R E V I E W

Sanfilippo syndrome: causes, consequences, and treatments

Anthony O Fedele

Lysosomal Diseases Research Unit,
South Australian Health and Medical
Research Institute, Adelaide, SA,
Australia

Abstract: Sanfilippo syndrome, or mucopolysaccharidosis (MPS) type III, refers to one of five autosomal recessive, neurodegenerative lysosomal storage disorders (MPS IIIA to MPS IIIE) whose symptoms are caused by the deficiency of enzymes involved exclusively in heparan sulfate degradation. The primary characteristic of MPS III is the degeneration of the central nervous system, resulting in mental retardation and hyperactivity, typically commencing during childhood. The significance of the order of events leading from heparan sulfate accumulation through to downstream changes in the levels of biomolecules within the cell and ultimately the (predominantly neuropathological) clinical symptoms is not well understood. The genes whose deficiencies cause the MPS III subtypes have been identified, and their gene products, as well as a selection of disease-causing mutations, have been characterized to varying degrees with respect to both frequency and direct biochemical consequences. A number of genetic and biochemical diagnostic methods have been developed and adopted by diagnostic laboratories. However, there is no effective therapy available for any form of MPS III, with treatment currently limited to clinical management of neurological symptoms. The availability of animal models for all forms of MPS III, whether spontaneous or generated via gene targeting, has contributed to improved understanding of the MPS III subtypes, and has provided and will deliver invaluable tools to appraise emerging therapies. Indeed, clinical trials to evaluate intrathecally-delivered enzyme replacement therapy in MPS IIIA patients, and gene therapy for MPS IIIA and MPS IIIB patients are planned or underway.

Keywords: lysosomal storage disease, Sanfilippo syndrome, mucopolysaccharidosis III

Introduction

Lysosomal storage disorders are a group of more than 50 inherited monogenic disorders. Each is caused by a deficiency of an enzyme responsible for the degradation of a metabolic product, whose accumulation results in lysosomal malfunction and disease.[1] Classification is primarily based on the nature of stored material, and secondarily by the enzyme whose activity has been impeded. In Australia, the combined incidence of lysosomal storage disorders is at least 12.9 per 100,000 live births, thus indicating a significant health and economic impact on the family and the community.[2]

Sanfilippo syndrome, or mucopolysaccharidosis (MPS) type III, refers to one of five autosomal recessive, neurodegenerative lysosomal storage disorders whose symptoms are due to the incomplete lysosomal degradation of heparan sulfate.[3] The subtypes are caused by the deficiency of: sulfamidase (MPS IIIA);[4] α-*N*-acetylglucosaminidase (NAGLU, MPS IIIB);[5] heparan acetyl CoA: α-glucosaminide *N*-acetyltransferase (HGSNAT, MPS IIIC);[6] *N*-acetylglucosamine 6-sulfatase (GNS, MPS IIID);[7] or

Correspondence: Anthony O Fedele
Lysosomal Diseases Research Unit, South
Australian Health and Medical Research
Institute, PO Box 11060, Adelaide,
SA 5001, Australia
Tel +61 8 8128 4923
Email anthony.fedele@sahmri.com

N-glucosamine 3-*O*-sulfatase (arylsulfatase G or ARSG, the currently putative MPS IIIE).[8] Phenotypic, enzymatic, and genetic classifications of MPS III subtypes are summarized in Table 1. A listing of reported prevalence is presented in Table 2.

There is currently no therapy available for MPS III. Animal models exist for all forms, whether spontaneous or generated via gene targeting.[8–18] These have contributed to a better understanding of MPS III, and will continue to deliver invaluable tools to evaluate emerging therapies.

Clinical symptoms of MPS III

Somatic symptoms in humans can include coarse facial features with broad eyebrows, dark eyelashes, dry and rough hair, and skeletal pathology that affects growth and causes degenerative joint disease, hepatosplenomegaly, macrocrania, and hearing loss.[19–22]

The primary characteristic of MPS III is however degeneration of the central nervous system (CNS), resulting in mental retardation and hyperactivity, typically commencing during childhood, although there is some heterogeneity with respect to severity and age of onset.

Pre-natal and early stages of post-natal development are usually normal. The initial stages of disease may begin between the ages of 1 and 3 years, which manifest as delayed cognitive development and/or aggressive behavioral problems, as well as hindered speech development.[19,20,23]

Behavioral difficulties may become increasingly severe between the ages of 3 and 5 years, commonly manifesting as a combination of hyperactivity, which is often violent and destructive, as well as sleep disturbances.[20,24–26]

Patients may remain in this state for between 5 and 10 years, after which there is a regression in behavioral disturbances. This is associated with a progressive and severe loss of intellectual processes (such as speech) and motor functions (including walking and swallowing). MPS III patients ultimately regress to a vegetative state until death, which can occur anywhere between the early teens in the most severe scenarios, to as late as the sixth decade in attenuated forms.[19,20,27–30]

Biochemistry of genes involved in heparan sulfate degradation

Heparan sulfate is a negatively charged polysaccharide or glycosaminoglycan (GAG) covalently bound to a number of proteins at the cell surface and in the extracellular matrix. It in turn consists of repeating disaccharide units of either L-iduronic acid or D-glucuronic acid and α-linked glucosamine.[3,8,31,32] The *O*-sulfation of any subunit can occur, while the α-glucosamine may be acetylated, *N*-sulfated, or unmodified.

Heparan sulfate GAGs exist in the cell as proteoglycans[33] that are catabolized within the lysosome. This step may commence with the action of the endohydrolase heparanase on full-length heparan sulfate GAG chains at the plasma membrane, or early endosomal vesicles. This activity yields heparan oligosaccharide fragments of approximately 10–20 monosaccharide residues, whose subsequent degradation occurs via sequential exolytic processes in the lysosome.[34]

Depending on the non-reducing end of these heparan oligosaccharides, this pathway may commence with the desulfation of 2-sulfated iduronic acid residues via the action of iduronate 2-sulfatase (Enzyme Commission [EC] 3.1.6.13),[35,36] and is followed by the hydrolysis of the subsequent terminal L-iduronic acid by α-L-iduronidase (EC 3.2.1.76).[37,38] Both enzymes are also involved in the degradation of dermatan sulfate and their deficiencies result in MPS II (MIM 309900) and subtypes of MPS I (MIM 607014, MIM 607015, and MIM 607016), respectively. At this stage, ARSG desulfates 3-*O*-sulfated *N*-sulfoglucosamine.[8,39] Sulfamidase then hydrolyses the sulfates bound to the amino group of glucosamine,[40,41] and the exposed amino group is

Table 1 Summary of the phenotypic, enzymatic, and genetic classification of the subtypes of MPS III

Subtype	Phenotype MIM number	Activity	EC number	Gene/locus	Gene/locus MIM number	Cytogenetic location
MPS IIIA	MIM 252900	Sulfamidase	EC 3.10.1.1	*SGSH*	MIM 605270	17q25.3
MPS IIIB	MIM 252920	α-*N*-acetylglucosaminidase	EC 3.2.1.50	*NAGLU*	MIM 609701	17q21.1
MPS IIIC	MIM 252930	Heparan acetyl CoA: α-glucosaminide *N*-acetyltransferase	EC 2.3.1.78	*HGSNAT*	MIM 610453	8p11.1
MPS IIID	MIM 252940	*N*-acetylglucosamine 6-sulfatase	EC 3.1.6.14	*GNS*	MIM 607664	12q14.4
MPS IIIE	NA	*N*-glucosamine 3-*O*-sulfatase	EC 3.1.6.-	*ARSG*	MIM 610008	17q24.2

Notes: MPS IIIE is currently a proposed disease insofar as ARSG deficiency in humans has yet to be uncovered. As such, it has not been assigned an MIM number for its phenotype. The EC number for *N*-glucosamine 3-*O*-sulfatase has not been updated beyond 3.1.6.
Abbreviations: MIM, Mendelian Inheritance in Man; NA, not applicable; EC, Enzyme Commission; MPS, mucopolysaccharidosis; NAGLU, α-*N*-acetylglucosaminidase; HGSNAT, heparan acetyl CoA: α-glucosaminide *N*-acetyltransferase; GNS, *N*-acetylglucosamine 6-sulfatase; ARSG, arylsulfatase G; SGSH, N-sulfoglucosamine sulfohydrolase.

Table 2 Summary of reported prevalence studies of MPS III

Country	Reference	Incidence (per 100,000 live births)			
		MPS IIIA	MPS IIIB	MPS IIIC	MPS IIID
Australia	Meikle et al[2]	0.78	0.43	0.07	0.09
France	Héron et al[23]	0.46	0.10	0.09	0.03
Germany	Baehner et al[147]	1.11	0.36	0.10	0.00
Greece	Héron et al[23]	0.00	0.78	0.15	0.00
The Netherlands	Poorthuis et al[148]	1.16	0.42	0.21	0.10
Northern Portugal	Pinto et al[149]	0.00	0.72	0.12	0.00
Sweden	Malm et al[150]	0.44	0.03	0.17	0.00
Taiwan	Lin et al[151]	0.08	0.28	0.03	0.00
United Kingdom	Héron et al[23]	0.82	0.21	0.06	0.02

Note: Note that ARSG deficiency manifested as MPS IIIE has yet to be detected in humans.
Abbreviations: MPS, mucopolysaccharidosis; ARSG, arylsulfatase G.

in turn acetylated by the activity of HGSNAT.[42,43] NAGLU is consequently able to recognize and remove the generated *N*-acetylglucosaminide from the neighboring uronic acid.[44,45] Desulfation of any 2-*O*-sulfated glucuronic acid residues will then occur with glucoronate 2-sulfatase,[46] a deficiency of which is yet to be detected. β-D-glucuronidase (EC 3.2.1.31) then hydrolyzes the terminal glucuronic acid.[47] This is also required to degrade dermatan sulfate, and its deficiency causes MPS VII (MIM 253220). Finally, GNS removes the sulfate group from sulfated *N*-acetlyglucosamine residues.[48]

The catalytic activation of all eukaryotic sulfatases, including those deficient in MPS IIIA, MPS IIID, and MPS IIIE, undergo post-translational modification in which a conserved cysteine within the active site is catalytically converted in the endoplasmic reticulum to a C_α-formylglycine by the enzyme sulfatase modifying factor 1 (SUMF1).[49,50] SUMF1 deficiency thus ablates the function of all sulfatases, which manifests as the symptoms of multiple sulfatase deficiency.[51]

Pathophysiology of MPS III

The order of events from heparan sulfate accumulation through downstream changes in the levels of other biomolecules within the cell and ultimately the clinical symptoms of MPS III, particularly with respect to CNS degeneration, is probably the least understood aspect.

Monosialic gangliosides GM_2 and GM_3 accumulate in lysosomes and other organelles (such as mitochondria and Golgi bodies) secondary to heparan sulfate accumulation,[18,52–55] either by direct GAG-mediated inhibition of lysosomal enzymes responsible for ganglioside degradation,[56] or, alternatively, by deregulated trafficking or synthesis of gangliosides.[53]

Not only is the reason for GM_2 and GM_3 accumulation still a matter of debate, but also their contribution to neuro-degenerative aspects of MPS III. It is speculated from studies with MPS III animal models that both heparan sulfate and gangliosides initiate an inflammatory response that may exacerbate damage within the brain.[12,18,57–59] This may occur as heparan sulfate released from lysosomes (probably via exocytosis) as well as ganglioside-engorged and damaged neurons are phagocytosed by microglia (immunologically active cells involved in CNS defense to damage).

The overall degradative function of lysosomes also relies on the capacity of the lysosomal membranes to associate with target membranes, such as those of autophagic vacuoles.[60] Interfering with the composition of lysosomal membranes has been shown to affect trafficking and fusion within the endo-lysosomal network. Using embryonic fibroblasts derived from mouse models of MPS IIIA and multiple sulfatase deficiency, it has been demonstrated that cholesterol abnormally accumulates in the endo-lysosomal membrane as a consequence of disease. This sequesters soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors, which are in turn required for the function of the fusion machinery in these membranes, thus inhibiting this function.[61] This impairment may have wide reaching consequences, such as the pathology of the mitochondrial system observed in the MPS IIIC mouse, caused by the accumulation of dysfunctional mitochondria otherwise eliminated by normal autophagy and lysosomal function.[18]

Genetics of MPS III

The mutations that have been sequenced in the alleles of the causative genes in patients diagnosed with MPS III are summarized in Table 3. A commentary of those deemed of interest due to frequency and/or phenotypic consequences follows.

MPS IIIA

The first patient known to be diagnosed with symptoms associated with an MPS caused by heparan sulfate accumulation was described by Sanfilippo et al.[62]

Later, it was observed that medium conditioned by the fibroblasts of a particular MPS III patient was able to reverse mucopolysaccharide accumulation in some but not all of a cohort of other MPS III fibroblasts.[63] This cross-correction was repeated with urine from these patients. This suggested that the disease could be caused by the absence of one of two factors, and as such was arbitrarily classified as types A or B, based on which agent was evidently deficient.

Table 3 Summary of mutation types found in the alleles of the causative genes of patients diagnosed with MPS III

Subtype	Gene	Number of mutation types									
		Total	Missense	Non-sense	Splicing	Small deletion	Small insertion	Small indel	Gross deletion	Gross insertion	Complex rearrangement
MPS IIIA	*SGSH*	137	93	11	2	17	9	1	3	1	0
MPS IIIB	*NAGLU*	153	90	14	5	23	13	1	4	3	0
MPS IIIC	*HGSNAT*	63	27	9	13	5	5	1	2	1	0
MPS IIID	*GNS*	23	3	4	3	4	4	1	2	0	2

Notes: Copyright ©2015 Cardiff University. All rights reserved. Adapted with permission from the Human Gene Mutation Database (HGMD Professional 2014.2) (http://www.hgmd.cf.ac.uk/ac/index.php).[152] Data from Stenson et al.[152] Accessed October 1, 2014. In this table, small refers to deletions and/or insertions involving 20 or less base pairs, while gross describes deletions or insertions involving 21 or more base pairs.
Abbreviations: MPS, mucopolysaccharidosis; *NAGLU*, α-*N*-acetylglucosaminidase; *HGSNAT*, heparan acetyl CoA: α-glucosaminide *N*-acetyltransferase; *GNS*, *N*-acetylglucosamine 6-sulfatase; *SGSH*, *N*-sulfoglucosamine sulfohydrolase.

Soon after, the increased mucopolysaccharide in MPS IIIA was purified from disease fibroblasts and shown to migrate with the mobility of heparan sulfate. In addition, a compound capable of reducing the accumulation of mucopolysaccharide was purified from the urine of healthy individuals and demonstrated to display *N*-sulfoglucosamine sulfohydrolase (*SGSH*, or sulfamidase) activity,[64] an absence of which was observed in fibroblasts, leucocytes, and lymphoblasts from MPS IIIA patients.[4,65] Eventually, the gene for MPS IIIA was identified.[41] The gene for sulfamidase (*SGSH*) is localized to chromosome 17 q25.3 and contains eight exons.[41,66] The cDNA codes for a product of approximately 55 kDa (excluding the signal peptide sequence). Within this, there are up to five asparagine-linked glycosylation sites.[41]

Specific sulfamidase mutations have been found in high frequencies in distinct geographical locations. The missense mutation p.R74C, which replaces a large, positively charged R group in the active site with one that is small and non-polar, occurs at a frequency of 56% of the Polish population.[67] The p.R245H amino acid change is found in 31% of mutant alleles in Australia, 35% of those in Germany, and 58% of those in the Netherlands.[67,68] This residue is not conserved among human sulfatases, and has little consequence on specific activity, but it does however affect the stability of the protein.[69,70] The p.S66W missense mutation is found in 29% of alleles in a cohort of Italian patients;[71] this is not conserved among sulfatases, but is close to the conserved cysteine within the active site that is catalytically converted to a C_α-formylglycine by SUMF1.[49,50] A substitution of the small R group of serine with the aromatic R group of tryptophan would thus be predicted to interfere with the active site, and studies of this variant demonstrated a compromise between stability and activity.[69,70]

MPS IIIB

Soon after the genetically heterogeneous nature of MPS III[63] and the factor deficient in MPS IIIA were discovered,[64] it was shown that MPS IIIB was caused by a critically reduced activity of α-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, (α-*N*-acetylglucosaminidase, or *NAGLU*).[72]

The gene for MPS IIIB was cloned around the same time as that for MPS IIIA.[45,44] *NAGLU* is on chromosome 17q21.1 and contains six exons. The cDNA codes for a polypeptide of 743 amino acids, which contains a cleavable signal sequence and six asparagine-linked glycosylation sites. The mature protein's 720 amino acids yield a molecular mass of approximately 80 kDa.

Unlike MPS IIIA, there are no common mutations in MPS IIIB. Rather, most of the known mutant alleles in MPS IIIB patients occur at low frequencies or not more than once. However, the p.F48L, p.G69S, p.S612G, and p.R643C missense mutations have been associated with a later-onset phenotype.[73–75]

MPS IIIC

Primarily due to the biochemical properties of the deficient protein, the gene for MPS IIIC remained more elusive. Originally, it was demonstrated that skin fibroblasts from MPS IIIC patients displayed a lack of HGSNAT activity.[6] The responsible enzyme was then localized to the lysosomal membrane and shown to catalyze a transmembrane acetylation of the terminal glucosamine residue of intra-lysosomal heparan sulfatase.[76–79] Over 20 years later, the gene was identified.[42,43] *HGSNAT* is on chromosome 8p11.1 and consists of 18 exons. The cDNA encodes a polypeptide of 635 amino acids, which contains eleven transmembrane domains and five asparagine-linked glycosylation sites. The amino-terminal 42 amino acids form a signal peptide crucial for integration into the lysosomal membrane, where it is then post-translationally modified into a 27 kDa α chain and a 44 kDa β chain.[80,81]

There is contrasting but compelling evidence for two different models of the mechanism of HGSNAT activity (Figure 1). One proposes that the enzyme binds acetyl CoA
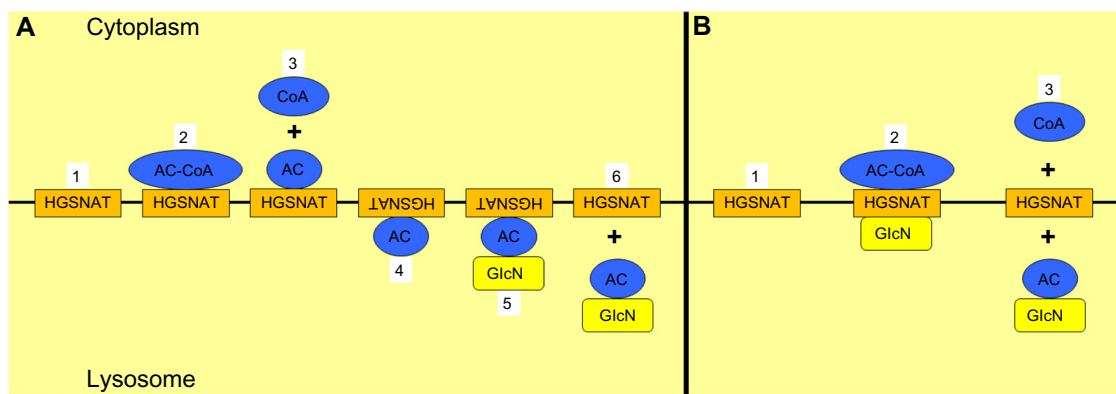
Figure 1 Proposed models for the mechanism of heparan acetyl CoA: α-glucosaminide *N*-acetyltransferase (HGSNAT) activity.
Notes: (**A**) *HGSNAT* (1) acquires acetyl CoA from the cytoplasmic side of the lysosomal membrane (2), and is itself acetylated at an active site histidine (3). A conformational change allows for the transfer of the acetyl group into the lysosome (4). Once heparan sulfate interacts with the active site, the terminal glucosamine residue of heparan sulfate (GlcN) acquires the acetyl group (5), thus forming *N*-acetylglucosaminide (6). Data from previous studies.[76–78,80] (**B**) HGSNAT (1) catalyzes its reaction via a random ternary order complex (2), so that the process requires only one step, and no direct acetylation of the enzyme as an intermediate (3). Data from previous studies.[79,81]

from the cytoplasmic side of the lysosomal membrane, and is itself acetylated at an active site histidine. A conformational change allows for the transfer of the acetyl group into the lysosome. Once heparan sulfate interacts with the active site, the terminal glucosamine acquires the acetyl group, thus forming *N*-acetylglucosaminide.[76–78,80] A second model suggests that catalysis occurs via a random ternary order complex, in one step, and with no direct intermediary acetylation of the enzyme.[79,81]

The missense mutations p.R344C and p.S518F account for 22% and 29%, respectively, of mutant alleles in the Netherlands.[20] Furthermore, the deleterious nature of nearly all known missense mutations can be established via their occurrence in amino acids present in a transmembrane domain and/or conservation among orthologs.[42,43,82] The protein and activity of the majority of the known missense mutations have been exogenously expressed in cell culture.[83,84] Transfection of 18 of these resulted in negligible HGSNAT activity, while the remaining four displayed protein and function comparable to those of wild type, and so are therefore currently considered clinically insignificant polymorphisms.

Interestingly, mutations in HGSNAT have been found in six patients with the non-syndromic form of retinitis pigmentosa, each of whom was diagnosed in the third to fifth decade of life.[85] These mutations result in reduced HGSNAT activities compared with healthy controls but sufficiently high to avoid neurodegeneration and other symptoms of MPS IIIC.[85] Retinitis pigmentosa was also reported in the two patients with late-onset MPS IIIC.[20,86] Their genotypes were identified as compound heterozygous for two missense mutations (p.G262R and p.S539C), both of which occur in amino acids predicted to be in the membrane,[42,43,82] and whose

separate overexpression in cell culture resulted in activity that was essentially undetectable.[83,84]

MPS IIID

Discounting MPS IIIE, which has not yet been described in humans, MPS IIID was the last to be classified, but the first to have its causative gene discovered. In 1980, skin fibroblasts from two patients with the clinical symptoms of MPS III and high urinary concentrations of heparan sulfate were demonstrated to lack the enzymatic activity required to release sulfate from *N*-acetylglucosamine 6-sulfate linkages. This was thus designated Sanfilippo type D (MPS IIID).[7]

GNS activity was subsequently purified and characterized[48,87] and Edman sequencing of the isolated form permitted cloning of the coding gene.[88,89] *GNS* is found on chromosome 12q14 and contains a total of 14 exons. The cDNA encodes for a polypeptide of 552 amino acids, and a protein of 78 kDa. In addition to a cleavable amino-terminal signal peptide of 36 amino acids, post-translational modification also results in cleavage by internal peptidases into a 32 kDa amino-terminal and 48 kDa carboxy-terminal species. It also contains 13 potential asparagine-linked glycosylation sites.

Similar to MPS IIIB, there are no common mutations in MPS IIID. It is noteworthy that there are relatively few missense mutations compared with the other MPS III subtypes (13%, see Table 3), and a dominance of deletions, insertions, and rearrangements.

MPS IIIE

The gene for MPS IIIE, *N*-glucosamine 3-*O*-sulfatase (*ARSG*), is localized to chromosome 17q24.2 and

contains eleven exons.[8,90,91] The cDNA encodes a protein of 525 amino acids, of which four are asparagine-linked glycosylation sites.[90,91]

Although ARSG has been molecularly characterized, and dog[17] and mouse[8] models of MPS IIIE exist, ARSG deficiency in humans has yet to be uncovered. These model organisms present with ataxia,[17,92] which is only additionally observed in a New Zealand Huntaway dog model of MPS IIIA.[13]

Transcriptional regulation of the causative genes for MPS III

The expression of most lysosomal genes is mediated by the transcription factor EB (TFEB), a member of the microphthalmia-associated transcription factor E subfamily of basic helix-loop-helix transcription factors.[93] This functions by binding to the Coordinated Lysosomal Expression and Regulation (CLEAR) element (a DNA motif consisting of the palindromic consensus sequence GTCACGTGAC) of associated genes, and increasing their transcription.[93] Stresses such as starvation and lysosomal storage induce TFEB translocation to the nucleus, thus facilitating transcriptional activity, and contributing to an increase in CLEAR-containing lysosomal genes and lysosomal biogenesis in general.[93–96]

This phenomenon has been demonstrated to involve the regulatory multi-subunit containing mammalian target of rapamycin complex 1 (mTORC1). In normal conditions (for instance, in the presence of full nutrients and basal lysosomal function), a complex including vacuolar H$^+$ (V)-ATPase, the Ragulator complex, and Rag GTPases is in an active state. In being so, it recruits mTORC1 to the lysosomal surface, where mTORC1 in turn becomes activated and phosphorylates TFEB, thus maintaining TFEB in the cytoplasm in an inactive form. However, in conditions of cellular stress, such as starvation or increased lysosomal storage, the Rag GTPases are inactivated, mTORC1 is detached from the lysosome, and TFEB is no longer phosphorylated. Consequently, TFEB translocates to the nucleus, thus facilitating its capacity as a transcription factor and increasing the expression of CLEAR element containing genes.[97–101]

The genes for MPS IIIA, MPS IIIB, MPS IIID, and MPS IIIE each contain at least two CLEAR elements (listed in Table 4) and their mRNA levels are increased in cell culture upon exogenous TFEB expression, and reduced via miRNA-mediated inhibition of TFEB.[93]

Interestingly, HGSNAT does not contain a CLEAR element, and there is no evidence to suggest that it is directly regulated by TFEB.[93,102–104] Furthermore, the mechanisms of HGSNAT transcriptional activation have not

Table 4 CLEAR element sequences present in the genes for MPS III

Subtype	Gene/locus	CLEAR element sequence	Location
MPS IIIA	SGSH	GCGCCACCTGACCG	−312
		CGCGCACGTGACCG	−8
MPS IIIB	NAGLU	GGCTCAGCTGACCT	−321
		CGGTCACGAGACGC	179
MPS IIID	GNS	ACCTCAGGTGATCC	−905
		CCGTCACGTGACCG	−29
		GGCTCACGTGATCG	13
MPS IIIE	ARSG	GTCCCACGTGGACC	6
		TGCGCGCGTGACCC	170
		GAGCCACGTGTGCC	184

Notes: Location refers to the position of the CLEAR element relative to the transcription start site, where the A of the ATG translation initiation start site of the coding sequence is nucleotide +1. Note that the gene for MPS IIIC (HGSNAT) does not contain any CLEAR elements. © 2014 The American Society for Biochemistry and Molecular Biology. Adapted from Moskot M, Montefusco S, Jakóbkiewicz-Banecka J, et al. The phytoestrogen genistein modulates lysosomal metabolism and transcription factor EB (TFEB) activation. *J Biol Chem*. 2014;289:17054–17069.[103]
Abbreviations: MPS, mucopolysaccharidosis; NAGLU, α-N-acetylglucosaminidase; HGSNAT, heparan acetyl CoA: α-glucosaminide N-acetyltransferase; GNS, N-acetylglucosamine 6-sulfatase; ARSG, arylsulfatase G; CLEAR, Coordinated Lysosomal Expression and Regulation; SGSH, N-sulfoglucosamine sulfohydrolase.

been characterized. However, the 5′ untranslated region of HGSNAT has been demonstrated to contain a SINE-VNTR-Alu (SVA) – B element insertion.[105] Although its influence on HGSNAT expression requires further investigation, SVA elements are known to be non-autonomous, hominid-specific, non-long terminal repeat (LTR) retrotransposons that possess the capacity to sporadically generate disease-causing insertions.[106]

Laboratory diagnosis of MPS III
GAG analysis

The detection of heparan sulfate in the urine of suspected MPS III patients, or, more specifically, the GAGs within, is often the first biochemical step in diagnosis.

The traditional method of urinary GAG quantification involves the use of dimethylmethylene blue. Here, dimethylmethylene blue associates with sulfated GAG and the absorbance of the complex at a wavelength of 520 nm can be measured on a spectrophotometer.[107,108] An elevation in urinary GAG will suggest a form of MPS.

Subsequent analysis can permit a further characterization of the accumulating product. High-resolution cellulose acetate electrophoresis of urinary samples can separate and identify distinct GAGs, thus providing an indication of MPS III from other forms.[109] Additionally, qualitative gradient polyacrylamide gel electrophoretic separation can confirm increased secretion of particular heparan sulfate chains, thus diagnosing the particular MPS III subtype.[110]

Furthermore, methods comprising tandem mass spectrometry are now being developed. One involves quantification after derivatization of sulfated *N*-acetylhexosamine and sulfated *N*-acetylhexosamine-uronic acid.[111] This method identifies specific oligosaccharides that accumulate as a result of a specific enzyme deficiency that act at the non-reducing end of heparan sulfate oligosaccharides.[34,111,112] This can identify all MPS subtypes, except for MPS IIIB and MPS IIIC. Additionally, extraction and depolymerization of heparan sulfate using methanolysis under acidic conditions, and analysis of the subsequent desulfated disaccharide reaction products by liquid chromatography/tandem mass spectrometry can be used.[113,114] This has the disadvantage that it cannot differentiate between MPS III types, but is simple and quick to run and thus a good first screen test. A liquid chromatography/tandem mass spectrometry-based technique that exploits the unique nature of the non-reducing end residue of the accumulated GAG upon the deficiency of a particular enzyme in the pathway has also been developed.[115]

Activity assay

Two types of artificial substrates that can be catalytically processed by the enzymes in skin fibroblasts and leucocytes otherwise deficient in MPS IIIA to MPS IIID are available: those that are radiolabeled[116–119] and those that are fluorogenic.[120–123] The basis of radiolabeled substrates is that the relevant enzymatic activity alters the charge of the substrate, thus permitting chromatographic separation. Regarding the latter, a fluorescent 4-methylumbelliferone product is released upon successful completion of the reaction, and can be detected by employing a fluorometric device.

An important fact of the fluorogenic substrate for the MPS IIIC assay is that HGSNAT acetylates it to a non-fluorescing intermediate, which is in turn hydrolyzed to the fluorescing form by β-hexosaminidase; so the presence of the latter in cells that are being evaluated is therefore imperative.[122] Also, for MPS IIIA and MPS IIID, regardless of the chosen substrate, activity of a second sulfatase should be tested, in order to categorically exclude multiple sulfatase deficiency.

Genomic DNA sequencing

This is achieved by employing oligonucleotide primers that span the intron/exon junctions of the exons of the gene of interest using the polymerase chain reaction with genomic DNA as the template. Products can be sequenced and cross-referenced to the standard wild-type sequence and known disease-causing mutations and polymorphisms.

This is the definitive method for diagnosing not only disease but also carrier status (the latter is particularly difficult to detect via GAG analysis and activity assays as the levels between carriers and unaffected controls are likely to be similar). However, this is assuming the mutation occurs within one of the boundaries that are being sequenced. Any changes beyond the scope of the amplifying oligonucleotides in concept cannot be screened. Also, if an entire exon is absent in heterozygosity, this cannot be detected via standard qualitative genomic DNA sequencing (which will still have the alternative allele as a template). This would justify the development and application of quantitative techniques for mutation screening, particularly for MPS IIID, where there is a proportionally higher occurrence of large rearrangements.[30]

Future diagnostic methods

Two motivations for the creation of new screening techniques are decreasing the age at which it can be applied, and increasing output and/or decreasing cost, either by a simpler and more rapid method to detect a particular disease, or by an increased scope in the number of diseases that can be diagnosed using the same test.

A chorionic villus sample taken at 9–10 weeks gestation or cultured cells from 14–16 weeks amniocentesis can be tested for MPS III-causing mutations via genomic DNA sequencing or enzymatic activity assays.[124] Increased heparan sulfate, although less reliably, has also been detected using electrophoresis in amniotic fluid taken at 16 weeks gestation from a pregnancy involving an MPS IIIA fetus.[125] In each case, the main disadvantage is the intrusiveness of the method of sample collection.

Therefore, steps are being taken to establish multi-tasked newborn screening strategies for MPS III (and other lysosomal storage disorders), whether via detection of protein, activity, or GAG levels. Recent developments have included: measuring the protein amounts of multiple specific enzymes in dried blood spots using a multiplex immune-quantification assay, including sulfamidase and NAGLU;[126,127] the synthesis of substrates for the enzymes deficient in MPS IIIA to MPS IIID that measure the reaction products in an in vitro activity assay via tandem mass spectrometry;[128] and the use of tandem mass spectrometry to measure and characterize the accumulated GAGs in dried blood spots after digestion into disaccharides.[129]

Another advance is whole genome or exome sequencing, which would, in theory, permit screening of the entire genome or protein-coding region. These may possess similar caveats as genomic DNA sequencing of a selected gene but could also provide a standard method to test any subject for all sequencing abnormalities.

Treatments in development for MPS III

Due to the lack of a therapy to rescue the primary insult and/or pathophysiology of MPS III, present efforts are generally directed toward regulating behavior and sleep disturbances. However, a number of therapies for MPS III are being developed and evaluated.

Enzyme replacement therapy

The ability for sulfamidase, NAGLU, and GNS to be secreted from and taken up by the lysosomes within cells has provided the basis for the foundation of therapies for MPS III.

The major obstacle thus far in the establishment of enzyme replacement therapy (ERT) is the inability of these same proteins to cross the blood brain barrier (BBB). Injection of human recombinant sulfamidase directly into the brain[130] or into cerebrospinal fluid via the cerebellomedullary cistern[131] of MPS IIIA mice has been successful in alleviating a number of symptoms but has also emphasized the importance of early detection on account of the irreversibility of certain (particularly neurodegenerative) aspects of the pathophysiology.

The problem of potential invasiveness of targeting the brain has been grappled by engineering forms of sulfamidase to cross the BBB. For instance, a mixture of increasing the dose of recombinant sulfamidase and modifying the chemical structure to remove mannose-6-phosphate glycans has been attempted.[132] Despite significantly increasing the effectiveness upon systemic delivery into the MPS IIIA mouse outside the CNS, it was less effective in the brain.

Gene therapy

The use of gene therapy is an extension of ERT insofar as it attempts to introduce the coding sequence of the protein of interest into the cells of the patient via the use of a viral vector, rather than synthesize it in and purify it from another host. Manipulated cells will not only themselves possess enzymatic activity, but will also secrete it into circulation to be taken up by un-altered cells.

Co-delivery of sulfamidase and SUMF1 via intraventricular injection of a recombinant adeno-associated virus (AAV) that drives the expression of both coding sequences resulted in increased sulfamidase activity in the mouse brain; a decrease in lysosomal storage and microglial activation and an enhancement of motor and cognitive capabilities were also observed.[133] This strategy is now at the human clinical trial stage.[134] Moreover, a therapy using a similar approach and with comparable results for MPS IIIB is being developed based upon successful and efficacious AAV-mediated human NAGLU delivery in MPS IIIB mice and Schipperke dog models.[135,136]

A recent advance has attempted to address a number of issues (repeated administration, invasiveness of delivery methods, and inability of the normal enzyme to cross the BBB) with one solution.[137] Sulfamidase engineered to be fused to both the signal peptide of the highly secreted iduronate-2-sulfatase protein and the BBB binding domain of apolipoprotein B was included in an AAV-based vector designed to target the liver, thus allowing it to serve as a "factory" for perpetual systemic delivery of the modified enzyme. Adult MPS IIIA mice then intravascularly received this once. Not only did the signal peptide enhance secretion, but also the BBB binding domain permitted rescue of sulfamidase activity in the brain, reduction of neuropathology, and a restoration of behavior.[137]

Genistein

Genistein is an isoflavone purified from soy that exhibits an ability to reduce GAG levels. Although the overall mechanism by which this occurs has not been entirely clarified, its properties as a kinase inhibitor serve to induce TFEB transcriptional activity.[103,104] For instance, it would seem that inhibition of the mTORC1-mediated phosphorylation of TFEB by genistein results in the translocation of TFEB to the nucleus from the cytoplasm, an increase in the expression of CLEAR-containing lysosomal genes, and an improvement in the removal of lysosomal storage products.[103,104]

Supplementation of growth medium with genistein resulted in reduction in GAG levels in skin fibroblasts derived from MPS IIIA and MPS IIIB patients.[138] Moreover, treatment of an MPS IIIB mouse model showed an initial reduction in GAG storage[139] and an improved clinical outcome when higher doses were provided.[140] Most importantly, a pilot study involving five MPS IIIA and five MPS IIIB patients were administered with a genistein-enriched soy isoflavone extract for 12 months, which reduced urinary GAG levels, enhanced hair morphology, and improved behavior.[141]

Substrate deprivation therapy

In the context of MPS III, the purpose of this strategy is to reduce the levels of heparan sulfate containing GAGs by decreasing the amounts that are initially generated. Treatment of MPS IIIA mice with the non-specific inhibitor of GAG synthesis Rhodamine B resulted in reduced urinary GAG, as well as levels in the liver and the brain, and improved

learning and memory as determined by water maze, with no long-term adverse effects.[142–144]

Development of a therapy for MPS IIIC

The rescue of HGSNAT activity has its own challenges. As with all disorders caused by faulty multi-pass transmembrane protein expression, approaches that rescue deficiencies of soluble proteins that can be secreted and taken up (such as ERT) are not applicable to HGSNAT deficiency. Some success has been achieved with molecular chaperones to rescue HGSNAT activity.[83,145] These are typically reversible competitive inhibitors of the enzyme in question, which are administered at sub-inhibiting concentrations. The basis of this is that the inhibitor is capable of associating with its interacting site and aiding the structural restoration of the misfolded protein. In doing so, it restores the biochemical deficiency, which ultimately manifests as the disease in question.[146] Modified U1 spliceosomal RNAs that recognize mutated donor sites of selected splice-site mutants and restore RNA splicing of HGSNAT have also been evaluated.[145] Yet, such therapies will even in theory be successful in restoring only a subset of mutants (in these cases, missense and donor splice site, respectively). This presents a conundrum not just for MPS IIIC, but all disorders arising from multi-pass transmembrane protein expression deficiencies.

Conclusion

All the genes whose deficiencies cause MPS III have been discovered, and their gene products have been biochemically characterized to varying degrees. Furthermore, a number of genetic and biochemical diagnostic methods have been developed and adopted by diagnostic laboratories. Although MPS III disorders are rare, they are sufficiently debilitating to patients and challenging to parents and carers to warrant attention and research. Despite the current lack of a completely effective therapy for MPS III, there is much promise in therapy development, to the point that clinical trials to evaluate intrathecally-delivered ERT in MPS IIIA and gene therapy for MPS IIIA and MPS IIIB patients are in either clinical trial or being planned.

Acknowledgments

Disclosure

The author reports no conflicts of interest in this work.

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