



Title	Human Genetic Disorders Caused by Mutations in Genes Encoding Biosynthetic Enzymes for Sulfated Glycosaminoglycans
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Citation	Journal of Biological Chemistry, 288, 10953-10961 https://doi.org/10.1074/jbc.R112.437038
Issue Date	2013-04-19
Doc URL	https://hdl.handle.net/2115/52882
Rights	This research was originally published in Journal of Biological Chemistry. Mizumoto, Shuji; Ikegawa, Shiro; Sugahara, Kazuyuki. Human Genetic Disorders Caused by Mutations in Genes Encoding Biosynthetic Enzymes for Sulfated Glycosaminoglycans. 2013. vol.288 pp.10953-10961. © the American Society for Biochemistry and Molecular Biology.
Type	journal article
File Information	HUSCAP minireview 2nd revision submitted 130224.pdf



Human genetic disorders caused by mutations in the genes encoding biosynthetic enzymes for sulfated glycosaminoglycans*

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Running title: *Bone and skin disorders involving chondroitin and dermatan sulfate (58/60)*

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Key words: chondroitin sulfate; dermatan sulfate; heparan sulfate; glycosaminoglycan; proteoglycan; glycosyltransferase; sulfotransferase; bone; cartilage; skin.

SUMMARY (97/100)

A number of genetic disorders are caused by mutations in the genes encoding glycosyltransferases and sulfotransferases, enzymes responsible for the synthesis of sulfated glycosaminoglycan (GAG) side chains of proteoglycans including chondroitin sulfate (CS), dermatan sulfate (DS), and heparan sulfate. The phenotypes of these genetic disorders reflect disturbances of crucial biological functions of GAGs in human. Recent studies have revealed that mutations in the genes encoding CS- and DS-biosynthetic enzymes cause various disorders of connective tissues. This review focuses on growing glycobiological studies of recently described genetic diseases caused by disturbances of biosynthetic enzymes for sulfated GAGs.

INTRODUCTION

Proteoglycans (PGs) having linear polysaccharides as side chains are widely distributed in extracellular matrices and at cell surfaces (1-3). Chondroitin sulfate (CS) and dermatan sulfate (DS) chains are classified as sulfated glycosaminoglycans (GAGs) and covalently attached to the core proteins of PGs (1-3). PGs function in embryonic development and play roles in the pathological development of a number of diseases through the GAG chains (3-7). GAGs are modified by sulfation at various positions of hydroxy groups in each

constituent sugar residue and by epimerization of uronic acid residues during the biosynthetic process, resulting in enormous structural diversity, which is fundamental to a wide range of biological events involving GAGs (4). Thus, it is imaginable that the heritable disturbance of the fine structure of GAGs may cause a variety of diseases.

The backbones of CS and DS consist of repeating disaccharide building units of *N*-acetyl-D-galactosamine (GalNAc) and uronic acid, D-glucuronic acid (GlcUA) or L-iduronic acid (IdoUA). CS/DS hybrid chains with both CS and DS structural elements are often found in mammalian tissues, and modified by sulfate groups at C-2 of uronic acids, and C-4 and/or C-6 of GalNAc residues with various combinations (4). In recent years, most, if not all, glycosyltransferases/epimerases/sulfotransferases and related enzymes for GAG biosynthesis have been cloned and characterized (Table 1, Figures 1 and 2) (3, 7, 8), although their regulatory mechanism(s) at the transcriptional level largely are not yet understood. However, in addition to well established mucopolysaccharidoses and lysosomal storage diseases (9), which are characterized by the accumulation of undigested GAG fragments in lysosomes due to defective catabolism by mutated glycosidases and sulfatases, several genetic diseases caused by mutations of the genes encoding biosynthetic enzymes have recently been described. Examples include

hereditary multiple exostoses resulting from mutations in the *EXT1* and *EXT2* genes encoding the glycosyltransferases responsible for HS biosynthesis (10, 11), chondrodysplasias caused by the mutations in the sulfate transporter and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase-2 (12), and Ehlers-Danlos syndrome (EDS) progeroid form caused by mutations in *B4GALT7* encoding β 4-galactosyltransferase-7, resulting in the defect of DS chains (13-18). Accumulating evidence suggests that CS/DS-biosynthetic enzymes in addition to the above mentioned genes are crucial to bone development and skin integrity in humans (Table 2). This review will overview the biosynthetic mechanism for CS/DS chains and focus on genetic diseases which have been recently characterized from a glycobiological point of view in terms of disturbances to the biosynthesis of functional CS/DS chains.

Biosynthesis of Chondroitin Sulfate and Dermatan Sulfate Chains

GAG-protein Linkage Region — The newly synthesized core proteins of PGs are initially modified by glycosylation to form a common GAG-protein linkage region tetrasaccharide, GlcUA β 1-3galactose β 1-3galactose β 1-4xylose β 1- (GlcUA-Gal-Gal-Xyl-), attached to the serine residue(s) of the GAG attachment sites of the PGs in the endoplasmic reticulum and Golgi compartments (2, 4, 7). Each specific glycosyltransferase, β -xylosyltransferase (XylT) (19, 20), β 1,4-galactosyltransferase-I (GalT-I) (21, 22), β 1,3-galactosyltransferase-II (GalT-II) (23), and β 1,3-glucuronosyltransferase-I (GlcAT-I) (24), which are encoded by *XYLT1* (and *XYLT2*), *B4GALT7*, *B3GALT6*, and *B3GAT3*, respectively, transfer to the serine residue or growing glycan from the corresponding uridine diphosphate (UDP)-sugars including UDP-Xyl, UDP-Gal, and UDP-GlcUA (Table 1). The GAG-protein linkage region tetrasaccharides, GlcUA-Gal-Gal-Xyl-O-, of CS and HS might be synthesized by the same set of enzymes including XylT, GalTs, GlcAT-I, some of which may form a multi-enzyme complex such as a so-called GAGosome for HS synthesis (25, 26). Further, the sugar residues in the GAG linkage region are frequently modified by 2-O-phosphorylation (the xylose residue), and

sulfation at C-6 (the first galactose) and C-4 or C-6 (the second galactose) (2). The enzymes responsible for the phosphorylation and sulfation have been identified as FAM20B and chondroitin 6-O-sulfotransferase-1 (C6ST-1), respectively (27, 28). Although the biological functions of these modifications remain unclear, they influence, at least *in vitro*, the glycosyltransferase activities of GalT-I and GlcAT-I.

Repeating Disaccharide Region of CS/DS — Following completion of the building of the tetrasaccharide, GlcUA β 1-3Gal β 1-3Gal β 1-4Xyl β 1-serine, the first GalNAc residue is transferred to the GlcUA residue in the linkage region by β 1,4-N-acetylgalactosaminyltransferase-I (GalNAcT-I), resulting in the initiation of the synthesis of the repeating disaccharide region of CS/DS chains (Fig. 1, Table 1) (29-32). Alternatively, the addition of a GlcNAc residue to the linkage region by α 1,4-N-acetylglucosaminyltransferase-I (GlcNAcT-I) evokes HS biosynthesis (Fig. 1) (33-41). Thus, the transfer of the first hexosamine residue, α -GlcNAc or β -GalNAc, which is the fifth saccharide from the reducing terminal, is crucial in determining the type of GAGs as HS or CS. The biosynthesis of a HS chain on core proteins requires a cluster of acidic and hydrophobic amino acids located near the Ser-Gly of the GAG-attachment site (41). In addition, the sulfation of Gal residues in the GAG-protein linkage region, GlcUA-Gal-Gal-Xyl-O-, has been reported (2). The potential sites for sulfation are C-6 of the first Gal and C-4 or C-6 of the second Gal residue, which occurs in the linkage region of CS/DS, but not HS/Hep (2). These observations indicate that the amino acids of the core protein at the GAG attachment site, the sulfation of the linkage region and/or unknown additional factors may be involved in the selective assembly of CS and HS chains. Thus, the molecular mechanism of the enigmatic differential biosynthetic assembly of HS and CS chains at the GAG attachment sites remains to be a black box.

Thereafter, polymerization of the CS backbone occurs to construct the repeating disaccharide region consisting of -3GalNAc β 1-4GlcUA β 1- by enzymatic activities designated as CS-GlcAT-II and GalNAcT-II, and catalyzed by an enzyme

complex CS-polymerase composed of various combinations of the chondroitin synthase family including chondroitin synthase (ChSy), chondroitin polymerizing factor (ChPF) and the other four family members (Table 1) (42-48). ChSy consists of 802 amino acids with homology to β 3-galactosyltransferase and β 4-galactosyltransferase family members on the amino- and carboxy-terminal sides, respectively, and is a bifunctional glycosyltransferase with GalNAcT-II and CS-GlcAT-II activities required for the formation of the disaccharide unit (42). On the other hand, ChPF possesses only weak GalNAcT-II activity (43), whereas Yada *et al.* independently reported that ChPF has the both GalNAcT-II and CS-GlcAT-II activities, resulting in the designation of ChPF as chondroitin sulfate synthase-2 (CSS2) (47). In spite of the dual enzymatic activities of ChSy, ChSy itself cannot achieve polymerization reactions to build up the repeating disaccharide units of CS. However, the association of ChSy with ChPF results in a dramatic augmentation of both the glycosyltransferase activities of ChSy (43). Furthermore, this enzyme complex can polymerize a CS chain onto the linkage region tetrasaccharide attached to the core protein (43). Thus, ChPF may function as a chaperone, which confers on ChSy the stronger glycosyltransferase activities, or stabilizes ChSy by forming an enzyme complex ChSy/ChPF (43-45).

In the case of DS chains, DS-epimerase converts β GlcUA into α IdoUA by epimerizing the C-5 position of GlcUA residues after the formation of a chondroitin polymer as a precursor backbone (49-52).

Subsequently, the chondroitin and dermatan chains fully develop through sulfation catalyzed by chondroitin/dermatan 4-O-sulfotransferases (C4ST/D4ST) (53-57) or chondroitin 6-O-sulfotransferase (C6ST) (58, 59), which transfer the sulfate group from a sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the C-4 or C-6 positions of GalNAc residues in each chain, respectively (Fig. 2, Table 1). Disulfated disaccharide units, [GlcUA(2-O-sulfate)-GalNAc(6-O-sulfate)], [IdoUA(2-O-sulfate)-GalNAc(4-O-sulfate)], [GlcUA-GalNAc(4-O-, 6-O-disulfates)], and [IdoUA-GalNAc(4-O-, 6-O-disulfates)], are found infrequently but in significant amounts in various mammalian tissues and cells, and formed by the successive actions of uronosyl

2-O-sulfotransferase (UST) (60) and GalNAc 4-sulfate 6-O-sulfotransferase (GalNAc4S-6ST) (61), which transfers sulfate to the C-2 position of a uronic acid residue flanking the GalNAc(4-O- or 6-O-sulfate) and to the C-6 position of GalNAc(4-O-sulfate) formed by C4ST, respectively (Fig. 2, Table 1). Sulfation patterns arranged by disaccharide units including these units and non-sulfated and/or monosulfated units [GlcUA-GalNAc(4-O-sulfate), GlcUA-GalNAc(6-O-sulfate), IdoUA-GalNAc(4-O-sulfate) and IdoUA-GalNAc(6-O-sulfate)] in CS, DS, and CS/DS hybrid chains vary among cell types, tissues, developmental stages, and pathological conditions, resulting in enormous diversity, which is the structural basis of the biological functions of CS/DS chains (4, 5).

Human Disorders Affecting Skeleton and Skin Caused by Disturbance of Chondroitin Sulfate and Dermatan Sulfate Syntheses

GalT-I (B4GALT7) deficiency—Mutations of the GalT-I gene cause Ehlers-Danlos syndrome (EDS)-progeroid type (Table 2) (13-18). EDS is a heterogeneous group of heritable connective tissue disorders characterized by joint and skin laxity and tissue fragility. Namely, six major types [classical type, hypermobility type, vascular type, kyphoscoliosis type, arthrochalasia type, and dermatospraxis type (62)] and several minor types including the progeroid type are known (supplemental Table S1).

The characteristics of EDS-progeroid type (GalT-I deficiency) include an aged appearance, developmental delay, short stature, craniofacial dysmorphism, generalized osteopenia, defective wound healing, hypermobile joints, hypotonic muscles, and loose yet elastic skin (13-16). Fibroblasts from these patients with p.Arg270Cys mutation in GalT-I show reduced galactosyltransferase activity compared with control subjects, and synthesize de-glycanated decorin and biglycan core proteins in addition to their PG forms (16). It has also been demonstrated that p.Ala186Asp mutation markedly reduces GalT-I activity *in vitro*, whereas its effects on the biosynthesis of CS/DS and HS are much less pronounced (17). In addition, a drastic decrease in GalT-I activity and GAG biosynthesis caused by p.Leu206Pro and p.Arg270Cys mutations has been reported (63, 64). Interestingly, the reduction in GalT-I

activity caused by the p.Arg270Cys mutation results in a reduction in the sulfation of HS chains and a retardation of wound closure *in vitro* (18). Taken together, the phenotypes of EDS progeroid form caused by GalT-I mutations are attributable to defects in mainly DS and partially HS and/or CS chains.

GlcAT-I (B3GAT3) deficiency ——— A family with recessive inheritance and five affected individuals with joint dislocations mainly affecting the elbow, and congenital heart defects including a bicuspid aortic valve, was reported. A mutation (p.Arg277Gln) in the *B3GAT3* gene coding for GlcAT-I was identified for this Larsen-like syndrome family (65). Larsen syndrome is characterized by dislocations of the hip, knee, and elbow joints, equinovarus foot deformity, and craniofacial dysmorphism that includes hypertelorism, prominence of the forehead, a depressed nasal bridge, and a flattened midface (66, 67). The p.Arg277Gln mutation causes a drastic reduction in GlcAT-I activity in the patients' fibroblasts (~5% of control fibroblasts) (65). Although the wild-type GlcAT-I is located in the *cis* and *cis*-medial Golgi in control fibroblasts, the amount of mutant protein is markedly reduced as demonstrated by immunofluorescent staining using anti-GlcAT-I antibody, indicating that the mutant GlcAT-I may be produced to a lesser extent, be degraded, or be susceptible to a protease as compared with the wild-type (65). Furthermore, the mutation results in a decrease in the biosynthesis of GAGs. The fibroblasts from patients produce not only a PG form of decorin, which is secreted by the fibroblasts and has a single DS chain, but also DS-free decorin presumably bearing the linkage region trisaccharide stub, Gal β 1-3Gal β 1-4Xyl (65). Further, the numbers of CS and HS chains on the core proteins at the surface of the fibroblasts are reduced to 65% and 53% of those in control subjects, respectively (65). These observations suggest that the mutant GlcAT-I (p.Arg277Gln) cannot transfer GlcUA to the common GAG-protein linkage region tri-saccharide Gal-Gal-Xyl, resulting in a partial deficiency of CS, DS, and HS that presents as connective tissue disorders with heart defects, Larsen-like syndrome, B3GAT3-type.

More recently, another mutation p.Pro140Leu was found in a consanguineous family from the Nias island in Indonesia (68). These patients had skeletal phenotypes

characterized by disproportionate short stature, but no heart phenotype in contrast to the p.Arg277Gln mutation. A recombinant enzyme of the p.Pro140Leu mutation showed significant reduction in the enzymatic activity, reflecting the mutation that lies within the donor substrate-binding sub-domain of the catalytic domain of GlcAT-I. However, cultured lymphoblastoid cells show that defective synthesis is more pronounced for CS than for HS.

CSGALNACT1 deficiency ——— Two possible mutations in the *CSGALNACT1* gene encoding a protein with GalNAcT-I and -II activities are found in patients with Bell's palsy and unknown type of hereditary motor and sensory neuropathy (HMSN) (69). HMSNs are heterogeneous neurodegenerative disorders characterized by a progressive loss of functions in the peripheral sensory nerves (70). Its symptoms commonly include weakness, falls, and sensory loss often associated with cavus or planus foot deformity (70). Degeneration of myelin sheaths and/or axons causes paralytic amyotrophy predominantly involving distal limbs in association with hypo- or a-reflexia. The recombinant mutant proteins for p.His234Arg and p.Met509Arg, exhibit no GalNAcT-II activity, implying these mutations in *CSGALNACT1* and/or CS-PGs may be associated with pathogenetic mechanisms of the peripheral neuropathies (69). To further understand the neuropathy involving CS, knockout mice, *Csgalnact1*^{-/-} may be useful, although currently, the mice are reported only to show an abnormal development in cartilage (71, 72).

CHSY1 deficiency ——— The Temtamy pre-axial brachydactyly syndrome is an autosomal recessive congenital syndrome characterized by bilateral, symmetric pre-axial brachydactyly and hyperphalangism, facial dysmorphism, dental anomalies, sensorineural hearing loss, delayed motor and mental developments, and growth retardation. The disease is caused by mutations in chondroitin synthase 1 (*CHSY1*), including p.Gly19-Leu28del, p.Gly51Alafs*30, p.Gln69*, and p.Pro539Arg (73, 74). The knockdown of *CHSY1* in zebrafish suggests that it is involved in the signaling of bone morphogenetic protein (BMP) during the bone development (73). Tian *et al.* reported syndromic recessive pre-axial brachydactyly with partial duplication of

proximal phalanges caused by the *CHSY1* mutations (74). Furthermore, Wilson *et al.* recently demonstrated that *Chsy* knockout (*Chsy*^{-/-}) mice manifest brachypodism with a striking patterning defect in distal phalanges, chondrodysplasia, and a decrease in bone density (75). Associated with the digit-patterning defect are a reduction in CS and a shift in cell orientation. The expression of *Gdf5* (growth and differentiation factor 5), a member of the BMP family is altered during the earliest stages of joint formation of the *Chsy*^{-/-} mouse (75), indicating that *Chsy1* restricts *Gdf5* expression. These observations suggest that CHSY1 and/or CS chains are indispensable regulators of joint patterning and skeletal development, and that the *Chsy*^{-/-} mouse is a good animal model for human brachydactyly caused by *CHSY1* mutations.

C6ST-1 (CHST3) deficiency ——— A loss-of-function mutation in chondroitin 6-*O*-sulfotransferase-1 (C6ST-1) causes human spondyloepiphyseal dysplasia (SED), Omani type, a severe chondrodysplasia with major involvement of the spine (76-81). The original patients with SED, Omani type caused by a missense mutation p.Arg304Gln, showed a short stature, severe kyphoscoliosis, osteoarthritis in elbow, wrist, and knee joints, secondary dislocation of the large joints, rhizomelia, fusion of carpal bones, and mild brachydactyly (76, 77). Several of their clinical features including ventricular septal, mitral, and/or tricuspid defects, aortic regurgitations, deafness, and metacarpal shortening differ significantly from the original description of the disease in Turkish siblings (p.Tyr141Met and p.Leu286Pro) (78, 79). 6-*O*-Sulfation on GalNAc residues in CS chains was barely detected in fibroblasts and urine obtained from the patients (78). Furthermore, Sperti-Furga and his colleagues have demonstrated that additional *CHST3* mutations cause autosomal recessive Larsen syndrome, chondrodysplasia with multiple dislocations, humero-spinal dysostosis, and Desbuquois syndrome (80, 81). These observations suggest that the degree of the 6-*O*-sulfation deficiency in CS varies depending on the substituted amino acids in C6ST-1. The clinical spectra are similar to those seen in other skeletal dysplasias caused by defective sulfation of GAGs. Different pathological phenotypes may result from a relatively narrow clinical features and age-related descriptions of the same

conditions.

D4ST-1 (CHST14) deficiency ——— Koshio *et al.* reported six unrelated Japanese showing characteristic craniofacial features, multiple congenital contractures, progressive joint and skin laxity, and progressive multi-system complications, features partially similar to those of EDS, kyphoscoliosis type (VI) caused by a deficiency of lysyl hydroxylase (82, 83). Although lysyl hydroxylase activity was normal in these patients, homozygosity mapping of two independent consanguineous families identified *CHST14* encoding dermatan 4-*O*-sulfotransferase-1 (D4ST-1) harboring four mutations (p.Lys69*, p.Pro281Leu, p.Cys289Ser, and p.Tyr293Cys) (84). A recombinant mutant D4ST-1 showed no D4ST activity (84). In addition, the fibroblasts from the patients showed a marked reduction in sulfotransferase activity (84). Surprisingly, CS chains but not dermatan were produced as a decorin side chain by the fibroblasts (84). In fact, 4-*O*-sulfations in CS and DS chains act as a block to prevent DS-epimerase from re-equilibrating between GlcUA and IdoUA (50). Hence, the defect in D4ST-1 allows a back-epimerization reaction converting IdoUA to GlcUA to form chondroitin, followed by sulfation with chondroitin 4-*O*-sulfotransferase (C4ST), resulting in an aberrant shift from DS to CS synthesis, which may affect the formation or maintenance of adequate collagen bundles in the patients' dermal tissues (84).

Dünder *et al.* and Malfait *et al.* independently reported that the mutations in D4ST-1 caused adducted thumb-clubfoot syndrome (ATCS) and EDS, musculocontractural type (EDS-VIB) without a mutation of lysyl hydroxylase (85, 86). ATCS is an autosomal recessive disorder showing characteristic clinical features such as adducted thumb, clubfoot, craniofacial dysmorphism, arachnodactyly cryptorchidism, atrial septal defect, kidney defect, cranial ventricular enlargement, and psychomotor retardation as well as thin and translucent skin, joint instability, and osteopenia from birth to early childhood (87, 88). Five of the eleven patients with ATCS died in early infancy or childhood, indicating that ATCS patients may have more severe manifestations than patients with EDS, type VIB.

CONCLUSION

The cloning of cDNAs for the genes encoding enzymes involved in the biosynthesis of GAG chains during the last 15 years has led to a better understanding of not only the biosynthetic mechanism but also the functions of CS, DS and HS chains *in vivo*, which have been clarified by using model organisms such as nematodes, fruit flies, zebrafish, and knockout mice (7, 8, 89-91). Moreover, recent advances in the study of human genetic diseases of skeleton and skin achieved by the cooperative efforts of clinicians, molecular geneticists, and glycobiologists have revealed the importance of CS/DS side chains of PGs. Further understanding of the molecular pathogenesis involving CS and DS chains is essential to facilitate the development of therapeutics for these diseases.

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FOOTNOTES

*This work was supported in part by the Matching Program for Innovations in Future Drug Discovery and Medical Care (to K. S.) from The Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT); by a Grant-in-aid for Young Scientists (B) 23790066 (to S. M.) from the Japan Society for the Promotion of Science, Japan; by the Drs. Hiroshi Irisawa and Aya Irisawa Memorial Research Grant from the Japan Heart Foundation (to S. M.); and by a Grant-in-aid for Encouragement from the Akiyama Life Science Foundation (to S. M.).

¹The abbreviations used are: CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate; GAG, glycosaminoglycan; PG, proteoglycan; GlcUA, D-glucuronic acid; IdoUA, L-iduronic acid; GalNAc, N-acetyl-D-galactosamine; DSE, DS-glucuronyl C5-epimerase; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; C4ST, chondroitin 4-O-sulfotransferases; D4ST, dermatan 4-O-sulfotransferase; C6ST, chondroitin 6-O-sulfotransferase; GalNAc4S-6ST, N-acetyl-D-galactosamine 4-sulfate 6-O-sulfotransferase.

FIGURE LEGENDS

FIGURE 1. Schematic presentation of the biosynthetic assembly of the GAG backbones by various glycosyltransferases.

Each glycosyltransferase requires the respective UDP-sugar as donor substrate. Following the synthesis of specific core proteins, the synthesis of the so-called GAG-protein linkage region, GlcUA β 1-3Gal β 1-3Gal β 1-4Xyl β 1-O- common to CS/DS and HS/Hep chains, is initiated by XylT, which transfers a Xyl residue from UDP-Xyl to the specific serine (Ser) residue in the endoplasmic reticulum, and is completed by the consecutive addition of each sugar by GalT-I, GalT-II, and GlcAT-I, which are common to the biosynthesis of both CS and HS, in Golgi apparatus.

Following the completion of the synthesis of the linkage region, the first β GalNAc residue is transferred to the naked GlcUA residue in the linkage region by GalNAcT-I, which initiates the assembly of the chondroitin backbone. Subsequently, the repeating disaccharide region, [-3GalNAc β 1-4GlcUA β 1-]_n, is elongated by alternate additions of GlcUA and GalNAc residues from UDP-GlcUA and UDP-GalNAc catalyzed by CS-GlcAT-II and GalNAcT-II activities, respectively, of a hetero-complex (CS-polymerase) of formed with chondroitin synthase and chondroitin polymerizing factor.

On the other hand, the addition of α 1-4-linked GlcNAc to the linkage region by GlcNAcT-I initiates the assembly of the HS repeating disaccharide region [-4GlcNAc α 1-4GlcUA β 1-]_n. Then, the chain polymerization of the HS chain is catalyzed by HS-GlcAT-II and GlcNAcT-II activities of HS polymerase, which is a hetero-complex of EXT1 and EXT2.

The molecular mechanism of the differential biosynthetic assembly of HS and CS chains at the GAG attachment sites remains to be elucidated as details have been discussed in the text, and therefore the transfer reactions of the 5th sugar (1st amino sugar) are shown in this Fig. by two distinct dotted arrows.

After the formation of the chondroitin and heparan backbones, GAG chains are matured by

sulfation at various positions and epimerization at GlcUA residues.

Each enzyme (glycosyltransferase and/or epimerase), its coding gene, and the corresponding inherited disorder are described under the respective sugar symbols from top at each line. Sulfotransferases involved in the chain modifications are not included in this figure, and illustrated in Figure 2 (see also Table 2 for the inherited diseases of sulfotransferases).

FIGURE 2. A schematic diagram of the biosynthetic modification of CS/DS chains.

After the formation of the chondroitin backbone, [-4GlcUA β 1-3GalNAc β 1-], each sugar residue is modified with a number of sulfate groups. Sulfation occurs mainly at positions 4 and 6 of GalNAc and position 2 of GlcUA catalyzed by various sulfotransferases. All sulfotransferases transfer a sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a universal donor substrate to a specific position of GlcUA or GalNAc residue. C4ST and C6ST transfer sulfate to position 4 or 6 of GalNAc residues, resulting in the formation of A-units [GlcUA-GalNAc(4-*O*-sulfate)] and C-units [GlcUA-GalNAc(6-*O*-sulfate)], respectively. Further sulfation is catalyzed by GalNAc4S-6ST or UST, which is essential for the formation of highly sulfated disaccharide units, E-units [GlcUA-GalNAc(4-*O*-, 6-*O*-sulfate)] and D-units [GlcUA(2-*O*-sulfate)-GalNAc(6-*O*-sulfate)], respectively.

After the formation of the chondroitin backbone, DSE converts GlcUA into IdoUA by epimerizing the C-5 carboxy group, resulting in the formation of the dermatan backbone, composed of iO-units [-4IdoUA α 1-3GalNAc β 1-]. The position 4 of GalNAc residues are sulfated by a distinct 4-*O*-sulfotransferase, D4ST, forming iA-units [IdoUA-GalNAc(4-*O*-sulfate)]. Further sulfations of DS chains are infrequently achieved by GalNAc4S-6ST or UST common to CS chains.

The abbreviation of "i" in iA, iB, and iE stands for IdoUA. DSE, DS-C5-epimerase; C4ST, chondroitin 4-*O*-sulfotransferase; C6ST, chondroitin 6-*O*-sulfotransferase; D4ST, dermatan 4-*O*-sulfotransferase; D6ST, dermatan 6-*O*-sulfotransferase; UST, uronyl 2-*O*-sulfotransferase; GalNAc4S-6ST, GalNAc 4-sulfate 6-*O*-sulfotransferase; 2S, 4S, and 6S, 2-*O*-, 4-*O*-, 6-*O*-sulfate.

Table 1. Human CS and DS-biosynthetic enzymes.

Enzymes (Activity)	Coding genes (synonym)	Chromosomal location	mRNA accession No.
<u>Linkage region</u>			
Xylosyltransferase (XylT)	<i>XYLT1</i>	16p12.3	NM_022166
	<i>XYLT2</i>	17q21.33	NM_022167
β 4Galactosyltransferase-I (GalT-I)	<i>B4GALT7</i>	5q35.2-q35.3	NM_007255
β 3Galactosyltransferase -II (GalT-II)	<i>B3GALT6</i>	1p36.33	NM_080605
β 3Glucuronyltransferase-I (GlcAT-I)	<i>B3GAT3</i>	11q12.3	NM_012200
Xylose 2- <i>O</i> -kinase	<i>FAM20B</i> (<i>gxl1</i>)	1q25	NM_014864
Galactose 6- <i>O</i> -sulfotransferase	<i>CHST3</i> (<i>C6ST-1</i>)	10q22.1	NM_004273
<u>Repeating disaccharide region</u>			
Chondroitin synthase (GalNAcT-II, CS-GlcAT-II)	<i>CHSY1</i>	15q26.3	NM_014918
	<i>CHSY2</i> (<i>CSS3</i>)	5q23.3	NM_175856
	<i>CHSY3</i> (<i>CHPF2, CSGLCA-T</i>)	7q36.1	NM_019015
Chondroitin polymerizing factor (GalNAcT-II, CS-GlcAT-II)	<i>CHPF</i> (<i>CSS2</i>)	2q35	NM_024536
Chondroitin <i>N</i> -acetylgalactosaminyltransferase (GalNAcT-I, GalNAcT-II)	<i>CSGALNACT1</i>	8p21.3	NM_018371
	<i>CSGALNACT2</i>	10q11.21	NM_018590
Dermatan sulfate epimerase	<i>DSE</i>	6q22	NM_013352
	<i>DSEL</i>	18q22.1	NM_032160
Uronyl 2- <i>O</i> -sulfotransferase	<i>UST</i>	6q25.1	NM_005715
Chondroitin 4- <i>O</i> -sulfotransferase	<i>CHST11</i> (<i>C4ST-1</i>)	12q	NM_018413
	<i>CHST12</i> (<i>C4ST-2</i>)	7p22	NM_018641
	<i>CHST13</i> (<i>C4ST-3</i>)	3q21.3	NM_152889
Dermatan 4- <i>O</i> -sulfotransferase	<i>CHST14</i> (<i>D4ST-1</i>)	15q15.1	NM_130468
Chondroitin 6- <i>O</i> -sulfotransferase	<i>CHST3</i> (<i>C6ST-1</i>)	10q22.1	NM_004273
<i>N</i> -Acetylgalactosamine-4-sulfate- 6- <i>O</i> -sulfotransferase	<i>CHST15</i> (<i>GalNAc4S-6ST</i>)	10q26	NM_015892

Table 2. Genetic disorders caused by mutations affecting the biosynthesis of CS/DS side chains.

Gene (coded enzyme or protein)	Disorders	MIM No.	Clinical features	References
<i>SLC26A2</i> (DTDST)	Achondrogenesis type IB Atelosteogenesis type II Diastrophic dysplasia	600972 256050 222600	Lethal chondrodysplasia with severe under-development of skeleton, extreme micromelia, death before or immediately after birth.	92, 93
<i>PAPSS2</i> (PAPS synthase-2)	Multiple epiphyseal dysplasia, AR type Spondyloepimetaphyseal dysplasia, Pakistani type (PAPSS2 type) Hyperandrogenism Brachyolmia, AR type	226900 612847	Epiphyseal dysplasia and early onset osteoarthritis. Short, bowed lower limbs, enlarged knee joint, kyphoscoliosis, and mild generalized brachydactyly. Androgen excess, premature pubarche, hyperandrogenic anovulation, low level of serum, dehydroepiandrosterone, short trunk, kyphosis and scoliosis.	12, 94, 95
<i>SLC35D1</i> (UDP-GlcUA/UDP-GalNAc transporter)	Schneckenbecken dysplasia	269250	Neonatal lethal chondrodysplasia, platyspondyly with oval-shaped vertebral bodies, extremely short long bones with dumbbell-like appearance, small ilia with snail-like appearance.	96
<i>B4GALT7</i> (GalT-I)	EDS, progeroid form	130070	Developmental delay, aged appearance, short stature, craniofacial dysmorphism, generalized osteopenia.	13-18
<i>B3GAT3</i> (GlcAT-I)	Larsen-like syndrome, B3GAT3 type	245600	Joint dislocations mainly affecting the elbow, congenital heart defects such as bicuspid aortic valve, aortic root dilatation.	65
<i>CSGALNACT1</i>	Hereditary motor and sensory neuropathy, unknown type Bell's palsy	—	Intermittent postural tremor, reduction in compound muscle action potentials, acquired idiopathic generalized anhidrosis, hemi-facial palsy.	69
<i>CHSY1</i>	Temtamy pre-axial brachydactyly syndrome Syndromic recessive pre-axial brachydactyly	605282	Short stature, limb malformation, hearing loss.	73, 74
<i>CHST3</i> (C6ST-1)	Spondyloepiphyseal dysplasia, Omani type Chondrodysplasia with multiple dislocations Humero-spinal dysostosis Larsen syndrome, AR type Desbuquois syndrome	143095	Short stature, severe kyphoscoliosis, osteoarthritis (elbow, wrist and knee), secondary dislocation of large joints, rhizomelia, fusion of carpal bones, mild brachydactyly, metacarpal shortening, ventricular septal defect, mitral and tricuspid defects, aortic regurgitations, deafness.	76-81
<i>DSEL</i> (DS epimerase-2)	Bipolar disorder Depressive disorder Diaphragmatic hernia Microphthalmia	611125	Alternating episodes of depression and mania or hypomania, congenital malformation of the diaphragm.	97-99
<i>CHST14</i> (D4ST-1)	EDS, Kosho type EDS, musculocontractural type	601776	Craniofacial dysmorphism, multiple contractures, progressive joint and skin laxities, multisystem fragility-related	82-88

EDS, type VIB
Adducted thumb-clubfoot syndrome

manifestations, contractures of thumbs and feet, defects of heart,
kidney and intestine.

SLC26A2, solute carrier family 26 (sulfate transporter) member 2; DTDST, diastrophic dysplasia sulfate transporter; PAPSS2, 3'-phosphoadenosine 5'-phosphosulfate synthase 2; SLC35D1, solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter) member D1; B4GALT7, xylosylprotein beta 1,4-galactosyltransferase 7; B3GAT3, beta-1,3-glucuronyltransferase 3; CSGALNACT1, chondroitin sulfate *N*-acetylgalactosaminyltransferase 1; CHSY1, chondroitin sulfate synthase 1; CHST3, carbohydrate (chondroitin 6) sulfotransferase 3; DSEL, dermatan sulfate epimerase-like; CHST14, carbohydrate (*N*-acetylgalactosamine 4-*O*) sulfotransferase 14.

AR and EDS stand for autosomal recessive and Ehlers-Danlos syndrome, respectively.

FIGURE 1

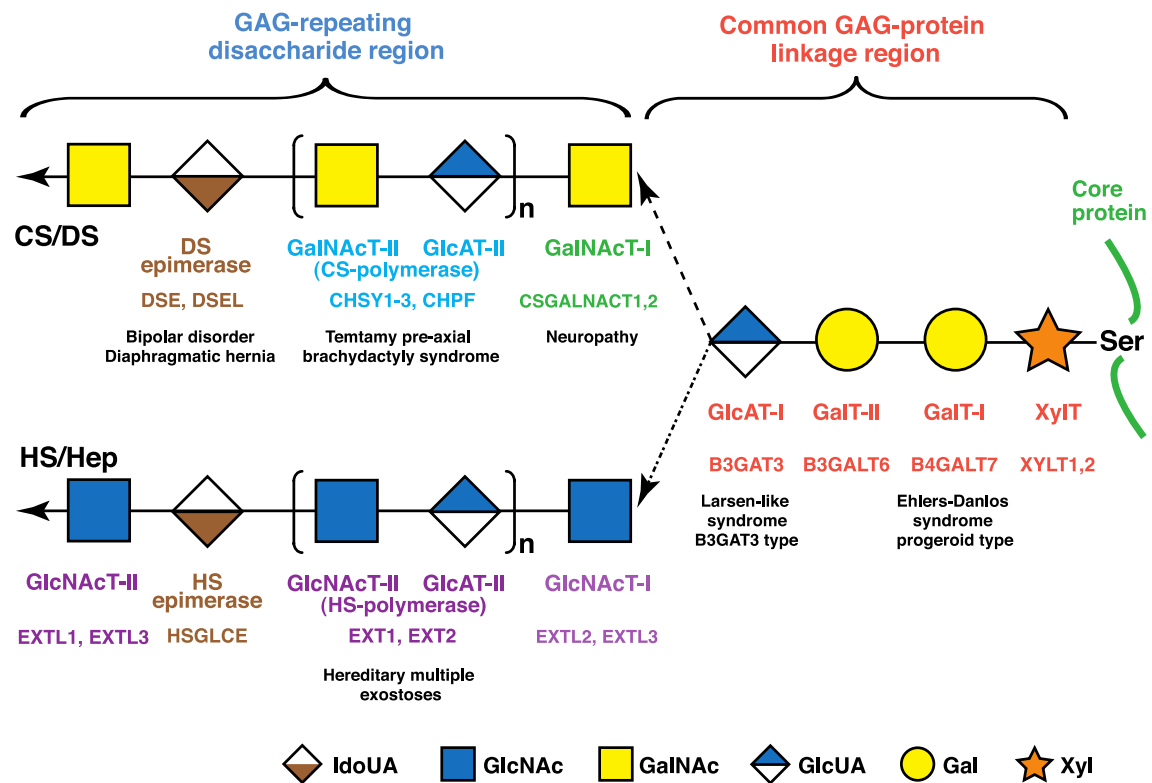


FIGURE 2

