normal Persons (mmole/molCr)
< 1	34.6	8.9±8.2
2–5	60	~ 3.6±3.8
11–31	29.6	~ 0.9±1.0
40–61	5.5	~ 0.4±0.3

B

Age (yr)	Affected with Pompe (μmole/mL)	Normal Persons (μmole/mL)
< 1	3.0	0.3±0.39

C

Age (yr)	Affected with Pompe (μmole/mL)	Normal Persons (μmole/mL)
NA	5.3 (2.7–10)	7.1 (3–30)

Adapted from An et al., 2000; Young et al., 2000; Rozaklis et al., 2002.

Several publications[13-16] clearly demonstrate that lysosomal enzymes are reasonably stable in dried blood samples and can be used to measure selected enzymes. This approach offers an alternative to evaluating analytes that may be elevated on the basis of an abnormality in lysosomal integrity secondary to the accumulation of a complex molecule.

Pompe disease represents a special case within the family of storage diseases. The α-glucosidase responsible for Pompe disease is typically measured in muscle tissue or cultured skin fibroblasts. There exists a second enzyme, a neutral α-glucosidase (or renal glucosidase) that is primarily expressed in renal tissue, but also present in white cells and interferes with the accurate quantitation of the acid α-glucosidase in lysosomes. The optimal pH of the neutral enzyme overlaps with the optimal pH of the acid glucosidase, and neutrophils have a higher proportion of this neutral enzyme than do lymphocytes. The neutral acid α-glucosidase is significantly less abundant in muscle or cultured fibroblasts, making these tissues a better biologic source for the evaluation of Pompe disease. In an attempt to overcome this problem, Umapathysivam et al[17] developed a monoclonal antibody that was specific for the acid form of the enzyme and showed that in the majority of patients affected with Pompe disease, the mature form of the acid α-glucosidase was either absent or present in very low concentrations. To directly measure the enzyme using the aid of the antibody, the antibody was coated onto plastic wells and used to trap the enzyme from blood. This proved to be effective. They demonstrated that enzyme activity could not be detected in blood samples obtained from infantile, juvenile, or the majority of adult-onset patients.[11] There was no overlap between values obtained from affected patients compared with those of normal controls or obligate heterozygotes for Pompe disease.

An expansion of the idea of using enzyme activity from dried blood spots as a means of assaying for LSD has been developed by the group at the University of Washington. They have used tandem mass spectroscopy as a common platform for measuring enzyme reactions.[18] Artificial substrates are synthesized for each of the lysosomal enzymes of interest, along with appropriate internal standards. Each of the substrates and products has a different mass (m/z) that can be uniquely identified by the tandem mass spectrometer. The method consists of taking a single 5-mm punch, using a common extraction buffer of sodium phosphate solution at pH 7.0 to solubilize the bound enzymes into a liquid phase. After extraction, 10 μL of the extracted enzyme solution can be used for each assay. Following an overnight incubation with the appropriate substrate, the internal standards are added, and the multiple reaction mixtures can be combined for further processing and injection into a mass spectrometer. Because of the mass differences of the products being assayed, a single injection is used to quantitate multiple enzymes simultaneously (multiplexing).

In a paper published in 2004, Li and authors report the results of 5 lysosomal enzymes that can be measured simultaneously.[19] These are the enzymes responsible for Fabry, Gaucher, Krabbe, Niemann-Pick A/B, and Pompe diseases. The assays were documented to be linear with regards to protein concentration of each assay, and linear over the time period of the incubations. An affected patient has approximately 4% residual activity, compared with a non-affected person. A summary of the results from this method is shown in **Table 4**, page 14.

Table 4. Lysosomal enzyme activity from a single 5-mm blood spot. The maximum observed activity is less than the minimum activity observed in random samples obtained from healthy newborns.[19]

Disorder (enzyme)	(n)	Enzyme Activity (μmole/hr/L) Affected Persons (maximum activity)	Normal Infants Median (range)
Gaucher (ABG)	6	0.18	3.31 (0.89–9.6)
Fabry (GLA)	5	0.17	2.23 (0.77–5.65)
Krabbe (GALC)	9	0.20	1.01 (0.42–1.53)
Niemann-Pick A/B (ASM)	5	0.32	4.41 (0.92–11.3)
Pompe (GAA)	5	0.33	3.12 (0.93–7.33)

Adapted from Li et al., 2004.

ABG=acid ß-D-glucosidase; ASM=acid sphingomyelinase; GAA=acid α-glucosidase; GALC=galactosylceramide ß-galactosidase; GLA=α-galactosidase A

The authors were able to obtain accurate quantitation of the α-glucosidase for the detection of Pompe disease because of the innovative use of an acarbose, which inhibits the neutral α-glucosidase retaining the activity of the acid α-glucosidase. The α-glucosidase assay performed on blood spots clearly distinguishes between infants affected with Pompe disease and healthy ones.

Conclusions

With the emerging technologies of ERT, BMT, and eventually gene therapy, selected LSDs can now be considered potential candidates for newborn screening programs. Enough preliminary evidence exists that the direct analysis of analytes or enzymes for selected LSDs may be an approach for their early detection. The LAMP-1 protein continues to hold promise for disorders involved with mucopolysaccharide metabolism and potentially the GM-2 gangliosidoses. The direct measurement of enzyme activity from newborn blood spots appears to have greater versatility and, with the development of the use of tandem mass spectrometry as a platform for measuring enzyme activity directly from blood spots, may offer an opportunity for easy introduction into existing newborn screening programs.

Screening newborns for LSDs will not be without its tribulations. As we have learned from previous screening programs, the introduction of new technology or new disorders to screening programs will unveil unanticipated surprises. We know pseudo-deficiency alleles exist that are likely to be detected by measuring enzymes directly, and extensive data are not yet available on the potential overlap of enzyme activity between some heterozygotes and affected infants. It is also possible that new disorders will become "unmasked" through newborn screening programs, similar to what happened with the detection of biopterin defects when screening for phenylketonuria. Because of these cautionary notes, any initiation of newborn screening programs for LSDs will need to be carefully evaluated and will require careful clinical confirmation.

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