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Review

Bone, joint and tooth development in mucopolysaccharidoses: Relevance to therapeutic options

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ABSTRACT

The mucopolysaccharidoses (MPS) are prominent among the lysosomal storage diseases. The intra-lysosomal accumulation of glycosaminoglycans (GAGs) in this group of diseases, which are caused by several different enzyme deficiencies, induces a cascade of responses that affect cellular functions and maintenance of the extra-cellular matrix. Against the background of normal tissue-specific processes, this review summarizes and discusses the histological and biochemical abnormalities reported in the bones, joints, teeth and extracellular matrix of MPS patients and animal models. With an eye to the possibilities and limitations of reversing the pathological changes in the various tissues, we address therapeutic challenges, and present a model in which the cascade of pathologic events is depicted in terms of primary and secondary events.

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1. Introduction

1.1. Mucopolysaccharidosis and glycosaminoglycans (GAGs)

The word mucopolysaccharidosis (MPS) literally means “a disease in which viscous polysaccharides are being stored”. There are eleven such diseases, each caused by genetic deficiency of a different lysosomal enzyme involved in the degradation of these polysaccharides. The diseases are numbered from MPS I to MPS IX and named after the physicians who first described the syndromes or discovered the underlying enzyme deficiency (Table 1) [1–9].

Chemically, the viscous polysaccharides are glycosaminoglycans (GAGs) that consist of long, un-branched chains of negatively charged amino sugars and uronic acids and have a very high capacity to bind water. Most are linked to a protein core (proteoglycan), form large complexes with hyaluronic acid, and are the ground substance of connective tissues. Depending on the precise composition of the polysaccharide chain, the glycosaminoglycans have names such as dermatan sulfate, heparan sulfate, keratan sulfate, chondroitin sulfate and hyaluronic acid (Table 1).

Connective tissue is composed of cells and extracellular matrix (ECM). The matrix is produced by the cells and consists of protein fibers (mainly collagen) and proteoglycans. It provides volume and function, which is specified largely by its chemical composition. For instance, the connective tissue located directly under the epithelium of the skin and under the endothelium of the large blood vessels is loosely organized so as to provide a soft cushion that can absorb subtle transformation by pressure. In non-structural organs such as the liver, the connective tissue literally holds the hepatocytes together in a functional network of cell strains and blood sinuses. Articular cartilage (joints) is very rich in GAGs and can contain up to 80% of water. The jelly-like substance is optimally structured to absorb pressure, but breaks up when traction is applied. It is thus unlike tendons, whose glycosaminoglycan content and cell density are low, but whose collagen content is high, the collagen fibers also being laid in a single direction to transduce force. The connective tissue matrix of bone and teeth is mineralized. GAGs also serve as key biological response modifiers [10–12].

Like any other biological substance in the body, GAGs are continuously renewed. They are degraded by enzymes produced by the connective tissue cells; in part extracellularly and in part intracellularly in the

Abbreviations: BMP, bone morphogenetic protein; BMT, bone-marrow transplantation; CS, chondroitin sulfate; C4S, chondroitin 4-sulfate; DS, dermatan sulfate; ECM, extracellular matrix; ERT, enzyme-replacement therapy; FGF(R), fibroblast growth factor (receptor); GAG, glycosaminoglycan; GD, Gaucher disease; GH, growth hormone; GUSB, β-glucuronidase; HSCT, hematopoietic stem-cell transplantation; HS, heparan sulfate; HSPG, heparan sulfate containing proteoglycans; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KS, keratan sulfate; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; LSD, lysosomal storage disease; MAPC, multipotent adult progenitor cell; ML III, mucolipidosis type III; MMP, metalloproteinase; MPS, mucopolysaccharidoses; MyD88, myeloid differentiation factor-88; OA, osteo arthritis; PG, proteoglycan; PTH, parathyroid hormone; RA, rheumatoid arthritis; STAT, signal transducers and activator of transcription; TIMP, tissue inhibitors of metalloproteinases; TGF-β, transforming growth factor β; TGN, trans golgi network; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α

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Table 1
Enzyme deficiencies and storage products in MPS.

Number	Eponym	Enzyme deficiency	Storage product
I	Hurler/ Scheie	α-L-iduronidase	Heparan sulfate/dermatan sulfate
II	Hunter	Iduronate 2-sulfatase	Heparan sulfate/dermatan sulfate
III-A	Sanfilippo type A	Sulfamidase	Heparan sulfate
III-B	Sanfilippo type B	α-*N*-acetylglucosaminidase	Heparan sulfate
III-C	Sanfilippo type C	Acetyl-CoA; α glucosaminide N-acetyltransferase	Heparan sulfate
III-D	Sanfilippo type D	*N*-acetylglucosamine 6-sulfatase	Heparan sulfate
IV-A	Morquio type A	Galactose 6-sulfatase	Keratan sulfate/chondroitin 6-sulfate
IV-B	Morquio type B	β-galactosidase	Keratan sulfate/chondroitin 6-sulfate
VI	Maroteaux–Lamy	N-acetylgalactosamine 4-sulfatase	Dermatan sulfate/chondroitin 4-sulfate
VII	Sly	β-glucuronidase	Heparan sulfate/dermatan sulfate/chondroitin 4-sulfate/chondroitin 6-sulfate
IX		Hyaluronidase	Hyaluronic acid

lysosomes after uptake through endocytosis. Lack of degradation due to a lysosomal enzyme deficiency leads to intralysosomal GAG storage, followed by loss of cellular functions, tissue damage and organ dysfunction. This process determines the clinical symptoms observed in patients with mucopolysaccharidoses.

This review describes the processes underlying normal development of bone, joints and teeth in relation to abnormalities seen in MPS patients and MPS animal models and the therapeutic challenges these abnormalities present.

1.2. Bones, joints and teeth in MPS patients

As well as joint and dental problems, most patients with mucopolysaccharidoses have bone problems that cause skeletal deformities. Some of these clinical features are illustrated in Fig. 1. The bone and joint abnormalities were summarized recently [13].

Due mainly to lysosomal deposition of GAGs in the chondrocytes [14], the extracellular matrix (ECM) of the articular cartilage, the synovia, and the surrounding tissues, MPS patients have stiff joints, contractures and poor mobility. Hyperlaxity of the joints can also occur. Together, these processes ultimately manifest as degenerative joint disease. Since the abnormalities develop early in life, they also interfere with normal growth, which explains the typical short stature of most MPS patients (Fig. 2).

Before we introduce the pathophysiology of MPSs, the following paragraphs describe the normal development of bones, joints and teeth first.

2. Normal development of bones, joints and teeth

2.1. Bones

The human skeleton consists of bones that meet at joints and are held together by ligaments. To enable movement, tendons connect bones with muscles. Cartilage is part of the skeleton. It is found in joints and in the growth plate of immature long bones. The skeleton also serves as a scaffold and cage that supports and protects vital organs.

Bone tissue is a reservoir for calcium, phosphate and other ions, and harbors the marrow, which is essential for blood-cell formation. Bone is maintained by osteocytes, each of which is directly connected with the circulation by thin cytoplasmic extensions running to the blood vessels through canaliculi (small tunnels in the calcified bone matrix). Together with osteoclasts, osteoblasts are instrumental in bone synthesis and bone remodeling. In a process called endochondral ossification, long bones grow due to the proliferation and differentiation of chondroblasts in the growth plate of immature long bones; newly formed cartilage is replaced by bone as fast as it is formed. Flat bones are formed by the differentiation of mesenchymal cells to bone-forming osteoblasts in a process known as intramembranous ossification.

Three types of cell are important for the formation, growth, renewal and maintenance of bone: osteoblasts, osteocytes and osteoclasts. Their roles and functions are detailed below.

Osteoblasts, the bone-forming cells, are of mesodermal origin. Originating from multipotent mesenchymal cells, they differentiate into osteocytes while synthesizing the organic components of the bone matrix (type I collagen, proteoglycans, and glycoproteins). The young bone is deposited along the remnants of the cartilage of the growth plate and along pre-existing bone as an osteoid layer that turns into bone after calcification.

The lifespan of an osteoblast ranges from one to two hundred days. Sixty to eighty percent dies by apoptosis [15].

Osteocytes, the cells surrounded by calcified bone, have a very long lifespan of one to fifty years. Maintaining the bones throughout the skeleton, they are about ten times more numerous than the osteoblasts, and a thousand times more numerous than the osteoclasts [15,16]. Housing in lacunae between lamellae of matrix, they maintain contact with each other and with cells at the bone surface through long, very thin dendritic processes that traverse the bone matrix [15]. A gel-like matrix surrounds the osteocytes and the dendritic processes. Through the canaliculi, oxygen, nutrients and waste products are transported to and from the osteocytes by hydraulic vascular pressure, partly by diffusion and partly by the convection induced by mechanical forces.

Osteoclasts are created by the fusion and differentiation of cells from the monocyte-macrophage cell lineage; they contain between five and fifty nuclei [17]. The lifespan of an osteoclast is approximately one to twenty-five days, and they die by apoptosis [15].

Osteoclasts are bone-resorptive cells that play a crucial role in normal bone turnover. They adhere tightly to the bone surface, where they create an extracellular lysosomal space [18], in which protons are secreted by the vacuolar H^+-ATPase pump [19,20]. The apical membrane of the polarized osteoclast opposing the bone is ruffled; into this membrane, lysosomal vesicles are inserted [21]. The bone minerals dissolve in the acidic environment of the extracellular space, paving the way for lysosomal proteases to degrade the organic components of the bone matrix [20,21]. The degradation products are endocytosed at the ruffled border membrane and delivered to the basal membrane by transcytosis [22,23].

2.2. Bone formation and remodeling

As stated briefly in the Introduction, there are two ways in which bones are formed. Both processes involve the transformation of pre-existing mesenchymal tissue into bone tissue. In the process of intramembranous or desmal ossification, mesenchymal cells directly differentiate into osteoblasts that produce the bone matrix, which subsequently acquires its strength through mineralization. By contrast, endochondral ossification involves the primary deposit of a cartilage template, which originates through mesenchymal cell differentiation and is gradually replaced by bone.

The growth of short bones and flat bones is achieved by intramembranous ossification. Compacted mesenchymal cells define an ossification center, which is surrounded by the periosteum, a thin layer of connective tissue with osteoprogenitor cells at the bone surface side and fibrogenic cells adjacent to the mesenchyme. The osteogenic cells differentiate into osteoblasts and continuously deposit new layers of lamellar bone against the preexisting bone [16,24]. The formation of the skull is a typical example of intramembranous ossification (Fig. 3).

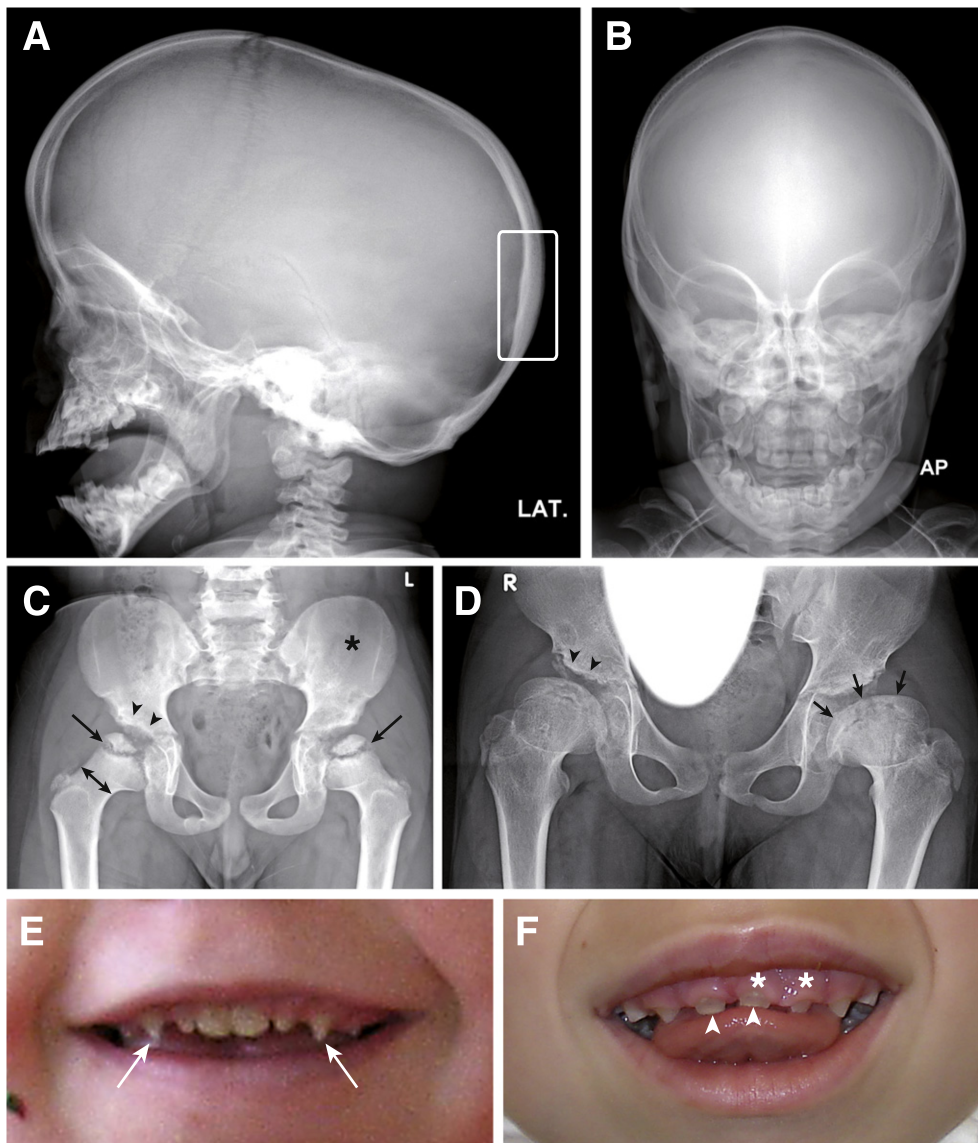

Fig. 1. A and B) Lateral and anterior–posterior radiograph of the skull of a two-year-old patient with MPS VI. Note the atypical shape of the skull, (dolichocephalic) with partial craniosynostosis involving mainly the sagittal and the lambdoid sutures (not visible) and the thickened skull (rectangle). C) Shows an X-ray of an eight-year-old patient with MPS VI. Note the irregularities of the epiphyses in the femoral heads. The growth plates of both femur heads are too small and lateralized (arrows). The neck of the caput femoris is broad and plump (two headed arrow) and in a valgus position. There is dysplasia of the acetabuli (arrowheads); the right acetabulum is steep and shallow. The asterisk shows flaring of the wing of the os ileum. D) Shows an X-ray of a ten-year-old patient with MPS VI. Note the deformed and flattened epiphyses of the femur (arrows), and the dysplastic acetabuli (arrowheads). E and F) Abnormal teeth in an eight-year-old and a six-year-old patient with MPS I. Both patients have hypoplastic peg-shaped teeth (arrows) and dysplastic teeth (arrowheads). The asterisks indicate gingival hyperplasia.

The curved plates of young bone are surrounded by a single layer of osteoblasts, and are deposited amid very loose mesenchymal tissue. During the process of embryonic development, the plates of bone are continuously reshaped as they grow. Osteoblasts and osteoclasts work in concert to obtain the proper curvature: old bone is carved away from the inside and new bone deposited on the outside. Defects in this process can lead to an abnormal shape of the skull, such as that seen in pycnodysostosis, which is caused by cathepsin K deficiency. The different plates of the skull fuse shortly after birth, forming an inflexible bony joint called synostosis.

A role in intramembranous ossification is played by bone-morphogenetic proteins such as BMP2, BMP4, and BMP7. These are thought to activate transcription factor *Cbfa1* in the mesenchymal cells, which are thus transformed into osteoblasts [25].

Rapid growth of long bones is achieved by endochondral ossification and requires the presence of a growth plate. The growth plates disappear toward adulthood, when lengthwise bone growth stops, though bone widening may still occur.

Skeletal long bones are initially deposited in the mesenchymal tissue as a cartilage template, whose shape resembles a miniature version of the bone to be formed. When the cartilage template reaches a certain size, a collar of bone is deposited around the mid portion, and blood vessels penetrate the cartilage structure. Chondrocytes in the center enlarge and die through apoptosis. Osteoprogenitor cells, which are transported to this region by the blood vessel, differentiate into osteoblasts and form a primary ossification center, which later becomes the diaphysis. Secondary ossification centers appear later at the thicker endings of the cartilage template. These become the epiphyses.

Until adolescence, the growth plate remains cartilage, and separates the diaphysis from the epiphysis. Cartilage is also maintained at the endings of the bones where joints are formed. In this area, the mesenchymal cells that have formed the cartilage templates of opposing

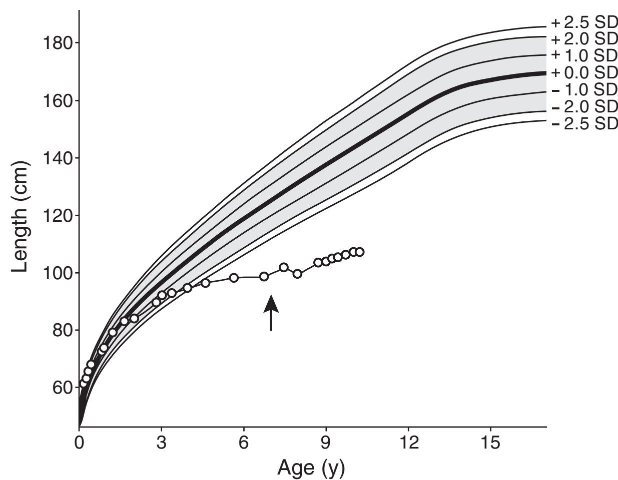

Fig. 2. The growth chart of a patient with MPS VI (open circles). Enzyme-replacement therapy was started at 7 years of age (arrow).

long bones migrate and differentiate, leaving either a cavity and forming a diarthrosis with free bone movement, or leaving a synarthrosis with little or no movement, such as syndesmosis, synchondrosis or synostosis. The cavity of a diarthrosis is filled with synovial fluid produced by the synovial membrane protruding into the joint from the periphery. The fluid provides lubrication to the joint and nutrients to the avascular articular cartilage [16,24].

The growth plate is divided into five zones: a resting zone, a proliferative zone, a hypertrophic zone, a calcified zone and an ossification zone. These start at the mid portion of the growth-plate, and extend in two directions, with major growth taking place toward the diaphysis, and minor growth toward the epiphysis (see Fig. 4) [16,24].

The hypertrophic chondrocytes are degraded by osteoclasts, and the osteoblasts, arising from osteoprogenitor cells, deposit uncalcified young bone (osteoid) against the remnants of the cartilage matrix. The mineralized bone trabeculae that are formed support the growth plate.

Many different factors are involved in endochondral ossification. GAGs have an important regulatory function. They interact in the FGFs, BMPs (as described below), TGF-β, and the wingless-type (Wnt) signaling pathways [26].

2.3. The extracellular matrix (ECM) of bone and cartilage; the role of GAGs

Fifty percent of the extracellular bone matrix consists of inorganic material: calcium, phosphorus, bicarbonate, citrate, magnesium, potassium and sodium. Calcium and phosphorus form hydroxyapatite

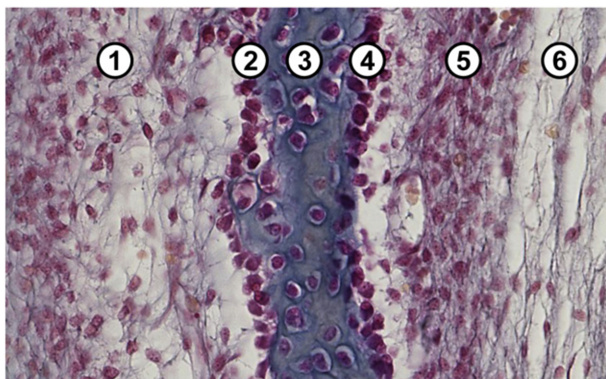

Fig. 3. Intramembranous ossification: 1 and 6) mesenchyme; 2 and 4) osteoblasts; 3) bone with osteocytes; 5) compacted mesenchyme with a blood vessel.

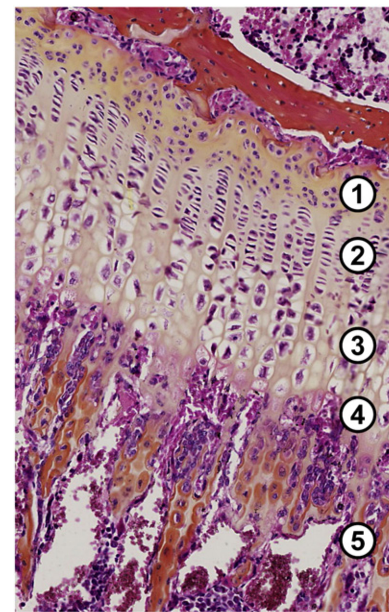

Fig. 4. The epiphyseal growth plate. 1) The resting zone: hyaline cartilage with chondrocyte progenitor cells. 2) The proliferative zone: chondrocytes undergo rapid mitosis and align into vertical columns. The columns are separated by septa of extracellular matrix consisting primarily of collagen (collagen type II) and proteoglycans. 3) The hypertrophic zone: chondrocytes mature and become hypertrophic. They contain large amounts of glycogen and start to secrete alkaline phosphates and collagen type X. The matrix is reduced to thin septa between the chondrocytes. 4) The calcified cartilage zone: concurrent with the death of chondrocytes, the thin septa of cartilage matrix are calcified by the deposition of hydroxyapatite. 5) The ossification zone: blood capillaries and osteoprogenitor cells invade the cavities left by the chondrocytes. The osteogenitor cells form osteoblasts, which are distributed in a layer over the calcified septa. The osteoblasts deposit young bone (osteoid) on top of the three-dimensional calcified cartilage matrix (Mescher AL 'Junqueira's Basic Histology', Brighton et al. [24]). The bone matrix consists of collagen type I, GAGs, and inorganic material (Ortega et al. [37]).

crystals. The organic material in the matrix of bone consists of collagen type I fibers; in the cartilage, it consists of collagen type II fibers. Large water-retaining proteoglycan aggregates (PG) fill the intervening spaces, interacting with the network of collagen fibers. The entire structure of cells and extracellular matrix is completed by various glycoproteins, such as chondronectin [16]. Homeostasis of the extracellular matrix depends on the balance between de-novo synthesis and degradation of the matrix components; for this the osteocytes and chondrocytes have the primary responsibility [27].

In cartilage the collagen fibers are organized in a lacy network that provides tensile strength [28,29]. Aggrecan is the most common PG in articular cartilage. Chondroitin and keratan sulfate are the side chains of aggrecan [30]. Other smaller proteoglycans are less abundant and have one or two GAG side chains, such as dermatan sulfate or heparan sulfate [31].

2.4. Proteoglycans, glycosaminoglycans, and hyaluronic acid as matrix components

Proteoglycans typically consist of a protein core to which long sugar chains (glycosaminoglycans) are covalently attached. The attachment sites are formed by serine residues in the core protein that occur at an interval of 12 amino acids.

Proteoglycans have a feather-like structure (Fig. 5). The glycosaminoglycans linked to the serine residues consist of repeating disaccharide units of sulfated and unsulfated uronic acids, and N-acetyl hexosamines. The composition of the repeating disaccharide units determines the name of the glycosaminoglycans, for example heparan sulfate (HS), dermatan sulfate (DS), chondroitin sulfate and keratan sulfate (KS). KS does not contain uronic acid moieties, but galactose instead.

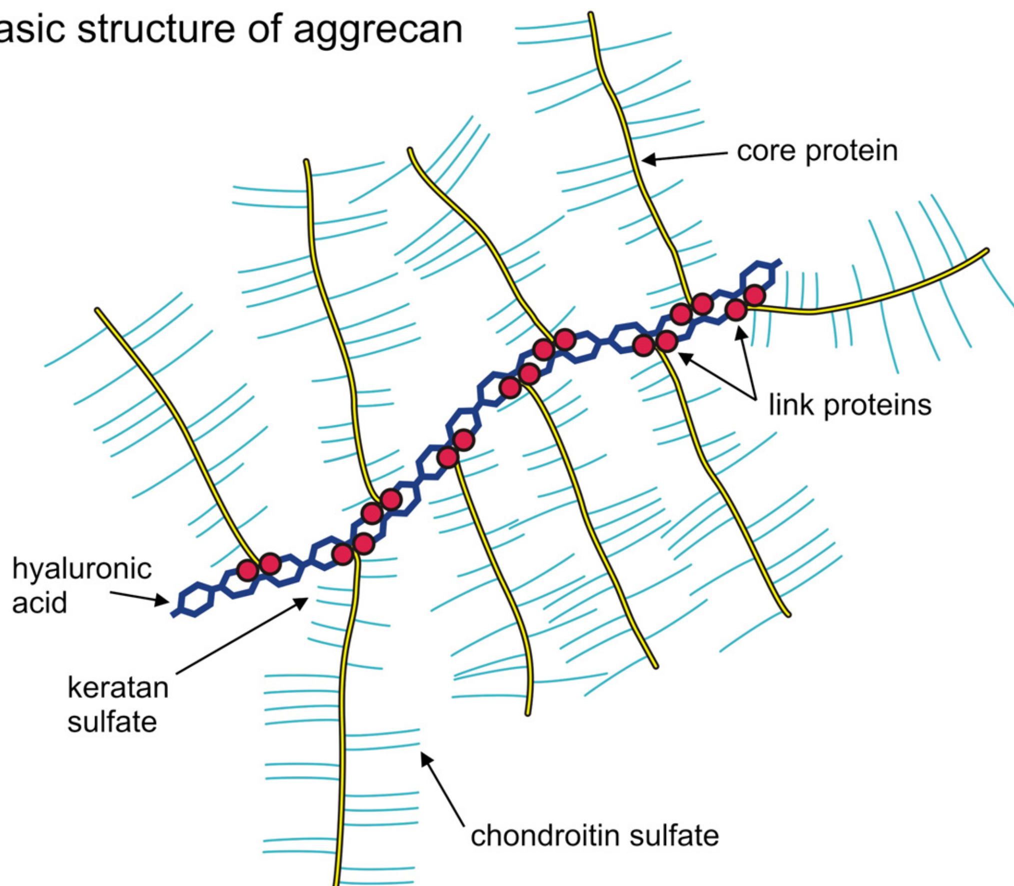

Fig. 5. Aggrecan is the complex of proteoglycans that all are connected to a central long chain of hyaluronic acid via link proteins (red dots). The proteoglycans in the large aggrecan complex are feather-like structures composed of a number of regularly spaced glycosaminoglycan chains, such as keratin sulfate and chondroitin sulfate (in light blue), which are covalently linked to a protein core (yellow).

One and the same core protein can contain different glycosaminoglycan side chains. The large proteoglycan structures are linked to a polysaccharide backbone, hyaluronic acid; together, they form an even more voluminous complex. In the extracellular matrix of articular cartilage, the long chain of hyaluronic acid is oriented in parallel with the collagen type II fibers.

The featherlike structure of proteoglycans overlays the collagen fibers, forming a tight molecular network. Because the negatively charged glycosaminoglycans have a capacity to bind and retain water, they form a jelly-like structure. The tightly packed negatively charged glycosaminoglycans move apart as far as possible. They are brought together by pressure; the more pressure is put on the cartilage, the higher the repelling force will be. This enables the cartilage of the joints to absorb shocks.

An intact proteoglycan network is essential for the integrity and assembly of the functional cartilage matrix. This was demonstrated in an artificial system of cultured chondrocytes [31] which showed that inhibition of GAG incorporation in newly formed cartilage matrix not only causes the newly synthesized GAGs to diffuse further away from the chondrocytes, but also reduces the cross-linking of the collagen.

Proteoglycans – mainly those inserted into the plasma membrane and containing few glycosaminoglycan side chains – also serve as key biological response modifiers. Some of these roles are addressed below. Summarized briefly, they act as 1) stabilizers, cofactors, and/or co-receptors for growth factors, cytokines, and chemokines; 2) regulators of cathepsin activity; 3) signaling molecules during embryogenesis and in response to cellular damage such as wounding, infection, and tumorigenesis; and 4) targets for bacterial, viral, and parasitic virulence factors (attachment, invasion, and immune system evasion) [10–12].

2.5. Bone remodeling and the extracellular matrix (ECM)

Bone remodeling is a continuous process of bone resorption (osteoclasts) and bone formation (osteoblasts). The average turnover (volume replacement) of bone is 10% per year, but there are large differences dependent on age and bone regions [15]. Bone tissue is able to adapt its structure and function in response to mechanical forces and metabolic demands [32]. A change in the balance between bone resorption (osteoclast) and bone formation (osteoblast) results in a corresponding loss or gain of bone tissue.

As cathepsins and matrix metalloproteinases (MMPs) are key enzymes in the turnover of the ECM, they are essential to the process of bone remodeling [27]. Cathepsin K is highly expressed by osteoclasts. It is a lysosomal enzyme that cleaves the triple helical region of type I and II collagen at multiple sites [27,33].

During endochondral ossification, cathepsin K degrades type II collagen (cartilage) in the hypertrophic zone to create space for the deposition of young bone by osteoblasts [34]. Some evidence has been presented that certain types of GAGs modulate the cathepsin K activity depending on their concentrations [35]. According to this model, GAG storage in MPS might affect the bone remodeling [36].

Because MMPs fragment the protein core of the proteoglycans, the extracellular matrix (ECM) disintegrates, resulting in a cascade of events. The release of biologically active components activates other proteases, and affects processes such as cell attachment, migration, proliferation, differentiation and apoptosis [37].

MMP activity is controlled by various mechanisms. For instance, MMP transcription is upregulated via growth factors and cytokines, and MMP translation and proenzyme activation are regulated by tissue inhibitors (TIMPs; tissue inhibitors of metalloproteinases), which

inhibit the translation of MMPs and form complexes with MMPs that influence proenzyme activation [38–40]. Precise regulation of MMP activity is crucial for maintaining the balance between tissue remodeling and destruction.

During endochondral ossification, at least three MMPs are highly expressed. When neovascularization of the cartilage anlage begins, MT1-MMP and MMP9 are expressed in the pre-osteoclasts and other chondroclastic cells of unknown origin. MMP13 is expressed in the terminal hypertrophic chondrocytes and the newly recruited osteoblasts [37].

2.6. Interaction of GAGs with bone morphogenetic proteins (BMPs) and fibroblast growth factor (FGF)

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor β (TGF-β) superfamily [41]. They interact with several regulatory pathways [42] and promote the differentiation of osteoclasts and chondrocytes from mesenchymal progenitor cells. In the growth plate they also promote chondrocyte hypertrophy and apoptosis. Strict regulation of the BMP activity is required to secure normal bone formation during postnatal life [41].

BMPs play an integral role in the development of the skeletal system, but also of the heart and the nervous system [43]. In humans, mutations in the BMP pathway are associated with skeletal disorders. For instance, a mutation in the Ib BMP receptor has been identified in a patient with brachydactyly, and mutations in the BMP antagonist noggin have been found in patients with symphalangism and synostoses [42].

BMPs are secretory proteins with the ability to promote bone formation, but can also induce formation of ectopic cartilage. Although the activities of BMPs and their antagonists are modulated by heparan sulfate containing proteoglycans (HSPG), it is not fully understood how their activity in the MPS storage diseases is influenced by these GAGs [43], whose sulfated residues are thought to bind to BMPs and their antagonists, thereby modulating receptor-mediated signaling.

The growth factor regulatory role of some of the GAGs is also shown by a chondroitin-sulfate-synthesis-deficient mouse model. Mice deficient for chondroitin 4-sulfotransferase have growth plate abnormalities resembling those in MPS VI mice, in which the TGF-β signaling is upregulated and the BMP signaling down-regulated, indicating that chondroitin sulfate balances the activity and localization of these two growth factors [44,45].

Fibroblast growth factor and BMP signaling have opposing functions in the growth plate. They interact through mutual antagonism. FGF ligands and FGF receptors (FGFR) are both expressed in developing skeletal and cartilage anlage. Several human craniosynostosis disorders have been linked to activating mutations in FGF receptors. Disruption of FGFR2 signaling in skeletal tissues results in skeletal dwarfism and lower bone-mineral density (BMD). Lower proliferation of osteoprogenitor cells is combined with reduced anabolic function of mature osteoblasts and diminished osteoblast differentiation [46,47].

FGF-2, a prototypical member of the FGF family that is involved in tissue morphogenesis and neurogenesis, binds to two kinds of cell-surface receptors: high-affinity FGF receptors (FGFRs), and low-affinity receptors (composed of HS proteoglycans) that act as extracellular FGF2 reservoirs and coreceptors. Formation of the FGF-2–FGFR–HSPG complex is necessary for mitogenesis and optimal biologic response to FGF-2 [48]. Dermatan sulfate (DS) also binds and activates FGF-2. The interaction between DS and FGF-2 has been studied only with respect to cellular proliferation: in its capacity to stimulate cell growth in vitro, DS exceeded HS [49].

Three studies have suggested that high concentrations of small, abnormally sulfated, HS chains (such as those present in Hurler syndrome) can have a detrimental effect on orderly hematopoietic stem-cell growth and differentiation [50–52].

2.7. Teeth

Teeth, too, are bony structures: their shaft consists of dentin, and the crown protruding in the oral cavity is covered with enamel. The inner part of teeth, the dental pulpa, is composed of loose connective tissue. Odontoblasts are the cells that form the organic matrix of the shaft (dentin) (Fig. 6), which consists of collagen type I, phosphoproteins, phospholipids and proteoglycans. Newly formed, not yet calcified dentin is called pre-dentin and has its equivalent in osteoid, un-calcified young bone [16].

During tooth development, ameloblasts are aligned across the cap of the primitive tooth and produce a layer of enamel, which also is a form of bone. In this way the crown is formed. The root is covered with a layer of cementum produced by the cementoblasts; this is connected to the bony socket of the jaw by the peridontal ligament, an array of collagen fibers that also holds the teeth in position [53].

2.8. Development of the teeth

The mandible and maxilla grow to accommodate the developing teeth. The enamel on the crown of the teeth is derived from ectoderm; all other parts differentiate from the surrounding mesenchyme. Tooth buds of the primary dentition appear around the tenth week of embryonic development; the buds for the second dentition start to develop four years after birth [53].

2.9. BMPs and FGFs and their role in tooth development

GAGs can affect BMP activity as described above. BMP4 plays an important role in tooth development from the moment the initial epithelial lamina is formed until the late bell stage [54]; during postnatal tooth development, it is highly expressed in both the odontoblasts and in ameloblasts.

Initial tooth development appears to require a BMP signal. Deletion of the BMP4 gene in odontoblasts and surrounding osteoblasts leads to permanent defects in tooth cytodifferentiation, and also in the supporting periodontal tissue (decreasing the rate of formation from pre-dentin, decreasing odontoblast maturation, affecting proper dentinal tubule formation, and reducing the expression of collagen type I and osteocalcin). In mice, dysmorphogenic odontoblasts were seen that failed to properly elongate and differentiate, thereby producing permanently thinner dentin, enlarged pulp chambers in the molars, and less bone tissue to support the teeth. Indirectly, deletion of the BMP4 gene also disturbed the process of enamel formation. Postnatally,

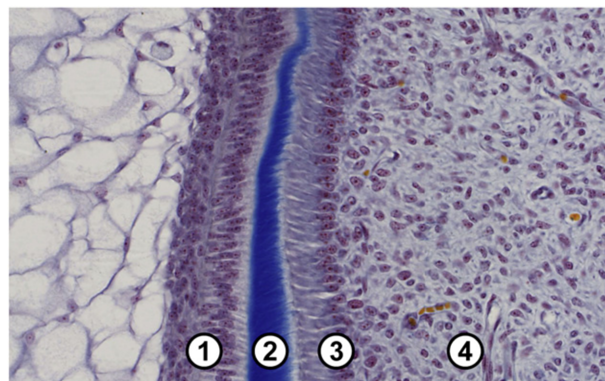

Fig. 6. Embryonic tooth formation in rabbit. 1) ameloblast; 2) dentin; 3) odontoblast; 4) mesenchyme.

odontoblast-derived BMP4 plays a key paracrine or endocrine role in amelogenesis [54].

The BMP2 gene appears to be involved in BMP4 activation. Its deletion in odontoblasts leads to disorganization of morphologically altered odontoblasts at the dentinal tubule stage and failure to mature in later stages of tooth development [54].

Fibroblast growth factors (FGFs) are involved in epithelial–mesenchymal tissue interaction. FGF signaling via FGFR2 in the epithelium is crucial for cell proliferation during tooth and palate development [55]. Interference of abnormal HS and DS (as seen in MPS) with the FGF pathway may cause abnormal growth and differentiation of teeth in MPS [48,52].

3. Bone, joint and tooth problems in mucopolysaccharidoses

Although the severity of bone disease varies by the type of MPS, most of the skeletal anomalies in MPS patients are likely to originate from aberrant cartilage and bone development. For instance, the complex skeletal pathology observed in six-week-old MPS I mice suggests that very early changes predispose for the dysostosis that becomes apparent later in life [56]. Growth in length relies on the perfectly orchestrated proliferation and differentiation of chondrocytes in the growth plate — a process which is irreversibly disturbed in MPS by the lack of GAG turnover.

Various bone problems in MPS patients can be explained by abnormal endochondral ossification: they include thoracolumbar kyphosis/scoliosis, odontoid hypoplasia, wide oar-shaped ribs, shortened long bones, coxa valga, dysplastic femoral heads, genu valgum, and bullet-shaped phalanges. Histological abnormalities have been reported in each of the 5 zones of the growth plate. These are described below. The main causes of osteopenia in MPS patients are probably abnormal bone remodeling (osteoblast/osteoclast dysfunction) and abnormalities in the growth plate, exacerbated by immobility in an advanced stage of the disease. Developmental deformities of the vertebral bodies and the femoral heads accelerate the normal degeneration of the joints caused by weight-bearing forces; they also induce inflammation.

Macrocephaly with thickened skull and short wide clavicles can be explained by abnormal intramembranous ossification and abnormal bone remodeling. Reports of MPS cases with craniosynostosis in the literature have speculated that GAGs may interact with the FGF receptor [46,47].

Dental complications in mucopolysaccharidoses (MPS I, IV and VI) can be severe. They include hypoplastic peg-shaped teeth with retarded eruption or unerupted dentition; thin enamel (often 'grayish'); a pitted surface; dentigerous, cystlike follicles, malocclusions; short mandibular rami with abnormal condyles (condylar defects); and gingival hyperplasia [57–59]. Affected MPS patients easily develop dental caries and need regular conservative dental therapy [60].

Secondary cellular responses, involving interactions of GAGs with BMP and FGF, may play a role in all bone, joint, and tooth problems seen in MPS patients.

3.1. Endochondral ossification and the epiphyseal growth plate in MPS patients and animals

3.1.1. Resting zone

Chondrocytes in the resting zone of the growth plates of patients with MPS I, II and IV are unusually large and contain granular material. They are named "foam" cells, as they contain numerous large cytoplasmic vacuoles filled with undegraded GAGs. Foci of loose connective tissue interrupt the architecture of the growth plate, creating irregularly shaped metaphyses. The remaining parts of the growth plate are fairly regular, with well-organized endochondral ossification [61].

In a MPS VI cat model, the resting zone of the tibia occupies a larger than usual space in which the chondrocytes are rather tightly packed (hyperplasticity) [62]. Clonal expansion of the chondrocytes in the resting zone, which is seen in MPS VI cats, can be explained by a response to GAG accumulation. GAGs are negatively charged and able to mobilize and bind mitotic growth factors.

Compared with the proliferative and hypertrophic chondrocytes, the articular chondrocytes and the chondrocytes in the resting zone have a long half-life. Pathology is therefore more likely to develop in the slowly dividing cells in the resting zone.

3.1.2. Proliferative and hypertrophic zones

In the MPS VII mice the number of chondrocytes in the proliferative zone is markedly decreased (60%); the proliferative capacity is only 55% of normal. The ECM directly surrounding the chondrocytes is very rich in chondroitin-4-sulfate (C4S). On the basis of these findings it has been hypothesized that C4S might interact with a cell membrane receptor and thereby reduce chondrocyte proliferation and bone growth [63].

In six-week-old MPS I, mice the growth plate is abnormally broad with distended resting, proliferative, and hypertrophic zones. The chondrocytes are hypertrophic. In contrast to the decreased proliferation in the MPS VII mice, the proliferative zone in six-week-old MPS I mice occupies a relatively high number of chondrocytes, and the columnar organization is relatively well preserved. In older mice, the columnar organization of the growth plate is disrupted, and the bone trabeculae begin to thin [56].

Similar defects in the process of endochondral ossification have been observed in MPS I patients, in cats with MPS I and MPS VI, and in mice with MPS VI [64]. For instance, chondrocyte maturation in distinct regions of the hypertrophic zone was disorganized in the iliac crest growth plate of MPS I patients, and in the growth plates in the femoral head and tibia of MPS VI cats [64]. The lower rate of bone growth in MPS patients and animal models may be attributable to delayed turnover of hypertrophic cells [62]. The lower number of hypertrophic cells in MPS VI rats has been ascribed to enhanced expression of TGF-β that inhibits the terminal differentiation of immature chondrocytes [38]. A decrease in the number of hypertrophic chondrocytes that are subsequently replaced by bone can explain the osteopenia in MPS VI rats.

3.1.3. The ossification zone

The accumulation of GAGs in MPS VII mice and dogs has been demonstrated in osteoclasts and osteoblasts located in the ossification zone [65].

In the iliac growth plate of humans with MPS I there were fewer longitudinal septa upon which cartilage mineralization and metaphyseal osteoblastic activity could occur. The primary spongiosum was irregular with poor speculation [64]. In the femoral growth plate of MPS VI cats, calcifying cartilage was disorganized by irregularities in the chondro-osseous junction, and there were osteoclast deficits [66]. Abnormalities in the cortical bone structure supporting the growth plate have been reported in a murine MPS I model [56]: at six weeks of age, the zone of provisional calcification and primary spongiosa was abnormally wide, indicating either that more matrix was produced or that it was less degraded than normal. Islands of un-ossified cartilage persisted amidst the newly formed bone, resulting in the loss of well-defined narrow trabeculae. Some of these findings could be explained by the lack of osteoclast activity, which normally degrade GAGs from the cartilage matrix, leaving a well-ordered scaffold consisting mainly of type II collagen fibers to which the osteoblasts can adhere.

The GAG storage in MPS I appears to compromise this process. Ossification starts before the GAGs have been removed from the matrix, and remnants of the cartilage anlage are retained within the newly formed bone. This leads to abnormalities in the composition and architecture of the growth plate, and thus to growth retardation. The abnormalities persist, which suggests that the lack of GAG degradation also affects the process of bone remodeling [56].

In a MPS VI cat model the abnormalities are not restricted to the cartilage of the growth plate but also pertain to osteoblast function, suggesting that bone formation is deficient [62]. Osteonectin, a

glycoprotein secreted by osteoblasts, with affinity for collagen and promoting bone mineralization, has been mentioned in this context. It is a substrate for MMP-2 and 9 that are both elevated in MPS bones and joints [38].

Like the osteoblasts, but to an even greater extent, the osteoclasts in MPS VII mice contain large foamy vacuoles [20]. The osteoclasts fail to form ruffled border membranes and seem to detach easily from the bone surface. These defects are intrinsic to the MPS VII osteoclasts and not the result of an abnormal bone matrix. They are corrected during bone-marrow transplantation, when the osteoclasts are replaced.

Osteoclast function may also be impaired by an excess of GAGs in the extracellular matrix, which inhibits cathepsin K activity and thereby cartilage resorption [35].

3.2. The role of signal transducers and activator of transcription (STAT)

Several studies have indicated the role of STAT in the process of endochondral ossification and MPS-related change. STAT stands for a family of transcription factors. The phosphorylation and activation of STAT1 by activated FGFR3 increases the expression of the cell-cycle inhibitor p21, reducing chondrocyte proliferation [46]. Metcalf et al. [63] have shown that mRNAs that encode several of the STAT transcription factors known to be selectively altered by inflammation were abnormally expressed in the growth plates of MPS VII mice.

3.3. Osteopenia and osteoporosis in MPS

The abnormal cartilaginous structures in the epiphyseal growth plate are an important cause of osteopenia in most MPS patients, but not in MPS III patients. Heparan sulfate accumulation causes neurological complications (psycho-motor retardation) and only minor skeletal abnormalities including coarse facies and joint stiffness [67].

The psycho-motor retardation in MPS I, II and III might also contribute to osteopenia or osteoporosis. The secondary inducers include immobilization, too little exposure to the sun, poor nutritional status, vitamin D deficiencies and treatment with anti-epileptica [68]. In MPS IV and VI patients, severe bone deformities (immobilization) and vitamin D deficiencies are also described [69].

3.4. The role of weight-bearing forces on abnormally shaped bones and joints

In MPS patients, abnormal bone structures (dysostosis multiplex) are seen very early in life. The acetabula of the hips in MPS I, II, IV and VI patients are short and steep and form an abnormal acetabular cup. The morphology of the femoral head and neck can be changed not only by accumulation of GAGs in the growth plate and bone cells, but also by abnormal weight-bearing forces. The latter is shown in Fig. 7, which demonstrates hallux valgus in a patient with MPS II. Hallux valgus is an abnormal angulation of the big toe involving the joint between the metatarsal bone and the proximal phalanx. It also occurs in the normal population (Fig. 8). The wrongly directed pressure on the bones can cause joint inflammation, affect the GAG content of the articular cartilage, and induce secondary alterations in the underlying bone. It also has a long-range effect on the cartilage structure of the joint between the proximal and distal phalanges. The proximal site of the joint between the metatarsal bone and the proximal phalanx has fully disappeared due to the inflammation. The distal site of this joint has a very low GAG content, although the gross structure is still preserved. In response to the primary pathologic changes, new bone is formed at the periphery of the joint (osteophyt); and immature cartilage and bone are formed just under the zone of articular cartilage. BMPs and other growth factors are likely involved in these processes. Although not directly involved, the cartilage of the joint between the proximal and distal phalange of this toe also has a very low GAG content. Thus, in this pathological condition mixed aspects of osteo arthritis and rheumatoid arthritis (OA and RA) are seen, which also occur in MPS. Recently Baldo et al. described this phenomena in a knee joint of an MPS I murine model [70] showing inflammatory infiltration and pannus formation with hypertrophy of synovial cells.

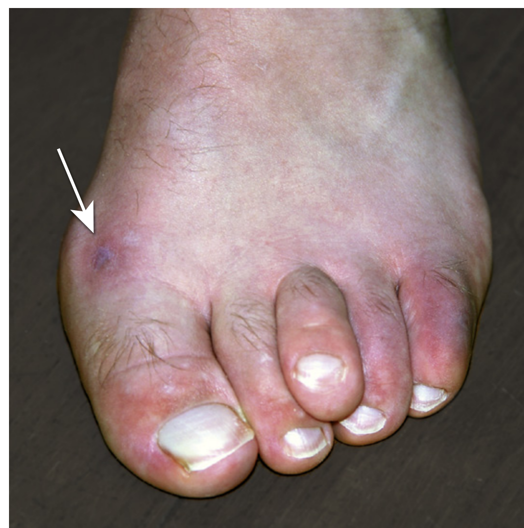

Fig. 7. Hallux valgus of the left foot of a 12 year old MPS II patient. Notify the redness of the skin over the joint between the metatarsal bone and the proximal phalanx, which is caused by inflammation (arrow).

Although the primary causes of hallux valgus in the general population and in MPS patients are totally different, the cascade of pathologic events and the consequences are partly similar.

3.5. Joints and inflammation

Simonaro et al. hypothesized that lysosomal and/or extracellular GAG storage in the MPS disorders induce inflammation and affect the growth of connective tissue cells and other cell types by activating the Toll-like receptor 4 (TLR4) signaling pathway [71]. Activation of this pathway can be induced either by 1) GAG fragments interacting with CD44, a hyaluronan-binding cell surface glycoprotein receptor (55 kDa), and myeloid differentiation factor-88 (MyD88); or 2) through lipopolysaccharide (LPS), which requires MyD88, lipopolysaccharide binding protein LBP, and CD14 [72–76]. Simonaro et al. have shown that the expression of MyD88, LBP, and CD14 is up-regulated in MPS connective tissue cells [71].

Large fragments of hyaluronic acid and oligosaccharides released by the breakdown of the extracellular matrix also signal through TLR4 via MyD88 and CD44, providing further support for the concept that this pathway is activated by GAG storage in the MPS disorders [73].

TLR4 activation in MPS animals resulted in the production of ceramide, a pro-apoptotic lipid and the release of numerous inflammatory cytokines and proteases [77]. Among the cytokines, TNF-α was markedly elevated both in the circulation and within the joint tissue of MPS animals [71].

There is great similarity in the secretion of pro-inflammatory cytokines like TNF-α, IL-1β and nitric oxide in the extracellular matrix of cartilage and bone of animals and humans with primary MPS syndromes compared with primary degenerative and inflammatory process like osteo- or rheumatoid arthritis. The cytokines are produced by MPS chondrocytes, synoviocytes, and macrophages [38].

Stimulation of MPS connective tissue cells by the inflammatory cytokines causes enhanced secretion of several of the matrix metalloproteinases (MMPs). Dramatically elevated expression of MMP-2 and MMP-9 and abnormally high expression of TIMP-1 accompany joint disease in MPS [38]. The imbalance of MMPs over tissue inhibitors of

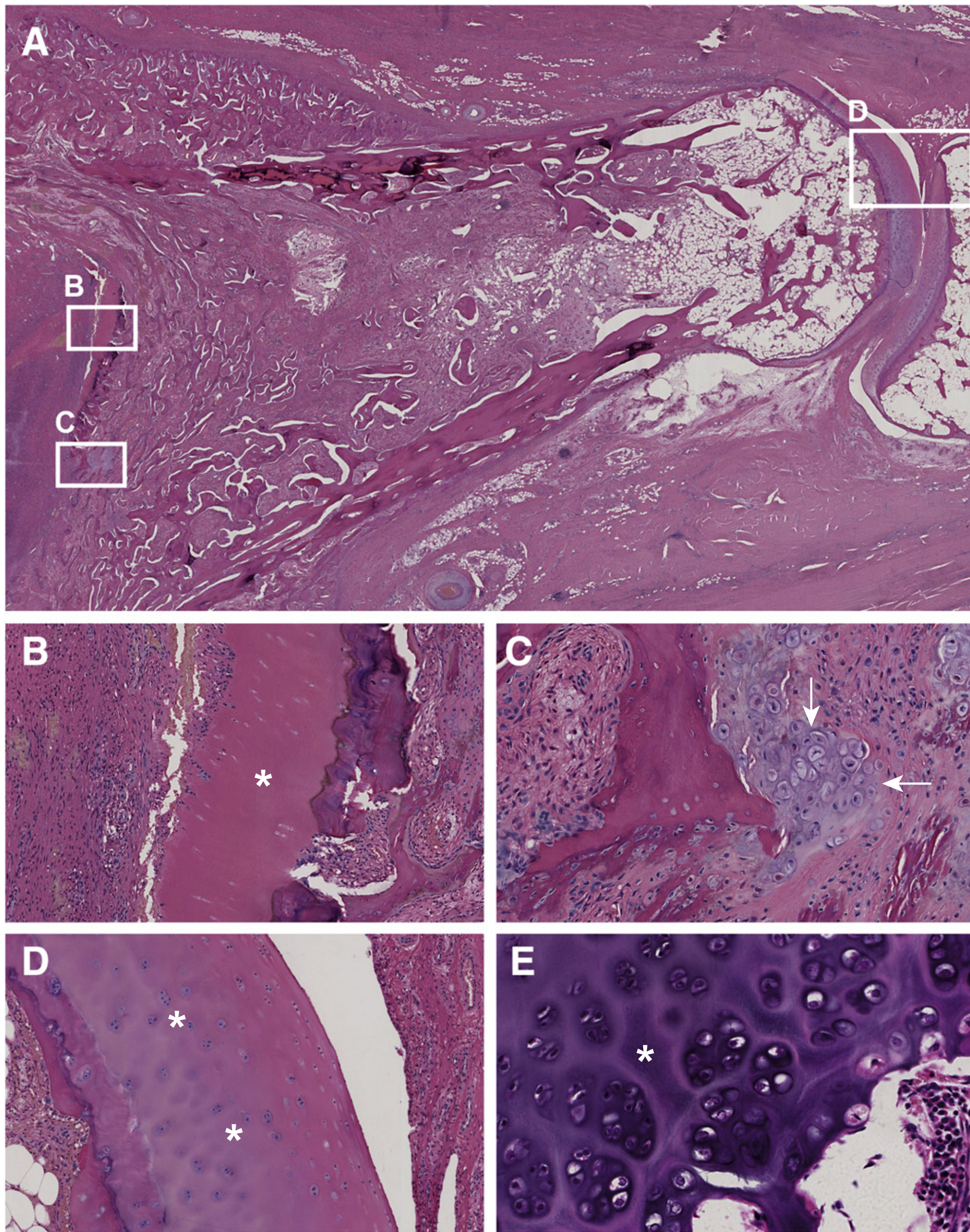

Fig. 8. Microscopic image of an amputated great toe of a 52 year old woman not affected by MPS (hallux valgus). A) Overview. B and C) show details of the joint between the metatarsal bone and the proximal phalanx, and D) shows a detail of the joint between the proximal phalanx and the distal phalanx. The joint between the metatarsal bone and the proximal phalanx has been destroyed by inflammation due to the abnormal pressure, and the GAG content in the cartilage has diminished (asterisk) (B). Also visible in this joint are the secondary alterations in the underlying bone; in this case abnormal cartilage formation (arrows) (C). Although the gross architecture is still preserved, the cartilage of the joint between the proximal phalanx and the distal phalanx has a very low GAG content (asterisks) (D). Image E shows healthy cartilage with normal GAG content (asterisk).

metalloproteinase, the TIMPs, precipitate features of both osteo arthritis as well as rheumatoid arthritis in joints of MPS patients.

Patients without MPS but with osteo arthritis (OA) and cartilage degeneration have changes in the structure and composition of the extracellular matrix, characterized by lower proteoglycan content and enhanced collagen degradation [78]. To compensate for the loss of matrix substance, the rate of synthesis of both matrix components is increased [79,80].

3.6. Dental problems

Guven et al. have investigated the ultrastructural and chemical properties of MPS I (Hurler) teeth. The dentin of the primary teeth was characterized by extremely narrow dentinal tubules with an irregular wave-like pattern. The enamel-dentin junction was poorly shaped, micro gaps occurred and the enamel displayed an irregular arrangement of prisms. Both the enamel and the dentin had an abnormal protein structure and the dentin protein content was low [57].

4. Secondary cellular effects and responses in MPS

4.1. Interaction of GAGs with BMPs and FGFs

Impaired BMP-4 signaling by GAGs in multipotent adult progenitor cells (MAPCs) in the human Hurler syndrome identifies a mechanism that might contribute to the progressive skeletal abnormalities. The same mechanisms may be involved in development of neurological problems [43].

It has been demonstrated that cell and matrix associated GAGs promote proliferation and block BMP-4-mediated differentiation of

several types of human malignant cells. The storage of GAGs in Hurler cells may have the same effect. GAGs impair the BMP-4 activity but do not change the expression of various BMPs or their receptors. The activity of BMP-4 can be restored by clearing the lysosomal GAG storage with exogenously supplied enzyme as demonstrated in cultured cells.

Both heparan sulfate (HS) and dermatan sulfate (DS) accumulate in Hurler syndrome and both are cleared by treatment with α-L-iduronidase, the role of DS cannot be ruled out as a contributor to impaired BMP-4 signaling [43] as observed in Hurler MAPCs.

HS in Hurler syndrome is structurally and functionally abnormal and has impaired ability to bind to and mediate the effects of fibroblast growth factor-2 (FGF-2) signaling [48]. This results in FGF-2-induced proliferation and survival of Hurler multipotent progenitor cells. Both the mitogenic and survival-promoting activities of FGF-2 were restored by substitution of Hurler HS by normal HS. Chondroitin 4-sulfate (C4S), one of the GAGs that store in MPS VII, can activate FGF signaling as well through some of the FGFRs [81].

4.2. Autophagy, polyubiquitination and mitochondrial function

The molecular pathways underlying pathology in lysosomal storage diseases (LSDs) are largely unknown [82]. Recycling of the building blocks of cells, organelles, (glycol)proteins, (glycol)lipids, carbohydrates, and other kind of macromolecular compounds is required for cellular homeostasis [83].

The ubiquitin–proteasome proteolytic system and the autophagosome to lysosome pathways are both used for large scale degradation of cellular components. The ubiquitin–proteasome system degrades short lived regulatory proteins that are for instance important for processes like cell-cycle progression, regulation of gene transcription and signal transduction [84]. The long-lived structures are mainly targeted to the lysosomes by autophagosomes [85].

Lysosomal storage leads to reduced functionality of the lysosomal compartment and consequently leads to build-up in the autophagocytic and endocytic pathways [86–88]. Dysfunction of autophagy as secondary event seems to play an important role in the pathophysiology of LSDs. It has been shown that lysosomal storage induces impaired autophagy. A recent study also showed mitochondrial dysfunction and inflammation to occur as secondary event in MPS VI [83]. Similar anomalies in association with dermatan sulfate storage have been observed in the visceral organs but not in the central nervous system of MPS VI rats accompanied by inflammation and cell death. Prevention of dermatan sulfate storage in these rats by gene therapy resulted in restoration of autophagy, as well as regression of inflammation and apoptosis.

4.3. Compromised lysosome membrane integrity

Compromised lysosomal membrane integrity is another problem resulting from the accumulation of undegraded or partially degraded substrates in the lysosomes of LSD patients [89,90]. By leading to leakage of ions and metabolites from the lysosomes to the cytosol, it causes alterations in cellular homeostasis.

Pereira et al. have shown that secondary alterations, such as changes in the Ca^{2+} and H^{+} homeostasis caused by lysosomal membrane permeability, can contribute to cell death and to the pathophysiology of MPS I mice [90]. For example, too high a calcium concentration in the cytosol compromises the fusion between endosomes and lysosomes. Too high an endosomal and lysosomal pH has a similar detrimental effect [91], as has also been demonstrated in Mucolipidosis type IV [92].

Large changes in lysosomal membrane integrity cause leakage of lysosomal enzymes and other macro-molecules from the lysosomes in the cytosol, where the presence of lysosomal proteases such as cathepsins may contribute to apoptotic cell death [89,93–96].

5. Relevance to therapeutic options

The following section describes the existing and potential therapeutic options for preventing or correcting short stature, osteopenia, joint stiffness and contractures in MPS patients.

5.1. Enzyme-replacement therapy

Enzyme-replacement therapy (ERT) is currently available for MPS I, II and VI [97]. So far, although ERT ameliorates bone and/or joint problems in MPS animal models if started very early, it has achieved little improvement of bone disease in humans [77]. In a cat with MPS VI, Auclair et al. injected the enzyme directly into the joint rather than intravenously [98] to improve the therapeutic efficacy in joints. This significantly reduced storage material in the articular chondrocytes and the synovial membrane. Within two months of treatment, GAG accumulation recurred [98]. Monthly injections had better effects than injections every three months. The method of intra-articular therapy is presently being tested in two MPS VI patients [99]. However, the correction of MPS storage in the articular cartilage and the growth plate remains challenging because the accessibility of the chondrocytes is restricted mechanistically by slow diffusion of the relatively large therapeutic enzymes through the molecular structure of the matrix.

With regard to age-related effects, McGill et al. reported a sibling study in which one sibling with MPS VI started ERT in the eighth week after birth and the other at 3.6 years. Therapeutic efficacy was assessed by comparing the two siblings' bone and joint problems. Although the child who had been treated very young preserved joint movement, did not develop scoliosis, and had no facial dysmorphy [100], the growth rate of both siblings was similarly decreased, and macrocephaly was not prevented in the period between birth and 3.6 years. In both siblings, radiological changes of the skeleton progressed, and degenerative changes took place in the joints [100].

Likewise, in the MPS VI cat model the best response occurred if therapy started directly after birth and if antibody development was prevented [101]. It seems that bones and joints respond better to ERT when the treatment is started early. Neonatal screening for MPS enzyme deficiencies might thus be an option for diagnosing MPS children as early as possible [102].

5.2. Bone-marrow transplantation and hematopoietic stem-cell transplantation

Although bone-marrow transplantation (BMT) or hematopoietic stem-cell transplantation (HSCT) in MPS I (ideally before 18 months of age) leads to several positive changes, it does not greatly reduce the skeletal abnormalities [103]. If no matched donor is available, cord blood from unrelated individuals can be used for transplantation [104]. However, despite early transplantation, transplanted children often require major orthopedic surgery for genu valgum, acetabular hip dysplasia and kyphoscoliosis later in life [103,105,106].

In MPS animal models, osteoblast and osteoclast function was restored by bone-marrow transplantation [20].

5.3. Gene therapy in MPS animals

Mango et al. examined the effect of liver-targeted retroviral gene therapy in β-glucuronidase (GUSB) deficient MPS VII mice and dogs. In mice, full correction of the femur length was not obtained, despite high level enzyme expression from the liver, not even if gene therapy was used in very young animals. This was explained by the poor accessibility of the growth plate [65].

As an alternative to ERT and BMT/HSCT, Byers et al. examined the effect of direct transduction of the synovial membrane of rats with a GUSB containing lentiviral vector [107]. The transduction was only partially successful: enzyme expression was obtained in the synovia for

8 weeks, but the chondrocytes and the fibroblasts of the ligament were not transduced. This was probably due to steric exclusion of viral particles by cartilage ECM. In theory, the treatment can only work if enough enzyme is produced by the synovial cells, which can then leak into the synovial fluid and reach the chondrocytes by diffusion through the ECM.

5.4. Short stature and growth hormone (GH)

Even though they did not have a growth-hormone deficiency, a number of MPS IV patients have been given growth hormone. There was no evidence that this improved their growth [60]. Eight children with MPS I-Hurler syndrome who had previously undergone HSCT also received GH treatment [108,109]; some of them had HSCT-induced GH deficiency, others did not, but all had growth failure. After one year of treatment with GH their growth failure was partially corrected. Potential complications of GH are Legg–Calvé–Perthes disease, scoliosis and carpal tunnel syndrome [110,111]. Although none of the transplanted patients had to discontinue GH treatment due to progressive scoliosis, one had a slipped capital femoral epiphysis, which can be caused by the GH therapy.

The orthopedic complications characteristic of MPS I (Hurler) can be exacerbated by GH treatment, though opinions differ with regard to the prevalence and progression of scoliosis or kyphosis during GH treatment. Some studies have indicated that the percentage and rate of progression of the scoliosis curve is generally higher than expected during GH therapy; others have reported little to no progression [112–115].

Hip abnormalities with a Perthes-like disease aspect (femoral head dysplasia, see Fig. 1A) are common in untreated MPS patients and in patients treated with ERT [116]. It is not known whether GH aggravates this problem, but it can be envisaged that the growth plate becomes disorganized when chondroblast proliferation is randomly stimulated by GH.

The occurrence of pubertal delay in MPS VI patients and precocious puberty in MPS III patients may both be related to primary abnormalities of the hypothalamic–pituitary–gonadal/thyroid hormone axes, and may potentially affect growth. However, gonadal and thyroid hormonal dysfunctions have seldom been demonstrated in MPS patients and argue against the existence of any substantial abnormalities of the hypothalamic–pituitary–gonadal/thyroid hormone axes [117,118].

5.5. Osteopenia and the use of growth hormone

Throughout human life, growth hormone (GH) and insulin-like growth factor-I (IGF-I) play important roles in the homeostasis of bone. GH acts directly on the target tissues, i.e. bone, skeletal muscle and many others tissues. Many of the effects of GH are indirectly mediated by circulating (liver-derived) or locally produced IGF-I.

Although GH treatment improves osteopenia in pediatric patients with growth hormone deficiency [119], the question is whether it also improves the osteopenia in MPS patients [120].

In a GH deficient rat model it was demonstrated that GH administration increases periosteal and endocortical bone formation. GH also mitigates trabecular bone loss by increasing bone formation [119]. Since trabecular bone loss has been demonstrated in most MPS animal models, there is good reason to investigate the effect of GH dosing in MPS patients, particularly in younger ones whose bone abnormalities and growth retardation are still limited.

5.5.1. Osteopenia and the use of parathyroid hormone

Parathyroid hormone (PTH) is best known for releasing calcium from bone; primary hyperparathyroidism causes bone resorption. Parathyroid hormone also has anabolic activity [121]. The anabolic properties of PTH manifest at a low, intermittent dose. Under this regimen, PTH positively affects bone volume and microarchitecture by stimulating bone formation. This effect was seen in postmenopausal women with osteoporosis and in young women with growth disturbances [121,122].

In the GH-deficient rat model (created by hypophysectomy), PTH increases bone formation mainly by reducing the osteoclast density per bone area, but has hardly any effect on growth in length [119].

Given that PTH lowers osteoclast density, it would be unlikely to have a positive effect on osteopenia in MPS patients. Given that PTH lowers osteoclast density, it would be unlikely to have a positive effect on osteopenia in MPS patients, since the number of osteoclasts can be low (MPS VI cats) and their function defective (MPS VII mice) [66].

5.5.2. Osteopenia and the use of bisphosphonates

Patients with Gaucher disease (GD), a lysosomal glycolipidosis, may also have severe osteopenia, even when treated with ERT. In Gaucher disease this has been attributed to “chronic macrophage activation”, inflammation and induction of accelerated bone turnover [103]. Bone-mineral density was improved by the administration of bisphosphonates, whose effect is attributed to the inhibition of osteoclast function [123].

Another lysosomale storage disorder characterized by substantial bone abnormalities is Mucolipidosis. Patients with type III Mucolipidosis (ML III, pseudo-Hurler polydystrophy), have skeletal manifestations resembling those of MPS patients. They have a distinctively high turnover of bone [124] caused by vigorous, osteoclast-driven, subperiosteal bone resorption pertaining to almost the entire periosteal surface. ML III patients treated with bisphosphonates have a dramatic clinical response, their pain decreasing and their mobility improving. Their bone density also increased, particularly in the metaphyseal regions.

Due to immobilization, children with psycho-motor retardation related to causes other than MPS may have severe osteoporosis. In our clinic, such patients are treated successfully with bisphosphonates. If we take these facts into consideration, it could be that MPS patients, too, will benefit from bisphosphonates. On the other hand, bisphosphonate inhibits osteoclast function, which seems already compromised in MPS [66].

5.5.3. Osteopenia and the use of BMPs

It has been demonstrated that BMP-2 can be used to treat osteoporosis [41]. BMP-2 can accelerate bone healing in animal models [125] and in humans promote intervertebral and lumbar posterolateral fusions [126]. It has also been shown to induce new dentine formation, to have a potential application in root canal surgery, and to be an effective bone inducer around dental implants for periodontal reconstruction [127].

Because of the stimulating effect of BMPs on osteoblast differentiation, their potential application in MPS deserves attention (this review).

5.5.4. Osteopenia and exercise

The bone and joint problems in hips and knees cause pain and restricted mobility, leading indirectly to osteopenia. Physical exercise improves bone mass in growing children. Although the precise mechanism whereby it influences bone metabolism is not known, a response to greater mechanical stress and to changes in endocrine parameters are both likely contributors [32]. Physiotherapy and exercise might therefore improve osteopenia in MPS patients.

5.6. Intervention at the level of secondary cellular events

As stated above, various secondary effects occur in the pathophysiolgy of MPS, including disturbed autophagy and polyubiquitination, mitochondrial dysfunction, inflammation, apoptosis, and loss of lysosomal membrane integrity. These secondary events can be prevented or resolved by reducing the lysosomal storage of GAGs. While this can be achieved by ERT or gene therapy [20,35,83,128], the lysosomal GAG load can also be limited by reducing GAG synthesis. Such substrate reduction is used in Gaucher disease and Niemann Pick disease type C [97]. Used as a substrate inhibitor in MPS III animal models, Rhodamine B has beneficially affected CNS function [97]. However, the effect of substrate inhibition on bone problems in this and other MPSs has not yet been demonstrated.

5.7. Inflammation

Research has been done on preventing the damage of bones and cartilage, which is caused by inflammation. Simonaro et al. showed the important role of TLR4 signaling in MPS bone and joint disease, and suggested that targeting TNF-α may have positive therapeutic effects [77].

6. Concluding remarks

Growth retardation, dysostosis multiplex, osteopenia/osteoporosis, stiff joints and abnormal teeth in MPS patients are the final result of lysosomal GAG accumulation in connective-tissue-forming cells such as mesenchymal cells, fibroblasts, chondrocytes, osteoblasts and osteocytes, osteoclasts, odontoblasts, ameloblasts and cementoblasts. The primary cause of cellular dysfunction is intralysosomal GAG storage, which directly affects the composition and metabolism of the extra-cellular matrix. These primary events evoke a cascade of pathological processes that have local effects on tissue and organ function, and distant effects on systemic functions. As the MPS-degrading enzyme deficiencies are determined genetically, the GAG storage starts in utero, eliciting a long-term effect on body structure and function. Skeletal malformations, dental dysplasia and hypoplasia are all pre-eminent examples of this process. To conclude this review, Fig. 9 summarizes the cascade of pathologic events, starting with primary GAG storage.

6.1. Primary and secondary cellular events

GAGs, one of the major components of the extracellular matrix, are synthesized and recycled by the connective tissue cells. They enter the cell by endocytosis and are degraded in the lysosomes. If one of the lysosomal enzymes involved in their degradation is missing or malfunctioning because of a genetic defect, GAGs accumulate in lysosomes. Intra-lysosomal GAG storage not only expands the volume of the lysosomal system, but also the functioning of the lysosomes as end-stations of the endocytic and autophagocytic transport pathways. The lysosomal membrane integrity is compromised, which has several consequences, such as dysfunction of the lysosomal membrane ATPase proton pump and leakage of proteases (cathepsins) into the cytosol. The high intra-lysosomal pH prohibits optimal functioning of lysosomal hydrolases, thereby contributing to secondary lysosomal storage. The release of lysosomal cathepsins and other lysosomal proteases into the cytoplasm has been associated with apoptosis. Dysfunction of autophagy leads to a series of cellular disturbances, including mitochondrial dysfunction. Abnormal vesicular traffic also affects endocytosis and thereby remodeling of the extra-cellular matrix. Such remodeling is also affected by the excess of proteoglycan, which can inhibit or stimulate cathepsin K activity.

Most of the secondary cellular events are not unique for MPS, but occur in several of the lysosomal storage disorders. A review of the pathological cascade in neuropathic lysosomal storage disorders has recently been published and it shows several similarities with the model presented here for the MPS [129].

6.2. Cell-type-specific and tissue-specific events

Lysosomal GAG storage in chondrocytes directly affects the growth and maintenance of cartilage. In early life, long bones grow by endochondral ossification; a disturbance of this process by chondrocyte malfunction leads to poor growth, skeletal malformations and also osteopenia, whereby fewer trabeculae are formed and less calcification takes place. Articular cartilage of the joints persists throughout life. The GAG storage in this tissue culminates in a pathology resembling osteo arthritis, as well as rheumatoid arthritis.

GAG storage in osteoblasts, osteocytes and osteoclasts hampers adequate bone formation and bone remodeling. Early anatomical abnormalities of bones can induce and enhance osteo arthritis and inflammation, such as that caused by abnormal weight-bearing forces. Inflammation itself is detrimental to chondrocyte function, as it leads to loss of extracellular matrix components (as in hallux valgus).

Lysosomal storage of GAGs in teeth-forming cells such as the odontoblasts, ameloblasts and cementoblasts seems to be the greatest cause of abnormally shaped and irregularly positioned dental elements.

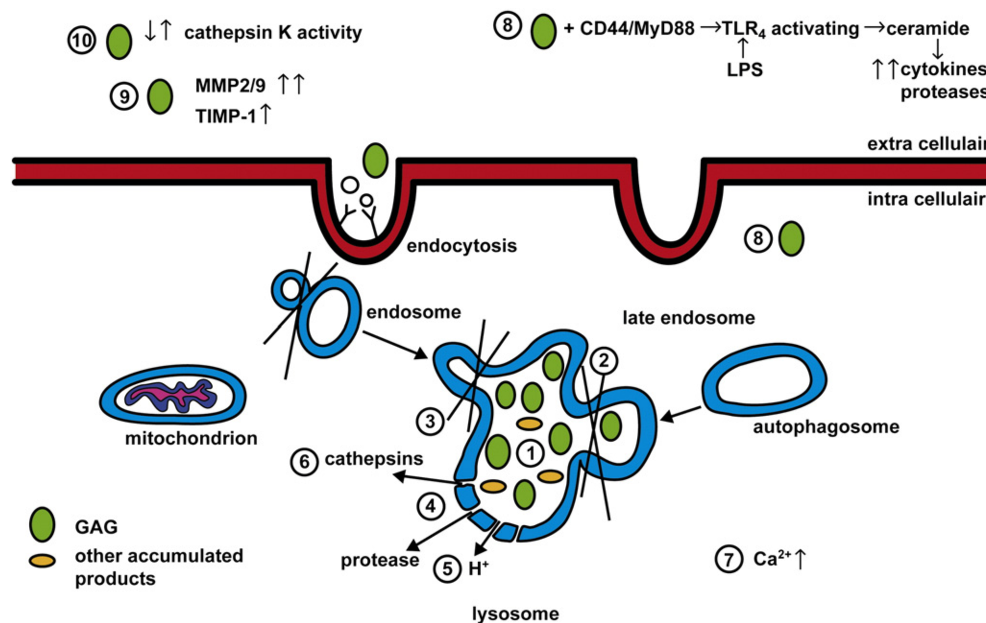

Fig. 9. Pathologic cascade in mucopolysaccharidoses. 1) Lysosomal GAG storage due to deficiencies of MPS-degrading enzyme. 2) Impairment of autophagosome–lysosome fusion. 3) Impairment of endosome–lysosome fusion, affecting remodeling of the extra-cellular matrix. 4) Compromised lysosomal membrane integrity and disturbances of ion homeostasis. 5) Leakage of lysosomal hydrolases and H^+ out of the lysosome elevates the lysosomal pH and diminishes lysosomal function. 6) Leakage of cathepsins and other proteases into the cytosol may contribute to apoptosis. 7) Elevated Ca^{2+} levels in the cytosol, compromises the fusion of endosomes and lysosomes. 8) Activation of the TLR4 pathway by GAGs leads to the production of ceramide, which in turn leads to the release of cytokines and proteases, which further elevates the TNFα level. 9) GAG-induced imbalance of MMP-2/9 and TIMP-1 causes degradation of the ECM. 10) Certain GAGs can modulate the cathepsin K activity, which might affect ECM remodeling.

But because the formation of teeth also requires interplay between teeth and bony elements of the mandibula and maxilla, GAG storage in the osteocytes probably contributes to the problem.

6.3. Secondary responses

Partially degraded and undegraded GAGs interact with several growth factors, such as BMPs, FGFs and the FGF receptor. Activation of the TLR4 signaling pathway has been demonstrated in osteoblasts, osteocytes, osteoclasts, chondrocytes and odontoblasts and in the ECM in association with inflammation.

Elevated expression of metalloproteinases and unbalanced elevation of metalloproteinase inhibitors causes osteo arthritis and rheumatoid arthritis, cumulating in articular cartilage degeneration. The proteoglycan content of the ECM is low and the collagen is degraded abnormally fast.

6.4. Primary and secondary interventions

Therapeutic interventions reducing the lysosomal GAG storage are also expected to resolve some of the secondary pathophysiologic events. While enzyme-replacement therapy seems the first logical option, its effect on bone, joint and tooth pathology is limited by the texture of these tissues. Cartilage is a-vascular, and large molecules can barely diffuse through the matrix. Bone cells are nourished by short connections with the blood vessels, but bone has a very slow turnover. In addition, the bone, joint and tooth problems are the result of long-term aberrant formation and maintenance of these tissues, which makes the abnormalities largely irreversible. Very early intervention is therefore mandatory. Surgical intervention can correct or minimize some of the joint, bone and dental problems at a later age. Standard approaches can help to resolve inflammation related morbidity.

Growing insight in the cascade of pathophysiologic events is slowly but steadily opening the doors that will enable us to improve the lives of MPS patients by manipulating the disease process at the primary or secondary levels.

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