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Review

Newborn Screening in Gaucher Disease: A Bright and Complicated Future

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Abstract

Gaucher disease (GD) is one of the most common lysosomal storage disorders resulting from biallelic mutations in the *GBA1* gene, causing a dysfunction of the lysosomal hydrolase, glucocerebrosidase (acid-β-glucosidase; E.C 3.2.1.45). Clinical manifestations are heterogenous and can include splenomegaly, anemia, and neurological impairments in the case of neuronopathic Gaucher disease types 2 and 3. Newborn screening, arguably the most important public health initiative to date, has been regularly conducted on newborns in the United States since the 1960s. The development of new low-cost screening methods and effective treatments are motivating the inclusion of GD and other lysosomal storage disorders in population-wide newborn screens. In this article, we review the history of newborn screening for GD, the screening methods used, and ethical considerations and challenges regarding the successful implementation of population-based newborn screening for GD.

Keywords

Lysosomal storage disorders; GBA1; genetic testing ethics; neuronopathic Gaucher disease; newborn screening implementation

1. Introduction

Newborn screening (NBS), since its inception in the 1960s with the advent of Guthrie's test for phenylketonuria (PKU), is arguably the most successful public health program in the United States (US) [1]. Lysosomal storage disorders (LSDs) encompass over 60 rare genetic diseases. During the past two decades, many new treatments, therapies and diagnostic methods have been developed for previously undiagnosed, untreated, and under-treated diseases. Gaucher disease (GD), one of the two most common LSDs, is an autosomal recessively inherited disorder resulting from mutations in the *GBA1* gene. GD is characterized by clinical heterogeneity, with limited genotype/phenotype correlation [2]. While the concept of NBS is over 60 years old, its application to the LSDs, and specifically GD, is relatively recent. In the last 30 years, GD has evolved from having no effective treatment to having several successful therapies, and is now a potential target for gene therapy [3]. Here we will discuss the history and aims of NBS through the lens of GD, with a focus on the current landscape of screening and the ethical considerations that influence that process.

1.1 NBS: Historical and Ethical Background

Worldwide, NBS screening differs greatly in both scope and practice. Ten principles around public health screening set by Wilson and Jungner in 1968 were the framework that guided the establishment and enhancement of global programs for many years [4]. The principles remain largely the same today, with emphasis on strong validity of potential screening tests, understanding of the medical disease or condition, and access to follow-up diagnostics and information. Of note, an important revision of the criteria was introduced in 2008, as the earlier 1968 report did not include a recognized treatment as an important criterion [5]. A comparison of criteria from 1968 versus 2008 is detailed in Box 1. Furthermore, various other guidelines developed after Wilson and Jungner's initial framework show a continued shift in focus toward programmatic and systemic implementation, encouraging the inclusion of conditions previously excluded from consideration based on the earlier criteria [6].

Jungner criteria (1968)

- The condition sought should be an important health problem.
- The natural history of the condition, including development from latent to declared disease, should be adequately understood.
- There should be a recognizable latent or early symptomatic stage.
- There should be a suitable test or examination.
- The test should be acceptable to the population.
- There should be an agreed policy on whom to treat as patients.
- There should be an accepted treatment for patients with recognized disease.
- Facilities for diagnosis and treatment should be available.
- The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
- Case-finding should be a continuing process and not a "once and for all" project.

Andermann et al criteria Revision (2008)

- The screening program should respond to a recognized need.
- The objectives of screening should be defined at the outset.
- There should be a defined target population.
- There should be scientific evidence of screening program effectiveness.
- The program should integrate education, testing, clinical services and program management.
- There should be quality assurance, with mechanisms to minimize potential risks of screening.
- The program should ensure informed choice, confidentiality and respect for autonomy.
- The program should promote equity and access to screening for the entire target population.
- Program evaluation should be planned from the outset.
- The overall benefits of screening should outweigh the harm.

Box 1 Principles of Public Health Screening in 1968 vs. 2008.

In the US, NBS is controlled at the state level, with the Recommended Uniform Screening Panel (RUSP), developed by American College of Medical Genetics in 2004 and adopted by the US Department of Health and Human Services in 2010, serving as a federal nationwide standard. At first, the report focused on three principles when evaluating diseases for inclusion: 1. Identification can be achieved at a time interval (24 to 48 hours after birth) at which the disorder would not ordinarily be clinically detected; 2. The test must have appropriate sensitivity and specificity; 3. There must be demonstrable benefits of early detection, timely intervention, and efficacious treatment [7]. There were no LSDs included in the initial RUSP. However, with improvements in assessment techniques and therapeutic development, as well as an improved inherent understanding of the natural history of these diseases, some LSDs are now included as viable targets for NBS [8-11]. The term 'demonstrable benefits' can be difficult to define as there are few studies regarding the impact of treatment in pre-symptomatic individuals and the impact of early disease diagnosis. Such studies would be invaluable and should be widely encouraged, to determine the true benefit of NBS screening.

1.2 The History of NBS for the LSDs

One of the earliest reported NBS efforts for an LSD was in a cohort of 37,104 Italian male neonates screened for Fabry disease (FD). The program found an incidence of 1:3,100 vs the historically reported number of 1:50,000 and determined that the incidence of late-onset Fabry disease was eleven times higher than the classic phenotype [12]. This early study highlighted the key aspects and challenges of NBS for the LSDs, including finding an increased incidence of disease, as well as describing a late-onset phenotype with complicated treatment considerations. FD ultimately was not added to the RUSP, due in part to its variable disease onset and the lack of

published data demonstrating the impact of preventive treatment early in life [13]. Two other LSDs, Neiman Pick disease type C and Krabbe disease, were similarly considered and rejected for inclusion in 2008-10. Pompe disease, while not included in 2008 due to factors including the lack of screening test specificity and a request for more data regarding the benefit and implications of diagnosing the late-onset disease, was later approved in 2013. It was formally added to the RUSP in 2015 after it was decided that earlier treatment benefits outweighed the potential harms [14]. In February of 2022, it was voted to include mucopolysaccharidoses type II (MPS II) in the RUSP, and on Aug 2, 2022, this was accepted by the Secretary of the Department of Health and Human Services [15]. Krabbe disease was scheduled to be reconsidered in May of 2022. Re-evaluation and inclusion of these LSDs recognizes the improvements made both in screening technologies and in the development of new therapeutic modalities.

1.3 Driving Forces for Adding NBS for Gaucher Disease

Expansion of NBS is historically driven by accurate, affordable testing, improved and novel therapies, and public/patient involvement, which is why population based NBS for GD has received renewed interest in the US and worldwide [16]. With the adoption of tandem mass spectrometry (MS/MS) for NBS in the 1990s, a far larger number of samples can be screened for multiple diseases simultaneously, reducing cost [17]. New assays developed for NBS, specifically the LSDs followed, including methods that first facilitated screening for five different enzymes on a single dried blood spot (DBS) [18], then expanding to include up to nine enzymes [19]. Furthermore, since the 1990's new treatments for GD have been introduced, including enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) and others are currently under development, including small molecule chaperone drugs and gene therapy [20, 21]. However, the neuronopathic manifestations of GD remain largely unaddressed by current therapeutic options, and the widely diverse clinical phenotypes associated with GD have complicated the discussion regarding the development and implementation of NBS initiatives for GD.

2. Methods of Newborn Screening for Gaucher Disease

The primary approaches used for newborn screening for GD and other LSDs from DBS to date are fluorometry and mass spectrometry. These methods for GD screening have already been adopted and added to the routine newborn screen for LSDs in several states in the US, as well as internationally. Screening results are considered positive when the enzyme glucocerebrosidase (acid-β-glucosidase) is absent or deficient; however, further diagnostic testing must be conducted to confirm the diagnosis of GD.

2.1 Assays for Gaucher Disease Screening

2.1.1 Fluorometric Methods

The Chamoles team pioneered utilizing rehydrated DBS to measure enzymatic activity in LSDs, successfully measuring enzymatic activity using standard fluorometry and radiometric assays [22-28]. Standard fluorometry involves the use of an artificial substrate for the enzyme of interest, conjugated with a fluorescent tag, such as 4-methylumbelliferone (4-MU) in the case of many LSDs. The enzymatic reaction by glucocerebrosidase releases the fluorescent 4-MU along with glucose. To

quantify glucocerebrosidase activity with this strategy, the artificial substrate 4-MU is added to the DBS in a well or test tube, incubated for 20 hours at 37 degrees Celsius for the enzymatic reaction to take place, and the 4-MU fluorescence is read with a plate reader [24]. Decreased 4-MU fluorescence indicates decreased enzymatic activity. In samples from patients with GD, a lower fluorescence readout corresponds to lower glucocerebrosidase activity. A team in China used this method to screen 80,855 newborns in Shanghai in 2017, identifying one newborn with GD, reflecting an incidence rate of 1:80,855 (see Table 1) [29]. Although this technique is cost effective for one or two enzymes, incubation times are lengthy, and one DBS punch can only be used for one screen. In practice, multiple LSDs may be of interest, and thus, the inability of standard fluorometry to multiplex is a significant limitation.

Table 1 Comparison of International NBS results for Gaucher disease.

	Total Screened	Confirmed Incidence[a]	Method	Level of Multiplexing	1st tier enzyme activity cutoff for GD (μmol/L/h)	2nd tier enzyme activity cutoff for GD, if applicable (μmol/L/h)
Taiwan (2011-2013) [30]	103,134	1:103,134; 0.0010% (n = 1)	MS/MS	4	≤7.5 (n = 141)	≤7.5 (n = 5)
Taiwan (2018-2019) [31]	73,743	1:73,743; 0.0014% (n = 1)	UPLC-MS/MS	8	≤2.5 (n = 27)	≤2.5 (n = 9)
Hungary [32]	40,024	1:13,341; 0.0075% (n = 3)	MS/MS	5	≤3.50 (n = 141)	≤3.50 (n = 5)
Mexico [33]	20,018	0 (n = 0)	MS/MS	6	≤1.45 (n = 0)	≤1.45 (n = 0)
Brazil* [34]	9,878	1:4,939; 0.0202% (n = 2)	DMF	4	≤3.9[b]	≤3.9[b]
Shanghai, China [28]	80,855	1:80,855; 0.0012% (n = 1)	Standard Fluometry	1	≤30.07 (n = 11)	≤15.4 (n = 3)
Northeast Italy [35]	44,411	1:22,205; 0.0045% (n = 2)	MS/MS	4	≤2.2 (n = 9)	≤2.2 (n = 2)
Austria [36]	34,736	1:17,368; 0.0058% (n = 2)	ESI-MS	5	≤4.0 (n = 4)	N/A

* Indicates small-scale screen findings.

[a] Determined by leukocyte/plasma enzymatic activity and/or genotyping.

[b] Indicates unreported screen results.

Due to its inefficient nature, standard fluorometry is scarcely used in modern newborn screens, but is a viable option for follow-up screens. Subsequently, digital microfluidics (DMF) has been developed by expanding on standard fluorometry concepts to simultaneously screen for multiple enzymes from one DBS. It uses nanoliter or microliter volumes of reagents and utilizes an array of electrodes under the influence of an electric field to automate the entire fluorescence assay on a chip in a disposable cartridge. In the NBS performed for LSDs in Missouri, enzymes are extracted from the DBS punches in 96-well plates for 30 minutes with an extraction buffer. Then, the supernatant of the sample extraction, reagent, and filler fluid are loaded into the cartridge. and placed in the benchtop instrument for around three hours [18, 37]. Currently the prevailing DMF system is the FDA-approved Seeker™ system manufactured by Baebies, Inc [38].

The benefits of DMFs are that it is a very simple, affordable, and quick assay. It takes advantage of spatial multiplexing instead of true multiplexing, so that each enzyme can be screened under its own optimized conditions, which allows for specificity in reaction conditions, such as acidity, substrate type, and inhibitors of choice for each disorder screened. However, the limitations of DMF and other fluorometric approaches are the use of an artificial substrate as opposed to the endogenous substrate. There is concern regarding the difficulty in developing pure artificial fluorometric substrates as well as limited availability of reagents from generic vendors, and this approach cannot accurately screen for individuals with the LSD Niemann-Pick A/B with the most common *ASM* mutation [39].

2.1.2 Mass Spectrometry Methods

The other approach to measure glucocerebrosidase enzymatic activity in NBS is using a mass spectrometer. Mass spectrometry measures enzymatic products or biomarkers instead of fluorescence. In mass spectrometric approaches, the marker for GD is the decrease in glucocerebrosidase enzymatic activity [31]. While there are promising studies of using glucosylsphingosine (Lyso-GL1) as a biomarker for individual Gaucher screening [40], it has yet to be tested in a large population-based screen, and further investigation is warranted, especially focusing on its inclusion in larger LSD screening panels. In tandem mass spectrometry (MS/MS), the enzymatic reaction is first performed in the “pre-MS/MS” step before injection into the mass spectrometer to detect enzymatic products. This is done in an assay cocktail of enzymatic substrates, internal standards, inhibitors of non-LSD enzymes, and reaction buffer, which is added directly onto a well or test tube with the DBS punch. This method is more developed due to the availability of commercial substrates. However, the enzymatic step is time consuming, and a cleaning step using solid-phase and/or liquid-phase extraction of the products must be performed after the enzymatic reaction, because the excess detergents, salts, and substrates in the reaction buffer contaminates the mass spectrometry analysis. Thus the “pre-MS/MS” step is time-consuming and labor intensive and is the primary limitation of the mass spectrometry approach. One mass spectrometer has the throughput of 7.8 digital microfluidic plate readers [41]. PerkinElmer has developed a 6-plex LSD MS/MS assay that is FDA-approved and commercially available which includes assays for Gaucher, Pompe, Krabbe, MPS-I, Niemann-Pick diseases, and Fabry diseases; it is currently the most common assay used in most states to screen for LSDs. This product has improved false-positive rates compared to fluorometric assays and offers a more streamlined “pre-MS/MS” step [41, 42].

To automate the contaminant cleaning process before injection into the mass spectrometer in MS/MS, one can inject the sample into a column in the ultraperformance liquid chromatography-tandem mass spectrometer after the enzymatic reaction. In the Illinois LSD NBS protocol, ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is performed by adding the 5-plex assay cocktail into the test tube or well containing the DBS, incubating for 17 hours, and then it is quenched, transferred, and injected into the UPLC-MS/MS instrument [37]. An 18-plex UPLC-MS/MS assay including GD and 14 other lysosomal storage disorders was developed in 2020 by expanding the commercially available 6-plex MS/MS assay by PerkinElmer [42]. It is currently being used in a prospective pilot study by the ScreenPlus Program in New York (see Table 2) [43, 44]. UPLC-MS/MS allows for a higher level of multiplexing, but there is concern about the additional costs from the chromatography.

Table 2 Comparison of Population-wide NBS results for Gaucher disease in the United States.

	Total Screened	Confirmed Incidence[a]	Method	Level of Multiplexing	1st tier enzyme activity cutoff for GD (μmol/L/h)	2nd tier enzyme activity cutoff for GD, if applicable (μmol/L/h)
Missouri [45]	308,000	1:61,600; 0.0016% (n = 5)	DMF	4	≤8.0[b]	≤4.0 (n = 37)
Illinois [46]	219,973	1:43,995; 0.0023% (n = 5)	UPLC-MS/MS	5	≤20.0[b]	≤17.0 (n = 117)
New York* [44]	65,605	1:4,374; 0.0229% (n = 15)	MS/MS	5	<20% DMA[b, c]	<15% DMA[c] (n = 17)
Washington* [47]	43,000	1:43,000; 0.0023% (n = 1)	UPLC-MS/MS	6	<10% DMA[c] (n = 3)	N/A

* Indicates pilot screening findings.

[a] Determined by leukocyte/plasma enzymatic activity and/or genotyping.

[b] Indicates unreported screen results.

[c] Cutoff was determined on a daily percentage basis compared to the population mean. DMA = daily mean activity.

Another mass spectrometric method not widely adopted, but with promising preliminary findings is electrospray ionization mass spectrometry (ESI-MS/MS). This method uses an electrospray ionization source for LC-MS/MS (a less advanced version of UPLC-MS/MS). A small-scale study using ESI-MS/MS for five LSDs (Gaucher, Pompe, Krabbe, Fabry, and Niemann-Pick diseases) on 212 patients showed 100% sensitivity and high specificity with this method [48].

Analysis time for 100 samples is around three hours, and, using this method, Austria screened 34,736 newborns and identified two cases of GD, corresponding to an incidence rate of 1:17,368 (see Table 1) [36].

2.2 Current Gaucher NBS Programs

To date, GD is included in the newborn screens in Missouri, Illinois, New Jersey, Tennessee, Oregon, and at certain hospitals in New York (https://newbornscreening.hrsa.gov/your-state). Missouri and Illinois have the most comprehensive documented history of population-wide Gaucher newborn screening in the US. Internationally, Taiwan, Hungry, Mexico, Brazil, China, Italy, and Austria have reported their Gaucher screening findings as shown in Table 1 and Table 2. The false-positive and positive predictive values are excluded because the enzyme activity cutoff of choice significantly impacts these values, which are modified over time as more screening results are being reported; additionally, with the development of the novel post-analytical tool Collaborative Library Integrative Reports (CLIR; https://clir.mayo.edu/) to adjust cutoff levels, these values are an unfair comparison of screening modalities [49].

2.2.1 States Including Gaucher Disease in the NBS Panel

Missouri has the longest ongoing prospective program of population wide Gaucher screening. They use a 4-plex DMF approach with the artificial 4-methyl-umbelliferyl-β-d-glucopyranoside substrate at a pH of 5.2 in their newborn screens for GD, with their four-year full-population screening results from January 2013 to January 2017 presented in the article by Hopkins et al. in 2018 [45]. The DMF cartridge used by the Missouri State Public Health Laboratory, the Seeker™ System, runs 44 samples per cartridge to screen for four LSDs (MPS I, Pompe, Gaucher, and Fabry diseases). Over four years, they screened 308,000 newborns, with a glucocerebrosidase activity cutoff of 5.5 µmol hydrolyzed substrate/liter blood/hour for newborns less than seven days old and 4.0 µmol/L/h for newborns seven days and older. The cutoff for the referral (second tier) screening was set as the 0.4th percentile of GCase activity based on the data from newborns in prepilot deidentified DBSs, with the provisional (first tier) screening cutoff set slightly higher than the referral cutoff [50]. Because the blood spots used in this report were around 6 months old, 4.0 µmol/L/h is the value indicated in Table 2. They reported a Gaucher incidence of 1:61,600 (n = 5), comparable with previously reported GD rates and the false positive rates were similar to other newborn screening assays in the state. Brazil also used the same method in a small-scale screen of 9,878 infants in 2018, in which they used the cutoff of 3.9 µmol/L/h and identified two Gaucher cases for an incidence rate of 1:4,939 (see Table 1) [34].

Illinois piloted their LSD screen in 2014 and expanded it to a state-wide screen in 2015. From November 2014 to August 2016, they performed a 5-plex LSD screen for Gaucher, Pompe, Fabry, MPS I, and Niemann-Pick diseases. Within that timeframe, they screened 219,973 infants with a reported incidence for GD of 1:43,995 (n = 5). They employed the UPLC-MS/MS method with a conservative cutoff of ≤17 µmol/L/h to avoid false-negatives. Cutoff values were determined by comparing enzymatic activity from DBS of normal infants compared to DBS of Gaucher patients [46].

2.2.2 International NBS for Gaucher Disease

Taiwan piloted its newborn screening for LSDs using standard fluorometry to screen for Pompe and Fabry diseases in 2005 and 2006, respectively [51], and then in 2015 shifted to the 4-plex MS/MS strategy using the PerkinElmer reagents to screen for Gaucher, Pompe, MPS I, and Fabry diseases [52]. They piloted the 4-plex assay by screening 103,134 newborns from September 2011 to January 2013 using a glucocerebrosidase activity cutoff of 7.5 μmol/L/h, which identified 141 subjects below the cutoff in the first-tier screen, and then five in the second. After sequencing, they found one newborn with two mutations and two newborns with one mutation, resulting in an incidence rate of 1:103,134. Two-tiered screening cutoff of 7.5 μmol/L/h was derived from the 0.5 percentile activity amongst 1,778 anonymous DBS using MS/MS [30]. They have since adopted an 8-plex UPLC-MS/MS screening assay in 2018 for lysosomal diseases to include four additional LSDs: MPS II, MPS IIIB, MPS IVA, and MPS VI [31]. This 8-plex strategy has been validated by a pilot study in the University of Washington (see Table 2) [53]. In the recent Taiwan report, they screened 73,743 newborns from March 2018 to April 2019, with 27 newborns having glucocerebrosidase activity below the first-tier screen cutoff and nine in the second tier. Sequencing identified one newborn with type 3 Gaucher, giving an incidence rate of 1:73,743. The enzymatic cutoff of 2.5 μmol/L/h was chosen for this screen, derived from 20% of the normal mean activity [31].

Hungary uses MS/MS for their 5-plex LSD screening assay (Gaucher, Krabbe, Pompe, Fabry, and Niemann-Pick A/B diseases). In 2012 they screened 40,024 newborns from one of the two newborn screening centers in Hungary. The cutoff of 3.50 μmol/L/h was chosen by averaging the lower 0.25 and lower 0.5 percentile glucocerebrosidase enzymatic activity from 1,000 samples. From the screen, they identified three confirmed cases with GD and seven GD carriers with the relatively high incidence rate of 1:13,341 [32].

Mexico used MS/MS to screen for six LSDs (Gaucher, Krabbe, Pompe, Niemann-Pick diseases, Fabry, and MPS-I) from July 2012 to April 2016 using the commercially available PerkinElmer reagents. They screened 20,018 DBS taken within 24-48 hours from birth, and found no Gaucher cases below the enzymatic cutoff of 1.45 μmol/L/h. Their cutoff was based on PerkinElmer guidelines by evaluating known positive and negative samples [33].

Finally, Northeast Italy screened four LSDs (Gaucher, MPS I, Pompe, and Fabry) in 44,411 newborns from September 2015 to January 2017 also using the PerkinElmer MS/MS kit. Setting an enzymatic activity cutoff of 2.2 μmol/L/h in a two-tiered screen (a second spot is requested for a second-tier screen if the value fell below the cutoff in the first-tier screen), they identified two newborns with GD for an incidence rate of 1:22,205. Cutoff was determined by analyzing 3,500 deidentified newborn DBSs and choosing a high-risk level of enzyme activities below 20% of the median of those samples [35].

2.3 Post-Screen Analyses

The screens for different LSDs generally have higher positive rates compared to other NBS, thus necessitating the development of post-screen analyses to validate positive test results [54, 55]. Thus, to enhance accuracy, second-tier screens are performed free of charge to the family following a positive primary test. This prevents anxiety and costs resulting from false positive results and allows for prompt treatment of false negative cases. Second-tier screens are tests run on the same DBS sample, but typically with different target analytes and different assays. Upon a positive primary

screen result for GD, the physician may elect to recall the DBS or to collect a new DBS for repeat biochemical testing [56, 57].

A promising tool to reduce false-positive rates, as well as to identify cases where a second-tier screen is necessary, is the post-analytical multivariate pattern recognition software Collaborative Library Integrative Reports by Mayo (CLIR; https://clir.mayo.edu/). This was developed to be used in conjunction with the primary and/or secondary screens to either provide a diagnostic result or to differentiate differential diagnoses of two different disorders. It is a free collaborative software for users that contributes positive reference data to allow for the constant expansion of the library. CLIR allows users to input newborn demographic data, such as birth weight and age at DBS collection, along with at least three enzyme activity values, to adjust screen results such that disease marker cutoffs are based on the individual, instead of using a universal cutoff for all newborns [58, 59]. It has been integrated since February 2016 in newborn screens for Krabbe, Pompe, and MPS I [60].

Upon detection of decreased glucocerebrosidase in the primary and optional secondary screens, two tests are performed to confirm diagnosis of GD: 1) Measurement of glucocerebrosidase enzymatic activity in leukocytes (Mayo ID: GBAW) and 2) LC-MS/MS to detect elevated concentrations of plasma glucosylsphingosine (Lyso-GL1) (Mayo ID: GPSY and GPSYW) [61]. If decreased glucocerebrosidase activity is determined, but the Lyso-GL1 levels are not elevated, total gene sequencing of the *GBA1* gene is required for a definitive diagnosis of GD (Mayo ID: GBAZ) [13].

3. Current Challenges

3.1 Mixed GD Phenotypes Present Challenges in Interpreting NBS Results

There are several key hurdles to developing and implementing a NBS program for GD in the US. There is currently no treatment that fully addresses the neuronopathic aspects of GD. In the most severe form of GD, acute neuronopathic GD (GD2), NBS will identify patients without a definitive treatment option. Similarly, although at the opposite end of the GD spectrum, NBS will identify asymptomatic individuals who may only become symptomatic late in life, if at all. While there is mixed evidence for parental support for expansion of NBS to include diseases where there is no treatment [62, 63], it is important to note that clinical trials are starting and ongoing for GD2 using several modalities including gene, chaperone, and adjuvant therapies. Since patients with acute neuronopathic GD are those most severely affected, early identification would be needed to prevent disease progression. Additionally, in neuronopathic GD, there is less genetic homogeneity, with many individuals carrying rarer and less well described *GBA1* variants, creating further difficulties in interpreting NBS results with a mixed clinical assessment.

Categorically, the identification of asymptomatic individuals with GD by NBS screenings ethically falls somewhere between that of a late-onset disorder and predictive testing of adult-onset disorders in pediatrics. The ACMG guidelines for reporting incidental findings were updated in 2013 to include adult-onset disorders for pediatric patients, prompting significant discussion about the ethical considerations of such testing [64]. The benefits of disclosing the diagnosis of an adult-onset disorder to the parents of pediatric patients have included enabling parents to prepare for the future, make informed decisions about the health of their child and diminish potential psychological harms associated with not reporting known genetic information [65-68]. Potential harms often mirror the arguments of potential benefits, with the exception that predictive testing violates a child's autonomy (as an adult-onset disorder can never be imminent) [69, 70] and increasing

parental anxiety due to the 'vulnerable child' syndrome [71, 72]. These universal arguments are echoed in the smaller LSD community, with benefits such as decreased time to diagnosis and prevention of morbidity, versus the potential economic and psychological harms [73-76]. This makes applying these arguments and precedents to GD problematic, as while it is thought that certain patients will need treatment or monitoring in their pediatric years, the true incidence is not known. A recent Delphi consensus group of GD experts supported NBS for GD since an earlier disease onset conveys a high risk of morbidity and there is often a lengthy diagnostic odyssey [21].

Also, while there is a clear need for prompt treatment initiation in symptomatic individuals, especially pediatric patients, there are limited established guidelines for the asymptomatic patients likely to be diagnosed through NBS, aside from recommendations for bi-annual or yearly evaluations [77-79]. There is data suggesting that many patients with non-neuronopathic GD may never need treatment. In one study conducted at a New York center, only 4 out of 32 pediatric patients identified through parental or prenatal screening progressed to needing therapy [80]. Similarly, in Israel, 4 out of 30 patients diagnosed from screening progressed to a stage where they required treatment at the time of publication [81]. In both studies the pathologic variant p.N409S (N370S) was the predominant *GBA1* variant observed, and p.N409S homozygosity was the most common genotype identified in. Without clear, evidence-based, dedicated guidelines for pre-symptomatic patients, and potentially with the lack of access to GD experts, parents/providers may feel pressured to treat patients that may not require therapy. Due to challenges arising from variable genotype-phenotype correlation and the complicated treatment of these rare diseases, the need for more geneticists and genetic counselors specialized in rare diseases is paramount [82]. Further training opportunites demonstrating the inclusion of clinical data, family history and NBS data with real-world treatment decisions would be of great benefit to clinicians making difficult early treatment determinations.

3.2 Systemic Implementation

Implementation of NBS can also be a lengthy process. Figure 1 demonstrates the time lag between the approval by committee, the addition of a disease to the RUSP and the adoption by the States. Additional federal funds were made available through the NewSTEPs New Disorders Implementation Project [58, 83]. While 15 states accessed this program, after five years only nine were able to fully implement GD NBS screening. The reported causes of delays were related to hiring, training, and retention of staff, acquiring equipment, and organizing information technology infrastructure, along with implementing clinical follow up [58]. If GD were added to the RUSP, it is likely that there would be a significant time lag in implementation, and additional funding, either state or federal, would be needed to limit the gap and provide for the increased need for follow-up, in order to guarantee timely access for both parents and providers.

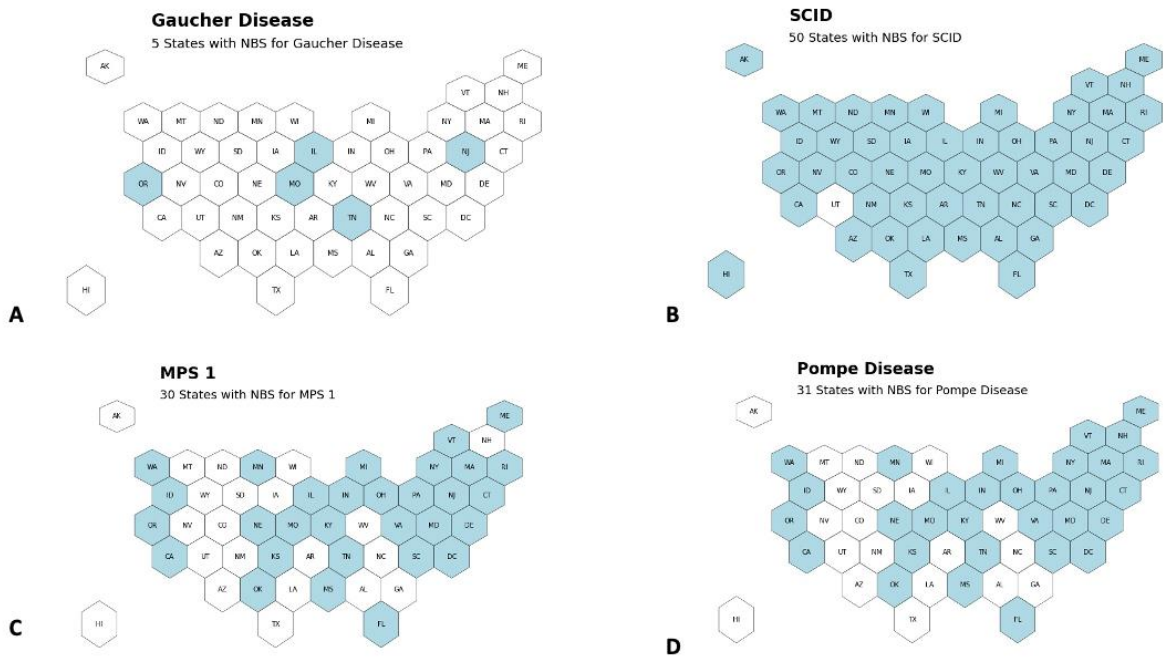

Figure 1 Current NBS in the United States. A: GD is currently included in the NBS panels in five states. B: Severe Combined Immune Deficiency (SCID), which was added to the RUSP in 2009 is now included in the NBS panel in 49 states and the District of Columbia. C: MPS 1, approved in 2015 and added in 2016 is included in the NBS panel in 30 states and D: Pompe disease, approved in 2013 and added in 2015 is currently on the NBS panel in 31 states. Information current as of 5/2021 retrieved from https://www.newsteps.org/resources/data-visualizations/newborn-screening-status-all-disorders.

Furthermore, the high cost of treatment for GD cannot be ignored. A recent study put the cost of treatment for children and adolescents between $243,381 and $546,758 dollars per year in the US [84]. The only treatment option currently available for children, ERT, requires ongoing intravenous infusions, which can be cumbersome and challenging in young children. This underscores the need to both accurately identify patients with GD in need of treatment and ensure equitable and timely access to therapy for those who require it.

4. Conclusion

Gaucher disease is a prototype disorder that highlights the current successes and challenges that face newborn screening as a public health program. NBS for GD can be done effectively and inexpensively with current technologies such as DMF and MS/MS. Recent improvements increasing levels of multiplexing and simplifying determination of the enzymatic activity are paving the way for GD to be included in the NBS panel in more locations, with many states and countries piloting GD screening and adding GD to their routine screens. Careful reporting from these initial programs with regards to incidence and characterization of new patient cohorts will be invaluable for the development of future programs. However, for wider adoption of population based NBS, careful attention to the development, implementation and clear and consistent follow-up guidelines for all

GD clinical phenotypes are key components that must be incorporated into any expansion, to ensure that the benefits continue to outweigh the harms.

Author Contributions

Emory Ryan conceived and designed the project, acquired and analyzed data and drafted and revised the manuscript. Tiffany Jong designed the project, acquired and analyzed data and drafted and revised the manuscript. Ellen Sidransky conceived and designed the project and drafted and revised the manuscript.

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Competing Interests

The authors have no competing interests to report.

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