MPS I: Early diagnosis, and treatment of bone disease
Kingma, S.D.K.

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Chapter
Epidemiology and diagnosis of lysosomal storage disorders; challenges of screening

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CHAPTER 2

ABSTRACT
The lysosomal storage disorders (LSDs) are a group of genetic disorders resulting from defective lysosomal metabolism and subsequent accumulation of substrates. Patients present with a large phenotypic spectrum of disease manifestations that are generally not specific for LSDs, leading to considerable diagnostic delay and missed cases. Introduction of new disease modifying therapies for LSDs has made early diagnosis a priority. Increased awareness, but particularly the introduction of screening programs allow for early diagnosis and timely initiation of treatment. This review will provide insight into the epidemiology and diagnostic process for LSDs. In addition, challenges for carrier screening, high-risk screening and newborn population screening for LSDs are discussed.
INTRODUCTION

The lysosomal storage disorders (LSDs) comprise a heterogenic group of more than 50 genetic disorders caused by progressive accumulation of specific substrates due to deficiency of hydrolytic enzymes, non-enzymatic lysosomal proteins or non-lysosomal proteins involved in lysosomal biogenesis. A wide range of disease manifestations can occur, including hydrops foetalis, neurocognitive decline, dysmorphia, hepatosplenomegaly and musculoskeletal abnormalities. Most LSDs are characterized by a broad phenotypic spectrum and may present from very early in life to late in adulthood. Due to the rarity of the diseases and the heterogeneity of disease manifestations, which are generally not specific for LSDs, lengthy diagnostic delays and missed cases are common. In this review, epidemiological studies that studied a large panel of LSDs, current diagnostic workup of patients suspected of LSDs and subsequent challenges for implementation of screening are discussed.

EPIDEMIOLOGY

Information about the incidence of LSDs is relatively limited. The results of the 6 largest epidemiological studies that studied birth prevalences of a relatively large panel of LSDs are presented in table 1. Birth prevalences of the neuronal ceroid lipofuscinoses (NCLs) and female Fabry carriers were only studied in some reports, and were therefore not included in this table. Combined birth prevalences of LSDs range from 7.5 per 100,000 in British Columbia to 23.5 per 100,000 live births in the United Arab Emirates (UAE) with the sphingolipidoses as the most prevalent group, followed by the mucopolysaccharidoses (MPSs).

Social isolation, immigration and epidemiology

When discussing introduction of screening programs for LSDs, reliable epidemiological data are essential, as birth prevalences may differ considerably per population group. Striking differences in birth prevalences between countries can be observed (table 1) and these can, indeed, at least partially be explained by differences in immigration patterns or isolation, for instance due to geographical, lingual, ethnic or religious preferences or customs. For example, in persons from Ashkenazi Jewish ancestry, strikingly high prevalences of several genetic diseases occur, including some LSDs, which has led to the introduction of highly successful screening programs. The remarkable high birth prevalences of MPS VI, GM1 gangliosidosis and fucosidosis in the UAE are another example and primarily due to ethnic isolation and founder effects, which is illustrated by the observations that 95% of genotyped patients were homozygous for their LSD causing mutation and that, indeed, most patients were from the same tribes or blood-related. Birth prevalences for most of the LSDs are comparable between British Columbia, the Czech Republic, Australia and The Netherlands, with MPS I, Gaucher disease and metachromatic leukodystrophy (MLD) as the most prevalent LSDs (mean birth prevalences around 1/100,000 live births). The population of British Columbia and Australia are primarily of European and particularly British origin.
suggesting a cause for the similarity in birth prevalences of LSDs. However, the increasing immigration rates from different countries and ethnic groups to Western countries, is likely to change the birth prevalences of genetic diseases, including LSDs, in the near future.  

### Clinical awareness and epidemiology

Extensive investigations in a region or population group and increased awareness may have major influences on epidemiological data in rare diseases, as only a few extra diagnosed cases may alter the birth prevalence.  

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*Table 1.* Birth prevalences of LSDs reported in different countries: total number of cases within a certain period of time divided by the total number of births in the same period (Australia) or total number of diagnosed cases born within a certain period of time divided by the number of births in the same period (The Netherlands, British Columbia, Portugal, the Czech Republic, and United Arab Emirates), expressed as cases per 100,000 live births.

* Fabry disease: number of male cases per 100,000 live male and female births.
cases may have a considerable effect on calculated birth prevalences. This is suggested to be a partial explanation for the high birth prevalences observed in Northern Portugal, as other regions of Portugal were excluded from most of the epidemiological analysis.

However, birth prevalences reported in published epidemiological studies all date from before the start of newborn screening (NBS) pilot studies and likely underestimate the true prevalences of many of the LSDs. Indeed, NBS (pilot) studies done in Hungary, Austria, Taiwan, Italy and the states of New York and Washington, reported on average 5–80 times higher birth prevalences than previously reported. The increase is primarily due to recognition of more attenuated and/or later-onset forms of the diseases, as demonstrated by Spada et al., who reported that 10 out of 11 Fabry patients diagnosed by a NBS pilot study in a part of Italy were of the late-onset type.

**DIAGNOSIS OF LSD**

Although the clinical presentation of LSDs depends on the type, quantity and site of storage of undegraded material, there are a number of overlapping clinical features, that are, however, not specific for LSDs. LSD patients thus often present a diagnostic challenge, which, in combination with the rarity of the different disorders, may lead to significant diagnostic delay. The most prominent signs and symptoms that should lead to appropriate diagnostic studies for LSDs are probably loss of earlier acquired cognitive and motor skills and/or combinations of signs and symptoms affecting different organs or organ systems. The combination of neurological signs and symptoms with musculoskeletal and/or cardiac signs and symptoms, and/or umbilical or inguinal hernia and/or ophthalmologic features including corneal clouding and retinal changes with a ‘cherry red spot’ should not only lead to inclusion of LSDs in the differential diagnosis but may also lead to more specific investigations (table 2; based on references). Figure 1 (based on references) presents a schematic approach for the initial diagnostic workup of patients with dysmorphia, musculoskeletal manifestations and/or progressive cognitive impairment as key clinical characteristics. Table 2 presents other clinical signs and symptoms that may be used in the differential diagnosis of LSDs. However, as LSDs are characterized by a broad spectrum of clinical manifestations, phenotypic severity and age of onset, and occurrence of atypical clinical manifestations have been reported, figure 1 and table 2 should be used only for guidance and not as absolute criteria.

The first step in the diagnostic workup generally consists of urinary analyses for specific undegraded macromolecules (figure 1). Usually, quantitative analysis of glycosaminoglycans (GAGs) using a dye binding assay is followed by electrophoresis, enabling separation of different GAGs. Urine oligosaccharide screens involve separation of urine sugars by thin-layer or high-pressure liquid chromatography. In addition, free sialic acid concentration in urine...
can be assessed by mass spectrometry. When a patient presents with musculoskeletal manifestations and/or progressive cognitive impairment, especially in combination with coarse facial features, these urinary tests can be used as a sensitive first diagnostic approach. However, metabolic screening of urine only covers distinct groups of LSDs and carries a risk of false negative results, especially in patients with attenuated phenotypes of MPS III or MPS IV. Therefore, if strongly suspected, normal urinary screens should still be followed by enzyme analysis.

Dysmorphic features, generally referred to as ‘coarse’, are a feature in a number of LSDs, in particular the more severe phenotypes of the MPSs and oligosaccharidoses. These coarse features include wide set eyes, a flattened wide nasal bridge, frontal bossing, enlarged gums...
and macroglossia, often in combination with thick and abundant hair. In mucolipidosis (ML) type II (I-cell disease), infantile sialic acid storage disease (SASD) and GM1 gangliosidosis, these features may already be observed in the newborn period.

A wide variety of neurological signs and symptoms may occur, either at presentation or during the course of the disease. Early symptoms can be ataxia, seizures, progressive cognitive and motor retardation. Pyramidal tract lesions and seizures may occur during the course of the disease in LSDs with progressive central nervous system disease. Only a few symptoms are sensitive and specific, such as vertical supranuclear gaze palsy, which is an early feature of Niemann Pick type C (NPC) disease. Several of the LSDs are clinically

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**Figure 1.** Algorithm for the diagnostic process of patients with several LSDs presenting with coarse facial features and/or musculoskeletal abnormalities (dysostosis multiplex and/or limited range of motion of large joints) and/or progressive cognitive impairment. GAGs glycosaminoglycans, ML mucolipidosis, MLD metachromatic leukodystrophy, MPS Mucopolysaccharidosis, MSD multiple sulfatase deficiency, NCL neuronal ceroid lipofuscinosis, NPA/B Niemann Pick type A/B, NPC Niemann Pick type C, SASD sialic acid storage disease.
characterized by only neurological symptoms, including GM1 and GM2 gangliosidoses, MLD, NCL and Krabbe disease.\(^\text{20,42}\)

Bone disease is a feature of several LSDs, and the constellation of radiographic abnormalities resulting from defective endochondral and intramembranous bone formation observed in the MPSs, ML II/III and galactosialidosis, is collectively referred to as ‘dysostosis multiplex’.\(^\text{45}\) In addition, patients with the attenuated phenotypes of the MPSs, oligosaccharidoses and ML II/III frequently present with limitation of motion range in large joints, perceived as joint stiffness (figure 1). Other types of bone disease observed in LSDs are osteonecrosis with pain crises in Gaucher disease, rickets in patients with cystinosis, skull deformities and osteolysis in cathepsin K deficiency (pycnodysostosis), and painful joint stiffness and swelling in Farber disease.\(^\text{37,45,48,49}\) Measurement of specific enzyme activity, generally followed by mutation analysis, is required to obtain a definitive diagnosis in LSDs. In some disorders, however, enzyme activity cannot be used for diagnosis. In NPC, diagnosis relies on a specific filipin staining assay in cultured skin fibroblasts in combination with mutation analysis.\(^\text{38}\) In patients suspected of Fabry disease, diagnosis based on enzyme testing and mutation analysis can be inconclusive, as a number of genetic variants of unknown significance resulting in partially deficient enzyme activity, may erroneously lead to a conclusive diagnosis.\(^\text{50}\) Although Fabry disease is an X-linked disorder, females may suffer from significant symptoms,\(^\text{51}\) however, diagnosis in females is even more complicated as the activity of the involved enzyme is often borderline or normal.\(^\text{41}\) The additional use of biomarkers and algorithms designed for the diagnosis of Fabry disease may help to improve the diagnostic approach in Fabry disease.\(^\text{50}\) Finally, deficiency of activator proteins or saposins, specifically required to assist a range of lysosomal enzymes, cannot be directly detected by enzymatic testing, and should be considered in patients highly suspected of a specific LSD but with normal enzyme activity in lymphocytes.\(^\text{35,43}\)

A number of additional studies, including magnetic resonance imaging (MRI) of the brain, cardiac ultrasound studies and ophthalmological examination may assist in the diagnostic workup (table 2).

**CHALLENGES OF SCREENING**

Early diagnosis, which is essential to allow timely initiation of disease modifying therapy, may be achieved through increased awareness among clinicians, as well as the general public. Although many ‘awareness campaigns’ have been initiated, especially for disorders for which enzyme replacement therapy (ERT) has become available, there are no data to support the effectiveness of such a strategy. Even more, studies failed to show a decrease in diagnostic delay for Pompe disease or MPS I over the last decade.\(^\text{21,22}\) This is probably due to the fact that physicians not specialized in LSDs will generally see none or only very
few LSDs during their working career as the overall prevalence of many disorders varies between <1:1,000,000 and 1:20,000 live births (table 1). An alternative strategy is diagnosis by screening.

**Carrier screening**

Genetic drift and endogamy have led in certain population groups to a high risk for carriership for a limited number of mutations leading to high prevalence of specific genetic diseases. The purpose of carrier screening is to inform couples about the risk for genetic disease in offspring and to help decision-making on marriage and reproduction as well as to allow specific prenatal testing. A highly successful example is carrier screening for Tay-Sachs (GM2 gangliosidosis) in the Ashkenazi Jewish population, which resulted in a dramatic decrease in birth prevalence of this disorder. Carrier testing for other diseases, including ML IV, Niemann Pick type A/B (NPA/B) and Gaucher disease, have been introduced in screening panels for people of Ashkenazi Jewish ancestry. However, there are ethical considerations that need to be addressed when introducing carrier screening in high risk populations. An Israeli carrier screening program for Gaucher disease demonstrated that 84% of the identified couples were at risk for offspring with mild or even asymptomatic Gaucher disease. They observed that screening for some mutations does not necessarily identify children requiring treatment, but can rather lead to questionable pregnancy terminations. This illustrates the importance of reliable data on natural history, genotype-phenotype correlations and epidemiology in different population groups before introducing carrier screening.

The rapid decrease in costs of next generation sequencing (NGS) and the improvement in coverage and thus in reliability of screening, paves the way for introducing carrier screening for a number of genetic diseases, including LSDs, in the general population by screening potential parents before conception (preconceptional screening). If both parents are identified to be carriers for mutations in the same gene, they may opt for prenatal testing or preimplantation genetic diagnosis. If such a technique becomes widely available, it might considerably change the prevalence of a number of severe LSDs. However, such screening approaches are highly controversial, and several ethical issues need to be addressed before preconception screening should be made available as a screening option.

**High-risk screening**

High-risk screening is performed to identify patients with low prevalence diseases based on the presence of a specific clinical sign or symptom. Almost all high-risk screening studies in LSDs have been performed for Fabry disease, as relatively high frequencies of Fabry disease may be detected among patients presenting with cardiomyopathy, early cryptogenic stroke or kidney failure of unknown cause. There are, however, two major
issues that need attention when considering high-risk screening for LSDs. Firstly, as patients will already exhibit disease signs and/or symptoms, this approach may lead to lower efficacy of treatment as irreversible organ damage may already be present. In Fabry patients, for instance, ERT is significantly less effective in patients with decreased renal function. Secondly, a recent systematic review showed that high-risk screening for Fabry disease resulted in the identification of individuals with genetic variants of unknown significance. High-risk screening may therefore lead to erroneously labelling of individuals as having a genetic disease and even to initiation of an ineffective, invasive and costly therapy.

Newborn screening

In the early 1960s, large scale newborn population screening began with Robert Guthrie’s pioneering studies on presymptomatic identification of phenylketonuria in dried blood spots. In 1968, Wilson and Jungner described a set of criteria for population screening in a report from the World Health Organization. These criteria include the availability of suitable tests and treatment, agreed policy on whom to treat, and adequate understanding of natural history. Wilson and Jungner’s principles are still the directive for the decision to

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<td>Wittmann et al.</td>
<td>2012</td>
<td>Hungary</td>
<td>40,024</td>
<td>Y</td>
<td>+ Gaucher</td>
<td>39.98</td>
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<tr>
<td>Hwu et al.</td>
<td>2009</td>
<td>Taiwan</td>
<td>171,977</td>
<td>N</td>
<td>+ Fabry</td>
<td>43.61</td>
<td></td>
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<tr>
<td>Duffner et al.</td>
<td>2009</td>
<td>US</td>
<td>&gt;550,000</td>
<td>N</td>
<td>+ Krabbe</td>
<td>4.55</td>
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<tr>
<td>Spada et al.</td>
<td>2006</td>
<td>Italy</td>
<td>37,104*</td>
<td>N</td>
<td>+ Fabry</td>
<td>32.34</td>
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Table 3. Newborn screening studies in dried blood spots for LSDs published between 2003 and 2014, that have included >10,000 newborns. Birth prevalences are expressed as cases per 100,000 live births. MPS Mucopolysaccharidosis, NPA/B Niemann Pick type A/B, US United States. * Only male newborns were screened.
include diseases in screening programs and over the past decades most developed countries have expanded their NBS programs. Because of the invariably progressive nature of LSDs and because diagnostic and therapeutic strategies have significantly improved, several LSDs have become attractive candidates for inclusion in NBS programs. Especially in the disorders for which adequate disease modifying treatment is available, diagnosis before the onset of irreversible clinical manifestations through NBS may greatly improve (event-free) survival. This is the case for several neuronopathic LSDs, such as MPS I and Krabbe disease, in which early haematopoietic stem cell transplantation (HSCT) may prevent or delay irreversible neurological damage and for disorders in which early initiation of ERT may be lifesaving such as in infantile Pompe disease, or prevent irreversible cardiovascular or renal disease, such as in Fabry disease.

The only LSD that is currently nominated by the Secretary of Health and Human Services in the United States (US) to be included in the Recommended Uniform Screening Panel, is Pompe disease. However, five states in the US have recently mandated NBS for several LSDs and NBS for Krabbe disease was already initiated in the state of New York in 2006. Some other countries have started (pilot) NBS programs for a number of LSDs and in Taiwan, Pompe and Fabry disease are now parts of the NBS program. Table 3 shows the results on large scale (>10,000 dried blood spots (DBS)) NBS (pilot) studies published in the last decade (2003-2014). Not unexpectedly, striking differences have been found between birth prevalences in these studies and earlier studies (table 1). It is evident that with the expansion of NBS programs for different LSDs, a number of methodological and ethical considerations still need to be addressed.

Laboratory techniques

A wide range of laboratory tests that may be used for diagnosis of LSDs within the scope of NBS programs has been reported over the recent years. Most assays are based on the measurement of enzyme activity using artificial substrates in DBS, either by tandem mass spectrometry (MS/MS), microplate fluorometry or digital microfluidic fluorometry. Alternative approaches are by measurement of accumulating substrates in blood or urine. For NBS programs, these assays need to be multiplexed and adapted for high-throughput screening. In the (near) future, NGS of the involved genes, applying techniques allowing full coverage of all exomes such as the single molecule molecular inversion probes technique, may replace screening based on enzyme activities or metabolite concentrations, as NGS as screening technique may ultimately proof to be more reliable, easier to multiplex and less expensive. Despite this tremendous technical progress, several challenges remain in selecting and improving appropriate screening methods and sensitivity and specificity of the different techniques will be further studied in ongoing and future NBS (pilot) studies.

Costs of screening, follow up and treatment
CHAPTER 2

The economic impact of expanding NBS panels with LSDs needs attention. Due to the major advances in high-throughput screening technology, including the introduction of NGS in the future, the costs of the screening process itself will probably not be prohibitive, even in lower-income countries. When trying to assess costs of screening, it is important to also take into account the costs of long-term follow up of patients identified by screening of whom not all will develop symptoms early in life, as well as the costs of disease modifying therapy. Therapies for LSDs are often very expensive, especially ERT. It appears logical that only those diseases for which access to treatment and reimbursement for treatment can be guaranteed, should be included in NBS programs.

‘Patients in waiting’ and genetic variants of unknown significance

One of the major concerns for inclusion of LSDs in NBS programs is the identification of individuals with attenuated or late-onset phenotypes as well as the detection of individuals with genetic variants resulting in decreased enzyme activity but with unknown clinical significance. The phenotypic spectrum of most LSDs is remarkably broad and ranges from patients with severe expression of the disease with early start of symptoms, who may benefit from early start of treatment, to individuals who remain completely asymptomatic until senescence and in whom early introduction of invasive, potentially dangerous and costly medication may be harmful and will unnecessarily increase medical costs. Prediction of the phenotype, in order to avoid overtreatment of patients with attenuated forms of a disease after identification by NBS, might be done by genotyping. This approach is used, for instance, in the screening program for Krabbe disease, initiated in 2006 in the state of New York, in order to identify patients at risk for infantile Krabbe disease that may benefit from early HSCT. However, genotype-phenotype correlations are not always sufficiently reliable in LSDs and other methods that allow very early differentiation between phenotypes need to be developed. This might be done by (combining) data on the genetic, enzymatic, clinical and biomarker data at the time of detection by metabolic screening, and these strategies should preferably be developed before inclusion of a disease in a NBS program.

In addition, those individuals that have been detected by NBS but are classified as ‘attenuated’ and therefore do not need immediate intervention (e.g. ERT or HSCT), need regular follow up in order to allow early diagnosis of symptoms and timely initiation of therapy. The time between diagnosis of the potential disease causing enzymatic deficiency in the newborn period and the development of symptoms may, however, be extremely long for some diseases (e.g. Pompe and Fabry disease), even up to a normal lifetime. This may lead to the phenomenon known as ‘patients in waiting’, a term cornered by Timmermans and Buchbinder in 2010 and discussed by Kwon and Steiner in relation to a report on an early diagnosed presymptomatic patient with Pompe disease. Being a ‘patient in waiting’ for a potentially severe, life threatening disorder for which there is disease modifying treatment
available may lead to complex psychosocial problems, as well as to costs of long-term follow up programs.

In addition to the identification of individuals with ‘very attenuated’ phenotypes who may become ‘patients in waiting’, NBS for LSDs may also result in identification of significant numbers of individuals with genetic variants of unknown significance, many of which will not convey a risk for clinical disease. This is particularly true for Fabry disease. NBS pilot studies for Fabry disease led to the identification of a remarkably large number (tables 1 and 3), depending on the study/population 13,15-19,64. This is for a large part due to the identification of patients with late-onset cardiac variants of Fabry disease, but also to the identification of individuals with genetic variants of unknown significance, some of which may never lead to clinical disease 50. There is, therefore, an urgent need for protocols and algorithms, which allow separation of individuals with, or at risk for, disease from those with neutral variants and, again, these protocols should preferably be developed before introduction of a disease in a NBS program. In addition, clear guidelines on the management of presymptomatic individuals with LSDs detected by NBS need to be developed, such as the recently published guideline of the American College of Medical Genetics (ACMG) Work Group 41.

Acceptable and effective therapy

One of the essential criteria for NBS is the availability of effective treatment 60. While the currently available disease modifying treatment options for LSDs in general significantly ameliorate the course of the disease, effectiveness varies considerably between patients and there can still be significant residual disease. For example, although early, presymptomatic, HSCT for Krabbe disease greatly increases (event-free) survival, most patients still develop progressive neurological manifestations, despite successful engraftment 78. In addition, ERT in infantile Pompe disease, even when started early, may not always prevent ventilator-free survival or lead to independent walking, partially due to the development of antibodies against the infused enzyme 79,80. Although immunomodulation may significantly improve the outcome of those patients 81, further studies are needed to establish the long-term efficacy of this approach. Antibody formation has also been shown to affect treatment efficacy in a number of other ERTs 82-84. It is therefore paramount that parents are informed not only about the benefits of treatment but also about the limitations in order to be able to make balanced decisions.

The challenges reported for introduction of LSDs in NBS programs illustrate that several fundamental issues still need to be addressed. Firstly, increased knowledge on natural history, studies on genotype-phenotype correlation and the development of diagnostic algorithms allowing separation of severe, attenuated and genetic variants with no risk for developing clinical disease are urgently needed for decision-making strategies in the context
of NBS. Secondly, knowledge on the long-term outcome of disease modifying treatments is needed to be able to better balance costs and benefits and to improve information to parents. Although it poses ample challenges, NBS is probably the only way to learn about the natural history of genotypic variants, whom to treat how and when, and to learn more about epidemiology of the diseases, and, last but not least, significantly improve outcome of these devastating disorders.

CONCLUSION

The LSDs are a group of genetic disorders resulting from defective lysosomal metabolism and subsequent accumulation of substrates. Patients present with a large phenotypic spectrum of disease manifestations that are generally not specific for LSDs, leading to lengthy diagnostic delays and missed cases. Introduction of new disease modifying therapies for LSDs have made early diagnosis a priority. Increased awareness, but particularly the introduction of different screening programs allows for early diagnosis and treatment, prenatal counseling and prevention of long and burdensome diagnostic odysseys. Before introduction or expansion of screening programs for LSDs, however, different methodological and ethical challenges need to be addressed. Firstly, as the prevalence of different LSDs differs considerably between population groups, reliable epidemiological data are needed to assess the potential benefits of screening for LSDs in different population groups. Secondly, as NBS will identify all forms of diseases, natural history studies on particularly late-onset diseases and the separation from genetic variants of unknown significance are needed. Thirdly, studies on phenotypic prediction, e.g. genotype-phenotype correlation and biomarkers or algorithms, are urgently needed to predict disease manifestations and therapeutic efficacy, preferably very early in life. Fourthly, studies on long-term efficacy of disease modifying treatments are needed, and in addition, consensus on which outcomes justify implementation of screening. Fifthly, guidelines for diagnostic confirmation and management of presymptomatic individuals are essential. Although implementing screening programs results in ample challenges, outcomes of current newborn (pilot) screening programs will likely contribute in meeting these challenges.

ACKNOWLEDGEMENTS

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REFERENCES


Lysosomal storage disorders; challenges of screening


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