Molecular Genetics and Metabolism 125 (2018) 322–331

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Minireview

Anatomical changes and pathophysiology of the brain in mucopolysaccharidosis disorders

Brian W. Bigger[a,*], David J. Begley[b], Daniela Virgintino[c], Alexey V. Pshezhetsky[d]

[a] Stem Cell & Neurotherapies Laboratory, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK
[b] Drug Delivery Group, Institute of Pharmaceutical Science, King's College London, London, UK
[c] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, Human Anatomy and Histology Unit, Bari University School of Medicine, Bari, Italy
[d] Departments of Pediatrics and Biochemistry, CHU Sainte-Justine, Research Center, University of Montreal, Montreal, QC, Canada

ARTICLE INFO

Keywords:
Mucopolysaccharidosis
Heparan sulfate proteoglycans
Neuropathology
Gangliosides

ABSTRACT

Mucopolysaccharidosis (MPS) disorders are caused by deficiencies in lysosomal enzymes, leading to impaired glycosaminoglycan (GAG) degradation. The resulting GAG accumulation in cells and connective tissues ultimately results in widespread tissue and organ dysfunction. The seven MPS types currently described are heterogeneous and progressive disorders, with somatic and neurological manifestations depending on the type of accumulating GAG. Heparan sulfate (HS) is one of the GAGs stored in patients with MPS I, II, and VII and the main GAG stored in patients with MPS III. These disorders are associated with significant central nervous system (CNS) abnormalities that can manifest as impaired cognition, hyperactive and/or aggressive behavior, epilepsy, hydrocephalus, and sleeping problems. This review discusses the anatomical and pathophysiological CNS changes accompanying HS accumulation as well as the mechanisms believed to cause CNS abnormalities in MPS patients. The content of this review is based on presentations and discussions on these topics during a meeting on the brain in MPS attended by an international group of MPS experts.

1. Introduction

Mucopolysaccharidosis (MPS) disorders are characterized by deficiencies in lysosomal enzymes involved in glycosaminoglycan (GAG) degradation. The resulting excessive intra- and extracellular GAG storage leads to widespread tissue and organ dysfunction, and reduced life expectancy [1, 2]. MPS enzymes act in a sequential fashion to reduce one, two, or all of the three major GAG polysaccharide chains to monosaccharides, including heparan sulfate (HS), keratan sulfate (KS) and dermatan sulfate (DS). Depending on the disease, the absence of a GAG degrading enzyme results in accumulation of specifically sulfated polysaccharides of those GAGs affected. The presence of somatic and neurological signs and symptoms of the different MPS disorders appear to correlate with the primary accumulating GAG (Table 1) [1, 3, 4]. KS and DS are the main GAGs stored in patients with MPS IV and VI, respectively [3]. These patients have severe skeletal dysplasia and somatic involvement, but normal cognitive development [1, 2, 5, 6]. HS is the main GAG accumulating in MPS IIIA-D, whereas in patients with MPS I, II, and VII both DS and HS accumulate (as well as KS in MPS VII) [2]. Patients with HS storage disorders, and more particularly those with a rapidly progressing phenotype, have significant central nervous system (CNS) involvement that can manifest as impaired cognition, hydrocephalus, behavioral difficulties, epileptic seizures, and/or sleeping problems [1, 3, 7–12]. Since primary CNS involvement is only observed in those disorders associated with impaired HS catabolism, accumulation of HS, and more fundamentally some aspects of the partially catabolized HS fragments such as altered chain length, sulfation patterning, and non-reducing end status, might be fundamental to the pathology of these CNS abnormalities. Notably, although attenuated MPS I and MPS II patients are often described as having no neurological symptoms, presumably due to residual enzyme production, recent studies have highlighted that attenuated MPS II patients develop cognitive

Abbreviations: BBB, Blood-brain barrier; CNS, Central nervous system; CSF, Cerebrospinal fluid; DAMP, Damage-associated molecular patterns; DS, Dermatan sulfate; FGF, Fibroblast growth factor; HGSNAT, Heparan-α-glucosaminide N-acetyltransferase; GAG, Glycosaminoglycan; GFAP, Glial fibrillary acidic protein; HPLC, High-performance liquid chromatography; HS, Heparan sulfate; KS, Keratan sulfate; MCP1, Monocyte chemoattractant protein 1; MEC, Medial entorhinal cortex; MPS, Mucopolysaccharidosis; MPS IH, MPS I Hurler; MS, Mass spectrometry; ROS, Reactive oxygen species; PET, Positron emission tomography; RP-HPLC, Reversed phase high-performance liquid chromatography; PVS, Perivascular space; SCMAS, Subunit c of mitochondrial ATP synthase; TLR, Toll-like receptor

* Corresponding author at: Division of Cell Matrix and Regenerative Medicine, 3.721 Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT, UK.
E-mail address: brian.bigger@manchester.ac.uk (B.W. Bigger).

https://doi.org/10.1016/j.ymgme.2018.08.003
Received 23 May 2018; Received in revised form 9 August 2018; Accepted 9 August 2018
Available online 10 August 2018
1096-7192/ © 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

Table 1
Biochemical classification of the MPS disorders and the occurrence of neurological and somatic symptoms [3, 108].

MPS type	Deficient enzyme	Main GAG stored	Neurological symptoms	Somatic symptoms
MPS I	α-L-iduronidase (EC 3.2.1.76)	HS, DS	Hurler: severe Hurler-Scheie and Scheie: mild to absent	Wide spectrum of severity
MPS II	Iduronate-2-sulfatase (EC 3.1.6.13)	HS, DS	Severe (rapidly progressing phenotypes) or mild to absent (slowly progressing phenotypes)	Wide spectrum of severity
MPS IIIA	*N*-sulfoglucosamine sulfohydrolase (EC 3.10.1.1)	HS	Severe	Mild to absent
MPS IIIB	α-*N*-acetylgluscoaminidase (EC 3.2.1.50)	HS	Severe	Mild to absent
MPS IIIC	AcetylCoA-α-glucosaminide *N*-acetyltransferase (EC 2.3.1.78)	HS	Severe	Mild to absent
MPS IIID	*N*-acetylglucosamine 6-sulfatase (EC 3.1.6.14)	HS	Severe	Mild to absent
MPS IVA	*N*-acetylgalactosamine-6- sulfatase (EC 3.1.6.4)	KS	None	Severe or mild
MPS IVB	β-galactosidase (EC 3.2.1.23)	KS	None	Severe or mild
MPS VI	*N*-acetylgalactosamine-4-sulfatase (EC 3.1.6.12)	DS	None	Severe or mild
MPS VII	β-D-glucuronidase (EC 3.2.1.31)	HS, DS	Severe (rapidly progressing phenotypes) or mild to absent (slowly progressing phenotypes)	Wide spectrum of severity
MPS IX[a]	Hyaluronidase (EC 3.2.1.35)	Hyaluronan	None	Periarticular soft tissue masses, short stature

DS: dermatan sulfate; GAG: glycosaminoglycan; HS: heparan sulfate; KS: keratan sulfate; MPS: mucopolysaccharidosis.
[a] Few cases described in literature

difficulties in the long term [12, 13], with a significant deficit in measures of attention [14]. In the MPS I registry, cognitive impairment was observed in 31% of Hurler-Scheie (MPS IHS) and 9% of Scheie (MPS IS) patients [15].

This review summarizes current knowledge on anatomical and pathophysiological changes associated with HS accumulation in the CNS of MPS I, II, III, and VII patients. In addition, a discussion on potential biomarkers that could be used to measure CNS disease progression in these patients is included. The content of this review is based on information from an expert meeting on the brain in MPS, held on April 28–30, 2016 in Stockholm, Sweden, attended by an international group of 39 MPS experts. Additional relevant literature was obtained from PubMed searches using search terms ("Mucopolysaccharidoses/anatomy and histology"[Mesh]) AND "Blood-Brain Barrier"[Mesh] (10 items), (("Mucopolysaccharidoses/anatomy and histology"[Mesh] OR "Mucopolysaccharidoses/pathology"[Mesh])) AND "Blood Vessels"[Mesh] (30 items), ((("Mucopolysaccharidoses/anatomy and histology"[Mesh] OR "Mucopolysaccharidoses/pathology"[Mesh])) AND ("brain"[Mesh])) (178 items). Publications not available in English were excluded. Searches were performed without date restriction. Additional publications were identified from reference lists within the most relevant MPS-related papers focusing on CNS anatomy and pathophysiology. The literature search was completed in December 2016.

2. Anatomical changes in the brain in MPS disorders

Post-mortem histological and neuroimaging studies have revealed several brain abnormalities frequently present in patients with MPS I Hurler (MPS IH), II, III [16], and VII [5, 17]. Multifocal or diffuse white matter lesions are almost universally present in all these MPS types (Fig. 1A) [5, 18] and have been shown to be more abundant in MPS II patients with cognitive impairment [19] and to increase in number with increasing disease duration [20]. Increased signal intensity in the periventricular white matter has been reported for MPS I, II, III, and VII [21–23]. Dilated perivascular spaces (PVS), also referred to as cribriform changes, are among the most frequently observed CNS abnormalities in MPS I, II, and III patients (Fig. 1B) [21, 24–28]. These changes can be seen throughout the brain, but are most abundant in the periventricular and subcortical white matter; their severity varies between and within MPS disorders [16, 17, 29]. Ventriculomegaly is also a frequent manifestation of MPS [30, 31]. It can be the result of communicating hydrocephalus involving the lateral and third ventricles, brain atrophy, or both (Fig. 1C and D). Ventriculomegaly associated with hydrocephalus is more prevalent in MPS I and II than in MPS III patients, and probably caused by inadequate cerebrospinal fluid (CSF) absorption [21–23, 25, 26, 28]. Brain atrophy, characterized by enlargement of the cortical sulci and an increase in CSF, is most prevalent in MPS II (Fig. 1C) [20, 22, 28] and III [21, 24, 25, 30]. In particular, decreases in gray matter over time, correlating with decreasing cognitive development quotient scores are observed in MPSIIIA and MPSIIIB patients [18, 32].

White matter lesions and brain atrophy in MPS patients are assumed to result from accumulation of storage material, including GAGs, in neurons and glial cells, which in turn has been suggested to trigger myelination abnormalities, gliosis, and cell death [16, 21, 25, 26, 30, 34, 35]. Dilated PVS are considered to be due to GAG deposition around the cerebral vessels [36] and/or in the meninges, which can lead to impaired CSF reabsorption and ventriculomegaly [5, 19, 20, 22, 23, 25]. Other mechanisms, such as inflammatory disease, and watershed ischemia in the deep white matter, may also contribute [37].

Abnormalities in the blood-brain barrier (BBB) may also contribute to CNS pathology in MPS patients. The BBB, which is formed early in brain development [38], helps to maintain CNS/CSF homeostasis and protects the CNS from harmful substances by limiting permeability through tight junctions and controlled regulation of transport routes [39]. Garbuzova-Davis et al. observed perivascular edema and microvascular leakage in the brain of MPS III patients, compromising BBB function and integrity [40] and probably contributing to CNS damage like white matter lesions and atrophy. It needs to be established whether similar changes to the BBB also occur in the other MPS disorders.

It should be noted that brain abnormalities have also been described for MPS types that are not typically associated with neurocognitive impairment. For example, dilated perivascular spaces and white matter lesions, not correlated with intellectual quotient scores, have been described in patients with MPS VI [24]. A brain MRI study in nine MPS IVA patients showed non-specific white matter changes in two patients and other abnormalities in seven patients, despite preserved cognition [41].

3. Cellular changes associated with storage of GAGs and gangliosides in MPS disorders

Information on neuropathological findings in patients with MPS is limited. Post-mortem histochemical and ultrastructural examination of brain tissue from MPS I, II, III, and VII patients showed neuronal abnormalities and gliosis throughout the brain resulting from lysosomal storage, with varying severity among anatomical brain regions [16, 33, 42]. Excessive storage materials in the brain have been found to result in enlargement of Purkinje cell dendrites in the cerebellum, neuronal cytoplasm distention (Fig. 2A and B), and development of

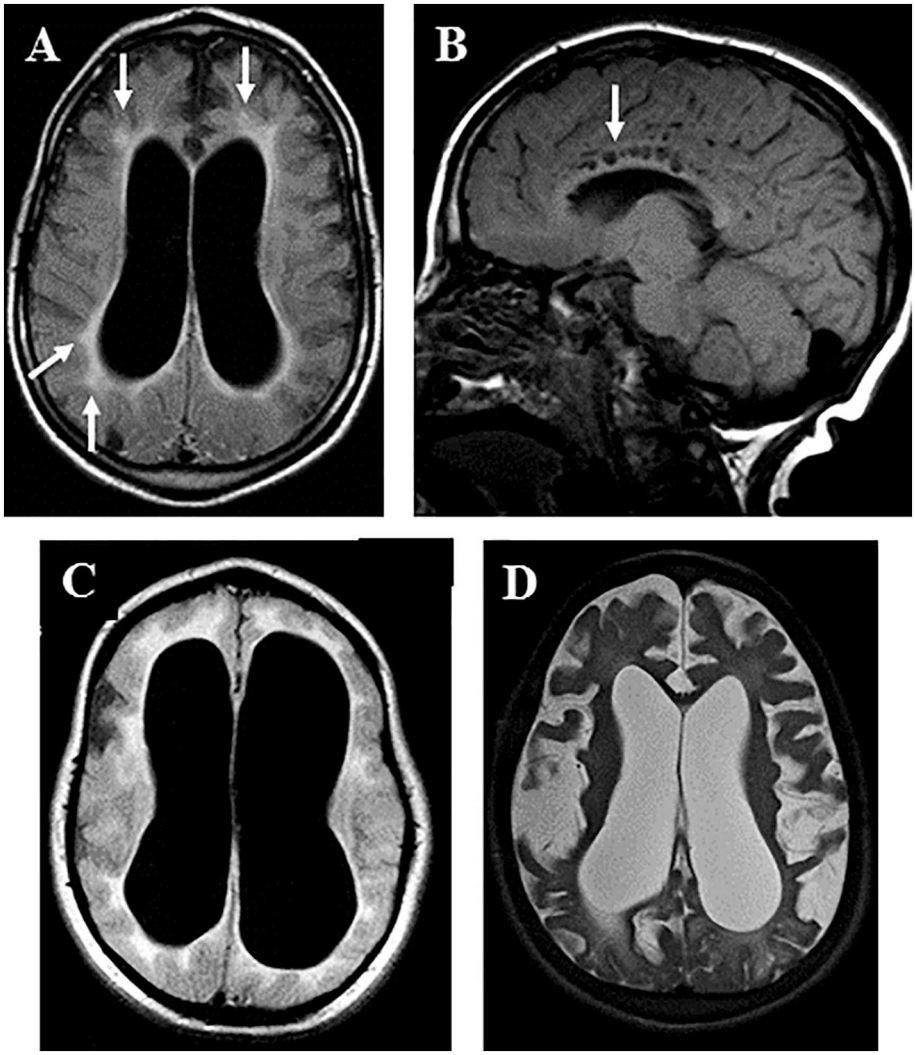

Fig. 1. Anatomical changes in the brain of MPS patients: (A) moderate white matter lesions in the periventricular white matter (arrows) and moderate ventriculomegaly; (B) enlarged perivascular spaces in the corpus callosum (arrow); (C) hydrocephalus; (D) brain atrophy (Reproduced from Nestrasil & Vedolin, 2017 [33] with permission from Elsevier).

'meganeurites' in the cerebral cortex, diencephalon, and brain stem [17, 30, 33, 35, 43–46]. Neuronal GAG accumulation, stained positive with toluidine blue [16] and ultrastructurally visible as clear vacuoles, abundantly occurs in Purkinje cells of the cerebellum, but is limited in the cerebral cortex, brain stem, and diencephalon of MPS I, II, and III patients [16, 29, 42]. Overall, neuronal cytoplasm is typically distended by material that stains with Sudan dyes and Periodic acid–Schiff (PAS), indicative of polysaccharides that may consist of GAGs, gangliosides, other polysaccharide sugars, and glycolipids [17, 29, 30, 46]. This is ultrastructurally visible as lamellated cytoplasmatic inclusions, such as concentric membranous bodies, zebra bodies [29, 30, 46], and dense granular material [16, 17, 30, 44, 46, 47]. In MPS I and II patients, Purkinje cells mostly contain concentric membranous and zebra bodies, while in MPS III dense granular material is present as well [44]. In addition, around 20% of neurons show signs of degeneration in patients, probably resulting in the reduced neuron density observed in several studies [30, 33, 42, 44, 48]. GAG and ganglioside levels in the brain of MPS I, II, and III patients [1, 35, 45, 49, 50] are up to 6 times greater than in healthy subjects [16, 30, 45]. Gangliosides may be produced as a secondary storage mechanism [45]. Mouse models of MPS I, II, MPS IIIA, IIIB, and IIIC all show abnormal behaviors, either cognitive in the case of MPS I [51], MPS II [52], MPS IIIA [53], and IIIC [54], or hyperactivity in the case of MPS IIIA, IIIB, and IIIC [53–55]. Using HS disaccharides fluorescently labelled with 2-aminoacridone and quantified with reversed phase high-performance liquid chromatography (RP-HPLC) against known HS standards, it has been shown that MPSI, II, IIIA, IIIB, and IIIC (personal communication Dr. B. Bigger) mice also show a 5–25 fold elevated storage of HS in the brain, with additional storage of monosialic gangliosides GM2 and GM3 [56–58]. Notably, neuronal loss is not usually seen in mouse models of disease [54, 56]. A direct link of accumulation of HS or gangliosides with cognitive impairment remains to be established.

Gliosis, cellular as well as fibrous (Fig. 2C), is often associated with neuronal abnormalities in patients [16, 33]. Varying degrees of astrogliosis and highly vacuolated macrophages/microglia [16, 29, 30, 33, 46] have been observed in the brains of MPS I, II, and III patients. Vacuolated macrophages/microglia are often also present in the meninges and dilated PVS [16, 17, 29]. In contrast to the neurons, glial cells mainly contain electron-lucent storage material [54], suggesting predominantly GAG storage in these cells. Lamellar inclusions can also be observed in glial cells, pericytes, and endothelial cells, although to a lesser extent than in neurons [16, 17, 30, 47]. Mouse models also demonstrate marked astrocytosis as shown by increasing numbers and distention of glial fibrillary acidic protein (GFAP) positive cells and microgliosis as shown by shortening of processes, thickening of cell bodies and presence of isolectin B4 or increased intensity of CD11b/

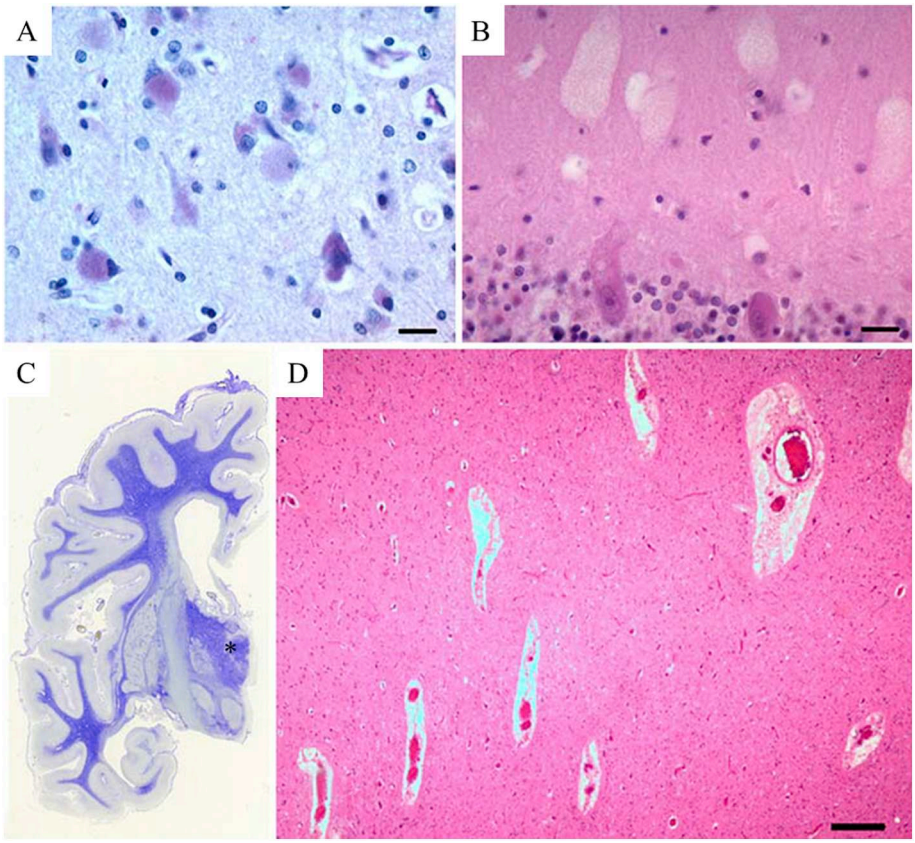

Fig. 2. Characteristic neuropathological changes in the brain of MPS patients with CNS involvement. (A) Neuronal swelling in the cerebral cortex, (B) dendritic swelling of Purkinje cells in the cerebellum, and (C) fibrous gliosis in the thalamus of MPS IIIB patients (asterisk). (D) Dilatation of the PVS in the white matter of an MPS II patient (Reproduced from Hamano et al., 2008 [3] with permission from Springer Science + Business Media).
A: periodic acid-Schiff staining; B and D: hematoxylin and eosin staining; C: Holzer staining. Scale bar in A and B = 20 μm; scale bar in D = 200 μm.
CNS: central nervous system; MPS: mucopolysaccharidosis; PVS: periventricular space.

CD68/Iba1 staining [54, 56].

GAG deposition around the cerebral vessels can result in dilated PVS (Fig. 2D) [36]. Moreover, GAG accumulation can also affect the cellular components of the BBB. Storage of GAGs and lamellated inclusions [16, 30] have been observed in endothelial cells and pericytes (personal communication Dr. D.J. Begley) of the BBB in MPS III patients [40]. Moreover, capillaries in the hippocampus, putamen, and cerebellum of MPS IIIA and D patients showed cell swelling, cell degeneration, perivascular edema, and accumulation of collagen, as well as varying reductions in tight junction proteins [40].

4. Neuropathological mechanisms triggered by altered GAG metabolism

Several animal models for MPS I, II, III, and VII are currently available [59], showing histochemical and ultrastructural changes in the CNS similar to those seen in human post-mortem tissues [43, 48, 54, 56, 60–70]. Particularly MPS mouse models have proven extremely valuable for pathogenesis studies. These animals have the advantage of being available in well-characterized strains, which can be bred easily and compared to unaffected control animals with the same genetic background [59]. Together with studies in humans, animal models have contributed to a better understanding of the neuropathological mechanisms underlying CNS abnormalities in MPS. In addition, they provide some explanation for the considerable differences in clinical presentations of MPS I, II, III, and VII, despite similar primary storage material (HS).

HS proteoglycans are involved in a wide variety of physiological processes [71]. At non-lysosomal sites, such as the cell surface and in the extracellular matrix, they play an essential role in cell signaling, distribution of growth factors, cytokines, and morphogens, and can influence cell motility and adhesion [71]. Excessive HS levels and abnormal sulfation in the CNS of MPS I, II, III, and VII patients may alter HS-dependent signaling [72], and trigger inflammation [69]; this could change intracellular processes and neurotransmission, which in turn affect CNS functioning [73]. This section will describe the pathological mechanisms that might be involved in the MPS-associated CNS abnormalities.

4.1. Altered cell signaling

The primary consequence of deficient HS degradation is the production and accumulation of HS oligosaccharide fragments [4, 16, 74], which may trigger several pathological mechanisms. Analysis of the MPS IIIA mouse brain showed the presence of a variety of oligosaccharides, ranging from di- to hexasaccharides [75]. In addition, a cell- and domain-specific distribution pattern for HS forms has been observed in the MPS IIIB mouse brain [76], although it should be cautioned that the phage display antibodies used in the latter study can be unreliable on repeated application as they recognize gross HS charged structures rather than specific epitopes. Significantly increased amounts and sulfation patterning of HS (Fig. 3) have been observed in brains of MPS I, IIIA, and IIIB mice [56, 73], as well as in MPS II (personal communication Dr. B. Bigger). and MPS IIIC [57]. In humans with MPS I, II, and III, studies have shown higher HS saccharide levels in urine, blood, and cultured skin fibroblasts from patients with more severe signs of CNS disease, such as cognitive decline and loss of speech [4, 77, 78].

Studies in mice and cell cultures have suggested that abnormal sulfation and size of HS fragments can affect the binding capacity of growth factors or morphogens and consequently alter neuronal functioning [73, 79]. For example, HS usually acts as a co-receptor in fibroblast growth factor (FGF) signaling [80]. Pathogenic HS in MPSI cannot properly interact with FGF-2 and the FGF receptor, thus disturbing neuronal proliferation and survival [81], which may contribute to the reduced development of neural progenitor cells and neuroplasticity in MPS [70, 82]. In addition, dysregulation of growth factor signaling has been suggested to increase glial cell death, which preceded

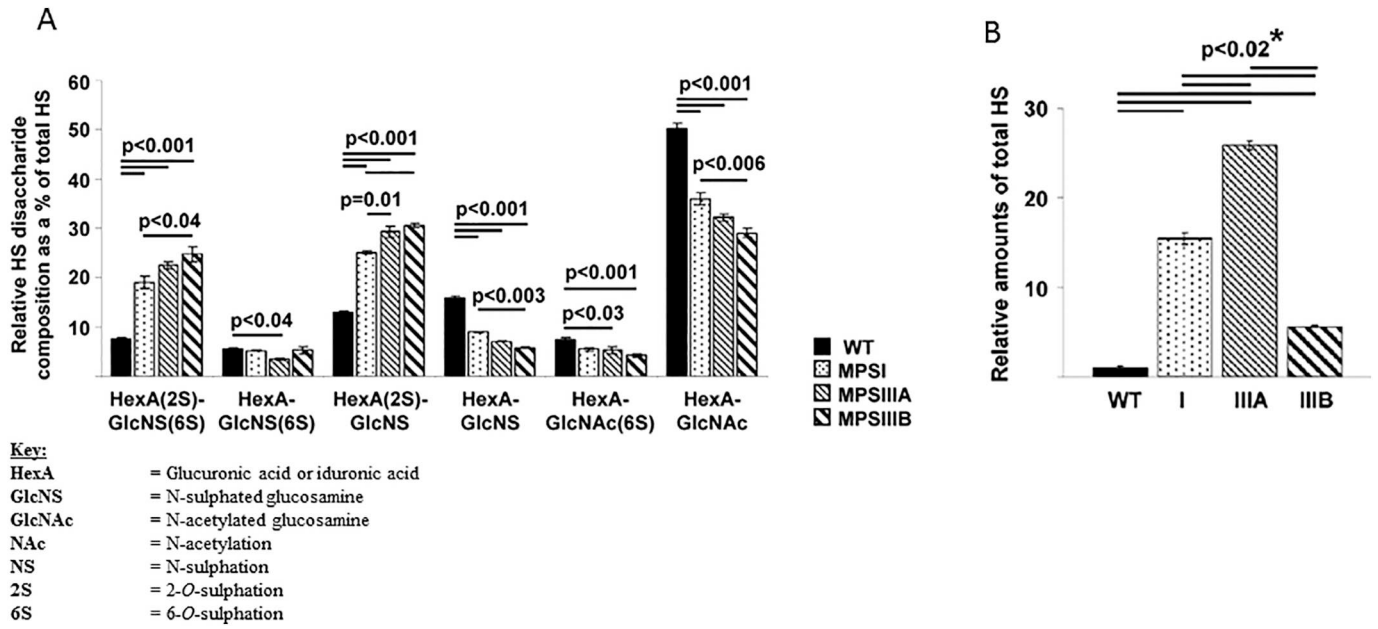

Fig. 3. (A) The amount and sulfation of HS and (B) relative amounts of total HS are increased in brains of MPS I, IIIA, and IIIB mice (Reproduced from Wilkinson et al., 2012 [56] with permission of Public Library of Science).
HS: heparan sulfate; MPS: mucopolysaccharidosis; WT: wild type.

neuronal degeneration in an MPS II mouse model [48]. Moreover, abnormally 2-*O*-sulfated HS in MPS I has been shown to reduce CXCL12-mediated migration of hematopoietic stem and progenitor cells after transplant [72] by sequestering CXCL12. Since all chemokines and cytokines likely have an HS binding site, this probably has an effect on chemokine and cytokine signaling in the brain. Partially degraded HS saccharides have been shown to enhance integrin-based focal adhesion activation of astrocytes and neural stem cells in MPS IIIB mice. This disturbed cell polarization and directed migration in the brain can affect cell plasticity and cell behavior during adult neurogenesis and induce neuropathological changes associated with MPS III [74].

Although MPS I, II, III, and VII are all associated with accumulation of HS, their phenotypical presentation and severity of neurological symptoms differ considerably. Behavioral abnormalities, for example, are more obvious in MPS II and III than in MPS I patients [12]. These differences might be due to differences in location and/or amount of GAG accumulation [16, 45] as well as disparities in GAG chain length, sulfation patterning, or chemical modifications at the non-reducing terminus of the partially degraded HS species [56, 76, 83]. In MPS II, partially degraded HS has sulfate moieties bound directly to the carbohydrate backbone, while in MPS III these are either *N*-sulfate (MPS IIIA), amino (MPS IIIC), or *N*-acetyl (MPS IIIB and D) moieties [3]. Węgrzyn et al. hypothesized that these modifications interfere differently with neuronal function, leading to the differences in behavioral problems [3]; however it is more likely to be an indirect effect of HS-protein interactions and downstream signaling pathways.

4.2. Pathological cascades triggered by primary storage

4.2.1. Neuroinflammation

A neuroinflammatory response, characterized by astrocyte and microglial cell activation and increased levels of inflammatory cytokines (including monocyte chemoattractant proteins, macrophage inflammatory protein, and interleukin-1α), has been observed in the brain of MPS I and III mouse models [48, 54, 56, 68, 84–86]. However, levels and types of increased inflammatory cytokines vary between MPS I, IIIA, IIIB, and IIIC mice. While monocyte chemoattractant protein and interleukin-1α are equally increased in all three disorders, macrophage inflammatory protein is much more elevated in MPS III [54, 56]. This could contribute to the differences in behavioral abnormalities.

Neuroinflammation may be caused by intrinsic cell mechanisms and/or by a response to neuronal abnormalities. Extracellular HS oligosaccharides have been shown to activate microglia through interaction with the toll-like receptor (TLR) 4 and adaptor protein MyD88 in MPS IIIB mice [69], which triggers production and release of pro-inflammatory cytokines and reactive oxygen species. Inactivation of this TLR4/MyD88 pathway suppresses early-onset neuroinflammation as seen by microgliosis, while astrocytosis remains unchanged, but does not reduce expression of disease markers like GAP43, GM2, and GM3, indicating that neurodegeneration was not delayed [69]. Notably, neuroinflammation was not suppressed over several months in double knockout mice, suggesting that inflammation in MPSIII at least, is not solely driven through the TLR4/MyD88 pathway and may be initiated via multiple damage-associated molecular patterns (DAMP)-mediated innate pathways [87]. Hence, although astrocyte and microglial cell activation have been shown to precede onset of clinical signs and neuronal loss in MPS III animal models [54, 61], it is not clear what role neuroinflammation has in disease pathology at this stage.

4.2.2. Oxidative stress

Lysosomes play a crucial role in the degradation and recycling of intra- and extracellular material. For example, they sequester intracellular material, such as mitochondria and poly-ubiquitinated proteins, through autophagy. Hence, accumulation of GAGs could lead to secondary storage of these intracellular materials [88, 89]. Abnormal mitochondrial numbers and morphology, and compromised energy metabolism have been observed in brain cells of MPS IIIC mice [54, 90]. Mitochondria are a major source of intracellular reactive oxygen species. Therefore, mitochondrial abnormalities could result in oxidative stress. Elevated mRNA levels for oxidative stress markers [54, 84–86, 91] and measures of superoxide ion production and oxidative products indicate that oxidative stress is present in the brain of MPS I and III mice [85, 90, 92, 93]. Accumulation of oxidative products and oxidative imbalance have also been reported for patients with MPS I, II, and IIIB [33, 94]. Oxidative stress can damage several molecules and activate deleterious pathways [95], which may contribute to MPS-associated neurodegeneration.

As experience from other disorders shows that activated microglia

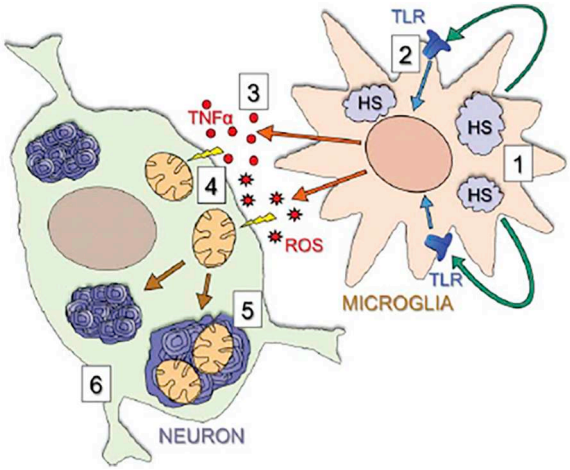

Fig. 4. Proposed mechanism underlying CNS involvement in MPS IIIC mice. 1) Accumulation of HS and HS-derived oligosaccharides accumulate in microglial cells. 2) Release of this material triggers 3) a TLR-induced inflammatory response. 4) The inflammatory mediators cause mitochondrial damage and, 5) together with the primary storage, block autophagy, and lead to accumulation of gangliosides and misfolded proteins in the neurons, 6) which finally trigger neuronal cell death. (Reproduced from Pshezhetsky et al., 2015 [90] with permission from Taylor & Francis).
CNS: central nervous system; HS: heparan sulfate; MPS: mucopolysaccharidosis; ROS: reactive oxygen species; TLR: toll-like receptor; TNFα: tumor necrosis factor alpha.

have the ability to induce mitochondrial damage through production of reactive oxygen species and oxidative stress [96, 97], a similar mechanism has been proposed for MPS IIIC as well (Fig. 4). However, Trudel et al. showed development of oxidative stress in MPS IIIB mice independent of TLR4-induced inflammation [85], suggesting that oxidative imbalance could be directly caused by HS storage rather than a secondary consequence of neuroinflammation.

4.2.3. Secondary accumulation

Accumulation of GAGs has been suggested to inhibit ganglioside degrading enzymes [49, 98], leading to the observed secondary accumulation of GM2 and GM3 gangliosides in the MPS disorders [16, 17, 30, 45, 47, 54, 56, 68, 99]. Abnormal storage of gangliosides has been observed in several brain regions of MPS I and III patients [45] and in animal models [54, 56, 99], including the cerebral cortex and hippocampus. Gangliosides can influence dendritogenesis during development. Therefore, it has been suggested that accumulation of gangliosides can trigger the changes in dendrite and axon morphology seen in MPS patients, leading to synaptic dysfunction [47, 98, 100]. In addition, GM2 ganglioside accumulation has been suggested to trigger neuronal cell death in the brain of Tay-Sachs and Sandhoff patients [101]. This mechanism may also underlie neurodegeneration in MPS patients (Fig. 4).

Several other substances have also been shown to accumulate in neurons of MPS I, II, and III mice. Hyper-phosphorylated tau protein was detected in the medial entorhinal cortex (MEC) and dentate gyrus of MPS IIIB mice, as well as beta amyloid [91, 102]. Accumulation of subunit c of mitochondrial ATP synthase (SCMAS) has been observed in MPS IIIB and IIIC mice, predominantly in the MEC and the somatosensory cortex [54, 90, 103]. Increased levels of glypicans, markers for metabolic stress, and proteins involved in autophagy (such as LC3) have also been observed in the MEC of MPS mice models [54, 91]. Glypican 1, SCMAS, and LC3 are much more abundant in the MEC of MPS IIIA and IIIB mice than in the MEC of MPS I and II mice [91], which could contribute to the differences in disease phenotypes. The increased levels of protein aggregates observed in MPS mice suggest inadequate lysosomal and/or proteasomal proteolysis, which could also lead to neurodegeneration (Fig. 4) [33, 91, 102]. Ohmi et al. suggested that the increase in glypican is crucial for these secondary defects in the MEC of MPS III mice. The proteoglycan glypican is the precursor of the glycan HS and might be metabolized differently in MPS III compared to MPS I and II brain [91].

4.3. Defective neurotransmission

As abnormal behavior and neuronal dysfunction are present in MPS III animals before loss of neurons is observed, they are presumed to be caused by defects in neurotransmission [54, 61, 104]. Decreased levels of pre- and post-synaptic proteins, in both inhibitory and excitatory synapses, have been reported in the cerebral cortex of MPS I and III mice [56, 105] and the hippocampus of MPS IIIC mice [106] as early as 10 days postnatally. In the hippocampal neurons of MPS IIIC mice, this decrease is associated with a decrease in synaptic spine density and altered morphology [106], while in the cortex of MPS IIIB mice, synapse density was reported to remain unaffected [105]. A study in MPS IIIB mice showed that the decrease in synaptophysin, the most abundant synaptic vesicle membrane protein, in the cortex is caused by HS oligosaccharide-induced degradation by the proteasome [105]. These findings of synaptic disorganization suggest that deficiencies in synaptic signaling [56, 106], including pre-synaptic and post-synaptic vesicular density affect neurotransmission, leading to behavior and cognitive deficits.

5. Pathophysiological biomarkers predicting neurodegeneration and treatment efficacy

There are currently no established objective means that can accurately predict clinical course and treatment effect in MPS patients. Classical diagnostic tests that measure GAG levels in urine might be used to some degree as biomarkers of disease severity, but are subject to several limitations ([1, 2, 107] and personal communication Dr. B. Bigger). While dye binding assays (dimethylmethylene blue or alcian blue) are not GAG-specific, qualitative tests using 2D chromatography can distinguish between different GAGs, but are time-consuming and give limited quantitative information. Moreover, urinary GAG levels can change with age and hydration status.

A number of other methods have been developed to quantify specific GAGs in urine, blood, tissues, or cells of MPS patients in an attempt to predict disease progression and therapeutic efficacy. Several groups used HPLC and mass spectrometry (MS) analysis to identify and measure levels of specific GAG disaccharide or trisaccharide units, with specific abnormal terminal end structures [73, 78, 108, 109]. Since abnormal sulfation patterns affect neuronal functioning, these patterns could in theory function as biomarkers for CNS disease (progression). Indeed, Fuller et al. showed that a ratio of α-L-iduronidase activity to two HS- and DS-derived trisaccharides provides a clear distinction between healthy controls, MPS I patients with CNS involvement, and MPS I patients without CNS involvement [78]. However, these techniques are complex, expensive, and require HPLC and MS instruments dedicated to carbohydrates. The ratio of urinary DS to chondroitin sulfate has also been suggested as a biomarker for disease progression and long-term treatment outcomes in MPS IH and II patients [110–113]. This ratio can be measured by means of 2D-electrophoresis and/or liquid chromatography tandem-mass spectrometry. The serum level of the GAG-regulated heparin cofactor II-thrombin (HCIIT) complex has been identified as a surrogate DS marker and a potential biomarker for MPS I, II, and VII. The concentration of this complex has been shown to correlate with disease severity and to quickly respond to treatment in MPS IH and II patients [112–116]. However, both biomarkers are unlikely to predict CNS disease or progression as their accumulation is measured in the urine or serum and not in the CNS and HCIIT responds primarily to DS and not HS storage [116].

The CNS and CSF could theoretically be a good source to measure

primary or secondary biomarkers for MPS disorders associated with neurodegeneration. Primary storage material (HS) in brain tissue, measured by direct or indirect methods, appears to be a relatively reliable biomarker in MPS mouse models. However, HS in CSF, which is typically the only source available from patients, may be less predictive as it is unclear how HS moves from the brain into the CSF compartment. Inflammatory cytokines, such as monocyte chemoattractant protein, macrophage inflammatory protein, and interleukin-1α [48, 54, 56, 68, 84–86, 117, 118], could also potentially be measured in the CSF. However, exposure to infectious agents or inflammation during disease could introduce variability in these CSF biomarkers. Moreover, as they can cross the BBB, they may move in and out of the CNS and CSF, which may lead to under- or overestimation of treatment effect. Measurement of lysosomal swelling, inflammation, proteasomal function, and oxidative stress would all require analysis of tissue [56]. Although CSF biomarkers may be predictive of the further disease course of MPS patients, they cannot identify neurodegeneration. To date, no available CSF biomarker can predict disease progression in a prospective manner.

Finally, some neuroimaging markers might be able to measure CNS involvement and treatment-related changes in the CNS of MPS patients. For example, the myo-inositol/creatine ratio, measured with magnetic resonance spectroscopy, has been shown to correlate with CNS disease in MPS II patients [19]. Myo-inositol functions as an osmolyte in the CNS; increased levels could relate to the increased volume of cells in MPS brains with CNS involvement [119]. In addition, the myo-inositol/creatine ratio has been suggested as a marker for astrogliosis [120–122], which could function as a biomarker for GAG-induced glial changes and neuroinflammation. Recent research also points to the potential utility of volumetric magnetic resonance imaging changes in gray matter and ventricular volume as biomarkers of CNS involvement in patients with MPS.

6. Discussion and conclusions

Accumulation of undegraded and partially degraded HS leads to a variety of secondary changes in the homeostasis of the brain of patients with MPS I, II, III, and VII, although it remains unclear if HS is directly responsible or is a bystander to secondary processes. Although different pathophysiological mechanisms have been investigated, currently available information is mostly based on animal models and the results do not allow identification of a single mechanism that can explain all the different CNS alterations observed in MPS. What is clear, is that in DS storage diseases such as MPS VI, despite similar secondary storage accumulation, global pathology and inflammation, there is no evidence of neurological disease, whereas in severe HS storage diseases, such as MPS III, cognitive deficits are typically present. If HS truly is at the heart of neurological disease pathology, how do we explain the different cognitive symptoms in MPS I and, for example, MPS III patients? Is this due to different HS species, or could it be another mechanism? Research to test the pathogenicity of specific GAG degradation fragments would be of benefit given a suitable assay. Given the wide variety of processes involving HS, changes in different pathways may interact with each other. Full transcriptomic and particularly proteomic signatures of diseased brain may go some way to understanding what storage substrates we are missing. In the past, transcriptomics have focused on total brain tissue; this has the disadvantage of combining the transcriptomics of several cell types. Increases in GFAP and monocyte chemoattractant protein 1 (MCP1), for example, that are typically two of the top hits described, are almost certainly just manifestations of proliferation of astrocytes and microglia respectively, rather than reflecting upregulation of these genes in neurons. We also need to consider linking neuroimaging outcomes with biomarker discovery. MRI appears to have limitations in defining cognitive changes in MPS diseases, due to the slow progression of the disease, except where neuronal loss has progressed to late stage, at which point it is largely irrelevant. MR spectroscopy could be of interest and positron emission tomography (PET) imaging may have a role to play, but the latter will require novel tracer development, as many of the current tracers available are inappropriate, which is a costly undertaking. Some pathological processes in MPS, such as lysosomal destabilization due to accumulation of large molecules, have also been described for other lysosomal storage disorders. Lysosomal destabilization may lead to deficiencies in some of the basic functions of lysosomes, such as autophagy, which in turn may induce secondary abnormalities such as compromised energy metabolism by mitochondria. Further studies are necessary to determine the sequence and interaction between pathophysiological mechanisms involved in neurological disease in MPS. This requires more specific transcriptomic studies of cell subtypes in the brain and elucidation of specifically altered pathways, ideally with transgenic mice. The question is whether there are specific brain areas that are most important to preserve in MPS disease. This research is hampered by the cross correctional ability of most of the enzymes, thus conditional knockouts in specific cell types have been of limited use to date. Membrane bound enzymes such as Heparan-α-glucosaminide *N*-acetyltransferase (HGSNAT) or those lacking secretional ability offer an approach to testing this.

Although specific molecules of the pathophysiological cascade have been suggested as useful biomarkers, there is currently no biomarker that allows for identification and monitoring of neurodegeneration in MPS disorders. While specific primary HS substrate levels may represent useful markers of response to treatment, they generally only give information about neurological disease when they are sampled directly from the brain, rather than the CSF, which is currently unfeasible in patients. Inflammatory biomarkers may prove to be variable in patient populations. Nonetheless, as there is clearly dysregulation of inflammation, both innate and adaptive, in MPS disease, an immune based biomarker could prove of some use in determining the effect of treatment on the brain, especially since inflammation has been linked to cognition in several neurodegenerative diseases. More research is therefore needed to identify other biomarkers that specifically provide information on the CNS.

Financial disclosures

Dr. Bigger is a shareholder and SAB member of Orchard Therapeutics and Phoenix Nest and received honoraria and grant support from Orchard Therapeutics, BioMarin as well as several MPS charities including the UK Society for Mucopolysaccharide Diseases;

Dr. Begley has received grant support and honoraria from Actelion, BioMarin Pharmaceutical Inc., Brains for Brain Onlus, Genzyme, GlaxoSmithKline, Hadley Hope Fund, Shire HGT, UK Engineering and Physical Science Research Council, UK Gauchers Association, UK Society for Mucopolysaccharide Diseases, Spanish Society for Mucopolysaccharide Diseases, Syngeneva, The Addi and Cassi Fund, and the European Union;

Dr. Virgintino received funding from the Foundation Puglia 2015;

Dr. Pshezhetsky received educational grants, travel grants, and honoraria from Genzyme/Sanofi, BioMarin, Alexion Pharma, Canadian Institutes of Health Research, JJB Foundation, and JLK Foundation.

Funding

This work was supported by BioMarin Pharmaceutical Inc.

Acknowledgements

The authors are grateful to Ismar Healthcare NV for their assistance in the writing of this manuscript, which was funded by BioMarin Pharmaceutical Inc. The expert meeting in Stockholm was also sponsored by BioMarin Pharmaceutical Inc.

References

[1] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill Medical Publishing Division, New York, 2001, pp. 3421–3452.

[2] J. Muenzer, Overview of the mucopolysaccharidoses, Rheumatology (Oxford) 50 (Suppl. 5) (2011) v4–v12.

[3] G. Węgrzyn, J. Jakóbkiewicz-Banecka, M. Narajczyk, A. Wiśniewski, E. Piotrowska, M. Gabig-Cimińska, A. Kloska, M. Słomińska-Wojewódzka, A. Korzon-Burakowska, A. Węgrzyn, Why are behaviors of children suffering from various neuronopathic types of mucopolysaccharidoses different? Med. Hypotheses 75 (2010) 605–609.

[4] G.V. Coppa, O. Gabrielli, L. Zampini, F. Maccari, V. Mantovani, T. Galeazzi, L. Santoro, L. Padella, R.L. Marchesiello, F. Galeotti, N. Volpi, Mental retardation in mucopolysaccharidoses correlates with high molecular weight urinary heparan sulphate derived glucosamine, Metab. Brain Dis. 30 (2015) 1343–1348.

[5] D.I. Zafeiriou, S.P. Batzios, Brain and spinal MR imaging findings in mucopolysaccharidoses: a review AJNR, Am. J. Neuroradiol. 34 (2013) 5–13.

[6] J. Muenzer, The mucopolysaccharidoses: a heterogeneous group of disorders with variable pediatric presentations, J. Pediatr. 144 (5 Suppl) (2004) S27–S34.

[7] M.C.O. Bax, G.A. Colville, Behaviour in mucopolysaccharide disorders, Arch. Dis. Child. 73 (1995) 77–81.

[8] J.A. Gilkes, C.D. Heldermon, Mucopolysaccharidosis III (Sanfilippo Syndrome)-disease presentation and experimental therapies, Pediatr. Endocrinol. Rev. 12 (Suppl. 1) (2014) 133–140.

[9] J.B. Holt, M.D. Poe, M.L. Escolar, Natural progression of neurological disease in mucopolysaccharidosis type II, Pediatrics 127 (2011) e1258–e1265.

[10] E.G. Shapiro, I. Nestrasil, K. Rudser, K. Delaney, V. Kovac, A. Ahmed, B. Yund, P.J. Orchard, J. Eisengart, G.R. Niklason, J. Raiman, E. Mamak, M.J. Cowan, M. Bailey-Olson, P. Harmatz, S.P. Shankar, S. Cagle, N. Ali, R.D. Steiner, J. Wozniak, K.O. Lim, C.B. Whitley, Neurocognition across the spectrum of mucopolysaccharidosis type I: age, severity, and treatment, Mol. Genet. Metab. 116 (2015) 61–68.

[11] E.G. Shapiro, L.A. Lockman, M. Balthazor, W. Krivit, Neuropsychological outcomes of several storage diseases with and without bone marrow transplantation, J. Inherit. Metab. Dis. 18 (1995) 413–429.

[12] E.G. Shapiro, S.A. Jones, M.L. Escolar, Developmental and behavioral aspects of mucopolysaccharidoses with brain manifestations - neurological signs and symptoms, Mol. Genet. Metab. 122S (2017) 1–7.

[13] E.G. Shapiro, K. Rudser, A. Ahmed, R.D. Steiner, K.A. Delaney, B. Yund, K. King, A. Kunin-Batson, J. Eisengart, C.B. Whitley, A longitudinal study of emotional adjustment, quality of life and adaptive function in attenuated MPS II, Mol. Genet. Metab. Rep. 7 (2016) 32–39.

[14] B. Yund, K. Rudser, A. Ahmed, V. Kovac, I. Nestrasil, J. Raiman, E. Mamak, P. Harmatz, R. Steiner, H. Lau, P. Vekaria, J.R. Wozniak, K.O. Lim, K. Delaney, C. Whitley, E.G. Shapiro, Cognitive, medical, and neuroimaging characteristics of attenuated mucopolysaccharidosis type II, Mol. Genet. Metab. 114 (2015) 170–177.

[15] M. Beck, P. Arn, R. Giugliani, J. Muenzer, T. Okuyama, J. Taylor, S. Fallet, The natural history of MPS I: global perspectives from the MPS I Registry, Genet. Med. 16 (2014) 759–765.

[16] A.S. Dekaban, G. Constantopoulos, Mucopolysaccharidosis type I, II, IIIA and V. Pathological and biochemical abnormalities in the neural and mesenchymal elements of the brain, Acta Neuropathol. 39 (1977) 1–7.

[17] C. Vogler, B. Levy, J.W. Kyle, W.S. Sly, J. Williamson, M.P. Whyte, Mucopolysaccharidosis VII: postmortem biochemical and pathological findings in a young adult with β-glucuronidase deficiency, Mod. Pathol. 7 (1994) 132–137.

[18] I. Nestrasil, L. Vedolin, Quantitative neuroimaging in mucopolysaccharidoses clinical trials, Mol. Genet. Metab. 122S (2017) 17–24.

[19] L. Vedolin, I.V.D. Schwartz, M. Komlos, A. Schuch, A.C. Puga, L.L.C. Pinto, A.P. Pires, R. Giugliani, Correlation of MR imaging and MR spectroscopy findings with cognitive impairment in mucopolysaccharidosis II AJNR, Am. J. Neuroradiol. 28 (2007) 1029–1033.

[20] L. Vedolin, I.V.D. Schwartz, M. Komlos, A. Schuch, A.C. Azevedo, T. Vieira, F.K. Maeda, A.M. Marques Da Silva, R. Giugliani, Brain MRI in mucopolysaccharidosis: effect of aging and correlation with biochemical findings, Neurology 69 (2007) 917–924.

[21] C. Lee, T.E. Dineen, M. Brack, J.E. Kirsch, V.M. Runge, The mucopolysaccharidoses: characterization by cranial MR imaging AJNR, Am. J. Neuroradiol. 14 (1993) 1285–1292.

[22] M.G. Matheus, M. Castillo, J.K. Smith, D. Armao, D. Towle, J. Muenzer, Brain MRI findings in patients with mucopolysaccharidosis types I and II and mild clinical presentation, Neuroradiology 46 (2004) 666–672.

[23] M.V. Kulkarni, J.C. Williams, J.W. Yeakley, J.L. Andrews, C.B. McArdle, P.A. Narayana, R.R. Howell, A.J. Jonas, Magnetic resonance imaging in the diagnosis of the cranio-cervical manifestations of the mucopolysaccharidoses, Magn. Reson. Imaging 5 (1987) 317–323.

[24] A.C.M. Azevedo, O. Artigalás, L. Vedolin, M. Komlós, A. Pires, R. Giugliani, I.V.D. Schwartz, Brain magnetic resonance imaging findings in patients with mucopolysaccharidosis VI, J. Inherit. Metab. Dis. 36 (2013) 357–362.

[25] T. Seto, K. Kono, K. Morimoto, Y. Inoue, H. Shintaku, H. Hattori, O. Matsuoka, T. Yamano, A. Tanaka, Brain magnetic resonance imaging in 23 patients with mucopolysaccharidoses and the effect of bone marrow transplantation, Ann. Neurol. 50 (2001) 79–92.

[26] O. Gabrielli, G. Polonara, L. Regnicolo, V. Petroni, T. Scarabino, G.V. Coppa, U. Salvolini, Correlation between cerebral MRI abnormalities and mental retardation in patients with mucopolysaccharidoses, Am. J. Med. Genet. A 125A (2004) 224–231.

[27] Z. Fan, M. Styner, J. Muenzer, M. Poe, M. Escolar, Correlation of automated volumetric analysis of brain MR imaging with cognitive impairment in a natural history study of mucopolysaccharidosis II AJNR, Am. J. Neuroradiol. 31 (2010) 1319–1323.

[28] E. Alqahtani, T.A.G.M. Huisman, E. Boltshauser, I. Scheer, T. Güngör, A. Tekes, G.H. Maegawa, A. Poretti, Mucopolysaccharidoses type I and II: new neuroimaging findings in the cerebellum, Eur. J. Paediatr. Neurol. 18 (2014) 211–217.

[29] M.Z. Jones, J. Alroy, J.C. Rutledge, J.W. Taylor, E.C. Alvord Jr., J. Toone, D. Applegarth, J.J. Hopwood, E. Skutelsky, C. Ianelli, D. Thorley-Lawson, C. Mitchell-Herpolsheimer, A. Arias, P. Sharp, W. Evans, D. Sillence, K.T. Cavanagh, Human mucopolysaccharidosis IIID: clinical, biochemical, morphological and immunohistochemical characteristics, J. Neuropathol. Exp. Neurol. 56 (1997) 1158–1167.

[30] M.G. Hadfield, N.R. Ghatak, I. Nakoneczna, H.R. Lippman, E.C. Myer, G. Constantopoulos, R.M. Bradley, Pathologic findings in mucopolysaccharidosis type IIIB (Sanfilippo's sydnrome B), Arch. Neurol. 37 (1980) 645–650.

[31] B. Lach, M.D. Haust, Nodular lesions of choroid plexus in Hurler disease, Fetal. Pediatr. Pathol. 30 (2011) 189–198.

[32] C.B. Whitley, M. Cleary, E.K. Mengel, P. Harmatz, E. Shapiro, I. Nestrasil, P. Haslett, D. Whiteman, D. Alexanderian, Observational prospective natural history of patients with Sanfilippo syndrome type B, J. Pediatr. 197 (2018) 198–206.

[33] K. Hamano, M. Hayashi, K. Shioda, R. Fukatsu, S. Mizutani, Mechanisms of neurodegeneration in mucopolysaccharidoses II and IIIB: analysis of human brain tissue, Acta Neuropathol. 115 (2008) 547–559.

[34] K. Nagashima, H. Endo, K. Sakakibara, Y. Konishi, K. Miyachi, J.J. Wey, Y. Suzuki, J. Onisawa, Morphological and biochemical studies of a case of mucopolysaccharidosis II (Hunter's syndrome), Acta Pathol. Jpn. 26 (1976) 115–132.

[35] A.S. Dekaban, V.M. Patton, Hurler's and Sanfilippo's variants of mucopolysaccharidosis. Cerebral pathology and lipid chemistry, Arch. Pathol. 91 (1971) 434–443.

[36] V.J. Parsons, D.G. Hughes, J.E. Wraith, Magnetic resonance imaging of the brain, neck and cervical spine in mild Hunter's syndrome (mucopolysaccharidoses type II), Clin. Radiol. 51 (1996) 719–723.

[37] T. Alden, H. Amartino, A. Dalla Corte, C. Lampe, P.R. Harmatz, L. Vedolin, Surgical management of neurological manifestations of mucopolysaccharidosis disorders, Mol. Genet. Metab. 122S (2017) 41–48.

[38] D. Virgintino, M. Errede, M. Rizzi, F. Girolamo, M. Strippoli, T. Wälchli, D. Robertson, K. Frei, L. Roncali, The CXCL12/CXCR4/CXCR7 ligand-receptor system regulates neuro-glio-vascular interactions and vessel growth during human brain development, J. Inherit. Metab. Dis. 36 (2013) 455–466.

[39] D.J. Begley, C.C. Pontikis, M. Scarpa, Lysosomal storage diseases and the blood-brain barrier, Curr. Pharm. Des. 14 (2008) 1566–1580.

[40] S. Garbuzova-Davis, S. Mirtyl, S.A. Sallot, D.G. Hernandez-Ontiveros, E. Haller, P.R. Sanberg, Blood-brain barrier impairment in MPS III patients, BMC Neurol. 13 (2013) 174.

[41] F. Borlot, P. Ricci Arantes, C. Robledo Quaio, J.F. da Silva Franco, C. Marques Lourenço, I. Gomy, D. Romeo Bertola, C.A. Kim, Mucopolysaccharidosis type IVA: evidence of primary and secondary central nervous system involvement, Am. J. Med. Genet. A 164A (2014) 1162–1169.

[42] M.D. Haust, B.A. Gordon, Ultrastructural and biochemical aspects of the Sanfilippo syndrome,—type III genetic mucopolysaccharidosis, Connect. Tissue Res. 15 (1986) 57–64.

[43] S.U. Walkley, M.E. Haskins, R.M. Shull, Alterations in neuron morphology in mucopolysaccharidosis type I, A Golgi Study Acta Neuropathol 75 (1988) 611–620.

[44] I. Ferrer, V. Cusí, M. Pineda, E. Galofré, J. Vila, Focal dendritic swellings in Purkinje cells in mucopolysaccharidoses types I, II and III. A Golgi and ultrastructural study, Neuropathol. Appl. Neurobiol. 14 (1988) 315–323.

[45] G. Constantopoulos, K. Iqbal, A.S. Dekaban, Mucopolysaccharidosis types IH, IS, II, and IIIA: glycosaminoglycans and lipids of isolated brain cells and other fractions from autopsied tissues, J. Neurochem. 34 (1980) 1399–1411.

[46] R.L. Kriel, W.A. Hauser, J.H. Sung, Z. Posalaky, Neuroanatomical and electroencephalographic correlations in Sanfilippo syndrome, type A, Arch. Neurol. 35 (1978) 838–843.

[47] S.U. Walkley, Secondary accumulation of gangliosides in lysosomal storage disorders, Semin. Cell Dev. Biol. 15 (2004) 433–444.

[48] E. Fusar Poli, C. Zalfa, F. D'Avanzo, R. Tomanin, L. Carlessi, M. Bossi, L.R. Nodari, E. Binda, P. Marmiroli, M. Scarpa, D. Delia, A.L. Vescovi, L., De Filippis, Murine neural stem cells model Hunter disease in vitro: glial cell-mediated neurodegeneration as a possible mechanism involved, Cell Death Dis. 4 (2013) e906.

[49] J. Baumkötter, M. Cantz, Decreased ganglioside neuraminidase activity in fibroblasts from mucopolysaccharidosis patients. Inhibition of the activity in vitro by sulfated glycosaminoglycans and other compounds, Biochim. Biophys. Acta 761 (1983) 163–170.

[50] Z. Patay, Diffusion-weighted MR imaging in leukodystrophies, Eur. Radiol. 15 (2005) 2284–2303.

[51] D.A. Wolf, A.W. Lenander, Z. Nan, L.R. Belur, C.B. Whitley, P. Gupta, W.C. Low, R.S. McIvor, Direct gene transfer to the CNS prevents emergence of neurologic disease in a murine model of mucopolysaccharidosis type I, Neurobiol. Dis. 43 (2011) 123–133.

[52] H.F.E. Gleitz, C. O'Leary, R.J. Holley, B.W. Bigger, Identification of age-dependent motor and neuropsychological behavioural abnormalities in a mouse model of

Mucopolysaccharidosis type II, PLoS One 12 (2017) e0172435.
[53] A. Langford-Smith, K.J. Langford-Smith, S.A. Jones, R.F. Wynn, J.E. Wraith, F.L. Wilkinson, B.W. Bigger, Female mucopolysaccharidosis IIIA mice exhibit hyperactivity and a reduced sense of danger in the open field test, PLoS One 10 (2011) e25717.
[54] C. Martins, H. Hulková, L. Dridi, V. Dormoy-Raclet, L. Grigoryeva, Y. Choi, A. Langford-Smith, F.L. Wilkinson, K. Ohmi, G. Dicristo, E. Hamel, J. Ausseil, D. Cheillan, A. Moreau, E. Svobodová, Z. Hájková, M. Tesarová, H. Hansíková, B.W. Bigger, M. Hrebícek, A.V. Pshezhetsky, Neuroinflammation, mitochondrial defects and neurodegeneration in mucopolysaccharidosis III type C mouse model, Brain 138 (2015) 336–355.
[55] A. Langford-Smith, M. Malinowska, K.J. Langford-Smith, G. Wegrzyn, S. Jones, R. Wynn, J.E. Wraith, F.L. Wilkinson, B.W. Bigger, Hyperactive behaviour in the mouse model of mucopolysaccharidosis IIIB in the open field and home cage environments, Genes Brain Behav. 10 (2011) 673–682.
[56] F.L. Wilkinson, R.J. Holley, K.J. Langford-Smith, S. Badrinath, A. Liao, A. Langford-Smith, J.D. Cooper, S.A. Jones, J.E. Wraith, R.F. Wynn, C.L.R. Merry, B.W. Bigger, Neuropathology in mouse models of mucopolysaccharidosis type I, IIIA and IIIB, PLoS One 7 (2012) e35787.
[57] J. Tordo, C. O'Leary, A.S.L.M. Antunes, N. Palomar, P. Aldrin-Kirk, M. Basche, A. Bennett, Z. D'Souza, H. Gleitz, A. Godwin, R. Holley, H. Parker, A.Y. Liao, P. Rouse, A.S. Youshani, L. Dridi, C. Martins, T. Levade, K. Stacey, D.M. Davis, A. Dyer, N. Clément, T. Björklund, R.R. Ali, M. Agbandje-McKenna, A.A. Rahim, A. Pshezhetsky, S.N. Waddington, R.M. Linden, B.W. Bigger, E. Henckaerts, A novel adeno-associated virus capsid with enhanced neurotropism corrects a lysosomal transmembrane enzyme deficiency, Brain 141 (7) (Jul 1 2018) 2014–2031, https://doi.org/10.1093/brain/awy126 (PubMed PMID: 29788236; PubMed Central PMCID: PMC6037107).
[58] H.F.E. Gleitz, A.Y. Liao, J.R. Cook, S.F. Rowlston, G.M.A. Forte1, Z. D'Souza, C. O'Leary, R.J. Holley, B.W. Bigger, Brain targeted stem cell gene therapy corrects Mucopolysaccharidosis type II via multiple mechanisms, Embo. Mol. Med. 10 (7) (Jul 2018), https://doi.org/10.15252/emmm.201708730 (pii: e8730, PubMed PMID: 29884617; PubMed Central PMCID: PMC6034129).
[59] M.E. Haskins, Animal models for mucopolysaccharidosis disorders and their clinical relevance, Acta Paediatr. 96 (2007) 56–62.
[60] M.E. Haskins, G.D. Aguirre, P.F. Jezyk, R.J. Desnick, D.F. Patterson, The pathology of the feline model of mucopolysaccharidosis I, Am. J. Pathol. 112 (1983) 27–36.
[61] S. Hassiotis, R.D. Jolly, K.M. Hemsley, Development of cerebellar pathology in the canine model of mucopolysaccharidosis type IIIA (MPS IIIA), Mol. Genet. Metab. 113 (2014) 283–293.
[62] B. Levy, N. Galvin, C. Vogler, E.H. Birkenmeier, W.S. Sly, Neuropathology of murine mucopolysaccharidosis type VII, Acta Neuropathol. 92 (1996) 562–568.
[63] M.Z. Jones, J. Alroy, P.J. Boyer, K.T. Cavanagh, K. Johnson, D. Gage, J. Vorro, J.A. Render, R.S. Common, R.A. Leedle, C. Lowrie, P. Sharp, S.S. Liour, B. Levene, H. Hoard, R. Lucas, J.J. Hopwood, Caprine mucopolysaccharidosis-IIID: clinical, biochemical, morphological and immunohistochemical characteristics, J. Neuropathol. Exp. Neurol. 57 (1998) 148–157.
[64] M.J. Wilkerson, D.C. Lewis, S.L. Marks, D.J. Prieur, Clinical and morphologic features of mucopolysaccharidosis type II in a dog: naturally occurring model of hunter syndrome, Vet. Pathol. 35 (1998) 230–233.
[65] R.D. Jolly, A.C. Johnstone, E.J. Norman, J.J. Hopwood, S.U. Walkley, Pathology of mucopolysaccharidosis IIIA in huntaway dogs, Vet. Pathol. 44 (2007) 569–578.
[66] C. Palmieri, U. Giger, P. Wang, M. Pizarro, H.L. Shivaprasad, Pathological and biochemical studies of mucopolysaccharidosis type IIIB (Sanfilippo syndrome type B) in juvenile emus (*Dromaius novaehollandiae*), Vet. Pathol. 52 (2015) 160–169.
[67] C. Russell, G. Hendson, G. Jevon, T. Matlock, J. Yu, M. Aklujkar, K.Y. Ng, L.A. Clarke, Murine MPS I: insights into the pathogenesis of Hurler syndrome, Clin. Genet. 53 (1998) 349–361.
[68] K. Ohmi, D.S. Greenberg, K.S. Rajavel, S. Ryazantsev, H.H. Li, E.F. Neufeld, Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1902–1907.
[69] J. Ausseil, N. Desmaris, S. Bigou, R. Attali, S. Corbineau, S. Vitry, M. Parent, D. Cheillan, M. Fuller, I. Maire, M.T. Vanier, J.M. Heard, Early neurodegeneration progresses independently of microglial activation by heparan sulfate in the brain of mucopolysaccharidosis IIIB mice, PLoS One 3 (2008) e2296.
[70] H.H. Li, H.Z. Zhao, E.F. Neufeld, Y. Cai, F. Gómez-Pinilla, Attenuated plasticity in neurons and astrocytes in the mouse model of Sanfilippo syndrome type B, J. Neurosci. Res. 69 (2002) 30–38.
[71] J.R. Bishop, M. Schuksz, J.D. Esko, Heparan sulphate proteoglycans fine-tune mammalian physiology, Nature 446 (2007) 1030–1037.
[72] H.A. Watson, R.J. Holley, K.J. Langford-Smith, F.L. Wilkinson, T.H. Kuppevelt van, R.F. Wynn, J.E. Wraith, C.L.R. Merry, B.W. Bigger, Heparan sulfate inhibits hematopoietic stem and progenitor cell migration and engraftment in Mucopolysaccharidosis I, J. Biol. Chem. 289 (2014) 36194–36203.
[73] R.J. Holley, A. Deligny, W. Wei, H.A. Watson, M.R. Niñonuevo, A. Dagälv, J.A. Leary, B.W. Bigger, L. Kjellén, C.L.R. Merry, Mucopolysaccharidosis type I, unique structure of accumulated heparan sulfate and increased *N*-sulfotransferase activity in mice lacking α-l-iduronidase, J. Biol. Chem. 286 (2011) 37515–37524.
[74] J. Bruyère, E. Roy, J. Ausseil, T. Lemonnier, G. Teyre, D. Bohl, S. Etienne-Manneville, H. Lortat-Jacob, J.M. Heard, S. Vitry, Heparan sulfate saccharides modify focal adhesions: implication in mucopolysaccharidosis neuropathophysiology, J. Mol. Biol. 427 (2015) 775–791.
[75] K. Mason, P. Meikle, J. Hopwood, M. Fuller, Distribution of heparan sulfate oligosaccharides in murine mucopolysaccharidosis type IIIA, Meta 4 (2014) 1088–1100.
[76] D.M. McCarty, J. Dirosario, K. Gulaid, S. Killedar, A. Oosterhof, T.H. Kuppevelt van, P.T. Martin, H. Fu, Differential distribution of heparan sulfate glycoforms and elevated expression of heparan sulfate biosynthetic enzyme genes in the brain of mucopolysaccharidosis IIIB mice, Metab. Brain Dis. 26 (2011) 9–19.
[77] J. Ruijter De, L. Ijlst, W. Kulik, H. Lenthe Van, T. Wagemans, N. Vlies Van, F.A. Wijburg, Heparan sulfate derived disaccharides in plasma and total urinary excretion of glycosaminoglycans correlate with disease severity in Sanfilippo disease, J. Inherit. Metab. Dis. 36 (2013) 271–279.
[78] M. Fuller, D.A. Brooks, M. Evangelista, L.K. Hein, J.J. Hopwood, P.J. Meikle, Prediction of neuropathology in mucopolysaccharidosis I patients, Mol. Genet. Metab. 84 (2005) 18–24.
[79] D.A. Pye, R.R. Vives, J.E. Turnbull, P. Hyde, J.T. Gallagher, Heparan sulfate oligosaccharides require 6-O-sulfation for promotion of basic fibroblast growth factor mitogenic activity, J. Biol. Chem. 273 (1998) 22936–22942.
[80] J.T. Gallagher, Multiprotein signalling complexes: regional assembly on heparan sulphate, Biochem. Soc. Trans. 34 (2006) 438–441.
[81] C. Pan, M.S. Nelson, M. Reyes, L. Koodie, J.J. Brazil, E.J. Stephenson, R.C. Zhao, C. Peters, S.B. Selleck, S.E. Stringer, P. Gupta, Functional Abnormalities of Heparan Sulfate in Mucopolysaccharidosis-I Are Associated with Defective Biologic Activity of FGF-2 on Human Multipotent Progenitor Cells Blood 106 (2005), (1956–1964).
[82] R.M. Walton, J.H. Wolfe, Abnormalities in neural progenitor cells in a dog model of lysosomal storage disease, J. Neuropathol. Exp. Neurol. 66 (2007) 760–769.
[83] M. Hochuli, K. Wüthrich, B. Steinmann, Two-dimensional NMR spectroscopy of urinary glycosaminoglycans from patients with different mucopolysaccharidoses NMR, Biomedicine 16 (2003) 224–236.
[84] G.R.D. Villani, N. Gargiulo, R. Faraonio, S. Castaldo, E. Gonzalez y Reyero, P. Di Natale, Cytokines, neurotrophins, and oxidative stress in brain disease from mucopolysaccharidosis IIIB, J. Neurosci. Res. 85 (2007) 612–622.
[85] S. Trudel, E. Trécherel, C. Gomila, M. Peltier, M. Aubignat, B. Gubler, P. Morlière, J.M. Heard, J. Ausseil, Oxidative stress is independent of inflammation in the neurodegenerative Sanfilippo syndrome type B, J. Neurosci. Res. 93 (2015) 424–432.
[86] A. Arfi, M. Richard, C. Gandolphe, D. Bonnefont-Rousselot, P. Thérond, D. Scherman, Neuroinflammatory and oxidative stress phenomena in MPS IIIA mouse model: the positive effect of long-term aspirin treatment, Mol. Genet. Metab. 103 (2011) 18–25.
[87] L.D. Archer, K.J. Langford-Smith, B.W. Bigger, J.E. Fildes, Mucopolysaccharide diseases: a complex interplay between neuroinflammation, microglial activation and adaptive immunity, J. Inherit. Metab. Dis. 37 (2014) 1–12.
[88] C. Settembre, A. Fraldi, D.L. Medina, A. Ballabio, Signals from the lysosome: a control centre for cellular clearance and energy metabolism, Nat. Rev. Mol. Cell Biol. 14 (2013) 283–296.
[89] A. Terman, T. Kurz, B. Gustafsson, U.T. Brunk, Lysosomal labilization IUBMB, Lifestyles 58 (2006) 531–539.
[90] A.V. Pshezhetsky, Crosstalk between 2 organelles: Lysosomal storage of heparan sulfate causes mitochondrial defects and neuronal death in mucopolysaccharidosis III type C, Rare Dis. 3 (2015) e1049793.
[91] K. Ohmi, H.Z. Zhao, E.F. Neufeld, Defects in the medial entorhinal cortex and dentate gyrus in the mouse model of Sanfilippo syndrome type B, PLoS One 6 (2011) e27461.
[92] G.R.D. Villani, C. Di Domenico, A. Musella, F. Cecere, D. Di Napoli, P. Di Natale, Mucopolysaccharidosis IIIB: oxidative damage and cytotoxic cell involvement in the neuronal pathogenesis, Brain Res. 1279 (2009) 99–108.
[93] G.K. Reolon, A. Reinke, M.R. Oliveira De, L.M. Braga, M. Camassola, M.É. Andrades, J.C.F. Moreira, N.B. Nardi, R. Roesler, F. Dal-Pizzol, Alterations in oxidative markers in the cerebellum and peripheral organs in MPS I mice, Cell. Mol. Neurobiol. 29 (2009) 443–448.
[94] V.G. Pereira, A.M. Martins, C. Micheletti, V. D'Almeida, Mutational and oxidative stress analysis in patients with mucopolysaccharidosis type I undergoing enzyme replacement therapy, Clin. Chim. Acta 387 (2008) 75–79.
[95] W. Droge, H.M. Schipper, Oxidative stress and aberrant signaling in aging and cognitive decline, Aging Cell 6 (2007) 361–370.
[96] N. Baregamian, J. Song, C.E. Bailey, J. Papaconstantinou, B.M. Evers, D.H. Chung, Tumor necrosis factor-α and apoptosis signal-regulating kinase 1 control reactive oxygen species release, mitochondrial autophagy, and c-Jun N-terminal kinase/p38 phosphorylation during necrotizing enterocolitis, Oxidative Med. Cell. Longev. 2 (2009) 297–306.
[97] E.B. Vitner, T. Farfel-Becker, R. Eilam, I. Biton, A.H. Futerman, Contribution of brain inflammation to neuronal cell death in neuronopathic forms of Gaucher's disease, Brain 135 (2012) 1724–1735.
[98] S.U. Walkley, M.T. Vanier, Secondary lipid accumulation in lysosomal disease, Biochim. Biophys. Acta 1793 (2009) 726–736.
[99] R. McGlynn, K. Dobrenis, S.U. Walkley, Differential subcellular localization of cholesterol, gangliosides, and glycosaminoglycans in murine models of mucopolysaccharide storage disorders, J. Comp. Neurol. 480 (2004) 415–426.
[100] S.U. Walkley, M. Zervas, S. Wiseman, Gangliosides as modulators of dendritogenesis in normal and storage disease-affected pyramidal neurons, Cereb. Cortex 10 (2000) 1028–1037.
[101] J.Q. Huang, J.M. Trasler, S. Igdoura, J. Michaud, N. Hanai, R.A. Gravel, Apoptotic Cell Death in Mouse Models of G_{M2} Gangliosidosis and Observations on Human Tay-Sachs and Sandhoff Diseases Hum Mol Genet 6 (1997), (1879–1885).
[102] K. Ohmi, L.C. Kudo, S. Ryazantsev, H.Z. Zhao, S.L. Karsten, E.F. Neufeld, Sanfilippo syndrome type B, a lysosomal storage disease, is also a tauopathy, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 8332–8337.
[103] S. Ryazantsev, W.H. Yu, H.Z. Zhao, E.F. Neufeld, K. Ohmi, Lysosomal accumulation of SCMAS (subunit c of mitochondrial ATP synthase) in neurons of the mouse

model of mucopolysaccharidosis III B, Mol. Genet. Metab. 90 (2007) 393–401.
[104] C.D. Heldermon, A.K. Hennig, K.K. Ohlemiller, J.M. Ogilvie, E.D. Herzog, A. Breidenbach, C. Vogler, D.F. Wozniak, M.S. Sands, Development of sensory, motor and behavioral deficits in the murine model of Sanfilippo syndrome type B, PLoS One 2 (2007) e772.
[105] S. Vitry, J. Ausseil, M. Hocquemiller, S. Bigou, R. dos Santos Coura, J.M. Heard, Enhanced degradation of synaptophysin by the proteasome in mucopolysaccharidosis type IIIB, Mol. Cell. Neurosci. 41 (2009) 8–18.
[106] C. Aragao de, L. Bruno, C. Han, G. Dicristo, P. McPherson, A.V. Pshezhetsky, Synaptic dysfunction in Sanfilippo syndrome type C, Mol. Genet. Metab. 117 (2016) S39.
[107] F. Andrade, L. Aldámiz-Echevarría, M. Llarena, M.L. Couce, Sanfilippo syndrome: overall review, Pediatr. Int. 57 (2015) 331–338.
[108] S. Tomatsu, T. Shimada, R.W. Mason, A.M. Montano, J. Kelly, W.A. LaMarr, F. Kubaski, R. Giugliani, A. Guha, E. Yasuda, W. Mackenzie, S. Yamaguchi, Y. Suzuki, T. Orii, Establishment of glycosaminoglycan assays for mucopolysaccharidoses, Meta 4 (2014) 655–679.
[109] E. Makino, H. Klodnitsky, J. Leonard, J. Lillie, T.C. Lund, J. Marshall, J. Nietupski, P.J. Orchard, W.P. Miller, C. Phaneuf, D. Tietz, M.L. Varban, M. Donovan, A. Belenki, Fast, sensitive method for trisaccharide biomarker detection in mucopolysaccharidosis type 1, Sci. Rep. 8 (2018) 3681.
[110] M.A. Saif, B.W. Bigger, K.E. Brookes, J. Mercer, K.L. Tylee, H.J. Church, D.K. Bonney, S. Jones, J.E. Wraith, R.F. Wynn, Hematopoietic stem cell transplantation improves the high incidence of neutralizing allo-antibodies observed in Hurler's syndrome after pharmacological enzyme replacement therapy, Haematologica 97 (2012) 1320–1328.
[111] R.F. Wynn, J.E. Wraith, J. Mercer, A. O'Meara, K. Tylee, M. Thornley, H.J. Church, B.W. Bigger, Improved metabolic correction in patients with lysosomal storage disease treated with hematopoietic stem cell transplant compared with enzyme replacement therapy, J. Pediatr. 154 (2009) 609–611.
[112] K.J. Langford-Smith, J. Mercer, J. Petty, K. Tylee, H. Church, J. Roberts, G. Moss, S. Jones, R. Wynn, J.E. Wraith, B.W. Bigger, Heparin cofactor II-thrombin complex and dermatan sulphate:chondroitin sulphate ratio are biomarkers of short- and long-term treatment effects in mucopolysaccharide diseases, J. Inherit. Metab. Dis. 34 (2011) 499–508.
[113] E.J. Langereis, N. Vlies Van, H.J. Church, R.B. Geskus, C.E.M. Hollak, S.A. Jones, W. Kulik, H. Lenthe Van, J. Mercer, L. Schreider, K.L. Tylee, T. Wagemans, F.A. Wijburg, B.W. Bigger, Biomarker responses correlate with antibody status in mucopolysaccharidosis type I patients on long-term enzyme replacement therapy, Mol. Genet. Metab. 114 (2015) 129–137.
[114] D.R. Randall, G.B. Sinclair, K.E. Colobong, E. Hetty, L.A. Clarke, Heparin cofactor II-thrombin complex in MPS I: a biomarker of MPS disease, Mol. Genet. Metab. 88 (2006) 235–243.
[115] D.R. Randall, K.E. Colobong, H. Hemmelgarn, G.B. Sinclair, E. Hetty, A. Thomas, O.A. Bodamer, B. Volkmar, P.M. Fernhoff, R. Casey, A.K. Chan, G. Mitchell, S. Stockler, S. Melancon, T. Rupar, L.A. Clarke, Heparin cofactor II-thrombin complex: a biomarker of MPS disease, Mol. Genet. Metab. 94 (2008) 456–461.
[116] K. Langford-Smith, M. Arasaradnam, J.E. Wraith, R. Wynn, B.W. Bigger, Evaluation of heparin cofactor II-thrombin complex as a biomarker on blood spots from mucopolysaccharidosis I, IIIA and IIIB mice, Mol. Genet. Metab. 99 (2010) 269–274.
[117] C.M. Simonaro, Y. Ge, E. Eliyahu, X. He, K.J. Jepsen, E.H. Schuchman, Involvement of the toll-like receptor 4 pathway and use of TNF-a antagonists for treatment of the mucopolysaccharidoses, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 222–227.
[118] P. Di Natale, C. Di Domenico, D. Di Napoli, Serum MIP-1 α level: a biomarker for the follow-up of lentiviral therapy in mucopolysaccharidosis IIIB mice, J. Inherit. Metab. Dis. 33 (2010) 159–165.
[119] S.K. Fisher, J.E. Novak, B.W. Agranoff, Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance, J. Neurochem. 82 (2002) 736–754.
[120] R. Katz-Brull, R.E. Lenkinski, R.A. Du Pasquier, I.J. Koralnik, Elevation of myoinositol is associated with disease containment in progressive multifocal leukoencephalopathy, Neurology 63 (2004) 897–900.
[121] H. Vrenken, F. Barkhof, B.M. Uitdehaag, J.A. Castelijns, C.H. Polman, P.J. Pouwels, MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter, Magn. Reson. Med. 53 (2005) 256–266.
[122] J.P. Kim, M.R. Lentz, S.V. Westmoreland, J.B. Greco, E.M. Ratai, E. Halpern, A.A. Lackner, E. Masliah, R.G. González, Relationships between astrogliosis and ^{1}H MR spectroscopic measures of brain choline/creatine and myo-inositol/creatine in a primate model AJNR, Am. J. Neuroradiol. 26 (2005) 752–759.