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Review

Newborn Screening for Mucopolysaccharidosis Type I: Past, Present and Future

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Abstract

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by a deficiency of the lysosomal hydrolase α-L-iduronidase. MPS I is characterized by a broad range of disease manifestations. This includes devastating neurocognitive and bone manifestations and a short life expectancy in severely affected MPS I patients. Neurocognitive manifestations are typically limited in more attenuated MPS I, but patients may still suffer from severe somatic and bone manifestations. Severe MPS I patients are primarily treated with hematopoietic stem cell transplantation (HSCT) and more attenuated patients with enzyme replacement therapy. HSCT should be initiated before irreversible disease manifestations, preferably before 9 months, but may be initiated in patients up to 2 years. Early diagnosis of MPS I is challenging at best, and newborn screening (NBS) has already been initiated in several countries to diagnose and treat patients early. This article summarizes the history, benefits, methods and challenges that have to be addressed before NBS can be used most effectively.

Keywords
Lysosomal storage disorder; Mucopolysaccharidosis type I; newborn screening; phenotype

1. Introduction to MPS I

Mucopolysaccharidosis type I (MPS I, OMIM 252800) is a lysosomal storage disorder (LSD) caused by a deficiency of the lysosomal enzyme α-L-iduronidase (IDUA, EC 3.2.1.76), encoded by the *IDUA* gene [Genbank NG_008103]. This leads to a defect in the degradation of the glycosaminoglycans (GAGs) heparan sulfate (HS) and dermatan sulfate (DS). HS and DS accumulation leads to progressive cellular and multi-organ damage. MPS I patients exhibit a wide phenotypic spectrum of disease manifestations including intellectual disability, skeletal disease, cardiac valve abnormalities and corneal clouding [1-3]. Severe MPS I manifests early in life, approximately at the age of 0.5 years [4, 5]. Newborn hearing screening may have failed, which may be followed by signs of upper respiratory tract obstruction, inguinal or umbilical hernia, kyphosis and other orthopedic deformities. After that, patients frequently develop coarse facial features, hepatosplenomegaly and frequent respiratory infections [4, 6-8]. Several symptoms are nonspecific and significant diagnostic delays have been described [5]. Although MPS I is characterized by a continuous phenotypic spectrum, patients are classified into two groups, because the most efficient therapeutic strategy depends on the clinical phenotype [1-3]. Patients with severe MPS I (historically classified as Hurler disease) suffer from severe central nervous system (CNS) manifestations and bodily manifestations. If untreated, severe MPS I patients die in the first decade. The clinical spectrum of attenuated MPS I patients (historically Hurler-Scheie and Scheie disease) is broad. CNS disease is typically limited, but patients may still suffer from severe and incapacitating somatic manifestations. Life expectancy may be limited and ranges from the second decade to almost normal [1-3].

Early initiation of treatment is essential because many of the symptoms are irreversible. Currently, two disease-modifying therapies are available for MPS I: hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT). In HSCT, transplanted healthy donor cells circulate in the bloodstream, may cross the blood-brain barrier, reach target organs, differentiate and continually produce IDUA. Because of its ability to prevent neurocognitive decline, HSCT is the standard therapeutic regimen for patients with severe MPS I [9]. Patients who are transplanted after 9 months may already show irreversible neurocognitive deficits [8]. Therefore, HSCT should be performed as early as possible, but may be initiated until the age of 2 years [8, 10]. For more attenuated MPS I patients, weekly ERT with recombinant IDUA is the recommended treatment. The recombinant enzyme does not cross the blood-brain barrier and cannot alleviate CNS symptoms [11, 12]. In severe MPS I patients, ERT may be used around transplantation until a suitable donor has been found or as an adjuvant. It has been shown to improve HSCT engraftment and decrease residual disease burden after HSCT [13].

Several countries implemented newborn screening (NBS) for MPS I to enable early diagnosis and treatment. This article summarizes the history, benefits, methods and challenges that must be addressed before NBS can be used most effectively.

2. History of NBS

The basis for NBS was established in the 1960s with the screening for phenylketonuria (PKU) and the definition of Wilson and Jungner's criteria. In 1961, Robert Guthrie developed a bacterial inhibition test to detect elevated phenylalanine concentrations in dried blood spots (DBS) to diagnose PKU patients [14]. In the next few years, 27 countries or states introduced NBS programs for PKU. In 1968, Wilson and Jungner [15] defined screening criteria to guide the selection of diseases for NBS. The criteria include that the disease should be an important health problem and that there is an understanding of the natural history, an acceptable diagnostic test, an agreed policy on whom to treat and an effective therapy available [15]. These criteria are still guidelines when candidate diseases are considered for implementation in NBS programs.

After the initial report of Guthrie *et al.* [14], in which DBS was used to diagnose PKU patients, the first enzyme activity assays that used DBS to diagnose LSD patients (Fabry disease and MPS I) were reported in 2001 by Chamoles *et al.* [16, 17]. DBS is easy to obtain, safe to transport and store, and the loss of enzyme activity in DBS seems minimal [17]. Chamoles *et al.* [17] used the 4-methylumbelliferyl-α-L-iduronide assay, which is generally used to measure residual enzyme activity to diagnose MPS I. After that, several efforts towards developing tandem mass spectrometry (MS/MS) and multiplexing the assay followed [18, 19].

Pilot programs on NBS for MPS I were already performed in the early years of this century [20]. In 2002, the US Department of Health and Human Services issued a list of primary and secondary disorders for which NBS programs should screen, the Recommended Uniform Screening Panel (RUSP). In 2016, MPS I was included in this list. This was followed by the initiation of several more (pilot) NBS programs, including in Japan, Taiwan, Italy, Brazil, The Netherlands, Mexico and the majority of the United States (US) [6, 21].

3. Benefits of Newborn Screening

MPS I is a severe and progressive disorder with an early onset in which the prognosis depends on the early initiation of treatment. Several efforts towards early diagnosis, as summarized below, have failed to improve the period between the onset of symptoms and diagnosis [5, 22].

The MPS I registry reported that severe MPS I patients had a median age of onset of 0.5 years, with a median age at diagnosis of 1 year [4]. More attenuated MPS I patients may have a diagnostic delay for years. A diagnosis may be delayed because the early symptoms of MPS I are typically nonspecific. It may take considerable time to exclude other more commonly observed disorders. Also, MPS I is a rare disorder with an incidence of approximately 0.11-3.8 cases per 100,000 live births [20, 23]. Therefore, many caregivers may never see a case of MPS I in their careers.

With the availability of HSCT and ERT, timely diagnosis of particularly severe MPS I patients has become essential. In severe MPS I patients, there is a strong correlation between the age of transplant and neurocognitive outcome [10, 24]. In addition, animal studies showed that some irreversible disease manifestations may be prevented by performing HSCT in the neonatal period [25-27].

In more attenuated MPS I patients, ERT has been shown to improve or stabilize somatic symptoms. This effect is more pronounced if ERT is started early [28-30]. Like HSCT, animal studies have demonstrated that some irreversible clinical manifestations such as musculoskeletal disease

and cardiovascular abnormalities may be prevented by neonatal initiation of ERT [26, 31-33]. These data emphasize the importance of early diagnosis and therapy.

3.1 Awareness

Increased knowledge of caregivers on the initial clinical manifestations of MPS may contribute to earlier diagnosis. Awareness related to referral and the decision-making process is also important. Several initiatives have been initiated to stimulate awareness and decrease diagnostic delays over the last decades. Rare diseases were part of the program of the European Commission in 1999. This resulted in several policies focused on improving the recognition and visibility of rare diseases. Also, several countries organize a yearly 'Rare Diseases Day.' Local initiatives that have been organized include continuing education programs for general physicians and specialists frequently confronted with patients suffering from yet unknown diseases [34]. Algorithms have been developed to prompt diagnostic investigations for LSDs [20, 35]. However, no reduction in diagnostic delay of MPS I has been observed in the years after these initiatives [5, 34].

3.2 Selective Screening

Selective or high-risk screening is the screening of individuals with a high risk of disease. For instance, selective screening by studying GAG and oligosaccharide excretion in the urine of patients with either intellectual disability, dysmorphisms, coarse facies or bone disease has been frequently discussed. This strategy is part of several diagnostic algorithms [20, 34].

There are also organized selective screening programs for patients with specific clinical manifestations. Some authors suggested that patients with an isolated MPS-related manifestation are unlikely to be diagnosed unless they are under a selective screening program [1]. The MPSs and other LSDs share many clinical characteristics and therefore, most selective screening programs aim to diagnose a group of diseases. Previous selective screening programs included pediatric patients with a hernia repair, with otorhinolaryngological surgery, or patients who visited a rheumatologist with musculoskeletal symptoms or suffered from carpal tunnel syndrome [1, 36-39]. The results of most selective screening programs were disappointing. Some selective screening programs never reported results, probably due to a lack of diagnosed patients [34, 39]. Colón *et al.* [36] described a selective screening program for patients with several clinical manifestations that may be compatible with MPS. Pediatricians were provided with information on clinical manifestations of the MPSs, followed by material for sample collection and dispatch. 180 patients were screened and 8 MPS patients were diagnosed, including 1 MPS I patient [36]. This study may have been more successful because it focused on multiple clinical manifestations.

The generally disappointing results of awareness and selective screening programs may have several reasons. Especially at a young age, MPS I patients may not have manifested some of the clinical manifestations yet. Several clinical manifestations may also be part of another more prevalent disease. Also, when an awareness program has been attended many years ago, the acquired awareness may be outdated due to the rarity of the MPSs [34].

It is important to continue efforts to timely diagnose patients with rare diseases. However, awareness and selective screening programs aim to diagnose patients that have already developed symptoms. Several symptoms in MPS I patients are irreversible; therefore, a strategy that enables the identification of patients before the onset of symptoms is preferable.

4. NBS Methodology

4.1 IDUA Activity as an NBS Strategy

The most commonly used first-tier strategy is quantifying IDUA activity in DBS. Most screening laboratories perform one of two methods that allow screening for multiple LSDs. The fluorometric assay of IDUA activity may be performed on a digital microfluidics platform (DMF) [19, 40, 41]. DMF has the advantage that each enzyme reaction is performed under individually optimized conditions such as pH, inhibitors and buffer. It has a shorter reaction time and lower costs due to lower reaction volumes and reagent savings as compared to microtiter plate fluorometry methods. Also, other LSDs may be added to the assay without needing more DBS samples. Due to these advantages, it is suitable for high-throughput screening [19]. The second method is the MS/MS method, for which different protocols exist. The method needs to be combined with either a solid-phase, liquid-liquid extraction phase or the addition of ultra-performance liquid chromatography (UPLC) column to eliminate contaminants before the sample is injected into the MS/MS [19]. Some authors claimed that MS/MS had superior performance and lower false-positive rates than DMF. However, a, a comparative study by Millington *et al.* [19] concluded that both platforms may be used for efficient NBS and that both platforms had equal performance and false positive rates.

The use of enzyme activity platforms in the scope of NBS has its challenges. Most importantly, NBS studies reported a high rate of false positives. Pseudo-deficiency alleles that do not cause clinical disease often cause low enzyme activity. Some NBS programs even reported that the amount of 'patients' with pseudo deficiencies was higher than that of patients with actual deficiencies [42]. There is a strikingly high rate of false-positives in some populations, such as African or African-American populations [41]. The high rate of false positives is independent of the screening methods that are described above [19]. In addition, enzyme activity does not correlate with phenotypic severity and cannot be used to guide decisions on therapeutic strategy.

Post-analytical tools may be integrated into screening algorithms to improve false-positive rates. Collaborative Laboratory Integrated Reports (CLIR, by Mayo Clinic, Rochester, USA) is a post-analytical tool that uses complex regression models, covariate-adjusted reference intervals and populated results [43]. This tool has been applied to NBS programs but has not been tested on a large scale for MPS I [40, 43, 44].

4.2 GAG Analysis as an NBS Strategy

There are multiple methods to quantify GAG accumulation in DBS [45]. Mass spectrometry analysis of GAGs is generally accepted as the superior strategy [45]. All methods are based on detecting short GAG fragments that are degraded using enzymatic or non-enzymatic depolymerization [46]. Herbst *et al.* [45] recently compared 4 MS/MS methods to quantify GAGs. The most frequently used method is the "internal disaccharide method." In this approach, GAG polymers from a DBS sample are degraded by bacterial enzymes after which the level of disaccharides is measured by LC-MS/MS [45]. They also studied 3 other methods that measure the non-reducing ends of GAG chains. The "SensiPro" approach and its modification "SensiPro Lite" require heparinase-degradation and derivatization of the reducing ends, after which LC-MS/MS measures the non-reducing ends. As compared to the other approaches, the SensiPro approaches require complex sample preparation. Lastly, in the "endogenous biomarker method," small non-

reducing end GAG fragments are measured that are present endogenously in patient samples and do not require degradation by bacterial enzymes [45, 46]. The first 3 approaches yielded similar results. The endogenous biomarker method showed the highest differentiation between MPS I and healthy controls because the biomarker was essentially undetectable in healthy controls. It even distinguished severe from attenuated MPS I patients, but only a few attenuated patients were included [45].

GAG analysis as a second-tier strategy significantly decreases the number of false positives by differentiating MPS I from pseudodeficiency or heterozygotes [40, 43, 45, 47, 48]. The techniques have a few limitations. The sample preparation may be relatively complex, it requires a long run time and the reagents may be relatively expensive [47]. Also, the techniques require mass spectrometers with detection sensitivities higher than typically used in screening laboratories [46]. Therefore, the technique is less suited as a first-tier strategy for NBS [47].

4.3 Genetic Analysis as an NBS Strategy

As the MPS I registry described in 2019, several genetic variants that cause MPS I correlate with phenotypic severity [49]. Therefore, genetic analysis is very important in the scope of NBS, both to diagnose MPS I patients and to classify the clinical phenotype. However, 12.4% of severe and 40% of attenuated MPS I patients had unique genotypes, genetic variants that have never been identified before [49]. With the implementation of NBS for MPS I, more unique variants may be expected. The poor genotype-phenotype correlation of several genetic variants and the unique variants illustrate the problem of relying on genotype to diagnose MPS I patients, even as a second-tier strategy. Indeed, a previous attempt to use genetic testing as a second-tier strategy still resulted in high false-positive rates [40]. Therefore, genetic testing is generally considered an appropriate strategy to perform after enzyme and GAG analysis as first and second-tier strategies [50].

5. Future Perspectives

NBS for MPS I has been studied in several pilot programs and is already implemented in several countries. Most results of NBS programs are shared publicly but little is known about the long-term outcome of MPS I patients diagnosed by NBS [42-44, 51-65]. Nationwide NBS programs have been implemented in Taiwan since 2015 and the age at diagnosis has decreased significantly, as reported by a retrospective multicenter study by Lin *et al.* [58]. The mean age at diagnosis of the MPSs as a group (MPS I, II, II, IV, VI) has decreased from 4.3 to 0.2 years old [58]. This allowed for the initiation of very early ERT and HSCT. A positive correlation between age at diagnosis and life expectancy was observed [58].

Previous and current NBS programs demonstrated that NBS for MPS I is feasible and beneficial for patients. NBS for MPS I will probably be implemented by more countries in the next few years. However, the programs also demonstrated many challenges that must be addressed. Firstly, there is a need for uniformity in the methods used for NBS to guarantee quality and limit the amount of false positives. Secondly, a method to predict the phenotypic severity is urgently needed to guide decisions on the most optimal therapy. Thirdly, there are several ethical issues related to NBS for MPS I. Fourthly, a uniform guideline on treatment and follow-up of patients diagnosed with NBS is lacking. Lastly, because some clinical manifestations are refractory to therapy, patients diagnosed with NBS may still suffer from significant residual disease.

5.1 Improvement of NBS Strategy

One of the most important goals achieved is the agreement on the need for GAG analysis as a second-tier test, followed by genetic analysis to reduce false positives. This method is already used in many NBS programs [40, 49, 50, 54, 66]. In addition, most results on NBS programs are shared publicly, which is essential to address accuracy and problems with different techniques or strategies. In time, the outcomes of patients that are diagnosed with NBS and treated with different therapeutic strategies may also be shared. There are also ongoing efforts to improve the accuracy of NBS for MPS I. For instance, GAG analysis is relatively time-consuming and costly compared to other biochemical tests [47, 67]. Studies on alternative second-tier tests include a recent report by Zhang *et al.* [68]. They described an immunocapture method coupled with mass spectrometry-based proteomics to measure IDUA protein. The method is not tested on a larger scale and the antibodies and specific instrumentation for the mass spectrometer may not be available in all screening laboratories [68]. Also, several screening laboratories in the US recently published an attempt to equalize their methods and cut-off levels, after which some adaptations to the methods were possible [69]. Future studies on uniformity of methods are important to guarantee quality and limit the number of false positives.

5.2 Phenotypic Severity

The phenotypic severity should be known to initiate the most effective disease-modifying therapy. With the implementation of NBS, MPS I patients will be diagnosed before the onset of phenotype-distinguishing disease characteristics. Current NBS studies reported that one of the most important problems with NBS is identifying patients with an uncertain clinical phenotype and identifying individuals with a positive NBS that cannot be identified as affected or unaffected [40, 42, 52, 55, 69].

Currently, the phenotype is determined by the age of onset of certain disease manifestations and by a subset of genetic variants that are associated with a particular phenotype [49, 70]. Previous studies showed that clinical manifestations are interpreted with a large variability [70]. Clinical manifestations in patients diagnosed with NBS are less specific, as these patients are younger. Therefore, other methods to determine or predict phenotypic severity should be studied.

5.2.1 Genetic Predictors

A study by the MPS I registry in 2019 described a close genotype-phenotype relation for several genetic variants [49]. Of all 380 severe MPS I patients with compound heterozygous or homozygous genetic variants in *IDUA,* 67.6% were predictive of null alleles and a severe phenotype. Attenuated patients were never homozygous or compound heterozygous for these specific variants. However, in the other 32.4% of severe MPS I patients, there was no clear correlation with the genetic variants. They described several factors that may hinder genotype-phenotype correlation. For instance, the earlier described unique genotypes were observed in 12.4% of severe and 40% of attenuated MPS I patients [49]. With the implementation of NBS for MPS I, more unique phenotypes will be observed due to the diagnosis of more patients and the identification of ‘patients’ that may otherwise not have been recognized as MPS I. Also, some variants have a functional heterogenous effect, such as c.1598C>G (p.Pro533Arg), c.53T>C (p.Leu18Pro) and c.979G>C (p.Ala327Pro) [49]. Genetic variants

that allow for some residual enzyme activity such as missense variants are susceptible to the effects of genetic modifiers and therefore, the role of these variants on the processing and catalytic properties of IDUA is very difficult to determine [7]. Now that NBS for MPS I is implemented in many countries, all genetic variants are observed more frequently. More information on genotype-phenotype correlation should become available when these patients are followed.

5.2.2 Biochemical Predictors

In addition to genetic predictors for phenotypic severity, biochemical predictors have been studied [6]. Several biomarkers correlated with phenotypic severity but in most, the correlation was incomplete or the study had a very small sample size [7, 45, 71, 72]. Some methods with better differentiation between phenotypes and a slightly higher sample size are summarized here. Firstly, an immune quantification method to measure IDUA activity was developed, which was plotted against measured GAGs. Severe MPS I patients could be distinguished from attenuated MPS I patients, but the assay is complex and the antibodies are not widely available [71]. The second study described levels of heparin cofactor II-thrombin (HCII-T) complexes in blood, a surrogate marker for GAG accumulation. HCII-T levels were significantly higher in severe MPS I patients than in attenuated patients and healthy controls [72]. The third study described an optimized 4-methylumbelliferyl-α-L-iduronide assay that distinguished between severe and attenuated MPS I patients. An algorithm was developed that combined the assay with genetic variants that correlate with severe MPS I and nonspecific symptoms that may already be present in neonates with severe MPS I. The algorithm enabled differentiation between the phenotypes [7]. With the implementation of NBS programs, more patients may be diagnosed and projects to study these biomarkers in large cohorts may be possible.

5.3 Ethical Considerations of Newborn Screening

5.3.1 Economic Considerations

Due to the advances in high-throughput procedures, the screening process costs are probably not the largest financial burden. However, the costs of follow-up and treatment are considerable. Some patients that NBS identifies may never develop disease manifestations but require long-term follow-up. In other patients where the clinical severity is unknown, ERT may be considered, which is very expensive. It is also important that an NBS program for MPS I is only initiated if treatment is available and reimbursement of therapy and follow-up can be guaranteed.

5.3.2 Psychological Considerations

NBS programs are originally implemented to enable early treatment by early diagnosis. Early diagnosis and HSCT in severe MPS I dramatically improve mortality and neurocognitive outcome. Identifying attenuated patients or patients with an uncertain clinical phenotype is not the aim of NBS and raises ethical concerns. Attenuated patients are also identified and may suffer from severe and irreversible disease manifestations such as skeletal disease, heart valve abnormalities and corneal clouding. There is some evidence of an effect of early ERT in preventing these manifestations [26, 28-33], but there may be a flexible window of time to start treatment. A risk-benefit consideration has to be made if very early treatment is in the patient's best interest [40].

The phenotypic spectrum of MPS I is broad and ranges from severely affected patients that should be treated as soon as diagnosed to 'patients' with unique genotypes that may never develop disease manifestations. Timmermans and Buchbinder [73] originally described the term 'patients-in-waiting'. 'Patients-in-waiting' are patients in which there is uncertainty about the clinical significance of NBS. NBS may provide results that oscillate between indicating a biochemical artifact and a potentially life-threatening disease. The patients and their families are waiting for a disease that may or may not manifest itself. Invasive investigations are often necessary, but the consequences on their lives and medical management are often uncertain [73, 74].

On the other hand, the emotional burden of a diagnostic odyssey before a diagnosis is made may also be considered. NBS programs eliminate this large problem previously addressed by other authors [1, 34, 40, 75]. The diagnosis may also bring patients and their families a sense of empowerment. It enables prenatal diagnosis for decisions on reproduction and may identify risks to other family members [40].

5.4 Guidelines on Treatment and Follow-Up

In Taiwan, in which NBS programs have been running for several years, 7 patients with a positive screening for MPS I were reported of which 3 received early ERT. The other patients had regular follow-ups [66]. Only a few reports on the clinical strategy after positive screening have been published and are summarized below.

It is clear that the early initiation of HSCT in severe MPS I patients dramatically improves mortality and neurocognitive outcome. Therefore, patients with low IDUA activity, high GAG levels and 2 genetic variants associated with severe MPS I, should receive HSCT as soon as possible [6]. The exact timing of HSCT, however, has to be defined by the transplant center in which age, weight, clinical symptoms and both the risk of the procedure and disease progression have to be considered [76]. In the meantime, patients may already be treated with ERT, which has been shown to improve HSCT engraftment and decrease residual disease burden after HSCT [13].

The decision-making process is more complicated for patients in which the phenotype is not certain. Clear and universal recommendations for follow-up and treatment are needed for these patients. ERT should be considered, but early ERT may mask the symptoms that would otherwise be directional for a certain phenotype.

Clinical examination for the earliest presenting manifestations, as well as biochemical assessment may help to predict the clinical phenotype and guide decisions on the most efficient therapeutic strategy. Several reports have summarized clinical findings typical for severe MPS I, including the age of onset [76, 77]. Patients should be examined for the presence of coarse facies, dysmorphism, hernias, cardiac murmurs, hepatomegaly, splenomegaly, respiratory symptoms, alteration of joint motion and the presence of kyphosis. Neurocognitive examination, heart ultrasound, audiometry, ophthalmologic examination and skeletal radiographs must be performed. In patients with uncertain clinical phenotype, these assessments should be conducted every 3-6 months, but clinical examination, assessment of neurodevelopment and growth should be performed every 3 months [76, 77]. Patients with no clinical symptoms, normal GAG levels and 1 or 2 pseudodeficiency genes may be classified as false-positives of NBS, but follow-up after 6 months may also be considered [76].

Hopefully, current NBS programs will provide information on genotype-phenotype correlation and very early onset manifestations. This information should be collected in the MPS registries to guide decisions in the future.

5.5 Manifestations That Are Refractory to Therapy

HSCT and ERT cannot fully prevent the development of some somatic complications, although these clinical manifestations seem milder in treated patients [78]. Patients suffer from devastating bone and joint disease; most need multiple and frequent surgical interventions despite treatment. Visual impairment due to corneal clouding is another incapacitating disease manifestation and older patients may suffer from retinal dystrophy or glaucoma [79, 80]. Other manifestations that are difficult to treat are valvular heart disease, hearing impairment and intellectual disability if these manifestations were already present before therapy [80].

An important reason for residual disease manifestations is that accumulation occurs very early in life. Evidence of accumulation can already be observed in MPS fetuses in the early stages of pregnancy [81]. Poor vascularization is another important reason for residual disease in bone, joints and cornea [6].

Because early initiation of current therapies prolongs survival, clinical manifestations refractory to treatment will probably become more prominent. However, some reports on the neonatal initiation of HSCT or ERT, described prevention of some of the manifestations that are currently refractory to therapy including MPS I bone disease [25-33]. Some gene therapy and genome editing studies reported promising results in MPS I mice [82-84]. Gentner *et al.* [85] described the interim results of MPS I patients that were treated with gene therapy. The patients received autologous hematopoietic stem and progenitor cells that were genetically modified to overexpress IDUA. Extensive metabolic correction and amelioration of skeletal disease were observed [85]. More studies on the effect of very early ERT and HSCT and studies on the effect of new therapeutic strategies on these refractory disease manifestations are needed.

6. Conclusion

NBS programs for MPS I are already implemented in several countries and other countries will probably follow in the next years. Early diagnosis of NBS and early treatment positively affect the outcome of MPS I patients. Substantial experience in NBS strategy has been gained after implementing many NBS (pilot) programs. Previous studies demonstrated clearly that a multiple-tiered approach is the most efficient in which enzyme activity analysis and GAG analysis should be followed by genetic analysis for variants in *IDUA* [40, 49, 50, 54, 66].

However, some fundamental challenges related to NBS should be addressed before NBS can be used most effectively. There is a lack of uniformity between the NBS methods that are currently running. Some efforts have been made to compare different methods or increase the uniformity of methods between laboratories [45, 46, 69], but more studies are needed. Uniformity of guidelines on diagnostic and therapeutic approaches for patients that are diagnosed with NBS is also lacking, which is recognized as one of the most challenging problems [40, 42, 66, 69, 76]. Themost suitable therapeutic strategy in MPS I depends on the clinical phenotype. Early onset clinical manifestations and genetic variants may indicate severe MPS I and HSCT may be initiated very early in a subset of patients. There are, however, a large number of patients in which the determination of the

phenotype is very complicated. More studies on strategies to early predict the phenotype before the onset of irreversible disease symptoms such as intellectual disability, should be initiated. Lastly, several manifestations such as MPS I bone disease are refractory to therapy. These clinical manifestations may be more prominent once other clinical manifestations are sufficiently treated and the life span increases. Studies on gene therapy and genome editing are currently being performed. Also, with the implementation of NBS, the effects of very early HSCT and ERT on preventing these manifestations will be clearer. Efforts towards addressing these challenges will provide data to increase the efficacy of NBS for MPS I.

List of Abbreviations

CLIR	Collaborative Laboratory Integrated Reports
CNS	central nervous system
DBS	dried blood spot
DMF	digital microfluidics platform
DS	dermatan sulfate
ERT	enzyme replacement therapy
GAG	glycosaminoglycan
HCII-T	heparin cofactor II thrombin
HS	heparan sulfate
HSCT	hematopoietic stem cell transplantation
IDUA	α-L-iduronidase
LSD	lysosomal storage disorder
MS/MS	tandem mass spectrometry
NBS	newborn screening
MPS	Mucopolysaccharidosis
PKU	phenylketonuria
RUSP	Recommended Uniform Screening Panel
UPLC	ultra-performance liquid chromatography
US	United States

Author Contributions

Sandra Kingma: designing, conducting, reporting, revising the work described in the article. An Jonckheere: designing, reporting, revising the work described in the article. François Eyskens: designing, reporting, revising the work described in the article.

Competing Interests

Sandra Kingma, An Jonckheere and François Eyskens declare that they have no conflicts of interest.

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