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REVIEW

Biosynthesis of glycosaminoglycans: associated disorders and biochemical tests

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Abstract Glycosaminoglycans (GAG) are long, unbranched heteropolymers with repeating disaccharide units that make up the carbohydrate moiety of proteoglycans. Six distinct classes of GAGs are recognized. Their synthesis follows one of three biosynthetic pathways, depending on the type of oligosaccharide linker they contain. Chondroitin sulfate, dermatan sulfate, heparan sulfate, and heparin sulfate contain a common tetrasaccharide linker that is O-linked to specific serine residues in core proteins. Keratan sulfate can contain three different linkers, either N-linked to asparagine or O-linked to serine/threonine residues in core proteins. Finally, hyaluronic acid does not contain a linker and is not covalently attached to a core protein. Most inborn errors of GAG biosynthesis are reported in small numbers of patients. To date, in 20 diseases, convincing evidence for pathogenicity has been presented for mutations in a total of 16 genes encoding glycosyltransferases, sulfotransferases, epimerases or transporters. GAG synthesis defects should be suspected in patients with a combination of characteristic clinical features in more than one connective tissue compartment: bone and cartilage (short long bones with or without scoliosis), ligaments (joint laxity/dislocations), and subepithelial (skin, sclerae). Some produce distinct clinical syndromes. The commonest laboratory tests used for this group of diseases are analysis of GAGs, enzyme assays, and molecular testing. In principle, GAG analysis has potential as a general first-line diagnostic test for GAG biosynthesis disorders.

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Introduction

Macromolecules composed of protein and carbohydrates are broadly classified as either glycoproteins or proteoglycans. Glycoproteins are proteins that contain one or more covalently linked saccharides lacking a serial repeat unit. The carbohydrate in a glycoprotein can represent between 1 and 80 % of its total weight (Cylwik et al 2013a; Cylwik et al 2013b). but typically is less than the protein moiety. By contrast, proteoglycans can contain 95 % or more carbohydrate, and their properties resemble those of polysaccharides more than those of proteins. The carbohydrate chains of proteoglycans are referred to as glycosaminoglycans (GAG) or mucopolysaccharides. Most glycosaminoglycans are highly sulfated and are attached covalently to a protein core through an oligosaccharide linker. A large number of core proteins is known, and to each, a variable number of GAG chains (one to over one hundred) of different types can be attached; this leads to enormous structural variation and tremendous functional versatility of proteoglycans (Esko et al 2009; Schaefer and Schaefer 2010). The macromolecular structure and highly polyanionic charge imparted by the sulfate and carboxyl groups allow GAGs to adsorb large amounts of water and to form solutions with high viscosity and elasticity. These properties contribute to their biological roles as lubricants, shock absorbers, and support elements in the connective tissue. GAGs are widespread in the extracellular matrix and at the cell surface, and function in embryonic development, as receptors for growth

factors and in regulation of cell growth and differentiation, in tumor growth and invasion, in the maintenance of tissue hydration, in the lubrication of joints and cartilage and in the stabilization of the structure of collagen fibers (Laurent et al 1996; Funderburgh 2000; Rabenstein 2002; Trowbridge and Gallo 2002; Sugahara et al 2003; Quantock et al 2010).

The human diseases traditionally associated with GAGs are mucopolysaccharidoses (MPS), a group of disorders caused by defects in the lysosomal enzymes that degrade GAGs. MPSs have well-characterized clinical presentations, including hepatosplenomegaly, bone dysplasia, facial dysmorphisms, and developmental regression, which result from the progressive and chronic accumulation of undigested GAGs in tissues. Diagnostic testing for MPSs is usually performed by means of specific enzymatic assays and by quantitation of total or specific GAGs. These tests are sensitive and specific, allowing for accurate routine diagnosis in experienced laboratories.

In contrast, inborn errors of metabolism of GAG synthesis (GAG-SIEM), with a few exceptions, have been reported to date mostly in single patients or small groups of patients. While many of these diseases involve the skeleton and connective tissue, other clinical manifestations are also possible. In addition, the clinical signs are often nonspecific, which makes it difficult for the clinician to determine the likelihood of a GAG biosynthetic disorder in individual patients.

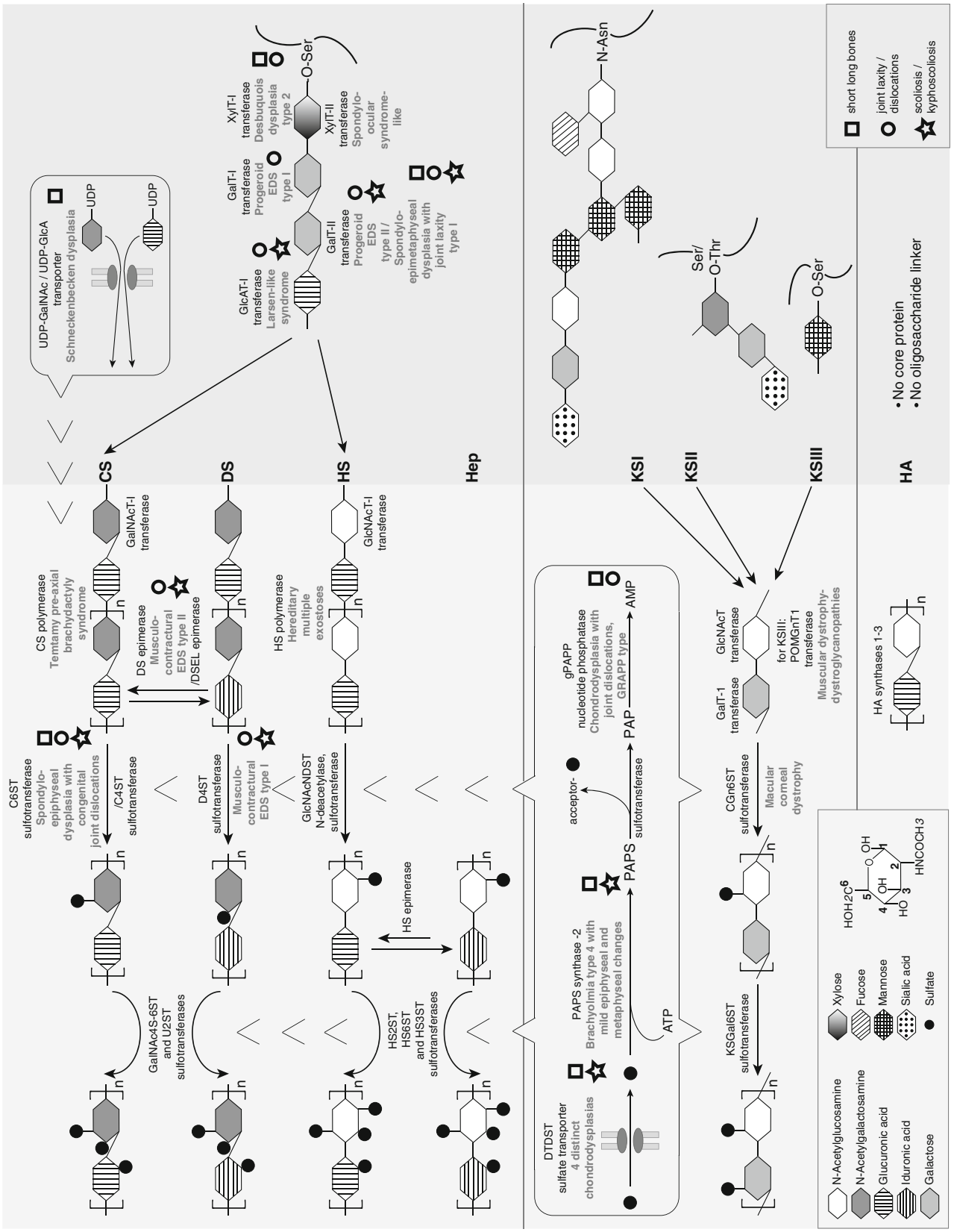
Several expert, detailed reviews describe the synthesis of subgroups of GAGs and their associated diseases (Funderburgh 2002; Uyama et al 2007; Mizumoto et al 2013; Cylwik et al 2013a; Cylwik et al 2013b; Mizumoto et al 2014; Vigetti et al 2014). Here we present a unifying overview of the biosynthesis of all classes of GAGs, describe the known GAG-SIEMs, propose a first clinical approach to diagnosis and discuss the potential for clinical biochemical testing.

The biosynthetic pathways of GAGs

The unifying characteristic of all GAGs is that they are long, unbranched heteropolymers of a repeating disaccharide unit, in which one sugar is a N-acetylhexosamine and the other is either a uronic acid (sugar containing a carboxyl group) or galactose. Based on the identity of the repeating disaccharide unit, six distinct classes of GAGs are recognized: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin sulfate (Hep), keratan sulfate (KS), and hyaluronic acid (HA, also known as hyaluronan). Two of the GAGs, CS and DS, contain N-acetylgalactosamine as one of the repeating sugars, while the second is either glucuronic acid (CS) or iduronic acid (DS). The other four GAGs are copolymers of N-acetylglucosamine and glucuronic acid (HS and HA), iduronic acid (Hep) or galactose (KS). Although HS and HA share the same repeating disaccharide unit, HA differs from HS and from all other GAGs in that it is unsulfated and it is not covalently linked to a core protein, but instead interacts non-covalently with proteoglycans *via* hyaluronan-binding motifs.

The biosynthesis of each individual GAG proceeds through one of three distinct pathways (represented schematically in Fig. 1), depending on the nature of the oligosaccharide linker it contains.

Fig. 1 Pathways of GAG biosynthesis, showing known inborn errors. ▶ Specific enzymatic activities and transporters required for GAG biosynthesis are indicated, together with associated diseases described to date. *Upper right box*. Individual monosaccharides are activated to the corresponding UDP-sugars in the cytosol, then are transported to the endoplasmic reticulum or the Golgi apparatus, where they are transferred to the growing polysaccharide chains by specific glycosyltransferases with α or β specificity. Depending on the type of oligosaccharide linker through which GAGs are attached to core proteins, their biosynthesis follows one of three different routes. *Upper panel*. Four GAGs (CS, DS, HS, Hep) use a common tetrasaccharide that is O-linked to specific serine residues in the core protein. Addition, by a specific glycosyltransferase, of a fifth sugar residue to the tetrasaccharide linker determines the GAG that will be synthesized: GalNAc for CS/DS, and GlcNAc for HS/Hep. Synthesis of these two GAGs is subsequently carried out respectively by CS polymerase, a bifunctional glycosyltransferase with GalNAc and GlcA specificities, and by HS polymerase, a heterodimer of two gene products, both with GlcNAc and GlcA specificities. Epimerization of GlcA to IdoA, a reversible reaction, generates DS from CS and Hep from HS. Before epimerization can take place in HS, specific GlcNAc residues are N-deacetylated and N-sulfated, and adjacent GlcA residues are then converted to IdoA by HS epimerase. The synthesis of CS, DS, HS, and Hep is completed by extensive sulfation, accomplished by sulfotransferases that transfer sulphur from PAPS, the universal sulphur donor, to specific positions of the sugar residues, as indicated in the figure. *Middle panel*. In KS, three different linkers can be used for attachment to core proteins. These linkers can also be extended with several other saccharides and are thus not exclusive to KS. The POMGnT1 enzyme catalyzes the addition of GlcNAc to O-mannosylated proteins. Polymerization of KS is performed by the concerted actions of two β-glycosyltransferases with substrate specificities for GlcNAc and Gal. Elongation appears to be dependent on 6-O-sulfation of the GlcNAc residues by the CGn6ST sulfotransferase. Additional sulfation by KSGal6ST sulfotransferase occurs at position 6 of the Gal residues. *Lower panel*. In contrast with other GAGs, HA lacks a core protein and oligosaccharide linker, is not sulfated and it is synthesized at the plasma membrane by three HA synthetase isoenzymes with GlcNAc and GlcA glycosyltransferase specificities. *Middle left box*. Mutations in the genes coding for the transporter responsible for the translocation of sulfate in the cytosol, PAPS synthase, and the Golgi-resident phosphoadenosine phosphate phosphatase, gPAPP, presumably affect the activity of multiple sulfotransferases, as indicated. *Lower left box* The numbering of carbon atoms in the sugar rings is shown, using N-acetylglucosamine as an example. Abbreviations: *UDP* uridine diphosphate, *GlcNAc* N-acetylglucosamine, *GalNAc* N-acetylgalactosamine, *GlcA* glucuronic acid, *IdoA* iduronic acid, *Gal* galactose, *Xyl* xylose, *GAG* glycosaminoglycan, *CS* chondroitin sulfate, *DS* dermatan sulfate, *HS* heparan sulfate, *Hep* heparin sulfate, *U2ST* uronyl 2-O-sulfotransferase, *CGn6ST* corneal N-acetylglucosaminyl-6-sulfotransferase, *POMGnT1* protein-O-mannose beta-1,2-N-acetylglucosaminyltransferase, *DTDST* diastrophic dysplasia sulfate transporter, *PAPS* 3′-phosphoadenosine 5′-phosphosulfate, *gPAPP* Golgi-resident phosphoadenosine phosphate phosphatase. See text for more details


UDP-GalNAc / UDP-GlcA transporter Schneckenbecken dysplasia
UDP
UDP
GlcAT-I transferase Larsen-like syndrome
GalT-I transferase Progeroid EDS type I
GalT-II transferase Progeroid EDS type II / Spondylo-epimetaphyseal dysplasia with joint laxity type I
XylT-I transferase Desbuquois dysplasia type 2
XylT-II transferase Spondylo-ocular syndrome-like
O-Ser
CS
DS
HS
Hep
GalNAcT-I transferase
GlcNAcT-I transferase
CS polymerase Temtamy pre-axial brachydactyly syndrome
DS epimerase Musculo-contractural EDS type II /DSEL epimerase
HS polymerase Hereditary multiple exostoses
C6ST sulfotransferase Spondylo-epiphyseal dysplasia with congenital joint dislocations
/C4ST sulfotransferase
D4ST sulfotransferase Musculo-contractural EDS type I
GlcNAcNDST N-deacetylase, sulfotransferase
HS epimerase
GalNAc4S-6ST and U2ST sulfotransferases
HS2ST, HS6ST and HS3ST sulfotransferases
DTDST sulfate transporter 4 distinct chondrodysplasias
PAPS synthase -2 Brachyolmia type 4 with mild epiphyseal and metaphyseal changes
PAPS
ATP
acceptor-
PAP
gPAPP nucleotide phosphatase Chondrodysplasia with joint dislocations, GRAPP type
AMP
sulfotransferase
KSGal6ST sulfotransferase
CGn6ST sulfotransferase
Macular corneal dystrophy
GalT-1 transferase
GlcNAcT transferase
for KSIII: POMGnT1 transferase Muscular dystrophy-dystroglycanopathies
KSI
KSII
KSIII
HA
HA synthases 1-3
N-Asn
Ser/ O-Thr
O-Ser
• No core protein
• No oligosaccharide linker
short long bones
joint laxity / dislocations
scoliosis / kyphoscoliosis
N-Acetylglucosamine
N-Acetylgalactosamine
Glucuronic acid
Iduronic acid
Galactose
Xylose
Fucose
Mannose
Sialic acid
Sulfate
HOH2C6
HNCOCH3

Biosynthesis of CS, DS, HS, and Hep

CS, DS, HS, and Hep are O-linked to specific serine residues in the core protein through a tetrasaccharide linkage region composed of xylose, two galactose residues, and glucuronic acid (reviewed in (Prydz and Dalen 2000; Sugahara and Kitagawa 2000; Mizumoto et al 2013)). This biosynthetic pathway can be divided in three stages. The first occurs in the cytoplasm, and consists of the activation of the individual monosaccharides to nucleoside diphosphate sugars (typically UDP-sugar), and of sulfate, to 3′-phosphoadenosine 5′-phosphosulfate (PAPS), the universal sulphur donor. In the second stage, activated sugar residues and PAPS are translocated into the endoplasmic reticulum or Golgi apparatus by specific transporters. Finally, UDP-sugars are incorporated into the growing polysaccharide chain by specific glycosyltransferases, and PAPS is used by sulfotransferases for sulfation of the sugar residues at defined positions. The first residue of the linker, xylose, is added to the serine residue of the core protein in the endoplasmic reticulum, while all subsequent elongation steps take place in the Golgi apparatus. Each saccharide of the linker is added by a specific β-glycosyltransferase (EC 2.4.2.26; EC 2.4.1.133; EC 2.4.1.134; EC:2.4.1.135).

Once the tetrasaccharide linker is completed, the incorporation of the fifth sugar residue will determine the type of GAG being synthesized: N-acetylgalactosamine dictates CS/DS, and N-acetylglucosamine, HS/Hep. As with each saccharide in the linker, the fifth sugar residue is added by a specific glycosyltransferase, either β-N-acetylgalactosaminyltransferase (EC 2.4.1.174) or α-N-acetylglucosaminyltransferase (EC 2.4.1.223). Several mechanisms might contribute to the regulation of the synthesis of one GAG over the other. First, the amino acid sequence around the serine residue to which HS is O-linked has a number of particular features: the Ser used for attachment is part of a Ser-Gly dipeptide, which can be repeated a few times, and which is next to a Trp residue and near to a cluster of acidic residues (Zhang and Esko 1994; Zhang et al 1995). Second, sulfation patterns of the linker region are also specific to certain GAGs, with both galactose residues being sulfated in CS/DS, but not in HS/Hep (Sugahara and Kitagawa 2000).

Starting with the sixth residue, further polymerization is carried out by CS polymerase, a bifunctional enzyme that displays N-acetylgalactosaminyltransferase (EC 2.4.1.175) and glucuronyltransferase (EC 2.4.1.226) activities or, alternatively, by HS polymerase, a heterodimer of two gene products, *EXT1* and *EXT2*, each with N-acetylglucosaminyltransferase (EC 2.4.1.224) and glucuronyltransferase (EC 2.4.1.225) activities.

Thus, the only uronic acid added during chain elongation of GAGs is glucuronic acid. To generate DS or Hep, the iduronate-containing GAGs, most, albeit not all, glucuronic acid residues in CS or HS are epimerized to iduronic acid by specific epimerases. In CS/DS, the conversion of glucuronic acid to iduronic acid is variable, ranging from one to nearly all of the glucuronic acid residues in the chain (Malmstrom et al 2012).

Two CS/DS epimerases have been described, one of which, DSE (EC 5.1.3.19), appears to be present in most tissues, whereas the second, DSEL, is found mostly in brain and kidney (reviewed in (Thelin et al 2013). The proportion of iduronic *versus* glucuronic acid might be regulated by a seven-amino acid motif present in the protein core of CS, but not DS-containing proteoglycans. This motif directs the nascent GAG to regions in the Golgi complex with lower epimerase activity (Seidler et al 2002). In the case of HS, N-deacetylation and N-sulfation of specific N-acetylglucosamine residues (EC 2.8.2.8) precede the epimerization reaction and generate N-sulfated glucosamine residues (Prydz and Dalen 2000). There is only one HS epimerase, which converts glucuronic acid residues adjacent to N-sulfated glucosamine residues to iduronic acid, and consequently, HS into Hep (Sheng et al 2012).

Sulfation of CS, DS, HS, and Hep

The biosynthesis of CS and DS is completed by extensive O-sulfation, accomplished by five sulfotransferases that transfer sulfate from PAPS, the universal sulfate donor, to specific positions on N-acetylgalactosamine, glucuronic, and iduronic acid residues (reviewed in (Mizumoto et al 2013). Fig. 1). Certain sulfotransferases are encoded by multiple genes, which have different expression patterns, levels of expression, and developmental timing (Uyama et al 2007; Mizumoto et al 2014). This results in a spatiotemporally specific sulfation of CS/DS, which might regulate the function and properties of this GAG. 4-O-sulfation in CS and DS prevents continued epimerization between glucuronic and iduronic acids, an otherwise reversible reaction (Malmstrom 1984). The normal ratio between CS and DS is altered when the responsible sulfotransferase is mutated (Miyake et al 2010).

As in the case of CS/DS, the biosynthesis of HS/Hep is completed by O-sulfation at various positions of their respective disaccharides, a process carried out by three sulfotransferases (Mizumoto et al 2014). Fig. 1.

The importance of the Golgi-resident phosphoadenosine phosphate phosphatase, gPAPP (EC 3.1.3.25), in the sulfation of GAGs has been demonstrated by studies in vitro and in a mouse model (Frederick et al 2008). *IMPAD1* encodes a member of the inositol monophosphatase family, and the encoded protein is localized to the Golgi apparatus and catalyzes the hydrolysis of phosphoadenosine phosphate (PAP) to adenosine monophosphate (AMP). Once PAPS, the universal sulphur donor for most sulfotransferase reactions both in the cytoplasm and in the endoplasmic reticulum and Golgi

complex, donates its sulfate group to an acceptor, PAP is formed as a reaction by-product (see Fig. 1). Build-up of PAP, such as occurs in the deficiency of gPAPP, exerts a negative feedback upon substrate sulfation. Inhibition of sulfotransferase reactions by PAP has been demonstrated in vitro (Vissers et al 2011). Biochemical analysis of gPAPP −/− mice revealed that cartilage was deficient in chondroitin 4-sulfate and lung exhibited decreased levels of both chondroitin sulfate and heparan sulfate (Frederick et al 2008).

Biosynthesis and sulfation of KS

Similar to the synthesis of CS, DS, HS, and Hep, biosynthesis of KS starts in the cytosol with the activation of the sugars by linkage to nucleotide diphosphate, and continues in the endoplasmic reticulum and subsequently in the Golgi apparatus. However, KS biosynthesis follows a different pathway, starting with the synthesis of one of three possible types of linker (Fig. 1). These linkers are either N-linked to asparagine or O-linked to serine or threonine residues in the core protein. Unlike the tetrasaccharide linker that is used exclusively for the synthesis of CS, DS, HS, and Hep, each of the three linkers used for KS can be elongated with a number of other saccharides. Polymerization of KS to these linkers is in fact the exception rather than the rule, and is directed by consensus sequences found in the protein core (Funderburgh 2002). The different KS linkers are tissue-specific: type I is mostly found in cornea, type II in cartilage, and type III in brain. Since these linkers can also serve for addition of molecules other than GAGs, their synthesis is not discussed further (reviewed in (Funderburgh 2002). Polymerization of KS is carried out by two glycosyltransferases, which alternately add N-acetylglucosamine and galactose. O-sulfation of both sugar residues of the copolymer at position 6 is accomplished by two different sulfotransferases with partly overlapping substrate specificities (EC 2.8.2.21). Evidence suggests that sulfation of N-acetylglucosamine residues in corneal KS is crucial for efficient polymerization, as it occurs preferentially on the terminus of the growing chain (Degroote et al 1997; Uchimura et al 1998). and mutation of the responsible sulfotransferase leads to a concomitant reduction in KS chain length (Plaas et al 2001).

Biosynthesis of HA

In contrast with all other GAGs, HA lacks a linker, is not covalently attached to a core protein, is not sulfated and its synthesis takes place at the plasma membrane. Three hyaluronan synthase isoenzymes (HAS1-3; EC 2.4.1.212) have been described (Itano et al 1999; Itano and Kimata 2002). which differ with respect to their catalytic efficiencies and their product size. The longest HA chains are synthesized by HAS2, with an estimated molecular mass of over 2×10^6 Da. Hyaluronan synthases are integral membrane proteins that use UDP-glucuronate and UDP-N-acetylglucosamine as substrates to synthesize HA, which appears to be exported to the extracellular matrix through membrane pores at the same time that it is synthesized (Itano and Kimata 2002). Knocking out *HAS2* in mice results in severe cardiac and vascular abnormalities and is embryonically lethal, suggesting a crucial role for its reaction product as a component of the extracellular matrix during embryogenesis (Camenisch et al 2000). By contrast, null mice deficient in either *HAS1* or *HAS3* are viable and fertile (Itano and Kimata 2002).

Physiological roles of the different GAGs

Chondroitin sulfate is involved in the regulation of assembly and maintenance of the extracellular matrix (ECM) and participates in cell proliferation through interaction with growth factors, including: fibroblast growth factors, hepatocyte growth factors, brain-derived growth factor, pleiotrophin, and midkine (Sugahara et al 2003; Kwok et al 2012; Mikami and Kitagawa 2013). CS is a major component of the ECM of cartilage. The tightly packed and highly charged sulfate groups of chondroitin sulfate generate electrostatic repulsion that provides much of the resistance of cartilage to compression (Gupta 2012; Mikami and Kitagawa 2013). Several CS-containing proteoglycans are known to regulate collagen fibrillogenesis and to play an important role in the physiology and biomechanical function of tendons and ligaments (Halper 2014). Recent data also indicate an important role for a CS-containing proteoglycan, biglycan, in cardiovascular disorders, including aortic valve stenosis and aortic dissection (Halper 2014).

Dermatan sulfate is expressed in many mammalian tissues. It is the predominant GAG of skin, but is also found in blood vessels, heart valves, tendons, and lungs (Gupta 2012). This tissue distribution coincides with the involvement of DS in cardiovascular disease, tumorigenesis, infection, wound healing, and fibrosis (Trowbridge and Gallo 2002).

Heparan sulfate is found at the cell surface and in the ECM and binds to a plethora of ligands, regulating a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation, cell-ECM attachment, cell–cell interactions, and cell motility (Sarrazin et al 2011). HS-containing proteoglycans can also act as receptors for proteases and protease inhibitors, regulating their spatial distribution and activity (Sarrazin et al 2011).

Heparin sulfate ("unfractionated heparin") is a highly sulfated glycosaminoglycan and has the highest negative charge density of any known biological molecule (Nelson and Cox 2004). Heparin is usually stored within the secretory granules

of mast cells and released only into the vasculature at sites of tissue injury. It is widely used clinically as an anticoagulant. It has been proposed that the main physiological role of heparin is regulation of mast cell mediators such as histamine and the mast cell–specific proteases (Forsberg and Kjellen 2001) against invading bacteria and other foreign materials (Nader et al 1999).

Keratan sulfate occurs as three different types, depending on the identity of the linker: type I in the cornea, II in cartilage, and III in the brain (Funderburgh 2002). The cornea is unique amongst collagen-rich connective tissues because it is transparent to visible light, a property conferred by regularly spaced and uniformly thin collagen fibrils, established and maintained by KS I containing proteoglycans (Quantock et al 2010).

Hyaluronic acid is widely distributed throughout connective, epithelial and neural tissues. It is one of the chief components of the ECM. It contributes significantly to cell proliferation and migration, and may also be involved in the progression of some malignant tumors (Stern 2009).

Inborn errors of GAG biosynthesis

Most GAG-SIEMs are very rare, some with only a few cases reported in the literature. The exceptions are those conditions with very specific clinical symptoms and signs, such as hereditary multiple exostoses, with a prevalence of 1/50,000 (Schmale et al 1994). as well as macular corneal dystrophy and diastrophic dysplasia, for which no reliable incidences have been established so far. Some of the GAG-SIEMs may actually be under-diagnosed or classified as collagenopathies.

The characteristics and tissue distribution of individual GAGs coincide to some extent with the observed clinical phenotypes. The major clinical features (Table 1) are short long bones, joint dislocations or laxity and scoliosis; additional suggestive features are skin laxity with or without atrophy and blue sclerae. Representative clinical features and specific presentations of GAG-SIEMs are depicted in Fig. 2. A more detailed clinical description and differential diagnosis are presented in Supplementary Tables 1 and 2.

To date, strong evidence for causality has been presented for 20 GAG-SIEMs, due to mutations in genes encoding 16 proteins involved in GAG biosynthesis (Table 1). Some of these conditions have been previously classified as O-glycosylation disorders, which is a general classification based on the particular chemical reaction taking place, rather than on a specific biochemical pathway. As such, all defects in the glycosyltranserases participating in the synthesis of GAGs can be considered O-glycosylation disorders.

In the following section, we describe each of the 20 proven GAG-SIEMs. Mutations in additional genes of GAG biosynthesis have been reported in other clinical conditions but causality is disputable; these are mentioned briefly. Finally, based upon available clinical descriptions, we propose a first clinical approach to GAG-SIEMs.

GAG-SIEMs due to defective linker biosynthesis

Six known conditions arise from enzymatic defects in the biosynthesis of the common tetrasaccharide linker that is necessary for the synthesis of HS/Hep and of CS/DS. The first sugar is attached by XylT transferases I and II, encoded by *XYLT1* and *XYLT2,* respectively. XylT transferases I and II have very similar functions and are co-expressed in many tissues with some temporal, spatial, and tissue-specific differences that may explain the distinct clinical phenotypes (Hinsdale 2014). The phenotype associated with XylT-I deficiency is described as the *Desbuquois dysplasia type 2 (MIM: 615777),* an autosomal recessive condition characterized by chondrodysplasia, laxity, and dislocation of large joints, severe pre- and postnatal growth retardation, and flat face with prominent eyes (Bui et al 2014). The small number of patients with XYLT-II deficiency showed spondylo-ocular syndrome-like osteoporosis induced fractures, cataracts, sensorineural hearing loss, and heart defects without shortened long bones or joint laxity (Munns et al 2015).

GalT-I and GalT-II, the enzymes that add the second and third sugars, present with almost identical clinical phenotypes: *Ehlers-Danlos syndrome, progeroid form types I (MIM: 130070) and II (MIM: 615349)*. The main characteristics of these two autosomal recessive disorders are bone, joint, and skin involvement with progeroid features, growth retardation, and developmental delay. Typically, cardiac examination is normal. In addition, type II EDS, progeroid form, presents platyspondyly, scoliosis, metaphyseal flaring as well as proptosis and blue sclerae (Sellars et al 2014; Cartault et al 2015).

Mutations in the gene encoding GalT-II can also cause a unique severe connective tissue syndrome with features of Ehlers-Danlos syndrome progeroid form, spondyloepimetaphyseal dysplasia, early-onset bone fractures, and intellectual disability, designated *spondyloepimetaphyseal dysplasia with joint laxity, type 1, with or without fractures (MIM: 271640)*. Characteristic skeletal abnormalities are progressive severe kyphoscoliosis, thoracic asymmetry with respiratory compromise resulting in early death, short ilia, elbow malalignment, and laxity and joint contractures (Beighton and Kozlowski 1980; Smith et al 1999; Malfait et al 2013; Nakajima et al 2013). Patients can also present congenital cardiac disease and dysmorphic features.

Deficiency of GlcAT-1, the enzyme that completes the synthesis of the linker, causes *Larsen-like syndrome (MIM: 245600)*. This autosomal recessive condition is characterized by scoliosis, osteopenia, laxity, and dislocation particularly of large joints (knee, hip, elbow), and by dysmorphic features:

Table 1 Main clinical features of GAG-SIEMs. +, present; −, absent. Gray-shaded boxes highlight major associations between clinical signs and biochemical groups

Group	Disease, inheritance, gene, MIM	Main Clinical Features			Other Clinical Features		
		Short long bones	Joint laxity, dislocations	Scoliosis, kyphoscoliosis	Blue sclerae	Skin laxity, atrophy	Specific features
(Linker) Glycosyltransferases	Spondyloocular syndrome, AR, *XYLT2* MIM: 605822	-	-	-	-	-	Osteoporosis Cataracts Hearing loss
	Desbuquois dysplasia type 2, AR, *XYLT1* MIM: 615777	+	+	-	+	+/-	
	EDS, progeroid form type I, AR, *B4GALT7* MIM: 130070	-	+	-	-	+	
	EDS, progeroid form type II, AR, *B3GALT6* MIM: 615349	-	+	+	+	+	
	Spondyloepimetaphyseal dysplasia with joint laxity, type 1, with or without fractures, AR, *B3GALT6* MIM: 271640	+	+	+	+	+	
	Larsen-like syndrome, AR *B3GAT3* MIM: 245600	-	+	+	+	+	
Transporter	Schneckenbecken dysplasia, AR, *SLC35D1* MIM: 269250	+	-	-	-	-	
Glycosyltransferases (Polymerases)	Temtamy preaxial brachydactyly syndrome (TPBS), AR, *CHSY1* MIM: 605282	-	-	-	-	+/-	Preaxial brachydactyly
	Hereditary multiple exostoses (HME), AD, *EXT1* and *EXT2* MIM: 133700	-	-	-	-	-	Multiple exostoses
	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), AR, *POMGNT1* MIM: 253280	-	-	-	-	-	Muscular dystrophy
Epimerases	EDS, musculocontractural type2 (EDSMC2), AR, *DSE,* MIM: 615539	-	+	+	+	+	
Specific sulfotransferases	EDS, musculocontractural type1 (EDSMC1), AR, *CHST14* MIM: 601776	-	+	+	+	+	
	Spondyloepiphyseal dysplasia with congenital joint dislocations, AR, *CHST3* MIM: 143095	+	+	+	-	-	
	Macular corneal dystrophy type I and II, AR, *CHST6* MIM: 217800	-	-	-	-	-	Corneal mascular dystrophy
Multiple sulfotransferases	Epiphyseal dysplasia, multiple, 4, AR, *SLC26A2* MIM: 226900	+	-	+	-	-	
	Diastrophic dysplasia, AR *SLC26A2* MIM: 222600	+	+	+	-	-	
	Achondrogenesis IB, AR, *SLC26A2* MIM: 600972	+	-	+/-	-	-	
	Atelosteogenesis type II, AR, *SLC26A2* MIM: 256050	+	-	+	-	-	
	Brachyolmia 4 with mild epiphyseal and metaphyseal changes, AR, *PAPSS2* MIM: 612847	+	-	+	-	-	
	Chondrodysplasia with joint dislocations, GPAPP type, AR, *IMPAD1* MIM: 614078	+	+	-	-	-	

large fontanelle, hypertelorism, downslanding palpebral fissures, microretrognatia, and cleft palate (Baasanjav et al 2011; von Oettingen et al 2014; Budde et al 2015; Jones et al 2015). The reduced GlcAT-I activity impairs skeletal as well as heart development. Associated heart malformations include mitral valve prolapse, ventricular septal defect, and bicuspid aortic valve (Baasanjav et al 2011).

Classical Larsen syndrome (MIM: 150250) is an autosomal dominant condition due to mutations in *FLNB* encoding filamin B. Interestingly, the causal proteins in these two

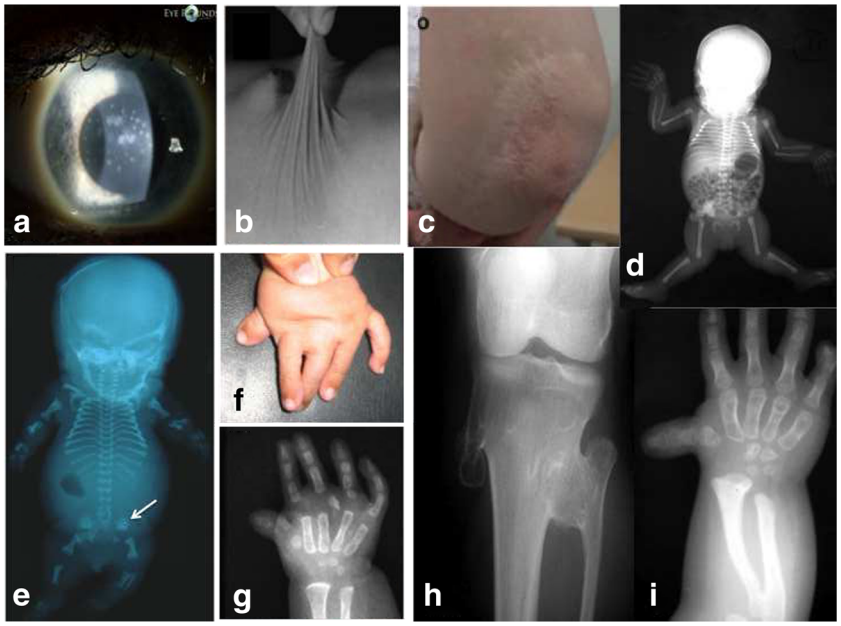


Fig. 2 Important clinical features and specific presentations of GAG biosynthesis defects. **a** Macular corneal dystrophy. Note diffuse clouding of the stroma and fleck-like opacities (reprinted with permission from EyeRounds.org, copyright University of Iowa). **b** Skin laxity in a *D4ST1*-deficient EDS patient (courtesy of Tomoki Kosho (2013). Discovery and delineation of dermatan 4-O-sulfotransferase-1 (D4ST1)-deficient Ehlers-Danlos syndrome, Current Genetics in Dermatology, Dr. Naoki Oiso (ed.), ISBN: 978-953-51-0971-6, InTech, DOI: 10.5772/55026, under the Creative Commons Attribution License (CC-BY). Available from: http://www.intechopen.com/books/current-genetics-in-dermatology/discovery-and-delineation-of-dermatan-4-o-sulfotransferase-1-d4st1-deficient-ehlers-danlos-syndrome). **c** Atrophic scars, *D4ST1*-deficient EDS patient (courtesy of Tomoki Kosho (2013). Discovery and delineation of dermatan 4-O-sulfotransferase-1 (D4ST1)-deficient Ehlers-Danlos syndrome, Current Genetics in Dermatology, Dr. Naoki Oiso (ed.), ISBN: 978-953-51-0971-6, InTech, DOI: 10.5772/55026, under the Creative Commons Attribution License (CC-BY). Available from: http://www.intechopen.com/books/current-genetics-in-dermatology/discovery-and-delineation-of-dermatan-4-o-sulfotransferase-1-d4st1-deficient-ehlers-danlos-syndrome). **d** Congenital joint dislocations. Shown are bilateral knee and radial head dislocations in a *CHST3*-deficient patient (reprinted with permission from Genereview, Andrea Superti-Furga and Sheila Unger, CHST3-related skeletal dysplasia, ncbi.nlm.nih.gov/books/NBK1116/) and copyright: University of Washington, Seattle). **e** Schneckenbecken dysplasia: micromelia, platyspondyly, thoracic hypoplasia, snail-like appearance of ilia (reprinted with permission from the BMJ Publishing Group, Furuichi T et al Identification of loss-of-function mutations of *SLC35D1* in patients with Schneckenbecken dysplasia, but not with other severe spondylodysplastic dysplasias group diseases, J Med Genet. 2009 Aug;46(8):562–8). **f** Preaxial brachydactyly of hand (courtesy of Temtamy and Aglan, *Orphanet Journal of Rare Diseases* 2008 3:15 doi:10.1186/1750-1172-3-15, under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0). **g** X-ray of hand in **f**, showing preaxial brachydactyly and hyperphalangism (courtesy of Temtamy and Aglan, *Orphanet Journal of Rare Diseases* 2008 3:15 doi:10.1186/1750-1172-3-15, under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0). **h** Hereditary multiple exostoses (reprinted with permission from of Radiopaedia.org, contributors: Dr. Y. Weerakkody and Dr. F. Gaillard, under the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported licence (http://creativecommons.org/licenses/by-nc-sa/3.0/). **i** Diastrophic dysplasia, the hitch-hiker thumb (reprinted with permission from of Radiopaedia.org, case of Dr. Paula Brill, donated by Radswiki.net, under the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported licence (http://creativecommons.org/licenses/by-nc-sa/3.0/)

conditions influence the interactions between cellular membranes and the extracellular matrix, and may be part of the same functional complex (Feng and Walsh 2004).

GAG-SIEM due to defective sugar transport

Schneckenbecken dysplasia (MIM: 269250), which results from deficiency of the UDP-GalNAc/UDP-GlcA transporter that supplies activated sugars for CS synthesis, is characterized by severe bone dysplasia (spine, iliac bones, limbs) without joint or skin involvement (Hiraoka et al 2007; Freeze and Schachter 2009; Furuichi et al 2009) and is lethal in the neonatal period. Midfacial hypoplasia and cleft palate are reported.

GAG-SIEMs due to defective GAG polymerases

There are three known conditions associated with enzymatic defects of GAG polymerization. *Temtamy preaxial brachydactyly syndrome (TPBS) (MIM: 605282)* is caused by the enzymatic deficiency in chondroitin synthase, the polymerase for CS. The main clinical symptoms implicate bone or joints of the extremities, without skin involvement, namely: bilateral symmetric preaxial brachydactyly, adducted thumb, hyperphalangism, symphalangism, syndactyly, and

clinodactyly. Some patients also present with neurosensory deafness and dysmorphic features like synophrys, hyperteloric appearance, macrophtalmia, prominent philtrum, and microdontia (Li et al 2010; Tian et al 2010).

Hereditary multiple exostoses (HME) (OMIM: 133700) is a disorder in which the HS polymerase heterodimer fails to form because of a mutation in either *EXT1* or *EXT2*. Clinically, the disease is limited exclusively to bone. Exostoses, which are benign cartilaginous-bony outgrowths, form next to growth plates (Fig. 2h), and can cause growth retardation and deformities, chronic pain, and impaired motion. The lifetime risk of osteochondrosarcoma is estimated as ~1 % (Nadanaka and Kitagawa 2008; Huegel et al 2013).

Because HS regulates the distribution and availability of several growth and signalling proteins, including members of the hedgehog, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and Wnt families, all of which are expressed in the growth plate (Kronenberg 2003; Huegel et al 2013). it is not surprising that HS influences many crucial processes in skeletogenesis and skeletal growth. The level of HS may be important for directing the ratio between peripheral and longitudinal bone growth (Jones et al 2013).

The POMGNT1 enzyme participates in O-mannosyl glycosylation, adding the first N-acetylglucosamine residue to the O-linked mannose linker in the synthesis of KS III, the brain-form of keratan sulfate, as well as in the synthesis of alpha-dystroglycan and other O-mannosylated proteins, in muscle (Yoshida et al 2001). Mutations in the *POMGNT1* gene encoding POMGnT1 transferase cause a phenotype spectrum of *Muscular dystrophy-dystroglycanopathy (MDDG)*: a severe congenital form with brain and eye anomalies *(type A3; MDDGA3, MIM: 253280),* formerly designated Walker-Warburg syndrome (WWS) or muscle-eye-brain disease (MEB); a less severe congenital form with mental retardation *(type B3; MDDGB3; MIM: 613151)*; and a milder limb-girdle form *(type C3; MDDGC3; MIM: 613157).* Muscle-eye-brain disease is characterized by: muscle dystrophy, cobblestone lissencephaly, cerebellar and brainstem anomalies, microphthalmia, cataracts, and optic nerve atrophy. Typically, there is no bone or joint involvement.

GAG-SIEMs due to defective GAG sulfation and epimerization

Synthesis of all GAGs except HA is completed by extensive sulfation at specific positions, catalyzed by multiple sulfotransferases. GAG-SIEM associated mutations have been described in three sulfotransferase genes. A very similar clinical phenotype arises from mutations in one of these three genes and in one gene encoding a GAG epimerase. Additionally, disorders have been associated with mutations in the genes coding for the DTDST sulfate transporter, PAPS synthase 2, and the gPAPP nucleotide phosphatase, all of which presumably affect the activity of multiple sulfotransferases by restricting substrate availability (see Fig. 1). Altogether, there are ten clinical entities resulting from any of these biochemical deficiencies.

*Ehlers-Danlos syndrome, musculocontractural types 1 (*EDSMC1*, MIM 601776) and 2 (*EDSMC2*, MIM 615539)* have similar clinical phenotypes. Both are caused by deficiency of a DS biosynthetic enzyme: D4ST sulfotransferase in EDSMC1 and DS epimerase in EDSMC2. Both syndromes have bone, joint, skin, cardiac, lung (pneumothorax), gastrointestinal, and genitourinary involvement (Muller et al 2013; Janecke et al 2015; Syx et al 2015). see Table 1. Some EDSMC2 patients also present with muscle weakness and cerebral atrophy (Muller et al 2013).

Spondyloepiphyseal dysplasia with congenital joint dislocations, (MIM: 143095) is due to deficiency of C6ST sulfotransferase, another enzyme of CS biosynthesis. The bone phenotype is more severe than in CS polymerase deficiency, involving spine and limbs, with additional congenital joint dislocation and osteoarthritis. Interestingly, there is also cardiac valve involvement (Rajab et al 2004; Thiele et al 2004; Hermanns et al 2008; van Roij et al 2008; Tuysuz et al 2009; Unger et al 2010).

Macular corneal dystrophy (MCD, MIM: 217800) is a stromal corneal dystrophy resulting from deficiency of corneal Gn6ST sulfotransferase encoded by the *CHST6* gene. Clinical signs are confined to the cornea (Funderburgh 2002; Quantock et al 2010; Pomin 2014). with progressive, initially central, superficial and irregular whitish fleck-like opacities that typically involve the limbus and the deep stroma down to the Descemet membrane. A diffuse haze of the entire corneal stroma progressively develops in parallel with the opacities. The cornea is much thinner than normal. As the disorder progresses, Descemet membrane becomes grayer and develops guttate excrescences (Weiss et al 2015). Severe visual impairment occurs between 10 and 30 years of age. Photophobia and painful recurrent erosions also rarely occur.

This pathology is correlated with undersulfation or complete absence of sulfation, and shorter chain length of corneal KS (Hassell et al 1980; Funderburgh 2002).

KS I diffusely accumulates throughout the corneal stroma. Unique among the stromal dystrophies is the primary involvement of Descemet membrane and endothelium in MCD. Electron microscopic corneal findings are similar in systemic mucopolysaccharidosis (MPS) and MCD, however, light microscopy and clinical examination reveals presence of flecks in MCD only (Weiss et al 2015).

Corneal grafting can restore vision in MCD, but the disease may recur in the graft after many years (Klintworth 2009).

DTDST sulfate transporter mutations result in four distinct medical conditions. *Multiple epiphyseal dysplasia type 4 (MIM: 226900), achondrogenesis type IB (MIM: 600972), atelosteogenesis type II (MIM: 256050), diastrophic dysplasia*

(MIM: 222600) are a spectrum of severe chondrodysplasias. Key findings include early lethality (achondrogenesis IB and atelosteogenesis type II), joint pain in hips and knees (multiple epiphyseal dysplasia type 4), joint contractures and "hitchhiker" thumb (diastrophic dysplasia, see Fig. 2i), cleft palate (all but multiple epiphyseal dysplasia type 4) and cystic swellings of the outer ear (multiple epiphyseal dysplasia type 4 and diastrophic dysplasia). The impairment in sulfate uptake into chondrocytes leads to depletion of cytoplasmic sulfate and of PAPS and to the synthesis of undersulfated proteoglycans (Superti-Furga 2003).

Brachyolmia 4 with mild epiphyseal and metaphyseal changes (MIM: 603005) is a consequence of mutations in the *PAPS2* gene, which is responsible for the synthesis of the universal sulfate donor. This condition is characterized by short and bowed lower limbs with early-onset osteoarthropathy and spine involvement, such as platyspondyly and scoliosis. Some patients have additional features including hyperandrogenism (Faiyaz ul Haque et al 1998; Noordam et al 2009; Miyake et al 2012).

Mutations in *IMPAD1,* encoding the Golgi-resident phosphoadenosine phosphate phosphatase, gPAPP, which processes PAP, a reaction by-product of the sulfation reaction (see previous section) are the cause of *chondrodysplasia with joint dislocations, GRAPP type (MIM: 614078),* which is characterized by congenital joint dislocations, chondrodysplasia with short stature, micrognathia and cleft palate, and a distinctive facies: high forehead, flat face and micrognathia (Vissers et al 2011; Nizon et al 2012).

Possible associations of GAG biosynthetic enzyme deficiencies and human disease

Mutations in additional genes in the GAG biosynthetic pathway, such as *GALNT1, DSEL*, and *HAS2,* have been described in association with other conditions, but causality has not been proven unequivocally.

Heterozygous mutations in the *GALNT1,* encoding GalNAcT-I transferase, were found in two patients with motor and sensory neuropathy (Saigoh et al 2011). However, there was no direct evidence for pathogenicity. One patient presented with intermittent postural tremor in childhood and motor and sensory neuropathy in adulthood; the other, with acquired idiopathic generalized anhidrosis and hemifacial palsy.

DSEL gene mutations were reported with diaphragmatic hernia (Zayed et al 2010). and with bipolar (Goossens et al 2003). and major depressive disorders (Shi et al 2011). A deletion encompassing the genes *CDH19, DSEL, TXNDC10, CCDC102B* found in the patient with diaphragmatic hernia was inherited from his mother who had a normal phenotype. The sequence of these genes was normal on the other allele. One other patient with congenital diaphragmatic hernia was found to be heterozygous for a *DSEL* gene mutation (c.1516G>A) predicted to be benign. The authors showed weak or absent expression of *DSEL* in murine diaphragm (Zayed et al 2010).

The 18q21q22 region was controversially linked with bipolar disorder and major depressive disorder (McMahon et al 1997; McQueen et al 2005; Nwulia et al 2007). Two cSNPs in *DSEL* (Y730C and I1113M) were present in heterozygous state in one and two patients respectively, in a cohort of 113 bipolar patients, and absent in 160 controls (Goossens et al 2003). However, there are no functional tests or segregation studies for the SNPs to support a pathogenic role for *DSEL* mutations in bipolar disease. An association was found between the recurrent early-onset major depressive disorder and a region 75 kb upstream of *DSEL*, but more confirmatory evidence is required to prove causality (Shi et al 2011).

The importance of *HAS2*, encoding HA synthase 2, in mouse heart development has been established (Camenisch et al 2000; Camenisch et al 2002). However no convincing report has proven that this gene is related to congenital heart disease in humans. A single patient from a cohort of 100 patients, with non-syndromic ventricular septal defect, carried a heterozygous *HAS2* mutation, which was absent in 250 controls (Zhu et al 2014). It was not specified whether the mutation occurred *de novo* or segregated in the family.

The association of HA with cancer is well-established (Stern 2009). Overexpression of any of the three HA synthases, which increases the HA level, has been associated with acceleration of tumor growth and metastasis (Necas et al 2008; Kobayashi et al 2010). Conversely, overexpression of hyaluronidases is also found in cancer (Stern 2009; Tian et al 2013). Hence, a balance between the production and the degradation of hyaluronic acid is perhaps implicated in maintaining a cancer free organism, but the mechanism is unknown.

Clinical approach to GAG-SIEM

Although the number of cases reported to date is limited, and thus clinical descriptions are probably incomplete for most of GAG-SIEMs, a few recurrent clinical features are apparent. Three major clinical signs are chondrodysplasia/short long bones, joint laxity/dislocations, and scoliosis/kyphoscoliosis. Other recurrent features are skin laxity and blue-colored sclera. The presence of more than one of these features in a single patient greatly increases the likelihood of a GAG synthetic defect. Also, certain GAG-SIEMs cause specific clinically identifiable syndromes. We discuss three main categories: patients with joint laxity and multiple dislocations, with or without skin laxity; patients with osteochondrodysplasia with or without scoliosis; and finally, patients with clinically distinct syndromes.

Evaluation should include a general physical examination including eye examination for blue sclerae, proptosis, myopia, lens dislocations, retinal detachment and anterior chamber abnormalities, examination of skin texture, translucency, extensibility and integrity; assessment of joint mobility and range, including determination of the Beighton score and a skeletal survey. Patients with clinical suspicion of an Ehlers-Danlos or cutis laxa-like disorder with additional features like contractures, failure to thrive, prematurely aged appearance, large fontanelle, hypertelorism, downslanting palpebral features, cleft palate, microretrognathia or normal molecular testing for typical causal genes, should be referred to a center experienced in the diagnosis and interdisciplinary management of these diseases.

Joint laxity and multiple dislocations with or without skin laxity

This clinical presentation is seen in ten of the 20 GAG synthesis disorders, seven of which also present skin laxity (see Table 1). Skin laxity in isolation, unassociated with joint laxity, has only been described in some patients with TPBS.

The differential diagnosis, apart from the GAG biosynthesis defects discussed, includes Larsen syndrome (*FLNB*, MIM: 150250), otopalatodigital syndromes (*FLNA*), Desbuquois dysplasia type 1 (*CANT1* MIM: 251450), spondyloepimetaphyseal dysplasia with joint laxity-2 (*KIF22, MIM:* 603546), immunodeficiency type 23 with skeletal dysplasia (*PGM3* MIM: 615816), Catel-Manzke syndrome (*TGDS* MIM: 616145), and Ehlers-Danlos syndromes (EDS). Except for EDS, these conditions are not characterized by skin laxity. Patients with joint laxity for whom standard molecular analyses are normal may be at particular risk for GAG-SIEMs, especially if skin laxity is present.

The skin in GAG-SIEMs is described as loose or redundant, resembling descriptions of cutis laxa rather than Ehlers-Danlos syndrome, in which the skin is hyperextensible but tight and elastic, returning quickly following extension. However, available clinical descriptions are not sufficient to draw a strong conclusion on this point. Cutis laxa is genetically heterogeneous. Hernias, emphysema and heart valve regurgitation are common, but not the osseous, ligamentous or other changes typical of GAG-SIEMs (Berk et al 2012).

Joint dislocations and progeria are seen in the progeroid forms of EDS (*B3GALT6* and *B4GALT7*), and dislocations on contractures are noted in musculocontractural types of EDS (*DSE* and *CHST14*). Dislocation of large joints constitutes a prominent sign of Larsen-like syndrome (*B3GAT3*).

Short long bones with or without scoliosis

Short long bones are present in many osteochondrodysplasias. Specific diagnosis of GAG-SIEMs and other osteochondrodysplasias is often possible from clinical and radiological features. Short long bones are a main feature of ten GAG-SIEMs, and additional joint laxity and dislocations are present in five of them (see Table 1).

When considering skeletal dysplasias with joint dislocations, it is clinically useful to consider them according to the severity of the skeletal dysplasia. Pertinent to the conditions discussed here and their differential diagnoses, we will attempt to rank these conditions from most to least severe. Desbuquois dysplasia type 2 (*XYLT1*) is very severe with prenatal onset of micromelia and congenital dislocation of large joints, followed by diastrophic dysplasia (*SLC26A2*), which is milder but easily recognizable at birth, and conditions with moderately short stature and joint dislocations, such as those caused by mutations in *IMPAD1, CHST3, B3GALT6*. In other conditions with joint laxity, while the long bones are not short, there are other signs of osteochondrodysplasia.

Scoliosis is present in 11 GAG synthetic disorders, never in isolation, but rather associated with short long bones (seven out of 11), joint laxity (seven out of 11) or with both (three out of 11, refer to Table 1). Joint laxity may impact on the progression of scoliosis. In patients without increased laxity, scoliosis may be accompanied by structural vertebral abnormalities including platyspondyly, vertebral body coronal cleft, ovoid vertebral bodies, and absence of ossification.

Clinically distinct GAG-SIEMs

Five GAG-SIEMs can be diagnosed based on their distinctive clinical and radiological findings: spondylo-ocular syndrome-like, temtamy preaxial brachydactyly syndrome, hereditary multiple exostoses, muscular dystrophy-dystroglycanopathy, and macular corneal dystrophy types I and II (Table 1, Fig. 2). The central nervous and corneal involvement in muscular dystrophy-dystroglycanopathy and macular corneal dystrophy types I and II are unique to date among GAG biosynthetic disorders. Osteoporosis induced fractures and cataract are distinctive for the newly described spondylo-ocular syndrome-like.

It is appropriate to emphasize that the clinical spectrum of each of the GAG-SIEM is incompletely known. The preceding clinical discussion provides a first sketch of this new group of inborn errors of metabolism, but at steps in GAG biosynthesis at which no inborn error is yet described, new and perhaps unexpected phenotypes may be discovered.

Biochemical testing for GAG-SIEM

Mutations in the many genes involved in GAG biosynthesis result in clinical features that are often nonspecific. Molecular or enzymatic assays for individual defects are

important for definitive diagnosis, but they are not convenient for screening of patients. In the clinical context of patients at increased risk of a large number of GAG-SIEMs, a simple and affordable laboratory test for use in screening would be welcome. However, such a biochemical test is not available at the present time. The clinician must thus rely on clinical molecular testing and biochemical testing performed in research laboratories to confirm the diagnosis. We discuss the different biochemical tests in blood, urine, and fibroblasts used in the identification of patients with GAG-SIEM (Supplementary Table 3). Tests performed in animal models or in other biological samples are not presented, as they are most often inconvenient for clinical diagnosis. These techniques are grouped in four categories: those involving incubation of cultured fibroblasts with an isotope-labeled substrate; techniques used for analysis of unlabeled GAGs from fibroblasts or culture supernatants; techniques used for the analysis of GAGs from urine or blood samples; and finally, those techniques with diagnostic potential for all GAG biosynthesis disorders through analysis of GAGs in urine or blood. With these techniques, it was possible to demonstrate in biological samples from GAG-SIEM patients a decreased expression, concentration or molecular weight of some proteoglycans, or an increased or decreased concentration of specific disaccharides derived from GAGs.

Fibroblast incubation with isotope-labeled substrate

With the exception of HA, GAGs are highly sulfated molecules. Consequently, incubation of fibroblasts in culture with sulfate molecules containing a radioactive or stable isotope of sulfur allows incorporation of this atom in the GAGs. In addition, ^{3}H-radiolabeled precursor sugars such as glucosamine can also be incorporated into the GAGs. Using these approaches, total GAGs can be measured directly in lysates of fibroblasts or can be analyzed individually following separation by gel electrophoresis (SDS-PAGE) or HPLC.

As GAGs are large molecules, techniques enabling their separation without enzymatic or chemical degradation to smaller fragments are mainly limited to SDS-PAGE. The use of HPLC requires a preliminary fragmentation step performed with enzymes that selectively degrade different GAGs: chondroitinase AC for CS, chondroitinase B for DS, heparanases for HS, keratanases for KS, heparinases for Hep, and hyaluronidases for HA. In most cases, the resulting disaccharides fragments are derivatized by reductive amination using the fluorophore 2-aminobenzamide, separated by HPLC, and detected by fluorescence. One group detected the resulting disaccharides directly by mass spectrometry (Dundar et al 2009). and the concentrations and proportions of unsulfated and sulfated CS- and DS- derived disaccharides were then used to demonstrate a biochemical enzymatic deficiency.

Primarily CS and DS have been studied, mainly because they are the most abundant GAGs in fibroblasts. Using such techniques, it was possible to biochemically characterize disorders of CS and DS biosynthesis, involving enzymatic defects in glycosyltransferases, sulfotransferases, and sulfate transporter (Quentin et al 1990; Rossi et al 1996; Superti-Furga et al 1996; Seidler et al 2006; Hermanns et al 2008; Dundar et al 2009; Bui et al 2014; Munns et al 2015).

Fibroblasts or culture supernatants

Direct analysis of GAGs from fibroblast lysates, or of GAGs secreted in the culture supernatant after incubation with serum-free media can be performed by several methods. In this case, there is consequently no in vitro labeling of the GAGs. As decorin is a proteoglycan containing approximately 95 % DS and 5 % CS, it is possible to estimate the biosynthesis of these two GAGs by specifically analyzing the GAG composition of decorin produced and secreted by fibroblasts. This type of analysis has been the most widely used to characterize GAG biosynthesis disorders.

In one method, altered migration of decorin was revealed in patients with deficient CS and DS synthesis following SDS-PAGE and immunoblotting, with or without prior digestion of the GAG fraction with chondroitinases. Enzymatic defects in glycosyltransferases, an epimerase and sulfotransferases have been demonstrated in this way (Miyake et al 2010; Baasanjav et al 2011; Muller et al 2013).

Alternatively, immunohistochemistry or immunofluorescence (FACS) techniques have been used to characterize defects in CS, DS biosynthesis, and also HS biosynthesis. In these cases, the primary antibodies were directed against the GAG residues, rather than the peptide fraction of the proteoglycan that contains them, followed by detection through immunofluorescence or immunohistochemistry. Defects in two glycosyltransferases and a polymerase could be thus characterized (Tian et al 2010; Baasanjav et al 2011; Munns et al 2015).

Another method consists of enzymatic digestion of GAGs with specific enzymes cleaving one or more types of GAGs into disaccharides, which can then be separated by HPLC and quantitated. With this technique, enzyme defects in the epimerization and specific sulfation of CS and DS were revealed (Thiele et al 2004; van Roij et al 2008; Miyake et al 2010; Muller et al 2013).

Urine or plasma-based assays

Because blood and urine are easier to obtain than fibroblasts and thus more convenient for diagnosis, they are the preferred specimens for biochemical testing in the clinical setting. Two studies have looked at GAGs in urine or blood of GAG-SIEM

patients, using enzymatic digestion followed by analysis of the resulting fragments by HPLC.

Analysis of disaccharides derived from enzymatic digestion of CS and HS in blood of patients with HS polymerase deficiency revealed that the amount of HS in plasma was decreased and the ratio of HS to CS was almost half of that in healthy individuals (Anower et al 2013). The results suggest that blood could be used as a diagnostic sample for this enzymatic deficiency.

Study of urinary GAGs in patients with C6ST deficiency (Thiele et al 2004) showed a reduction of 6-sulfated CS in urine from patients and an increase in the precursor disaccharides (unsulfated or sulfated at positions 2 and 4).

Urine or plasma: potential tests for all GAG biosynthesis disorders

Many of the techniques presented earlier could be adapted for the analysis of the other GAGs. As sulfation of GAGs is the final step in the synthesis of CS, DS, KS, HS, and Hep, techniques making use of the incorporation of isotopically labeled sulfate molecules into GAGs could potentially be applied to all these GAGs and consequently to all enzyme deficiencies. Regarding techniques involving analysis of fibroblasts or culture supernatants, the investigation of proteoglycans by SDS-PAGE and immunoblotting could be adapted for proteoglycans containing other types of GAGs, by using antibodies directed against those proteoglycans.

Finally, techniques using enzymatic digestion with specific glycosidases or chemical cleavage of GAGs, followed by analysis of the produced disaccharides by HPLC, could be applied to all GAGs including HA, which is the only unsulfated GAG. Several reports suggest that this approach is feasible. With digestion by specific glycosidases and HPLC analysis with mass spectrometry detection, it was possible to quantitate urine and plasma concentration of CS, DS, HS, and KS from controls and mucopolysaccharidosis patients (Tomatsu et al 2014a). Using chemical cleavage of GAGs by methanolysis, followed by HPLC and mass spectrometry detection, CS, DS, and HS were also quantitated in urine from controls and mucopolysaccharidosis patients (Zhang et al 2015). HA could also be quantitated in normal urine, along with CS and DS, by digestion with specific glycosidases and HPLC analysis with fluorescence detection (Toyoda et al 1991). It is worth mentioning that although mass spectrometry has been used to quantitate normal or increased concentration of GAGs in normal controls and patients with mucopolysaccharidoses (Oguma et al 2007). reviewed in (Tomatsu et al 2014b), only one group reported the use of mass spectrometry to quantitate decreased concentrations of GAGs in human samples, as seen in the context of GAG-SIEMs (Dundar et al 2009). Decreased and increased concentration of GAG-derived disaccharides by fluorescence detection, and the use of disaccharides ratios have proven useful for diagnostic confirmation of GAG-SIEMs. Current mass spectrometry techniques could perform as well or even better to quantitate decreased GAGs-derived disaccharides, but their application to detect low concentration of GAGs and discriminate between normal and GAG-SIEM samples has not yet been extensively validated.

Consequently, using the appropriate method, it should be possible, in the future, for the clinician to have access to a biochemical test allowing screening of GAG biosynthesis disorders from urine or blood samples.

Conclusion and perspectives

GAG biosynthesis has peculiar biochemical properties and widespread effects on cell signalling, prenatal development and connective tissue biology. Hereditary defects of GAG biosynthesis are a clinically diverse, incompletely described group of inborn errors. Biochemical testing is not currently available for routine diagnosis, and as such, careful documentation of all clinical signs, followed by targeted or non-targeted molecular analysis is probably the most suitable approach to diagnosis at the present time. Current efforts to develop a diagnostic test applicable to all or several of these conditions are discussed. Precise diagnosis permits genetic counseling and prenatal diagnosis if desired. Current treatment of GAG biosynthetic defects is limited to monitoring for potential complications, symptomatic treatment, and orthopaedic surgery. It is hoped that recognition of this group of inborn errors, and of the clinical features arising from abnormal GAG synthesis, will catalyze discussion of GAG biology and of potential therapies for this important new group of rare inborn errors.

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Compliance with ethics standards

Conflict of interest None.

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