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## Newborn Screening for Lysosomal Storage Disorders: *Quo Vadis?*

Roy W.A. Peake<sup>1,\*</sup> [Associate Medical Director], Deborah L. Marsden<sup>2</sup> [Assistant Professor], Olaf A. Bodamer<sup>3</sup> [Park Gerald Chair in Genetics and Genomics, Associate Chief], Michael H. Gelb<sup>4</sup> [Professor and Boris and Barbara L. Weinstein Endowed Chair in Chemistry], David S. Millington<sup>5</sup> [Emeritus Medical Research Professor of Pediatrics, Adjunct Professor of Biochemistry], and Frits Wijburg<sup>6</sup> [Head of the Division of Metabolic Diseases]

<sup>1</sup>Department of Laboratory Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA <sup>2</sup>Division of Genetics and Metabolism, Boston Children's Hospital, Harvard Medical School, Boston, MA <sup>3</sup>Division of Genetics and Genomics, Boston Children's Hospital, Harvard Medical School, Boston, MA <sup>4</sup>Department of Chemistry, University of Washington, Seattle, WA <sup>5</sup>Duke University Health System, Durham, NC <sup>6</sup>Academic Medical Centre, Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands

Screening newborns for lysosomal storage disorders (LSDs)<sup>7</sup> has gained credence due to the increasing number of therapeutic options available and evidence that early intervention significantly improves outcomes. LSDs are progressive conditions that are typically asymptomatic at birth, potentially making them ideal candidates for newborn screening (NBS). Over the past decade, the development of high-throughput assays with multiplexing capabilities for use with dried blood spot (DBS) samples has facilitated several pilot NBS programs for LSDs worldwide. Such programs have been invaluable in providing knowledge of these disorders, particularly in regards to their true incidence and the overall feasibility of widespread population screening for LSDs. Despite these endeavors, implementation of

\* Address correspondence to this author at: Department of Laboratory Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115. roy.peake@childrens.harvard.edu.

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Nonstandard abbreviations: LSDs, lysosomal storage disorders; NBS, newborn screening; DBS, dried blood spot; SACHDNC, Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (of the US Department of Health and Human Services); RUSP, recommended universal screening panel; MPS-I, mucopolysaccharidosis I; MPS-IH, Hurler syndrome; FDS, US Food and Drug Administration; ERT, enzyme replacement therapy; MS/MS, tandem mass spectrometry; DMF, digital microfluidics.

NBS for LSDs is still debated and there are lingering concerns in some quarters. In the US, the US Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) is responsible for reviewing evidence and making recommendations for proposed conditions to be added to the Recommended Universal Screening Panel (RUSP). However, each state determines whether or not it will follow the recommendations. Outside the US, the process is less systematic, and regional newborn screening committees determine which disorders should be added to a panel. The original criteria of Wilson and Jungner (WHO; 1968) for population screening should be used wherever possible as a guide. In general, disorders are considered if they meet criteria based on the following: screening should provide clear benefits to the patient; testing capabilities should be in place; and effective treatments should be available. Despite the technical advances achieved in assay development, there are concerns about the appropriateness of NBS for some LSDs on the basis of the interpretation of results, the need for confirmatory testing, availability of effective therapies, and the overall costs involved. At present, the SACHDNC has recommended that Pompe disease and mucopolysaccharidosis I (MPS-I) be added to the RUSP, and the Secretary of Health and Human Services has approved this recommendation. Several US states are currently screening for one or more LSDs, with many more states close to implementing their programs. In this Q&A, we invited a panel of experts to share their views on the current status of NBS for LSDs and the challenges that lie ahead.

### **Should we be screening for LSDs, and if so, which disorder(s) would benefit most from NBS?**

**Olaf Bodamer**



While a strong argument can be made for NBS as a seminal public health measure in general, there needs to be careful consideration and evaluation for each condition added, including LSDs. False-positive test results, lack of adequate confirmatory testing, and

identification of late-onset presentations will place an unnecessary emotional and financial burden on the family and society at large, without obvious health benefit to the individual.

In my opinion, a case can be made for 2 LSDs to be included in NBS programs, which follows the recent recommendation from the US Secretary of Health and Human Services. There is mounting evidence that early diagnosis and timely initiation of therapy will significantly improve health outcomes in infants with either the severe form of MPS-I, known as Hurler syndrome (MPS-IH), or infantile-onset Pompe disease. In fact, bone marrow and/or stem cell transplantation in MPS-IH within the first 6 to 12 months of life will, (a) preserve cognitive development; (b) guarantee near normal quality of life, and (c) reduce many comorbidities, although there are proven limitations for skeletal and cardiac manifestations. Initiation of enzyme replacement therapy in infants with infantile-onset Pompe disease diagnosed through NBS in Taiwan has resulted in normal cardiac and pulmonary function and near normal fine and gross motor development in this otherwise inevitably fatal disease. However, one drawback is the identification of asymptomatic newborns with low enzyme activities and genotypes suggestive of milder (later-onset) forms of Pompe disease or MPS-I. There is currently no evidence-based consensus on follow-up or time of initiation of therapy in these individuals. Frequently, genotype–phenotype correlation is challenging and does not allow an accurate prediction of the clinical course.

There is limited benefit for other LSDs to be included in NBS programs at this moment in light of absence of supporting evidence. Additional carefully planned multi-program pilot studies are needed to gather systematic data on individual LSDs that may be considered for inclusion in NBS programs.

### Michael Gelb



My quick answer is yes, for those LSDs for which an established treatment exists that halts the progression of the disease and/or improves long-term outcomes for the patients. This is

particularly relevant for disorders where outcomes are more favorable if treatment is started before the disease becomes too severe.

For nontreatable diseases in general, my answer is no. Studies in Taiwan have convincingly shown that early treatment for severe Pompe disease leads to much better outcomes, although most expect that such patients will show disease progression after several years. This is the best we can do while we wait for gene therapy. However, I do not treat patients with LSDs nor do I parent a child with an LSD. These are complicated issues, and I am glad we have the SACHDNC to hash this out. I just wish there were more parents on this committee because scientists and doctors cannot answer all of the questions. On speaking with parents of affected individuals, the general consensus is that they would cherish even 5 to 10 “good” years with their child versus what they currently endure.

### David Millington



From a historical viewpoint, most of the LSDs are far removed from the original criteria proposed by Wilson and Jungner to justify the addition of new conditions to an NBS program. In the US, some programs have been coerced into mandating screening for LSDs without prior consultation with the parties responsible for its implementation, with the result that unreasonable pressure has been imposed on a system already under stress owing to lack of resources provided for NBS in many states. I believe that it is reasonable to consider screening for those conditions that have an early neonatal onset form that is treatable. Moreover, I believe we should initiate NBS only for conditions that have been recommended by the SACHDNC, which at this time has approved only 2 LSDs, Pompe disease and Hurler syndrome (MPS-IH), for inclusion in the RUSP. I am not fundamentally opposed to screening for other LSDs that have US Food and Drug Administration (FDA)-approved treatments, but it seems to me that it would be prudent to first conduct pilot prospective studies for evidence-based review by the SACHDNC. There are, in my view, more than enough challenges associated with screening for these 2 conditions for the time being. There

is good reason to believe that new therapies for LSDs, including combination therapies, may offer a more convincing rationale to screen for additional LSDs in the future.

### Frits Wijburg



The criteria of Wilson and Jungner for population screening, first published in 1968, are still widely, and in my opinion justly, used to determine which conditions should be included in NBS panels. The most important of these criteria are, (*a*) the condition is an important health problem; (*b*) availability of a suitable test for diagnosis; (*c*) a recognizable latent or early symptomatic state; (*d*) an adequate understanding of the condition's natural history, and (*e*) availability of an acceptable treatment for patients with recognized disease. LSDs are invariably progressive, generally affecting several organs or organ systems, resulting in significant morbidity and a restricted lifespan. Therefore, almost all LSDs fit the first criterion. In addition, numerous studies have shown the feasibility of sensitive and specific high-throughput biochemical testing in DBS for a number of LSDs. I have no doubt that such assays will be available for many additional LSDs in the near future. Therefore, meeting criterion *b* will not be an insurmountable problem. Furthermore, since almost all of the LSDs are characterized by an early asymptomatic or paucisymptomatic postnatal period, there is a window for commencing treatment before irreversible damage has occurred, thereby meeting criterion *c*. Unfortunately, knowledge on the natural history of many LSDs is limited. The rarity of the disorders, in combination with remarkable variability in phenotypes, varying from intrauterine death to first symptoms emerging in senescence, make it highly challenging to assess the phenotype at an early stage of the disease. In addition, for most LSDs there is incomplete understanding of genotype–phenotype correlations, and a lack of biomarkers for assessing or predicting disease severity or disease activity. This precludes many LSDs from introduction in NBS panels since criterion *d* is not satisfied. Finally, although an impressive number of phase I/II and III trials are now planned and executed for a number of disorders, an effective disease-modifying treatment is still available for only a small number of LSDs. Currently, more than 45 different LSDs are

recognized, and an effective therapy, such as enzyme replacement therapy (ERT), substrate reduction therapy, or hematopoietic stem cell transplantation, is available for only approximately 10 disorders. However, not all of these disorders meet criterion *d* and thus they lack reliable ways to predict phenotypic severity.

In my opinion, NBS is at present justified for Pompe disease and MPS-I. For the infantile-onset phenotype of Pompe disease, it has been shown that very early introduction of ERT significantly reduces mortality and morbidity. However, patients with the much more prevalent late-onset form of Pompe disease, who may develop clinical symptoms only in adulthood or even senescence, will also be detected by NBS. These patients may need many decades of follow-up before clinical symptoms develop, leading to eventual treatment with ERT. It is therefore essential to have excellent long-term follow-up programs in place for such patients, including supportive and psychosocial care. NBS for MPS-I is justified since early hematopoietic stem cell transplantation improves the outcome in the severe Hurler phenotype of this disorder (MPS-IH). The identification of patients with the attenuated, nonneuronopathic phenotype of MPS-I is less challenging compared with that for Pompe disease, as the latter patients develop progressive symptoms during childhood that respond to early introduction of ERT. In regards to the other LSDs for which treatment is available, greater knowledge should be obtained before they sufficiently meet Wilson and Jungner criteria and justify their inclusion in NBS programs.

## **Over the past decade, several pilot NBS programs for LSDs have been conducted. What is the most important lesson we have learned from these pilot programs?**

### **Olaf Bodamer**

The most important lesson learned is the identification of an unexpectedly high incidence of LSDs, including MPS-I, Fabry, Pompe, and Gaucher diseases, with a predominance of nonsevere (later-onset) phenotypes. The clinical course of the latter is frequently difficult to predict in the absence of genotype–phenotype correlation and suitable biomarkers, necessitating coordinated clinical follow-up. The New York State NBS Program has developed such a follow-up program for asymptomatic infants with high suspicion of later-onset Krabbe disease across multiple clinical centers and specialties. The pilot NBS programs have also provided additional valuable insights into test methodologies and performance and utility of confirmatory diagnostics, including second-tier molecular testing, as well as clinical follow-up.

### **Michael Gelb**

The most important point is that NBS for LSDs by direct measurement of lysosomal enzyme activities in DBS is feasible. I can say with certainty that the tandem mass spectrometry (MS/MS) enzyme assays lead to an acceptable number of screen-positive samples, such that there are essentially no false negatives and the number of follow-up analyses is manageable. In our pilot studies of 9 LSDs at the Washington State NBS laboratory (approximately 80000 live births per year), the number of screen positives per LSD totaled fewer than 20 per

year, and for the majority were less than 10 per year. Clearly, follow-up is manageable with such small numbers. The second point, possibly known before the pilots were conducted, is that genotypes are helpful only as a second-tier analysis because of the large numbers of variations of unknown pathogenic significance. It is clear that the potential use of next-generation sequencing for first-tier NBS for LSDs is a nonstarter at the present time, although this may be a possibility in 20 years when we understand the genotype-phenotype correlations better.

### David Millington

I believe we need to be clear about the nomenclature here; a true pilot NBS program is prospective, has already-established algorithms for follow-up of presumptive positive cases, and refers at-risk cases for diagnostic work-up and treatment as necessary. Studies that use de-identified samples and/or are retrospective, although able to provide valuable information, are not strictly comparable with prospective NBS studies. This is particularly true for performance metrics, and should therefore be categorized as prepilot-phase research studies. NBS programs are generally obligated to conduct both prepilot and pilot studies before implementation of a new screening test. From both the pilot and prepilot LSD studies reported thus far, the most important lesson we have learned is that the prevalence of LSDs is higher, and in some cases such as Fabry disease, much higher than previous estimates based on clinical information. The majority of cases detected for both Pompe and Fabry diseases are later-onset forms of the disorders that pose particular challenges in terms of when and how aggressively to treat affected patients. As a further complication, especially with Pompe and MPS-I, prospective screening pilot studies have revealed a high prevalence of individuals with pseudo-deficiency alleles that result in low enzyme activity by in vitro testing using synthetic substrates, but are clinically unaffected. Screening for LSDs thus imposes substantial challenges for the healthcare providers who have to make sense of this and explain it to their clients.

### Frits Wijburg

Over the last decade, we have learned many important lessons from the pilot studies on NBS for Fabry disease. These studies invariably detected an amazingly high prevalence of individuals with low enzyme activity, suggestive of Fabry disease, however often in combination with genetic variants that were previously not reported. This has led to the recognition of new phenotypic subgroups in Fabry disease, including a late-onset form and the late-onset cardiac variant of Fabry disease. Studies also revealed that low enzyme activity in combination with genetic variants does not equal the presence of Fabry disease. It has indeed proven to be a major challenge to construct algorithms that allow a reliable distinction between “true Fabry disease” and non-disease-causing biochemical and genetic variants. Such algorithms should include mutation analysis, biomarker studies in blood and/or relevant tissues, and assessment of clinical signs and symptoms. Valid decisions on commencing lifelong, invasive, and expensive treatment can only be made on the basis of such strict criteria.

The detection of variants of unknown clinical significance is also a consideration. However, this is also a concern for other diseases with a long screening history, such as

phenylketonuria (e.g. individuals with mild hyperphenylalaninemia) and medium-chain acyl-CoA dehydrogenase deficiency (the so-called medium-chain acyl-CoA dehydrogenase screening variants) and should not obstruct inclusion of disorders in NBS programs.

## **Some LSDs have extremely poor clinical outcomes with limited response to treatment (e.g. Krabbe disease), or have no FDA-approved treatment available (e.g. Niemann-Pick type A/B). How is NBS justified for such disorders?**

### **Olaf Bodamer**

Therapeutic and/or management strategies that reduce morbidity and mortality and thereby change the natural disease course are important criteria to consider prior to implementation of NBS. The example of infantile-onset Krabbe disease demonstrates that the availability of a potentially curative approach may not guarantee optimal health outcomes, but a reduction in morbidity and mortality, and ultimately an improvement in quality of life. This is a huge achievement in an otherwise devastating disorder. LSDs without FDA-approved therapies should not be considered for inclusion in NBS unless there are other clinical management strategies with proven medical utility to reduce morbidity and/or mortality.

### **Michael Gelb**

NBS for Niemann-Pick type A/B should not be implemented at the present time. Twelve years ago, our team published a paper showing the power of MS/MS for multiplexing 6 LSDs for use in NBS programs. This 6-plex assay included Niemann-Pick type A/B as an example only. In my view, this 6-plex assay was taken too literally by the biotech community, which led to a reagent distribution program via the CDC without my involvement. On hearing about the 6-plex assay, various parent advocacy groups lobbied for its use, resulting in the implementation of NBS for Niemann-Pick type A/B in a few states.

Krabbe disease is complex, and I am unable to fully address this in a few sentences. Here is a summary with some of the most relevant points. NBS for Krabbe disease is a catch-22 situation. For example, I do not believe we could have evaluated NBS for Krabbe disease by a pilot study of 100000 samples; rather, a sample size of around 1000000 would be required. However, such numbers would only be possible if an NBS program goes live. The New York Krabbe disease consortium deserves a special mention because they have done a spectacular job. The New York NBS program has taught us an enormous amount about Krabbe disease, and we are learning more about the genotypes and how to best follow the high-risk patients. None of this would have been possible without live NBS for Krabbe disease. I strongly disagree with the view that Krabbe disease is a bad NBS candidate because the current treatment is bone marrow transplantation. For many years, this approach has been an acceptable form of treatment for leukemia, and Krabbe disease is certainly an equal, in terms of disease severity. The treatment is not optimal yet, but I have seen several treated individuals who are doing very well compared to their affected siblings. We should remember that Krabbe disease is a horrific disorder, and perhaps we should tolerate a



treatment that works only some of the time, in comparison with cancer, where treatment may be effective for a few years, or just months, and only in a small minority of patients.

### David Millington

In my opinion it is not justifiable to screen for these conditions unless or until there is consensus among the experts that there is a viable treatment option. Programs are best advised to use discretion; just because the technology to screen for multiple LSDs exists does not automatically justify doing so. The purpose of the SACHDNC, as previously discussed, is to provide a mechanism for evidence-based review of each condition by a respected panel of experts and make recommendations based on the available data. However, even the 2 LSD conditions approved thus far did not receive a unanimous “yes” vote from the Committee.

The counter argument is that multiplex LSD testing for 5 or more LSDs provides an opportunity to identify more affected individuals and understand the true incidence of rare conditions, including those with limited treatment options. NBS may also help facilitate diagnoses that may otherwise take months or years to identify. There is still, however, a lack of consensus among healthcare professionals as to the effectiveness of NBS, even for those conditions already approved by the SACHDNC.

### Frits Wijburg

My opinion is that such disorders should not be included in NBS panels. An essential Wilson and Jungner criterion for justifying population screening is the availability of an acceptable disease-modifying treatment. Introducing this group of disorders in population NBS programs may easily cloud the purpose of NBS, and may lead to decreased acceptance of NBS in the population. However, early diagnosis of genetic disorders by NBS has several advantages. It allows informed family planning and targeted prenatal testing in future pregnancies. In addition, it prevents the long diagnostic odyssey that is often observed with LSDs. Furthermore, screening for these disorders will advance knowledge on the natural history of different phenotypes. Finally, patients diagnosed by NBS may be recruited for new therapeutic studies as soon as treatment becomes available. An option for screening for these disorders may involve presenting 2 sets of programs to the parents: one for treatable disorders and one for disorders for which no effective treatment is yet available. However, explaining the pros and cons of choosing screening programs to new parents may prove extremely challenging.

## **In terms of a complete, multifactorial care pathway, involving laboratory screening, diagnosis, treatment, follow-up, and management of these disorders, are we truly ready for NBS for LSDs and is it cost-effective?**

### Olaf Bodamer

Numerous challenges must be overcome prior to NBS for LSDs. First, there is a shortage of clinicians trained in biochemical genetics in general, and with expertise in LSDs in particular, which has a negative impact on accessibility to confirmatory testing and clinical

follow-up. In addition, there is an uneven distribution of NBS referral centers, with predominance in metropolitan areas and relative shortage in rural areas. This will be one of the biggest challenges for the years, if not decades to come. Second, confirmatory testing may not be available in a timely fashion due to lack of diagnostic laboratories with expertise in LSDs, health insurance barriers, and low socioeconomic status of families. Third, management and follow-up consensus-based guidelines for the asymptomatic infant with an LSD are generally unavailable, with the exception of the American College of Medical Genetics and Genomics guidelines published in 2011 and guidelines published by individual states. Health economy studies have shown cost-effectiveness for a number of NBS conditions, including phenylketonuria and congenital hypothyroidism, but are not yet available for many other conditions that are currently included in NBS panels, including LSDs. There is concern that early initiation of costly, potentially lifelong therapies such as ERT may tilt the health-economy balance when outcomes-related data are not available.

### Michael Gelb

I am not a healthcare cost-analysis expert; however, I wish to make the following points. The cost of “dealing” with LSDs (including NBS, patient management, and treatment costs) has essentially zero impact on the amount of money we spend on healthcare in the US. Rather than carefully analyzing the cost– benefit ratio for LSD NBS (in itself, an almost impossible task), perhaps more attention should be directed towards the spiraling costs associated with many other areas within our healthcare system. As an example, a new immunotherapy for stage IV melanoma that costs \$150000 per year per patient, is effective in approximately 40% of patients for a few years, and has been approved by the FDA *is* covered by insurance. It is therefore difficult to argue against the treatment of a newborn with a horrific LSD for about the same price. In regards to NBS for LSDs and treatment vs cancer screening and treatment, in my opinion neither is cost-effective. However, almost nothing in medicine is cost-effective, so why should we hold NBS for LSDs to a different standard? It is also worth noting that, at present, we spend several hundred thousand dollars a year to care for a nontreated patient with a serious LSD. To put these costs in perspective, it is difficult to believe how a typical resident of New York state is unable to pay 20 cents a year for Krabbe disease NBS.

Are we ready for LSD NBS follow-up? In most places no, but it is unlikely that we will proactively get ready *before* we have NBS, as has been the case in the past for other disorders. However, a great deal of credit should go to the New York, Missouri, and Illinois NBS laboratories for their endeavors in successfully delivering NBS for LSDs. Some NBS laboratories (e.g., Missouri) tend to outsource some of the follow-up testing to reference laboratories, whereas larger NBS laboratories (e.g., New York), tend to perform most of the follow-up testing in-house. It is imperative that all of the diseases screened for are followed-up, and NBS programs should not be implemented without having this in place.

### David Millington

From past experience, one could reasonably argue that we have never been truly ready for the consequences of NBS for any condition. Unexpected and unanticipated consequences have followed each and every NBS test that has been introduced, and no matter how well

prepared we may think we are, the same will be true for LSDs. There have already been unexpected outcomes from the limited experience to date, and we can certainly expect further surprises. The cost-effectiveness of screening for LSDs has not been addressed in a systematic manner, and may need to be considered on a case-by-case basis. Unlike most of the disorders screened for on the RUSP, the current treatment options for LSDs are invasive, very expensive, and far from perfect. It is not even clear that they are sustainable over the lifetime of a patient. There is reason to believe that as more treatment options such as gene replacement, small molecule, and combined therapies become available, the outcomes for affected patients should improve and treatment may become less costly and invasive. Nevertheless, as the natural history of early-onset forms of an otherwise lethal condition changes under treatment, longer-surviving patients will require more specialist care from multiple clinical disciplines, further increasing the cost burden of disease.

### Frits Wijburg

Setting up such complete pathways for care is essential for all disorders included in NBS programs; for decades, these have successfully been introduced for many other genetic metabolic diseases. Therefore, I do not see any reason to doubt that this can be achieved for LSDs. The costs of screening are unlikely to be prohibitive for inclusion of LSDs in NBS programs since assays will be multiplexed and automated. As long as follow-up and decisions on treatment initiation will follow evidence based protocols, NBS for these disorders will be cost-effective. Early diagnosis will avoid unnecessary costs associated with the so-called diagnostic odyssey and, in addition, early initiation of treatment will significantly improve outcomes, resulting in less lifelong costs of supportive care.

### **Several screening methods have been proposed for LSDs. Which approach offers the most in terms of high-throughput capability, screening performance, cost-effectiveness, and overall suitability to large-scale NBS programs?**

#### Michael Gelb

There is a lot of nonfactual information out there. Here are the current facts, all of which are published or available from NBS laboratories that have purchased the technologies: (a) For the LSDs being discussed for NBS, direct enzyme assay in DBS is the most suitable approach because DNA sequencing is not ready for first-tier analysis, as mentioned previously. Lysosomal enzyme abundance assays miss cases where nonfunctional but stable enzymes are produced. Also, for many LSDs, biomarker analysis gives an unmanageable number of false positives, or is not suitable for high-throughput analyses. (b) Large-scale pilot studies carried out by the Washington and New York NBS laboratories by MS/MS, and in the Missouri state NBS laboratory by digital microfluidics fluorometry, have shown that MS leads to a 2- to 4-fold reduction in the number of screen positives compared with fluorometry, even when identical criteria are used for establishing screen cutoffs (note, studies carried out more than 2 to 3 years ago are not relevant since they were performed with technologies that are no longer available). (c) The difference in total cost and space requirements for MS vs digital microfluidics fluorometry, including all equipment,

servicing, and reagents, is small (<30%). (d) Why would you invest in fluorometry when many of the assays now being discussed and emerging in the future can only be carried out using MS (X-linked adrenoleukodystrophy, MPS-IVA, Niemann-Pick type C)? Finally, it is worth mentioning that such assays would not be possible but for the pioneering work of the late Professor Nestor Chamoles, who demonstrated that lysosomal enzymes are still active in dried blood spots.

### David Millington

Screening methods for LSDs currently used in prospective NBS programs are all based on measurement of enzymatic activity in extracts from DBSs using synthetic substrates. In my view, a program considering screening for only 1 or 2 conditions, such as Pompe and MPS-I, could opt for the benchtop fluorometric assay method that has already been successfully used for several years to screen for both Pompe and Fabry disease in Taiwan. This method uses standard microtiter-plate equipment already in use in NBS programs, is cost-effective, and can be deployed rapidly and inexpensively. It is worth noting that the Taiwan program has provided the only opportunity thus far to compare MS/MS with bench top fluorometry for Pompe disease within the same program, and in fact there was no performance advantage realized after switching to MS/MS. For programs that decide to or are likely to add more LSDs to their NBS panel, the best option for cost-effective high-throughput multiplex screening for LSDs in my opinion is the digital microfluidics (DMF) platform currently deployed in the Missouri NBS Program. The fact that DMF requires nanoliters of DBS extract for each assay, that each assay is individually optimized for pH and other buffer conditions, that results are available within a few hours, and that it is very inexpensive to deploy, are compelling reasons to consider this option. MS/MS is currently the only viable alternative for multiplex screening for LSDs, but even for a modest program (100000 newborns per year) the capital outlay for equipment, infrastructure, and personnel requirements, not to mention ongoing service contracts and maintenance and replacement costs for the additional tandem mass spectrometers required (those currently used for analyte screening are already running at or near capacity) can run into the millions of dollars and may be prohibitive for adoption by many programs. These costs, which are by comparison trivial for DMF, are difficult to justify even if there is a significant and demonstrable advantage to MS/MS-based assays. However, all the available evidence from the prospective screening studies for LSDs in the Illinois, New York, Missouri, and Taiwan programs, including that presented publicly at the recent American Public Health Laboratories Pompe workshop and *WORLD.Symposium*, indicates that MS/MS has no such advantage over DMF. This is not surprising, primarily because the largest source of variance is the blood spot itself. Multiple factors contribute to low enzyme activities, and the fact is that in the real-world environment of prospective NBS, population cutoffs for any condition have to be sufficiently aggressive, at least in the initial pilot phase of screening, to avoid if possible the pitfall of false-negative results. There are proven ways to limit the negative impact of high initial presumptive positive rates; for instance, the use of 2-tier cutoffs and second-tier DNA testing on the original DBS to eliminate most carriers and pseudo-deficiencies from referral, evaluating the original DBS for other disease biomarkers, and repeating newborn screens for those with values in the equivocal or indeterminate range. No doubt, other strategies will be developed. Regardless, there is scant evidence to support the contention that the performance

metrics of any one LSD screening method justifies its exclusive consideration above other options. This leaves us ultimately with the cost factor. I would argue that money is better spent in the follow-up, to ensure that diagnostic testing, counseling, and treatment are available to all those identified by NBS to be at risk for an LSD, than on unnecessarily costly technology to screen them in the first place. That said, NBS for X-linked adrenoleukodystrophy, the latest condition to be approved by the SACDNC, currently requires MS/MS technology. Unless the target analyte for X-linked adrenoleukodystrophy can be included in the current amino acid/acylcarnitine test menu, or a less expensive screening test is developed, programs may still need to invest in additional MS/MS equipment if they wish to screen for this condition.

## **Is there any evidence to suggest that an enzyme activity level or genotype can accurately predict whether a child will develop early-onset, adolescent, or adult-onset phenotype for some LSDs?**

**Michael Gelb**

This is my favorite question of all. The dogma in the field is that the level of residual lysosomal enzymatic activity is not a good predictor of the severity of an LSD. In my opinion, some careful thinking strongly suggests otherwise. To the best of our knowledge, in all cases in which mutations lead to null enzyme activity (for example, stop codons, frameshifts and large deletions), the patient suffers from an early-onset LSD, so there is no evidence for compensating enzymes. For many LSDs, even a few percent of normal enzymatic activity is sufficient to prevent symptoms. Therefore, it is clearly an enzyme assay resolution problem. Fluorometric and radiometric assays simply are not good enough to detect small differences in residual enzymatic activity at the low end. Conversely, MS/MS assays display an analytical range that is more than 2 orders of magnitude larger than that for the established assays, and we can now “spread the scores” more, so to speak. For example, recent collaborative work has shown that an LC-MS/MS assay of the Pompe disease enzyme in leukocytes provides an enzymatic activity that is 3- to 4-fold lower for infantile-onset patients compared to late-onset patients. This is in contrast with the fluorescence assay, where both cohorts are indistinguishable. For Krabbe disease, infantile Krabbe disease patients have 4- to 5-fold less enzyme activity than high-risk newborns that are so far asymptomatic. My hope is that these new high-resolution enzymatic activity assays will allow us to go beyond diagnosis toward the realm of disease prediction. If this can be achieved, we can set some of the NBS high-risk patients free at an early age and thus reduce the biggest fear of all for NBS of any disease. This is the number one goal right now, and I am very excited about the results so far and the power of mass spectrometry.

**David Millington**

I assume that this question is referring to the diagnostic testing that follows an abnormal newborn screen. The severity of a disease can be inferred from the residual enzyme activity in a purified leukocyte extract from whole blood. In addition, for disorders with a substantial registry of known disease-causing mutations (e.g., Pompe disease), enzyme activity may be augmented with genetic information. However, it is well known that even among family

members affected with the same disease genotype, outcomes can vary dramatically. Predictions based on genotype and enzyme activity do not take into account multiple underlying genetic and environmental factors that may modify the disease process, and for this reason I would not trust that information alone to make such a critical decision as when and how aggressively to treat a patient. Conversely, noninvasive tools to assess the extent of damage caused by these progressive disorders, such as is caused by glycogen accumulation in muscle fibers in Pompe disease, for example, are improving and may eventually be expected to facilitate such decisions. In the case of MPS-IH, genotype–phenotype correlation is even more challenging because, like Pompe disease, there is a high prevalence of familial mutations but a more limited library of known disease-causing mutations. Because enzyme activities are very low for both MPS-IH and attenuated MPS-I, we will have to rely primarily on clinical evaluation for the timing of therapeutic intervention. These arguments carry over to the other LSDs because all have variable phenotypes.

**LSDs are often variable in their natural history, making results interpretation challenging. In addition to early-onset forms requiring early treatment, NBS will also identify asymptomatic carriers and late-onset forms of some disorders. What is the best strategy for follow-up and management of these patients?**

**Olaf Bodamer**

Follow-up and management of asymptomatic infants with late-onset LSDs require comprehensive clinical protocols aimed at identification of subtle disease manifestations and ultimately time of initiation of therapy. These protocols require guidance from a multi-talented group composed of different specialties, charged ideally with developing evidence-based or, in the absence of evidence, consensus-based guidelines, either at the state or national level. Professional organizations such as the American College of Genetics and Genomics or the respective NBS societies play a pivotal role in the development of such guidelines.

**Frits Wijburg**

This is indeed a challenge and something that needs to be carefully worked out before starting screening for any disorder. Protocols, preferably based on (inter)national consensus, will guide follow-up and management of such individuals. These protocols should be based on current knowledge while realizing that the full phenotypic spectrum, as well as knowledge on both the clinical and analytical sensitivity and specificity of enzymatic testing, biomarkers and genotypes, will only accumulate after initiation of screening. Individuals with a confirmed diagnosis but who have, based on biochemical or genetic testing, probably or certainly a late-onset phenotype, should be followed up on a regular basis. Special attention is needed for the psychological burden on families, and later on the identified individuals, resulting from an asymptomatic diagnosis of an LSD (a potentially devastating progressive multisystem disorder) and a diagnosis of a late-onset phenotype (with sometimes even the possibility of being asymptomatic until demise in late senescence).

Follow-up should therefore not be strictly medical, but needs to include supportive psychosocial care.

**There is concern that conditions are “haphazardly” being added to state NBS panels, due to advances in the state of the art and increased political pressure from various advocacy groups. Is NBS for LSDs another example of this approach?**

**Olaf Bodamer**

The addition of conditions, including LSDs, to NBS panels should follow a vigorous review process and thorough evaluation of all available information through key stakeholders, including advocacy groups, policy makers and payers. In the absence of supporting evidence, carefully planned multilaboratory pilot studies should be completed in large diverse populations to inform about NBS test characteristics, confirmatory testing, and clinical follow-up programs. Ideally legislation should not only guarantee NBS for a particular condition, but also address confirmatory testing, clinical follow-up, and available therapies.

**Frits Wijburg**

Yes, this has happened, and I think that this is a serious threat to the future of NBS for LSDs. Including disorders in screening panels without appropriate knowledge of the natural history, protocols for timing of treatment initiation in different phenotypes, efficacy of treatment in different phenotypes, and consensus (and, if possible, evidence-based) protocols for follow-up of all identified individuals with all phenotypes will ultimately lead to disappointment in the program and a dramatic loss of cost-effectiveness. Decisions on including disorders in NBS panels should not be influenced by politics or pharmaceutical companies as they may all have their own agendas. Careful, independent, evaluation of scientific evidence and consensus between experts, in collaboration with patient organizations, is, in my opinion, the only way to avoid mistakes and prevent failure of the NBS program. Introduction of any genetic metabolic disorder in NBS programs, including LSDs, should only be considered through this approach.

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