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LSDS WITH NEUROLOGIC INVOLVEMENT

Newborn screening for neuropathic lysosomal storage disorders

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Abstract Interest in newborn screening (NBS) for lysosomal storage disorders (LSDs) has increased significantly due to newly developed enzyme replacement therapy (ERT), the need for early diagnosis, and advances in technical developments. Since the central nervous system cannot be treated by ERT, neuronopathic LSDs are generally not the primary target of NBS. An exception is Krabbe disease, in which hematopoietic stem cell transplantation before the onset of symptoms has benefits. However, NBS for LSD relies on measuring enzyme activities, so the most severely affected individuals (usually patients with neuronopathic subtypes) will be detected together with patients with less severe disease. In the near future, NBS is likely to be developed for diseases such as Gaucher, Niemann-Pick A/B, and certain mucopolysaccharidoses. The ability to predict phenotypes (neuronopathic or not) by enzyme activity and genotyping will therefore be critical for adequate patient management. This article reviews the status of LSD screening and issues concerning detection of neuronopathic LSDs by screening.

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Abbreviations

BBB	blood-brain barrier
CHT	congenital hypothyroidism
DBS	dried blood spot
ERT	enzyme replacement therapy
GAA	acid alpha-glucosidase
GALC	galactocerebrosidase
HSCT	hematopoietic stem cell transplantation
IOPD	infantile-onset Pompe disease
LSD	lysosomal storage disorders
MGA	maltase-glucoamylase
MPS	mucopolysaccharidosis
MS/MS	tandem mass spectrometry
NBS	newborn screening
PKU	phenylketonuria

Introduction

Newborn screening (NBS) is a very successful and specific medical practice in health care (Kaye et al. 2006) first used for diseases that lead to insidious brain damage, such as phenylketonuria (PKU) and congenital hypothyroidism (CHT). In the case of PKU, the brains of newborns and infants are highly susceptible to the toxic metabolites. Brain damage in PKU is irreversible, thus, severe neurologic sequelae, especially mental retardation can result if treatment is delayed until after clinical presentation (Cederbaum 2002). A simple phenylalanine method for detecting PKU in large populations of newborn infants was first introduced by Dr. Guthrie in 1963 (Guthrie and Susi 1963). The bacteria inhibition assay, in which bacteria are grown on growth medium with the addition of an analog, was later applied to

other diseases involving amino acid metabolism (homocystinuria and maple syrup urine disease) and galactosemia. Immunoassay techniques have also been used by newborn screening labs to detect CHT and congenital adrenal hyperplasia (Dussault et al. 1975; Pang et al. 1977). This panel of NBS assays, plus screening for biotinidase deficiency, has been used in many countries for more than 30 years (Kaye et al. 2006). Advances in NBS have occurred over the past decade with the introduction of tandem mass spectrometric (MS/MS) analysis of acylcarnitines and amino acids to detect various disorders affecting fatty acid, amino acid, and organic acid metabolism (Chace et al. 1993; Turecek et al. 2007). There has also been substantial progress in NBS for cystic fibrosis. Screening usually employs measurement of immunoreactive trypsinogen (IRT) as the first screen, followed by a variety of strategies, including repeating the IRT assay or cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis, as well as recall for a sweat test (Massie and Clements 2005). Early treatment of cystic fibrosis results in improved pulmonary outcomes in adolescence (McKay et al. 2005).

Methods of NBS for LSDs

There have been a number of important technical developments that allow NBS for lysosomal storage disorders (LSDs). For routine NBS, one 3-mm paper disc punched from dry blood spots (DBS) is used for each assay. However, one 3-mm paper disc contains only 3.2 μl of blood, whereas routine laboratory diagnosis of LSDs employs several milliliters of blood. Chamoles et al. (2001a) discovered that lysosomal enzymes are stable in enzyme assays in which reaction rates are linear during an extended incubation period of 20 h (Chamoles et al. 2001a, b). Enzyme stability over an extended incubation time allowed development of assays using small sample volumes, such as that extracted from a DBS. Therefore, LSDs can now be diagnosed using DBS, and NBS for LSDs can be integrated into a standard screening menu.

The discovery of inhibitors in assaying acid α-glucosidase (GAA) activity is a crucial step toward NBS for Pompe disease (Li et al. 2004; Zhang et al. 2006). In the assay, either glycogen or an artificial substrate (4-methylumbelliferyl-β-D-glucopyranoside) is used with an acidic buffer (pH 3.8). However, maltase-glucoamylase (MGA) digests the substrates at the same pH. Therefore, it is necessary to isolate lymphocytes from peripheral blood to decrease the interference from MGA. The availability of an inhibitor of MGA makes it possible to assay GAA in whole blood or in DBS with no need for lymphocyte isolation. Two inhibitors, maltose (Chamoles et al. 2004) and acarbose (Li et al. 2004), were developed separately; however, acarbose is used more widely (Zhang et al. 2006).

Also, newly designed substrates have resulted in the development of assays for LSDs. Gelb and colleagues developed a series of substrates for assaying lysosomal enzymes, and products of different enzyme assays are quantified using MS/MS (Li et al. 2004). Multiplexing of several lysosomal enzyme assays is possible because the products have different masses. After separate incubations for each enzyme, the products are mixed together, taken through a clean-up procedure, reconstituted in matrix, and analyzed simultaneously by MS/MS (Zhang et al. 2008). Yet another multiplex assay applying immune quantification using microbead suspension array technology has also been shown to detect 11 different LSDs in a single analysis (Meikle et al. 2006).

Despite the advances, challenges remain with these methods. Enzyme assays can be disturbed by environmental conditions during shipment and storage. Fluorescent substrates are convenient but sometimes have high background luminescence, and whereas MS/MS is highly accurate, it requires expensive equipment and extensive sample preparation. The immunoassay is multiplex but carries the risk of missing patients with positive cross-reacting immunologic material. Molecular genetic analysis (as applied to Krabbe screening in New York) (Duffner et al. 2009a) can be used to confirm a diagnosis, but identification of new mutations can make interpretation difficult. The cost of most of these methods are comparable with traditional NBS tests, except for molecular genetic analysis, which is much more expensive.

Mechanisms to incorporate LSD detection into NBS programs

Interest in NBS for LSDs has increased significantly due to the newly developed enzyme replacement therapy (ERT) (Brady 2006). Previously, palliative care and bone marrow transplantation were used to manage and treat patients with LSDs. The first ERT was designed for Gaucher disease. The deficient enzyme was initially purified from placental tissue (Furbish et al. 1977) and later produced by cultured cells. After the success of ERT for Gaucher disease, a similar therapeutic model was developed over the ensuing years to treat Fabry disease (Schiffmann et al. 2000; Eng et al. 2001), mucopolysaccharidosis (MPS) type I (Kakkis et al. 2001), MPS type II (Muenzer et al. 2002), MPS type VI (Harmatz et al. 2004), and Pompe disease (Kikuchi et al. 1998; Van den Hout et al. 2001). Therefore, the methodology used to establish a diagnosis for these diseases and begin patients on treatment has become important for physicians and laboratories.

Initial experiences in treating patients with LSDs soon revealed that much of the damage that occurs with the later stages of disease could not be reversed by ERT. In Gaucher disease, many visceral manifestations are reversible; however, such is not the case with other LSDs, including MPS and Pompe disease. Irreversible bone complications often occur in MPS and Gaucher disease (Weisstein et al. 2004; Vom Dahl et al. 2006). In addition, cardiac valve damage in MPS and renal damage in Fabry disease are both irreversible (Breunig et al. 2006; Yano et al. 2009), as is muscle damage in Pompe disease (Thurberg et al. 2006). LSDs are caused by deficiencies of lysosomal hydrolytic enzymes, and intralysosomal accumulation of undigested materials occurs very early in life or before birth (Vogler et al. 2005; Kooper et al. 2006), but symptoms appear only after the development of significant tissue or organ dysfunction. Therefore, treatment for LSDs should start as early as possible, and NBS would be useful for presymptomatic diagnosis of LSDs.

Three LSDs are currently screened for by various NBS programs around the world. Pompe disease screening was selected for Taiwanese screening programs because of its higher prevalence than in other populations, 27.4% of all cases in a multinational survey for infantile-onset Pompe disease (IOPD) (Kishnani et al. 2006), and unsatisfactory outcomes in treating IOPD patients with ERT (Chien et al. 2009). Krabbe disease was selected by the state of New York because hematopoietic stem cell transplantation (HSCT) during the newborn stage improves outcomes (Escolar et al. 2005). Fabry disease was chosen by some programs because early treatment is obviously beneficial, although there is no urgency to treat patients in infancy (Spada et al. 2006; Hwu et al. 2009; Lin et al. 2009). Screening for infantile-type Pompe and Krabbe disease may fit with the classic Wilson and Jungner criteria for NBS, except that the long-term outcome and cost–benefit ratio are not known at this time. However, these screening tests also detect newborns affected by late-onset variants of the diseases who will only need treatment decades later, and this raises a concern, as shown in a recent U.S. Department of Health and Human Services Health Resources and Services Administration (HRSA) review of Pompe screening (http://www.hrsa.gov/heritabledisorderscommittee/reports/).

Current practice in LSD screening

Pompe disease is the first LSD in which NBS has effectively improved outcomes (Kemper et al. 2007). Babies affected by Pompe disease are usually identified because of muscle weakness and respiratory distress at 3–4 months of age, and it may take another 1–2 months to establish the diagnosis (Kishnani et al. 2006). However, some patients treated at an early age are not responsive to ERT in their skeletal muscles, and histological findings in the muscles explain the finding (Thurberg et al. 2006). When the disease process becomes advanced, the distended lysosomes in the muscles rupture into the cytoplasm, and autophagy is abnormally activated (Thurberg et al. 2006; Raben et al. 2007). Both processes destroy the cellular organelles, and cells can no longer be rescued by ERT. Cross-reactive immunologic material (CRIM)-negative status is predictive of overall survival, invasive ventilator-free survival, and poorer clinical outcomes in infants with Pompe disease treated with recombinant human GAA (rhGAA) (Kishnani et al. 2010).

Screening for IOPD relies on measurement of GAA activity in DBS in the presence of acarbose as an inhibitor. Newborns with low GAA activity are requested to return to the hospital, and a routine chest X-ray can establish the diagnosis for classic IOPD. Before screening, a portion of IOPD patients became respirator dependent after ERT (Kishnani et al. 2007; Nicolino et al. 2009). Since the initiation of screening, five CRIM-positive Taiwanese IOPD patients have been treated starting before 1 month of age for 14–33 months, and all can walk and none are respirator dependent (Chien et al. 2008; Chen et al. 2009; Chien et al. 2009). There are several reports concerning NBS for Fabry disease (Spada et al. 2006; Hwu et al. 2009). Newborns with Fabry disease are identified, and undiagnosed family members can be treated. However, NBS discovered a large number of individuals carrying later-onset Fabry mutations, and it is difficult to predict their phenotypes (Hwu et al. 2009). Screening for MPS is also under evaluation (Wang et al. 2007; Blanchard et al. 2008).

As mentioned earlier, the need for early treatment has prompted the development of NBS for LSDs. Since the central nervous system cannot be treated by ERT, neuronopathic LSDs are generally not the primary target of NBS. However, NBS for Krabbe disease, a primarily neuronopathic disease, has been performed since 2006 (Duffner et al. 2009a). Krabbe disease, or globoid-cell leukodystrophy, is due to a deficiency of the lysosomal enzyme galactocerebrosidase (GALC). The accumulation of galactolipids results in demyelination in the brain. In the infantile form, symptoms usually appear before 6 months of age and death occurs before 2 years of age. HSCT has been performed for 11 asymptomatic newborns and 14 symptomatic infants with infantile Krabbe disease (Escolar et al. 2005). Newborns show higher rates of survival with progressive central myelination and continued gains in developmental skills and cognitive function, whereas children who undergo transplantation after symptom onset have minimal neurologic improvement. The results are encouraging, although a few early-treated patients had

mild-to-severe delays in gross motor function. In August 2006, the state of New York began screening all newborns for Krabbe disease using GALC activity as a first-tier test and DNA mutation analysis as a second-tier test. They used a multidisciplinary approach to evaluate infants who had positive results on NBS and suggested HSCT to treat infantile Krabbe disease (Duffner et al. 2009a). In the first year of screening, 40 babies were referred for diagnostic testing. Molecular analysis of the GALC gene predicted two babies with infantile Krabbe disease and two with late-onset Krabbe disease. The former two babies received HSCT, and the latter two were followed expectantly (Caggana et al. 2008). Long-term outcome data, however, are emerging that suggest residual progressive neurological involvement exists in patients who have been treated (Duffner et al. 2009b; Steiner 2009).

Screening for other neuronopathic LSDs

MPS I is traditionally divided into the neuronopathic Hurler type (MPS IH) and the milder nonneuronopathic Scheie type (MPS IS); there will also be patients with severities in between (MPS IHS). ERT is the recommended treatment for MPS IS (also for MPS IHS) (Wraith et al. 2004; Clarke et al. 2009), whereas HSCT is the treatment of choice for MPS IH. NBS for MPS I is similar to that for Krabbe disease, although decision making is less urgent. HSCT for MPS IH needs to be done before 2 years of age or before mental developmental indices fall below 70 (Peters et al. 1996; Staba et al. 2004). If NBS is performed for MPS I, genotyping should be done for any newborns with a low level of iduronidase activity. There are two common mutations in MPS IH: p.Q70X and p.W402X (Scott et al. 1995; Li et al. 2002). All nonsense and frameshift mutations should lead to a complete deficiency of iduronidase activity from those alleles. However, MPS I is a recessive disease; therefore, the significance of both alleles needs to be clear before a phenotype can be assigned. Newborns with a predicted MPS IH phenotype are treated with HSCT, and ERT before HSCT may be also helpful (Tolar et al. 2008). For newborns predicted to have MPS IS (or MPS IHS) phenotype, ERT is administered either immediately after diagnosis or after the appearance of initial symptoms. However, what will happen if the prediction is wrong? HSCT should be an effective treatment for either MPS IS or MPS IHS, but patients are put at unnecessary risk of HSCT if it is not indicated. However, if MPS IH is mistaken for MPS IS or IHS, then only ERT will have been administered. It is possible that developmental delay will be detected at a later age and treatment with HSCT will be reconsidered. However, the phenotype of patients with MPS IH may be modified by ERT at a very young age, and it is possible that the best opportunity for HSCT will be missed.

Both Niemann-Pick A and Gaucher II, the severe subtypes of these disorders with neurological involvement, have no treatment. Screening for any disease is likely to detect affected individuals with the most severe type to milder types, or even variants. NBS for LSDs relies on measuring enzyme activities. Generally, an individual will have symptoms only when one specific enzyme activity drops to 10–20% of the normal mean value or lower. However, for any measurement, accuracy decreases when the detected levels are low; thus, it is usually not reliable to distinguish phenotypes with enzyme activity. The most severely affected individuals (the neuronopathic types) will certainly be identified by NBS. If NBS is done for Niemann-Pick and Gaucher disease, genotyping and phenotype prediction will be necessary. If the prediction is Niemann-Pick A or Gaucher II, no treatment should be given. If those patients are treated by ERT (an ERT is under development for Niemann-Pick disease) (Schuchman 2007), ERT should be discontinued when neurodegeneration is detected. However, the decision to not treat a newborn who appears normal will be difficult; similarly, it will be difficult to withdraw treatment after the establishment of attachments between parents and their babies.

The situation is a little different for MPS II. In patients with the severe form, somatic manifestations (dysostosis multiplex) and neurodegeneration progress relentlessly and patients usually die in the second decade of life (Martin et al. 2008). Other patients have milder disease manifestations, sometimes normal mentality, and survive into adulthood. Currently, all MPS II patients in the United States and Europe, as well as in some other countries, are treated regardless of severity. However, when patients with the severe type are treated by ERT from a young age, discontinuing treatment is a difficult decision.

Special problems that neuronopathic LSDs pose to NBS

One specific problem that LSDs pose to NBS is slow disease progression in a portion of affected patients. Onset of clinical symptoms can be obscure, and uncertainty exists as to when treatment should be initiated. Neuronopathic LSDs pose further challenges to NBS because CNS symptoms may not be treatable. An untreatable disease may not fit the criteria for NBS, but screening does provide the opportunity to offer genetic counseling before the family decides to have another child. The most difficult problem occurs when NBS detects both the neuronopathic and nonneuronopathic subtypes of a disease. Achieving a balance between benefit by detecting the treatable subtype and harm from finding the untreatable subtype is difficult.

Outlook and conclusion

It has become clear that systemic administration of recombinant lysosomal enzymes, even starting shortly after LSD detection by NBS, does not effectively treat neurological manifestations of LSDs because the blood-brain barrier (BBB) is complete when a baby is born, and large molecules such as lysosomal enzymes cannot enter the brain (Saunders et al. 1999). Therefore, if newborns with predicted neuronopathic LSDs are treated, their prognosis may be poor. However, potential new therapies to treat CNS manifestations are under development, especially for treating patients shortly after detection by NBS. Data have shown that recombinant lysosomal enzymes can be infused directly into the brain through the cerebrospinal fluid space or cerebral ventricles (Dickson et al. 2007; Munoz-Rojas et al. 2008). Additionally, there are small molecular substrate inhibitors or pharmacologic chaperones that can pass through the BBB (Pastores 2006; Beck 2007; Chien et al. 2007; Platt and Jeyakumar 2008). Research on gene and stem cell therapy is also ample (Beck 2007).

In conclusion, neuronopathic LSDs pose a challenge, but not an obstacle, to NBS. NBS for LSDs has just begun, and there is a great deal to be learned from each disease, including false-positives, false-negatives, and cost–benefits ratios. Therefore, NBS for LSDs must be performed with great care, and there needs to be a close collaboration among disciplines concerning diagnosis, treatment, reimbursement, and ethical issues in relation to LSDs.

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