Newborn Screening for Lysosomal Storage Disorders in Illinois: The Initial 15-Month Experience

Barbara K. Burton, MD[1], Joel Charrow, MD[1], George E. Hoganson, MD[2], Darrell Waggoner, MD[3], Brad Tinkle, MD[4], Stephen R. Braddock, MD[5], Michael Schneider, MD, PhD[6], Dorothy K. Grange, MD[7], Claudia Nash, MS[8], Heather Shryock[8], Rebecca Barnett, BA[8], Rong Shao, PhD[9], Khaja Basheeruddin, PhD[9], and George Dizikes, PhD[9,10]

Objectives To assess the outcomes of newborn screening for 5 lysosomal storage disorders (LSDs) in the first cohort of infants tested in the state of Illinois.

Study design Tandem mass spectrometry was used to assay for the 5 LSD-associated enzymes in dried blood spot specimens obtained from 219 973 newborn samples sent to the Newborn Screening Laboratory of the Illinois Department of Public Health in Chicago.

Results The total number of cases with a positive diagnosis and the incidence for each disorder were as follows: Fabry disease, n = 26 (1 in 8454, including the p.A143T variant); Pompe disease, n = 10 (1 in 21 979); Gaucher disease, n = 5 (1 in 43 959); mucopolysaccharidosis (MPS) type 1, n = 1 (1 in 219 793); and Niemann-Pick disease type A/B, n = 2 (1 in 109 897). Twenty-two infants had a positive screen for 1 of the 5 disorders but could not be classified as either affected or unaffected after follow-up testing, including genotyping. Pseudodeficiencies for alpha-L-iduronidase and alpha-glucosidase were detected more often than true deficiencies.

Conclusions The incidences of Fabry disease and Pompe disease were significantly higher than published estimates, although most cases detected were predicted to be late onset. The incidences of Gaucher disease, MPS I, and Niemann-Pick disease were comparable with previously published estimates. A total of 16 infants could not be positively identified as either affected or unaffected. To validate the true risks and benefits of newborn screening for LSD, long term follow-up in these infants and those detected with later-onset disorders will be essential. *(J Pediatr 2017;190:130-5).*

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During the past decade, there has been increasing interest in newborn screening for lysosomal storage disorders (LSDs). New treatments have become available for many of these conditions, and new laboratory methods for detecting them in dried blood spots (DBSs) have been developed. Newborn screening for a number of LSDs has been ongoing in Taiwan since 2005,[1] and pilot programs have been initiated in a number of other countries.[2,3] In 2005, New York became the first US state to screen newborns for an LSD when newborn screening for Krabbe disease was initiated.[4] Since then, legislation has been passed in several states mandating newborn screening for various LSDs, and in 2013, Missouri became the first state to screen all newborns for multiple LSDs, including Pompe disease, Gaucher disease, Fabry disease, and mucopolysaccharidosis (MPS) type I.[5] In 2014, the Advisory Committee on Heritable Disorders in Newborns and Children voted to add Pompe disease to the Recommended Uniform Screening Panel, and in 2015 followed this with a vote to add MPS I. These recommendations were accepted by the Secretary of Health and Human Services, making Pompe disease and MPS I the first LSDs for which uniform screening of all newborns is recommended.[6] Since then, many states have either initiated pilot

CK	Creatine kinase	IOPD	Infantile-onset Pompe disease
DBS	Dried blood spot	LOPD	Late-onset Pompe disease
GAA	Alpha-glucosidase	LSD	Lysosomal storage disorder
GAG	Glycosaminoglycan	MPS	Mucopolysaccharidosis
GBA	Beta-glucosidase	MS/MS	Tandem mass spectrometry
IDUA	Alpha-L-iduronidase	VUS	Variant of unknown significance

From the [1]Department of Pediatrics, Division of Genetics, Birth Defects & Metabolism, Ann & Robert H. Lurie Children's Hospital, Feinberg School of Medicine of Northwestern University; [2]Department of Pediatrics, Division of Genetics, University of Illinois College of Medicine; [3]Department of Pediatric, Department of Human Genetics, University of Chicago, Chicago, IL; [4]Department of Genetics, Division of Clinical Genetics, Advocate Children's Hospital, Park Ridge, IL; [5]Department of Pediatrics, Division of Medical Genetics, Saint Louis University School of Medicine, St. Louis, MO; [6]Department of Pediatrics, Division of Medical Genetics, Carle Clinic, Champaign, IL; [7]Department of Pediatrics, Division of Genetics and Genomic Medicine, Washington University School of Medicine, St. Louis, MO; [8]Genetics Program, Illinois Department of Public Health, Springfield; [9]Newborn Screening Laboratory, Illinois Department of Public Health, Chicago, IL; and [10]Division of Laboratory Services, Tennessee Department of Health, Nashville, TN

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screening programs or are currently developing plans to do so. In 2014, Illinois initiated a pilot screening program for 5 LSDs, including Pompe, MPS I, Gaucher, Fabry, and Niemann-Pick diseases. This was followed by statewide screening in June 2015. This report presents the results obtained in 219 793 infants screened between November 1, 2014, and August 31, 2016.

Methods

Before the initiation of the newborn screening pilot, the LSD subcommittee of the Genetic and Metabolic Diseases Advisory Committee of the Illinois Department of Public Health began holding regular meetings by teleconference to develop follow-up protocols for the evaluation of infants with positive screening tests for the various disorders. In addition, information sheets for primary care physicians and disease-specific forms for obtaining short- and long-term follow-up data on screen-positive infants were created. Once screening began, the subcommittee continued to hold monthly meetings to review progress, discuss positive cases, and identify issues to be resolved.

All laboratory testing was conducted at the Newborn Screening Laboratory of the Illinois Department of Public Health in Chicago. A modification of the published method of Spacil et al was used to measure multiple enzyme activities in DBS specimens and for detection and quantification of enzyme products by tandem mass spectrometry (MS/MS).[7] Substrates were purchased from PerkinElmer (Waltham, Massachusetts). The multiplex assay includes buffer, substrates, and internal standards for the 5 LSD-associated enzymes (Gaucher, Pompe, Fabry, MPS I, and Niemann-Pick diseases), along with inhibitors of non-LSD enzymes competing for the substrates associated with Pompe, Fabry, and MPS I diseases. The enzyme reaction is incubated with the DBS for 17 hours at 37°C. Whereas other MS/MS-based assays for LSDs use solid-phase and/or liquid-phase extraction of the enzyme products to remove detergents, salts, and excess substrates before injection into the mass spectrometer, these manipulations were eliminated by substituting in-line ultraperformance liquid chromatography (UPLC). After quenching the overnight incubation with acetonitrile, an equal volume of water is added, and a portion of the supernatant is removed and applied directly to the UPLC column (without drying and reconstitution) by a 96-well, refrigerated autosampler. During a 2.5-minute injection-to-injection cycle, detergent and salt are diverted to waste, while the product–internal standard pairs are injected together into the mass spectrometer (eliminating possible artifacts due to ion suppression). Finally, the column is regenerated for the next injection. The current method allows analysis of at least five 96-well plates (450 specimens plus controls) in each 24-hour period by a single MS/MS instrument (ACQUITY TQD; Waters, Milford, Massachusetts) with excellent dynamic range and separation of positive from negative specimens.

Assay validation was performed, and screening cutoffs were established using DBSs from presumably normal infants and DBSs obtained from patients with each of the target disorders. Pilot screening was initiated for infants born in a limited number of hospitals in November 2014. During the pilot phase, DBSs were analyzed both in the Illinois Department of Public Health laboratory and in the PerkinElmer commercial laboratory. Results from the PerkinElmer laboratory were reported to the referring hospitals and physicians. On June 1, 2015, screening became statewide. The cutoffs used are shown in **Table I**. A 2-tiered cutoff system is in use. When a result is obtained in the borderline range, a repeat DBS is requested. If the result is again borderline or positive, then referral to a designated consultant is recommended. If the initial (or any) specimen tests in the positive range, then immediate referral to the consultant is recommended without sending a repeat DBS.

Table I. Cutoffs for defining positive results

Enzymes	Disorder	Borderline*	Positive*
GLA	Fabry	>13 and ≤18	≤13
GAA	Pompe	>18 and ≤22	≤18
ASM	Niemann-Pick	>11 and ≤15	≤11
GBA	Gaucher	>17 and ≤20	≤17
IDUA†	MPS I	>28 and ≤31	≤28

GLA, alpha-galactosidase A; *ASM*, acid sphingomyelinase.
*Results calculated as % of daily median.
†On August 1, 2016, cutoffs for MPS I were changed to borderline, >14 and ≤18, and positive, ≤14, owing to the addition of the inhibitor lactone to the assay mixture.

Positive test results were communicated to physicians and referring hospitals by Illinois Department of Public Health Genetics Program staff in Springfield. After telephone notification of a physician, an information sheet on the disorder and list of consultants to which the patient could be referred for further testing were provided. Designated consultants are physicians who are board-certified in clinical genetics, clinical biochemical genetics or medical biochemical genetics, have at least 1 year's experience in the treatment of patients with LSDs, and have expressed interest in being listed. Genetics Program staff were responsible for obtaining the short-term follow-up data on all infants with positive screening test results and the long-term follow-up data on those with positive diagnoses from the designated consultants.

Because of follow-up considerations, Illinois is not currently collecting assay data for Krabbe disease, even though the Krabbe substrate is included in the multiplex assay mixture. This arrangement is accomplished by closing the particular MS/MS recording channels in which the Krabbe data would be recorded otherwise. As these follow-up considerations are resolved, results for Krabbe disease will be recorded and reported. Newborn screening for both Krabbe disease and MPS type II is legislatively mandated in Illinois in addition to the 5 disorders for which screening is currently ongoing. MPS II newborn screening has not yet been initiated, owing to issues related to substrate availability. It is anticipated that screening for both of these additional disorders will be initiated in 2017.

Results

The overall outcome of screening of the first 219 793 infants in Illinois is summarized in **Table II**. A total of 521 infants