MPS I : early diagnosis, bone disease and treatment, where are we now?

**MPS I: early diagnosis, bone disease and treatment, where are we now?**

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List of abbreviations

| | |
|---|---|
| AAV | adeno-associated-virus |
| ALP | alkaline phosphatase |
| BGLAP | bone gamma carboxyglutamate protein; osteocalcine |
| BMP | bone morphogenic protein |
| CCR | C-C chemokine receptor |
| CNP | C-natriuretric peptide |
| CNS | central nervous system |
| Col | collagen |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeat |
| CS | chondroitin sulfate |
| DBS | dried blood spots |
| DS | dermatan sulfate |
| ECM | extracellular matrix |
| EGF | epidermal growth factor |
| ERK | extracellular signal-regulated kinases |
| ERT | enzyme replacement therapy |
| FDA | US food and drug administration |
| FGF | fibroblast growth factor |
| GAG | glycosaminoglycan |
| GSK3B | glycogen synthase kinase B |
| GUSB | β-glucuronidase |
| HCII-T | heparin cofactor II-thrombin |
| Hh | Hedgehog |

| | |
|---|---|
| HS | heparan sulfate |
| HSCT | haematopoietic stem cell transplantation |
| IDUA | α-L-iduronidase |
| Ihh | Indian Hedgehog |
| IL | Interleukin |
| IL-Ra | Interleukin receptor antagonist |
| KS | keratan sulfate |
| LSD | lysosomal storage disorder |
| MCP | monocyte chemoattractant protein |
| MEF | myocyte enhancer factor |
| MIP | macrophage inflammatory protein |
| MS/MS | tandem mass spectrometry |
| NBS | newborn screening |
| NRE | non-reducing ends |
| MMP | metalloproteinases |
| MPS | Mucopolysaccharidosis |
| NFκB | Nuclear Factor kappa-light-chain-enhancer of activated B cells |
| OSX | Osterix |
| PKU | phenylketonuria |
| PTHrP | parathormone related protein |
| RUNX | Runt related transcription factor |
| SDF | Stromal cell derived factor |
| Shh | Sonic Hedgehog |
| SOX | Sry box transcription factor |
| TLR | Toll like receptor |

| | |
|---|---|
| TNF | Tumor necrosis factor |
| VEGF | Vascular endothelial growth factor |
| Wnt | Wingless |
| WT | wildtype |

SUMMARY

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder characterized by α-L-iduronidase deficiency. Patients present with a broad spectrum of disease severity ranging from the most severe phenotype (Hurler) with devastating neurocognitive decline, bone disease and early death to intermediate (Hurler-Scheie) and more attenuated (Scheie) phenotypes, with a normal life expectancy. The most severely affected patients are preferably treated with haematopoietic stem cell transplantation, which halts the neurocognitive decline. Patients with more attenuated phenotypes are treated with enzyme replacement therapy. There are several challenges to be met in the treatment of MPS I patients. Firstly, to optimize outcome, early recognition of the disease and clinical phenotype is needed to guide decisions on therapeutic strategies. Secondly, there is thus far no effective treatment available for MPS I bone disease. The pathophysiological mechanisms behind bone disease are largely unknown, limiting the development of effective therapeutic strategies.

This article is a state of the art that comprehensively discusses 3 of the most urgent open issues in MPS I: early diagnosis of MPS I patients, pathophysiology of MPS I bone disease, and emerging therapeutic strategies for MPS I bone disease.

**Take-home message**

Important challenges to be addressed in the treatment of MPS I patients are: early diagnosis and prediction of phenotypic severity, elucidating pathophysiological mechanisms behind MPS I bone disease, and the development of an effective therapeutic strategy for MPS I bone disease.

## INTRODUCTION

Mucopolysaccharidosis type I (MPS I, OMIM 252800) is a lysosomal storage disorder (LSD) caused by a deficiency of the lysosomal hydrolase α-L-iduronidase (IDUA, [Genbank NG_008103]). It results in the accumulation of the glycosaminoglycans (GAGs) heparan sulfate (HS) and dermatan sulfate (DS) in virtually all body tissues. MPS I is characterized by a wide phenotypic spectrum and is historically classified in three subtypes: MPS I Hurler, Hurler/Scheie and Scheie. However, more practical is the classification in two groups: severe (MPS I-H) and attenuated (MPS I-H/S and MPS I-S), which is used in this report.

Severe MPS I has a distinct phenotype, characterized by progressive central nervous system (CNS) disease. Somatic manifestations include severe musculoskeletal, pulmonary and cardiac disease, inguinal and umbilical hernias and corneal clouding. If untreated, severe MPS I patients have a significantly reduced life span [1]. Attenuated MPS I has a broad phenotypic spectrum with less precise disease characteristics. The phenotype ranges from mild or no neurocognitive impairment but severe somatic manifestations (formerly MPS I Hurler/Scheie) to patients with no intellectual disability and mild somatic manifestations (formerly MPS I Scheie). Life expectancy ranges from the 2nd or 3rd decade to an almost normal life expectancy [1–3].

The pathophysiology of MPS I, and in particular of bone and joint manifestations, is largely unknown. MPS I cells are shown to be filled with enlarged lysosomes containing undegraded GAGs [4]. In addition, GAGs have important regulatory functions. GAGs influence cell migration, proliferation and differentiation. Also, GAGs function as co-receptors for proper interaction of extracellular ligands, including growth factors and chemokines, with their receptors [5]. The secondary effects of GAG storage are poorly understood, but include the storage of secondary metabolites, inflammation as a result of the potential of GAGs to stimulate immune cells, and impaired autophagy [6–9]. In addition, pathophysiological

mechanisms underlying MPS I bone disease include effects of GAGs on elastogenesis, growth factors and osteoclast activity [10–13].

Currently, there are two disease modifying treatment options available for MPS I: haematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT). In HSCT, healthy donor cells are transplanted, after which IDUA is continually produced by the haematopoietic cells, which cross-corrects the other cells in the body. After HSCT, donor stem cells circulate in the bloodstream, cross the blood-brain-barrier, differentiate and produce IDUA, thereby preventing CNS impairment [14].

HSCT is the treatment of choice for severe MPS I, if diagnosed before the age of approximately 2.5 years [15]. HSCT may also be considered in some attenuated patients that show neurocognitive manifestations [15]. It significantly reduces mortality, stabilizes neurocognitive function and significantly reduces the course of somatic manifestations. Due to its considerable risk of mortality and procedure-based morbidity, attenuated phenotypes are rather treated with ERT. However, its safety has increased in recent years, and a 95% overall survival rate has been reported [16,17].

Patients with attenuated MPS I are preferably treated with ERT. Weekly ERT with recombinant IDUA (laronidase) has been shown to improve cardiac and respiratory function, reduce hepatosplenomegaly, and improve quality of life [18,19]. The enzyme binds to mannose-6-phosphate receptors on the cell surface and is delivered to the lysosome where it fulfils its function [14]. The recombinant enzyme can, however, not cross the blood-brain barrier, and therefore, HSCT is the treatment of choice in patients in whom neurocognitive decline is expected (severe MPS I).

MPS I related morbidity and mortality may be significantly decreased if some diagnostic and therapeutic challenges are being met. Firstly, if treated early, neurocognitive function in MPS I patients will stabilize. This necessitates early diagnosis. Several countries have studied or already implemented newborn screening (NBS) (pilot) programs for MPS I [20]. Secondly, after diagnosis, early recognition or prediction of the clinical phenotype is necessary to guide decisions on the optimal therapeutic strategy. Lastly, current therapeutic strategies are not effective in treating MPS I bone disease. Lack of knowledge on pathophysiological mechanisms limit the development of new therapeutic strategies for MPS bone disease. In this report, NBS for MPS I and efforts towards the development of strategies that may predict the MPS I phenotype are discussed. Furthermore, pathophysiologic mechanisms and possible therapies for MPS I bone disease are discussed.

## CHALLENGES IN EARLY DIAGNOSIS OF MPS I

Timely initiation of HSCT or ERT has been shown to significantly decrease morbidity and mortality in MPS I. Early diagnosis of MPS I is essential to allow timely initiation of these therapies. There are several strategies for early diagnosis of MPS I.

Early diagnosis may be achieved by increased awareness among physicians that are confronted with yet undiagnosed MPS I patients. Newly diagnosed MPS I patients frequently have a medical history of recurrent respiratory infections, upper airway obstruction or hernia. Programs to educate otorhinolaryngologists, pediatricians and surgeons may lead to a clinical suspicion of MPS I at an early stage in patients that present with these early onset symptoms. Several awareness programs have been initiated after ERT became available for MPS I [21]. Also, clinical algorithms have been published to raise suspicion of MPS I and enable earlier diagnosis [22].

The International MPS I registry is a registry that has been initiated in 2003 and collects data on natural history and treatment outcomes of MPS I patients. The MPS I registry showed in 2012 that time to diagnosis did not reduce in recent years. Thus, awareness programs that have been initiated in the years before the study seem to have failed in reducing the time to diagnosis in MPS I patients [21]. Recently, a new report from the MPS I registry confirmed again that time to diagnosis has not decreased in recent years. Time to initiation of treatment after diagnosis, however, has significantly decreased [23]. In addition, a single center study from The Netherlands reported no significant reduction in diagnostic delay of MPS I, despite several awareness programs over a period of 20 years in The Netherlands [24].

Early diagnosis can also be achieved by screening. Selective screening or high risk screening targets patients that are at risk for a certain disease. For instance, patients with recurrent airway infections, patients with inguinal or umbilical hernia and patients with corneal clouding have a higher risk of MPS I [21,24]. Screening patients that visit an otorhinolaryngologist, surgeon or ophthalmologist with these manifestations, may identify MPS I patients earlier.

Awareness programs and selective screening aim to diagnose symptomatic patients. These patients may have already developed irreversible neurological deficits. Therefore, a strategy that identifies patients before the onset of symptoms is preferable.

***Newborn screening programs***

A superior strategy that diagnoses MPS I patients before the onset of clinical symptoms is NBS.

In 1961, Dr. Robert Guthrie from Buffalo, New York identified a method to diagnose phenylketonuria (PKU) in dried blood spots (DBS). After that, the first pilot NBS study for PKU was introduced in 29 states and some years later, 27 states had initiated NBS programs for PKU [25]. To date, NBS has been expanded to several diseases in most developed countries, including fatty acid oxidation disorders, organic acid disorders and galactosemia.
The inclusion of diseases in NBS programs is primarily assessed by Wilson and Jungners criteria [26]. These criteria emphasize that the disease should be an important health problem for which adequate diagnostic and therapeutic strategies are available [26]. MPS I is a good candidate for NBS, as early initiation of treatment has shown to significantly alter the disease course [27]. The effect of early HSCT in preserving neurologic function in severe MPS I

patients has been well established [27]. Also, sibling studies have shown significant improvement in neurocognitive function after early treatment with ERT in attenuated MPS I patients [28].

In 2016, the US Department of Health and Human services added MPS I to the Recommended Uniform Screening Panel due to evidence of effective laboratory techniques to diagnose MPS I and due to the benefits of early identification and treatment [29]. Among other countries such as Taiwan, 22 US states are universally screening for MPS I [30]. In 2015, the Dutch minister of Health ordered MPS I to be included in the NBS program in the Netherlands [31], after reports on the increasing success and reduced mortality of HSCT [32,33].

Several countries have studied NBS for MPS I by performing pilot studies, including Brazil, Mexico and Italy. Table 1 shows the results of the largest NBS (pilot) programs that have been published after 2003 and included more than 10,000 DBS.

*Challenges in diagnosis by NBS*

There are several open issues that have to be addressed to maximize the benefit of NBS for MPS I.

Firstly, mutations of unknown significance will be found, including pseudodeficiencies. For instance, Elliott *et al.* [34] reported a positive rate of 13.6/100,000 live births (Table 1). The positive cases, however, included 2 gene carriers and 3 newborns with mutations (A79T, D223N) associated with pseudodeficiency [35]. Also, 1 mutation (G33H) was described which clinical significance has not been proven yet, to the authors knowledge. Therefore, if the mutations that will not cause an MPS I phenotype (A79T, D223N and the gene carriers) are excluded, the birth prevalence is estimated to be 2.33/100,000 live births. Most studies use

enzyme activity assays as a first-tier strategy. GAG analysis and/or mutation analysis are frequently used as a second tier test to avoid false-positives [36]. This was also the method of Elliott *et al.* [34], but the high positive rate may be due to the relatively high cut-off level of IDUA activity (10% of normal values). It is known that even with low IDUA activity, most subjects still retain capacity to degrade GAGs, and MPS I patients often have undetectable IDUA activities. Also, the positive cases in Elliott *et al.* [34] had the same IDUA activity as 2 MPS I carriers.

Clinical management after detecting a mutation of unknown significance poses a significant challenge. These patients require regular follow-up, but it is not clear when these patients should be started on therapy. In addition, there is no consensus when the diagnosis of MPS I may be rejected in patients that do not develop symptoms. In the meantime, patients with mutations of unknown clinical significance are labeled as having a 'disease'. They may be condemned to years of emotional burdening by waiting for a disease that may or may not manifest [37]. Detecting a mutation of unknown significance may also cause social-economic consequences, such as issues with health insurance companies and the costs of medical follow-up. In the future, some countries may implement genetic screening as a first-tier strategy for NBS. Depending on the technique of genetic screening (selective or not selective), a decrease or an increase in the amount of mutations of unknown significance may be expected.

A second open issue is the early determination of the clinical phenotype of patients diagnosed by NBS. As the optimal treatment strategy for MPS I patients depends on the clinical phenotype, methods to early determine or predict the phenotype are urgently needed to enable early initiation of the appropriate treatment strategy. This open issue is discussed in the next section of this article.

Lastly, current treatment options of MPS I bone disease remain limited. Early diagnosis and treatment will lead to increased survival, but probably also increased morbidity associated with skeletal manifestations. Therapy for MPS I bone disease will pose an important challenge. This necessitates studies on the effect of very early initiation of current therapeutic strategies. In addition, studies to elucidate the largely unknown pathophysiological mechanisms underlying MPS I bone disease are needed to enable identification of new therapeutic strategies. These open issues are discussed in the last sections of this article.

***Early recognition or prediction of clinical phenotype***

As early initiation of either HSCT or ERT significantly improves clinical outcome of MPS I patients, early diagnosis and after that, early recognition or prediction of the phenotype is essential. Several countries have already implemented NBS programs for MPS I. Other countries will probably follow in the next few years. This emphasizes the need for a method to early predict the disease phenotype to timely enable decisions on optimal therapeutic strategies and prevent irreversible neurological deficits in patients diagnosed by NBS.

Currently, classification of phenotypic severity is based on the expert assessment of clinical manifestations. Age of onset and clinical evolution of intellectual disability, joint stiffness, joint disease, contractures, kyphosis, cardiomyopathy, and large head or frontal bossing are used by experts to classify patients and decide on subsequent therapy [38]. Expert opinion of phenotypic severity, however, appears to be variable. An expert panel has attempted to assess phenotypic severity in a group of MPS I patients, using a scale from 0 to 10, based on signs and symptoms at presentation. The authors attempted to develop a numeric scale to enable objective phenotypic differentiation. Due to considerable variability in the assessment of MPS

I patients between the experts, the attempt to develop a numeric scale failed [38]. In an effort to predict the phenotypic severity early, early onset and even more unspecific symptoms have to be used. These results suggest that assessing phenotypic severity solely by clinical characteristics would probably be unrealistic. A combination of early onset symptoms and other biomarkers may, however, provide a tool to reliably predict a phenotype before more severe symptoms and irreversible manifestations have occurred.

In earlier attempts to predict the phenotype of MPS I patients early, clinical symptoms, mutation analysis, biochemical methods, or a combination of those methods have been studied.

*Early onset manifestations*

As NBS will diagnose MPS I patients shortly after birth, only symptoms with a very early onset (Table 2) may be useful in distinguishing or predicting the clinical phenotypes.

The median age of onset varies significantly in previous published studies. The MPS I Registy reported a median age of onset between 0.8-1.6 years for the severe phenotype, and sometimes many years later for the more attenuated phenotypes [39]. In a relatively small cohort of 55 severely affected MPS I patients, however, 98% of patients had developed symptoms before the age of 6 months. In this report, attenuated MPS I patients were not studied [40]. It is clear that more data on the natural history of MPS I is needed, in particular symptoms that may occur in the early months, or preferably, weeks of life.

Early onset symptoms (before the age of 6 months) may include kyphosis, upper airway obstruction, coarse facial features, inguinal/umbilical hernias and hepatosplenomegaly [41].

These symptoms appear earlier in severe MPS I patients, as compared to attenuated MPS I patients (Table 2). In addition, in the study of Kiely *et al* [40], more than 25% of severe MPS I patients failed the newborn hearing test and several had congenital birth defects such as cardiac defects, orthopedic deformities and inguinal hernias. Also, most patients developed upper and lower respiratory infections (90%) and feeding difficulties (70%) in the first 6 months of life.

Respiratory infections and feeding difficulties are, however, very nonspecific symptoms and are reported in 33% and 25% of the general population at the same age [40]. It may be difficult to assess if such a symptom results from MPS I. Also, some manifestations such as kyphosis have been reported as difficult to recognize at an early age by parents and care-givers [11]. However, for patients that are already diagnosed with MPS I by NBS, symptoms indicative of a severe phenotype such as kyphosis may prove to be recognized more easily.

HSCT before the age of 9 months may preserve neurocognitive function [40], but if performed later, long-term deficits are expected and patients remain with significant morbidity [33]. It is likely, however, that even the patients that received HSCT before the age of 9 months have already developed some irreversible disease manifestations. It takes considerable time (approximately one year) for donor stem cells to reach the brain after HSCT, replace the existing microglial cells and secrete IDUA effectively. This delayed delivery probably contributes to the slow improvement or even worsening of CNS symptoms in some patients after HSCT [14]. As MPS I patients may be diagnosed by NBS shortly after birth, the large diagnostic gap between diagnosis and determination of a clinical phenotype is a delay that might be ameliorated in the future.

Little is known about symptoms in MPS I before the age of 1-2 months. To the authors knowledge, only one small study has been published in which clinical symptoms before the age of 4 weeks were retrospectively reviewed in 20 patients with MPS I (Table 2). A significant difference between severe MPS I patients and more attenuated patients in the incidence of upper respiratory tract obstruction and inguinal hernia was observed [11].

As the value of symptoms that may predict phenotypic severity is not clear yet, further studies are needed on very early onset symptoms to help discriminate patients with a severe MPS I phenotype soon after diagnosis by NBS. After that, as many symptoms are nonspecific or not easily recognized, guidelines or algorithms must be drafted up to combine or weigh the significance of certain manifestations.

*Mutation analysis*

Over 200 pathogenic variants of the IDUA gene have been reported thus far. Correlations between genotype and phenotype and the possibility of predicting the phenotype by mutation analysis have been studied extensively [35,42–44]. In 2019, the MPS I registry published a report on MPS I genotype-phenotype relationships in 1007 MPS I patients. A clear correlation was described in 68% of severe MPS I patients if the mutation was either homozygous or compound heterozygous for two variants that were predicted to severely affect translation or transcription of the IDUA gene (for example, nonsense and frame-shift mutations). Attenuated patients were never homozygous or compound heterozygous for these specific variants. Also, 10 missense and intronic variants were exclusively present in severe MPS I patients and 15 missense variants were exclusively present in attenuated patients [35].
Some factors, however, significantly limit the possibility of genotype to predict the MPS I phenotype. Firstly, in unique genotypes, e.g. mutations that were observed in only one patient

yet, the influence on a phenotype has not been elucidated yet. In the report of the MPS I registry in 2019, unique phenotypes were present in 12% of patients with a severe MPS I phenotype, and 40% of patients with an attenuated phenotype [35].

Secondly, the role of many mutations on the processing and catalytic properties of the IDUA enzyme is unknown yet. Missense mutations, or other mutations that allow for some residual enzyme activity may be more susceptible to the effects of modifiers such as single nucleotide polymorphisms (SNPs) or epigenetic factors [11]. Some missense mutations indeed appear to have variable effects on phenotypic severity, even if present homozygous [35].

Finally, SNPs, which are thought to be largely phenotypically neutral, may predispose to disease or may influence a disease phenotype. The contribution of SNPs has been studied little. Ou *et al.* [45] studied the relation between SNPs and MPS I phenotype using bioinformatic tools to narrow down SNPs that might influence disease, but could not demonstrate a relationship yet.

Therefore, for some MPS I patients it is possible to classify the disease phenotype based on genotype alone. This emphasizes the importance of other methods for phenotype prediction.

*Biochemical analysis*

Biochemical methods to predict phenotypic severity in MPS I patients diagnosed by NBS should meet some specific conditions. Firstly, the method should clearly distinguish the different MPS I phenotypes. Secondly, the method should be validated. Lastly, the method should be easy to perform and be available in different laboratories. Biochemical methods that have been studied earlier to discriminate between severe and attenuated MPS I patients include enzyme studies, studies of storage products, or other metabolites.

Enzyme assays

To diagnose MPS I patients, the 4-methylumbelliferyl-α-L-iduronide IDUA activity assay is generally used to measure residual enzyme activity in fibroblasts or leukocytes. Because enzyme activity is often too low to distinguish between the different phenotypes of MPS I [46], the possibility of optimizing this assay has been explored. Steps to enhance the ability to differentiate between phenotypes have included the use of higher protein amounts from MPS I fibroblasts, higher substrate concentration and longer incubation time. Two studies indeed reported better discrimination between the phenotypes after optimization. A complete partition of the phenotypes, however, could not be demonstrated [11,47].

Some other enzyme assays to differentiate between the phenotypes have been studied. Fuller *et al.* [48] measured IDUA activity using an immune quantification method and plotted this against stored GAGs, thereby multiplying the differentiating effect. They indeed discriminated patients with severe MPS I from attenuated MPS I. However, the assay is quite complex and antibodies are not widely available. Therefore, it is difficult to repeat in other laboratories. Other authors proposed enzyme kinetic data. In these studies no clear distinction between the phenotypes was observed [46,49].

GAG assays

Quantification of GAGs in urine has been shown to be useful for screening MPS I and is currently used as a biomarker to estimate therapy efficacy. In studies to date, it has been shown not to correlate with disease severity [50] and has been hypothesized to rather reflect storage in the kidneys and not total body storage [51]. Other methods, such as measuring GAG synthesis by sulfate incorporation have been studied, however, with a very small sample size (2 cell lines from severely affected patients and 2 from attenuated MPS I patients) [46].

GAG degradation occurs in an ordered manner from the non-reducing end of the GAG chain. Deficiency of a certain GAG degrading enzyme in the lysosome results in accumulation of characteristic non-reducing ends (NRE), e.g. the terminal carbohydrate structures [52]. Quantification of NRE provides a disease-specific biomarker [53,54]. Herbst *et al*. [54] studied NRE in DBS of 13 severe and 2 attenuated MPS I patients using several tandem mass spectrometry (MS/MS) approaches. One of these methods (endogenous disaccharide) showed a clear separation between healthy control and MPS I patients but also separation between severe and attenuated MPS I patients [54]. Because only 2 attenuated MPS I patients were studied, its potential to predict disease phenotype has to be studied further.
Secondary storage, the accumulation of metabolites other than based on the deficient enzyme, such as chondoitin sulfate (CS) and keratan sulfate (KS) in addition to HS and DS in MPS I, has been previously demonstated [52,55,56]. The above descibed GAG/NRE analysis methods may also be useful to assess secondary storage as a method to predict phenotypic severity [52].

Another approach for GAG quantification is the use of a surrogate marker, such as accumulation of heparin cofactor II-thrombin (HCII-T) complexes in blood. GAGs, in particular DS are known to play a central role in the activation of the HCII-T complex, which is involved in blood clotting. HC-II has been shown to be elevated in MPS I patients and to decrease after initiation of therapy. In a small group of 18 MPS I patients, severe patients had significantly higher serum HCII-T levels as compared to attenuated patients [51]. Further studies are needed to examine the use of HCII-T levels in a large group of MPS I patients with different phenotypes. Also, clear protocols for sample preparation for the assay are needed as the detection of HCII-T levels has been shown to be highly dependent on careful sample preparation and HCII-T levels already have a wide range among MPS I patients. These factors might complicate the use of this method [57].

Other biomarkers

Biomarkers that reflect secondary pathophysiological mechanisms due to GAG accumulation may also be used to differentiate between phenotypes. Raymond *et al.* [58] studied biomarkers in cerebrospinal fluid of severe MPS I patients. They observed an increase in HCII-T, GAGs and lumbar puncture opening pressure in severe MPS I patients compared to healthy control. In addition, the concentration of several cytokines were increased (monocyte chemoattractant protein 1; MCP-1, stromal cell derived factor 1a; SDF-1a, interleukin receptor antagonist; IL-Ra, macrophage inflammatory protein 1b; MIP-1b, interleukin 8; IL-8 and Vascular endothelial growth factor; VEGF) in severe MPS I patients compared to healthy control. Another study compared the concentration of cytokines in healthy control, severe and attenuated MPS I. The authors observed no statistical significant differences and rather large standard deviations [59]. Further research is needed to elucidate the value of these markers to discriminate between phenotypes.

Despite extensive research on biochemical predictors to differentiate between MPS I phenotypes, most studies have been unsuccessful, or subsequent studies have not been published. Therefore, it is likely that biochemical methods may only be successful as part of a method to predict phenotypic severity, as is applicable on all methods described earlier in this report.

*Combination of different methods*

A few research groups developed tools by combining different methods to more accurately predict the clinical phenotype of MPS I patients. Firstly, a theoretical algorithm was proposed consisting of mutation analysis and disease altering SNPs [45]. A second theoretical algorithm

consisted of IDUA activity in leucocytes, IDUA activity in fibroblasts and mutation analysis, to be used after implementation of NBS [47]. These algorithms have to be tested in MPS I patients before their potential can be determined. A last algorithm combined mutation analysis, enzyme activity in fibroblasts using an assay that was optimized for very low enzyme activities, and clinical characteristics that may be present in the first 4 weeks of life. The resulting algorithm had a sensitivity and specificity of 100% to diagnose severe MPS I patients, but was, due to the rareness of MPS I, only validated in a group of 14 MPS I patients. Such an algorithm has to be validated in a larger group of patients [11].

To accurately predict phenotypic severity in MPS I patients diagnosed by NBS, tools that combine different methods, such as the discussed approaches, probably have the largest change of success. Further research is urgently needed as more countries are implementing NBS for MPS I. In addition, these algorithms should be updated regularly when, for instance, new mutations are discovered or superior techniques are identified.

## CHALLENGES IN TREATING MPS I BONE DISEASE: PATHOPHYSIOLOGY

The skeletal manifestations of MPS I patients are generally referred to as dysostosis multiplex. This includes short stature, thoracolumbar kyphosis, flattened vertebral bodies, odontoid hypoplasia, oar-shaped ribs, short and thickened clavicles, bullet shaped phalanges, a large skull, dysplastic femoral heads, coxa valga and genu valgum. MPS I bone disease is one of the most incapacitating manifestations in MPS I patients [41].

Although current therapeutic strategies significantly alter the disease course, there is no effective therapeutic strategy available for MPS I bone disease. To enable the development of new therapeutic strategies for MPS I bone disease, understanding pathophysiological mechanisms and the reason why current therapies fail to succeed is essential. In this section, pathophysiological mechanisms underlying MPS I bone disease are discussed.

### *Bone development and the growth plate*

Bone formation begins after mesenchymal cells condensate into clusters of cells. During intramembranous bone formation, mesenchymal cells differentiate into osteoblasts without the involvement of chondrocytes (i.e. the skull). However, most bones are formed by endochondral bone formation. During this process, a cartilage model is formed and gradually replaced by bone matrix. In the condensates, mesenchymal cells differentiate into chondrocytes that produce collagen 2a1 and the proteoglycan aggrecan. The chrondrocytes start to proliferate, resulting in increased bone growth. Chondrocytes in the center stop proliferating and due to enlargement of the cells (hypertrophy), the bone grows further. Hypertrophic chondrocytes are master regulatory cells that synthesize extracellular matrix (ECM), produce collagen 10a1, attract blood vessels and direct perichondrial cells to become

osteoblasts. The cartilage forms a scaffold for osteoblasts to form a bone collar, which is followed by chondrocyte apoptosis and further ossification.

These steps are regulated by morphogens. Morphogens are signaling molecules that form a concentration gradient, which organizes an area of surrounding cells into a pattern. The response of the cells and organization depends on the concentration of the involved morphogen. Growth factors are morphogens that have been shown to be involved in the pathophysiology of MPS I bone disease and include bone morphogenic proteins (BMPs), fibroblast growth factors (FGF), wingless (Wnt) and the hedgehogs (Hh). Of the Hhs, Indian hedgehog (Ihh) is the master regulator of bone development. It is closely related to Sonic hedgehog (Shh) which is a regulator of limb outgrowth.

Ihh is synthesized by hypertrophic chondrocytes. It stimulates chondrocyte proliferation and osteoblast activity. It regulates its own activity by stimulating the production of parathormone related protein (PTHrP). PTHrP is produced by resting chondrocytes, and stimulates chondrocytes to keep proliferating, thereby delaying the production of Ihh. This step is executed by the stimulation of the transcription factor SOX9. After sufficient proliferation and due to increased distance to the source of PTHrP that stimulates proliferation, chondrocytes stop proliferating. Downregulation of SOX9 leads to upregulation of the transcription factor RUNX2. Subsequent upregulation of MEF2C, leads to differentiation into hypertrophic chondrocytes and again, Ihh production. FGF and BMP have opposite effects on proliferation of chondrocytes, Ihh production and terminal differentiation [60,61]. Upregulation of RUNX2, OSX, and MEF2C leads also to osteoblastic differentiation, stimulation of BGLAP (osteocalcine) and ALP (alkaline phosphatase), and mineralization of bone.

*Growth plate abnormalities in MPS I*

MPS I cells are characterized by enlarged lysosomes, which are filled with undegraded GAGs. Growth plates of MPS I mice show disorganized enlarged chondrocytes (Fig. 1A). The growth plate and in particular the hypertrophic region is thickened, as compared to wildtype (WT) mice. In addition, the distribution of GAGs in MPS I mouse growth plates is altered [13]. MPS I mouse growth plates also showed signs of inflammation (presence of leucocytes), loss of cartilage proteoglycan content and collagen content (less than 50% in comparison to WT) [62]. The growth plates of MPS I patients show similar abnormalities to growth plates of MPS I mice [63].

***GAGs and growth factors***

GAGs are linear polysaccharides comprised of repeating disaccharide units, that are linked to proteins, thereby forming proteoglycans. Proteoglycans are a major part of the ECM of all organs. GAGs form a matrix that is capable of water resorption and desorption, which is able to withstand compression.

GAGs function as co-receptors at the cell membrane for growth factors, among several other substrates. GAGs (in particular HS) that are bound to the cell membrane may undergo proteolytic cleavage by metalloproteinases (MMPs) and heparinases. After release, the growth factors are transported through the ECM either bound to GAGs or by translocation from one GAG binding site to another (Fig. 1B) [64,65]. These processes enable growth factors to be transported over several cell diameters, until they reach GAGs that serve as co-receptors on target cells [64–66].

The synthesis and expression of GAGs are strictly regulated during bone development. An increased amount of loose GAGs in the ECM may either promote transportation of growth factors, but may also serve as a barrier, preventing passive diffusion of growth factors through

the ECM (Fig. 1B). Because partially degraded GAGs accumulate in the MPSs, altered interaction between accumulated GAGs and growth factors probably contribute to disease manifestations.

*Structural abnormalities of GAGs and growth factors*

The interaction between growth factors and GAGs has proven to be complicated and is dependent on many factors. In addition to the quantity of GAGs, the distribution of growth factors is dependent on the structure and sulfation pattern of GAGs. Allen *et al*. [67] studied the assembly of FGF, HS and the FGF-receptor in mouse embryos and demonstrated that structural differences in HS may influence morphogen (e.g. growth factor) gradient formation. They also showed that the assembly of FGF, HS and the FGF-receptor varied with each developmental stage. In addition, experiments using gain or loss of HS sulfation demonstrated that highly sulfated HS may, depending on the growth factor and its receptor, either enable or disable the formation of a growth factor complex and thereby activate or deactivate growth factor signaling pathways (Fig. 1C) [68–70]. For instance, heavily sulfated HS showed to strongly facilitate the interaction of FGF with its receptor. In contrast, the effect of sulfation on BMP signaling was the opposite because sulfated HS is bound to the BMP antagonist Noggin, which prevents BMP to interact with its receptor [69].

The partly degraded GAGs that accumulate in MPS I mice and patients are shown to be more heavily sulfated compared to control [71]. Therefore, altered structure and sulfation pattern of GAGs may be part of the pathophysiology behind MPS I bone disease.

***MPS and growth factors***

As growth plates of the different MPSs share many characteristics, studies on growth plate abnormalities in the other MPSs may provide important information.

MPS II is also characterized by HS and DS accumulation. Bellesso *et al.* [72] studied FGF signaling in several models of MPS II. In a zebrafish model, perturbation of FGF signaling was observed using immunohistochemistry. Also, gene expression of the downstream FGF signaling molecules *ERK1* (extracellular signal-regulated kinase 1) and *DUSP6* were decreased in MPS II mice, zebrafish and fibroblasts from MPS II patients.

In MPS VII, HS, DS and CS accumulate. In bones from MPS VII mice, delayed bone formation has been demonstrated using μCT (micro Computed Tomography). Also, gene expression of markers for hypertrophic chondrocytes (such as *RUNX2*, *PTH1R*, *MEF2C*) and markers for bone formation (*ALP* and *BGLAP*) were delayed in MPS VII mice compared to WT mice [61,73]. Peck *et al*. [73] studied metabolic profiling on bone samples of MPS VII mice in the early stages of bone development. They showed signs of impaired hypertrophic differentiation. In addition, changes in the BMP pathway, e.g. upregulation of BMP2 and 4 and downregulation of BMP inhibitors, which normally occur between 9 and 14 days of age in WT mice, were largely absent in MPS VII mice [73].

Finally, in whole growth plates from MPS VII mice, the expression of *Ihh*, a promotor of proliferation, was downregulated [74]. These alterations in growth factor signaling and function probably also occur in MPS I.

*MPS I and growth factors*

Only a few studies on growth factors in MPS I have been performed yet.

Firstly, Pan *et al.* [75] studied binding of FGF2 in MPS I multipotent progenitor cells. They showed that binding of FGF2 to its receptor was decreased in MPS I cells, compared to binding in healthy control cells. The authors also showed that GAGs from the medium of MPS I cells perturbed the interaction and function of FGF2. Enzymatic removal of MPS I GAGs and replacement by GAGs from normal cells was shown to restore defective FGF2

induced proliferation and survival of MPS I cells [75]. In a second study, enzymatic removal of GAGs from MPS I cells has been shown to also enhance BMP4 signaling [76]. Finally, a large study on growth factors in MPS I demonstrated altered distribution of GAGs and growth factors in several models for MPS I bone disease [13]. GAG and growth factor distribution were studied using immunohistochemistry methods in human MPS I chondrocytes and growth plates from MPS I mice. Altered distribution of GAGs with different sulfation patterns were observed in the ECM of MPS I chondrocytes. This was confirmed in bones from MPS I mice. In addition, growth plates from MPS I mice showed altered distribution of FGF2 and Ihh. As alterations in GAG distribution may influence growth factor signaling, FGF2 signaling was measured by phosphorylated ERK protein levels. Chondrocytes from MPS I patients that were incubated with FGF2 showed increased FGF2 signaling, as compared to healthy control chrondrocytes incubated with FGF2. Finally, the influence of growth factors on bone growth was studied by incubating bones from wildtype and MPS I mice with FGF2 in a bone culture system. Wildtype bones exhibited decreased growth after incubation with FGF2. FGF2 did, however, not affect growth of MPS I bones. These results show that MPS I bones react differently on treatment with FGF2, as compared to WT bones [13].

These studies suggest that altered interaction and distribution of growth factors and GAGs contribute to the pathophysiology of MPS I bone disease. Targeting this pathophysiological mechanism may provide a new therapeutic strategy for MPS bone disease.

### *Other pathophysiological mechanisms*

#### *GAGs*

GAGs regulate the organization of basal membranes and interact with a variety of extracellular ligands such as growth factors, adhesion molecules, tyrosine kinase receptors and Toll-like receptors (TLRs). GAG storage has been demonstrated in lysosomes, but also in

other subcellular localizations such as the Golgi apparatus, endoplasmatic reticulum, and in the ECM. GAG accumulation has a direct effect, but also causes a wide range of secondary effects such as secondary storage and inflammation, as well as impaired autophagy, elastogenesis and skeletal remodeling [5,66].

*Secondary storage*

Accumulation of secondary metabolites, such as CS, KS, gangliosides and cholesterol have been demonstrated in LSDs. In MPS I fibroblasts, secondary storage of CS was demonstrated [55]. In MPS I mice, KS accumulation has been demonstrated which, interestingly, correlated with the severity of bone lesions [56]. As KS and CS are abundant in bone, secondary accumulation may significantly worsen the skeletal phenotype [56].

Secondary storage may be caused by either increased GAG synthesis, decreased degradation or altered cellular trafficking. The lack of normally structured GAGs may lead to enhanced biogenesis of GAGs to compensate for this effect [77]. In addition, enzyme studies have demonstrated that GAGs that accumulate may inhibit lysosomal enzymes [52]. Oxidative stress and elevated lysosomal pH (discussed in more detail below) may lead to the loss of function of several lysosomal enzymes responsible for degradation of metabolites, and thus enhanced and secondary storage [78].

*Inflammation*

GAGs have been shown to influence immunological mechanisms. Studies in brain from MPS I and III mice reported neuro-inflammation, which was characterized by microglial activation, astrocytosis and increases in pro-inflammatory cytokines [7]. GAGs are able to activate TLR4. TLR4 activation, probably by GAGs that are excreted into the ECM by MPS I cells, leads to

activation of the intracellular NFκB pathway resulting in cytokine production and activation of the innate immune system [79,80]. Kellerman *et al.* [81] showed in MPS I mice that the activation of inflammatory mechanisms led to the activation of reactive oxygen species and nitrogen [81]. In chondrocytes from MPS VI rats, in which DS accumulates, Simonaro *et al.* [82] demonstrated increased levels of Tumor Necrosis Factor (TNF) α, nitrogen oxide and apoptosis, increasing with the age of the animals. Similar as in the animal models, increased TNFα levels have been demonstrated in plasma of MPS I patients [83]. These inflammatory processes in chondrocytes and cartilage probably lead to cartilage destruction [84].

*Autophagy*

Another pathophysiological mechanism underlying MPS I bone disease is impaired autophagy. During autophagy, particles in the cytosol that are destined for degradation are secluded into the autophagosome, which fuses with the lysosome leading to degradation and recycling of the components.

Enlargement of lysosomes due to GAG accumulation may lead to loss of the integrity of lysosomal membranes, thereby triggering a pathophysiological cascade that allows for elevation of pH and leakage of lysosomal proteases in the cytosol. This may lead to impaired fusion of lysosomes with autophagosomes and impaired autophagy, in addition to oxidative stress and apoptosis [41,66]. Indeed, abnormal autophagy and increased amounts of autophagosomes have been demonstrated in several animal models of MPS [85].

Impaired autophagy, in addition to altered endocytosis and vesicular trafficking, has been suggested to lead to altered ECM and bone tissue remodeling as a pathophysiological mechanism underlying bone disease in MPS [66].

*Elastogenesis*

Impaired elastogenesis may also contribute to musculoskeletal problems in MPS I. Elastin binding protein (EBP) is a chaperone for tropoelastin and facilitates the assembly of tropoelastin upon growing elastic fibers in the ECM. Decreased expression of EBP has been demonstrated in MPS I fibroblasts, and in healthy control fibroblasts that were incubated with additional DS. Therefore, defects in elastic fiber assembly in MPS I have been attributed to DS accumulation [86]. Biomechanical studies on MPS I mouse bones indeed showed decreased elasticity and lower maximum load values (measured by the ability to bear weight) [87].

*Enzymes involved in bone remodeling*

Bone development requires skeletal remodeling. Skeletal remodeling involves osteoblasts to produce cartilage and bone at some sites, and osteoclasts to resorb cartilage and bone at other sites. Both increased and decreased osteoclast activity can have major functional consequences.

The activity of cathepsin K, the main bone degrading enzyme within osteoclasts is regulated by GAGs. The GAGs CS and KS are shown to have a stimulating effect, and HS and DS have an inhibiting effect [88]. Accumulation of HS and DS may lead to decreased cathepsin K mediated cartilage degradation and a delay in endochondral bone formation. This effect has been demonstrated in MPS I mice [12].

The activity and expression of other enzymes abundant in osteoclasts, such as MMPs, has also shown to be altered in the MPSs [80,89,90]. MMP expression and subsequent chondrocyte apoptosis were upregulated in animals with MPS VI and VII due to by inflammatory responses such as TNFα production [80]. In MPS I mouse growth plates, MMP2 and MMP9 levels were shown to be increased and correlated with the areas in which proteoglycan staining was reduced. As MMPs are known for the ability to degrade ECM components such

as elastin and proteoglycans, the authors hypothesized that MPS I bone disease may be due to enhanced activity of these destructive proteinases [62].

In addition to defects in enzymes produced by osteoclasts, other observations related to bone remodeling have been reported. Firstly, the ruffled border, a membrane structure required for bone resorption, was less developed in osteoclasts from MPS I mice. Secondly, osteoclast number per bone perimeter and osteoclast surface per bone surface were reduced in MPS I mice [91]. Thirdly, in a cross-sectional study, osteocalcin (synthesized by osteoblasts) levels were increased in serum of MPS I patients, as compared to healthy control [92]. Fourthly, the dysregulation between production and resorption of bone may lead to the presence of excess cartilage in the primary calcification zone, further disrupting bone structure [41]. Finally, other factors that are hypothesized to influence bone remodeling and MPS I bone disease are vitamin D deficiency, other nutritional deficits and immobilization [92].

***Disrupted balance***

The simultaneous and coordinated action of secreted growth factors, GAGs and other above described factors, regulate differentiation and bone growth. The above discussed mechanisms all influence each other, resulting in complicated pathophysiological mechanisms underlying bone disease, which are depicted in Fig. 2.

CHALLENGES IN TREATING MPS I BONE DISEASE: EMERGING THERAPIES

MPS I bone disease is one of the most incapacitating features in MPS I patients and patients frequently require multiple surgical interventions. Current therapeutic strategies, ERT and HSCT, have a limited effect on the progression of MPS I bone disease [93]. Current therapies function by circulation of IDUA, either infused (ERT) or produced by transplanted cells (HSCT). Cartilage, which is avascular, receives nutrients by diffusion. The distance that enzymes such as IDUA have to travel to reach cartilage is large and therefore, there is limited penetration of the expressed enzyme into cartilage. The joint capsule normally contains blood vessels, but in MPS I, it may have a more fibrous structure and decreased vascularization due to the skeletal lesions. In addition to cartilage, ligaments and tendons are also poorly vascularized and difficult to treat by current treatment strategies such as ERT [94]. In addition, osteoblasts may have a decreased uptake of IDUA compared to other cells, such as fibroblasts. This has been demonstrated in an *in vitro* study by Tsukimura *et al.* [95]. Also, irreversible bone lesions occur very early in life, maybe even before birth [96]. Therefore, even relatively early initiation of therapy may already be too late to prevent bone disease. Lastly, because current therapies effectively treat most of the other clinical signs and symptoms, patients survive longer. With increased survival, MPS I bone disease, refractory to treatment, becomes more prominent. MPS I bone disease thus becomes an even greater problem and studies on therapies that aim to overcome the above discussed obstacles are needed.

### *Early HSCT*

HSCT effectively for prevents/treats most of the disease manifestations of MPS I, including neurological disease. Significant bone disease persists, however, even with complete engraftment and normal enzyme activities. In 2014, Weisstein *et al.* [97] studied the long term

effects of HSCT in patients between 9 months and 2.5 years, and demonstrated that HSCT had no significant effect on MPS I bone disease. A more recent study was performed by Guffon *et al.* [98] on long term disease burden in 25 severely affected MPS I patients that received HSCT between the age of 12-57 months. Despite HSCT, all patients had significant residual skeletal disease and all patients needed corrective surgical intervention [98]. Several other studies confirmed that skeletal abnormalities progress after HSCT [99,100].

Two studies on the effect of neonatal HSCT in MPS I mice reported almost complete prevention of bone disease [101,102]. A third study showed that after neonatal HSCT, MPS I mice had a significant improvement of parameters of bone structure and remodeling measured by μCT. A combination of neonatal HSCT and weekly ERT was more effective at correcting bone structure and remodeling parameters. The authors remarked, however, that they did observe abnormalities that might lead to alteration of bone remodeling if the mice were followed for a longer period [103].

***Early ERT***

ERT has been shown not to alter the clinical course of MPS I bone disease. Very early initiation of ERT, however, may (partially) prevent MPS I bone disease. The only evidence comes from case history studies on siblings started on ERT at different ages. These studies showed that the earlier ERT is initiated, the better the outcome, probably because younger children still retain some capacity to normalize growth [104,105]. Not all sibling studies, however, reported clear improvement in growth after early start of ERT [28]. Studies on ERT administered in the neonatal period in MPS I mice showed an improved effect on some difficult-to-treat organs such as blood vessels and heart valves, but no alteration of the course of MPS I bone disease. These studies, however, administered ERT either weekly or once

every two weeks, which may not be frequently enough [103,106]. Two studies in canine models of MPS I showed attenuation of bone disease after neonatal ERT [107,108].

A phase I clinical trial to assess the safety and feasability of in utero ERT for LSDs, including MPS I, is currently recruiting (NCT04532047) [109]. ERT may be a valuable option to treat fetusses/neonates with MPS I before more permanent treatment can be initiated.

***Modification of ERT***

Modification of ERT, by chemically altering the enzyme to enable prolonged circulation [110] or targeting the enzyme to a component of bone matrix, may increase the effect of ERT on MPS I bone lesions. Targeting IDUA to bone may be accomplished by using hydroxyapatite-binding sites, terminal deca-aspartate motifs, or the drug alendronate that has a high affinity to bone. Also, the use of nanoparticles as drug-carriers have been suggested, but not yet investigated for MPS I [94,111].

Another alternative is intra-articular ERT. Intra-articular ERT has been shown to be more effective than intravenous ERT for MPS I bone disease in dogs [112] but is, due to the many joints that are involved in MPS I bone disease, probably not a feasible approach for patients.

***Gene therapy***

Gene therapy is often considered as the ultimate treatment for most inborn errors of metabolism. There are two types of gene therapy. In the first type, a viral vector carrying a transgene that encodes the deficient enzyme is injected and introduced into the cells of the patient *in vivo*. In the second type, patients cells are harvested, the transgene is introduced *ex vivo* and the cells are transferred back to the patient. The purpose is to provide a functional

copy of the deficient gene in part of the patients cells, resulting in slowing or reversing the disease.

Liver directed neonatal gene therapy (*in vivo*) using a retroviral vector completely prevented bone disease and some other manifestations in a MPS I mouse model [113]. A subsequent study in MPS I dogs, however, showed that neonatal gene therapy reduced but did not eliminate skeletal disease [114]. Other studies also showed a marked improvement of bone disease after neonatal gene therapy, with effects that were more pronounced in mouse models as compared to canine MPS I models [115–117].

*Ex vivo* gene therapy of autologous haematopoietic stem cells has the benefit of a reduced risk of adverse reactions associated with transplantation of donor cells. Using this approach, supranormal enzyme activities may be aimed. A study in adult MPS I mice that received *ex vivo* gene therapy reported supranormal IDUA activity in haematopoietic stem cells and almost complete prevention of neurologic and bone disease [118]. Studies in MPS I dogs, however, showed undetectable *in vivo* enzyme expression due to immune responses and gene deactivation [119,120]. A phase I/II clinical trial on *ex vivo* gene therapy in MPS I patients is currently running (NCT03488394) [109]. Preliminary results have shown rapid achievement of supranormal levels of IDUA after gene therapy, extensive metabolic correction and amelioration of skeletal disease [121,122].

Other approaches for gene therapy may include *in utero* gene therapy or targeted gene therapy, but these approaches are not yet successful. *In utero* gene therapy in MPS I dogs has failed to sustain enzyme expression in previous experiments [123,124]. There have been some studies on targeting gene therapy to bone, for instance by adapting vectors to have an

increased affinity for hydroxyapatite. In a model of MPS IVA mice, the modified gene therapy regimen had an increased expression in bone, as compared to non-modified gene therapy [125].

### *Genome editing*

Genome editing is a genetic engineering tool that may be used to modify DNA or RNA sequences. The aim is to remove a DNA sequence of interest and replace it with another DNA sequence using artificially engineered nucleases or 'scissors'. The nucleases generate double stranded breaks at a selected position in the DNA, which are repaired by joining with a new sequence that for instance codes for IDUA. The position of this double stranded break may not be the mutation site (which is a specific sequence for every mutation) but rather a 'safe harbour' locus, which is a repurposable place in the genome [126–129]. In addition to gene therapy, genome editing is a very promising approach towards treating MPS I bone disease.

The Zinc-finger nuclease approach (ZFN) was used *in vivo* using an AAV8 (adeno-associated-virus 8) vector in MPS I mice. A corrective copy of the *IDUA* gene was inserted at the albumin locus in hepatocytes leading to sustained expression and IDUA secretion. MPS I mice showed correction of GAGs and prevention of neurological disease, but the effect on bone disease was not studied [127]. A phase 1/2 study of this approach in patients with attenuated MPS I is currently ongoing (NCT02702115) [109].

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat)-Cas9 approach was used *in vivo* by injecting a liposomal complex containing the CRISPR/Cas9 plasmid and a plasmid carrying the *IDUA* gene that were targeted towards the safe-harbour ROSA26 locus. Newborn MPS I mice receiving the treatment had a modest increase in serum IDUA activity, GAG reduction in different tissues and partial prevention of bone disease. The treatment,

however, had no effect on neurological manifestations [128]. An alternative approach is *ex vivo* genome editing. Gomez-Ospina *et al.* [129] genetically modified haematopoietic stem cells with CRISPR-Cas9 to target IDUA to the safe-harbour locus CCR5 (C-C chemokine receptor type 5). This was followed by transplantation of adult MPS I mice. The modified cells secreted supranormal IDUA levels. MPS I mice showed improved neurological manifestations and almost completely normalized bone parameters assessed by μCT [129].

***Small molecule therapies***

Therapies that consist of small molecules may have a better penetration in poorly vascularized cartilage, and may prove a possible therapeutic strategy for bone manifestations in MPS I. Small molecule therapies include substrate reduction therapy (genistein, rhodamine B, odiparcil), chaperone therapy and stop codon read-through therapy.

*Genistein*

Genistein, a naturally occurring soy isoflavone, is the most studied substrate reduction strategy and is available over the counter. Genistein may reduce GAG synthesis by inhibiting tyrosine kinase activity of epidermal growth factor (EGF) receptors. The first report on the effect of genistein in decreasing GAG synthesis in fibroblasts by Piotrowska *et al.* [130], was quickly followed by a series of studies on the effects of genistein in MPS III. Variable effects of genistein were observed in subsequent studies in cells, mice and clinical studies [130–136]. Finally, a double-blind, randomized, placebo controlled trial on the effect of a high dose of genistein in MPS III patients showed no significant adverse reactions, however, also no significant benefit to support the use of genistein in MPS III patients [137,138].

In MPS I, the first studies in fibroblasts also demonstrated a reduction of GAG synthesis [130]. Another study, however, demonstrated increased GAG synthesis and storage in cultured MPS

I chondrocytes [139]. A subsequent study demonstrated significant adverse effects in MPS I mice after genistein treatment, e.g. decreased skeletal growth and scrotal hernia/hydrocele. This may be due to the unspecific effects of genistein on multiple (growth factor) pathways. These results discourage the use of genistein in MPS I patients, and emphasize that genistein should be used with caution in the other MPSs [140].

*Rhodamine B*

Rhodamine B is a chemical fluorescent dye that has been used in cosmetics. After the discovery that Rhodamine B inhibited GAG synthesis in fibroblasts from human lip tissue [141], studies in MPS models were initiated. It has been studied sparsely, but rhodamine B seems to have only mild side effects in humans [142]. It has been shown to decrease GAG synthesis in *in vitro* and *in vivo* models for MPS III [143]. Derrick-Roberts *et al.* [142] studied its use in MPS I mice that were treated from the age of 4 weeks old (juvenile). They studied skeletal disease by μCT of L5 vertebrae and observed an improvement of trabecular bone and bone mineral volume, but no correction. Other bone parameters were unchanged upon treatment [142].

*Odiparcil*

Odiparcil is a β-D-xyloside, a primer (comparable to a scaffold) for GAG synthesis and is used as an oral antithrombic agent [144]. It is thought to function as a 'decoy-substrate' that promotes the production of soluble GAGs that are more easily eliminated via urine [142,145]. It has been shown to reduce cartilage thickening in MPS VI mice [142]. It has been shown safe in adults with MPS VI and currently, a phase I/II trial in pediatric MPS VI patients is planned [145]. The use of odiparcil for MPS I has to be elucidated yet. It has been shown to primarily prime CS, to a lesser extent DS and primes HS only weakly [146,147]. However, its structure can

affect the type of GAG chain that it primes [147] and modifications might be a possibility for future research. In addition, it has been shown to affect HCII-T [144], which is a biomarker in MPS I as earlier discussed.

*Chaperone therapy*

Another possible therapy may be chaperone therapy. Chaperones are small molecules that have the potential to improve folding of misfolded proteins or to protect misfolded proteins from degradation. Most severe MPS I patients, however, have nonsense mutations and very little residual enzyme. Improved folding may probably not significantly affect enzyme activity and clinical manifestations in MPS I patients.

*Stop codon read-through therapy*

Severe MPS I patients predominantly have nonsense mutations. Therefore, stop codon read-through therapy may be an interesting alternative. Compounds such as chloramphenicol and aminoglycosides have the ability to suppress premature stop codon mutations and allow the protein to be fully translated [148,149], but may be toxic. Less toxic compounds such as the drug PTC124 (ataluren) have also been studied. PTC124 has been shown to reduce GAG storage in brain and liver of MPS I mice [150]. A clinical study was ended early, however, due to problems with recruiting patients [94]. There are efforts towards developing compounds that may enhance the effectivity of aminoglycosides and limit its toxicity. The small molecules CC-885 and CC-90009 may induce the degradation of GTPases that stimulate translation termination. A recent study showed that in MPS I fibroblasts that were incubated with an aminoglycoside and one of these compounds, premature stop codon read-through and IDUA activity were both enhanced [151].

### *Anti-inflammatory therapy*

Another promising therapeutic strategy for MPS I bone disease is the use of anti-inflammatory agents. Pentosan polysulfate (PPS) is a sulfated polysaccharide polymer isolated from beech trees has a pro-chondral and anti-inflammatory effect. It is a FDA (US food and drug administration) approved drug for interstitial cystitis and its use is being examined for osteoarthritis and for the MPSs. Simonaro *et al.* [152–155] examined both the use of oral and subcutaneous PPS in different animal models for MPS. In MPS VI rats, it has been shown to reduce GAG accumulation and decrease inflammatory markers, and if administered subcutaneously, is able to partially rescue the bone phenotype [152,153]. In MPS I dogs, a reduction of inflammation and GAGs in urine and tissue were observed, however, bone phenotype after subcutaneous administration was not examined [154]. Subcutaneous PPS treatment has been shown to be safe in a small number of adult MPS I patients (phase 2 study), and resulted in a significant reduction of urinary GAGs and an improvement of joint mobility [155]. PPS has an anti-inflammatory effect and thereby reduces secondary inflammation caused by GAG accumulation. The mechanism of GAG reduction by PPS is, however, not fully understood yet [155].

The use of the TNFα inhibitor infliximab has been shown to decrease inflammation and improve joint pathology in MPS VI rats [156]. Infliximab was shown to be safe and to improve range of motion in a small pilot study in one MPS I and one MPS II patient [157].

***Novel therapies***

*Enzymes*

Enzymes responsible for GAG degradation have been suggested as a potential therapeutic target. A study of Sawamoto *et al.* [158] described the use of keratinase, to reduce skeletal disease in a mouse model of MPS IV. Other enzymes, such as heparinase for HS accumulation and chondroitinase B for DS accumulation, may provide a novel therapeutic strategy for MPS I. The use of these enzymes, however, may also lead to the disturbance of physiological processes that are dependent on GAGs.

*Growth factor therapy*

Because growth factors play a major role in MPS I bone disease, targeting growth factor regulation may be a future therapeutic strategy for, probably specifically, bone disease [13].

Growth factor therapy may include monoclonal antibodies against growth factor receptors or agonists or antagonists for specific growth factors. Monoclonal antibodies are already successfully used to treat some forms of cancer [159]. Most of these therapies, however, aim to eliminate cells and are probably not suitable as a long-term therapy for MPS I patients.

The administration of growth factors, or probably more effective, small molecules that stimulate growth factor signaling pathways, have also been suggested as a possible therapy [13,73]. For instance, GSK3B inhibitors such as lithium as an indirect agonist of Wnt/β-catenin signaling may enhance bone formation. In addition, BMP agonists are small molecules that have been investigated previously, however, not yet in MPS [73].

*C-type natriuretic peptide*

C-type natriuretic peptide (CNP) has been shown to promote growth in several types of skeletal disease, such as achondroplasia and glucocorticoid induced growth retardation. Yamashita *et al.* [160], studied its effect in MPS VII mice. Using a gene delivery system, they overexpressed *CNP* and/or *GUSB* (which codes for β-glucuronidase, defective enzyme in MPS VII). Mice with *CNP* overexpression showed a significant increase in length in comparison to MPS mice without treatment or with only *GUSB* overexpression. The authors observed that *GUSB* overexpression reduced swelling in the resting zone of the growth plate, acceleration of cell proliferation and decreased bone sclerosis, and *CNP* overexpression thickened the proliferative and hypertrophic zones of the growth plates [160]. To combine ERT with a stimulator of endochondral bone formation such as CNP may be a possible approach to specifically target skeletal tissues that are not sufficiently treated by ERT [160].

***Future perspectives***

In addition to the emerging therapeutic strategies described before, some inspiring ideas on future therapeutic strategies that have been studied limitedly or not yet in MPS I are worth mentioning.

Targeting GAGs on the cell surface or in the ECM as an approach to block GAG interactions may reduce the influence of GAG accumulation. For this approach, binding peptides, competing xylosides or other GAG agonists that block receptors may be used [66]. De Pasquale *et al.* [161] incubated MPS I fibroblasts with a recombinant protein to bind excess HS and DS in the ECM and observed a normalization of GAG content and FGF2 signaling. The authors suggest that this 'substrate masking' approach may be a promising therapeutic strategy to prevent the secondary effects of GAGs, such as the effect on growth factors [161].

Because impaired autophagy is an important pathophysiological mechanism underlying MPS I, this might be an interesting therapeutic target. Resveratrol, a polyphenol present in grape peels, has recently been suggested as a autophagy influencer that may benefit MPS I patients [162].

Because of the broad pathophysiology of MPS I bone disease, it is likely that current therapeutic strategies (HSCT, ERT) have to be combined with other therapies that specifically target clinical manifestations that are currently unresponsive to therapy. The need for these therapies, for instance growth factor therapy may be limited to keystages of skeletal development in MPS patients [73].

## CONCLUSION

MPS I patients exhibit devastating disease manifestations, such as severe neurocognitive decline and incapacitating bone disease. Early diagnosis and treatment initiation are important to prevent irreversible disease manifestations such as neurological and bone disease. Several countries have already implemented NBS programs for MPS I and other countries will probably follow in the next few years. There are, however, several challenges that have to be addressed to maximize the benefit of NBS for MPS I. In the current article, we discuss three of the most urgent challenges in more detail: early diagnosis and prediction of the MPS I phenotype, pathophysiological mechanisms of MPS I bone disease and treatment of MPS I bone disease.

The optimal treatment strategy for MPS I depends on the clinical phenotype. Using NBS, MPS I patients will be diagnosed before the onset of phenotype distinguishing disease manifestations. Therefore, methods to early determine or predict the phenotype are urgently needed to enable early initiation of the appropriate treatment strategy and to prevent irreversible disease manifestations. Genotype or phenotype alone seem insufficient to reliably predict the disease phenotype. Studies on early onset clinical characteristics in MPS I patients that are diagnosed by NBS, but also studies on biochemical and genetic markers are essential. In the first section of this article, we describe efforts towards implementing NBS and the prediction of phenotypic severity in MPS I patients. In the future, the development of a tool that combines genetic, biochemical and clinical parameters is probably the best strategy to enable early prediction of the phenotype.

Current treatment options for MPS I bone disease are limited. Early diagnosis and treatment will lead to increased survival and probably increased morbidity due to skeletal manifestations. The pathophysiological mechanisms of MPS I are largely unknown, limiting the development of new therapeutic strategies. In the second section of this article, we provide an overview on the current knowledge of pathophysiological mechanisms underlying MPS I bone disease. Targeting pathophysiological mechanisms such as growth factors, autophagy or enzymes involved in bone remodeling may provide new therapeutic strategies for bone disease.

In the third and last section, we describe the effects of current and emerging therapies on MPS I bone disease. HSCT and ERT have been shown to significantly decrease morbidity and mortality in MPS I patients, but MPS I bone disease is not treated effectively. Studies on the effect of very early initiation of HSCT and ERT in patients diagnosed by NBS should be started as soon as possible. Because MPS I bone disease develops very early in life, however, it is unlikely that these strategies will completely prevent MPS I bone disease. Some emerging therapies, such as gene therapy and anti-inflammatory therapy have produced promising results. In addition, there are several pathophysiological mechanisms that have been unexplored as therapeutic targets for MPS I bone disease. If, in the future, very early HSCT or gene therapy is not sufficiently effective for MPS I bone disease, these therapies may be combined with one or more emerging therapies specific for MPS I bone disease. In addition, phenotype-based or even personalized therapeutic strategies may be developed to more optimally treat MPS I patients.

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| Study | Year | Country | Number of patients | Genetic tests | False-positive rate | Birth prevalence* |
|---|---|---|---|---|---|---|
| Gragnaniello *et al* [36] | 2020 | Italy | 160,011 | + | 0% | 1.25 |
| Hall *et al* [163] | 2020 | Georgia | 95,332 | + | 0.018% | 0 |
| Stapleton *et al* [164] | 2020 | Japan | 18,222 | - | 0% | 0 |
| Wasserstein *et al* [165] | 2019 | New York | 35,816 | + | 0.036% | 0 |
| Taylor *et al* [29] | 2019 | North Carolina | 62,734 | + | 0.028% | 1.59 |
| Chuang *et al* [166] | 2018 | Taiwan | 294,196 | + | 0.004% | 1.35 |
| Donati *et al* [167] | 2018 | Italy | 64,907 | + | 0.012% | 1.50 |
| Minter Baerg *et al* [168] | 2018 | Kentucky | 55,161 | + | 0.002% | 1.81 |
| Burlina *et al* [169] | 2018 | Italy | 44,411 | + | 0.027% | 2.25 |
| Hopkins *et al* [170] | 2018 | Missouri | 308,000 | + | 0.04% | 0.65 |
| Burton *et al* [171] | 2017 | Illinois | 219,973 | + | 0.068% | 0.45 |
| Navarrete-Martinez *et al* [172] | 2017 | Mexico | 20,018 | + | 0.009% | 0 |
| Bravo *et al* [173] | 2017 | Brazil | 10,567 | + | 0.019% | 0 |
| Elliott *et al* [34] | 2016 | Washington | 43,000 | + | 0.014% | 2.33 |
| Liao *et al* [174] | 2014 | Taiwan | 60,473 | - | 0% | 0 |
| Lin *et al* [175] | 2013 | Taiwan | 35,285 | + | 0.048% | 5.67 |
| Scott *et al* [176] | 2013 | Washington | 106,526 | + | 0.006% | 2.82 |

**Table 1.** NBS studies in DBS for MPS I published after 2013 that have included more than 10,000 newborns. * Birth prevalence is expressed as MPS I patients per 100,000 live births.

| Early onset symptoms <1 month old | Early onset symptoms <6 months old |
|---|---|
| Upper respiratory tract obstruction | Coarse facial features |
| Inguinal hernia | Inguinal hernia |
| Congenital cardiac disease | Umbilical hernia |
| Kyphosis | Hepatosplenomegaly |
| Failed newborn hearing screen | Frequent respiratory infection |
| Orthopedic deformities | |

**Table 2.** Clinical manifestations with an onset before the age of 1 and 6 months.

**Figures**

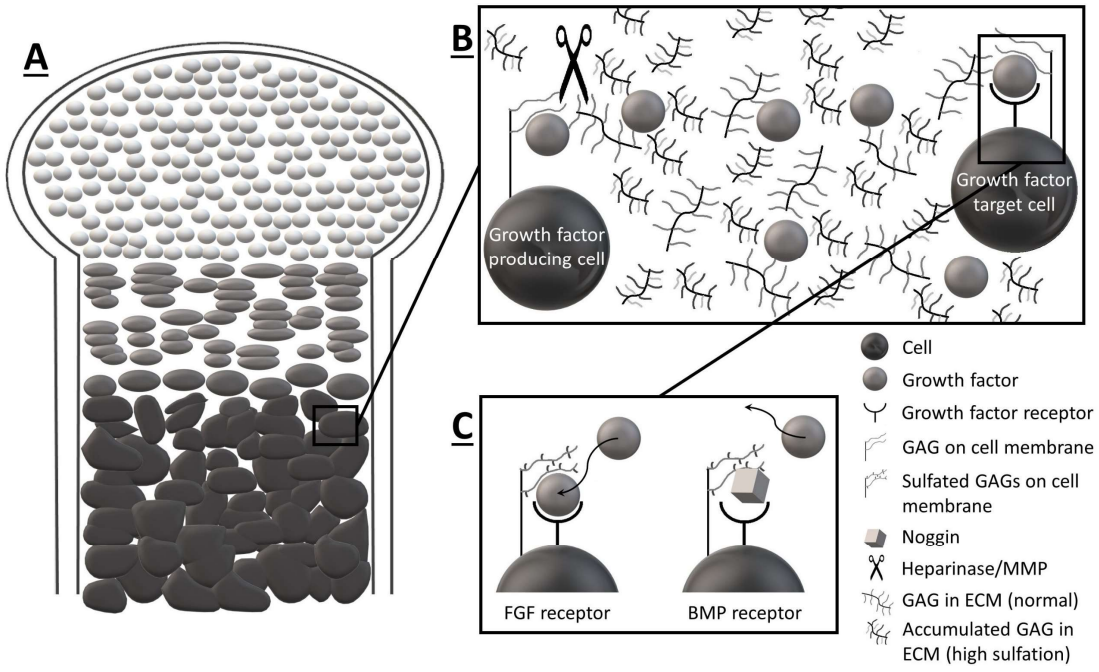


**Figure 1.** Schematic representation of an MPS I growth plate and mechanisms that may contribute to alterations in GAG and growth factor distribution and signaling. A normal growth plate is characterized by long rows of proliferating chondrocytes and a thinner hypertrophic zone. A MPS I growth plate (A) shows short rows of proliferating chondrocytes, more chaotically distributed. The hypertrophic zone is thickened and densely packed with swollen chondrocytes. Schematic and hypothetical representation of growth factor distribution in the ECM of an MPS I growth plate (B). GAGs bound to the cell membrane of growth factor producing cells function as co-receptor for growth factors. GAGs may be cleaved by MMPs and heparinases, after which the growth factor is transported through the ECM. The growth factor remains either bound to the GAG or is translocated from one GAG binding site to another, until the growth factor reaches the target cell. The ECM of a MPS I growth plate is filled with undegraded GAGs with an altered structure, shorter with increased sulfation, which may either promote transportation of growth factors or serve as a barrier. GAGs as co-

receptor on the cell membrane that are highly sulfated (C) may alter binding of growth factors to its receptor. Heavily sulfated HS has been shown to facilitate the interaction of FGF with its receptor. In contrast, sulfated HS is bound to the BMP antagonist Noggin, which prevents BMP to interact with its receptor.

BMP; bone morphogenic protein, ECM; extracellular matrix, FGF; fibroblast growth factor, GAG; glycosaminoglycan, HS; heparan sulfate; MMP; matrix metalloproteinase, MPS; mucopolysaccharidosis.

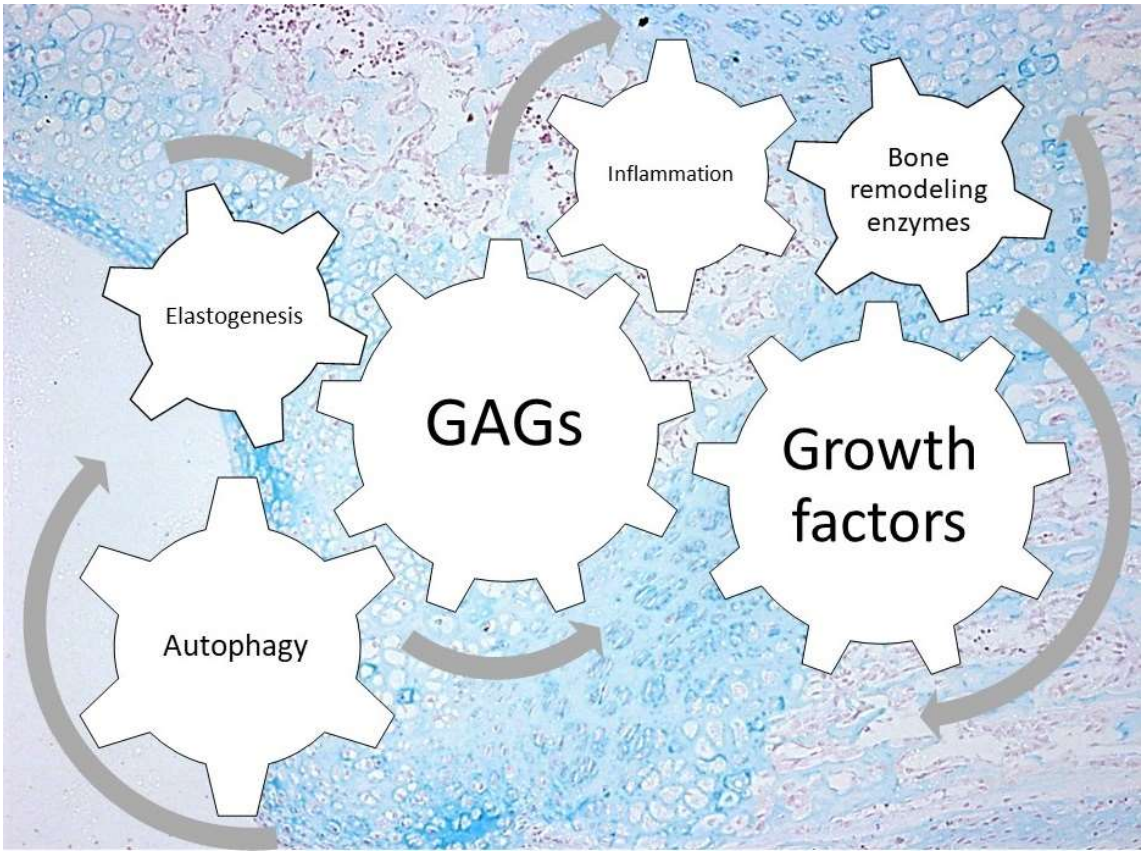


**Figure 2.** Pathophysiological mechanisms underlying MPS I bone disease. GAGs glycosaminoglycans.