Lysosomal storage diseases: Diagnostic confirmation and management of presymptomatic individuals

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Disclaimer: This guideline is designed primarily as an educational resource for health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

Purpose: To develop educational guidelines for the diagnostic confirmation and management of individuals identified by newborn screening, family-based testing after proband identification, or carrier testing in at-risk populations, and subsequent prenatal or postnatal testing of those who are presymptomatic for a lysosomal storage disease. **Methods:** Review of English language literature and discussions in a consensus development panel comprised an international group of experts in the clinical and laboratory diagnosis, treatment and management, newborn screening, and genetic aspects of lysosomal storage diseases. **Results:** Although clinical trial and longitudinal data were used when available, the evidence in the literature is limited and consequently the recommendations must be considered as expert opinion. Guidelines were developed for Fabry, Gaucher, and Niemann-Pick A/B diseases, glycogen storage type II (Pompe disease), globoid cell leukodystrophy (Krabbe disease), metachromatic leukodystrophy, and mucopolysaccharidoses types I, II, and VI. **Conclusion:** These guidelines serve as an educational resource for confirmatory testing and subsequent clinical management of presymptomatic indivduals suspected to have a lysosomal storage disease; they also help to define a research agenda for longitudinal studies such as the American College of Medical Genetics/National Institutes of Health Newborn Screening Translational Research Network. ***Genet Med* 2011:13(5):457–484.**

Key Words: *newborn screening, lysosomal storage disease, enzyme replacement therapy, presymptomatic, consensus guidelines*

BACKGROUND

The lysosomal storage diseases (LSDs) comprise a heterogeneous group of almost 50 disorders that are caused by genetic defects in a lysosomal acid hydrolase, receptor, activator protein, membrane protein, or transporter, causing lysosomal accumulation of substrates that are specific to each disorder. The accumulation is progressive, ultimately causing deterioration of cellular and tissue function. Many disorders affect the central nervous system (CNS) and most patients have a decreased lifespan and significant morbidity. The LSDs are often categorized according to the type of substrate stored (i.e., mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, gangliosidoses, etc.).[1]

Most lysosomal proteins are the products of housekeeping genes expressed throughout the body, but storage occurs only in those cells with an available substrate (e.g., GM_2 ganglioside is present predominantly in the CNS and deficiency of hexosaminidase A, which acts on the GM_2 ganglioside and can be measured in the blood, causes Tay Sachs disease, a CNS condition). In all cases, the diagnosis must be established by specific enzyme assays and by mutational analysis. Urinary mucopolysaccharides and oligosaccharides, although useful for screening, can be normal and increased nonspecifically in healthy neonates.[2]

Although each disorder is rare, LSDs as a group have a frequency of one in 7000–8000 live births.[3,4] The frequency estimate may be low as more individuals with mild disease and/or adult-onset forms of the diseases are being identified.

All LSDs are inherited in an autosomal recessive fashion, except for Fabry, Hunter (mucopolysaccharidosis type II [MPS II]) and Danon diseases, which are X-linked. Some disorders are more prevalent in certain geographic areas or among particular population groups (e.g., Gaucher, Tay-Sachs, Niemann-Pick type A, and mucolipidosis IV are more common in Ashkenazi Jews), largely as a result of ancestral founder mutations.[5–7] For many diseases, such as Fabry, most kindreds have private mutations.

Highly effective preconception carrier screening programs for populations at risk for Tay-Sachs disease have been in place since 1971,[6,8] leading to a great reduction in the number of affected children born. Carrier screening of Ashkenazi Jews has been expanded to include several other hereditary disorders found at higher frequency in this group.[9]

A single clinically defined disorder may be caused by more than one enzymatic defect, such as Sanfilippo disease (MPS III), that can be caused by a deficiency in any one of four hydrolases. Conversely, a disorder caused by a single enzyme deficiency usually gives rise to a spectrum of manifestations depending on the amount of residual enzyme activity and currently unknown modifiers. The age of onset, severity of symptoms, organ systems affected, and CNS manifestations can vary markedly, sometimes even within families. Although specific mutations or types of mutations can be associated with certain outcomes, genotype-phenotype correlations are typically not strong as with Gaucher disease (GD) patients with the same mutations who may present in childhood or be asymptomatic throughout adult life.[10] For women with X-linked lysosomal storage disorders such as Fabry disease, the severity and extent of disease manifestations may be determined primarily by the degree of X-chromosomal inactivation,[11] although evidence of random inactivation has been shown.[12]

Diagnosis and ascertainment

Probands are typically ascertained because of clinical signs and symptoms, often after the disease is advanced and interventions less efficacious. Presymptomatic individuals, the subject of this article, may be ascertained through screening of family members of the proband, carrier screening, prenatal testing, populations at risk for a genetic disorder, or newborn screening (NBS). As will be discussed for each disorder, diagnosis depends on enzymatic or molecular definition of mutations, or both.

Treatment of LSDs

Because of their wide-ranging medical and psychosocial ramifications, LSDs require an ongoing multidisciplinary, team approach to treatment. Comprehensive management generally

combines disease-specific therapy (if available) with symptom-specific measures. The team leader should be someone (generally a biochemical geneticist) who is experienced in treating LSDs, is aware of disease-specific complications and nuances of therapy, and keeps up to date with recent advances. Each patient's team should include other relevant medical specialists familiar with LSDs. Once a diagnosis is established, genetic counseling is essential to provide patients and their families with an understanding of mode of inheritance, identify at-risk family members, and discuss recurrence risks. Patient and parent support groups are invaluable sources of emotional support and practical advice.

Hematopoietic stem cell transplantation (HSCT) has been used successfully in the management of some LSDs. The rationale behind HSCT is that a reconstituted hematopoietic system from a healthy, matched donor will contain stem cells that can produce the missing enzyme. The small amounts of secreted enzyme are available to be taken up by mannose-6-phosphate receptors on other cells, endocytosed, and delivered to the lysosome. The major drawback to HSCT is its high morbidity and mortality, although both have improved over time, particularly with the use of refined conditioning regimens and cord blood as a stem cell source. Graft failure is more common in HSCT for some of the LSDs. The advantage of HSCT is that cells can integrate into many tissues, including the CNS. The disadvantages include the low level of correction and the time required for integration of the cells into other tissues, factors that currently preclude HSCT from being curative.

Specific treatments for LSDs are evolving rapidly with the involvement of an expanding number of biotechnology companies. Most widely used is enzyme replacement therapy (ERT), which supplies the missing enzyme exogenously through repeated intravenous infusions. With ERT, larger doses of enzyme can be administered than are attainable through HSCT; however, the blood-brain barrier (BBB) cannot be crossed, precluding the use of ERT for CNS disease. Even in patients with significant CNS involvement, ERT may be useful for reducing the morbidity associated with the somatic manifestations. The usefulness of ERT in the pre- and peri-HSCT period is being studied, and intrathecal ERT is being tested for MPS I and II. ERT is currently commercially available for Gaucher, Fabry, MPS I, II, VI, and Pompe diseases (PDs) and is undergoing clinical trials for MPS IVA and Niemann-Pick type B.

ERT is not without its challenges. Many patients do not produce native enzyme (and are cross-reacting immunologic material [CRIM]-negative) or make native enzyme that differs significantly from administered enzyme, and consequently make antibodies to the exogenous enzyme, which may reduce efficacy and often causes adverse infusion reactions. Fortunately, the infusion reactions are usually easy to treat, many patients develop tolerance over time, and allergic reactions are rare.

Oral therapies are available for two LSDs and more are being tested. Cysteamine is used successfully to preserve renal function in cystinosis.[13–15] Substrate reduction therapy (SRT) with *N*-butyldeoxynojirimycin (OGT-918, miglustat, Zavesca; Actelion, Basel, Switzerland) reduces production of glycosphingolipids by inhibiting glucosylceramide synthase, the first step of their biosynthesis. SRT is approved for use in GD, although side effects preclude its more widespread use,[16,17] and Niemann-Pick type C in Europe. A new-generation agent (Genz-11638; Genzyme Corporation, Cambridge, MA) is being tested that may have fewer side effects. For SRT to reduce lysosomal storage, there must be residual enzyme activity, which is always the case in GD but not in other disorders. Unfortunately, SRT does not reduce substrate turnover, resulting in cellular depletion of these evolutionarily conserved (and presumably important) glycolipids, a fact that may ultimately limit the utility of this therapeutic approach.

Oral small molecule chaperones are compounds that improve the folding and trafficking of lysosomal proteins with specific missense mutations. Clinical trials for Fabry disease are underway (Amicus Therapeutics, Camden, NJ). PTC124 (Ataluren®, PTC Therapeutics, South Plainfield, NJ) causes the ribosome to read-through nonsense codons and yet allows the ribosome to end translation normally at the correct stop codon. This drug, currently in testing for other conditions, could be useful for some patients with LSDs caused by nonsense mutations.

Gene therapy holds the promise of a cure for LSDs. However, many hurdles must be overcome before gene therapy can be applied to the LSDs including delivery to the correct cells, random integration, sustained expression, and immune reactions.

There is currently great variability in clinical practice for LSD treatment both within and among countries. Specific areas of controversy include when (and even if) to start specific therapies, what dose to use, how to monitor patients, when to stop treatments, and what adjunctive therapies should be used. Some of the variability is based on legitimate financial concerns given the expense of many specific therapies, but much has to do with the lack of long-term longitudinal studies with sufficient numbers of patients. Many available data comes from case reports, case series, clinical trials involving small numbers of patients, and voluntary patient registries as part of industry's postmarketing commitments to the drug regulatory agencies.

For many countries, expense is a large consideration in the treatment of LSDs. Insurance plans may have a lifetime cap for drug expenses that can be rapidly exhausted with most of the available therapies. Some health systems demand that each new therapy be demonstrated to be cost-effective, a difficult challenge for these rare disorders. Some have designed special funding programs for rare disease treatments. Less affluent countries are unable to afford the drugs or routinely use a low dose. Some help is provided to many patients without resources by assistance programs from the drug companies; however, most individuals worldwide receive supportive and palliative care, at best.

Caring for presymptomatic individuals, however, diagnosed highlights the current limitations in our diagnostic evaluations and decision making. In part, the difficulty is due to the often poor correlations of residual enzyme activity and genotype with the clinical phenotype. HSCT is a consideration for some disorders that may have CNS involvement. To be effective, HSCT has to be performed well before evidence of CNS involvement. Because phenotype-genotype correlations are imperfect, it will always be uncertain whether a particular newborn will need HSCT or not. Because HSCT has significant associated mortality and long-term morbidity, deciding if and when to transplant will be a major area of clinical difficulty, as discussed in the context of the individual disorders. Other areas of difficulty include the often variable clinical response to therapy, the long time required for improvement or stabilization to be evident for those who become affected, and the general lack of large natural history studies for comparison. Most disorders lack useful and accepted biomarkers for therapeutic decision making.

Newborn screening

Early detection of LSDs can be important for patients and their families and constitutes a major rationale for instituting NBS. For several disorders, it is clear that earlier initiation of therapy can make a substantial difference in outcome. The

LSDs are sufficiently rare that most practitioners are unaware of their signs and symptoms, leading to diagnostic odysseys and delayed diagnoses. By the time patients are diagnosed, they may have suffered irreversible damage, limiting the effectiveness of treatment. Many patients remain undiagnosed. A second affected child is often born before the first is diagnosed. There is much to be learned about what can be realistically achieved with earlier detection (e.g., the response of skeletal disease in MPS VI) as well as the true incidence and extent of each disease.

Testing from dried blood spots (DBSs) is now possible for several LSDs using the same blood spot sample and high-throughput platforms, making population screening technically feasible (Table 1).

However, only few data are available that address sensitivity and specificity of these assays. Nevertheless, the Centers for Disease Control and Prevention has already produced freely available quality control DBS material for several LSDs,[21] making high-throughput screening programs feasible. NBS for some LSDs has or will begin shortly as pilot programs (Pompe and Fabry diseases in Taiwan and Fabry disease in Washington State) or as additions to established NBS programs (Krabbe disease [KD] in New York State and Krabbe, Fabry, Pompe, Niemann-Pick, and Gaucher diseases in the States of Illinois and Missouri; Austria has piloted two studies on Fabry and Pompe diseases, respectively). At the same time, Pompe and Krabbe diseases were nominated to the US Advisory Committee on Heritable Disorders of Newborns and Children for inclusion in NBS. The Advisory Committee on Heritable Disorders of Newborns and Children did not consider the evidence to be sufficient to be able to recommend their inclusion at the current time.

As with any screening program, there are many ethical considerations in screening for LSDs. Variants of uncertain significance will certainly be identified. Adult-onset variants will be identified, perhaps in greater numbers than the early infantile forms of these diseases, and some patients with these may never develop symptoms or require therapy. Identification of both novel and adult-onset variants can lead to problems with insurability, labeling someone as vulnerable from birth, excluding from military service, etc. Consumers vary in their desire to detect late-onset disorders in the neonatal period and the acceptance of anxiety that some will face during a diagnostic evaluation for a positive screen. However, experience suggests that parents of patients and older patients with delayed diagnoses are almost universal in their support for early detection. Legislative changes will be needed to protect identified individuals from discrimination and ongoing counseling and support for patients and families will be required to minimize the psychosocial effects of early detection for adult onset LSDs. In this regard, in the United States, the Genetic Information Nondiscrimination Act provides legal protection against discrimination for health insurance or employment for individuals with a presymptomatic genetic condition.[22,23]

Any NBS system requires an organized network of centers for definitive diagnostic tests, genetic counseling, and treatment. Generally, care of LSD patients is coordinated by biochemical geneticists or metabolic disease specialists at centers equipped to handle the complex, multidisciplinary needs of LSD patients. Such trained individuals and centers are currently in short supply. Large geographic regions are entirely lacking in the necessary expertise. Even within centers, caring for LSD patients is time consuming, often requires expertise and facilities for the treatment of children and adults, and involves a significant amount of unreimbursed time from physicians and their staff. Many private payers will not authorize follow-up visits at LSD centers, under the erroneous belief that any physician is capable and willing to deal with complex therapies and their side effects, coordinating multidisciplinary care and dealing with anxious families. Even if the patient can be seen by the appropriate specialist, they may only make recommendations for testing and treatment that is then up to the primary care physician to arrange, something many are ill-equipped or unwilling to do. Many patients must travel great distances to receive weekly or biweekly drug infusions, even if a local infusion center is available and long after home therapy could be appropriate.

Table 1 Comparison of two newborn screening assays for specific LSDs that can be determined from the same newborn screening sample

Disorder	Immune-quantification[18]	MS/MS[19,20]
Fabry disease	Yes	Yes
Gaucher disease	Yes	Yes
Krabbe disease	Pending	Yes
Metachromatic leukodystrophy	Yes	Pending
MPS I	Yes	Yes
MPS II	Yes	Yes
MPS IIIA	Yes	—
MPS VI	Yes	Yes
Mucolipidosis type II/III	Yes	—
Multiple sulfatase deficiency	Yes	—
Niemann-Pick disease type A/B	Yes	Yes
Pompe disease	Yes	Yes

MS/MS, tandem mass spectrometry; MPS, mucopolysaccharidosis.

Another essential component of a LSD screening program is an experienced laboratory for rapid and accurate enzymatic and molecular testing. The laboratory must incorporate appropriate quality assurance and proficiency testing programs including sample sharing between laboratories. There are currently only a few laboratories around the world with the required expertise and experience.

A final important part of a NBS program is a well-designed, monitored, longitudinal follow-up program. This will allow definition of natural history and response to therapies, providing answers to the many outstanding questions not addressed by small pilot programs, case series, and industry-sponsored registries. Such a follow-up network should have a biological repository of samples to serve as a resource for identification and validation of biomarkers and modifier genes. These are precisely the charges of the new American College of Medical Genetics (ACMG)/National Institutes of Health (NIH) Newborn Screening Translational Research Network.

Purpose

This guideline is intended as an educational resource. It highlights current practices and therapeutic approaches to the diagnosis and management of individuals who may have a LSD that is identified by NBS, family screening through a proband, or because of carrier testing in at-risk populations and subsequent prenatal or postnatal testing. Rather than discussing all LSDs, this guideline focuses on select LSDs for which a NBS

test and some specific treatment are available or may become available in the near future. The goal is to provide some guidance for confirmatory testing and subsequent management as well as to define a research agenda for longitudinal studies, such as the Newborn Screening Translational Research Network being initiated by the ACMG with funding from NIH's Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Target audience

This guideline is directed at a wide range of providers, although care is commonly provided by metabolic disease specialists/biochemical geneticists and neuromuscular experts.

MATERIALS AND METHODS

Consensus development panel

An international group of experts in the (a) clinical and laboratory diagnosis, (b) treatment and management (cardiac, respiratory, gastrointestinal/dietary, musculoskeletal, neurologic, psychosocial, general medical, and supportive and rehabilitative), (c) NBS, and (d) genetic aspects of LSDs was assembled to review the evidence base and develop a guideline on the diagnosis and management of the presymptomatic LSD patient.

Following a meeting during which published material and personal experience were reviewed by the panel, experts in the various areas reviewed the literature (predominantly English language identifiable with a PubMed search) in these areas and drafted their appropriate guideline sections. All members of the panel reviewed and approved the final guidelines. Consensus was defined as agreement among all members of the panel. For the most part, the recommendations must be considered as expert opinion because additional levels of evidence were not available in the literature. Where available, evidence from clinical trials is used to guide recommendations. The guideline was reviewed by the ACMG Board and approved on August 23, 2010.

RESULTS: GUIDELINES FOR SPECIFIC LSDs

Pompe disease or glycogen storage disease type II (OMIM# 232300)

Synonyms

Acid maltase deficiency, acid α-glucosidase (GAA) deficiency.

Background

PD is due to intralysosomal accumulation of glycogen secondary to deficiency of GAA (EC 3.2.1.20). The resulting clinical phenotypic spectrum ranges from infantile to adult-onset. PD was first recognized by Dr. Pompe in a 7-month-old infant[24] and later named as PD.[25] PD was the first inborn error of metabolism to be recognized as a LSD.[26] The overall prevalence of PD is estimated to be approximately 1:40,000 in the Netherlands and in New York City.[27–29] The prevalence of infantile-onset PD is estimated to be 1:138,000 births in the Netherlands, 1:33,000 in Taiwan based on NBS, and it seems to be more frequent overall in the Chinese and Afro-American populations.[27,28,30,31]

Clinical phenotype

All patients with PD have variable but progressive, intralysosomal glycogen storage in skeletal, heart, and smooth muscles with resulting organ damage and ultimate organ failure. The rate of glycogen accumulation depends on residual enzyme activity, environmental factors (nutrition), muscle fiber type, physical activity, and as yet unknown genetic modifiers.[32] Patients with the same haplotypes around the mutant gene may in fact exhibit different clinical phenotypes.[33] Although PD is often classified into two separate categories—infantile-onset and late-onset—based on age of onset of symptoms, PD is a clinical disease spectrum.[34–37]

Infantile-onset PD. Patients with infantile (classic) PD present with progressive left ventricular hypertrophy and generalized muscular hypotonia (floppy infant) and typically die within the first year of life because of cardiorespiratory failure.[38–41] Significant cardiomyopathy may already be present in utero and readily detected by prenatal ultrasound. In addition, the electrocardiogram (ECG) may show conduction abnormalities including a short PR interval, characteristic tall QRS complexes, and Wolf-Parkinson-White syndrome in some patients.[34,37,40,42] Additional symptoms include macroglossia, hepatosplenomegaly, and feeding difficulties.[34,35,43] Patients usually present with disease symptoms at approximately 3 months of age and death occurs at a median age of 6.0–8.7 months.[40,43]

Late-onset PD. The leading clinical symptom in patients with late-onset PD ("nonclassic" childhood, juvenile, or adult-onset) is progressive muscle weakness due to initial involvement of the muscles of the proximal lower limbs and the paraspinal muscles. There is a significant early involvement of the diaphragm and accessory respiratory muscles, which leads to respiratory failure necessitating assisted ventilation, in some instances, even when patients are still ambulatory.[41] Occasionally, respiratory failure may be the presenting clinical symptom associated with frequent upper airway infections, orthopnea, sleep apnea, and morning headaches.[37,41] Cardiac involvement is typically not observed in late-onset PD although some patients do have rhythm abnormalities due to underlying Wolf-Parkinson-White syndrome and cardiac hypertrophy can be noted in some.[44–46] Vascular involvement of large intracranial blood vessels due to glycogen storage in smooth muscle cells leading to cerebral aneurysms has been reported.[37,47,48]

Current diagnostics

Biochemical markers. The use of acarbose to inhibit activity of the isoenzyme maltase glucoamylase made it possible for the first time to measure GAA activity reliably in leukocytes and DBSs.[49–51] GAA activity can be either measured using fluorometry or tandem mass spectrometry (MS/MS).[49,50,52,53] Although there is a correlation between GAA activity in fibroblasts and clinical phenotype, the clinical phenotype may not be readily predicted through enzyme analysis in different tissues.[54]

Serum creatine kinase, transaminases, and lactate dehydrogenase are increased in most patients with PD but may occasionally be within normal limits in those with adult-onset PD.[34] Muscle biopsies for primary diagnostic purposes are obsolete as the false-negative diagnostic rate may be significant.[34,41] Urinary hex 4 is a breakdown product of glycogen and is typically increased in the majority of patients with PD. Levels of excretion are higher in infants and those with significant disease burden. Levels have correlated with muscle biopsy glycogen content. It is useful for monitoring the clinical response to treatment.[55,56]

Molecular analysis. Two hundred eighty-nine different pathogenic mutations in *GAA* are known including nonsense,

missense, small deletions, insertions, and nonpathogenic mutations. Details on mutations and associated phenotypes can be found at http://www.pompecenter.nl/?Moleculaire_Aspecten.[57]

A new tool that estimates the severity of a particular *GAA* sequence variant has been introduced. The severity of a given *GAA* sequence variant is reflected in the quantity and quality of GAA precursor (110 kD) and modified precursor molecules (95 kD, 76 kD, and <20 kD) following transfection of COS cells.[57,58]

Molecular testing is the preferred technique for prenatal diagnosis, provided the genotype of the index patient is known. Alternatively, enzyme analysis in chorionic villi may be used.[41]

Ascertainment

A PD NBS pilot program in Taiwan used acarbose and 4-methylumbelliferyl-b-D-glucuronide (4-MUG) to measure GAA activity in DBS.[59] The screening program covers approximately 45% of the Taiwanese population, and the same laboratory provides Pompe diagnostic services for all of Taiwan. Between October 2005 and March 2007, more than 130,000 newborn infants were screened, and PD was diagnosed in four infants during their first month of life. In contrast, three infants were diagnosed during the same time period based on clinical symptoms alone between the age of 3 and 6 months. All infants except one in the screening group had infantile-onset PD and were started on ERT.[59] The recall rate for repeat blood tests was 0.82% and for clinical recall 0.091%.[59,60]

The use of MS/MS for enzyme analysis in DBS for the diagnosis of Fabry, Gaucher, Krabbe, Niemann-Pick, and Pompe diseases, respectively, has been evaluated.[51–53] The MS/MS technique for GAA analysis in DBS was further evaluated and validated on more than 10,000 anonymous newborn infants in Austria and 29 known patients with PD.[52] The recall rate in this study was 0.03%.[52]

Antibodies against epitopes of lysosomal proteins including GAA have also been used for detection in neonatal screening samples, although a formal validation on a larger number of samples has not been done.[61] Patients with PD and structurally intact epitopes may not be readily detected by this method.

Therapy

Alglucosidase alfa (recombinant GAA [rhGAA], Myozyme®/Lumizyme®; Genzyme Corporation) has been shown to be effective in the treatment of patients with early- and late-onset PD.[18,35,36,62–65] The individual response to ERT may vary due to development of rhGAA specific antibodies, age of presentation, rate of progression of disease, muscle fiber type, defective autophagy, and underlying genotype.[32,35] The development of rhGAA antibodies may be more frequent in patients with absent GAA protein (CRIM-negative) and have an impact on the prognosis of patients with infantile-onset PD.[32] Induction of immune tolerance to reduce rhGAA antibody formation has been evaluated in *GAA* knockout mice.[66,67] Success with a tolerance-inducing regimen including treatment with anti-CD20 monoclonal antibody (rituximab) plus methotrexate and intravenous gamma globulin has been reported in a CRIM-negative infant.[68] Clinical trials are ongoing in infants.

Neurological symptoms in infantile-onset PD were not readily observed due to early death within the first year. The advent of ERT and the increased survival rate in infants treated early have uncovered neurological manifestions of PD related to cochlear dysfunction and delayed myelination, and bulbar involvement.[69–71] The long-term outcome of surviving infants on ERT is unfolding.

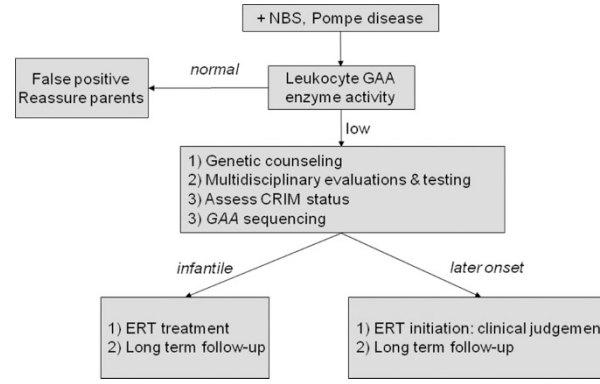

Fig. 1. Diagnostic algorithm for Pompe disease. NBS, newborn screening; GAA, acid α-glucosidase; CRIM, cross-reactive immunologic material; ERT, enzyme replacement therapy.

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 1.

1. Confirm the diagnosis by demonstrating GAA deficiency in a blood-based assay (DBS, leukocytes, and lymphocytes) or fibroblasts. Enzyme analysis in a blood-based assay is preferred due to the faster turnaround, lower costs, and reduced invasiveness.
2. Assess CRIM status by Western blot/mutation analysis for patients with infantile presentation (cardiac involvement in infancy).
3. Mutation analysis of the *GAA* gene.

Clinical follow-up and intervention.

1. Laboratory tests including serum creatine kinase, transaminases, lactate dehydrogenase, and urinary hex4.
2. Chest radiograph, ECG, and 2D echocardiogram.
3. Clinical evaluation including swallow, pulmonary, and neurological examination.
4. Prompt initiation of ERT in patients with infantile PD.
5. Evaluations every 6–12 months in the remaining patients.

It is important to identify patients with infantile PD as early as possible because ERT needs to be initiated as early as possible. The management of patients with infantile PD should be done at specialized centers with the appropriate expertise and back-up facilities. Under no circumstances should ERT be given at home or in peripheral potentially understaffed hospitals. Infantile patients are an anesthesia risk for infusion port placement and could develop airway problems should an infusion-related reaction occur. Close cardiology follow-up is required as cardiac remodeling occurs with ERT. A frank discussion with the parents is warranted regarding poor outcomes in CRIM-negative patients who typically do poorly on ERT alone.[72] The role of immune modulation in tolerance induction is emerging and data look promising.[68] Long-term issues should also be discussed.

Fabry disease (OMIM# 301500)

Synonyms

Anderson-Fabry Disease, angiokeratoma corporis diffusum, α-galactosidase A (α-gal A) deficiency.

Background

Fabry disease is a X-linked inherited lysosomal storage disorder caused by deficiency of the enzyme α-gal A (E.C. 3.2.1.22).[73] Affected patients have insufficient ability to degrade the membrane glycosphingolipid ceramide trihexoside (GL-3). The subsequent deposition of GL-3 in body tissues leads to the symptoms of the disease. No ethnic predilection exists for Fabry disease, which occurs in approximately 1:40,000 male births.[3] However, studies from select populations have shown a Fabry disease prevalence of 1:100–1:1000 male dialysis patients, 1:20–1:30 of "idiopathic" hypertrophic cardiomyopathy cases, and 1:20 male (1:40 female) patients with cryptogenic strokes.[74–79]

Clinical phenotype

Fabry disease causes significant morbidity and mortality in both hemizygous males and heterozygous females. The mean age of presentation for affected boys is 6–8 years; the typical presenting symptom is acute, episodic pain crises followed by chronic acroparesthesias.[80–82] GL-3 accumulation in the vascular endothelium and other cells leads to hearing loss, myocardial microvascular ischemia, dysrhythmias, hypertrophic cardiomyopathy, valvular insufficiency, gastrointestinal symptoms, hypohidrosis, temperature and exercise intolerance, dysregulation of vascular tone and autonomic functions, obstructive lung disease, progressive renal insufficiency leading to kidney failure, and increases the risk of cerebrovascular accidents and myocardial infarctions.[83–93]

Early death in hemizygotes occurs typically in the late fifth to early sixth decade from kidney failure, strokes, and cardiac events.[94–95] Heterozygous females, previously thought to be asymptomatic "carriers," can have significant symptomatology, generally at a later age than hemizygous men.[96–98] There is a "cardiac variant" of attenuated Fabry disease with hypertrophic cardiomyopathy as the predominant symptom, although these patients may develop milder symptoms in other organ systems.[99]

Current diagnostics

Biochemical markers. Reduced leukocyte α-gal A enzyme levels will be found in hemizygotes. As GL-3 storage begins prenatally, boys will have increased GL-3 levels in plasma and urinary sediment. LysoGL-3 may be a useful biomarker for the monitoring of treatment efficacy.[100] Heterozygote leukocyte enzyme activity and tissue GL-3 levels vary, are often in the "normal" range, and do not correlate with presence or severity of Fabry symptoms.[98,101]

Molecular analysis. Most pathogenic *GLA* mutations are "private" and nonrecurrent; more than 300 mutations have been described. In general, mutations that result in prematurely truncated α-gal A, which are approximately 45% of those reported, will result in a classical Fabry phenotype in a hemizygote.[102] Missense mutations that result in very low leukocyte α-gal A levels will also result in a classical phenotype. Because Fabry disease shows marked intrafamilial variability, predicting symptom severity, age of onset, and rate of progression is quite difficult even for a hemizygote with a mutation known to cause a classical phenotype. Mutations with residual α-gal A enzyme activity thought to consistently produce an attenuated phenotype (e.g., N215S)[103,104] have been reported in patients with classical disease.[105] For heterozygotes, intrafamilial variability, lack of correlation between biochemical markers and phenotype, and lyonization make presymptomatic prediction of phenotypic severity impossible. One pseudodeficiency allele, D313Y, has been described with low plasma α-gal A activity and slightly reduced leukocyte enzyme activity.[106] One study estimated the frequency of the D313Y allele to be 1 in 220 X-chromosomes, implying a 1 in 660 frequency in males.[107]

Ascertainment

Variant forms of Fabry disease with significant residual enzyme activity, including those who may not develop any symptoms, may be particularly common in NBS, up to 1:3,100–1:4600 male births, in one study.[108] Taiwan has also established a NBS program for Fabry disease and identified 42 male and 3 female infants with α-galactosidase mutations of 110,027 screened for a prevalence of 1:2400 live births and 1:1600 male births.[109] No data have been published regarding the sensitivity, specificity, false-positive rate, and positive predictive value of NBS for Fabry disease.

Therapy

Enzyme replacement therapy. Two versions of recombinant human α-galactosidase A (rhαGAL): alfa (Replagal®; Shire, Cambridge, MA) and beta (Fabrazyme®; Genzyme Corporation) have been developed. Results for clinical trials conducted on both versions have been published; in the United States, only rhαGAL beta was approved for treatment of Fabry disease, whereas, both forms are available in Europe, Australia, and Canada.[110–113] ERT with rhαGAL is the standard of care for symptomatic patients with Fabry disease.[86,114]

ERT with rhαGAL significantly reduces plasma GL-3 and tissue GL-3 storage in myocardium, kidney, and skin. Those treated with rhαGAL also demonstrated significant reduction in pain scores.[115] Subsequent studies have indicated that ERT also stabilizes renal function if initiated in patients with urinary protein excretion <1 g/24 hours. ERT also slows progression of renal insufficiency in those with significant proteinuria, improves pulmonary and gastrointestinal symptoms, and reduces renal, cardiac, and CNS events.[116–122] Women treated with ERT demonstrated reduced left ventricular hypertrophy as well as plasma and urinary GL-3[123]. ERT in children also reduced plasma and urinary GL-3 levels.[124] However, ERT cannot completely mitigate valvular disease, acroparesthesias, and risk for cerebrovascular accidents.

Adjunctive therapies such as statins and aspirin for reduction of thromboembolic risk factors, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers for treatment of proteinuria and hypertension, and various antiepileptic medications for the treatment of neuropathic pain are recommended as part of the comprehensive care of a patient with Fabry disease.[86]

Pharmacologic chaperone therapy. Clinical trials are being conducted in selected patients with missense *GLA* mutations using a competitive inhibitor of the α-gal A enzyme. In low concentrations, this inhibitor stabilizes misfolded (but functional) α-gal A as the enzyme is synthesized in the endoplasmic reticulum of the cell, allowing for transport into the lysosome where it can properly degrade GL-3.

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 2.

1. NBS will detect primarily hemizygotes; because of the variability in α-gal A enzyme activity in heterozygotes, it will likely fail to detect a substantial percentage (40–60%) of female infants with Fabry disease.[98,125]
   a. Because of this variability, any females identified by NBS will need molecular testing for confirmation.

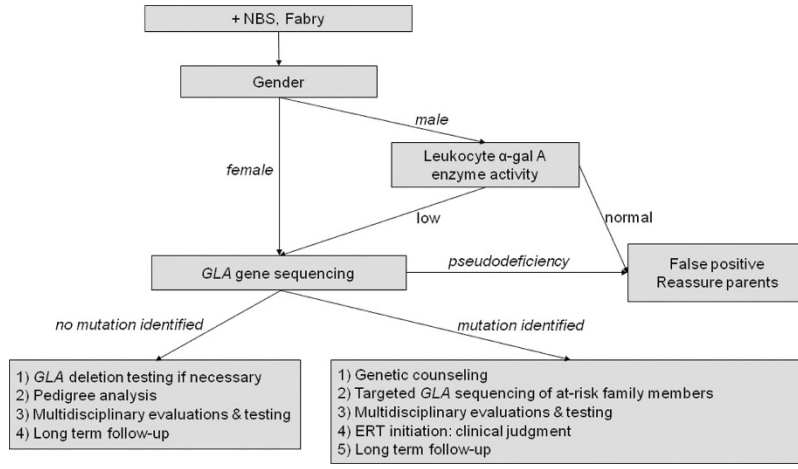

Fig. 2. Diagnostic algorithm for Fabry disease. NBS, newborn screening; α-gal-A, α- galactosidase A; ERT, enzyme replacement therapy.

   b. A male infant who screens positive for Fabry disease should have confirmatory testing performed by analyzing leukocyte α-gal A enzyme activity.
2. If the enzyme activity is low (in males) or a *GLA* mutation is found (in females), the infant should be referred for evaluation and genetic counseling at a metabolic center.
3. Confirmatory *GLA* sequencing should be performed in any male infant with low α-gal A enzyme activity, given the predicted high frequency of the D313Y pseudodeficiency allele.
   a. A detailed pedigree should be constructed to determine at-risk family members and testing offered, because most mutations are familial. If a mutation is not identified, pedigree analysis, measurement of biomarkers such as urinary GL-3, and molecular examination for deletions may clarify the patient's status.

Clinical follow-up and intervention. Management recommendations for ERT initiation and multidisciplinary follow-up have been published for both pediatric and adult Fabry patient.[86,126] Once the diagnosis of Fabry disease has been confirmed:

1. Baseline diagnostic studies (ECG, echocardiogram, ophthalmologic examination, renal function tests, plasma and/or urine GL-3) should be obtained. Affected members identified as a result of screening should also undergo identical evaluations; adults should also undergo additional testing as recommended.[86]
2. In global practice, there is wide variability in the usage of ERT even for hemizygotes, with some starting therapy at a young age even without symptoms and others waiting until end organ damage is evident. The decision to initiate ERT should be made according to the clinical judgment of the managing metabolic physician in conjunction with the family of the patient.
3. The infant should be seen by the metabolic specialist at 6-month intervals and monitored for onset of Fabry symptoms.

Gaucher disease

Synonyms

GD type 1, Nonneuronopathic GD (OMIM# 230800); GD type 2, acute neuronopathic GD (OMIM# 230900); GD type 3, chronic or subacute neuronopathic GD (OMIM# 231000); acid-β-glucosidase deficiency.

Background

GD is the most common lysosomal storage disorder, characterized by lysosomal accumulation of undegraded glucosylceramide because of deficiency or insufficient activity of the enzyme acid-β-glucosidase (glucocerebrosidase, glucosylceramidase, EC 4.2.1.25).[127] GD is a pan-ethnic disorder. Estimates concerning disease prevalence in the general population vary between 1:40,000[3] and 1:60,000.[128] In the Ashkenazi Jewish population, particularly, a high number of patients are observed with a calculated disease prevalence of approximately 1:800.[10] As a very rare variant, GD can also be caused by a deficiency of the nonenzymatic sphingolipid activator protein SAP C (or saposin C).[129–132]

Clinical phenotype

Based on characteristic patterns of clinical signs and age of onset, GD is subdivided into three main disease variants: type 1 (nonneuronopathic), type 2 (acute neuronopathic), and type 3 (subacute neuronopathic).[10] Although this categorization facilitates clinical management to a certain degree, it is important to realize that GD, like other lysosomal storage disorders, consists of a continuous spectrum of disease variants with "asymptomatic" and less severely affected type 1 patients at one end and severely affected type 2 and lethal in utero forms at the severe end of the clinical scale.[1,3] A detailed list of subtype- and system-specific disease manifestations of GD is given in Table 2.

In general, the type 1 patients who present in childhood tend to have more pronounced visceral and bony disease manifestations than those that present in adulthood.[127] Type 1 patients can experience growth retardation, delayed puberty, leukopenia, impairment of pulmonary gas exchange, and destruction of vertebral bodies with secondary neurologic complications.[10] There is an increased risk for multiple myeloma[133] and Parkinson disease.[134]

Table 2 Symptoms of Gaucher disease subtypes

	Type I (nonneuronopathic)	Type II (acute or infantile)	Type III (subacute or juvenile)
General	95% of Gaucher cases; childhood–adult onset; some symptomatic	1% of Gaucher cases; neonatal–infantile onset; rapidly progressive, fatal course	4% of Gaucher cases; infantile–childhood onset; subacute, slowly progressive
Visceral	Hepatomegaly (>80% of patients), splenomegaly (>90% of patients), interstitial lung disease, and pulmonary hypertension	Hepatomegaly, splenomegaly, hydrops fetalis (neonatal presentation), and interstitial lung disease	Hepatomegaly, splenomegaly, and interstitial lung disease
Hematopoietic	Anemia and thrombocytopenia	Anemia and thrombocytopenia	Anemia and thrombocytopenia
Orthopedic	Bony pain crisis, osteopenia, aseptic necrosis of femoral head, bony lytic lesions, bony infarctions, and pathological fractures	Arthrogryposis in severe cases, and generally death before bony abnormality	Bony pain crisis, osteopenia, aseptic necrosis of femoral head, bony lytic lesions, bony infarctions, and pathological fractures
Neurologic	No CNS involvement[a] and no cognitive regression	Bulbar palsies, hypertonicity, abnormal ocular saccades, and cognitive impairment	Oculomotor apraxia, myoclonic epilepsy, generalized tonic-clonic seizures, and cognitive impairment

[a]Except for an increased risk of Parkinson disease.

Some authors have proposed a subdivision of type 3 GD into three variants, depending on the most prominent disease symptoms. Variant 3a is characterized by rapidly progressive neurological manifestations (oculomotor apraxia, cerebellar ataxia, spasticity, refractory myoclonic seizures, and dementia) with variable visceral symptoms, whereas the 3b variant shows more pronounced visceral and bony symptoms with less severe, slowly progressive CNS involvement. A "3c" variant has been reported primarily in patients of Druze descent, with mild visceral disease, slowly progressive neurological manifestations, and unique to this subtype, cardiac valvular calcifications and corneal opacities.[10,127]

Current diagnostics

Biochemical markers. GD is most commonly diagnosed by demonstrating insufficient acid-β-glucosidase enzyme activity in peripheral blood leukocytes or DBSs on filter paper. Alternatively, cultured skin fibroblasts or, in the case of prenatal diagnosis, amniotic fluid cells and chorionic villi can be used as tissue source.[10] The measurement of β-glucosidase cannot reliably predict the disease phenotype or identify heterozygotes for GD.[10,127] In addition, patients with saposin C deficiency will be missed by determination of β-glucosidase enzymatic activity.[129,131,135]

Abnormally low enzymatic test results can be further corroborated by the demonstration of increased glucosylceramide levels.[136] Reflecting the high levels of macrophage activation in GD patients, chitotriosidase[137] and CCL18/PARC/MIP-418[138] show moderate to massive elevations in almost all patients. Although these biomarkers are not specific for GD and cannot be used to predict the subtype, their increase is usually far more pronounced than in other disorders with macrophage involvement. Apart from their role as supportive diagnostic tool, they can be used to monitor the efficacy of specific therapies (see below), although the correlation between the level of each biomarker and severity of active disease is limited or at least a matter of debate.[10] However, 5–6% of all GD patients are homozygous for a common 24-bp deletion in exon 10 of the chitotriosidase gene, which renders the enzyme inactive.[139] Alternative ancillary biomarkers comprise increased activities/concentrations of tartrate-resistant acid phosphatase, angiotensin converting enzyme, and plasma ferritin.[10,128]

Molecular analysis. Sequencing of the *GBA* gene is the definitive method to diagnose GD. Within the Ashkenazi Jewish population, four common mutations (p.N370S, p.L444P, c.84insG, and c.IVS2 + 1) account for 90% of the disease-causing alleles; these same mutations account for 50–60% of disease causing alleles in non-Jewish patients.[10] The p.L444P mutation accounts for nearly all disease-causing alleles in the Norrbottnian Swedish population, and the p.D409H mutation is responsible for the GD type 3c found in Druze kindreds. Recombinant (Rec) alleles contain several point mutations (including p.L444P) that arise as a result of gene rearrangements between *GBA* and a nonfunctional *GBA* pseudogene. Therefore, targeted mutation analysis of the p.L444P mutation cannot distinguish between isolated p.L444P mutations and Rec alleles, potentially leading to errors in genotype designation. A more detailed list of genotype-phenotype associations is given in Table 3. There are no known pseudodeficiency alleles for acid β-glucosidase.

Ascertainment

NBS programs for GD are expected to begin this year in at least two states in the United States. Given the high carrier frequency in Ashkenazi Jews, population-based prenatal carrier screening and testing of at-risk individuals in GD pedigrees

Table 3 Phenotype-genotype correlations in Gaucher disease (GD)

Genotype	Phenotype
p.N370S/any	GD type 1
p.L444P/p.L444P	GD type 3a or 3b
p.D409H/p.D409H	GD type 3c
p.L444P/recombinant	GD type 2
Recombinant/recombinant	GD type 2

have identified children and even identified older, currently "asymptomatic" GD type 1 individuals.

Therapy

GD type 1. To date, two options are available for the specific therapy of patients with GD type 1. The reference treatment is ERT and it was GD that served as model disease to establish the efficacy of this therapeutic approach.[10] The proof of concept studies date back to the early 1990s and used a modified human placental enzyme (alglucerase) to restore GBA activity in patients with GD.[140–142] In 1993, the recombinant successor enzyme (imiglucerase; recombinant human GBA; Cerezyme®, Genzyme Corporation) was introduced and numerous studies document safety and efficacy concerning major peripheral symptoms within the first year of treatment, whereas the response to bone abnormalities is less effective and may take at least several years.[10,143–145] Approximately 15% of treated patients develop IgG antibodies against the recombinant enzyme and approximately half of these patients show mild to moderate allergic adverse events, particularly during the first year of treatment.[10] In the majority of patients, antibodies disappear when ERT is continued with the same dosage,[126,146,147] and only a few patients develop therapy-limiting inhibitory antibodies.[10] A second form of ERT for Gaucher was recently approved for use (velaglucerase alfa, VPRIV®; Shire, Wayne, PA).[148] Finally, a third ERT product is being studied (taliglucerase alfa, UPLYSO®; Protalix Biotherapeutics, Carmiel, Israel).[149]

An alternative to ERT is SRT with *N*-butyl-deoxynojirimycin (Miglustat; Zavesca®; Actelion Pharmaceuticals, Basel, Switzerland).[16,17] SRT was shown to be effective concerning hepatosplenomegaly, anemia, and thrombocytopenia; by contrast, improvements of bone disease were delayed and limited.[144,150] Comparison of independent dose finding studies of both drugs suggest that SRT is similarly effective as a low-dose treatment with ERT, but less effective than standard- or high-dose enzyme replacement.[127] Therefore, SRT is currently only recommended as second-line therapy for adult patients with GD type 1, which either show severe side effects on ERT or refuse to receive ERT at all and have mild to moderate disease.[127] The profile of adverse effects on SRT comprises mild to moderate diarrhea (85–90% of patients), which usually resolves within the first year of treatment and is amenable to dietary changes and drug treatment, an initial weight loss of 6–7% (60% of patients), (sensory) peripheral neuropathy, transient tremor (30%), and possibly cognitive impairment.

GD types 2 and 3. Because of its rapid clinical progression, there is no specific therapy available for patients presenting with a GD type 2 phenotype. For patients with GD type 3, several therapeutic approaches have been tested in the past. In the pre-ERT era, a number of patients underwent HSCT, but long-term results have been poor.[10,151] In conjunction with the significant mortality risk associated with this treatment, HSCT is no longer recommended or performed for type 3 GD.

When ERT was established, studies with standard and high-dose treatment were performed despite the fact that only trace amounts of the currently used enzyme preparation cross the intact BBB, if at all.[152] The results were heterogeneous: some authors observed beneficial effects and an overall deceleration of mental and neurological deterioration,[153] whereas others could not demonstrate any significant therapeutic influence on the natural course of the neurological symptoms.[154,155] Notably, no study showed any advantage of high-dose regimens when compared with the standard treatment.[154,156] Finally, studies combining ERT and SRT were initiated, based on the rationale that miglustat passes the BBB.[157] Again, the results were ambivalent. Two case studies revealed stabilization[158] or even improvement[153] of neurological signs in symptomatic patients with GD type 3 and, over a 3-year observation period, demonstrated prevention of further neurological manifestations in a young child whose only initial manifestation was disturbed saccadic eye movements.[158] By contrast, a multicenter study investigating the efficacy of a combination treatment in a bigger patient cohort was recently terminated ahead of schedule as a result of disappointing intermediate results.

Future therapeutic approaches

Phase II clinical trials of a small molecule chaperone for acid β-glucosidase (Amicus Therapeutics, Camden, NJ) were recently completed, with disappointing results. A phase II clinical trial with another SRT (Genz-112638; Genzyme Corporation) aims to reduce the profile of side effects and has recently completed its primary endpoint. Further studies are ongoing.

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 3.

1. Leukocyte acid β-glucosidase enzymatic activity repeated.
2. If the GBA activity is low on the repeat specimen, GBA molecular confirmation and further evaluations should occur at a metabolic center as per the published recommendations[159–163] (https://www.lsdregistry.net/gaucherregistry/).

Clinical follow-up and intervention. Guidelines for the treatment of pediatric, adult, and female pregnant patients with GD type 1, and patients with GD type 2 have been published[159–163] (https://www.lsdregistry.net/gaucherregistry/). After confirmation of, and genetic counseling regarding the GD diagnosis:

1. Evaluations for anemia/thrombocytopenia, hepatosplenomegaly, and bony involvement should be performed.
2. For patients predicted to have neuronopathic GD, or for patients whose genotype cannot accurately predict phenotype, the degree of neurological impairment should also be assessed.
3. Gaucher biomarker and anti-GBA antibody levels should be measured before initiation of ERT.
   a. Type 3 GD patients should be started on treatment immediately;
   b. Treatment in type 1 GD patients should begin if two or more manifestations listed in the Table 2 are present.[159]
   c. Because of the lack of currently effective treatment for type 2 GD, only supportive care is recommended at this time.
4. Infants should be monitored at regular intervals (at least quarterly) to assess response to treatment and for development of additional Gaucher manifestations that may require additional interventions.

Krabbe disease (OMIM# 24520)

Synonyms

Globoid cell leukodystrophy.

Background

KD is caused by the deficiency of galactocerebrosidase (GALC; EC 3.2.1.46), a lysosomal β-galactosidase that is responsible for cleavage of galactosyl moieties from a variety of substrates including galactosylceramide, monogalactosyldiglyc-

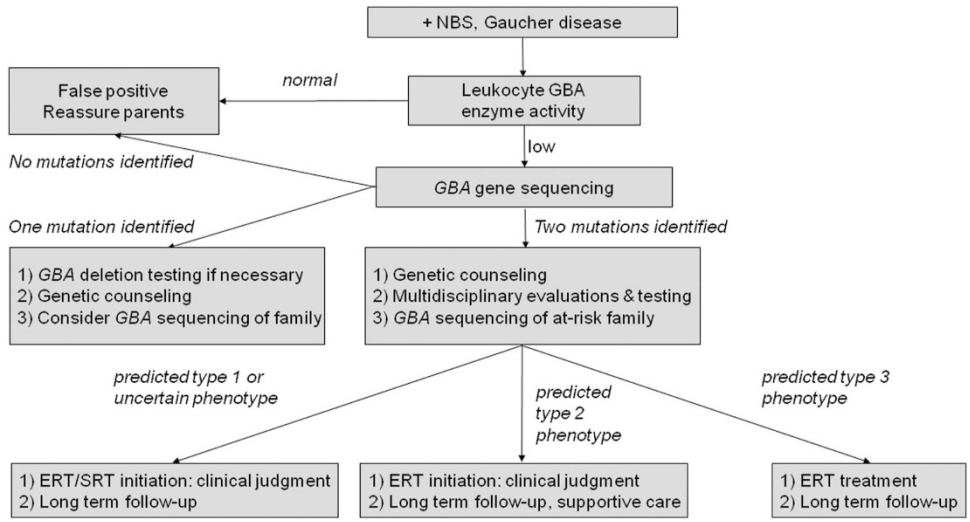

Fig. 3. Diagnostic algorithm for Gaucher disease. NBS, newborn screening; GBA, Acid-β-glucosidase; ERT, enzyme replacement therapy.

eride, and psychosine.[164] The name "globoid cell leukodystrophy" derives from the storage of myelin fragments and galactosylceramide in multinucleated macrophages (globoid cells) around blood vessels of affected white matter. KD is inherited as an autosomal recessive trait and more than 70 mutations, including missense, nonsense mutations, and small deletions in the *GALC* gene have been identified to date.[164,165] The resulting clinical phenotype is due to progressive damage of the white matter of the peripheral and CNSs and comprises a spectrum from early infantile KD (EIKD) to late-onset KD (LOKD).[166] The incidence of KD in Europe and the United States is estimated to be 1:100,000 newborns.[166,167] Based on these data before the onset of NBS for KD in New York, it was estimated that close to 90% of patients with KD may have the infantile form of disease. However, based on the data from the New York State NBS Program, the overall incidence of KD is approximately 0.91:100.000 and 0.26: 100,000 for EIKD based on the New York State case definition criteria (personal communication, JJ Orsini, 2009).[168]

Clinical phenotype

Early infantile-onset KD. Infants with EIKD typically present within the first months of life with progressive irritability, spasms upon noise stimulation, recurrent episodes of unexplained fever, blindness, and deafness.[166,169] The disease course is rapidly progressive, leading to frequent seizures, hyperpyrexia, hypersalivation, complete loss of social contact, and loss of bulbar functions. Death typically occurs within the first 2 years of age because of respiratory complications.[166] Peripheral neuropathy is always present in EIKD but may not be observed in LOKD. A detailed description of the natural history of KD from the Hunter's Hope Krabbe Family Database has been recently reported.[170]

Late-onset KD. Visual impairment, ataxia, and irritability, respectively, may be the first presenting symptoms in LOKD although age of onset may be highly variable.[171] All patients with EIKD show abnormal nerve conduction studies (NCSs), whereas approximately 90% of patients with EIKD have abnormal brainstem auditory evoked responses (BAER), 65% have an abnormal electroencephalogram, and 53% have abnormal flash visual evoked potentials (VER).[169,172] In contrast, only a small percentage of patients with LOKD show abnormal neurophysiologic studies.[172] Cranial magnetic resonance imaging (MRI) may show demyelination of white matter without any sign of peripheral nerve involvement.[173] Diffusion tensor imaging studies may help to identify early involvement of motor tracts in asymptomatic neonates with KD.[174]

Current diagnostics

Biochemical markers. Diagnosis of KD is made by demonstration of low GALC activity in leukocytes or DBSs.[164]

Molecular analysis. Confirmation of the diagnosis can be made by molecular analysis of the *GALC* gene.[164,165] Genotype-phenotype correlation is limited and may only be possible if the clinical impact of a particular genotype is known in a larger set of patients with KD.[165,175] In principle, homozygosity for the 30-kb deletion may predict EIKD.[166] Occasionally, patients with LOKD carry two severe mutations that abolish enzyme activity completely.[176] There is variability in presentation even with the same genotype.[167]

Ascertainment

New York State Laboratories, Wadsworth Center Albany, New York, started NBS for KD using MS/MS technology in August 2006. Through June 2009, 769,853 newborn infants were screened (personal Communication, JJ Orsini, 2009).[51,177,178] Out of a total of 140 recalls (recall rate 0.018%), two infants were identified to have EIKD and were transplanted; one died of transplant complications. Five additional infants were confirmed to have low enzyme activity but were not transplanted and are currently followed up very closely. An additional 13 and 36 infants were found to have moderately low or borderline low enzyme activity, respectively. All infants identified with low enzyme activity are being followed up by the Krabbe Disease Consortium in New York State.[178]

Therapy

The only therapy at present is early allogeneic hematopoietic stem cells (HSCs) or cord blood transplantation.[179,180] Escolar et al.[180] reported on the use of cord blood transplantation after

myeloablative chemotherapy in 11 asymptomatic newborns and 14 symptomatic infants with EIKD. Presymptomatic infants before transplantation continued to show psychomotor development and gain of milestones. Symptomatic infants only showed minimal neurologic improvement after transplantation.[180] A review of 25 cases of presymptomatic infants transplanted for EIKD from different transplant centers from the United States and Canada demonstrated an overall mortality rate of 15%.[168] Despite successful engraftment, most transplanted infants developed signs of neurological disease related to KD.[168]

Recommended follow-up procedures

Diagnostic confirmation. The diagnosis should be confirmed by demonstrating (1) GALC deficiency in leukocytes and (2) mutation analysis of the *GALC* gene.

Clinical follow-up and intervention

1. Early (preferably younger than 30 days of age) bone marrow/stem cell transplantation from cord blood should be considered in any case predicted to have EIKD (e.g., homozygosity for the 30-kb deletion, compound heterozygosity for the 30-Kb deletion, and another severe mutation with very low GALC activity). In most cases, the genotype cannot predict phenoytype.
2. Other individuals requires follow-up at regular, 6–12 monthly intervals.
3. Although there are no data on the appropriate follow-up studies, they could reasonably include the following: (a) neurologic examination, (b) cranial MRI, (c) neurophysiologic studies (BAER, VER, electroencephalogram, and NCS), (d) lumbar puncture (for cerebrospinal fluid protein), if subtle neurological signs are present, and (e) diffusion tensor imaging studies that may help to identify early involvement of motor tracts in asymptomatic neonates with KD.[174]

Metachromatic leukodystrophy (OMIM# 250100)

Synonyms

Arylsulfatase A (ARSA) deficiency.

Background

Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder caused by insufficient enzymatic activity of ARSA (E.C. 3.1.6.8). This enzymatic defect results in moderate to massive accumulation of sulfated glycolipids, in particular, galactosylceramide-3-O-sulfate (sulfatide), in the brain, peripheral nervous system, and kidneys.[181,182] Although the age of onset and dynamics of disease progression vary, MLD is primarily characterized by progressive neurodegeneration of the central and peripheral nervous systems.

MLD is a panethnic disorder; depending on the population studied, incidents estimates for the most common subtype, late infantile MLD, vary considerably between 1:40,000 (Sweden, Washington State) and 1:170,000 live births (Germany).[183] Of note, certain ethnic populations show significantly higher incidence rates such as the Habbanite Jewish population (1:75), Alaskan Eskimos (1:2500), and Navajo Indians (1:6400).[183]

Two other biochemical defects have been identified that result in a MLD or MLD-like phenotype. Several patients described with a MLD phenotype were found to have a deficiency of the nonenzymatic sphingolipids activator protein saposin B (OMIM# 249900).[184–186] Multiple sulfatase deficiency (MSD), caused by mutations in the sulfatase activator enzyme sulfatase modifying factor 1 (SUMF1) (OMIM# 272200), not only results in progressive demyelination of the central and peripheral nervous systems but is also accompanied by ichthyosis and features of MPS.[182,183]

Clinical phenotype

Based on age of disease onset, MLD has been divided into three main subtypes: late infantile, juvenile, and adult MLD. As in other lysosomal storage disorders, this classification facilitates clinical management but ignores the fact that MLD comprises a phenotypic continuum. The phenotypes and natural histories of each subtype are summarized in Table 4. Although disease progression in late infantile MLD is more uniform in both presentation and dynamics, the juvenile and adult forms are considerably more variable. Patients with the latter two types may manifest with primarily neurologic symptoms of clumsiness, gait disturbance, worsening of coordination, and fine motor skills, or with primarily psychiatric symptoms of bizarre behaviors, emotional lability, personality changes, or even psychotic episodes. Although disease progression toward complete loss of all cognitive skills and function is observed in most patients, some experience periods of disease stability punctuated by episodic deterioration.[182]

Sulfatide deposition occurs in the gallbladder, leading to papillomatous transformation that can be noted on abdominal ultrasound. Cerebrospinal fluid protein levels are generally increased, exceeding 50 mg/dL, in most MLD cases except the adult onset type. BAER and VER testing demonstrate impairment of hearing and vision. NCS velocities are slowed, reflecting peripheral demyelination and neuropathy. Demyelination of the CNS is evident on brain MRI initially as symmetric, nonenhancing periventricular and subcortical T2 white matter prolongation. With disease progression, cortical atrophy and ventriculomegaly become apparent. A scoring system for MRI has been developed for MLD.[187]

Current diagnostics

Biochemical markers. Deficient or insufficient residual activity of ARSA in peripheral blood leukocytes or cultured fibroblasts are a necessary, but not sufficient, condition for the diagnosis of MLD. ARSA "pseudodeficiency" is a relatively common variant that is found in 1–2% of the European and Euro-American individuals who have 5–15% of normal ARSA enzymatic activity but no sulfatide excretion or evidence of pathologic storage. These individuals never develop any disease-related clinical symptoms throughout their lives.[188,189] Because of the high prevalence of ARSA "pseudodeficiency," any positive biochemical test result must obligatorily be corroborated by a second analytical test system, such as *ARSA* sequencing or measurement of urinary sulfatides (or molecular analysis), because patients with all types of MLD excrete increased levels of these compounds.[182,190,191] Enzymatic assays using artificial substrates are inappropriate to predict possible disease phenotypes.[183] Although MLD can usually be distinguished from MSD based on phenotype alone, the measurement of a second sulfatase enzyme activity should be considered. In contrast to other lysosomal storage disorders, no other biomarkers are currently available for MLD.

Molecular analysis. The diagnosis of MLD can also be confirmed by molecular genetic analysis of the *ARSA* gene. To date, more than 140 disease relevant mutations have been identified (for details, see the Human Genome Mutation Database http://www.hgmd.cf.ac.uk/ac/gene.php?gene = ARSA). Several recurrent mutations have been observed that account for up to

Table 4 Natural history of metachromatic leukodystrophy (MLD) subtypes

	Late infantile type	Juvenile type	Adult type
General	40–60% of MLD cases, onset 6 months to 4 years, uniform disease course, and death within 5 years of onset	20–35% of MLD cases, onset 4 to 16 years, more variable disease progression, and death within 10–20 years of onset	15–25% of MLD cases, onset following puberty, variable velocity of progression, death within 5–20 years of onset
Initial symptoms	Peripheral neuropathy, muscle weakness, appendicular hyptonia, and hypo- or areflexia	Decline in school performance, emotional, behavioral disturbances, dysarthria/ataxia, and hyperreflexia	Decline in school/job performance, emotional lability, disorganized thinking, and hallucinations/delusions
Subsequent symptoms	Mental regression, visual and auditory impairment, loss of ambulation, evolution to hypertonia, dysarthria/ataxia, bulbar paresis/dysphagia, and seizure disorder (25%)	Mental regression, optic atrophy, loss of ambulation, spastic quadriplegia, incontinence, bulbar paresis/dysphagia, and seizure disorder (50%)	Clumsiness, incontinence, spastic quadriplegia, choreiform movements, dystonia, bulbar dysfunction, and seizures rare
End stage	Vegetative state, complete loss of interaction, and death from aspiration pneumonia	Vegetative state and decerebrate posturing	Vegetative state

60% of disease-relevant alleles in certain populations.[182,183,192] *ARSA* mutations characterized in more detail have been divided into two groups: (1) "null alleles" such as c.459 + 1g>a (25% of disease alleles) and c.1204 + 1g>a that result in complete loss of enzymatic activity and (2) "R alleles" such as p.P426L (25% of disease alleles) and p.I179S (12.5% of disease alleles) that allow the synthesis of ARSA enzyme with residual catalytic activity of up to 5% of normal.[182,193] Based on this classification, genotype-phenotype correlations have been proposed[194] and further corroborated[192,195,196] to predict, in limited fashion, the clinical presentation and natural history (see Table 5). Although the predictive value of this correlation is excellent for patients homozygous for two null alleles, patients with one and two R alleles show considerable phenotypic variability, implicating other genetic and/or environmental factors that contribute to the disease course.[182,183,192] Consequently, reliable prognostication in these cases is not possible.

To date, two pseudodeficiency-related sequence variations have been identified that can occur independently or together in *cis*. One, c.*96A>G, destroys the polyadenylation signal 95 bp downstream of the translation termination codon[197,198] and results in a markedly decreased synthesis of a catalytically normal enzyme. The other, p.N350S, abolishes the *N*-glycosylation site of the ARSA enzyme and causes aberrant targeting of the protein away from the lysosome.[198]

Ascertainment

To date, one high-throughput screening system for the reliable detection of ARSA deficiency in DBS has been proposed, but no NBS programs have actually begun to screen for MLD.[199] A high false-positive rate is anticipated as a result of the high prevalence of pseudodeficiency alleles in many populations and will be problematic for any MLD NBS program. Given the high frequency of pseudodeficiency alleles, a homozygous pseudodeficient genotype is approximately 400 times, and a MLD/pseudodeficient genotype 30–50 times more common than a true MLD/MLD genotype.[182]

Table 5 Genotype-phenotype correlations in metachromatic leukodystrophy (MLD)

Genotype	Phenotype
Null/null	Late infantile MLD
Null/R	Juvenile MLD (less frequently adult MLD
R/R	Adult MLD (less frequently juvenile MLD)
p.P426L/p.P426L	Adult MLD (neurologic subtype)[181,194]
p.I179S/other	Adult MLD (psychiatric subtype)[181,194]

Therapy

Late infantile MLD. Therapeutic options are at present very limited in MLD. For late infantile MLD, no approved specific therapy exists at all and treatment efforts are restricted to palliative and/or supportive measures including the prevention or delay of secondary complications.[181,183,193] Early HSCT at a presymptomatic stage is completely ineffective and is not recommended.[193,200]

Juvenile and adult MLD. Because of the less rapid disease progression, HSCT has been established for several years as the only specific therapeutic option for juvenile and adult forms of MLD.[181,201,202] Notably, HSCT harbors substantial risks and its real long-term effects are still unknown[182,183]. According to the current experience, when performed before onset of clinical symptoms, HSCT is able to stabilize cerebral demyelination and arrests or slows, disease progression in later-onset forms of MLD.[182,201,202] On the other hand, HSCT does not arrest or ameliorate disease progression in the peripheral nervous system,[201,202] and patients with successful HSCT have developed severe, peripheral neuropathy-related motor deficits several years after transplantation.[193]

Future therapeutic approaches

A number of alternative therapeutic concepts are currently being developed and investigated. Phase I studies of intrathecal recombinant human ARSA have been completed, and phase II studies are recruiting patients at the time of this writing. Other modalities include cotransplantation of HSC and mesenchymal stem cells,[203,204] umbilical cord blood transplantation,[205,206] ex vivo HSC gene therapy, in vivo and cell-based gene therapies, and coexpression strategies with recombinant ARSA and the formylglycine-generating enzyme.[181,193]

Recommended follow-up procedures

Diagnostic confirmation. The high frequency of pseudodeficiency alleles must be kept in mind when counseling a family whose newborn has been screened positive for MLD or an individual detected because of a prior affected family member/carrier screening in high-risk populations. Consequently, confirmation of the diagnosis must include (1) analysis of urinary sulfatides and (2) *ARSA* gene sequencing.

Clinical follow-up and intervention.

1. Presymptomatically identified MLD patients should be followed at regular intervals by both a neurologist and a metabolic physician.
2. Those predicted to have juvenile and late-onset MLD should be referred for a HSCT evaluation, recognizing that even early HSCT is ineffective for peripheral demyelination.
3. Periodic brain MRI imaging to monitor the status of CNS demyelination should be performed to allow for scoring and monitoring of response to therapy.

Care of late infantile MLD patients is currently limited only to palliative and supportive measures. Given the frequency of null alleles and the lack of treatment for the potentially high percentage of newborns to be identified with the late infantile type, it is questionable whether NBS should be considered for this disorder at the present time.

Niemann-Pick disease, types A (OMIM# 257200) and B (OMIM# 607616)

Synonyms

Lysosomal acid sphingomyelinase deficiency, sphingomyelin lipidosis.

Background

Deficiency of lysosomal acid sphingomyelinase (ASM; E.C. 3.1.4.12), encoded by the sphingomyelin phosphodiesterase-1 (*SMPD1*) gene, results in types A and B Niemann-Pick disease (NPA and NPB, respectively). Undegradeable sphingomyelin accumulates primarily in CNS neurons and reticuloendothelial cells. Collectively, both types occur in approximately 1 in 250,000 live births; NPA is seen more frequently and NPB less so in the Ashkenazi Jewish population with an incidence of 1:40,000 live births, whereas NPB is more common in individuals of Northern African descent.[207]

Clinical phenotype

Full details regarding symptomatology of NPA and NPB are given in Table 6. In general, NPA is characterized by neonatal-onset disease, neurodegeneration, and early death.[208] NPB has a more variable presentation, but age of onset is typically in later childhood or adulthood. Primary symptoms are related to hepatosplenomegaly and impaired pulmonary function due to accumulation of sphingomyelin in reticuloendothelial and pulmonary tissues.[208,209] With a few rare exceptions, cognition is spared.[211]

Current diagnostics

Biochemical markers. ASM activity assayed from fibroblasts or leukocytes is <5% of normal controls in NPA patients and between 2% and 10% of normal in those with NPB.[212] Because of the overlap in enzymatic activity between NPA and NPB, enzyme assay alone is unreliable in predicting phenotype. For similar reasons, enzyme activity cannot differentiate carriers from normal individuals. Postmortem studies in brains of patients with Niemann-Pick disease demonstrate markedly increased sphingomyelin levels in NPA and normal sphingomyelin in NPB.[213]

Affected patients may have increased serum transaminases, reduced fasting levels of high-density lipoprotein cholesterol, and increased low-density lipoprotein. Patients also demonstrate progressive anemia and thrombocytopenia. The characteristic finding in biopsy specimens from liver, lung, or bone marrow is the "foam cell," a large cell of histiocytic origin that is swollen with stored lysosomal lipid. Infiltration and accumulation of foam cells into body tissues leads to the visceromegaly, pulmonary compromise, and marrow dysfunction seen in both forms of the disorder.

Molecular analysis. Sequencing of the *SMPD1* gene is the most reliable method to confirm a diagnosis of NP. In the Ashkenazi Jewish population, three founder mutations p.R496L, p.L302P, and fsP330 account for more than 95% of mutant alleles and are associated with the NPA phenotype.[207] Non-Jewish NPA patients generally have "private" *SMPD1* mutations. NPB occurs in all ethnic backgrounds but is rarer in Ashkenazi Jews and more frequent in Northern Africans. The p.[Delta]R608 mutation predicts a NPB phenotype, even when found in trans with a NPA mutation,[214] and is thought to be protective against cognitive impairment. A few other mutations are also thought to be neuroprotective.[211] Individuals with at least one p.Q292K mutation had later-onset neurologic abnormalities such as mental retardation, expressive language delay,

Table 6 Symptoms of Niemann-Pick Types A (NPA) and B (NPB)

	NPA[207]	NPB
Constitutional	Onset in early infancy, failure to thrive, and early death at 27 months of age (mean)	Onset in childhood/adulthood and death from pulmonary disease[208]
CNS	Hypotonia at 7 months of age (mean), cognitive progression up to 8 months (mean), cognitive stagnation and regression, loss of deep tendon reflexes, eventual loss of interaction with environment, and dysphagia and aspiration	No neurocognitive impairment,[209] hyporeflexia, and peripheral neuropathy
Ophthalmologic	Retinal "cherry red spot"	Retinal "cherry red spot"
Gastrointestinal	Neonatal cholestatic jaundice, hepatosplenomegaly, feeding difficulty, and vomiting	Massive hepatosplenomegaly, hypersplenism, liver cirrhosis, and portal[210] hypertension
Pulmonary	Recurrent respiratory tract infections, infiltrative interstitial lung disease, and aspiration pneumonia	Infiltrative lung disease and chronic hypoxia

areflexia, and abnormal retinal findings.[211] This mutation seems to be more prevalent in the Czech and Slovak populations.[215]

Ascertainment

Early ascertainment is currently only through prenatal carrier or family-based testing. No pilot NBS programs have been established for NPA/NPB.

Therapy

Hematopoietic stem cell transplantation. Allogeneic or cord blood stem cell transplantation is ineffective at preventing neurocognitive regression in NPA, despite full donor engraftment.[216,217] Allogeneic stem cell transplantation was reported in three NPB patients.[93,219–221] Significant transplant-related complications were reported in all patients: poor linear growth, inadequate weight gain, and chronic graft-versus-host disease requiring immunosuppressive therapy. One patient experienced hepatic veno-occlusive disease with her first HSCT, developed graft failure, and required a second HSCT.[93] Although all three patients had normalization of leukocyte ASM enzyme activity, another patient experienced stagnation and regression of developmental milestones. At the time of the report, she was 18 years old, wheelchair bound, gastrostomy feeding dependent, with no verbal communication.[219] All three showed resolution of pulmonary involvement and hematopoietic abnormalities and incomplete improvement in visceromegaly.

Enzyme replacement therapy. Clinical trials are in progress to determine the efficacy of ERT with recombinant human acid sphingomyelinase in patients with NPB (Clinical trials identification number NCT00410566).

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 4. An infant with a positive newborn screen for NP should first have leukocyte ASM activity, transaminases, bilirubin levels, and lipid profile assayed. The infant and family should then be referred for evaluation and genetic counseling at a metabolic center. If the ASM activity is low, then *SMPD1* gene sequencing should be obtained to determine the causative mutations. Mutations with clear phenotypic correlations will allow for prediction of type A or B disease. *SMPD1* targeted gene sequencing should be recommended for any identified at-risk family members.

Clinical follow-up and Intervention. Once NP has been confirmed, the infant should be evaluated by an ophthalmologist with a dilated funduscopic examination. Plain radiographs of the chest and abdominal ultrasound should be performed at regular intervals to document the extent of pulmonary involvement and hepatosplenomegaly. The metabolic physician should evaluate the infant on a monthly basis, documenting weight gain, linear growth, pulse oximetry, and developmental progression. Eventually, the infant will need evaluation and regular follow-up by neurology and pulmonology as the disorder progresses. Because no curative treatment currently exists, only symptomatic and supportive care can be provided. Lipid lowering drugs (e.g., statins) are ineffective.

MPS type I

Synonyms

MPS I-H, Hurler syndrome, severe MPS I (OMIM# 607014); MPS I-HS, Hurler-Scheie syndrome, intermediate MPS I (OMIM# 607015); MPS I-S, Scheie syndrome, attenuated MPS I (OMIM# 607016).

Background

MPS I is caused by a deficiency of α-L-iduronidase (EC 3.2.1.76), encoded by the *IDUA* gene. α-L-iduronidase participates in the degradation of heparan and dermatan sulfate, two glycosaminoglycans (GAGs) found in nearly all body tissues. Consequently, α-L-iduronidase deficiency results in a disease that involves multiple organ systems resulting from the accumulation of undegradable GAG material throughout the body. The population frequency of MPS I is estimated to be approximately 1 in 100,000 births, with MPS I-H the most common and MPS I-S the rarest of the subtypes.[222,223] MPS I-H is especially common in the Irish Traveler population, with an incidence of 1 in 371 live births.[223]

Clinical phenotype

Disease manifestations of MPS I span a continuum of severity and age of onset, with Hurler syndrome representing the most severe end of the clinical spectrum with the earliest onset and presence of neurocognitive regression, Scheie syndrome the attenuated end of the clinical spectrum with later age of onset, and Hurler-Scheie syndrome used to describe patients with intermediate disease severity and symptom onset. A detailed list of subtype-

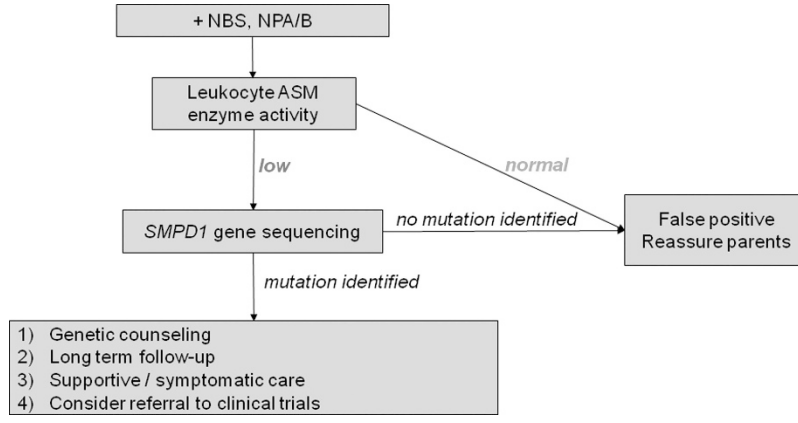

Fig. 4. Diagnostic algorithm for Niemann-Pick A (NPA) and B (NPB). NBS, newborn screening; ASM, lysosomal acid sphingomyelinase.

and system-specific disease manifestations of MPS I is given in Table 7. Emphasis must be made on the nonuniform nature of symptom severity; in other words, a patient with "intermediate" MPS I based on lack of cognitive involvement may have severe orthopedic disease and cardiac valvular dysplasia, for example.

Current diagnostics

Biochemical markers. α-L-iduronidase activity in MPS I is markedly reduced compared with normal controls. As a general rule, patients with MPS I-H have undetectable α-L-iduronidase activity whereas patients with MPS I-HS and MPS I-S have residual α-L-iduronidase activity. Evidently, as little as 0.4% of normal enzyme activity is sufficient to produce a mild phenotype.[224] Enzymatic activity alone is unreliable for prediction of phenotype because some MPS I-H patient fibroblasts had more enzyme activity than those from MPS I-HS patients; similarly, there were MPS I-HS cell lines with more activity than MPS I-S cells.[224,225] Enzymatic analysis is also insufficient for carrier testing because of overlap in activity between normal individuals and heterozygotes.[19]

Molecular analysis. Certain *IDUA* mutations allow for prediction of the phenotype. Homozygosity or compound heterozygosity for the p.Q70X and p.W402X nonsense mutations predict a MPS I-H phenotype. p.Q70X and p.W402X are also the two most common mutations in Caucasian MPS I patients, accounting for 60–70% of mutant alleles in those populations.[226] The presence of two nonsense mutations is predictive of a MPS I-H,[226] although one 20-year-old homozygous p.W402X patient was described as having MPS I-S without further description of her phenotype.[227] The p.R89Q missense and the c.678-7g>a (IVS5-7g>a) splice site mutations predict a mild phenotype.[228–232] All three subtypes of MPS I have been reported in patients with the homozygous p.P533R mutation; both MPS I-H and MPS I-HS have been reported with p.P533R compound heterozygotes with other "severe" mutations.[226] A rare p.A300T pseudodeficiency allele has been reported in one family.[233]

Ascertainment

Early ascertainment is currently only accomplished through family-based testing. As of yet, no NBS programs for MPS I have been established.

Therapy

ERT for MPS I-HS and I-S. Results for clinical trials with recombinant human α-L-iduronidase (laronidase) (rhIDU, Aldurazyme®; Genzyme Corporation) have been published.[234,235] Weekly ERT with 0.58 mg/kg/dose of rhIDU improved forced vital capacity and reduced symptoms of airway obstruction, apnea/hypopnea index, and duration of nighttime desaturation episodes. Exercise tolerance was increased, as patients receiving laronidase had significant improvement in the distance traveled during the 6-minute walk test compared with placebo. Liver and spleen volumes were reduced to near normal levels. Patients also demonstrated improvement in weight gain and linear growth velocity. Some improvement was also seen in restriction of joint mobility.[234] A similar efficacy profile was noted in MPS I-H patients receiving ERT.[235] Urinary GAG excretion was reduced by 55–60% to levels at or below the upper limit of normal.[235,236] ERT does not seem to adequately treat the orthopedic manifestations of MPS I, especially with regard to spinal cord compression and vertebral dysplasia. Clinical trials are underway to determine whether intrathecal rhIDU

Table 7 Symptoms of mucopolysaccharidosis I (MPS I)

	"Severe" MPS I, MPS IH	"Intermediate" MPS I, MPS IHS	"Attenuated" MPS I, MPS IS
General	Early (<12 months) onset, rapid disease progression, hepatosplenomegaly, hernias (inguinal, umbilical, and hiatal), and death in first decade if untreated	Intermediate onset, hepatomegaly, and hernias (inguinal, umbilical, and hiatal)	Childhood onset, hernias (inguinal, unbilical, and hiatal), and normal life expectancy
Cognition	Normal early development, developmental delay/plateau, and neurocognitive decline	Learning disability possible and attention deficit possible	Typically no symptoms
Neurologic	Communicating hydrocephalus	Cervical spinal cord compression and cervical instability	Cervical spinal cord compression and cervical instability
Ophthalmologic	Corneal clouding and open-angle glaucoma	Corneal clouding and open-angle glaucoma	Corneal clouding, open-angle glaucoma, and retinal degeneration
Otolaryngological	Chronic recurrent rhinitis, persistent nasal discharge, obstructive sleep apnea, recurrent acute otitis media, and mixed hearing loss	Chronic recurrent rhinitis, persistent nasal discharge, obstructive sleep apnea, recurrent acute otitis media, and mixed hearing loss	
Cardiac	Valvular dysplasia and insufficiency, cardiomyopathy Cor pulmonale (especially with sleep apnea), myocardial infarction	Valvular dysplasia and insufficiency Cor pulmonale (especially with sleep apnea)	Valvular dysplasia and insufficiency
Orthopedic	Vertebral dysplasia, Kyphosis/lumbar gibbus, hip dysplasia/dislocation, global restriction of joint mobility, carpal tunnel syndrome, short stature, and osteopenia/osteoporosis	Vertebral dysplasia, kyphosis/lumbar gibbus, hip dysplasia/dislocation, global restriction of joint mobility, Carpal tunnel syndrome, short stature, and osteopenia/osteoporosis	Lumbar spondylolisthesis, lumbar spinal compression, joint stiffness, Carpal tunnel syndrome, milder short stature, and osteopenia/osteoporosis

infusion is effective for these manifestations (Clinical Trials identification number NCT00215527).

Nearly all patients developed IgG antibodies to laronidase. Development of antibody was not associated with changes in urinary GAG levels, and titer levels decreased with continued infusions. Adverse effects of laronidase infusion were usually infusion reactions (flushing, fever, and headache) or anaphylactoid reactions (urticaria, rash, nausea, abdominal pain, and edema) and were managed by temporary reduction in infusion rate and administration of antihistamine and antipyretic medication.

ERT and HSCT for MPS I-H. Multiple studies documenting neurodevelopmental and somatic disease outcomes after HSCT for MPS I-H have been reported.[237–245] Although HSCT creates significant morbidity stemming from postconditioning immunocompromise, pneumonitis, graft-versus-host disease, and hepatic veno-occlusive disease, and is subject to graft failure or chimerism, it is currently the only known treatment modality that prevents mental retardation. Survival and engraftment rates have steadily improved to 85–90% in recent series.[240–245] One series saw no effect of pre-HSCT ERT on survival or engraftment,[244] whereas another noted a reduction in pulmonary complications and successful engraftment and survival of all seven patients treated with combined therapy.[245] Other groups eschew ERT before transplant unless the patient has significant cardiopulmonary disease, citing the possibility of anti-α-iduronidase antibodies interfering with successful engraftment.

HSCT performed before 24 months of age and the onset of significant developmental delay (developmental quotient < 70) has the highest probability of rescuing neurocognitive outcome; engrafted survivors may experience speech delay and learning disability.[238–241,246] Good developmental outcomes have been reported in "late" transplants, and some "early" transplant patients have significant developmental delay. Stem cell transplant from donors without *IDUA* mutations seems to correlate with higher levels of posttransplant α-L-iduronidase activity, GAG clearance, and better developmental outcomes than stem cells from heterozygous MPS-I donors.[239,247]

In addition to improvement of neurocognitive outcomes, HSCT successfully eliminates hepatosplenomegaly and glycosaminoglycanuria, improves joint mobility, slows development of cardiac valvular dysfunction, and reduces airway obstruction and frequency of otitis media.[238,240] Coarse facial features "soften" with time. Outcomes for other parameters are mixed. Hearing loss remained in approximately half of patients. Some demonstrated resolution of corneal clouding, but others required corneal transplantation. Glaucoma developed in some patients as well.[238,240] HSCT does not adequately treat the orthopedic complications of MPS I-H, presumably because of poor penetration of α-L-iduronidase into growth plate cartilage. Cervical spine instability, progressive kyphosis, spinal cord compression, carpal tunnel syndrome, and painful hip dysplasia were present in nearly all transplanted patients and required orthopedic surgery intervention.[238,240] Growth velocity is initially normal and slows down due to persistent vertebral body dysplasia.[238,240,241] The final adult height is usually 1–3 standard deviations below the mean.[238]

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 5.

An infant who has a positive MPS I newborn screen should have the following:

1. Follow-up testing with leukocyte α-iduronidase enzyme activity. If low α-iduronidase activity, the infant should then be referred to a metabolic center for (a) further evaluation. (b) genetic counseling regarding the specific diagnosis, and (c) other subspecialty evaluations.

Clinical follow-up and intervention. Management of a MPS I patient requires a multidisciplinary approach; detailed, system-specific guidelines for the treatment of MPS I have been published.[248] In addition to regular follow-up by a metabolic specialist, patients should also have the following:

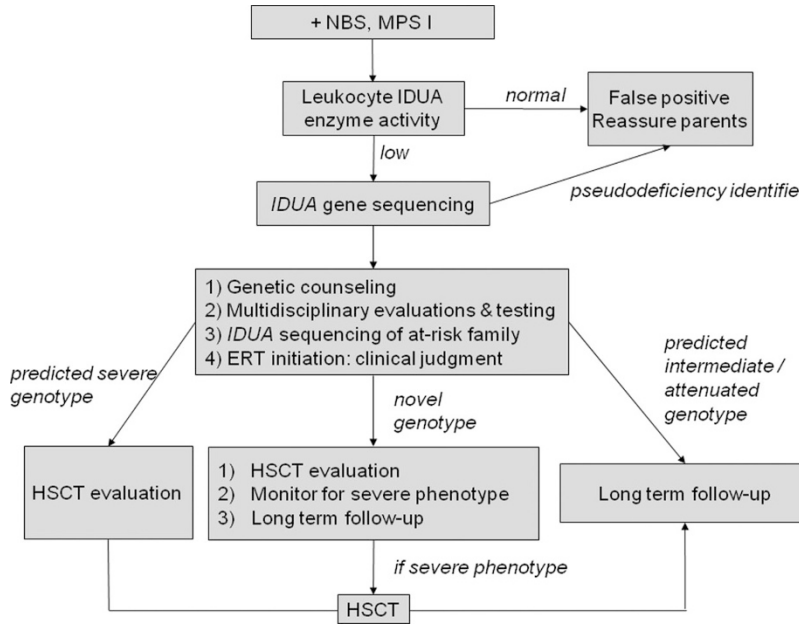

Fig. 5. Diagnostic algorithm for mucopolysaccharidosis type I (MPS I). NBS, newborn screening.

1. Evaluations from ophthalmology, otolaryngology, cardiology, orthopedic surgery, pulmonology, neurodevelopmental specialists, and pediatric neurosurgery as necessary.
2. Plain radiography will demonstrate a constellation of skeletal findings known as dysostosis multiplex: J-shaped sella turcica, "spatulate" ribs with anterior widening, wedge-shaped dysplastic biconcave vertebral bodies, shortened and rotated radius and ulna, proximal pointing of the metacarpals, and coxa valga. Severity of the dysostosis tends to correlate with disease severity.
3. Periodic audiometry.
4. Polysomnography.
5. Echocardiography.
6. Electrocardiography.
7. Abdominal ultrasound.
8. Imaging studies of the brain and spine.[248]

Follow-up and intervention of patients confirmed to have MPS I must ensure that those who are to develop severe disease receive HSCT early enough to preserve neurocognitive outcome, whereas those with milder disease do not receive HSCT unnecessarily.

Because α-iduronidase enzyme activity level is unreliable for phenotypic prediction, and no current biomarkers are widely available for predictive purposes, treatment decision-making depends on genotype-phenotype correlations drawn from *IDUA* mutations. However, this method also has significant limitations. The p.P533R *IDUA* mutation accounts for 5–10% of mutant alleles; patients with p.P533R mutations have encompassed the entire MPS I disease spectrum. New *IDUA* mutations for which phenotypes have not been reported will also undoubtedly be discovered. These scenarios present a difficult challenge to management as secure prognostication of MPS I phenotype will be impossible. The experience gained from caring for patients in this category must be gathered to guide future treatment for future newborns diagnosed with unclear MPS I phenotypes.

All confirmed infants with MPS I should have a detailed multidisciplinary initial evaluation as detailed in Muenzer et al.[248] Further disease management is dependent on the predicted MPS I phenotype.

MPS I-H. If MPS I-H is predicted,

1. Hematology/oncology referral should be made to initiate the HSCT process.
2. Surgery referral, for implantation of a central venous catheter, should also be made.
3. Initiation of ERT should be made based on the clinical judgment of the coordinating metabolic specialist.
4. The infant should also have regular neurodevelopmental assessments performed to follow the developmental trajectory during and after the treatment process.

MPS I-HS and MPS I-S. Because the milder forms of MPS I have later symptom onset:

1. ERT does not need to be initiated immediately in the neonatal period.
2. Monitoring of the infant by the metabolic specialist at least every 3 months.
3. ERT should be initiated based on the clinical judgment of the specialist and discussion with the infant's family. Similar to MPS I-H cases, ERT should be administered through peripheral venous access pending implantation of central venous access by the pediatric surgeon, if necessary.

MPS I patients with mutations that are novel or unable to predict phenotype (such as p.P533R):

1. The infant should be monitored regularly by the metabolic physician and other subspecialists.
2. If possible, the patient should be evaluated for potential HSCT and, if a donor is found, "put on hold" for transplantation based on the developmental outcome of the patient.
3. Neurodevelopmental assessments should take place every 3–4 months.
4. HSCT performed if the infant is beginning to demonstrate markers of MPS IH such as developmental delay, severe organomegaly, and/or skeletal manifestations.

MPS type II (OMIM# 309900)

Synonyms

Hunter Syndrome; iduronate sulfatase deficiency.

Background

MPS type II is an X-linked MPS caused by a deficiency of iduronidate 2-sulfatase (IDS, EC 3.1.6.13), encoded by the *IDS* gene. Similar to α-L-iduronidase, IDS is also involved in the breakdown of heparan and dermatan sulfate, catalyzing the enzymatic step prior to α-L-iduronidase. Consequently, symptoms of MPS II are similar to MPS I, involving storage of GAG material in multiple organ systems. The population frequency of MPS II is estimated to be approximately 1 in 76,000–320,000 male live births.[221,222]

Clinical phenotype

Nearly all affected patients are male, but rare symptomatic females have been described.[249] MPS II patients also display a spectrum of severity, but onset of symptoms is later and velocity of disease progression slower than MPS I. Patients with severe MPS II have cognitive regression and are typically diagnosed between 18 and 36 months. Attenuated patients are recognized between 4 and 8 years and have learning disabilities or normal intelligence, but no neurodegeneration.[250,251] Patients with neurocognitive involvement tend to have difficult, aggressive behavior. Those with complex *IDS* gene arrangements are at higher risk for generalized tonic-clonic seizure disorders. Corneal clouding, if present, is visible only with slit-lamp examination and does not interfere with vision. Glaucoma is also a rare manifestation of MPS II. Patients may experience night blindness and papilledema due to GAG storage around the optic cup. A characteristic thickening of the skin with a "pebbly" appearance is seen in many patients. Ultimately, problems associated with upper airway obstruction, cardiopulmonary disease, and orthopedic sequelae are responsible for early death in MPS II patients.

Current diagnostics

Biochemical markers. The level of plasma IDS enzyme activity, while low in affected patients, cannot predict disease severity. Enzymatic analysis also cannot be used to determine carrier status.[252,253] The enzyme requires activation via post-translational modification of cysteine 59 to formyl-glycine. The enzyme that performs this modification is encoded by the *SUMF1* gene; mutations in *SUMF1* cause MSD. Therefore another sulfatase such as ARSA or arylsulfatase B (ARSB) should be concurrently assayed with IDS activity to assess for MSD.

Molecular analysis. The most common recurrent *IDS* mutations (20%) are gross rearrangements, most of which arise from recombination events with an *IDS* pseudogene.[254–256] Most of these patients manifest the severe phenotype; those with extensive deletions demonstrate a contiguous-gene deletion phenotype and are more likely to develop seizures and other "atypical" symptoms.[257]

Very few recurrent point mutations have been reported; of those, genotype-phenotype correlations are difficult to draw due to the reports' lack of complete phenotypic data and inconsistencies in defining severity of disease. Some reported "intermediate" patients as having mental retardation, whereas others did not.[258,259] Individuals throughout the spectrum of phenotype severity have been reported for the p.A85T[257,259–262] mutation. The p.P86L[258,263] and p.R468Q[258–260,262–265] mutations have all been reported with a "severe" phenotype. Although the p.R172X[259,260,265–267] and p.R468W[233,259,268,269] mutations are usually reported as "severe," they have been associated with "intermediate"[256] or "mild" phenotypes.[270,271] The p.R443X mutation is generally reported as an "intermediate" phenotype, although the severity of cognitive involvement in most cases is uncertain.[256,258,259,265,267,272,273]

Therapy

Hematopoietic stem cell transplantation. HSCT successfully eliminated hepatosplenomegaly, reduced cardiac valvular thickening, improved joint mobility, and normalized coarse facies.[274–276] However, the major problem with HSCT for MPS II is its inability to preserve neurocognitive outcome[277–282] and the high mortality rate. All HSCT cases for "severe" MPS II followed by the North American Storage Disease Collaborative Study Group demonstrated declines in IQ to below 50.[283] These disappointing results, coupled with the development of ERT, do not make HSCT a currently acceptable treatment modality for any form of MPS II. However, citing the later age of the transplanted patients with "severe" disease, some groups are assessing the efficacy of early HSCT on MPS II patients whose older siblings manifested severe disease.

Enzyme replacement therapy. Results for clinical trials with recombinant human IDS (idursulfase, Elaprase™; Shire, Cambridge, MA) have been published.[284,285] Weekly ERT with 0.5 mg/kg/dose of recombinant IDS improved forced vital capacity and increased exercise tolerance as measured by the 6-minute walk test. Of the patients with hepatosplenomegaly at baseline, 80% had normalized at the end of the 53-week trial. Overall, the weekly ERT group demonstrated 25% reduction in liver and spleen volumes. Significant improvement in elbow joint mobility was also demonstrated by those receiving weekly ERT. Urinary GAG excretion normalized in 40% receiving idursulfase, and the weekly group excreted 52% less from baseline.

IgG antibodies to idursulfase developed in 47%. Although those with antibodies had a lower reduction in urinary GAG excretion, no differences in clinical outcomes were noted compared with those without antibodies. The percentage of patients with antibodies declined to 32% by the end of the pivotal trial. Infusion-associated reactions were similar to those experienced by MPS I patients receiving laronidase and also decreased in frequency after 12 weeks.

Ascertainment

Early ascertainment is currently only through family-based testing. Currently, there are no NBS programs for MPS II.

Recommended follow-up procedures

Diagnostic confirmation.

A suggested diagnostic algorithm is presented in Figure 6.

1. An infant who has a positive MPS II newborn screen should have confirmatory testing performed with simultaneous plasma IDS and leukocyte ARSA or ARSB enzyme activities.
   a. If only IDS is low, then genetic testing for MPS II should be performed.
   b. If both are low, then *SUMF1* should be sequenced to confirm MSD.
2. Once a diagnosis of MPS II or MSD has been confirmed, then a metabolic evaluation with genetic counseling should be performed.

Identification of females. NBS programs will find female infants with low IDS activity. Confirmatory testing should also be performed:

1. To distinguish false positives from MPS II heterozygotes and MSD females.
2. Genetic counseling and careful review of the family pedigree to identify other at-risk family members should be performed.
3. MPS II heterozygotes should be followed periodically by a metabolic physician for reinforcement of recurrence risk counseling and monitoring for development of Hunter syndrome symptoms.
4. Because some heterozygotes have enzyme activity in the "normal" range, not all carrier females can be identified with NBS.[253]

Clinical follow-up and intervention. Management guidelines for patients with MPS II have been published.[236]

1. All confirmed MPS II boys should be referred for genetic counseling, multidisciplinary evaluations, and diagnostic studies similar to those for MPS I.
2. Regular neurodevelopmental assessments should be performed to follow the developmental trajectory.
3. The decision to initiate ERT should be made according to the clinical judgment of the metabolic specialist.
   a. For a MPS II infant with severe disease, any discussion between specialist and parents about ERT initiation must include clear counseling about the inability to reverse cognitive outcomes. This is an extremely controversial issue, given the cost of ERT, its limitations, and the consequences of increasing mobility in a mentally retarded child with potential behavioral problems.
   b. Clear parameters for discontinuation of ERT must also be delineated before initiation.

MPS type VI (OMIM# 253200)

Synonyms

Maroteaux-Lamy Syndrome, ARSB deficiency.

Background

MPS VI is caused by mutations in the *ARSB* gene that encodes *N*-acetylgalactosamine-4-sulfatase (ARSB, EC 3.1.6.12). The incidence of MPS VI has been estimated to be approximately 1 in 300,000 live births.[221] Affected individuals cannot adequately degrade GAGs that contain *N*-acetylgalactosamine-4-sulfate and consequently excrete increased levels of urinary dermatan sulfate.

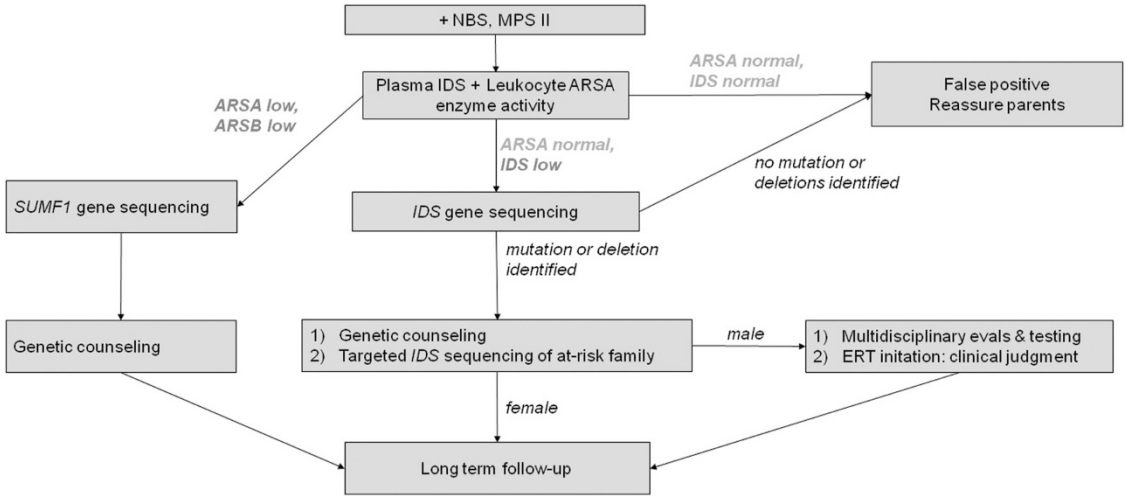

Fig. 6. Diagnostic algorithm for mucopolysaccharidosis type II (MPS II). NBS, newborn screening.

Clinical phenotype

Age of presentation and velocity of disease progression are variable, but affected patients typically come to medical attention at 6–24 months of age with deceleration of growth velocity, macrocephaly, macroglossia, facial coarsening, and hepatosplenomegaly. Symptoms are similar to the somatic manifestations of severe MPS I, with progressive corneal clouding, open-angle glaucoma, skeletal abnormalities, painful hip dysplasia, restriction in joint mobility, and cardiac valvular dysfunction. Otolaryngological complications include recurrent otitis media, conductive hearing loss, and upper-airway obstruction. Communicating hydrocephalus and spinal cord compression occur from accumulation of GAGs in the CNS.[286] Cognitive development and intelligence are normal and primarily limited by physical impairment of motor skill acquisition and learning. Cardiopulmonary complications arising from infection or perioperative airway difficulties are the primary causes of premature death in early adulthood.

A severe neonatal form with coarse facies, dysostosis multiplex, and cardiac disease has been reported.[287,288] Similarly, later-onset or attenuated forms of MPS VI have also been reported.[289–291]

Current diagnostics

Biochemical markers

Fibroblast or leukocyte ARSB activity in patients with MPS VI is generally <10% of the lower limit of normal, but amount of residual enzyme activity does not correlate with the severity of the phenotype.[291] Enzymatic analysis permits the distinction between affected patients, mutation carriers, and normal individuals. ARSB requires posttranslational modification of cysteine 91 into formyl-glycine by sulfatase-modifying factor for catalytic activity. Therefore, activity of another sulfatase, generally ARSA and/or iduronate sulfatase, should be concurrently assayed with ARSB activity to exclude the possibility of MSD.

Molecular analysis

Although most patients carry at least one "private" *ARSB* mutation, some genotypes allow for prediction of phenotype. Patients with homozygous truncating mutations had severe phenotypes, as did patients with the p.R315Q, p.S384N, and p.L72R mutations, which accounted for 19.8% of all alleles discovered. Patients carrying at least one p.Y210C, p.C405Y, p.D83Y, p.R152W, or p.R434I mutation had attenuated phenotypes.[292] No pseudodeficiency alleles are known.

Ascertainment

Early ascertainment is currently only through family-based testing. No pilot NBS programs have been established for MPS VI.

Therapy

Hematopoietic stem cell transplantation. Long-term follow-up of MPS VI patients who have undergone HSCT indicates efficacy in improving joint mobility, facial coarseness, obstructive sleep apnea, hepatosplenomegaly, cardiomyopathy, and possibly valvular thickening. No cervical cord compression was noted during the period of follow-up. The transplanted patients demonstrated progression of corneal opacity, continued short stature, and developed orthopedic complications (progressive kyphosis and carpal tunnel syndrome). HLA-identical donors were used in each case; all patients reported successfully engrafted, had reconstitution of leukocyte ARSB activity, and excreted less GAGs after transplant. Nonfatal graft versus host disease occurred in nearly all patients reported.[293–295] Success with umbilical cord transplant has also been reported.[296]

Enzyme replacement therapy. Results of clinical trials for recombinant human arylsulfatase B (rhASB, Naglazyme®; Biomarin Corporation, Novato, CA) have been published.[297–299] The 1 mg/kg/week dose produced a greater reduction in urinary GAG excretion compared with the 0.2 mg/kg/week dose. Although visceromegaly in MPS VI is not as prominent as other types of MPS, the five (50%) patients with hepatomegaly in the 48-week open-label phase II trial demonstrated reduction in liver size, with four demonstrating normal age-weight adjusted liver size.[298] In the same study, the three patients with the most severe abnormalities in nocturnal pulse oximetry experienced improvement in average oxygen saturation and decrease in time spent below 90% saturation. In the 24-week phase III double-blind, randomized, placebo-controlled study, the rhASB group demonstrated a significant improvement in the 12-minute walk test distance compared with the placebo group. Urinary GAG

excretion was reduced 75% from baseline in the rhASB group, whereas remaining essentially unchanged in the placebo group. Those treated with rhASB also demonstrated improvement in the 3-minute stair climb. No significant changes were observed in joint range of motion, energy level, and hand dexterity.[299] Preliminary long-term rhASB observations show stabilization of left ventricular ejection fraction, reduced left ventricular wall thickness, and in patients initiated on rhASB therapy in the first year of life, improved linear growth. ERT does not seem to have an effect on corneal clouding or the development of cervical myelopathy (personal communication, P Harmatz, 2009).

Nearly all patients developed IgG antibodies to rhASB; one patient was found to have antibodies with neutralizing activity in vitro. Development of antibody was not associated with changes in urinary GAG levels. Adverse effects of rhASB infusion were usually infusion reactions (rigors, fever, and shortness of breath) or anaphylactoid reactions (urticaria, rash, nausea, abdominal pain, and edema) and were managed by premedication with antihistamines, antipyretics, or corticosteroids. One episode of apnea occurred that was attributed a combination of obstructive sleep apnea and sedation from diphenhydramine.

Recommended follow-up procedures

Diagnostic confirmation. A suggested diagnostic algorithm is presented in Figure 7.

1. An infant who has a positive MPS VI newborn screen should have confirmatory testing with leukocyte ARSB and ARSA enzyme activity.
   a. If ARSB activity is low and ARSA normal, *ARSB* gene sequencing should be performed to confirm the diagnosis of MPS VI.
   b. If both sulfatase activities are low, MSD should be confirmed using molecular analysis of the *SUMF1* gene.
2. Once the diagnosis of MPS VI or MSD has been confirmed, the patient should then be referred to a metabolic center for evaluation and genetic counseling regarding the specific diagnosis.

Clinical follow-up and intervention. Similar to the other types of MPS, a multidisciplinary approach is needed to care for a MPS VI patient.

1. All confirmed MPS VI infants should have a multidisciplinary baseline and follow-up evaluations as detailed in the management guidelines.[300]
2. A skeletal survey that includes flexion/extension views of the cervical spine, lateral view of the entire spine, and frog-leg view of the hips should be obtained.
3. An early orthopedic surgery referral should be made if a gibbus is present or imaging demonstrates vertebral anomalies that predict early development of kyphoscoliosis.
4. MRI of the brain and spine should be obtained to assess CNS involvement. Although airway compromise is less of a concern in an infant with MPS VI compared with an older child, the anesthesiologist or otolaryngologist securing the airway for the procedure must have experience in dealing with the potential airway challenges presented by the disorder.
5. The family should be counseled regarding the risks and benefits of HSCT versus ERT. If HSCT is desired, the patient should be referred to a hematology/oncology specialist for transplant evaluation. If they choose ERT, it should be initiated as soon as possible after confirmation of the diagnosis.
   a. To obtain consistent venous access for the weekly rhASB infusions, the infant should be referred to a pediatric surgeon for placement of an indwelling central venous catheter.
   b. While awaiting placement of central access, ERT using peripheral venous access should be given so that initiation of treatment is not delayed.
6. The infant should be followed regularly by the metabolic physician to document growth, development, and symptoms of MPS VI. Early identification of neonates with MPS VI involvement offers a unique opportunity to determine whether early initiation of treatment prevents or slows the progression of the serious somatic effects of the disorder.

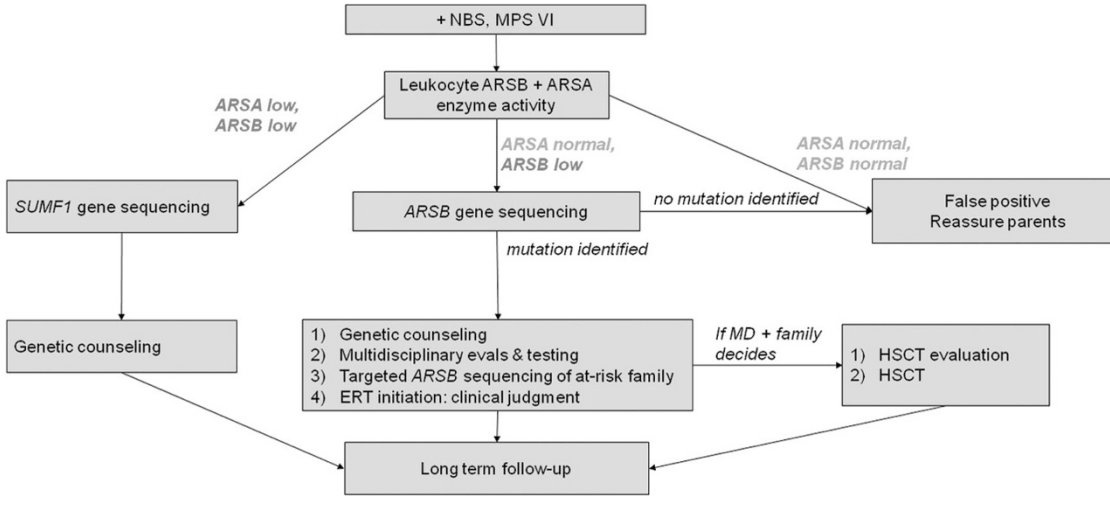

Fig. 7. Diagnostic algorithm for mucopolysaccharidosis type VI (MPS VI). NBS, newborn screening.

DISCUSSION

Family-based studies and new technologies for NBS have made the diagnosis of presymptomatic individuals with LSD's possible. These guidelines provide a framework for the initial evaluation and management of several disorders. There are significant limitations we faced in composing these guidelines.

LSDs are rare and complex conditions. There is limited natural history data for most conditions and little long-term follow-up data on the efficacy of different therapeutic approaches. The evidence bases for these rare disorders are poorly organized and statistically weak. Efforts to capture diagnostic and long-term follow-up data to improve understanding are urgently needed. Biospecimen repositories are needed for future research studies of biomarkers, modifier genes, etc. In this regard, the creation of ACMG/NIH Newborn Screening Translational Research Network[301] is timely and will play an important role in improving our knowledge base in the coming years.

Patients with LSD often need multidisciplinary care that should ideally be provided through a team approach including medical genetics, hematology, cardiology, neurology, ophthalmology, anesthesiology, etc. As NBS for LSDs becomes more widespread, there will be an increasing need for physicians trained in the care of these patients, particularly biochemical geneticists.

Laboratories used for enzymology and molecular diagnostics should be experienced and of high quality as evidenced by participation in quality assurance and proficiency testing programs. They should be capable of providing rapid turn-around of results (local laboratories are desirable).

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