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

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter
General discussion and future perspectives
Over the last decades, improved understanding about the pathophysiology and natural history of Mucopolysaccharidosis type I (MPS I), in combination with the availability of disease-modifying treatments, has greatly improved survival of MPS I patients. However, as all patients still display significant residual disease despite therapy, it is clear that more work needs to be done in order to optimize treatment. This thesis comprises studies on two of the important challenges in MPS I: early diagnosis, and treatment of MPS I bone disease.

**NEWBORN SCREENING**

Early initiation of treatment is probably one of the most important factors that may improve clinical outcome in MPS I patients. Pre-symptomatic diagnosis and initiation of treatment, made possible by newborn screening (NBS), may result in the most optimal treatment outcome. Other advantages of diagnosis by NBS are its potential to prevent the often long and burdensome diagnostic odysseys in MPS I and to allow timely reproductive choices and prenatal counseling. NBS (pilot) programs for several lysosomal storage diseases (LSDs) have been initiated in a number of countries, including for MPS I in Taiwan and the United States (chapter 2). On April 14th 2015, the Dutch Minister of Health decided to include MPS I in the current neonatal screening program after recommendation from the Health Council of the Netherlands. There are, however, several issues that complicate the introduction of MPS I in a NBS program. Firstly, the optimal treatment strategy currently depends on the clinical phenotype, and therefore, early phenotypic prediction is essential for MPS I patients diagnosed by NBS. To date, phenotypic severity is often assessed on the basis of clinical signs and symptoms, when irreversible organ damage may already be present. Therefore, the development of tools or biomarkers that may accurately predict the phenotype before the onset of symptoms, is essential. Secondly, the effect of current treatment options on MPS I bone disease remain limited.

**Early assessment of phenotype**

Early prediction of phenotypic severity in MPS I patients can be complicated. Mutation analysis, enzyme activity and clinical characteristics of MPS I patients alone all have limited value in predicting disease severity. Although homozygosity or compound heterozygosity for nonsense mutations corresponds with a severe Hurler phenotype, most missense mutations are associated with a more variable clinical outcome. This variable clinical phenotype is due to several factors, which may include the involvement of secondary biochemical pathways in the pathogenesis of clinical signs and symptoms, such as secondary accumulation of gangliosides or glycosaminoglycans (GAGs) not typically associated with MPS I (chapter 4). Also, over 30 polymorphisms in the IDUA gene have been reported. Although by itself regarded as non-pathogenic, these polymorphisms may modify the severity of MPS I and contribute to the variability in IDUA activity seen in healthy subjects. Also, it seems likely that the introduction of NBS for MPS I will result in the identification of many novel
mutations with unknown effect, which will further complicate phenotype prediction based on genotype. Assessment of phenotypic severity based on signs and symptoms at clinical presentation is difficult, and expert opinion on phenotypic severity appears to be remarkably variable. As newborns diagnosed by NBS will probably lack most of the characteristic symptoms, assessment of the phenotypic severity will be even more difficult.

We combined mutation analysis, enzyme activity measurements and clinical manifestations before the age of 4 weeks in an algorithm (chapter 3), which enables the prediction of phenotypic severity of newborn MPS I patients, and allows timely decisions on the optimal treatment strategy. Before applying this algorithm in a clinical setting, it needs further validation in other cohorts of patients. Also, studies on the effect of certain mutations, combinations of mutations and polymorphisms on IDUA protein levels and IDUA activity should be initiated to improve the predictive value of these mutations. Furthermore, clinical symptoms in an algorithm may be subjective, and studies aimed at objectifying clinical symptoms or modification of the algorithm to include for instance, predictive biomarkers, may be preferable. Finally, especially after introduction of MPS I in NBS panels, such an algorithm needs to be continuously adapted in order to incorporate novel mutations. The early initiation of treatment in patients detected through NBS, however, will completely change the natural course of the disease, which will no longer allow or halt studies on genotype-phenotype correlations.

After NBS implementation

In chapter 2, we describe challenges of NBS for LSDs in general. Findings from (pilot) programs and experience with the introduction of NBS for other inborn errors of metabolism have clearly shown that this will probably lead to the identification of individuals with very attenuated phenotypes and individuals with genetic variants of unknown significance resulting in lowered enzyme activity. Natural history studies on these groups of patients are urgently needed to learn who to treat and when to start treatment, thus avoiding overtreatment in patients who may remain asymptomatic until old age. Also, within the scope of NBS for MPS I, a longitudinal follow-up program for patients diagnosed by NBS is needed. This program should collect data on natural history and treatment responses but also clinical and biochemical data in the first months of life. This may provide essential information for early prediction of the disease severity, for improving our prediction algorithm, and to guide decisions on timing and choice of appropriate treatment strategies. For this aim, the MPS I registry (www.mpsiregistry.com), which is an international database that tracks outcomes of MPS I patients, might be helpful. However, the observational nature of the MPS I registry has led to incomplete datasets, which makes assessment of treatment outcomes in different patient groups difficult. A recent retrospective multicenter study on the outcome of haematopoietic stem cell transplantation (HSCT) in MPS I Hurler patients
showed that essential data on treatment efficacy can, in part, be obtained by international collaboration and rigorous analysis of available data. Such studies might also be pursued for the more attenuated phenotypes of MPS I, and on the outcome of patients on enzyme replacement therapy (ERT). An alternative approach is the use of new international, prospective, and mandatory databases. However, institution of such databases will face significant legal hurdles. In addition, financing the maintenance of these databases and systems for source data verification, may prove to be difficult.

CHALLENGES IN TREATING BONE DISEASE

The current disease modifying therapeutic strategies for MPS I, which are HSCT and ERT, effectively treat many of the clinical signs and symptoms. With increased survival, however, disease manifestations that are refractory to treatment, such as MPS I bone disease, become more prominent. Therapeutic efficacy is greatly limited because irreversible bone lesions occur at a very early age, and may already be present before birth. In addition, large proteins such as lysosomal enzymes have difficulties in reaching target cells in the poorly vascularized cartilage. Studies on therapies for MPS I bone disease that aim to overcome these obstacles, are needed.

Pathophysiology of bone disease

The pathophysiological cascades initiated by accumulating GAGs are largely unknown, which hampers the development of new therapeutic strategies. We studied (chapter 4 and 5) secondary pathological effects of GAG accumulation that likely contribute to MPS I bone disease. Our observation that distribution of GAGs and growth factors are altered in MPS I chondrocytes and MPS I mouse bones (chapter 5), suggest that targeting growth factor regulation may be a future therapeutic strategy for MPS I bone disease. Growth factor therapy, such as the use of monoclonal antibodies against growth factor receptors, is a standard and successful therapy for some forms of cancer. These therapies, however, aim to eliminate cells and the adverse effects are only acceptable for diseases that are otherwise life threatening. Furthermore, treatment in MPS I would probably be very long-term as compared to cancer treatment. Drugs with a better safety profile or with only a partial stimulating or inhibiting effect on certain growth factor receptors may be preferable for treatment or prevention of MPS I bone disease. The effects of such therapies may be studied in the future, however, the involvement of other growth factors in the pathophysiology of MPS I bone disease needs to be elucidated first.

Early start of current therapies

ERT

Initiation of ERT very early in life may (partially) prevent MPS I bone disease. However, the
only evidence comes from case history studies on siblings started on ERT at different ages\textsuperscript{8,9}. A study on ERT started directly after birth in MPS I mice, however, showed an improved effect on other difficult-to-treat organs such as blood vessels and heart valves, but no alteration of the course of MPS I bone disease\textsuperscript{10}.

**HSCT**
A study on the long term effect of HSCT in patients engrafted between the ages of 9 months and 2.5 years old, showed that HSCT does not alter the natural history of MPS I bone disease\textsuperscript{11}. A recent study on HSCT in neonatal MPS I mice, however, reported almost complete prevention of bone disease\textsuperscript{12}. These results indicate that the upper age limit of 2.5 years for HSCT\textsuperscript{13}, may be too high to prevent MPS I bone disease.

The efficacy of HSCT to ameliorate GAG storage has shown to be superior to the effects of ERT\textsuperscript{14}, which is probably due to the fact that ERT is administered only weekly. In contrast, with HSCT, IDUA is produced continually by haematopoietic cells, which may lead to a better pharmacokinetic profile and increased penetration of hard-to-treat organs, such as cartilage. The success of reducing mortality after HSCT, with 95\% overall survival, as described by Aldenhoven \textit{et al.}\textsuperscript{15}, opens up the possibility of treating more attenuated patients. If performed very early, this may prevent MPS I bone disease, which is a frequent and incapacitating manifestation in MPS I patients with all phenotypic severities, but will also improve other clinical manifestations. Also, HSCT is significantly less expensive than (life-long) ERT and ideally a once-only procedure, thus reducing costs and potentially improving the quality of life of patients.

**Emerging therapies**

\textit{Modification of ERT}
Modification of conventional ERT, such as chemical alteration of the enzyme, leading to prolonged circulation\textsuperscript{16}, or the targeting of the enzyme to a component of the bone matrix, which is studied in MPS VI and VII mice, may increase the effect of ERT on MPS I bone lesions\textsuperscript{17,18}. Also, intra-articular ERT seems more efficacious than conventional ERT for MPS I bone disease in dogs\textsuperscript{19}, but is, due to the many joints that are involved in MPS I bone disease, probably not a feasible approach in patients.

\textit{Mesenchymal stem cell transplantation}
As cartilage cells are derived from mesenchymal stem cells, transplantation of mesenchymal stem cells in addition to haematopoietic stem cells, may improve the efficacy of current transplantation protocols to prevent MPS I bone disease. Koç \textit{et al.}\textsuperscript{20} studied the potential of allogeneic mesenchymal stem cell (MSC) infusion in MPS I Hurler patients with a median
age of 15 years that previously received HSCT. They concluded that MSC infusion is safe and improved some of the residual MPS I bone disease. As MPS I bone disease is present very early in life, performing MSC transplantation at an early age, may significantly ameliorate MPS I bone disease.

**Gene therapy**

Gene therapy, which involves the transfer of a gene that encodes the deficient enzyme into cells of the body, is often considered as the ‘holy grail’ for the treatment of inborn errors of metabolism and studies in MPS I have been initiated. Preclinical studies on liver directed neonatal gene therapy have shown promising results for most MPS I manifestations. Skeletal disease, however, was ameliorated, but not prevented completely.\(^2\)

Gene therapy of autologous haematopoietic stem cells before transplantation is another promising approach. A study on the effect of this strategy in MPS I mice showed supranormal IDUA activity in haematopoietic stem cells and almost complete prevention of MPS I neurologic and bone disease.\(^2\) Recently, this therapeutic strategy has shown to be safe and successful in halting the progression of the neurodegenerative diseases X-adrenoleukodystrophy and metachromatic leukodystrophy.\(^2\),\(^3\),\(^4\)

**Small molecule therapies**

Small molecule therapies, aimed at better penetration of the poorly vascularized cartilage, which are currently under investigation for the MPSs, are substrate reduction therapy (genistein), chaperone therapy and stop codon read-through therapies.

Genistein, a natural occurring isoflavone, is the most investigated substrate reduction therapy for the MPSs and is available over the counter. The first report on the effect of genistein in decreasing GAG synthesis in MPS I, II and III fibroblasts,\(^2\) was quickly followed by a clinical trial in MPS III patients by the same group.\(^2\) Subsequently, a lot of different cell, animal and clinical studies followed, particularly for MPS III, and variable effects of genistein were observed (described in chapter 6 and 7). We investigated the effect of genistein on MPS I bone disease (chapter 6 and 7). Surprisingly, we observed increased GAG synthesis and storage in cultured MPS I chondrocytes and significant adverse effects in MPS I mice after genistein treatment. We hypothesize that this effect is due to the very unspecific effect of genistein on multiple pathways, such as growth factor signaling. These results discourage the use of genistein as a treatment for MPS I, and emphasize that genistein should be used with caution in the other MPSs. Currently, a double-blind, randomized, placebo controlled trial on the effect of a high dose of genistein in MPS III patients is executed in the United Kingdom, which will hopefully elucidate potential beneficial and adverse effects of genistein in MPS III patients.\(^2\)
Another emerging therapy is chaperone therapy, consisting of small molecules that have the potential to improve folding of misfolded proteins or protect misfolded proteins from degradation. However, patients with the severe Hurler phenotype frequently have nonsense mutations with very little residual enzyme. Therefore, improved folding or protection from degradation of such small amounts of protein will probably not have a clinically significant effect. However, for patients with a more attenuated phenotype, chaperone therapy may be an interesting option in the future.

Due to the high prevalence of nonsense mutations in the severe Hurler phenotype, stop codon read-through therapy could be a future therapy. Compounds such as chloramphenicol and aminoglycosides have the ability to suppress premature stop codon mutations and allow the protein to be fully translated\textsuperscript{28,29}. More recently, the nonsense suppression drug PTC124\textsuperscript{®} (ataluren), which is currently used as an investigational drug in patients with cystic fibrosis and Duchenne muscular atrophy, has been put forward as a potential therapy for MPS I. It has been shown to reduce GAG storage in brain and liver of MPS I mice\textsuperscript{30} and clinical trials in MPS I patients are currently planned\textsuperscript{31}.

Anti-inflammatory therapy

A very promising therapeutic strategy for MPS I bone disease is the use of anti-inflammatory agents, which reduces secondary inflammation processes caused by GAG accumulation. Pentosan polysulfate sodium (PPS) is a FDA approved drug for interstitial cystitis and has anti-inflammatory and chondrogenic effects. Treatment of MPS VI rats with PPS led to reduced inflammation, partial rescue of the bone phenotype and improved mobility\textsuperscript{32,33}. The first clinical trial on weekly subcutaneous injections of PPS in patients with MPS I is currently planned\textsuperscript{34}.

Because of the broad pathophysiology of MPS I bone disease, it is likely that the most efficient approach is to combine different treatment modalities.

FUTURE PERSPECTIVES

It is clear that treatment for bone disease can only be effective if initiated at a very early stage of the disease. Therefore, implementing NBS for MPS I may prove indispensable. Clinical trials on the effect of very early (neonatal) HSCT and ERT to prevent MPS I bone disease are essential and should be initiated soon after implementation of NBS.

Because bone lesions may already be present before birth, starting treatment directly after birth might, however, not completely prevent MPS I bone disease. The possibility and efficacy of prenatal treatments, such as gene therapy and HSCT, are being investigated in MPS VII mice\textsuperscript{35,36}. Prenatal treatment, however, requires prenatal diagnosis. Prenatal
counselling, which is currently done by enzyme or molecular testing on chorionic villi or amniocytes, is usually only considered in families of MPS I patients. Some studies on less invasive strategies for prenatal diagnosis of the MPSs have been performed, such as nuchal translucency for diagnosing MPS VII patients, and maybe even MPS II patients. Probably more applicable for MPS I, the detection of skeletal abnormalities that may already be present before birth, using sensitive imaging techniques for prenatal diagnostics, might be a future diagnostic approach. Another approach is preconception screening, to investigate whether the parents are carriers for MPS I causing mutations. Finally, fetal DNA and RNA can be detected in maternal blood from 5 weeks gestational age, thereby proving a method for early non-invasive prenatal diagnosis. Because maternal blood is a mixture of small amounts of fetal nucleic acids within a wide background of maternal nucleic acids, it is difficult to distinguish fetal nucleic acids from maternal nucleic acids. Therefore, current studies are based on the analysis of specific paternally inherited traits that are not present in the maternal genome. In the future, however, single nucleotide polymorphisms (SNPs) may be used to distinguish fetal nucleic acids from maternal nucleic acids and to diagnose maternally inherited disorders.

For an ultra-orphan disease as MPS I, collecting and sharing data is important to gain as much information as possible, complete studies as quickly as possible, and prevent unnecessary studies. Therefore, all the earlier mentioned efforts should be undertaken in a collaborative and international approach to ensure optimal research on future treatment of MPS I and prevention of MPS I bone disease.
REFERENCES


with rhIDUA is safe, well-tolerated, and reduces articular GAG storage in the canine model of mucopolysaccharidosis type I. Mol Genet Metab 2014;112:286-293.


35. Karolewski BA, Wolfe JH. Genetic correction of the fetal brain increases the lifespan of mice with the severe multisystemic disease mucopolysaccharidosis type VII. Mol Ther 2006;14:14-24.


