Newborn Screening of Lysosomal Storage Disorders
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BACKGROUND: Newborn screening is a state-based public health program established as a means for the early detection and treatment of certain medical conditions to minimize developmental disability and mortality. The program was initiated more than 40 years ago to detect and prevent phenylketonuria. Recent technological advances have expanded the scope of newborn screening to include more than 30 inborn errors of metabolism. Consideration is now being given to inclusion of screening for lysosomal storage disorders (LSDs).

CONTENT: Some lysosomal storage disorders (LSDs) express early in infancy or childhood and are treatable. Initiation of treatment in presymptomatic patients or in symptomatic patients before important symptoms are present may improve the long-term outcome. Therefore, early diagnosis is critical. Based on the availability of therapy and development of a screening method, 6 of the more than 40 known LSDs are candidates for newborn screening in the US: Gaucher disease, Pompe disease, Fabry disease, Niemann-Pick disease, mucopolysaccharidosis I, and Krabbe disease. This report reviews the history of newborn screening, the technology that has allowed for expanded screening during the last decade, LSDs and their treatment, and the evolving methods that might allow additional expansion of newborn screening to include certain LSDs.

SUMMARY: Recent and evolving technological advances may be implemented for newborn screening for LSDs. This screening will identify presymptomatic newborns, allowing for early treatment and prevention or limitation of morbidity otherwise associated with these inherited rare diseases.

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Newborn screening for inborn errors of metabolism is a state-based public health initiative established to identify disorders that can affect the long-term health of newborns. Early recognition of these disorders allows for immediate treatment, before the onset of irreversible impairment, and, in many cases, prevention of catastrophic health outcomes, including death.

Phenylketonuria (PKU)2 was the first disorder screened in newborns. A relationship between PKU and mental retardation was demonstrated in the 1930s, and Bickel and coworkers later showed that restricted intake of phenylalanine attenuated neurologic deficits in PKU patients (1). In 1959, Guthrie developed a simple bacterial inhibition assay (BIA) to monitor the blood phenylalanine concentrations in affected children. This test was refined to require just a few drops of blood obtained from a heel stick and blotted on a filter paper, now known as the Guthrie card or Guthrie specimen (2).

The era of routine newborn screening began in 1962 when Massachusetts piloted state-wide use of the Guthrie assay for PKU screening. The incidence of PKU at that time was thought to be about 1 in 20 000; however, early results from the Massachusetts program, involving approximately 53 000 infants, indicated that the frequency was much higher, approximately 1 in 6000 (3). These impressive results led to a Massachusetts state law requiring newborn testing for PKU, and national attention focused on the justification for neonatal screening. In 1968, the World Health Organization commissioned a report from Wilson and Jungner (4) to define criteria for inclusion in screening programs. Although these criteria were initially intended for large population-screening programs (such as for malignant disease), they have been broadly adapted for newborn screening. The criteria require that a disease be relatively frequent, that there is an effective treatment available to prevent long-term morbidity, that there is a reliable test suitable for high-throughput screening, and that screening and treatment are less costly to society than the care of untreated patients.

With the success of PKU screening, newborn testing for other treatable disorders was introduced. Guthrie subsequently pioneered BIAs to detect maple syrup
urine disease and homocystinuria; a bacterial assay for galactosemia was also introduced (5, 6). The Guthrie specimen method has also been used to develop alternative techniques for identifying diseases in infancy. An RIA to detect thyroxine concentrations was developed to screen for presymptomatic congenital hypothyroidism, and immunoassays are also used to detect congenital adrenal hyperplasia and cystic fibrosis (3, 7). Electrophoresis techniques enable screening for detection of hemoglobinopathies, and enzyme assays have been established for biotinidase deficiency (8, 9).

The development of tandem mass spectrometry (MS/MS) led to a major shift in the approach to newborn screening. Initial MS/MS studies focused on analysis of acylcarnitines in human fluids to detect organic acidemias and medium-chain acyl–coenzyme A dehydrogenase deficiency, a fatty acid oxidation disorder (10–12). However, optimization of the system has facilitated the simultaneous screening for various disorders of organic acid degradation, fatty acid oxidation, the urea cycle, and amino acids. This technology also offers a lower false-positive rate than traditional BIAs (13). In all, more than 30 inborn errors of metabolism can be rapidly detected by MS/MS.

The efficacy of newborn screening using MS/MS has been examined. Pilot programs in Australia and New England suggest a higher incidence compared with clinical diagnosis (13–15). In Australia, Wilcken and colleagues evaluated the rates of diagnosis of 31 metabolic disorders among 362 000 newborns screened by MS/MS vs the rates of clinical diagnosis in 6 preceding 4-year birth cohorts (15), and found more confirmed cases of inborn errors of metabolism than were diagnosed clinically. Detection was particularly more frequent for disorders of fatty acid oxidation and several organic acids.

The benefit of improved early diagnosis during the newborn period was also investigated in New England (14). Preliminary data suggest that in New England, patients in whom these disorders are identified by MS/MS have an improved clinical outcome with fewer hospitalizations and no neurological complications compared with those patients in whom the disorders were diagnosed clinically (13). In addition, assessment of parental stress demonstrated that parents in the clinical group reported significantly higher levels of stress compared with the MS/MS-screened group (13). Although false-positive results are low, such results may increase stress levels in parents, even when the results of follow-up evaluations are normal (16). Results of a more recent review of the literature and current communication practices suggest that much of this anxiety can be reduced by effective education and communication with the parents at the time of the initial evaluation after an abnormal newborn screen result (17).

Overall, these results suggest that the expansion of newborn screening using MS/MS has led to an increase in the early detection of certain rare metabolic disorders and improved clinical outcomes (14, 15).

Newborn screening is not mandated at the federal level. Instead, each state determines which disorders will be screened and which screening tool will be used; these decisions seem to be based largely on the economic cost of screening and treating identified disorders, because these variables have an impact on local economies.

Success with the expansion of newborn screening prompted an interest in identifying other diseases that could be detected and treated early in infancy/childhood before the onset of irreversible clinical impairment and possible death. However, because of the potential increased monetary and time costs, concern for the lack of trained specialists in the community at large, and increased burden on the medical infrastructure as well as increased screening difficulty, the American College of Medical Genetics has attempted to define a standardized newborn-screening panel by developing a core panel of 5 fatty-acid disorders, 9 organic-acid disorders, and 5 amino-acid disorders in addition to PKU (18). The use of this screening panel has now been adopted by all US states. In this recommendation, the American College of Medical Genetics noted that although the lysosomal storage disorders (LSDs) did meet the broad criteria for inclusion in the screening panel, at that time there was no available test suitable for high-throughput newborn screening. Currently, all US states also screen for congenital hypothyroidism, congenital adrenal hyperplasia, and galactosemia. All states uniformly screen the population for the hemoglobinopathies, sickle cell disease, sickle C disease, and S-β-thalassemia (19).

Adding new diseases to a newborn-screening panel requires much consideration, including cost-benefit analysis and confirmation of the availability of an established screening method that can detect the disorder(s) in newborns with acceptable sensitivity, specificity, and positive predictive value. Development of a screening tool requires identification of target analytes and determination of appropriate cutoffs for analyte concentrations. Reagents, such as substrates and internal standards, must be tested and be available commercially. QC procedures, including determination of the stability of the targeted analyte, and assay standardization, must be developed. To address these issues, the Advisory Committee on Heritable Disorders in Infants and Children (20) was formed in 2003 to advise the Secretary of Health and Human Services on the appropriate application of universal newborn-screening tests, technologies, policies, guidelines, and standards for effectively reducing morbidity and mor-
tality in newborns and children who have, or are at risk for, heritable disorders. Multidisciplinary groups, including medical and technical experts and patient advocates, can submit candidate disorders for inclusion in standardized recommendations. Committee recommendations are made after review of an external evidence-based opinion.

LYSOSOMAL STORAGE DISORDERS

The lysosome is an intracellular organelle that contains many acid hydrolases that degrade macromolecules (proteins, complex carbohydrates, nucleic acids, lipids, sulfates, and phosphates). The end products are either reused by the cell or eliminated from the body. The absence or loss of function of 1 enzyme along the pathway results in the accumulation of an intermediate metabolic product.

The LSDs represent a heterogeneous group of more than 40 genetic disorders, many resulting from a mutation in a gene encoding an intralysosomal enzyme (21, 22). However, other essential proteins involved in lysosomal metabolism and export of storage products have also been shown to underlie some LSDs. These include enzyme coactivators, membrane proteins, transporter proteins, and enzymes that process other lysosomal proteins. Regardless of etiology, all LSDs share the common pathogenesis of accumulated metabolic substrate in the lysosome. The progressive accumulation of these products leads to cellular distortion and dysfunction (21, 23). Widespread cellular destruction eventually causes tissue and organ dysfunction.

Almost all LSDs are inherited as autosomal recessive traits, except for the X-linked Fabry and Hunter (mucopolysaccharidosis type II [MPS II]) diseases and Danon disease (23, 24). Individually, the incidence of these inherited diseases is rare, ranging between 1 in 50 000 and 1 in 1:4. However, collectively, LSDs are far more common, approximately 1 in 7000 to 8000 births (25). Certain populations have a higher incidence of a particular LSD compared with the general population. Ashkenazi Jewish descendents are 50 to 60 times more likely to inherit mutations causing Gaucher, Tay-Sachs, Niemann-Pick type A, and mucolipidosis IV (23, 27, 28). Salla disease and aspartylglucosaminuria are more prevalent among those of Finnish descent, and type 3 Gaucher disease is most frequent among persons with Swedish ancestry (26, 29, 30).

The LSDs are primarily classified according to the nature of the stored material, but some are grouped by the protein deficiency (25). Broad categories include MPS, GM, gangliosidoses, neutral glycosphingolipidoses, glycoproteinoses, mucolipidoses, leukodystrophies, glycogen storage diseases, disorders of neutral lipids, and disorders of protein transport or trafficking (22, 23).

Diagnosing an LSD can prove difficult because of the variability in clinical expression (22, 31). The severity and extent of disease are often inconsistent, even within families, and patients with identical genotypes can have significantly different phenotypes (23). Disease phenotype may differ in age of onset, complexity of the storage product, rate of substrate accumulation, and tissue distribution (23). These observations of disease heterogeneity are related, in part, to the complexity of enzyme kinetics. Each tissue type has a set threshold of enzymatic activity, below which pathological changes manifest (22). Threshold activities are dependent on substrate flux, cellular turnover, and metabolic demand. Complicating the kinetic model additionally is the residual activity of the defective protein; small variations can greatly influence the rate of substrate accumulation and disease development (31). Genetic background, environmental factors, and lack of the final metabolic product of the affected pathway can also influence pathological expression (22, 23, 31).

Many LSDs have central nervous system (CNS) involvement, with or without somatic features. Tissues that normally have a high flux of the accumulating substrate are most affected. Clinical features suggestive of an LSD include developmental delay, progressive regression after a period of normal development, ataxia, seizures, weakness, and dementia (21). An LSD diagnosis should also be considered in the presence of course facial features, bone abnormalities, corneal clouding, unexplained joint stiffness, unexplained bone pain or burning neuropathic pain, psychiatric problems, nonimmune fetal hydrops, or organomegaly (21, 23).

A variety of diagnostic tests are available for analyzing blood, urine, or skin fibroblasts. Clinical signs and symptoms direct the choice of test(s). For some disorders, measuring the activity of the suspected defective enzyme is the most efficient way to determine the diagnosis (23). Given the variable signs and symptoms that might be present, a battery of tests may be necessary to arrive at a definitive diagnosis. Diagnostic tools used include brain MRI, DNA analysis, measurement of sialic acid content, electroretinogram, and ultrastructural analysis of biopsied material. Enzymatic and ultrastructural analyses of amniocytes or chorionic villus cells are used for the prenatal diagnosis of many LSDs.

TREATMENT FOR LSDs

Once diagnosed, therapeutic and supportive treatments must be considered and implemented. Ideally, therapy should be administered before the onset of irreversible pathology. At present, the only mechanism...
for identifying presymptomatic patients is to test siblings of patients already diagnosed with the disorder.

Several LSD treatment modalities addressing the underlying biochemical aberration are either currently available or under investigation. Presently, 2 enzyme delivery systems, hematopoietic stem cell transplantation (HSCT) and intravenous recombinant enzyme replacement therapy, are available.

HEMATOPOIETIC STEM CELL TRANSPLANTATION
For 2 decades, HSCT has shown efficacy in presymptomatic or mildly affected patients with certain LSDs (21). Donor bone marrow, obtained from HLA-identical siblings or unrelated noncarrier matches, provides healthy stem cells that produce the missing or defective enzyme. There is evidence of disease stabilization with this procedure (32). Early clinical trials using HSCT suggested penetration of the blood-brain barrier (32). Experiments in animal models revealed that donor cells are a source for therapeutic activities of the defective enzyme, and that over time donor macrophages replace microglial cells in the brain (32, 33). In humans, HSCT has been used with varying rates of success in patients with MPS I (Hurler), MPS II (Hunter), MPS VI (Maroteaux-Lamy), Gaucher, Wolman, metachromatic leukodystrophy, and Krabbe disease (32). Each LSD responds differently to HSCT, and timing of transplantation relative to symptom onset seems to be critical for some disorders (32). For Krabbe disease and metachromatic leukodystrophy, transplantation before clinical signs attenuates CNS pathology compared with no treatment or transplantation after clinical signs are evident (34). Complications after HSCT are frequent and have limited the usefulness of this treatment option. Most complications are due to graft-vs-host disease, toxicity of the conditioning regimen, and the high rate of graft failure (23).

ENZYME REPLACEMENT THERAPY
Enzyme replacement therapy supplements the defective enzyme with active enzyme, administered intravenously at regular intervals. Recombinant DNA techniques have allowed high-throughput production of some lysosomal enzymes. Transformed via the mannose-6 receptor pathway (or in the case of Gaucher disease, by macrophage mannose receptors), the exogenous enzymes are internalized by somatic cells, in which they degrade accumulated substrate, thereby diminishing the deleterious effects of the disease. Reombinant proteins cannot cross the blood-brain barrier, and enzyme replacement therapy has little or no effect on CNS manifestations.

The US Food and Drug Administration has cleared enzyme-replacement therapy for Gaucher disease, Fabry disease, MPS I, MPS II, MPS VI, and Pompe disease. Clinical trials are currently assessing the effect of intrathecal enzyme replacement to treat the CNS symptoms in MPS I and II. Clinical trials for Niemann-Pick B [acid sphingomyelinsase deficiency (ASM)] have recently been initiated, along with trials of alternate recombinant forms of glucocerebrosidase (for Gaucher disease). Preclinical work is underway in MPS III. A small-molecule oral-substrate reduction therapy has been recently become available for Gaucher disease, and clinical trials are underway for a different substrate inhibitor for Gaucher disease. Chaperone therapy using a small molecule to stabilize a misfolded enzyme, allowing for normal targeting to the lysosome, is in clinical trial for Fabry, Gaucher, and Pompe diseases. In preclinical animal studies, these small molecules have been shown to cross the blood-brain barrier, and may ultimately offer some opportunity for treatment of CNS involvement in some LSDs.

NEWBORN-SCREENING TECHNOLOGIES FOR LSDs
For several of the LSDs for which an effective therapy is available or in development, age of onset is early in infancy or childhood. Because of the wide spectrum of symptoms, diagnosis can often be challenging, and a delay between onset of symptoms and diagnosis is common. Misdiagnosis or delayed diagnosis can have devastating consequences, because efficacy of many available LSD treatments relies on early diagnosis. This need has prompted the pursuit of a reliable technology to screen for LSDs in presymptomatic infants and children. Several technologies are being developed and perfected for application to a newborn screening program that uses the blood filter paper specimen.

Because of the heterogeneity of mutations, mutation analysis is not suited for high-throughput screening. Likewise, there is a lack of both general and specific protein and metabolic markers in the blood for this group of disorders, limiting the use of biochemical testing (35). Screening of muscle biopsy samples or cultured fibroblasts is too invasive for large-scale application. However, several viable approaches to LSD newborn screening have been reported in the literature.

ENZYMATIC ASSAYS
Determination of α-L-iduronidase deficiency for diagnosing MPS-I is performed routinely on homogenates of leukocytes or cultured fibroblasts. Chamoles and colleagues adapted standard enzymology methods to assay α-L-iduronidase activity from dried blood spotted on filter paper (36). Samples were obtained from healthy adult volunteers, random newborns, known patients with severe or mild MPS-I, and obligate carriers of MPS-I. A 3-mm-diameter standard paper punch of each blood sample was added to elution liquid.
containing 4-methylumbelliferyl-\(\alpha\)-L-iduronide substrate, and the fluorescence of the enzyme product, 4-methylumbelliferone, was measured; results were compared with the fluorescence from a 4-methylumbelliferone calibrator \((36)\). Storage at 4 °C or \(-20\) °C for 21 days did not significantly alter enzymatic activity. Only minimal enzymatic activity was lost after 20 days of storage at room temperature, allowing adequate time for transportation of samples to a specialized testing facility.

Methods for detection of several other LSDs, including Pompe, Fabry, Sandhoff, Gaucher, Niemann-Pick, and Tay-Sachs diseases by use of this revised enzymatic assay for dried blood spots on filter paper have been reported \((37–40)\). The main drawback with this approach is that each assay uses the same indicator of enzyme activity (e.g., 4-methylumbelliferone), so multiplexing is prohibited. For example, to test for Fabry disease, 4-methylumbelliferyl-\(\alpha\)-D-galactopyranoside is used as the substrate \((37)\). Because the fluorescence of the resulting enzyme product is the same as in the MPS-I experiments, these 2 reactions cannot be run simultaneously and would require separate setups. The assays for Gaucher, Niemann-Pick, Pompe, Tay-Sachs, and Sandhoff diseases also yield 4-methylumbelliferone as the fluorescing enzymatic product \((38–40)\).

Another group has taken an alternative approach to develop an enzymatic diagnostic test for Pompe disease \((41)\). Using antibodies directed against acid alpha glucosidase (GAA), Umphilysivam and coworkers were able to “capture” endogenous GAA from dried blood spots, which they then assayed for activity. Microtiter plates coated with anti-GAA antibodies were incubated with dried blood from adult and newborn controls, Pompe patients, or obligate heterozygotes. After incubation, the activity of captured GAA was determined by using 4-methylumbelliferyl-\(\alpha\)-D-glucosidase as the substrate. After additional incubation, fluorescence of 4-methylumbelliferone was measured. Enzyme activity of 16 of the 17 Pompe patients (1 infantile onset, 3 juvenile onset, and 12 adult onset) was undetectable, and 1 patient had only 0.04 \(\mu\)mol \(\cdot\) L\(^{-1}\) \(\cdot\) h\(^{-1}\), well below the lower limit of the control ranges. Interestingly, 2 Pompe patients had GAA protein concentrations that were within the reference interval, indicating that enzymatic activity is a better screening marker than enzyme protein concentration.

Although this method is reportedly simple, inexpensive, and noninvasive, there are some drawbacks that would impede its being adopted as a universal/widespread newborn-screening tool. Similar to the Chamoles methods, it perpetuates the single-assay, single-disease model of newborn screening. Unique antibodies against individual deficient LSD enzymes would have to be developed. Hypothetically, microtiter plates could be coated with several different primary antibodies to capture different endogenous enzymes. However, if all the substrates produce the same fluorescent enzyme product (4-methylumbelliferone), then multiplexing is not possible.

In Taiwan \((42)\), where Pompe disease is more frequent than in the world-wide population, a pilot program was carried out in which newborns from 1 geographic area \((n = 206 088)\) were screened for GAA activity; infants born in areas where screening was not routinely carried out, and in whom a diagnosis of Pompe disease was made clinically after the onset of symptoms, were used as controls. The screening identified 6 affected infants, 5 with the early onset disease and 1 with later onset disease. The 5 severely affected infants were started on enzyme-replacement therapy by 30 days of age. Although clinically asymptomatic at diagnosis, all 5 infants had evidence of cardiac dysfunction and muscle glycogen deposition. In the unscreened infants, the clinical diagnosis of Pompe disease was made later, on average at 4 months of age. The infant with later-onset disease (no evidence of cardiac involvement in the newborn period) was started on enzyme-replacement therapy at 14 months of age because of progressive motor weakness. Initiation of earlier treatment of infants in the newborn-screening group resulted in normal cardiac function, growth and acquisition of age-appropriate milestones.

Also in Taiwan, Hwu et al. \((43)\) have reported results from a pilot program for newborn screening for Fabry disease using the fluorometric enzyme assay in 171 977 consecutive infants. These investigators found an incidence of Fabry disease of approximately 1 in 1250 males, of which 86% carried mutations associated with a later-onset phenotype. Of 81 689 females screened, only 2 heterozygotes were detected.

Spada et al. \((44)\) used the fluorometric enzyme method to screen 37 104 male newborns in the Piedmont region of northern Italy, and performed follow-up mutation analysis and in vitro expression of the mutants. The incidence in this population was 1 in 3100, predominantly with mutations consistent with later onset disease (such as cardiac variant Fabry disease). These studies suggest that Fabry disease may be underdiagnosed.

**MULTIPLEXED IMMUNE QUANTIFICATION**

Meikle and colleagues \((45)\) developed a multiplexed immune-quantification assay for 11 different LDSs. This method used a combination of monoclonal and polyclonal antibodies raised against each specific deficient enzyme and saposin C. Immune quantification was performed by using a microbead suspension array with detection of fluorescence to determine the amount of each protein calibrated against a standard curve. CV analysis was satisfactory. Control samples
were obtained from healthy Australian adults and newborns. Ranges outside of the reference intervals were determined by assaying blood-spot samples from affected adults and newborns. Diagnostic sensitivity and specificity were acceptable for all of the LSDs except Pompe disease (90% sensitivity and >90% specificity) and Gaucher disease (50% sensitivity and 98% specificity). One MPS II patients had iduronate-2-sulfatase concentrations close to the adult reference interval and 1 Fabry patient had α-galactosidase A concentrations higher than the newborn control reference interval; however, better discrimination for these patients, as well as Gaucher and Pompe patients, could be obtained by using other protein markers, such as a ratio of iduronate-2-sulfatase to sphingomyelinase. It was also suggested that other second-tier assays such as immune-capture or specific immunofluorescent assays using artificial substrate may further differentiate samples close to the cutoff.

Drawbacks to this method include the expense of buying or developing the specific antibodies that are not commercially available. Also, the use of ratios, although often used to improve the diagnostic sensitivity of newborn screening for other inborn errors of metabolism detected by MS/MS, has not been well established for use in the LSDs.

FUNCTIONAL DETECTION OF LSDs: MS/MS OF ENZYMATIC PRODUCTS

Gelb and colleagues designed a method to directly analyze the activity of endogenous lysosomal enzymes by using electrospray ionization (ESI)-MS/MS (46-49). Initially developed using human cell lysates, the method was adapted for dried blood spots samples. The method was first tested to detect galactocerebroside β-galactosidase (GALC) activity, which is deficient in Krabbe disease (48).

Several substrates for GALC were considered. The substrate β-Gal-C\(_{9}\)-Cer was chosen because of its 5-fold higher GALC activity. In addition, this substrate breaks down to a nonnatural product, C\(_{9}\)-Cer, which can be derived only from activity of GALC on the synthetic substrate, avoiding interference from natural ceramides present in the biological sample. An internal standard, C\(_{10}\)-Cer, was also constructed. Both C\(_{9}\)-Cer and C\(_{10}\)-Cer yield the same fragment ion (m/z 264.3) after collision-induced dissociation, and therefore, the product and the internal standard can be distinguished in the Q1 quadrupole. Quantification of C\(_{9}\)-Cer by using a known amount of internal standard, indicates the GALC activity in the sample.

Dried blood samples were obtained from 16 healthy individuals and 5 with Krabbe disease. A sample was added to an assay mixture containing the substrate and internal standard, and the mixture was incubated. Detergents and solvents were removed before ESI-MS/MS. The samples were introduced to a Sciex API-III Plus tandem–mass spectrometer. The enzymatic reaction product was quantified by determination of the ion abundance ratio of product (C\(_{9}\)-Cer) to internal standard (C\(_{10}\)-Cer) for each sample. Imprecision analysis showed that the GALC assay was highly precise. In addition, GALC enzymes derived from dried blood spots seemed to be sufficiently stable to allow for sample transportation.

A pilot program for Krabbe-disease screening (49), which used the MS/MS method as a single assay, was started in New York state in August 2006. Cutoffs for enzyme activity were developed from a study of 139 074 anonymous newborns, 56 known carriers, and 16 known Krabbe patients. An algorithm was used in which samples with enzyme activity below the cutoff were further analyzed by second-tier testing with mutation analysis. Only infants with 1 or more gene variation were referred for confirmatory testing; 100% of positive controls were detected.

As of June 2008, 555 000 newborns had been screened and 10 infants identified as being at high-to-moderate risk of developing early infantile-onset disease. Two infants with mutations previously seen in infantile Krabbe disease were referred for HSCT by 28 days of age. One succumbed to early complications, and the other was developmentally delayed but had not developed the classical features of the disease. Two others did not undergo HSCT, and remained clinically free of disease at the time of the report, at 16 and 8 months of age (50).

This technique was expanded for use in simultaneous assay of multiple lysosomal enzymes derived from the same dried blood spot sample (47). Substrates and internal standards were developed for Gaucher, Fabry, Pompe, and Niemann-Pick diseases, and more recently, for MPS-I. Substrates for acid β-D-glucosidase (for Gaucher disease) and acid sphingomyelinase (for Niemann-Pick) yielded nonnatural ceramides that could be differentiated from their corresponding internal standards. The internal standards of the GAA (for Pompe disease) and α-galactosidase A (for Fabry disease) assays were chemically identical but isotopically distinguishable from the reaction products.

Initially, no single assay buffer could support all enzymatic reactions in 1 well; each enzyme performed optimally under different conditions, depending on pH, detergent concentration, and buffer composition. Therefore, all enzymatic reactions were performed separately, and then purified by solvent-to-solvent and solid-phase extractions to remove buffer components. The GAA assay solution also contained acarbose to inhibit an interfering acid α-glucosidase (maltase glucoamylase) that might be present in blood samples.
Samples were obtained from healthy adult and infant noncarriers, affected patients, and heterozygous carriers. The detection rate for affected patients was 100%. All affected patients demonstrated reduced maximal enzyme activities that were at least an order of magnitude lower than the maximal activities of healthy controls, permitting unambiguous differentiation of affected and healthy individuals. Activities of acid β-D-glucosidase, acid sphingomyelinase, GAA, and α-galactosidase A were also distinguished from the activities of heterozygote carriers; no GALC heterozygotes were enrolled in the study.

The sample-to-sample and within-run reproducibilities of this approach were determined. The CVs were satisfactory. The ESI-MS/MS analysis of lysosomal enzyme activities offers certain advantages for incorporation into a newborn-screening program. The method is capable of screening the activity of up to 6 lysosomal enzymes on a single platform. Several challenges remain to streamline this technique for newborn screening. Although the methods are amenable to automation, some of the steps have never been performed in newborn-screening laboratories, and their use would require training of personnel and purchase of new reagents (51). To remove buffer components, the original 5 assays used a silica gel–based method; however, the α-L-iduronidase–reaction product bound too tightly to this gel, and the use of a reversed-phase C18-silica filter plate had to be adopted instead (52). The requirement of at least 2 types of solid-phase extraction might further complicate the methods. Importantly, it is not known if the lysosomal enzyme samples are compatible with samples for amino acid and acylcarnitine analysis, and it is possible that components of the lysosomal enzyme samples could suppress the ionization of amino acid and acylcarnitine derivatives (47). It is also likely that separate MS/MS systems would be needed for this analysis, which would increase the associated cost (51).

A pilot program for Pompe disease was carried out in Austria (53) to evaluate the MS/MS platform. Approximately 10,000 anonymous newborn samples were tested, along with samples from 229 healthy adults and 29 known Pompe patients (14 infant onset, 15 adult onset). Although no new positive samples were detected, all of the affected patients were detected and the method was considered suitable for high-throughput newborn screening. A pilot program with similar aims has recently been implemented for Fabry disease in Washington state.

To facilitate the introduction of this method for newborn screening, the Centers for Disease Control and Prevention are developing QC procedures and will distribute substrates and internal standards at no charge to interested newborn-screening programs worldwide.

The expansion of newborn screening has been a major public health achievement, and has markedly improved the morbidity and mortality for many inborn errors of metabolism, although for some, the treatment is imperfect. The introduction of newborn screening for the LSDs presents new challenges, and much debate continues on how to provide this sophisticated new technology, as well as follow-up and therapeutic intervention. These new assays must be validated in large numbers of newborns to increase our understanding of the true diagnostic sensitivity and specificity. False-negative results are difficult to assess, given the often-delayed diagnosis in many affected patients. Rapid, readily available confirmatory testing is necessary. For situations in which the initial enzyme result falls within an at-risk range, several pilot programs have developed algorithms, including molecular analysis. This may be a suitable model for other LSDs. Mutation analysis may, in many cases, enable health care providers to distinguish the acute early onset LSDs that require early intervention from the slower, later onset diseases.

For disorders such as early onset Pompe, Krabbe, and MPS-I, early presymptomatic detection is critical for survival or the limitation of severe disability. In the absence of a family history, presymptomatic detection of an LSD can be achieved only through a newborn-screening program. Newborn screening using the MS/MS method has now been mandated to begin by 2012 in 4 US states (Illinois, Missouri, Mississippi, and New Mexico), and will likely become part of other state and regional programs throughout the industrialized world within the foreseeable future. Efficacy of the currently available therapies, and detection of disorders in newborns of diseases with later onset, often in adulthood, may also generate much debate. To address some of these issues, the American College of Medical Genetics is developing a Newborn Screening Translational Research Network that will enroll large international cohorts in standardized protocols to determine long-term outcomes and optimal therapeutic interventions.

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