SANFILIPPO SYNDROME CLINICAL GUIDELINES

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01.
Diagnostic pathophysiology. Genotype-phenotype correlation.

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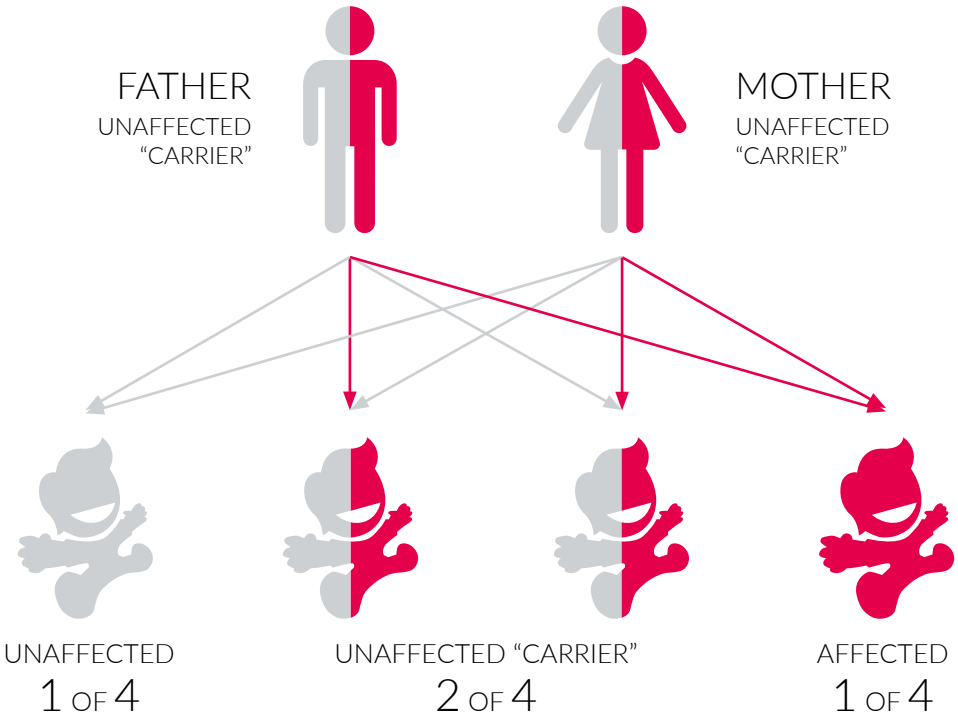
Mucopolysaccharidosis type III (MPS III) or Sanfilippo disease is one of 40 currently described lysosomal diseases. It is considered the most common MPS disorder[1], with an estimated incidence that varies according to the subtype and the geographic region, although the average prevalence of this disease is one in every 70,000 live births[2]. Nonetheless, this incidence may be an underestimation of the true prevalence of the different types of MPS III due to the difficulties that are involved in the correct diagnosis of mild forms. The disease is inherited in an autosomal recessive manner (Figure 1).

The four MPS III subtypes (A, B, C and D) are classified based on four different enzyme deficiencies in the heparan sulfate degradation pathway, which in turn are caused by different mutations (Table 1). This disease was first described by the paediatrician Silvestre Sanfilippo[3]. However, it was discovered in 1961 when the clinical manifestations of a female child with hepatosplenomegaly, a normal skeletal evaluation, and excretion of large amounts of heparan sulfate in her urine were published[4]. In 1963, Sanfilippo et al. described eight

Figure 1. **Recessive autosomal gene.**



children with intellectual disability and elevated excretion of a single glycosaminoglycan (GAG) heparan sulfate[3]. Most of the children who only excreted heparan sulfate had a less coarse facial appearance and milder somatic and radiographic manifestations compared to Hunter and Hurler patients (MPS II and MPS I, respectively), who presented with more severe manifestations. MPS III –Sanfilippo disease is characterised predominantly by a severe degeneration of the central nervous system.

Table 1. **Classification of MPS III – Sanfilippo disease**

Subtype MPS III (MIM #)	Gene (MIM *)	Deficient enzyme	Accumulated GAG
MPS III-A (#252900)	SGSH (*605270)	Heparan N-sulfatase	HS
MPS III-B (#252920)	NAGLU (*609701)	α-N-acetylglucosaminidase	HS
MPS III-C (#252930)	HGSNAT (*610453)	Heparan-α-glucosaminide N-acetyltransferase	HS
MPS III-D (#252940)	GNS (*607664)	N-acetylglucosamine 6-sulfatase	HS

MIM #: Online Mendelian Inheritance in Man (https://www.omim.org); HS: Heparan sulfate

1.1 PATHOPHYSIOLOGY

MPS III - Sanfilippo disease is caused by the lysosomal accumulation of heparan sulfate (HS), which belongs to the glycosaminoglycan (GAG) family. This is caused by a congenital deficiency in one of the four enzymes that are involved in its sequential degradation: heparan N-sulfatase (SGSH, MPS III-A; MIM #252900), α-N-acetylglucosaminidase (NAGLU, MPS III-B; MIM #252920), heparan-α-glucosaminide-N-acetyltransferase (HGSNAT, MPS III-C; MIM #252930) and N-acetylglucosamine 6-sulfatase (GNS, MPS III-D; MIM #252940)[5]. HS degradation occurs in the lysosomes of the cells.

HS chains are linked to a core protein that forms part of the HS proteoglycans (HSPG). A wide variety of HSPGs can be found, both on the cell surface and in the extracellular matrix, and these include syndecanes, glypicans, and perlecans, depending on the core protein and the number of HS chains that are attached to it [6]. HSPGs are involved in many different cell systems and functions, including cell migration, the vesicle secretion system, the endocytic system, cell adhesion and motility, the basal membrane structure, and the recognition of different factors and molecules as receptors or coreceptors [7].

HSPGs are essential components of the cell surface and extracellular matrix, and these provide structural support to glial and neuronal cells. It is worth mentioning that these regulate several

signalling pathways, control the proliferative capacity of neural progenitors, are essential for brain patterns and neurogenesis, and that they participate in the neuronal migration, axon guidance and synaptogenesis processes [8,9,10].

The accumulation of HS causes an alteration in the lysosomal environment given that the excess of non-degraded molecules can bind to various hydrolases, therefore reducing their activity, and resulting in a secondary accumulation of gangliosides (gangliosides GM2 and GM3)[11] and other GAGs, both inside and outside the lysosome that may contribute to the CNS pathology. Furthermore, HS deposits are found, not only within the lysosome, but also in other subcellular locations, therefore affecting the functionality of the CNS[12]. In addition, the storage of undegraded molecules affects intracellular traffic and flux in the endolysosomal and autophagic pathways[13]. It is possible that fragments of HS released into the extracellular matrix interfere with many HS functions, favouring the development of the disease. HSPGs act as ligands for several factors such as FGF and BMP4, the signalling of which is impaired due to the unbalanced turnover of HSPGs associated with the disease[14].

Recently, Yubero et al. observed a combined deficiency of Coenzyme Q10 and pyridoxal phosphate (PLP) (coenzyme and active form of vitamin B6) in patients with MPS III[15]. This secondary Coenzyme Q10 (CoQ10) deficiency could be related to this disease's complex pathophysiology[15]. Lysosomes have a high concentration of CoQ10 located in their membrane. This has been shown to play an important role in the acidification of the lysosomal lumen[16].

A decrease in the cellular concentration of CoQ10 results in an increase in reactive oxygen species (ROS), leading to oxidative stress, which causes lysosome and consequently cellular damage[17].

This lysosomal storage disease presents severe neurological manifestations. It has been observed that the main affected organ in MPS III patients is the central nervous system, while the somatic manifestation is relatively mild in contrast to other types of mucopolysaccharidosis[2]. Although the pathological mechanism in MPS III is not yet known, several mechanisms that may contribute to the neurodegeneration process have been described.

Firstly, an interaction between neuroinflammation, microglial activation, and adaptative immunity in MPS[18] has been suggested. In this line of research, several studies in animal models of MPS III-A and III-B have demonstrated the role of the immune system in the pathology of MPS III. HS has been shown to trigger an immune response in MPS III and, likewise, it has been shown to be involved in microglial activation in the central nervous system in the mouse model for MPS III-B[19].Microglial cells, such as astrocytes and the entire immune system in the brain are activated by the interaction of undegraded HS fragments with their respective TLR4 (Toll-like receptor4)[19,20,21,22,23]. Therefore, the mononuclear phagocytes resident in the brain (microglia) appear to be key in the neuroinflammatory processes that are associated with MPS[24], as once activated, these induce the synthesis of inflammatory cytokines, including TNF-α (tumour necrosis factor α) and IL-1β (interleukin 1β). At the same time, the inhibition of the

TLR4 signalling pathway in MPS III-B mice, despite causing a delay in inflammation in the brain, does not actually slow down the progression of the neurodegenerative process. Other studies have demonstrated the ability of HS to initiate an adaptive immune response by activating T lymphocytes in MPS III-B mice[25] which leads to the synthesis of inflammatory cytokines. All of these complex neuroinflammation and systemic inflammatory immune response processes appear to be responsible for the severe and progressive neurodegeneration that is observed in MPS III subtypes A and B.

In a mouse model of Sanfilippo B disease, a clear accumulation was found in the storage vesicles within the microglial cells[26]. These types of cells are essential for the brain's defence, and their alteration can result in the release of toxic products that, together with neuroinflammation, can contribute to neurodegeneration in patients with Sanfilippo syndrome.

Secondly, the deposition of GM2 and GM3 gangliosides, which takes place in the neurons of the cerebral cortex and cerebellum[27] has previously been described as the cause of neuronal apoptosis in Tay-Sachs and Sandhoff diseases, where the accumulation of GM2 is caused by genetic defects in hexosaminidases A and B, respectively[28,29]. Furthermore, considering that gangliosides, cholesterol and GPI-linked proteins (glycosylphosphatidylinositol) form clusters in lipid rafts, a possible involvement of rafts in the brains of mice with MPS III-A and III-B has been suggested. In fact, the increase in GM3 observed in a mouse model for MPS III-B would reflect an enrichment of these substances in rafts in the plasma membranes of neurons in the mid-entorhinal cortex region in the mouse's brain with MPS III-B[30]. Additionally, it has been suggested that the absence of gangliosides may have detrimental effects in MPS III[31]. As a result they have observed a significant reduction in life expectancy, as well as an increase in neurodegeneration in double mutant mouse models MPS III-A or III-B with knock-out of GalNAc transferase (Galnt3), a crucial enzyme for the synthesis of gangliosides, when compared with only mutant mouse models of MPS III-A or III-B[29].

Likewise, it has been demonstrated that ganglioside storage results in a reduction in calcium uptake by the endoplasmic reticulum (ER), together with the consequent increase in cytosolic calcium levels that trigger neuronal apoptosis, therefore favouring neurodegeneration. Decreased ER calcium can activate the unfolded protein response, which also triggers apoptosis, contributing to severe neurodegeneration[32].

Thirdly, neurodegeneration could be caused by the presence of protein aggregates[33,34,35]. In a post mortem test of the brains of patients with MPS III-B, a reduction in the density of GABAergic interneurons was observed, which could be related to mental illness. Likewise an accumulation of α-synuclein was detected, which could be related to the neurodegeneration of swollen-inflamed neurons[34]. In studies with the mouse animal model of MPS III-B, hyperphosphorylated tau protein (Ptau) and amyloid beta protein were detected in neurons in the mid-entorhinal cortex and dentate gyrus regions[35]. Moreover, elevated protein levels that are associated with dysfunction in autophagy were observed in these two brain regions; modified proteins with nitrotyrosine; which is a marker of oxidative stress that is observed in the brain of MPS III-B; and modified proteins linked to O-GlcNAc (N-acetylglucosamine), which

is a marker of metabolic stress, as well as an increased level of HS proteoglycans, known as glypicans, outside the lysosome compartment[30]. It has been suggested that this accumulation of glypicans outside the lysosome could be harmful to neurons because they are potentially involved in the formation of Ptau and beta amyloid aggregates, which are toxic and difficult to degrade[30]. Accumulation of these glypicans and the other markers identified in MPS III-B has also been observed in mice with MPS III-A. However, its levels were lower in the case of mice with MPS I and MPS II; and none was detected in the MPS VI mouse animal model, in which the heparan degradation pathway was not blocked[30]. Although there are differences between MPS III and Alzheimer's disease (for example, in MPS III there is no extracellular plaque formation); both have similar pathologies, such as the presence of Ptau and beta amyloid. Tau hyperphosphorylation probably represents one of the late events in pathogenesis that leads to neurodegeneration.

Fourthly, the accumulation of fragments of HS, which in MPS III-A and III-B are excessively and abnormally sulfated, can cause counter signalling reactions in brain neurons. In particular, they induce an overexpression of the GM130 protein and consequent alterations in the Golgi apparatus[36]; an increase in proliferation and the resulting neuritis[37], as well as alterations in cell polarisation and in neural cell migration[38], with all of these potentially contributing to neuropathology. Most of the knowledge about the physiological mechanism of MPS III comes from studies performed on animal models such as the knock-out mouse model for MPS III-B[39] and MPS III-A[40].

Future studies may demonstrate whether the pathophysiolocal mechanism is the same in the four MPS III subtypes, despite the existence of genetic and biochemical differences and regardless of the severity of the clinical manifestation. More recent mouse model studies for MPS III-C have confirmed signs of general inflammation in the brain, including the activation of astrocytes, microglia, and cytokine production, similar to those previously described for the other MPS. Furthermore, Martin et al.[41] observed a mitochondrial dysfunction in neurons and a neuronal loss that would explain why MPS III-C manifests itself predominantly as a neurodegenerative disease. Nonetheless, the precise sequence of events that commence with HS accumulation and that result in a widespread brain pathology and neuronal malfunction, loss, and death is yet to be determined.

In an MPS III-A mouse model, a high number of autophagosomes were found, as a consequence of the deterioration of the autophagy-lysosomal function, which probably leads to cell death[42].

Finally, it is important to mention that a link between Sanfilippo syndrome and Parkinson's disease was suggested when the mutations that cause Sanfilippo syndrome were linked to an increased risk of developing Parkinson's disease with the presence of alpha-synuclein aggregates in the brains of patients.

1.2 BIOCHEMICAL AND GENETIC DIAGNOSIS

Given the fact that MPS III manifests itself primarily as a neurological disease and it has few somatic clinical manifestations, its early diagnosis can prove difficult[43]. Current diagnosis laboratory practices for MPS III have been summarised below, with the goal of facilitating early patient identification and diagnosis. The diagnostic approach is based on clinical suspicion, radiological examinations, and detecting the presence of GAGs in urine (in particular, the increased excretion of HS). By evaluating the corresponding enzymatic activity (according to subtype) in leukocytes or fibroblasts, and by using molecular genetic studies, it is possible to obtain the definitive diagnosis. In Figure 2 an algorithm for the diagnosis of MPS III has been included[44,45].

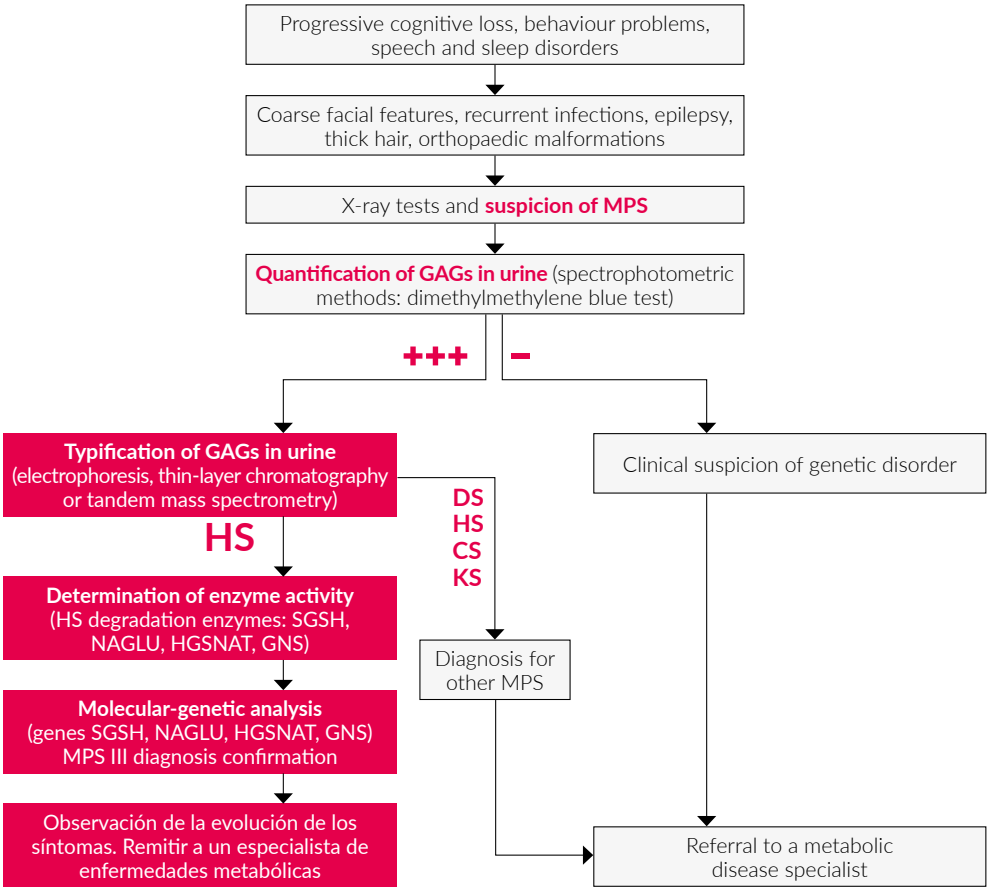
1.2.1 Determination of GAGs in urine

When a clinical suspicion of MPS III exists, we must firstly request a quantitative test to determine the presence of GAGs in urine using spectrophotometric methods with dimethylmethylene blue (DMB)[46,47]. GAG determination is an inexpensive and non-invasive test. Furthermore, at atmospheric temperature, GAGs remain very stable, therefore urine samples do not need to be frozen for shipment to the laboratory. The DMB test is based on binding the sulfate radical of the GAGs to dimethylmethylene blue and quantifying the GAG-S-DMB complex using a spectrophotometer[48]. The test's sensitivity varies depending on the technique used and the desired diagnostic threshold, although several studies place it at 100%, giving it a specificity of 75-100%[49]. Recent studies have also shown that the DMB test can be performed with dry urine samples on filter paper without any loss of precision, therefore making it much easier to send samples to laboratories[50]. This quantitative determination of GAGs in urine is a simple, non-invasive, inexpensive test, which is particularly useful in screening suspected MPS. For this reason, it is recommended that this test should be performed on all patients with developmental delay and/or abnormal behaviours. Although other quantitative tests can be performed to determine the presence of GAGs in urine (Alcian blue test, Azur A and B, carbazole reaction and turbidity test with cetylpyridinium), these tests are less sensitive or much more laborious than the DMB test, and therefore their use in the diagnosis of MPS III is not recommended. Additionally, semiquantitative urine tests that use cationic filter paper stains (for example, the Berry spot and Ames spot tests) give relatively high false-positive and false-negative percentages and are outdated[51,52,53].

It is interesting to note that a negative result in the test performed to determine the presence of GAGs in urine does not rule out MPS III, because in some patients with attenuated forms of the disease there may be an overlap in the levels of GAGs excretion with healthy controls, meaning therefore that the increased excretion of HS in MPS III may be overlooked[54,55]. There are reported cases of false positives and false negatives, specifically in MPS III patients with intermediate or mild phenotypes[56] and, of course, in heterozygotes[57]. Furthermore, the GAGs excretion in urine tends to decrease with age and among patients with more attenuated forms of the disease; therefore, it is less likely that older patients and/or those with more attenuated phenotypes will show levels as high as those in younger and more affected children[58,59]. Another factor that must be taken into account is the dilution of the urine sample. When determining the presence of GAGs, collecting the second urine of the day is advised given

Figure 2. **Diagnostic algorithm for MPS III - Sanfilippo disease** (modified from references[44,45]).

+++ : Elevated levels of GAGs in urine / **-** : Normal levels of GAGs in urine / **HS**: Heparan sulfate / **DS**: Dermatan sulfate / **CS**: Chondroitin sulfate / **KS**: Keratan sulfate / **SGSH**: Heparan N-sulfatasa / **NAGLU**: α-N-acetylglucosaminidase / **HGSNAT**: Acetyl-CoA: α-glucosaminide-N-acetyltransferase / **GNS**: N-acetylglucosamine 6-sulfatase.



that the first usually contains more false positives, or a combination of several urine samples may be taken. Finally, considering that the levels of GAGs in the urine decrease with age, before stabilising at approximately 15 years old, in order to interpret the concentration of GAG in urine, these results must be compared with the normal range for each age. Therefore, clinicians must be careful when comparing the results of GAG tests that have been performed in different laboratories given that the normal values and units of measurement may vary between laboratories.

The test for the quantitative determination of GAGs in urine is sensitive but nonspecific. Therefore, all urine samples that test positive in the quantitative GAG analysis must be confirmed through electrophoresis or thin layer chromatography, techniques that offer improved sensitivity and specificity. By using these techniques it is possible to identify the abnormal pattern of GAGs, which in the case of patients with MPS III corresponds to a band indicating an excess of HS, without the presence of either dermatan or keratan sulfate[60]. In addition, tandem mass spectrometry is another method that can be used for the detection and quantification of specific types of abnormal GAG species in urine[61,62,63].

Using this technique for GAGs analysis proves useful for screening; however, it must not be used to give a definitive diagnosis. Furthermore, a clear disadvantage is the need for expensive equipment that is only available in some laboratories.

GAG determination by tandem mass spectrometry can be performed according to several different perspectives, by enzymatic digestion, by butanolysis and by methanolysis. There are disadvantages and advantages to each of these procedures. In the case of enzymatic digestion, GAGs release unmodified disaccharides, which are the true analysed markers, nonetheless, this is an indirect measurement. On the other hand, butanolysis seeks to ionise the GAGs by adding butanol molecules allowing for proper identification. The methanolosis procedure is similar, with ionisation being performed with methanol. Butanolysis is recommended for MPS III given that it permits optimum HS quantification[64]. Nevertheless, since 2016, methanolysis has made it possible for the four different types of GAG (HS, Dermatan Sulfate, Queratan Sulfate and Chondroitin Sulfate) to be quantified[65].

In recent times, Lawrence et al. have been developing a mass spectrometry method, which can be used for analysing non-reducing end (NRE) carbohydrates as biomarkers for MPS Settembre[66,67].

1.2.2 Determination of enzymatic activity

The gold standard technique for diagnosis is the determination of the enzymatic activity in skin fibroblast cultures, leukocytes, plasma or serum. The specific diagnosis of MPS III-A, -B, -C or -D is confirmed by demonstrating a decrease or absence of one of the four enzymatic activities that are involved in the degradation of HS in the patient's leukocytes or fibroblasts. This reduction should be less than 10% when compared with this activity in healthy individuals, with normality of the other sulfatases. Given that multiple sulfatase deficiency disease also presents a reduction in the activity of heparan N-sulfatase, N-acetylglucosamine 6-sulfatase,

and other sulfatases, it is necessary to perform the biochemical analysis of at least one other sulfatase in order to confirm the diagnosis of MPS III, therefore ruling out the multiple sulfatase deficiency[68].

Different methods exist for the enzymatic determination in MPS III: radioactively labelled oligosaccharide assays[69,70,71,72,73], spectrophotometric assays[74,75] and fluorescence-based assays[76,77,78,79,80]. At present, fluorescence-based assays are used, therefore allowing the enzymatic activities to be determined using the corresponding fluorogenic substrates (4-methylumbelliferyl-), which are commercially available. Enzyme assays have higher sensitivity than urine sample-based methods. It is worth considering that the enzymatic determination for MPS III-B through dried blood spot testing is only valid for screening, and positive results must be confirmed in plasma samples[81]; future studies will confirm the use of this technique in routine clinical practice[82]. Moreover, the fluorimetric enzyme assays for MPS III-A, -C and -D do not yield fluorescent products, therefore requiring the addition of a hydrolytic enzyme coupled with enzymatic reaction in order to generate the fluorescent 4-methylumbelliferone substrate.

For this reason, patients with deficiency in terms of betahexosaminidase activity may give false positives for MPS III-C[80], and in the case of MPS III-A and -D (both caused by sulfatase deficiencies), the second sulfatase activity must be measured in order to rule out multiple sulfatase deficiency[83]. New methods are currently being developed for the quantification of enzyme activities in MPS III through fluorimetry and tandem mass spectrometry[84], however these techniques are not yet available for the four MPS III subtypes. Analytical techniques are in the process of being developed, and these allow for detection in samples impregnated on paper through multiplex assays that include several MPS[85].

1.2.3 Molecular genetic analysis

Diagnosis for the identification of the causative mutations of each subtype of MPS III is confirmed by conducting molecular studies. In this technique, the coding regions (exons) and flaking intronic regions (exons) of each of the four genes that are responsible for MPS III are amplified and sequenced, and the results are subsequently compared with reference sequences and databases containing known mutations and polymorphisms. Molecular genetic testing can include a combination of gene-targeting tests (multigene panel) and comprehensive genomic testing (exome sequencing, array exome, genome sequencing) depending on the presenting phenotype. Molecular genetic testing must be offered to the families of all patients in order to ensure that they are offered genetic counselling. Genotype/phenotype correlations have been described for a limited number of genetic variants that may aid prognosis (more details in the next section of this chapter).

The mutations that have been described to date can be found in the Institute of Medical Genetics's Human Gene Mutation Database (HGMD®) (Cardiff, United Kingdom)[86]. The SGSH gene (OMIM: 252900), which codifies sulfamidase or heparan N-sulfatase, the deficiency of which results in MPS III-A, is located on chromosome 17q25.3[87]. To date, 142 mutations have been described, the majority of which are missense mutations (missense or

non-missense mutation) (64.1%). Nonsense mutations (with premature stop), small insertions and small deletions have also been found. In MPS III-B, the NAGLU gene (OMIM: 609701), which codifies α-N-acetylglucosaminidase is located on chromosome 17q21[88]. 154 mutations have been described in this gene, including missense, nonsense, deletions, insertions, and splicing. The HGSNAT gene (OMIM: 610453), which codifies acetyl-CoA:α-glucosaminide-N-acetyltransferase is located in the pericentromeric region of chromosome 8 8 (8p11.2-p11.1), and its mutations are involved in MPS III-C[89]. To date, 66 mutations have been described (51.5% of which are missense mutations). The deficiency of the N-acetylglucosamine 6-sulfatase enzyme that triggers MPS III-D is due to mutations in the GNS gene (OMIM: 607664), which is located on chromosome 12q14.3[90,91]. A total of 23 mutations have been described, with a very high percentage of large deletions, only seven of which are missense. The proportion of MPS type III attributed to pathogenic genetic variants in each of the genes is 6% for the GNS gene (~84% of which are detectable through sequencing, and 16% are detectable through duplication/deletion analysis), 4% for the GNSNAT gene (~98% detectable through sequencing)[92], 30% for the NAGLU gene (~90% detectable through sequencing)[93], and 60% for the SGSH gene (~98% detectable through sequencing)[94].

1.2.4 Prenatal diagnosis

The prenatal test in which the enzymatic activity assay is applied to amniotic fluid or chorionic villus cells is available in a very limited number of specialised centres worldwide, therefore, where possible, molecular genetic analysis is preferred for prenatal diagnosis[95]. When using this method, the mutated alleles of the family undergoing the test must be known a priori, in order to simplify the molecular diagnosis through chorionic villus sampling, which therefore requires a smaller sample amount. Additionally, if the MPS III subtype is known, but there is a lack of information on mutations, a direct enzyme assay can be performed. In advanced stages of pregnancy, qualitative GAG analysis can be performed in amniotic fluid[96]. Before performing these tests, it is important that the patients are offered adequate genetic counselling[97].

1.2.5 Carrier diagnosis

Carrier diagnosis is a service that is frequently requested by families with MPS III. At present, it is possible to provide definitive information on the status of the carriers as long as the mutations of the family requesting this are known. Molecular diagnosis based on the DNA mutation analysis is the only definitive test for determination. If the mutation is unknown, other biochemical and/or enzymatic methods would have to be used, nonetheless, there are certain limitations to these techniques[98,99].

1.2.6 Diagnosis by gene panel sequencing

Genetic diagnosis by gene panels includes GNS, HGSNAT, NAGLU, SGSH, and it is possible to extend panelisation to other genes of interest that are associated with phenotypic characteristics that overlap with MPS type III (such as the GNPTAB genes [N-Acetylglucosamine-1-Phosdotrasferase alpha /beta subunits; OMIM: 607840], IDUA [Alpha-L-Iduronidase; OMIM: 252800], IDS [Iduronate 2-Sulfatase; OMIM: 300823], SUMF1 [C-Alpha- Formylglycine generating enzyme, OMIM: 607939] and RAI1 [gene induced by acid, OMIM: 607642]. This technique is more likely to identify the genetic cause of the condition

at a reasonable cost, although the identification of variants of uncertain significance (a family segregation study will be required in order to ensure the pathogenic criteria of the identified variant), and pathogenic variants in genes that do not explain the underlying phenotype is limited (more information on genotype/phenotype correlation is required for these variants).

1.2.7 Future prospects: neonatal screening and whole exome sequencing

Over the last decade a potential neonatal screening assay for MPS III based on the automated determination of GAGs in dry paper urine samples has been available[100]. However, the specificity and sensitivity of this MPS test are yet to be evaluated in a pilot study. In addition, this test is not currently used in neonatal screening programmes, given that congenital metabolic disorders that do not have approved therapies, such as MPS III, do not meet the inclusion criteria for these screening programmes. By reviewing these criteria[101], and with the introduction of new therapies in the clinical trial phase for MPS III, this could change. In addition, the progress that is being made in the development of new technologies based on tandem mass spectrometry for the determination of enzymatic activities or the quantification of oligosaccharides derived from GAGs[102], will offer new possibilities that could allow for neonatal screening to be considered for one or more of the MPS III subtypes. For example, pilot neonatal screening programmes for other MPS are currently under development[103,104].

Lastly, advances that are being made in the methodology for sequencing the whole exome, which corresponds to the protein coding regions in the genome, offer future possibilities for screening and diagnosis. Although this technique still presents certain limitations[105], there are some cases of patients with MPS III that have been identified using this methodology[106,107]. In the case of MPS III, the mutation spectrum of the four subtypes consists primarily of isolated mutations, whereas mutations caused by large deletions or reading frame changes are not as common. Therefore, whole exome sequencing could completely change the diagnostic algorithms for MPS III, allowing for the simultaneous screening of several congenital neuropathic disorders, identifying previously unknown or private mutations, which will help in the understanding of phenotypic variants by identifying genetic modifiers[108].

Undoubtedly, the future development of neonatal dry blood spot screening and high-throughput methods for sequencing the whole exome will represent relevant changes for the diagnosis of MPS III.

1.3 GENOTYPE-PHENOTYPE CORRELATION

Most of the mutations identified in MPS III are private or novel – unique to individual families, which therefore makes it difficult to establish genotype-phenotype correlation as well as to screen the general population. Moreover, the interpretation of the clinical phenotype, which results from the different mutations, is very difficult for this type of disease in which the primary characteristic is the degeneration of the central nervous system and the majority of the clinical evaluations are the result of independent observations that are not always comparable. Furthermore, the prediction of genotype-phenotype correlations is complicated due to the presence of polymorphisms, which could modify the residual enzymatic activity, thereby influencing the clinical phenotype. In fact, the phenotype of MPS III varies considerably from severe to intermediate or attenuated. In order to make a personalised prediction of clinical severity for a given patient, detailed records for each patient, which indicate the genotype, the determination of the amount and activity of the enzyme in fibroblasts derived from the patient, a detailed description of the phenotype, the natural history and precise measurements of the quantity and nature of the accumulated compounds are critical.

There are countless mutations that are responsible for the four MPS III subtypes (HGMD® database)[86], although in some cases one or several mutations may be predominant in a given geographic region or within a particular ethnic population. Several studies have shown that subtype B is more prevalent in southern Europe[109,110,111,112] whereas subtype A is more prevalent in Northern Europe[113,114,115,116]. Some of the mutations described can clearly be classified as null, that is to say that they result in the total absence of enzymatic activity. These include deletions of large DNA fragments, changes in location within the chromosome, frameshift mutations, mutations in splicing consensus regions, and premature stop mutations (nonsense). A priori it is expected that the presence of two null alleles will cause greater disease severity, but if one of the alleles has some residual enzyme activity this could result in a milder form. In general the clinical effect (phenotype) of missense mutation (missense or non-missense mutation) can only be predicted based on previous experience with those mutations. Even knowledge of an enzyme's structure and catalytic centre is not always useful in predicting the effect of missense mutations, given that many of the amino acid changes/substitutions can cause problems in the transportation of the newly synthesised protein from the ER to the lysosome, without interfering with the enzyme's catalytic activity. Therefore, in order to establish correlations between the type of mutation and the severity of the biochemical and clinical phenotype, which proves highly useful for the clinical prognosis, population studies must be combined with functional studies in which the mutations identified in the patients are introduced as this will allow for the characterisation of the effects of each mutation on enzyme activity, folding, stability processing and intracellular traffic.

1.3.1 MPS III-A

MPS III-A is the most common subtype in Northern Europe[113,114,115,116]. Several reviews of the existing correlation between genotype and clinical manifestations in patients with MPS III-A have been published[117,118,119]. These confirm the highest phenotypic variability that is associated with genotype. In addition, these studies of the molecular characterisation of the SGSH gene in patients with MPS III-A have indicated a high incidence of certain mutations in different

geographical origins, which could be very useful when determining the molecular diagnosis of this subtype[117]. As such, common mutations have been identified due to their geographical distribution in the SGSH gene: the p.R245H mutation is more frequent in the Australian, Dutch and German populations[120]; p.S66W in the Italian population, especially in Sardinia[121]; c.1079delC in the Spanish population[122,123] and the p.R74C is more common in Poland[124].

The p.S66W, p.R74C, p.R245H, p.Val361SerfsTer52 (c.1080delC) and p.Q380R mutations are associated with the severe classical phenotype[118,120,124,125,126,127]. On the other hand, the presence of p.G122R, p.R206P, p.S298P, p.I322S and p.E369K mutations are considered to be responsible for a more attenuated Sanfilippo phenotype[117,128,129]. In particular, patients with p.S298P mutation in one or more alleles present with a more attenuated phenotype, with a significantly longer conservation of psychomotor functions and longer survival, even in combination with severe p.S66W, p.R245H, p.Val361SerfsTer52 and p.Q380R mutations[118]. Furthermore, the missense mutations p.L12Q, p.180L and p.T421R appear to result in a very mild MPS III-A phenotype[118].

The prediction of the genotype-based phenotype is hindered by the presence of polymorphisms such as p.V226A, p.V361I and p.R456H[120,121] in the SGSH gene, which could modify the residual activity of the mutated enzyme[117]. The MPS III-A is the most frequent subtype in Spain[130], which goes against the results of previous studies that indicated that this MPS III subtype was more common in Northern Europe[113,114.115.116]. In this retrospective study in which 55 patients participated (62%, MPS III-A; 20%, MPS III-B and 18%, MPS III-C), the natural history of MPS III in Spain was established. As in other studies, a great diversity in terms of clinical manifestations was observed due to allelic heterogeneity. Most of the mutations for MPS III-A were previously described[122,123], although three new mutations were identified[130].

1.3.2 MPS III-B

Most of the mutations found in the NAGLU gene are private/novel, therefore indicating the high molecular heterogeneity of subtype B, which is more common in southern Europe. In Greece, subtype B was found to be more prevalent than A88[111,131]. In Portugal, where MPS III-B is also the most common subtype, the p.R234C mutation was identified as a founder mutation, which is common in patients from Spain and Portugal, therefore suggesting its unique and relatively recent origin in the Iberian Peninsula[132].

All of the described mutations occur once or with relatively low frequency, therefore reflecting the broad phenotypic spectrum that is observed in MPS III-B[110,117]. Nonsense (premature stop) mutations, including p.R297X and p.E336X, are associated with severe phenotypes[93,110,117,133]. Most of the missense mutations that have been identified are unique, and it has been observed that the p.Y140C, p.P521L, p.R565W, p.R643C and p.R674H mutations appeared with relative frequencies of 3.4 – 5.4% in patients with MPS III-B94. The p.V334PF, p.P521L, p.R565W and p.R674H mutations have been identified in patients with severe phenotype[93,110,133,134]. Additionally, the p.F48L, p.G69S, p.L497V, p.S612G, p.R643C and p.E634K mutations seem to reduce the clinical severity of the MPS III-B phenotype, for that reason, enzymes with these mutations possess a certain amount of residual activity[93,117]. Finally, the mutations with

deletions and insertions that have been identified in the NAGLU gene are associated with severe phenotypes[93], which is probably due to increased instability and/or absence of residual enzyme activity.

As in the case of MPS III-A, several polymorphisms have been identified in MPS III-B, including a missense change, p.G737R[110,133] which could potentially modify disease severity[117].

In the Spanish population, several new mutations have been identified in the NAGLU gene[135]. Among them, the p.W168X and p.R234C mutations were found in more than one patient. More recent studies have suggested that the p.R234C mutation has its origin in the Iberian Peninsula[132]. Recently, Delgadillo and al., identified a new causative mutation of MPS III-B in the retrospective study of the natural history of MPS III in Spain (11 patients with MPS III-B)[130] as well as the previously described mutations. The high allelic heterogeneity makes it difficult for a clear genotype-phenotype correlation to be established.

1.3.3 MPS III-C

In 2009, Feldhammer et al. conducted a review of all the mutations described in the HGSNAT gene and ten new mutations were identified[136]. Although the spectrum of mutations in patients with MPS III-C showed great heterogeneity and there was no obvious genotype-phenotype correlation, it was possible to identify certain mutations with high frequency in specific populations, suggesting a founder effect of the p.R344C and p.S518F missense mutations in the Netherlands (22% and 29.3%, respectively)[137], of the c.525dupT insertion mutation in Portugal (83%)[138], and of the c.852-1G>A mutation in southern Italy[139], which would be very useful when conducting molecular studies of MPS III-C in these countries. Other mutations have been described, which are p.R384X, c.493+1G>A, p.R344H and p.S541L, and although these present a relatively high frequency in families with MPS III-C they are characterised by a fairly wide geographic distribution[136].

Most patients with MPS III-C have severe clinical phenotypes that are accompanied by a total or near total loss of HGSNAT enzymatic activity. It has been observed that both the severity and the clinical evolution are highly variable even among siblings, which therefore complicates the prediction of the clinical phenotype for each patient. Even so, two mutations, p.G262R and p.S539C, have been described that are probably associated with an attenuated phenotype[137]. For MPS III-C, several polymorphisms have also been identified, p.P237Q, p.V481L, p.K523Q and p.A615T, which result in changes in an amino acid without affecting the enzyme's activity, and therefore would not be of clinical relevance[136].

In Spain, Canals et al. identified nine pathogenic mutations (seven of them new) in the HGSNAT gene in eleven patients with MPS III-C (seven of Spanish origin, one of Argentine origin and three of Moroccan origin)[140]. The most frequent mutations were c.372-2A>G and c.234+1G>A. Haplotype analysis suggested a single origin for both. Each of the seven new mutations that were identified appeared in just one patient and four of them were missense mutations without residual enzyme activity (0 - 1.19%)[140].

1.3.4 MPS III-D

LMPS III-D is the rarest MPS III subtype. In 2003, two mutations in the GNS gene responsible for MPS III-D70,71 were identified for the first time, although the gene had already been identified in 1988[141]. In 2010, Valstar et al. conducted a review of the 22 mutations that have been identified so far in 31 patients from 26 families[142]. In 2015, a total of 23 mutations were identified in the GNS gene (HGMD® database)[86]; a very high percentage of which were large deletions, an aspect that has not been observed in genes that are involved in other lysosomal diseases. Nonetheless, it is still difficult to establish genotype-phenotype correlations for MPS III-D. It can be predicted that nonsense mutations and insertions or deletions that cause reading frame changes would be considered pathogenic associated with a worse prognosis of the disease.

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02.
MPS III. Clinic. Clinical forms. Differential diagnosis.

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Mucopolysaccharidosis (MPS) type III or Sanfilippo syndrome is clinically characterised by severe central nervous system dysfunction, with only mild somatic involvement[1]. The existing disparity between severe brain degeneration (with progressive cognitive and behavioural impairment) and relatively mild skeletal, viscera, and facial features is unique among the MPS diseases[2].

Most individuals with MPS III present a severe form of the disease, in which three different phases can be distinguished: an initial phase of psychomotor retardation, in particular in terms of language, which begins when the patient is between 1 and 4 years; a phase of slow progressive cognitive decline and prominent behavioural disturbances, which begins when the patient is between 3 and 6 years; and a final phase of neurodegeneration with severe dementia and motor impairment in adolescence, which often leads to death in the late second or early third decade of the patient's life. Other symptoms include sleep disorders, recurrent diarrhoea, repeated ENT infections, impaired hearing and vision, and epilepsy. However, considerable variability has been observed in terms of the onset and severity of the disease in all types of MPS III (A, B, C and D), and even in patients from the same family[3-5].

2.1 ONSET SYMPTOMS

Children appear normal at birth and seem to develop without problems in the first year of their life[6]; although in the study by Meyer et al[3], 67% of the patients presented symptoms within the first year, and in the study by Buhrman et al[7], 40% presented symptoms in the first 12 months and 74% in the first 24 months.

The onset of the disease is variable, generally beginning with psychomotor retardation, behavioural problems, or a combination of both when the child is between 2 and 6 years[8]. Other initial findings may include the presence of coarse hair, hirsutism, sleep disorders, and slight hepatomegaly (common in young patients, but not in teenagers and adults)[1]. At the time of diagnosis, language delay is much more significant than motor delay[4,9]. The patients present with language delay, with poor articulation and content, with some patients never learning to speak[1].

Cleary and Wraith[10] described 62 patients with MPS III (47 type A, 12 type B and 3 type C). The mean age of diagnosis was 4.9 years. The initial symptoms in the majority of the patients were a combination of psychomotor retardation, language delay, and recurrent ENT infections. More than half of the patients presented with behavioural disorders and diarrhoea, and 16 suffered from hearing loss. Meyer et al[3] studied 71 patients with MPS IIIA; in the initial phase, they observed that 66.7% of the patients presented language delay (2 patients never spoke) and 33.3% presented motor delay. The most frequent onset symptoms were sleep disorders (38%), behavioural disorders (38%), diarrhoea (31%), recurrent infections (23%), language delay (20%), and hernias (20%). In the only Spanish case study that has been published[11], the most frequent onset symptoms were language delay (85%), coarse facies (78%), hyperactivity (65%) and recurrent otitis (46%). In the study conducted by Buhrman et al[7], the most common onset symptoms were language delay (48%), dysmorphic facies (22%), hearing loss (20%), motor delay (13%), developmental delay (11%), behavioural problems (9%), somatic symptoms (4%) and otitis (4%).

2.2 BEHAVIOURAL DISORDER

Progressive behavioural disorder is characteristic of Sanfilippo syndrome. It usually begins around the age of 3-5 years, and consists of hyperactive, chaotic, anxious and sometimes aggressive behaviour[8]. Children with MPS III can be stubborn and isolated, and they may find it difficult to interact with other children. Self-stimulating behaviours[12] and pica (biting or licking objects) are very common[9]. These children often have tantrums and exhibit destructive behaviour, which can prove dangerous to their siblings, and therefore they require constant supervision. The combination of a severe cognitive disability with significant behavioural disorders in individuals with normal physical strength makes managing these children particularly difficult[1]. Some patients are so difficult to control that they end up being admitted to an institution[13]. Behavioural disorders were present in 84/87 patients with MPS IIIA. Some patients with an attenuated form of the disease did not show signs of a behavioural disorder until adulthood[6]. Behavioural problems decrease with age, eventually disappearing due to the progression of neurodegeneration, leading to a complete loss of initiative[5,8,14]. Cross et al[15] compared a group of children with MPS III with another group of children with intellectual disabilities caused by other disorders. Between the ages of 10 and 15, children with MPS III had fewer behavioural problems and adaptive skills.

Rumsey et al[16] studied a group of 21 patients with severe MPS IIIA, with 13 children in this group meeting the criteria for autism spectrum disorder according to the ADOS scale (Autism Diagnostic Observation Schedule), especially those children older than 46 months. In this group social and affective alterations were the most frequent, however these children did not present with restrictive interests or repetitive behaviours, except for biting objects.

2.1.1 Sleep disorders

Sleep disorders and insomnia are very common (80-90%)[6,17] and include: trouble getting to sleep, waking up early, waking up on a frequent basis during the night, reduced sleep efficiency and tiredness during the day[8,18,19]. In many patients, sleep problems occur occasionally with them managing normal sleep between episodes[6]. Sleep disturbances generally begin at 3-5 years. This disorder is typically associated with behavioural problems, such as sleepwalking at night, crying suddenly, biting the bedding, getting into their parents' bed and starting singing, laughing, and exhibiting other hyperactive nocturnal behaviours[20,21]. Sleep disturbances in children with MPS III may be related to various factors: alterations in the circadian rhythm of melatonin levels, neurocognitive impairment, airway obstruction, and, in some cases, nocturnal epileptic seizures[21].

2.3 NEURODEGENERATION. REGRESSION OF NEURODEVELOPMENT

Patients with MPS III generally continue to acquire neurocognitive skills until they are 3 years old, although they do so at a slower rate than normal. In patients with severe phenotypes, development stabilises at around 3 years, and a phase of more or less rapid deterioration of their cognitive and motor functions begins when they are between 4 and 6 years[3,12,22], leading to 6 year old children presenting with cognitive abilities that correspond to a normal 2-year-old's level[5]. There is a great variability in the rate of regression, even among siblings, with some showing rapid loss of function, while others have a much slower disease progression[8]. One third of the patients with MPS IIIA presented with regression before they were 4 years old and 78.9% did so before the age of 6[3]. Language delay may be evident in children aged 2 years, before cognitive decline begins[7,23]. Any speech and comprehension skills acquired by the child are subsequently lost. The regression of intellectual functions, especially of language, precedes the regression of motor functions. Receptive language lasts longer than expressive language, and cognitive function is the most affected area, however, their adaptive behaviour remains intact for a longer time[3,7,14,24,25].

Patients with Sanfilippo disorder frequently suffer seizures in the second or third phase of the disease, which tend to be easily controllable[2]. 16/62 patients suffered seizures in the study by Cleary and Wraith[2], the majority when they were 8 years or older, both generalised and mixed, 39% in the study by Van de Kamp et al[9], 52% in the study by Meyer et al[3], 66% in the study by Valstar et al[6], and 45% in the study by Delgadillo et al[11]. The frequency increases with age: 73.9% of those over 15 years of age had experienced seizures[3]. Seizures occur earlier in patients with a severe phenotype[6].

Neurological problems are progressive. The patient's gait becomes clumsy and their coordination is poor. Pyramidalism and involuntary athetoid movements appear. They may drool constantly and have problems with chewing and swallowing. In this last phase, patients appear "isolated", losing contact with their environment as a result of progressive dementia[1,14,25]. Finally, the patient is bedridden, remaining in a vegetative state.

Individuals with the more severe form of Sanfilippo do not generally survive beyond the age of 20, while those with milder forms of the disease can live considerably longer. The mean age at death in patients with MPS IIIA was 15.2 years (range, 8.5-25.5 years) in the study by Meyer et al[3], and 15 years (range, 11.5-26 years) in the study by Delgadillo et al[11]. The survival rate varies between the types and within each one of them. In a study in the United Kingdom, it was observed that the mean age (± standard deviation) of death was 15.22 ± 4.22 years in MPS IIIA, 18.91 ± 7.33 years in MPS IIIB and 23.43 ± 9.47 in MPS IIIC[26].

2.4 EXTRANEUROLOGICAL SYMPTOMS

Although somatic involvement is absent or mild in many patients with MPS III, a wide variety of systemic symptoms have been described in the literature, with some individuals presenting with prominent systemic manifestations. 93% of the children in the study by Buhrman et al[7] presented with significant somatic disease. Barone et al[27] presented the case of an 18-month-old child with Sanfilippo B who presented with intense somatic involvement, including macrocephaly, coarse facies, marked hepatosplenomegaly, umbilical and inguinal hernias, and multiple dysostosis, despite presenting with normal psychomotor development at that time (although, with subsequent neurodegeneration).

2.4.1 Coarse facies. Facial appearance

Coarse facies is not a prominent component of Sanfilippo syndrome, especially in the early stages, and some patients maintain a normal appearance even in adult life[1], nonetheless, many children develop a coarse facial appearance as they age[7,9,10]. In the study conducted by Meyer et al[3], only 7% (5/71) of the patients with MPS IIIA presented coarse facies at onset, and 86% in the evolution. However, in the study by Delgadillo et al[11], 78% had coarse facies early on. The nasal bridge may be slightly flattened, and the lips might be slightly thicker than normal in these patients. Many children with Sanfilippo suffer hirsutism, presenting coarse hair, bushy eyebrows, and a low hairline[2]. 48% of the subjects included in the study by Van de Kamp et al[9] and 73.2% of those included in the study by Meyer et al[3] had macrocephaly. In figures XX we can see the facial appearance of some children with MPS III.

2.4.2 Musculoskeletal disorder and height

Some patients present with contractures, which tend to be mild and mainly affect the elbows[8]. In the study by Valstar et al[6], 53% of patients with MPS IIIA presented with contractures, especially in their elbows, hands and feet. The patients can also experience stiff knees and hips although this does not generally limit mobility until later on. In advanced stages, distal lower limb spasticity may worsen gait.

The skeletal involvement of MPS III is minimal, with only slight dysostosis multiplex, predominantly affecting the skull, vertebrae, and hips[9]. 73% of patients presented a thickening of the cranial vault. Mild or moderate abnormalities of the spine (kyphosis or scoliosis) may be present. More than half of the patients' presented abnormalities in the dorsolumbar vertebrae (ovoid, hooked or small)[9].

The musculoskeletal abnormalities caused by MPS III are less severe than those caused by other MPS, nonetheless, some individuals with Sanfilippo present symptoms of musculoskeletal abnormalities, mainly due to spinal or hip disorders. In a study of 18 children with MPS III, with a mean age of 10.3 years, 3 presented with significant scoliosis (21-99°), 2 had hypoplasia of L1, 8 had hip dysplasia, 4 had bilateral osteonecrosis of the femoral heads, 3 equine (one of them with fixed contractures), 1 had undergone a surgical procedure for carpal tunnel syndrome, and another for a spring thumb[28]. The patient's hip may be underdeveloped, with a radiography similar to that of Perthes disease, which can cause pain and difficulty walking. The presence of radiological alterations and hip pain was reviewed in 33 patients with MPS III.

Eight patients (24%) presented signs of osteonecrosis of the femoral head, 6 of them bilateral, and 7/8 experienced hip pain. None of the 14 patients with an attenuated form presented any abnormalities. Therefore, osteonecrosis of the femoral head is common in patients with severe Sanfilippo and its evaluation should be considered in the follow-up of these patients[29].

The height of patients with this disease is almost always normal before the age of 10, but it is affected in half of children over 13 years of age[9]. Growth is delayed in children older than 6 years, however this delay is less intense than in other MPS. The severity of MPS III correlates with the patient's growth pattern and final height (however, growth seems to be accelerated in the first 6 years in patients with a severe phenotype)[30].

2.4.3 Early puberty

Some children with Sanfilippo disease and central precocious puberty have been described in the literature[31,32]. The 5 described patients were male. 4 were treated with GNRH agonists, which seemed to improve the patients' final height and behavioural problems[32].

2.4.4 Hernias

Recurrent hernias are reported, with a variable frequency depending on the study, ranging between 8% and 62%[3,4,9,10].

2.4.5 Hepatomegaly

Patients may present with slight hepatomegaly, especially in childhood[2], with a variable frequency depending on the study, which ranges from less than 50% to 90%[3,6,9]. Splenomegaly is less frequent and prominent.

2.4.6 Digestive involvement. Diarrhoea.

Episodes of diarrhoea are common and some patients suffer persistent diarrhoea[9,10]. In general, this condition improves with age[1]. Constipation is common in older patients[6]. A 5-year-old boy who presented with psychomotor retardation and elevated transaminases for 18 months, and who was finally diagnosed with MPS IIIA[33] has been described in the literature.

2.4.7 Dental alterations

In the study by Buhrman et al[7],39% of the patients presented with abnormal dentition.

2.4.8 Cardiac involvement

Cardiac abnormalities in Sanfilippo syndrome are rare, but these can be serious. In the study by Cleary and Wraith[10], 5/62 patients presented with echocardiographic abnormalities, one of which presented with severe hypertrophic cardiomyopathy that ended up being fatal. In the study by Meyer et al[3] 38% of the patients with MPS IIIA who underwent a cardiological study were diagnosed with valve disease (especially the mitral valve). In a study by Valstar et al[34] that considered patients with MPS IIIB, 10/44 had mild valvular heart disease, and 2 had left ventricular hypertrophy with severe aortic stenosis. Some cases of patients with MPS III with severe valve failure[35,36] and conduction disturbances have been published in the literature[37,38]. The prevalence of valvular anomalies is similar to those found in MPS I and II, however, the

presented symptoms are milder[39], although these worsen with age[40]. n a study of 30 patients with MPS III (16 under 18 years), the left ventricular ejection fraction was found to be normal in children and slightly altered in adults; and aortic and mitral valve alteration was found in 43% and 33% patients respectively, and first degree AV block was detected in 15.6%[41]. Cardiomyopathy has been described as an onset symptom in some individual with mild or non-neuronopathic MPS III (see below).

2.4.9 ENT and respiratory affectation

Respiratory infections and otitis are common in children with Sanfilippo syndrome[2].In the study by Cleary and Wraith[10], 26/62 patients underwent ENT surgery before diagnosis, and in the study by Delgadillo et al[11], 23/55 underwent an adenoidectomy and 15/55 underwent a tonsillectomy prior to diagnosis. In the study by Buhrman et al[7], 91% of the children underwent a tympanocentesis at a mean age of 24 months, and 72% of the patients underwent an adenoidectomy and/or tonsillectomy procedure.

2.4.10 Hearing and vision

A reliable evaluation of hearing and vision is often impossible in MPS III patients due to the behavioural and cognitive problems presented.

Hearing loss is common in patients with moderate-severe impairment[1] and it can be progressive. In the study performed by Meyer et al[3], 45% presented with impaired hearing. In the first 5 years there is a high frequency of hearing loss caused by otitis media; afterwards, it is likely that sensorineural hearing loss predominates, although a complete hearing evaluation is difficult to perform and auditory potentials must be used[7,42].

The most common ocular abnormality in MPS III is retinitis pigmentosa, with associated changes in the electroretinogram[43]. In a study conducted by Valstar et al[6], retinitis pigmentosa was observed in 6 out of 18 patients with Sanfilippo A for whom it was possible to perform the ophthalmoscopy procedure, all of whom were older than 20 years; however, the 12 patients with a normal fundus were under 20 years of age. In 2 of the 6 patients with retinitis the preliminary examination was normal, therefore retinitis pigmentosa could be interpreted as a sign of neurodegeneration[6,44]. Retinitis pigmentosa was also observed in 6 patients with MPS IIIB[34] and in 3 patients with Sanfilippo C[4,44]. Rare cases of corneal opacity, optic atrophy or papilledema have been documented in the literature[43]. Some mild or non-neuronopathic cases of MPS III may present retinitis pigmentosa as an initial symptom (see below).

2.5 NEUROIMAGING

A cranial computed tomography performed at the beginning of the regression shows mild-moderate cortical atrophy in most patients. Progression to severe cortical atrophy occurs in the final stages of the disease[1].

The most frequently described brain magnetic resonance imaging (MRI) findings in patients with Sanfilippo syndrome are: increased perivascular spaces in the corpus callosum, basal ganglia and white matter, hyperintensities in white matter and cerebral atrophy[45–48], similar to those found in other MPS[48].

In a study by Barone et al[45], 3 children with MPS III were observed using MRI for 3 years, and it was determined that brain atrophy and white matter alterations may precede the onset of evident neurological symptoms, and that the progression of neurological involvement tends to be accompanied by different degrees of progressive atrophy. Another serial evaluation with 3 children with MPS IIIB who underwent MRI observation showed similar findings[46]. In neither of the studies did the intensity of the changes in MRI correlate with clinical severity[45,46]. In a child with Sanfilippo A, moderately sized enlarged perivascular spaces were observed in the corpus callosum, and likewise, anomalies of the clivus and cervical vertebrae were reported[47].

2.6 DIFFERENCES BETWEEN TYPES

Recognising the clinical variability in MPS III is important for clinical diagnosis, prognostic and genetic counselling, and, for adequate therapeutic evaluation in the near future. It is difficult to distinguish individual patients with any of the 4 types of MPS III based on clinical criteria, given the existence of considerable clinical heterogeneity within each of the types, and even within the same family.

In the study by Cleary and Wraith[10] differences between types A and B were not observed, nonetheless, most authors point out that type A is more severe than type B. However, there are adults with MPS IIIA and seriously ill patients with type B. Types C and B also appear to be clinically heterogeneous[1]. Type A is the most frequent in Northern Europe and in Spain and type B seems to be more frequent in South-Eastern Europe[8,11].

Van de Kamp et al[9] studied 76 patients with Sanfilippo syndrome in the Netherlands (36 with type A, 23 with type B and 14 with type C), concluding that type A has an earlier onset, more severe manifestations and earlier death than types B and C.

Out of the 55 Spanish patients with Sanfilippo who were included in the study by Delgadillo et al[11], 34 were type A, 11 were type B, and 10 were type C. The crises generally occurred earlier in type A than in types B and C. Loss of language occurred at around 5 years in types A and B and later in C. Loss of ambulation also occurred later in type C than in types A and B.

Valstar et al[6] described 92 patients with MPS IIIA (32 alive and 60 deceased). A wide phenotypic variability was observed, the mean age of onset was 2.5 years (range 0.5-7 years),

loss of language occurred at 7.5 years on average (range, 2—51 years), and the mean age at which gait loss occurred was 13 years (range, 5-51 years). Based on the clinical evolution, 3 phenotypic groups can be considered: severe, intermediate and attenuated. Patients with a severe phenotype, which is the most common, are totally dependent in the second half of their first decade, and these patients generally die in the first 2 decades of their life. Individuals with intermediate MPS IIIA regress more slowly and can live into adult life. Those with an attenuated form of the disease can reach higher levels of development, with some language and ambulation, and survive well into adulthood.

There is also a broad spectrum of disease severity among patients with MPS IIIB. In a Dutch study of 44 patients[34], only 9 patients (21%) presented the classic form of the disease, with psychomotor delay, especially in terms of language delay, occurring at a mean age of 3 years, loss of ability to speak, occurring at a mean age of 7.5 years (range, 5-10 years), loss of gait, occurring at a mean of 12 years (range, 8-18 years), and death in the first 2 decades of life. The other had an attenuated course (see below).

Globally, type C is less severe than type A. A Dutch study analysed 29 patients with MPS IIIC[4]. The patients' psychomotor development was normal in the first year with the first symptoms occurring at a mean age of 3.5 years (range, 1-6 years). These symptoms consisted of psychomotor retardation and behavioural problems. Two patients had an attenuated form and intrafamilial variability was observed. The symptoms of patients with MPS IIIC were similar to those with MPS IIIA, but with a slower evolution. Three patients over the age of 30 had retinitis pigmentosa, and no patients under the age of 16 suffered from epilepsy. Verbal communication tends to be lost before the age of 10 years in patients with MPS IIIA[3], and by 15 years in patients with MPS IIIC[3]. The majority of patients with MPS IIIA lose the ability to walk by the age of 15, while the majority of patients with MPS IIIC lose their gait when they are between 20 and 30 years[3,4,10]. The mean age at death was 34 years (range, 25-48).

Type D is very rare and also heterogeneous, with onset occurring between 15 months and 11 years of age. Its clinical presentations are diverse with some patients dying at 14-17 years[49] and other patients surviving into their fourth decade[50]. Kaplan et al[51] described two siblings with MPS IIID, aged 11 and 3 years. These patients presented with coarse facies, hirsutism, limited elbow extension, and slight global developmental delay in addition to language delay, but no neurological deterioration had been detected yet. Tylki et al[52] described a child with Sanfilippo D who began their second year of life with hyperactivity and language delay, slight hirsutism, inguinal hernia, slight hepatomegaly and multiple dysostosis, without neurological regression at least up to 11 years of age. Ozand et al[53] described the case of a girl with MPS IIID with an acquired language disorder, suggestive of receptive verbal agnosia, without dysmorphism or skeletal alterations, and with cognitive regression and alterations in white matter and brain atrophy in CMR. Valstar et al[54] described 12 patients with MPS IIID. The clinical signs and symptoms were similar to those of patients with other MPS III. Initial psychomotor development was normal, with the onset of behavioural problems at 4 years, followed by developmental stagnation, language impairment and subsequent motor impairment.

2.7 ATTENUATED SHAPES OF MPS III

In the attenuated end of the MPS III spectrum, the onset is usually at around 4 years of age with psychomotor and/or language delay. Afterwards, the mild cognitive deficit may remain stable through adolescence and perhaps even into adulthood before progressing. Behavioural problems are also present in patients with an attenuated phenotype, although these appear at a later stage and are easier to treat. The third phase and death can take place between the fourth and seventh decades of the patient's life[34,55]. Diagnosis is especially difficult and it is almost always late in these forms of MPS III.

Lindor et al[13] studied two adult siblings with MPS IIIA who presented with attenuated manifestations. Between 1 and 4 years, the sister presented with oppositional and aggressive behaviour and delayed language development, although her subsequent language development was adequate. At 7-8 years of age, cognitive impairment was observed and she was sent to a special education school. When she was 24 years of age, she began with physical and verbal aggression and was admitted. She presented mild coarse facies, slight contractures of the elbows and hips, and mild symptoms of multiple dysostosis (thickened skull and widened ribs). Her 30 year old brother who suffers intellectual disability, and who is somewhat more impulsive and rougher than his sister, was also included in this study. In the first years of his life he showed delays in terms of language and sociability, and when he was 5 years of age he was diagnosed with hearing loss and special educational needs. Gabrielli et al56 described a 20 year old woman, who presented with the onset of impaired growth and moderate intellectual disability at 6 years of age, without any reports of intellectual regression or behavioural disorders to date. In the study performed by Meyer et al[3], 7/71 patients with MPS IIIA, who were over 12.5 years of age, partially preserved language, cognitive and motor functions up to a maximum of 23.8 years of age. Valstar et al[6] described 8 patients from 6 families with attenuated MPS IIIA phenotype. In one case, the onset of behavioural disturbances in a man aged 33 years of age led to his diagnosis of mild intellectual disability. Two siblings aged 9 and 11 years of age, studied after a cousin had been diagnosed with MPS III, presented an intelligence quotient of 70 and 92. An exceptionally atypical case of a woman with MPS IIIA has also been described, who presented with hypertension aged 45 years before developing severe biventricular cardiomyopathy and dying aged 56 years after a heart transplant. She did not present neurological alteration, nor any other somatic symptoms, facial dysmorphia or alterations in the CMR related to MPS[57].

Many attenuated cases of MPS IIIB have been described, especially in the Netherlands. In a study by Valstar et al[34], 33 attenuated patients were described out of a total of 42 (79%), with much slower cognitive and motor regression, and survival well into adulthood. The first symptom of the disease, which in this case was slight psychomotor delay, was observed at a mean age of 4 years, however after this the patients showed developmental stagnation and stable intellectual disability for many years. Language loss occurred at a mean age of 35 years (range, 8-68 years) and loss of ambulation occurred at 42.5 years of age (range, 18-68 years). Almost all patients survived into adulthood. The coexistence of behavioural disorders, present in almost all patients, was key to reaching the diagnosis. 12 patients from 2 families had been included in a previous study[9,55,58,59]. In the study developed by Van de Kamp et al[9], 14/23

Sanfilippo B patients from 3 families had an attenuated phenotype, with the majority of these patients presenting with dementia in the third or fourth decade of their lives and prolonged survival. Van Schrojenstein-de Valk et al[58] described 7 patients aged between 30 and 43 years with attenuated MPS IIIB, who presented with dementia and late-onset behavioural changes. Moog et al[55] published a study on 20 patients with the attenuated form of MPS IIIB. All of the patients included in this study had intellectual disability with onset in the first decade (mean age 5 years), with slow progressive deterioration. Most patients presented prominent behaviour problems, which were difficult to treat, and developed physical problems such as heart disease, arthritis, swallowing difficulties, visual disturbances and seizures. They suffered loss of mobility between 36 and 68 years of age. 6 died between the ages of 28 and 69 years. The diagnosis was made in the 3rd-7th decade of life except for 3 patients. Verhoeven et al[59] described a 57 year old woman with attenuated MPS IIIB, who had presented with psychomotor retardation and behavioural disorders at the age of 6 years, before presenting with slowly progressive dementia in the fourth decade of her life. A 66-year-old brother, who had been institutionalised for 54 years, presented a similar clinical condition.

Berger-Platinga et al[44] described two sisters with mild Sanfilippo C, who were asymptomatic until 28 and 36 years of age, when they began to present progressive mental deterioration, behavioural disorders and visual alteration due to retinitis pigmentosa. Due to their mental and behavioural deterioration, they required admission to a residence at 42 and 46 years of age, respectively.

The MPS III phenotype is broader than previously described. Nijmeijer et al[60] presented 12 patients from 6 families (11 MPS IIIA and 1 MPS IIIB), with a median age at diagnosis of 43 years (3-68), with mild or non-neuronopathic phenotype, these patients had completed secondary education and maintained an independent life in adulthood. Of the 4 index patients, 2 were diagnosed due to the presentation of retinal dystrophy, hypertrophic cardiomyopathy, and neurocognitive impairment, with the others being diagnosed by family tracing. At a mean age of 47 years (19-74) 9/12 presented normal cognitive functions, 9 had retinal dystrophy and 8 had hypertrophic cardiomyopathy. These patients exhibited late-onset mild cognitive impairment and in some patients, a non-neuronopathic phenotype consisting of retinal dystrophy and/or cardiomyopathy was present. The 2 cases of attenuated late-onset MPS IIIC[44] and the case of MPS IIIA with adult-onset cardiomyopathy also belong to this mild or non-neuronopathic phenotype[57].

2.8 INTRAFAMILIAL VARIABILITY

Intrafamilial variability has been described in all types of MPS III, underlining the need for caution to be taken in family counselling as well as the limitations when siblings are used as controls to assess the outcome of treatment.

McDowell et al[61] described 2 siblings with MPS IIIA, with similar physical findings, but marked differences in terms of their mental conditions. The less affected sister acquired early sphincter control and presented “educable mental retardation”. By 12 years of age, although she had lost some academic skills and developed behavioural disorders, her social and language skills

remained largely intact. Nonetheless, her brother never acquired sphincter control, lost his language at 2 years of age, and presented cognitive regression. At 11 years of age, he had a severe behavioural disorder and a total lack of communication.

In a family with MPS IIIB, whose case study was published by Di Natale et al[62], the less-affected 23-year-old brother works in a store with an IQ of 44; and, his 26-year-old sister does not have any contact with the environment due to progressive dementia and she presents coarse facies, severe hearing loss, uncontrollable hyperactivity, destructive behaviour and aggressiveness.

In a study of 2 interrelated families, 6 definite patients and 2 probable patients with attenuated Sanfilippo B were described, with a wide phenotypic variability. In general, the patients' language development was quite normal, and it was either lost late or was not lost; however, one patient never spoke and another lost speech at 6 years of age. Mental decline was generally late, and the majority went to primary school, nonetheless one patient was retarded from the first year of their life and another was institutionalised aged 11 years. Locomotion was quite normal, except in one patient who never walked well. Two of the patients had typical coarse facies, while the rest did not[63].

In 3 families with Sanfilippo C, with 2 affected siblings, the difference in the age at which each sibling completely lost their speech varied between 6 and 16 years of age, and the loss of gait varied between 4 and 10 years of age[4].

2.9 CLINICAL DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS (see table 2).

As with all lysosomal diseases, Sanfilippo syndrome can present with a wide spectrum of severity, with some patients experiencing their symptoms as early as the first year of life, whereas the onset in other patients doesn't occur until the third or fourth decade of life. There may be a significant delay in diagnosis from the start of the disease, due to mild somatic and radiographic involvement and the high incidence of false negatives in urinary GAG screening[1]. Delay in diagnosis is very common in patients with a slowly progressive or attenuated phenotype, and this can range from 1.5 to 9 years of age depending on the subtype[64]. Today, with the possibility of effective treatments, early diagnosis is more important than in the past[64].

In general, Sanfilippo syndrome should be considered in a child who presents with language delay or impairment, especially if this is associated with characteristic somatic findings or behavioural disorders. It is not uncommon for patients with MPS III to begin with an isolated language delay while experiencing normal development in other areas, which can lead to a misdiagnosis of idiopathic language delay. In other cases, patients present with a more global developmental delay that may be diagnosed as an autism spectrum disorder or an idiopathic psychomotor delay[64]. Sanfilippo syndrome should be considered in a child who exhibits language delay or cognitive regression along with acquired autistic social behaviour[16].

In the second phase of MPS III, psychomotor retardation is accompanied by behavioural and sleep disorders. Hyperactivity is very apparent and this can easily be mistaken for attention deficit hyperactivity disorder or autism spectrum disorder. Characteristically, children with Sanfilippo respond poorly to stimulant medication and behavioural treatments[64]. Other diseases with prominent language delay and behavioural disturbances in childhood include creatine defects, 4-hydroxybutyric aciduria, adenylosuccinate lyase deficiency and some mitochondrial diseases (especially those associated with hearing loss)[65].

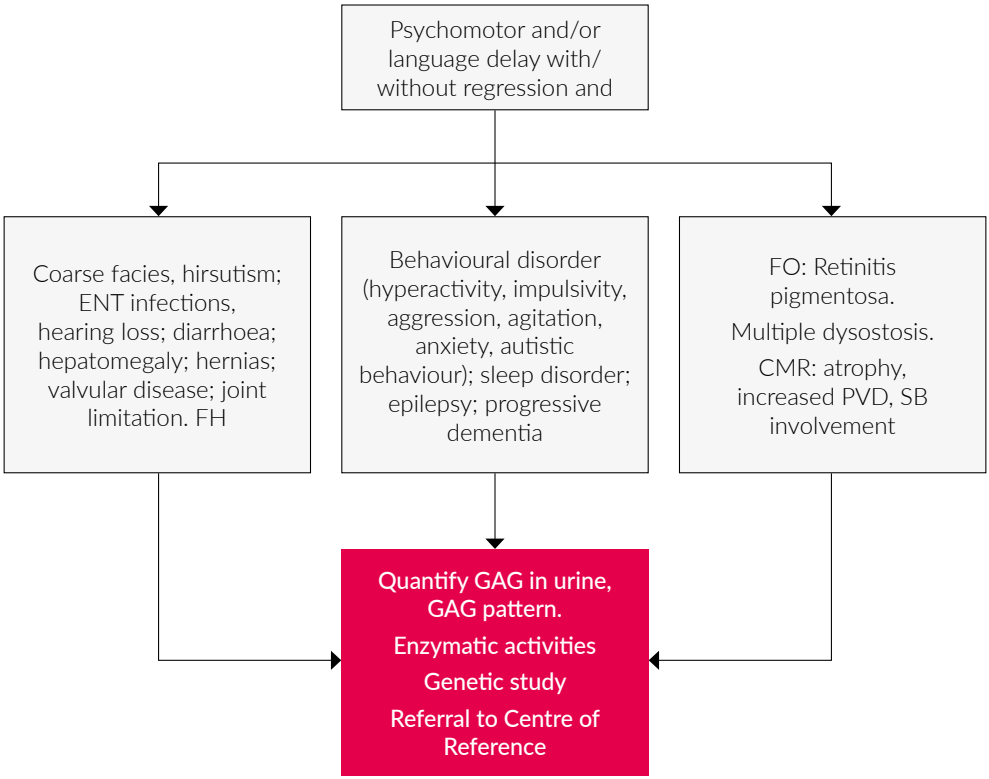
Given the existence of attenuated cases, it is necessary to suspect MPS III in patients with psychomotor retardation, even in the absence of regression, and Sanfilippo syndrome should be included in the differential diagnosis of patients with static psychomotor retardation, especially when this occurs in combination with behavioural problems[34]. MPS III should also be considered in adults with a history of mental retardation that present with progressive dementia[55,58,59], especially if this is accompanied by behavioural disorders and retinitis pigmentosa[44]. In cases that present with a mild or non-neuronopathic phenotype, the key to diagnosis may be the presence of late neurocognitive deterioration, retinal dystrophy, cardiomyopathy, or a combination of these symptoms[60].

The presence of characteristic coarse facies in a child with psychomotor and/or language retardation, with or without behavioural disorders, suggests the diagnosis of MPS III. If a patient presents with signs and symptoms of what is known as “Hurler phenotype” (coarse facies, visceromegaly, hernias, valvular disease, hearing loss, ENT infections, multiple dysostosis, joint limitation), Sanfilippo, other MPS, or other lysosomal diseases should be considered[66]. The first approach should be the quantitative assessment of GAG and the enzymatic activity of MPS in the patient’s urine and blood samples. If any of these symptoms are present alongside hirsutism, episodic or permanent diarrhoea, sleep disorders, epilepsy or the existence of other cases in the family, in a child or adult with intellectual disabilities and/or behavioural disorders, the presence of MPS III must be investigated.

The typical MPS findings on CMR (increased perivascular spaces, patchy white matter involvement, or brain atrophy) may lead to diagnosis in a child with compatible symptoms, however, the absence of abnormalities does not rule out this diagnosis.

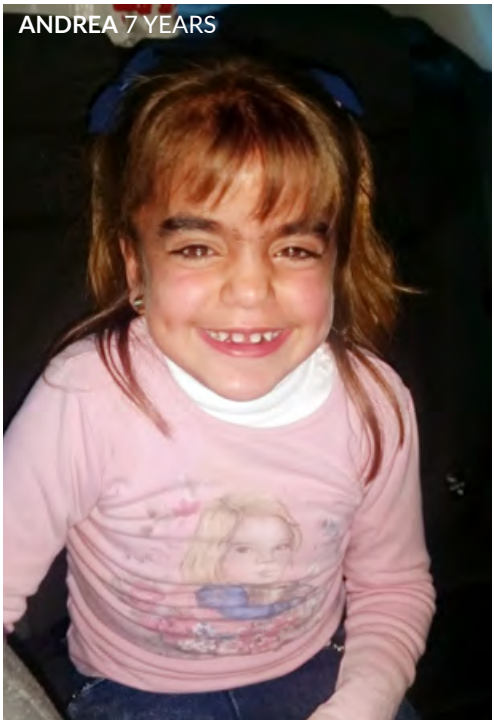
Table 2. **MPS III diagnostic algorithm** (adapted from Wijburg[64]).

FH: family history. **FO**: fundus oculi.



Figures 3-9. **Facial appearance of children with Sanfilippo syndrome (MPSIII A).**

Figures 10-12. Facial appearance of children with Sanfilippo syndrome (MPSIII B).



Figures 13-14. **Facial appearance of children with Sanfilippo syndrome (MPSIII C).**

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03.
Neuropsychology of Sanfilippo syndrome.

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3.1 INTRODUCTION

Sanfilippo disease or mucopolysaccharidosis type III (MPS III) is a lysosomal disease, which is derived from enzymatic deficiencies in the heparan sulfate degradation pathway. MPS III, unlike other MPS, only presents a mild somatic disease; the characteristics and symptoms of which occur in half of the patients, and may include coarse facial features, hepatomegaly, hearing loss and an enlarged tongue[1]. It is primarily a neurodegenerative disease[2] that manifests with severe intellectual disability, developmental regression, behavioural problems, sleep disorders, and autistic traits. This deterioration occurs simultaneously with neuronal loss, which is reflected in the increase in ventricular volume and in the reduction in the volume of cortical grey matter and the amygdala[1].

The progression of MPS III tends to be very rapid, however, some patients present with an attenuated form of the disease, and there have been reports of early dementia in adults, with or without a previous history of intellectual disability. In children, MPS III progresses in the same way as other neurodegenerative diseases, with them presenting stunted cognitive development, which is initially reported as a delay in acquisitions, followed by a period of stagnation in which no new learning occurs and the subsequent loss of previously acquired capacities[3-4].

3.2 PROGRESSION OF NEUROPSYCHOLOGICAL ALTERATIONS IN MPS III

The progression of the disease, which was initially described in three stages, has subsequently been confirmed through different natural history studies[5-8]. The diagnosis is usually made when parents report increased behavioural disturbances, loss of language skills, or delays in the development of their children[9]. Table 1 shows the signs and symptoms in the different stages of the disease.

In the **first stage** which occurs when the patient is between 1-3 years of age, and usually prior to diagnosis, the clinical pattern shows delayed development, especially in terms of language. Different studies have shown[6,7,10] that a speech delay is evident at 18 months, occurring with a similar frequency in the 4 MPS III subtypes[7]. It has been observed that hearing loss occurs at the same time as language delay, which would explain why language is affected before cognition[11]. With regard to language development, no differences have been observed in patients with the 4 MPS III subtypes, with only 64.5% of patients acquiring the ability to associate two words, and only 43% achieving this before the age of 3 years[7], most of these patients never manage to develop a complete expressive language[5].

In the **second stage,** which takes place from the age of 3 years, serious behavioural and sleep disorders are observed[6,7,10] which are the first indicator of the progression of the disease. Subsequently a regression in the patients' linguistic, cognitive and motor skills is observed[5]. As a result of the rapid deterioration of MPS III patients, it has been observed that the proportion of children who remain in standardised education is lower in patients with subtype A, followed

by patients with subtypes B, D and C[9]. A study carried out in Spain indicated that patients start in special education when they are around 6 years[8].

The **third stage,** which begins when the patient is around 10 years of age, usually begins with the loss of language functions (gradually speech becomes unintelligible), loss of mobility and progressive dementia, which is manifested in isolation and reduced contact with their environment[6,7,10]. This slow deterioration and loss of skills leads these patients to take on a vegetative state[9]. Only a small proportion of MPS III patients over the age of 12 years (9.9%) still preserve some motor and language skills (walking with assistance and unintelligible speech) until adulthood[5]. As with other degenerative diseases, the loss of speech occurs prior to the loss of motor functions and it happens earlier in patients with subtypes A and B than in those with subtype C[6,7,10]. Loss of social interaction occurs significantly earlier in patients with subtype A than in those with subtype C. No differences have been observed between subtypes A and B[6,7,10]. From 10 years, the intensity of the problems progressively decreases, between 10 and 15 years, 60% of patients are irresponsive most of the time, and only 10% present behavioural problems[12].

As with the rest of MPS, Sanfilippo disease presents a considerable level of heterogeneity, and, as such, not all patients will follow this pattern of deterioration, with severe and attenuated forms being observed even within the same MPS subtype[13]. Table 2 shows the ages of onset of neurological signs and symptoms in rapidly progressive phenotypes. Cerebral atrophy often precedes dementia, but there is no correlation between the severity of the findings in imaging tests and the phenotype[14]. While dementia has been reported in adult patients with MPS IIIB, this has already been observed in MPS IIIA patients by the age of 6 years[15]. It is believed that the progression of dementia occurs much faster in subtype A patients than in those with subtypes B and C, nonetheless, the clinical phenotype of patients with the same MPS III subtype is variable, which has been attributed to the high number of mutations that have been identified in patients[7]. With the exception of the mutations that have been associated with attenuated forms of MPS IIIA[5] and MPS IIIB[16] clinical expressions, the correlations between phenotype and genotype have not yet been established.

3.3 COGNITIVE-BEHAVIOURAL PHENOTYPES OF MPS III SUBTYPES

3.3.1 SANFILIPPO SYNDROME SUBTYPE A

This is the most widely studied subtype due to its higher frequency, and, likewise, it is also the most serious, with an earlier onset and rapid progression[3]. It has been suggested that the behavioural phenotype of this subtype presents similar characteristics[17] to those described in patients with **Síndrome de Klüver-Bucy**[18], associated with amygdala dysfunction in many species[19]. Klüver-Bucy syndrome in children is characterised by hyperactive behaviour, irritability, aggressiveness, disinhibition, and sleep disorders. Patients with this syndrome present poor social interactions and visual contact, with some children showing signs of visual agnosia, distractibility caused by visual stimuli, hyperorality, and the absence or decreased emotional attachment to their parents[20]. Although there are very few reports on children, with

the majority of studies covering those in pubertal and prepubertal periods[21], this syndrome has also been described in young children. The literature includes cases of patients presenting with herpes simplex encephalitis[22], hypoxic-ischaemic encephalopathies[23], bi-temporal congenital malformations and epilepsy[24].

The behaviours characteristic of Klüver-Bucy syndrome are the same as the problematic behaviours that are present in patients in the second stage of MPS IIIA[17]. Parents report "orality", with their children putting objects in their mouth, although they do not actually end up ingesting them[25]. The biggest concern is their lack of awareness of danger, meaning that their caregivers must constantly be paying attention to them. A reduced capacity for empathy and interest in others has also been indicated in comparison with their peer group. They often present oppositional and disobedient behaviours, and they do not respond to punishment and can also present aggressive behaviours. These characteristics distinguish them from children with other MPS with equal cognitive impairement[26,27]. The similar behavioural phenotype observed among patients with Klüver-Bucy syndrome and those with MPS IIIA has been associated with marked atrophy of the amygdala, which may be responsible for the decrease in fear and lack of awareness of danger that these children usually present[17].

In terms of the cognitive development trajectory of patients with MPS IIIA[28], two types of progression have been described; a rapid progression that is observed in patients whose diagnosis occurs before the age of 6, and a slow progression in patients diagnosed after the age of 6. In the **group with rapid progression,** the reduction in cognitive development occurs when the patient is between 3 and 3 and half years, the decrease in capacity occurs after their 4th birthday, and the largest decrease in their development quotient (DQ) occurs up to the age of 6 years, with a decline of -14.6 points per year. Within the **group with slow progression,** a consistent trend has not been presented over time, with a decline in DQ of -3.7 points per year. In the group with a quicker progression, a much more rapid decrease in the volume of grey matter has been reported. The drop in DQ correlates with the loss of volume of grey matter, with both of these considered as sensitive markers of disease progression[28].

In MPS IIIA patients the decline in motor skills is more variable than the loss of cognitive skills. The patient's fine motor skills deteriorate at a more rapid rate than their gross motor skills[29]. Parents report a decline in their child's gross motor skills at a later stage than the direct measures suggest. This has been explained by the possible presence of ideomotor apraxia in patients, which means that they are capable of spontaneously performing activities that involve gross motor skills, but they are unable to imitate, which may be due to a difficulty in following instructions and their deterioration.

In terms of the evolution of children with MPS IIIA[30] the disease progresses with age, leading to an increase in social and behavioural problems, difficulties in regulating emotions, and a lack of awareness of danger. Hyperactivity, orality, irritability and aggression tend to decelerate as the patient ages, and, in particular their levels of hyperactivity decrease after the age of 6 years. The progression of their fearlessness nature has been closely linked to a reduction in the volume of the amygdala[30]. The decrease in hippocampal volume has also been associated with

the worsening of social and emotional interactions[30]. This finding is consistent with studies that have suggested hippocampus involvement in bonding processes[31] and in social emotions[32].

The progressive loss of language and poor social interactions that are characteristic of Sanfilippo Type A syndrome are similar to those of children with **trautism spectrum disorders (ASD).** In fact, when the patient is around 4 years old, their social and affective dysfunctions are serious enough for them to satisfy the diagnostic criteria. However, restricted interests or repetitive behaviours are largely absent, and traits typical of the autism spectrum become more prominent as the disease progresses[33]. When children with MPS IIIA with autism spectrum traits (mean age 6 years) are compared with younger patients (mean age 3 years) without autistic symptoms, the former present a reduced volume of the left amygdala[34]. Studies that have longitudinally evaluated autistic behaviours in subtypes A and B have also shown that autism spectrum traits manifest at a different age than they do in children diagnosed with autism[35]. In early diagnosed MPS IIIA (before 5 years) behavioural problems and autistic symptoms are reported less frequently than in patients with a later diagnosis[7].

3.3.2 SANFILIPPO SYNDROME TYPE B

There is limited knowledge on the progression of MPS IIIB. Some studies have[36] reported that the classic severe phenotype, with a similar course to type A, is only observed in a small proportion of patients (21%). The rest of the patients (79%) present a much more attenuated form, with a slow regression of both intellectual and motor skills, with the majority of patients surviving to adulthood. The first symptoms of the disease are reported as mild developmental delays around the age of 4 years. There is a slowdown and subsequent stagnation in terms of development. The patients with an attenuated phenotype present stable levels of intellectual disability for many years. Certain "missense" mutations were found exclusively in patients with the attenuated phenotype[36]. Some authors have suggested the need for metabolic studies to be conducted in order to confirm the presence of MPS IIIB in patients with a developmental delay concurrent with behavioural problems, even if they do not present a progressive decline in terms of their intellectual abilities[36]. Cases of slow progressive deterioration have been described in which the diagnosis of MPS IIIB was made in the sixth decade of life[37]. The loss of grey matter volume resulting in the thinning of the cortical mantle, as in patients with MPS IIIA, is the MRI pattern that is associated with cognitive impairment in MPS IIIB patients[38]. Recent studies that have been conducted in order to assess the natural history of advanced stage MPS IIIB patients have reported that cognitive, adaptive behaviour and grey matter volume measures are sensitive markers of disease deterioration[41].

In adult patients with slowly progressive MPS IIIB, behavioural problems such as motor restlessness, irritability, aggressiveness, excessive sensitivity to touch or temperature changes, anxiety, crying spells, stereotyped speech, timorousness, and lower participation in social interactions have been observed[39]. Preliminary studies[40] that have analysed the behavioural phenotype of advanced stage MPS IIIB patients that present severe cognitive alterations (mean age of 12.94 years old, SD of 7.01), have demonstrated the similarity with the phenotypes of MPS IIIA patients with a similar cognitive level, with these patients presenting autism spectrum traits and other symptoms similar to those reported in Klüver-Bucy patients, including loss of fear and a poor attention span.

3.3.3 SANFILIPPO SYNDROME TYPES C AND D

Patients with MPS IIIC and IIID tend to present more attenuated forms of the disease. Very few studies have analysed the progression of impairment in MPS IIIC. Psychomotor development is reported as normal in all patients in the first year of their life. Intellectual regression accompanied by loss of speech precedes motor impairment by more than 10 years[42]. The deterioration of motor and verbal functions appears to occur more slowly[42] than initial studies have suggested[43], although the course of the disease is highly variable. Cases of dementia in MPS IIIC adult patients have been described with them preserving their intelligence until adulthood before beginning a slow progressive deterioration[44]. Despite the older age at which MPS IIIC patients are diagnosed, autism spectrum disorders are less common than in patients with subtypes A and B[7].

There are no conclusive studies on patients with MPS IIID, since only isolated cases have been mentioned in the literature that do not allow for their natural history to be recorded, although it has been determined that their survival is longer[45,46].

3.4 NEUROPSYCHOLOGICAL EVALUATION IN MPS III

Neuropsychological evaluation establishes a cognitive/behavioural baseline at the time of diagnosis and this is a useful tool used to functionally **monitor the progression of the disease and the effects of different treatments**[4]. In the context of multidisciplinary consultations, the patient is first evaluated in order to identify the need for rehabilitative treatments and educational support depending on the stage of the disease. In order to be able to provide guidance on the management of a behavioural problem it is first necessary to objectively determine the age of development and the degree of deterioration. Given the rare nature of this disease, it is recommended that this evaluation be performed by a clinical neuropsychologist with experience in the paediatric evaluation of degenerative diseases and in the management of young children with severe behavioural disorders or with physical limitations.

The development of new treatments for children with neurodegenerative diseases such as MPS III is crucial. In order for these potential new treatments to be developed and evaluated, sensitive and reliable measurements of the progression of the disease are required. Cognition develops in parallel to cerebral development and this occurs rapidly during the period in which the disease is active, therefore it is important for these evaluations to record the progression of the disease and any changes produced by the treatments. Recent consensus publications have included the criteria that should be applied when selecting cognitive and adaptive tests in children with MPS diseases, advocating the use of international protocols that make it possible for comparable data to be obtained from different trials or studies[47-48]. The tests used in the evaluation must provide reliable and valid psychometric properties. It is important that these assessments obtain adequate normative data with recent revisions in order to prevent the occurrence of Flynn effect[49], that is to say, the increase in time ranges for cognitive scores. A wide range of levels must be used, with scores in a very low range, and the studies must also make use of age-equivalent scores in order to establish the level of development and maturing age, thereby making it possible to determine whether the child's development is progressing,

or if they are stagnating or deteriorating. Standardised scores within 3.3 standard deviations below the mean, which are common in cognitive tests, do not allow for the changes in these children to be evaluated, and, as such, scores that collect their developmental ages are needed. Finally, the fact that these assessments can be used in different cultures, that they are easy to administer, and that their application time is short is also important as this helps to prevent the easy fatigue presented by these children when executing cognitive tasks.

According to the aforementioned international consensus[47-48], in **children under 3 years,** the test that has proved to be most sensitive to changes in the development of children is the Bayley-III scale of infant development[50]. Other scales that have also been used include the Griffiths[51] and Mullen[52], however, neither of these two scales are available in the Spanish language. The Mullen scale has also not been revised since the 80s, and due to the Flynn effect, it has been suggested that this scale may overestimate the cognitive level of children. The Merrill-Palmer development scales[53] have been adapted into the Spanish language for its application in the clinical setting, although it has not been used in research studies. This scale provides very attractive assessment materials for children, it is very quick and easy to use and allows for the use of equivalent ages.

In children **older than 3 years to adulthood**[47-48], the Wechsler scales[54-56] have been widely used in the clinical setting. They present adequate normative data, reliability and validity, and they have been implemented in the Castilian population. However, the main problem that has been observed, is that these are long tests that require a demanding collaboration that children with MPS III are not always able to offer. To establish a cognitive level, tests such as the DAS-II (Differential Ability Scales)[57], are also used. This particular test has been adapted for use in Spain through the creation of the BAS-II intellectual aptitude scales[58], however, the main problem that has been observed is that it provides very unattractive material. In children with MPS III, the international Leiter-III manipulative scale[59] has also been used, but its reliability and validity are lower than those offered by the aforementioned tests. The most widely used consensus test is the second edition of the KABC-II (Kaufman Assessment Battery for Children)[60]. This test offers good reliability and validity and can be used in a wide range of ages, however it has not been adapted to the Spanish language; nonetheless, it uses a non-verbal scale, favouring the speed of its application, and it does not require language for its administration.

In patients with progressive deterioration, their lack of collaboration may be secondary to their inability to perform tasks. It cannot always be interpreted as secondary as this lack of collaboration may be due to behavioural problems. If regulating their behaviour proves particularly difficult, or if they are already presenting signs of advanced deterioration, the use of cognitive tests may be practically impossible, and it may be necessary to implement an approach based on scales of development and/or adaptive behaviour. The most widely used scales are the Vineland Adaptive Behaviour Scales, the third edition is currently available[61]. This provides indices that address particular domains: communication, life skills, socialisation, motor skills, and maladaptive behaviour, and subindices have also been established for each domain, with equivalent developmental ages being determined for the subindices. These correlate

well with the level of cognitive development assessed with the development scales and tests of general ability, making them a valuable instrument when selecting the most appropriate assessment strategies for each specific patient, with this last adaptation providing recent regulatory data. In a clinical setting, Spain has adapted the Battelle Development Inventory[62], which uses a screening survey to establish the patient's age of cognitive development and it can be used in patients with severe impairment and in those who are unable to collaborate in direct tests.

The SBRS (Sanfilippo Behaviour Rating Scale)[29] has recently been developed specifically for MPS III patients, and it allows for the assessment of the most frequent behavioural, emotional regulation and communication difficulties presented by patients with MPS III. The assessment of behavioural problems focuses on attention problems, oppositional/aggressive behaviours, ASD traits, and more specific Klüver-Bucy-type behaviours. The emotional regulation scale analyses the characteristics of the episodes in which the patients present a lack of control. Finally, the communication skills of the child are assessed.

3.5 NEUROPSYCHOLOGICAL INTERVENTION IN MPS III

Early diagnosis can help reduce the initial burden for caregivers[63]. However, early diagnosis does not occur frequently, given that, as it is a rare disease with different phenotypes, families often consult multiple specialists. The deceleration or stagnation in the patient's development, or the loss of acquired skills must be correctly recognised, which is why it is important to provide adequate training to clinical psychologists who attend early care programmes, allowing them to use objective tests to report the initial stagnation in evolutionary development. The initial presentation of language delays and alterations in social communication[28], which are symptoms similar to those described in ASD, can lead to a misdiagnosis and delay the eventual diagnosis of MPS III, which has implications both in terms of genetic counselling and in the patient's opportunity to participate in therapeutic trials in the early stages of the disease[64]. Alterations in language and social communication in MPS III patients emerge at a later date, despite the patient having presented typical and normalised development and they are less frequently associated with restricted and stereotyped behaviours and interests.

The care of patients with Sanfilippo syndrome is mainly provided by their parents, nonetheless, siblings, relatives, teachers and health professionals can also provide support[65]. It has a considerable effect on their day-to-day lives, and it can also affect the physical and psychological well-being of the caregivers. They must cope with behavioural disturbances, communication difficulties, sleep problems or somatic complications, resulting in a considerable reduction in their quality of life and high levels of anxiety and depression[65]. The **emotional impact on caregivers** is a consequence of psychological stressors such as their concern for the well-being and future of their children, but it is also determined by the physical exhaustion resulting from the care they are having to give. In addition, families are restricted in their normal activities, which can lead to feelings of social isolation[66]. The level of impact on the quality of life of both the patient with MPS III and their families differs depending on the age, symptoms and behaviour of the patients, and their changing care

needs can lead to a "stress cycle" that is associated with the emergence and evolution of symptoms, with new needs being generated as the disease progresses[63]. In the advanced stages of the disease it is important for the family to be provided with psychological support in the context of palliative care for the patient[67]. It is rare for new behavioural problems to appear, on the contrary, these tend to decrease as a result of the patient's increasingly low adaptive functioning, with levels of communication, socialisation and daily life skills that are lower than those of a 2 year old child[67].

In order to mitigate the impact of the disease, it is necessary to establish the help that is required not only by the patients themselves, but also by their caregivers. Clinicians must develop a good relationship with families, based on direct and regular contact. In these interactions, they must be provided with counselling and a clear explanation of the cognitive and behavioural symptoms that emerge at the time of diagnosis, managing the expectations of the parents with regards to the diagnosis and progression of the disease, and lastly, the early introduction of palliative care.

3.6 CONCLUSIONS

The progression of Sanfilippo syndrome results in the dysfunction of the central nervous system, neurocognitive decline and marked behavioural alterations, the pattern of which may provide the key to understanding the neurobehavioural pathology of this disease[30]. As the patient's condition deteriorates, they simultaneously experience neuronal loss that is reflected in the increase in ventricular volume and the reduction in the volume of cortical grey matter and the amygdala[1].

To date, no longitudinal studies have been conducted with different types of MPS III and phenotypic severity (severe and attenuated forms). However, follow-up neuropsychological studies in patients with neurodegenerative diseases such as Sanfilippo syndrome could provide prognostic markers and help clarify the risks-benefits of future treatment options. The prospect of new therapeutic trials in this rare disease emphasises the need for reliable and quantitative markers to be established in order to assess potential benefits. Very few studies carefully analyse the evolution of cognitive and behavioural deterioration in patients, and international consensus has advocated the need for multinational studies to be conducted in order to be able to recruit the largest number of patients, making it possible to present data with higher statistical power[68].

Different studies have demonstrated that despite the deterioration of patients and their behavioural problems, the majority of cases are susceptible to a cognitive/behavioural evaluation if it is carried out by professionals with experience in degenerative diseases in childhood. It is important for quantitative and specific neuropsychological measures to be developed in order to clarify the progression of Sanfilippo syndrome in its different stages, as knowing the pattern of behaviour changes would make it possible for the families of these children to be provided with support and guidance. Finally, interdisciplinary clinical consultations, which include clinical neuropsychology that promote the integration of care should be a standard, in order to provide the best possible clinical practice[63].

Table 3. **Signs and symptoms associated with the three phases of MPS III.**
Not all symptoms and signs occur in the same patient.

SIGNS / SYMPTOMS	PHASE 1	• Language delay • Delay in psychomotor development
	PHASE 2	• Behavioural disorders - Hyperactivity/Impulsivity - Decreased attention span - Aggression - Hyperorality - Emotional lack of control - Lack of awareness of danger - ASD traits • Sleep disorders • Speech regression • Progressive cognitive difficulties • Motor skills regression
	PHASE 3	• Profound intellectual disability • Loss of speech and communication • Loss of motor skills • Cessation of behavioural difficulties • Progressive isolation • Progressive loss of response to the environment

Table 4. **Ages of onset of neurological signs and symptoms in rapidly progressive MPS III phenotypes.**

NEUROCOGNITION	• Stagnation at 2-3 years old • Subsequent progressive deterioration • From 6 years old the developmental age is less than 2 years
SPEECH AND LANGUAGE	• Deterioration at around 2 years old • It is the most frequent initial alteration, it occurs before cognitive deterioration • Affected by hearing loss that can develop when the patient is around 2-3 years old • Only 50% of patients acquire the ability to associate two words before 3 years old
MOTOR SKILLS	• Fine motor skills: - Impairment at the same time as cognition - Stagnation at 2-3 years old - Loss of skills after 4 years old • Gross motor skills: - Preserved until 5-6 years old
BEHAVIOUR	• Behavioural problems begin between 2-4 years old: - Aggressive behaviour - Decreased attention - Loss of fear - Sleep problems with altered circadian rhythm - ASD traits do not present until 3 years old, they do not usually show repetitive behaviours apart from hyperorality • Associated with intellectual decline and developmental regression • They tend to decrease due to progressive deterioration after 8-9 years old
BRAIN ABNORMALITIES	• Age at which variable is detected (Me= 5 years): - Ventriculomegaly - Increase in perivascular spaces - Atrophy - Crisis/Epilepsy

Me = mean

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04.
Follow-up of mucopolysaccharidosis type III or Sanfilippo syndrome.

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The progressive nature of mucopolysaccharidosis (MPS) requires the continuous assessment of the clinical condition, which primarily includes assessing the patient's sight, hearing, joint mobility, cardiopulmonary function, neurological function, and intellectual level. Therefore, in the follow-up of these children, a multidisciplinary team must assess the progression of the symptoms, in order to determine the most appropriate treatment for each particular case[1-3].

However, unlike other MPS, Sanfilippo syndrome is characterised by a severe degeneration of the central nervous system, generally with mild somatic disease, therefore, in the majority of cases, check-ups will not need to be as frequent as for other types of MPS. The follow-up protocol will be established for each patient on a case-by-case basis, depending on the organs that are affected and the disease severity[2,4]. Table 5.

4.1 Medical history. Record of the problems that have occurred since the last check-up (every 6 months)

The following information is to be recorded in the patient's medical history: infections that have occurred in recent months, whether or not the patient has been prescribed any medication, whether they have required hospital admission, and whether the patient has undergone any surgical procedure.

4.2 Physical examination (every 6 months)

The patient's vital signs (blood pressure, heart rate, respiratory rate and oxygen saturation), as well as certain anthropometric data (weight, height, head circumference) are to be recorded. A detailed paediatric general examination will then be conducted, which will include the visceromegaly measurement (in the mid-axillary line) and the assessment of joint mobility angles.

4.3 Neurological and functional assessments (every 6 months)

The neurological assessment should include an account of the patient's psychomotor development milestones and daily activities, as well as any possible changes in their gait, endurance, fine motor skills, sphincters, behaviour, hearing and sight. Special emphasis must be placed on sleep and behavioural disorders in order to determine the most appropriate therapeutic management.

Epileptic seizures become frequent as the disease progresses. The type and frequency of these must be recorded in order to determine the most appropriate antiepileptic regimen[5,8].

The neurological examination must be thorough and it must be adjusted depending on the patient's age. This examination will include the assessment of the patient's strength, pyramidal signs, and the examination of their superficial and deep sensitivity.

Although spinal cord compression is not common in MPS III, signs of upper motor neuron involvement, loss of proprioception, reduction of the resistance for walking or unusual gait, bowel or bladder dysfunction, osteotendinous reflexes and clonus abnormalities must be assessed on a periodic basis through anamnesis and examination.

ADDITIONAL TESTS

1. **Craniocervical MRI.** This should be interpreted by expert radiologists who must look for signs of atrophy, white matter involvement, dilation of the Virchow-Robin spaces or ventricular enlargement. Since anaesthesia will be required in most of the cases, one set of basal anaesthesia will be requested, and this will be repeated taking the patient's clinical situation into account.[9-10].

2. **Motor and sensitive conduction velocity of the median and ulnar nerves:** this test will be conducted in order to assess the presence of carpal tunnel syndrome. This entity is less frequent in children with MPS III than in those with other types of MPS, nonetheless, in the majority of cases the patient will be asymptomatic or will present with minor clinical signs. Since this test will generally require sedation due to minimal cooperation from these patients, the most reasonable option would be for this procedure to be performed when there are compatible clinical symptoms, or when anaesthesia is already going to be administered for another reason, provided that the patient will benefit from a possible surgical release.

3. If seizures occur, an **EEG** should be performed every 6-12 months.

4.4 ENT assessment (every 6-12 months)

Hearing loss is common in patients with MPS III, however, it is often very difficult to assess due to the presence of behavioural disorders and cognitive impairment. For this reason, a periodic ENT follow-up with audiometry or truncal auditory evoked auditory potentials is very important in order to indicate, where necessary, the placement of a transtympanic drain and/or hearing aids[11].

A nocturnal polysomnography should be performed if obstructive sleep apnoea syndrome (OSAS) is suspected, in order to assess the need for adenotonsillectomy[7,9,12].

4.5 Cardiological assessment (every 12-24 months)

Cardiac involvement in children with MPS III is less frequent and it tends to be less severe than in children with other MPS, therefore cardiac check-ups can be more spaced out. In general, check-ups are recommended every 1-2 years, although the frequency should be determined for each individual patient, taking into consideration their age and condition. It should include an ECG and an echocardiogram. Apart from this, additional cardiological examinations should be performed prior to any major surgical procedures[8,13-17].

4.6 Orthopaedic assessment (every 12 months)

Although bone problems are less common in MPS III than in other types of MPS, some patients with MPS III develop kyphoscoliosis, equinus or equinovarus deformities, carpal tunnel syndrome and trigger finger[18]. Osteonecrosis of the hip is a frequent complication (especially in children who present with a more severe phenotype), and in the case of delayed diagnosis

this may result in prosthesis placement[19]. Periodic clinical examination can be used to monitor the progression of bone deformities. Radiography of the hip, spine and long bones should be performed basally and the frequency will be determined for each individual patient taking into consideration the severity of their alterations. Surgical indication should be established depending not only on orthopaedic findings, but also taking into account the patient's clinical condition and the degenerative nature of this disease[18].

Many children may require physiotherapy. In the course of its evolution, they may also require orthoses, adapted chairs and the administration of botulinum toxin to treat their spasticity.

4.7 Ophthalmic assessment (every 12-24 month)

These patients do not usually develop corneal opacity, nonetheless they may have pigmentary retinopathy or optic nerve atrophy[9,20,21].

The ophthalmological examination should include visual acuity, refraction, examination of the cornea using slit-lamp, ophthalmoscopy and intraocular pressure measurement, as well as electroretinogram if there is evidence of retinopathy. The visual evoked potentials test will be used if optic nerve damage is suspected[22].

4.8 Neuropsychological assessment (every 12 months)

Periodic neuropsychological examination (motor area, cognitive, behavioural) is essential in order to define the evolution of these patients, and to determine the appropriate speech therapy, psycho-pedagogic and school support[12]. This assessment should be conducted by neuropsychologists who are specialised in neurodegenerative disorders. The frequency of these assessments will be determined for each individual patient depending on their condition, but as a general rule, these will be conducted once a year.

4.9 Psychiatric assessment

Assessment and follow-up by a child psychiatrist with experience in these types of disorders may be of great help in the management of behavioural and sleep disorders. The frequency of check-ups will depend on each child's clinical conduction[8].

4.10 Anaesthetic assessment

Anaesthetic procedures present a high risk for patients with MPS, due to the presence of altered airway anatomy, GAG accumulation and progressive lung disease (restrictive and obstructive). Postoperative recovery may be slow and complications such as airway obstruction may occur. As a result, patients with MPS should be anaesthetised in well-equipped centres by experienced anaesthetists. In addition, a cardiological, respiratory and airway evaluation must be conducted prior to any procedure that requires sedation or anaesthesia.

4.11 Nutritional assessment (if required)

In late stages of the disease, impaired swallowing (weight loss, risk of aspiration) may occur and nutritional supplements or inserting a gastrostomy may be necessary[7].

4.12 Dental examination (every 2 years)

Although less frequent than in other types of MPS, these patients have a higher gum alteration, caries or dental abscesses than the general population, therefore regular check-ups are recommended, especially if there is irritability of unknown origin[12,23].

4.13 Additional tests

- **Blood tests** with haemogram, liver and kidney function, ions, nutritional markers (ferritin, vitamin B12, vitamin D) every 12 months.

- **Abdominal sonograph,** every 12-24 months.

Table 1. **Multidisciplinary monitoring.**

BP: blood pressure / **ECG:** electrocardiogram / **HC:** head circumference / **HT:** heart rate / **MRI:** magnetic resonance imaging / **NCV:** nerve conduction velocity / **RR:** respiratory rate / **satO2:** oxygen saturation.

	BASAL	EVERY 6 MONTHS	EVERY YEAR	EVERY 2 YEARS	ACCORDING TO CLÍNIC
Medical history	●	●			
Physical exam	●	●			
Weight, height, HC	●	●			
BP, HT, RR, SatO2	●	●			
Neurological assessment	●	●			
Neuropsychological assessment	●		●		
Audiometry-ENT assessment	●		●		
Psychiatric assessment and follow-up					●
Cardiology-ECG and sonography	●		●		
Ophthalmology	●			●	
Orthopaedic assessment:	●		●		
Radiography (hips, skeleton)	●				●
Electrophysiology: - EEG					●
Electrophysiology: - NCV					●
Polysomnography					●
Craniocervical MRI	●				
Abdominal sonography	●		●		
Blood test	●		●		
Nutritional assessment					●
Dental examination				●	

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05.
MPS III. Behavioural and sleeping disorders.

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5.1 SLEEPING DISORDERS

Sleeping disorders are very common in children with Mucopolysaccharidosis type III (MPS III). According to Bax and Colville, the prevalence of sleeping disorders in MPS is high, up to 71%, reaching values of 86-87% in MPS III[2,3,4], and these disorders are related to daytime behavioural problems[1].

Sleeping disorders in these patients include[5]:
- difficulty falling asleep
- waking up during the night
- insomnia
- wanting to walk around the house
- shouting, soft singing and laughing during the night
- waking up early
- episodes of daytime sleepiness.

Actigraphy sleepe studies have shown a higher latency in sleep onset in patients with MPS III compared with children of the same age[3]. There is no evidence of the description of the quantity, quality and circadian rhythm in the sleep in patients with MPS III.

Sleep problems consist of an irregular sleep or wakefulness pattern, which is manifested in difficulties in initiating and maintaining sleep at the beginning of the night. This could explain why some patients do not respond to conventional hypnotics, nonetheless, therapies that look to resynchronise sleep patterns, such as behavioural therapy, light therapy and/or melatonin have proved useful[3,5].

Melatonin levels are altered in patients with MPS III, suggesting that when treating sleep problems, focus should be placed on regulating the sleep and wakefulness pattern. Actigraphy has proven to be well-tolerated by these patients and as such it has been recommended as a measure of response to treatment[4].

Several studies have suggested that these sleep problems are caused by an alteration in circadian rhythm as a result of night-time melatonin levels, which are lower than the higher levels recorded in the mornings[5]. Circadian melatonin production appears to be altered in patients with MPS type III. Therefore, urinary 65-Mel levels are higher in the daytime and lower at night[5]. In addition, several studies have demonstrated the relationship between sleep problems and cognitive impairment in these disorders.

INTERVENTION PLAN

Most of the treatments have proved ineffective (around 67–91.5% of MPS III)[4], however some non-pharmaceutical measures do exist:
- Fixed bedtime.
- Minimal interaction with them if they wake up in the middle of the night, bringing the child straight back to bed again and not allowing them to get up again before a fixed time.

According to Fraser's 2005 study, these measures are 80% effective. 37% of MPS III patients have used these behaviour modification techniques, which have proved effective in dealing with the sleep problems in 58% of said patients.

Changes to the set-up of the child's room: parents should change or remove furniture from the child's room in order to make it safer. They can either use a camera or webcam or share the room with them during the night (this explains why many children go back to sleeping with their parents) to allow for closer supervision.

Behavioural measures require consistency and clarity in any explanations that are being given to the child, and their cognitive deficiencies and low attention span must be taken into consideration. In addition, a certain level of intellectual functioning is required in order for a reward system to be put in place. When their behaviour is caused by pain, an unfamiliar environment, or unpleasant sensations caused by light and sounds in the room, it is important for these to be identified and prevented through analgesia or distraction techniques[16].

The response to behavioural therapy combined with medication (whether melatonin or the use of atypical antipsychotics) tends to be good, and, likewise, functional behavioural analysis, as conducted in patients with Autism Spectrum Disorders (ASD)[16] proves useful.

Treatment with antipsychotics is not recommended because of the high risk of side effects. Furthermore, the patient's response to antipsychotics is unpredictable and the use of these medications may even worsen cases of hyperactivity and aggressiveness. Anxiolytics and hypnotics have also been tried on patients with MPS to attempt to tackle sleeping disorders, however it is understood that these could have paradoxical effects in such patients, leading to hyperactivity or excessive sedation.

Stimulants such as methylphenidate could be used to treat hyperactivity in non-severe form of MPS I and II, and it could even improve cognitive function and prevent drowsiness. Nonetheless, these prove largely ineffective in patients with MPS III and/or with significant cognitive impairment. In MPS III, phenothiazines such as trimeprazine or alimemazine and some antipsychotics such as risperidone[6, 13,16], are considered to be partially effective in controlling hyperactivity, anxiety, aggressiveness and agitation.

The response to hypnotic medication is variable among patients with MPS III. According to the largest study on MPS III, which was conducted in 2005 by Fraser, and which covered 141 patients, the most commonly used medications in clinical practices were: antihistamines, melatonin and benzodiazepines. Melatonin proved the most effective, which is consistent with the circadian rhythm disturbance in MPS III.

Other studies have indicated that high doses of hypnotics are required, and that these may result in hyperactivity or drowsiness the next day. The combination of behavioural treatments with psychopharmacological treatment was used by 50% of parents, with this measure producing the best results[7].

5.2 BEHAVIOURAL PROBLEMS

Neuropathic involvement does not only appear in Mucopolysaccharidosis III (MPS III), but also in other MPS, and it is characterised by developmental delays, neurocognitive regression, behavioural changes, sleep disorders and epilepsy[8,9].

Manifestations of behavioural problems are the main symptom of severe MPS type II and III and these are closely linked to:
- Overall developmental delays.
- Regressions in terms of neurocognitive and/or psychomotor development (in the context of disease progression).

Hyperactive behaviour is considered as one of the earliest indicators of involvement of the Central Nervous System (CNS) in patients with MPS type II. In patients with MPS III, the presence of hyperactive and impulsive behaviour is common from the initial phases, facilitating dangerous behaviour that could result in bodily injury (due to falls or hitting against furniture).

A lack of fear and reduced attention span, language and communication skills result in a greater tendency to isolation, which is reminiscent of autistic symptomatology, especially in patients with MPS type IIIA and IIIB[17].

Severe sleep problems, a tendency to irritability with or without tantrums and mood swings with mood fluctuations and instability are also covered in the treatment programmes.

Epilepsy is also common in MPS III. Its incidence increases as the disease evolves (advanced stage) and is accompanied by a progressive neurocognitive regression. The seizures tend to be tonic-clonic, nonetheless, there may also be tonic, focal myoclonic and other non-convulsive paroxysmal movements[8,9].

When treating these neuropathic forms of MPS, physicians aim to correct the enzyme deficiency and prevent the accumulation of glycosaminoglycans (GAGs) in the brain. Knowledge of the presentation and clinical course of the neuropathic forms of MPS can be advantageous for early diagnosis, therefore allowing for early intervention.

It should be noted that MPS type III is considered a neurodegenerative disorder, which is characterised by regression in terms of intellect, motor skills and behavioural problems, reaching dementia in the final stages.

It is understood that patients with the most severe forms experience stagnation when they reach the approximate age of 3 years. This is followed by a rapid deterioration in terms of language, cognitive development and adaptive function, with motor functions tending to be more preserved.

Less severe forms of MPS III present slow progression, with long periods of stability in terms of cognitive function and a slow intellectual deterioration, even in adolescents.

Patients with rapid disease progression lose a standard deviation of IQ of up to 14.6 per year by the age of 6.

In patients who present slower progression (attenuated forms), IQ reductions have been recorded at 3.7 per year. Cognitive decline occurs in parallel to brain atrophy and to decreases in grey matter volume.

In MPS type III, neuropsychiatric manifestations are classified in a three-phase model:

Phase 1 or initial: at the age of 2, there is developmental delay, especially in terms of language development. Almost half of the patients will never gain sphincter control. Behavioural problems. Physical development is normal.

Intermediate phase: at around 2-4 years, further cognitive impairment, sleep problems and behavioural abnormalities, presenting an increase in the frequency and intensity of tantrums. These patients show symptoms of anxiety, experiencing panic attacks in unfamiliar environments and often have phobias. They also demonstrate hyperactivity, a decreased attention span and impulsivity similar to ASD symptoms[17].

Phase 3 or late stage: at around 10 years, this phase is characterised by fewer behavioural changes, increased motor difficulties, spasticity, loss of balance and feeding problems due to choking. In addition, in this phase patients suffer progressive intellectual decline until they experience dementia and become bedridden before eventually reaching a vegetative state[9,10].

The Sanfilippo Behaviour Rating Scale (SBRS) has been developed in order to quantify and monitor behavioural changes in patients with Sanfilippo Syndrome. It includes the following four domains: movement, the lack of fear, social/emotional issues and executive dysfunction, and it can be useful when measuring behavioural changes and disease progression[11].

Around the age of 4 years, patients with MPS III may show ASD-like symptoms, such as lack of communication and social interaction, along with hyperorality. They also present restricted interests and repetitive behaviours[10].

INTERVENTION PLAN

These patients benefit from speech therapy, occupational therapy and physiotherapy. Environmental changes and behavioural interventions may be useful when managing sleep and behavioural problems.

When behaviour modification therapy is combined with psychotropic drugs in a MPS patient there is a risk of side effects, with antipsychotics also resulting in paradoxical effects such as over-activation or excessive sleepiness. Melatonin is effective in the management of sleep problems given the altered circadian rhythm observed in children with MPS III[8,12].

A recent publication by Hoffmann et al. 2020[12], concluded that family involvement in the treatment process is fundamental, and it should be based on an organised and stratified plan:

- **First step:** based on ways to cope with the patients' abnormal behaviours. For this purpose it is recommended that families keep diaries of the patient's behaviours and the strategies employed.

- **Second step:** the pharmacological strategy for dealing with both sleep problems and hyperactivity. In this phase, it is important to consider potential side effects, with melatonin being used for sleep disorders and atypical antipsychotics such as risperidone and/or aripiprazole[13,14].

- **Third step:** External support and monitoring of coping and resilience strategies. Many of these are used in children with special needs due to intellectual disabilities[15].

One of the most successful behavioural techniques is functional behaviour analysis, which looks to identify the relationship that we wish to change or improve. For this purpose, parents and professionals can perform the daily ABC (Antecedent-Behaviour-Consequence) for a period of time based on one of the child's problematic behaviours (such as aggressiveness or hyperorality or finger biting). This diary will also record environmental factors (only occurring at home or at school).

The use of this diary is important as it allows for the behaviours or factors that reinforce the abnormal behaviour to be identified (where, when and with whom), for example by caregivers paying more attention to those behaviours, or, on the contrary preferring not to undertake an activity that they find unpleasant. Identifying what happens before the abnormal behaviour (antecedent) may be the key to preventing the behaviour from occurring.

Aggressive behaviours can be caused by anxiety, pain or a change in routine or environmental factors. Therefore by providing the patient with a quieter environment in their room (with less noise and lighting) it is possible to reduce their anxiety levels, and, likewise, it is possible to give them an analgesic for the pain or distract them with another activity. It is important to provide clear and consistent messages and instructions to the child about what is considered desirable and undesirable behaviour. Non-verbal (gestures) or visual cues can be used to provide this information given that many of these children have severe communication and language problems.

Sometimes a reward can be offered (an activity that the child enjoys, such as using a tablet to watch cartoons or a certain food that they like, taking the presence of hyperorality into account). Inappropriate behaviours can also be ignored or techniques such as "time-out" can be used, in which the child is taken to a safe place for a few minutes, taking attention away from the inappropriate behaviour. Inappropriate behaviour can also be modelled into appropriate behaviour through adult guidance and rewards. For example, to prevent shouting, the child may only be rewarded if they do not scream.

If children have a short attention span and a very low cognitive ability, rewards should be given immediately to make them learn by direct association. However, behavioural measures do not always work, and can result in exhaustion and worries for parents and caregivers[16].

It is clear that most of the studies and research have concluded that the treatment of MPS III should be multidisciplinary with family involvement and the use of non-pharmacological measures as the treatment of choice for behavioural and sleep problems[12,13,14,16,18].

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06.
Treatment of Sanfilippo syndrome.

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In recent years, advances in the treatment of some lysosomal diseases have resulted in a significant change in their prognosis and in the quality of life of patients. Nonetheless, finding an effective therapy for mucopolysaccharidosis type III (MPS III) patients is proving difficult due to the neurodegenerative nature of this disease, and the challenges involved in passing drugs through the blood-brain barrier[1]. Nonetheless, several therapies, which target the central nervous system are currently in the testing phase.

There are several different approaches that can be followed when treating patients with mucopolysaccharidoses:

- supportive treatment, a multidisciplinary team approach involving the different specialists
- replacement of the deficient enzyme, either by external administration through a haematopoietic progenitor cell transplantation or by using chaperones that stabilise the protein
- Substrate reduction therapy through the inhibition of synthesising enzymes
- gene therapy

In this chapter we will review the alternative treatments that are currently available for patients with MPS III, and new molecules that have been proposed as possible modifiers of the evolution of patients[2-4]. The most relevant options are listed in Tables I and II.

6.1 SUPPORTIVE TREATMENT

Despite the therapeutic advances that are likely to emerge in the coming years, multidisciplinary treatment will remain the most important therapeutic option in order to ensure that the patient maintains the best quality of life possible. These measures should look to address both the different aspects of the condition, and the patient's general needs for integration into daily life. In the early stages, patients with MPS III will mainly require neurological follow-up, however, afterwards specialist care will also be needed.

Behavioural disorders are one of the most prominent problems in MPS patients, usually occurring earlier in patients with MPS IIIA, and, likewise, sleep disorders are very common. Due to their relevance, both of these problems are discussed in a specific chapter.

Despite the fact that bone problems are less prevalent and less severe in MPS III patients than in those with other MPS, their prevention and correction requires follow-up by orthopaedists, traumatologists and rehabilitation specialists. Special attention must be placed on the hip, given that a high prevalence of avascular necrosis of the femoral head has been reported. This condition requires specific diagnosis and treatment, with the placement of prostheses in advanced cases. It is also important to monitor the appearance of carpal tunnel syndrome. Both of these conditions may result in pain and discomfort that patients are unable to explain and that can have a negative impact on their behaviour.

It is essential that all patients are provided with proper nutritional support throughout the course of the disease. This includes planning an adequate diet that ensures that the patient's caloric, vitamin and mineral needs are met, as well as assessing the need for artificial feeding tracts such as nasogastric or gastrostomy tubes if the patient has trouble swallowing. Likewise, follow-ups must take into account the patient's tendency to diarrhoea, which is normally due to bowel dysfunction secondary to the deposition of GAGs, which can significantly worsen the quality of life.

In most patients, the existence of upper airway problems, which often involve obstructive apnoeas, may require tonsillectomy and adenoidectomy as well as transtympanic drainage. It is important to monitor auditory function given that hypoacusis will have a negative impact on language. Likewise, the possibility of the patient presenting with ophthalmological disorders (retinal changes and optic nerve abnormalities) means that regular monitoring is necessary.

Psychological support must be provided, not only for the patients themselves, but also for their families. They will all have to adapt to the different stages of the process, and, as such, they must be prepared to face the considerable health, family and social burden that is caused by this disease. Furthermore, psychomotor development control measures must also be implemented in the early stages in order to maximise the child's developmental capacity.

All of the specialists that are required to cover all of the possible aspects of the disease: pulmonologists, cardiologists, endocrinologists, etc. must be included in the treatment programme. It will be important for the patient follow-up to be well-coordinated given that a large number of visits may interfere significantly with the organisation of the patient's daily life.

6.2 ENZYME REPLACEMENT THERAPY

Enzyme replacement therapy consists of periodically administrating the marked deficient enzyme with a biochemical signal. This therapy is based on the concept of checking that an enzyme administered exogenously to a cellular medium is able to penetrate cells through the mannose-6 phosphate receptors and act correctly. This phenomenon is known as cross-correction. It has also been noted that 1-5 % of the metabolic activity is able to correct the metabolic defect in the cell line. Over the last 20 years, different techniques have been used to develop appropriate enzymes for the treatment of various lysosomal diseases.

The limitations on enzyme replacement therapy (ERT) in MPS III are related to the difficulty for drugs to pass through the blood-brain barrier that covers the nervous system. Different strategies are currently being designed, which are looking at ways to overcome this obstacle. On the one hand, the possibility of directly administering the drugs to the central nervous system, either directly (intraparenchymatous) or in the cerebrospinal fluid (intrathecal or intracerebroventricular). On the other hand, the possibility of associating the modified enzymes to a molecule to help them cross the barrier when administered intravenously. Once in the central nervous system, these recombinant enzymes must be taken up by the cells. In order for this to happen, in the case of MPS IIIA, a signal must be carried, usually a ligand of the mannose 6-phosphate receptor. Promising pre-clinical studies in mice, dogs, cats and monkeys based on these different methodologies have paved the way for human clinical trials.

Clinical trials with ERT in MPS IIIA to date include the following:

- Safety, Tolerability, Ascending Dose and Dose Frequency Study of rhHNS Via an IDDD in MPS IIIA Patients (NCT01155778). This was a safety and efficacy study sponsored by Shire, in which the recombinant SGSH enzyme was administered through an intrathecal drug delivery device. 12 patients aged 3 years or older were recruited and they received the experimental treatment in increasing doses once monthly for 6 months. The safety profile of the recombinant enzyme was considered to be adequate, although 7 patients experienced serious adverse events, most related to the intrathecal device (migration, disconnection or breakage). Subsequently, patients were enrolled in an extension study (NCT01299727) for the established dose. However, the study was put on hold given that the pre-specified efficacy criteria concerning neurocognitive evolution had not been met[5,6].

- A Study to Assess the Safety and Tolerability of SOBI003 in Paediatric MPS IIIA Patients (NCT03423186). This trial is currently active and is sponsored by SOBI. This study is based on a weekly intravenous administration of a recombinant enzyme in three cohorts at different doses. The main objective is to assess the safety of SOBI003. The secondary objectives include pharmacokinetics, immunogenicity and efficacy. Efficacy is based on neurocognitive, behavioural and quality of life evolution, as well as imaging tests (brain and abdominal MRI). Patients recruited in the first dose group showed good tolerability after completing infusions for 24 weeks. An extension study (NCT03811028) is currently being conducted for an 80 week period.

With regards to ERT in MPS IIIB, it is important to note that there is an added difficulty in crossing the blood-brain barrier given that the NAGLU enzyme has few mannose-6-phosphate residues. For this reason, in order to increase its cellular uptake, it fuses with insulin-like growth factor type 2, which also binds to the mannose 6-phosphate receptor but at a different junction. Clinical trials that have been conducted to date using this approach include:

- An Open-Label Study in Previously Studied, SBC-103 Treatment Naïve MPS IIIB Subjects to Investigate the Safety, Pharmacokinetics, and Pharmacodynamics/Efficacy of SBC-103 Administered Intravenously (NCT02618512). A study sponsored by Alexion Pharmaceuticals, which included 11 participants aged between 1 and 10 years, assessed the safety, tolerability, pharmacokinetics and efficacy of the intravenous administration of SBC-103, a recombinant human NAGLU enzyme that is capable of crossing the blood-brain barrier. The experimental product was well tolerated by patients with MPS IIIB and it led to a reduction of heparan sulfate in cerebrospinal fluid. However, no dose had any effect on preventing brain atrophy or neurocognitive impairment. It is interesting to note that SBC-103 was not detected in CSF, which seems to suggest that it may not have reached the CNS[7].

- A Treatment Study of Mucopolysaccharidosis Type IIIB (NCT02754076). This is an active trial that was initially sponsored by Biomarin, and which is currently sponsored by Allievex. This dose-escalation study evaluates the safety and efficacy of BMN250 or tralesinidase alfa, a recombinant human NAGLU enzyme that is administered weekly intraventricularly through an Omaya-type catheter. This trial, which began in 2016, looked to gradually increase the

initial dose (30, 100 and 300 mg) until the maximum tolerated dose was reached, before administering said dose for 48 months. The experimental product was well tolerated, although adverse events associated with the intraventricular reservoir, including intraventricular reservoir malfunction and infection were described. The heparan sulfate levels in cerebrospinal fluid decreased to a normal range in all 7 patients who were treated within the first 3 weeks. After 24 weeks of treatment, the liver and spleen volume decreased to the normal range in 9/9 and 7/9 patients, respectively. Neurocognitive development stabilised in 5/7 treated patients. An extension study (NCT03784287) in which a weekly intraventricular administration of 300 mg will continue for 240 weeks began in 2018 and it is currently active[8].

6.3 HAEMATOPOIETIC PROGENITOR TRANSPLANTATION

Since the 1980s, haematopoietic cell transplantation has been used as an effective therapy for various inborn errors of metabolism, especially lysosomal and peroxisomal diseases. The efficiency of this therapy is based on the provision of enzymes to the different tissues through the migration of donor cells from the cardiocirculatory system. The enzyme can be transferred from one cell to another by a cross-correction phenomenon mediated by mannose-6-phosphate receptors. Monocytic-macrophagic cells can cross the blood-brain barrier and transform into glial cells that are capable of synthesising enzymes. There are currently two donation options: haematopoietic cells from the bone marrow of a healthy donor (preferably related) or haematopoietic cells from the umbilical cord. The main advantage of this therapy is the definitive correction of the defective enzymes in those tissues in which the donated cells are successfully implanted.

However, there are certain disadvantages that prevent it from being used as a general treatment for all lysosomal diseases. On the one hand, the benefits provided by this treatment depend on the severity of the disease and the time of transplantation. On the other hand, its effects differ depending on the tissues: it is very effective in organs with a reticuloendothelial system, it has minimal bone impact, and the CNS cells require a period of 6-12 months for the deterioration to stabilise. Cells that cross the barrier do not appear to be able to synthesise sufficient enzyme in order to stop the neurological progression. Therefore, it is not efficient for MPS with neurological involvement, and, as such, it is not considered as a therapeutic option.

Nevertheless, there are publications that report on transplantations in MPS III patients, which have shown no beneficial effects, even in pre-symptomatic stages at the time of transplantation[9,10]. The most recent cases include two MPS III (A and B) patients who underwent umbilical cord cell transplantation before the onset of neurological symptoms. The urinary GAG levels decreased and the somatic involvement improved in both patients. However, they suffered neurological deterioration similar to untreated children. In another study, a patient who underwent a transplantation at the age of 2 years presented delayed cognitive impairment 8 years after transplantation (at the age of 10 years she was only able to speak in simple sentences) and severe behavioural problems. Similar results have been observed in more recent cases.

6.4 SMALL MOLECULES

6.4.1 Substrate reduction therapy

Substrate reduction therapy consists of inhibiting the synthesis pathway of GAGs that are unable to be degraded, therefore preventing their accumulation. These treatments are usually administered orally, using small molecules that can cross the blood-brain barrier. Different molecules have been tested, with genistein molecule being the most commonly used.

Genistein is a soy isoflavone that inhibits a tyrosine kinase of the epidermal growth factor receptor. The activity of this enzyme in the receptor is necessary for GAG synthesis. In early in vitro studies, the addition of genistein to fibroblast cultures from MPS IIIA and IIIB patients resulted in a significant reduction in GAG levels. Subsequently, the ability of genistein to reduce GAG deposits in both the peripheral tissues and the central nervous system was proved in mouse models, and this resulted in positive changes in the animals' behaviour. In 2007 and 2008, trials with Polish patients were presented. The patients were treated with daily doses of genistein, 5 mg/kg/day, and, as well as reducing the elimination of GAGs, an improvement in cognitive and behavioural functions was also observed[11]. At the 3-year follow-up, these changes were only maintained in a low percentage of the children. Further studies comprised of larger numbers of patients who were treated with 5 or 10 mg/kg doses have failed to confirm the beneficial effects of genistein[12]. Several studies with a high dose of pure genistein (150 mg/kg/d) are currently being conducted in order to assess the safety and efficacy of this treatment in MPS III patients. After at least 12 months of treatment, initial results in 22 patients with severe MPS II and MPS III have shown that the treatment is well tolerated and safe. In analytical tests and clinical controls that were performed every 3 months, no major biochemical alterations were observed, except for a slight elevation of transaminases, alkaline phosphatase, amylase or lipase[13]. Despite a minor reduction in the heparan sulfate and derivatives levels, no clinical differences were observed in the 21 patients who underwent this treatment[14]. Despite its dubious effectiveness, due to the safety of this treatment many MPS III patients are treated with different doses of genistein.

Rhodamine B is a dye that is used in cosmetics, which can be used to inhibit the synthesis of GAG chains by blocking the formation of glycogen precursors. In human fibroblasts, rhodamine B has been able to reduce GAG synthesis, and in animal MPS IIIA models, this has resulted in a decrease in the size of organs and cerebral accumulation, as well as an improvement in terms of cognitive abilities. Studies in four successive generations of mice treated with rhodamine B have not demonstrated toxicity or teratogenicity. There are no further planned studies in patients nor have there been any new updates in recent years, except in an animal model of MPS I in which rhodamine B has also proved effective in reducing skeletal and neurological involvement. It is possible that at the doses necessary to treat patients, rhodamine may have considerable toxic effects[15].

Recently, other gene therapy approaches have been reported. Silencing RNAs that inhibit the gene expression (EXTL2 and EXTL3) required for the synthesis of GAGs have been synthesised

in in fibroblasts from MPS IIIC patients. The in vitro results are encouraging and these are currently being tested in the treatment of the animal model[16].

6.4.2 Chaperones

A different path that can be used to increase enzyme activity is the use of chaperones. This is based on the idea that some mutations do not cause a quantitative but a qualitative defect of the enzyme, that is to say, that they change the configuration, therefore resulting in inactive and misfolded proteins. These proteins are degraded by proteases and removed from the cell. Chaperones are small molecules that look to ensure the functionality of a protein, and they are able to act at different levels: as substrate analogues, receptor agonists or antagonists, modulators, and as ligands for epitopes that ensure the conformation of the enzyme. In this context, it has been proven that glucosamine corrects the folding defect that is caused by some mutations in MPS IIIC, restoring enzyme activity. Several molecules have been proposed for MPS IIIB, which in low doses could act as chaperones, but these are still in the initial experimental phase[17].

6.4.3 Anti-inflammatory treatments

Inflammation is one of the pathogenic mechanisms of neurolysosomal diseases, including MPS III. For this reason, research has also been conducted into anti-inflammatory treatments due to their potential effects.

High doses (200 mg/kg) of aspirin was tested in vivo in MPS IIIA mice using intraperitoneal injections three times a week, and after 6 months of treatment they presented with an increase in gene expression for inflammation-related genes.

Pentosan polysulfate (PPS) is an anti-inflammatory drug, approved by FDA as a prochondrogenic molecule, which has shown immunomodulatory effects in pre-clinical studies of different MPS subtypes. In MPS IIIA mice, a reduction of neuroinflammation and an improvement in neurological evolution was observed at the very early stages of subcutaneous dosing[18].

Lastly, Anakinra is an IL-1 antagonist that has been approved as an anti-inflammatory drug. An ongoing clinical trial (SOBI) is currently looking at possible effects in patients with MPS III.

6.4.4 Other molecules

Trehalose is a 2 carbon sugar molecule that is able to cross the membranes and reach the nervous system. In cellular studies, trehalose regulates the autophagy mechanism and increases cell survival. Therefore, it appears to have neuroprotective effects[19]. Current studies are assessing its usage in the treatment of some degenerative and eye diseases. One of the biggest problems is the amount that is required in order for it to have a possible positive effect on the patients, and the frequency of administration taking into consideration its half-life.

Coenzyme Q is an antioxidant molecule that participates in mitochondrial respiratory chain reactions. As in other metabolic diseases, there is evidence that MPS IIIA and IIIB patients

present low coenzyme Q levels in fibroblasts. Furthermore, in vitro supplementation could increase enzyme activity and decrease the accumulation of GAGs, therefore improving cell survival[20]. The effect on patients has not yet been tested.

New molecules are being tested *in vitro*, from a pathophysiological approach to the different pathogenic pathways of the disease, however there are no significant results yet.

6.5 GENE THERAPY

Gene therapy involves obtaining a functional gene that produces the enzyme that is required to replace the deficient one. Different methods of this therapy have been attempted: addition of a foreign gene, inhibition of a specific area of the gene, or complete inhibition of the mutated gene in order to achieve a gain-of-function mutation. These techniques can be applied *in vivo*, by introducing the appropriate gene into the affected cells or tissues, or *ex vivo* through the genetic change to the haematopoietic cell lines that are subsequently transplanted into the patient. Nowadays the most commonly used viral vectors in the administration of gene therapy are adeno-associated viruses (AAV) derivatives (*in vivo* gene therapy). Lentiviruses are the most commonly used viral vectors in *in vivo* gene therapy.

This therapeutic approach is the most physiological one, and it boasts two main advantages: firstly, the observation that a small amount of enzyme (1-10 %) is sufficient for proper cell function, and, secondly, the cross-correction phenomenon by means of which the enzyme can be transferred from one cell to another and become normofunctional.

Nevertheless, there are still important limitations at present. One such limitation is the ability to transfer a sufficient quantity of the active gene or the appropriate cell population for the affected tissue. Numerous vectors have been tested. Some appear to be able to cross the blood-brain barrier through intravenous administration, while others are directly injected into the nervous system at the intracerebral or intracisternal level. A second problem is the level and duration of expression of the transgene product when administered at its site of action. These cells may be lost either spontaneously or due to an immunological reaction, and the potential toxicity on the administration through vector is not well established. With regards to AAV vectors, severe toxicity associated with the high-dose administration of AAV9 serotype (2×10^{14} viral genomes) (vg)/kilogram (kg) of body weight in monkeys and pigs was described for the first time in 2018. An increase in transaminases was observed in the treated monkeys, and in one of them, acute liver fulminant failure was observed. The degeneration of the dorsal-root ganglia was manifested through problems of balance and walking (ataxia) in pigs. Subsequently, serious adverse events in humans related to high doses of AAV vector gene therapy have also been described in several trials for neuromuscular diseases (AveXis spinal muscular atrophy, Solid Biosciences and Pfizer Duchenne muscular dystrophy, Audentes myotubular myopathy): at high doses above 2×10^{14} vg/kg, patients presented with severe immunological reactions at the systemic level (plateletopenia, anaemia, renal failure and heart failure) and even death after severe infection (sepsis) associated with progressive liver dysfunction[21].

In MPS IIIA, the first studies in a mouse model administered with intraventricular injections of sulphamidase, in addition to a modifying factor administered to increase enzyme activity transmitted by adenovirus vector (AAV5) showed a good uptake throughout the nervous system, a reduction in accumulation, and an improvement in motor and cognitive function. This has later been confirmed and refined in several studies in larger animal models (dog), with different vectors (AAV8, AVV9, AVV10) and different methods of administration (intravenous, intracerebral, intracisternal and intraventricular) with equally encouraging results. It has been observed that the effect is progressive during at least the first 12 months of administration.

For MPS IIIB, the positive effects of the simultaneous intravenous and intraventricular injection of the NAGLU enzyme with the adenovirus were described in an animal model. Subsequently, a similarly positive effect of a single intravenous administration of the AVV9 vector in a mouse model and in a larger animal model (monkey) were demonstrated.

In the case of MPS IIIC, a study in which a modified capsid AAV9 vector was administrated was successfully tested in the murine animal model with good evolution of the disease. Two-month-old MPS IIIC mice received a striatal injection with 5.2 x 109 vg/mouse, subsequently presenting with corrected pathological behaviour, increased clearance of GAGs, and corrected neuroinflammation in different brain regions[22]. This pre-clinical programme is currently being developed by Phoenix Nest.

With regards to the clinical application, several gene therapy clinical trials are currently being conducted for MPSIIIA and MPSIIIB, with both in vivo and ex vivo gene therapies. Trials with in vivo approaches include:

- A pilot open-label phase I/II clinical trial sponsored by Lysogene (NCT01474343) was initiated in 2011. The AAVrh10 vector carrying the hSGSH and hSUMF1 transgenes (AAVrh.10-SGSH-IRES-SUMF1) was injected intracerebrally using a 12-needle stereotaxic device into four MPS IIIA paediatric patients. Immunosuppressive therapy including mycophenolate mofetil and tacrolimus started 15 days before the surgical procedure, and the patients continued to undergo this therapy during follow-up, with it being gradually reduced in order to inhibit the loss of transduced cells. The injected product was well tolerated without any serious adverse effects, and, likewise, there were no infectious events associated with the immunosuppressive regimen. Clinical efficacy endpoints including brain MRI, neurocognitive/behavioural testing and biomarkers were not met in all patients, however the youngest patient showed the greatest improvement[23]. Lysogene later conducted a phase II/III clinical trial (NCT03612869) with another AAVrh10 vector encoding hSGSH, administered intracerebrally. 20 MPS IIIA patients aged over 6 months with a DQ>50 were enrolled in this clinical trial. The first patient was injected in February 2019, and 19 out of 20 patients have been enrolled until June 2020. This study is currently been placed on hold after lesions were observed through the MRI at the points at which the experimental product was injected[24].

- Abeona Therapeutics is also sponsoring two phase I/II clinical trials to evaluate the safety and efficacy of a single intravenous injection of scAAV9-U1a-hSGSH. The Transpher A trial

(NCT02716246) will enrol 22 MPS IIIA patients aged from birth to 2 years, or older than 2 years with DQ ≥60. This is a dose-escalation study consisting of three cohorts from low dose (0.5×1013 vg/kg), medium dose (1×10^{13} vg/kg), to high dose (3×10^{13} vg/kg). Preliminary data at 24, 12 and 6 months after all three doses had been injected observed an acceptable safety profile in all patients with a reduction in both heparan sulfate levels and liver and spleen volume. In the cohort of patients who were enrolled before 36 months of age, preliminary data indicates the stabilisation or improvement of their adaptive behaviour and/or cognitive function[25]. Study ABT003 (NCT04088734) is another clinical trial, which will use the same experimental product: scAAV9-U1a-hSGSH in patients with middle or advanced stage MPSIIIA with a DQ of less than 60. Patients will receive a single dose, which corresponds to the high dose from study ABT001.

- Esteve is currently conducting a phase I/II clinical trial for MPS IIIA patients with the intracisternal administration of an AAV9 vector encoding the SGSH enzyme gene (EudraCT 2015-000359-26).

- Uniqure Biopharma and Institut Pasteur conducted a clinical trial (NCT03300453) to assess the safety and efficacy of an AAV5 vector in MPSIIIB. The vector was administered intracerebrally at 16 injection sites through eight trepanations to a total dose of 4×10^{12} vg. Four 20, 26, 30 and 53-month-old patients were recruited between 2012 and 2014. 30 months after the injection, the product and administration procedure were well tolerated. Neurocognitive progression improved in all patients compared to the natural history, with the youngest patient treated presenting the best results. NAGLU activity in the cerebrospinal fluid increased in 15 % to 20 % of patients. However, the heparan sulfate levels did not change. In addition, neutralising antiAAV5 antibodies were not found throughout the follow-up period[26]. No potential beneficial effects of chronic immunosuppression on MPSIII-associated neuroinflammation were observed, as was the case in the similar Lysogene MPS IIIA trial.

- The Transpher B trial (NCT03315182), sponsored by Abeona, assessed the safety and efficacy of ABO101, a self-complementary AAV9 vector with the NAGLU gene, which was administered intravenously. The study design and inclusion criteria were similar to those used in the Transpher A trial, recruiting patients from birth to 2 years, or older than 2 years with a DQ ≥60. It is a dose-escalation trial: low cohort 2×10^{13} vg/kg; medium cohort 5×10^{13} vg/kg; high cohort 1×10^{14} vg/kg. A good safety profile has been noted to date and a sustained reduction in heparan sulfate in cerebrospinal fluid, urine GAGs and liver volume[27].

Another approach that is currently being evaluated is the combination of gene therapy with haematopoietic stem cell transplantation as a form of vector administration[11,28].

This *ex vivo* gene therapy approach requires for haematopoietic precursors to be collected from a donor (heterologous procedure) or the patient (homologous procedure), which will then be transduced through a lentiviral vector. The patient then undergoes myeloablative conditioning with chemotherapy before the transduced haematopoietic stem cells are reinjected.

- The Gene Therapy With Modified Autologous Autologous Haematopoietic Stem Cells for Patients With Mucopolysaccharidosis Type IIIA trial (NCT04201405), sponsored by the University of Manchester in collaboration with Orchard Therapeutics, plans to enrol up to five MPS IIIA patients aged from 3 to 24 months in order to assess the safety and efficacy of OTL-201. One patient was treated before this trial with a "special" licence using a haematopoietic progenitor transplant with autologous CD34+ cells transduced using a lentiviral vector. Twelve months after transplantation there was evidence of continued transplant engraftment with leukocyte SGSH enzyme activity that was 25 times higher than normal values, accompanied by a rapid decrease in the levels of heparan sulfate in cerebrospinal fluid, blood and urine. A similar ex vivo approach is being applied for MPS IIIB. Previous studies in animal models of MPSIIIB showed supraphysiological levels of enzyme activity in leukocytes maintained over the long term, as well as the normalisation of heparan sulfate levels in the blood, urine and cerebrospinal fluid. In addition, there was improved survival and normalisation of hyperactive behaviour and neuroinflammation[28]. A pre-clinical collaboration programme between the University of Manchester and Orchard is currently developing the lentiviral vector LV.CD11b.hNAGLU for a human clinical trial.

6.6 CONCLUSIONS

Given the primarily neurological nature of MPS III, developing an effective therapeutic approach is proving more difficult than in other lysosomal diseases with systemic expression. Nevertheless, the prognosis is expected to change substantially over the coming years if these new therapies that are currently in the trial phase achieve favourable results.

Table 1. **MPS III treatment summary.**

TREATMENT		RESULTS IN CELLS	RESULTS IN ANIMALS	RESULTS IN PATIENTS
Enzyme therapy	MPS IIIA	Positive	Positive	Table 2
	MPS IIIB	Positive	Positive	Table 2
Stem cell transplantation				Inconclusive results
Small molecules	Genistein	Positive	Positive	Not effective
	Rhodamine in MPS IIIA	Positive	Positive	Not effective
	RNA silencing in MPS IIIC	Positive	Not tested	
	Chaperone	Positive	Not tested	
	Anakinra	Positive	Positive	Clinical trial
	Pentosan polysulfate	Positive	Positive	Not tested
	Coenzymae Q	Positive	Not tested	
	Trehalose	Positive	Not tested	
Gene therapy		Positive	Positive	Table 2

Table 2. **Therapies in clinical trials.**

TREATMENT		PHASE	RESULTS
Enzyme therapy	Intrathecal MPS IIIA Shire	Completed	Not effective enough
	Intravenous MPS IIIA Sobi	Active. Not recruiting	Pending
	Intrathecal MPS IIIB Allievex	Active. Not recruiting	Pending
	Intravenous MPS IIIB Alexion	Completed	Not effective enough
Gene therapy	Intracerebral MPS IIIA Lysogene	Temporarily paused	Pending clinical results
	Endovenous MPS IIIA Abeona	Recruiting	No side effects Pending clinical results
	Intrathecal MPS IIIA Esteve	Active. Not recruiting	No published information
	Lentivirus MPS IIIA Orchard	Recruiting	
	Endovenous MPS IIIB Abeona	Recruiting	No side effects Pending clinical results

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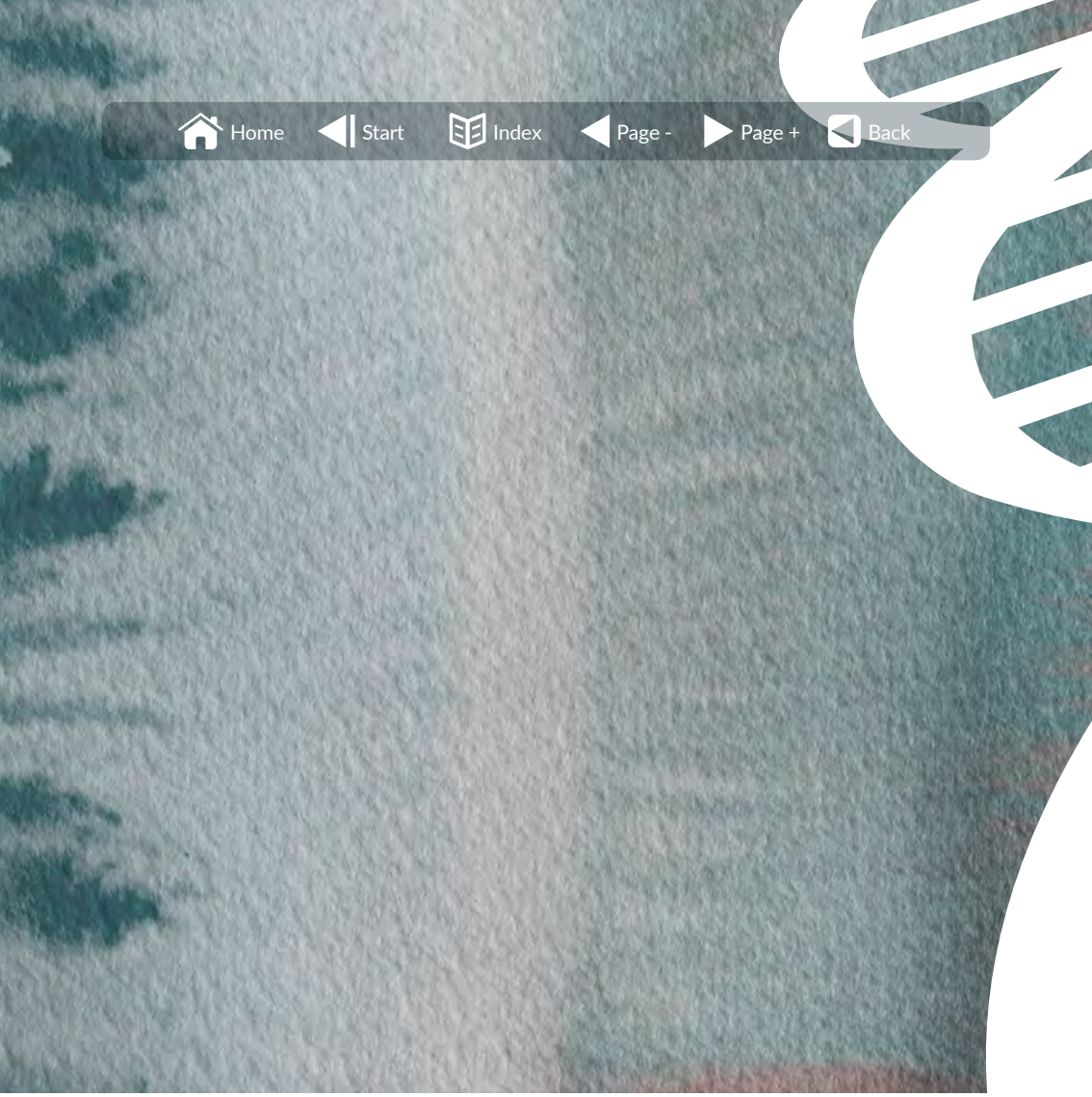

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