Review Article

Human Genetic Disorders and Knockout Mice Deficient in Glycosaminoglycan

Shuji Mizumoto,1 Shuhei Yamada,1 and Kazuyuki Sugahara2

1 Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, 150 Yagotayama, Tempaku-ku, Nagoya 468-8503, Japan
2 Laboratory of Proteoglycan Signaling and Therapeutics, Frontier Research Center for Post-Genomic Science and Technology, Graduate School of Life Science, Hokkaido University, West-11, North-21, Kita-ku, Sapporo, Hokkaido 001-0021, Japan

Correspondence should be addressed to Shuji Mizumoto; mizumoto@meijo-u.ac.jp and Kazuyuki Sugahara; k-sugar@sci.hokudai.ac.jp

Received 9 May 2014; Accepted 8 June 2014; Published 13 July 2014

Academic Editor: George Tzanakakis

Copyright © 2014 Shuji Mizumoto et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Glycosaminoglycans (GAGs) are constructed through the stepwise addition of respective monosaccharides by various glycosyltransferases and maturated by epimerases and sulfotransferases. The structural diversity of GAG polysaccharides, including their sulfation patterns and sequential arrangements, is essential for a wide range of biological activities such as cell signaling, cell proliferation, tissue morphogenesis, and interactions with various growth factors. Studies using knockout mice of enzymes responsible for the biosynthesis of the GAG side chains of proteoglycans have revealed their physiological functions. Furthermore, mutations in the human genes encoding glycosyltransferases, sulfotransferases, and related enzymes responsible for the biosynthesis of GAGs cause a number of genetic disorders including chondrodysplasia, spondyloepiphyseal dysplasia, and Ehlers-Danlos syndromes. This review focused on the increasing number of glycobiological studies on knockout mice and genetic diseases caused by disturbances in the biosynthetic enzymes for GAGs.

1. Introduction

Glycosaminoglycans (GAGs) are covalently attached to the core proteins that form proteoglycans (PGs), which are ubiquitously distributed in extracellular matrix and on the cell surface [1–7]. GAGs are linear polysaccharides that form the side chains of PGs and have been classified into chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and heparin based on their structural units. The backbone of CS consists of repeating disaccharide units of N-acetyl-d-galactosamine (GalNAc) and d-glucuronic acid (GlcUA) (Figure 1). DS is a stereoisomer of CS and composed of GalNAc and l-iduronic acid (IdoUA) instead of GlcUA (Figure 1). They are often distributed as CS-DS hybrid chains in mammalian tissues [8]. On the other hand, HS and heparin consist of N-acetyl-d-glucosamine (GlcNAc) and GlcUA or IdoUA (Figure 1). The glucosamine (GlcN) residues in HS and heparin are modified by not only N-acetylation but also N-sulfation. These GAG chains are modified by sulfation at various hydroxy group positions and also by the epimerization of uronic acid residues during the biosynthetic process, thereby giving rise to structural diversity, which plays an important role in a wide range of biological roles including cell proliferation, tissue morphogenesis, infections by viruses, and interactions with various growth factors, cytokines, and morphogens [7–18].

Glycosyltransferases, epimerases, sulfotransferases, and related enzymes in the biosynthesis of GAGs have been cloned and characterized (Tables 1–4 and Figures 2 and 3) [6, 7, 14, 19]. Furthermore, genetic analyses using model animals including zebrafish, fruit flies, and nematodes have led to new findings on different phenotypes [4, 8, 9, 12, 13]. Human genetic disorders including bone and skin diseases caused by mutations in the genes encoding the biosynthetic enzymes for GAGs have recently been reported [7, 14, 20]. This review focused on recent advances in knockout mice for GAG biosynthesis, as well as cartilage and connective tissue disorders caused by disturbances in the biosynthesis of functional GAG chains.
2. Biosynthesis of 3′-Phosphoadenosine 5′-Phosphosulfate

The sulfation of GAGs is required for the exertion of their physiological functions. Sulfotransferases catalyze the transfer of sulfate from the donor substrate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to the corresponding acceptor substrates [21]. PAPS is synthesized from ATP and inorganic sulfate in the cytosol, and the reaction takes place in two sequential steps [21–23]. ATP sulfurylase first catalyzes the reaction between ATP and inorganic sulfate to form the biosynthetic intermediate, adenosine 5′-phosphosulfate (APS) [22, 23]. The formation of the active sulfate, PAPS, is then catalyzed by APS kinase, which involves a reaction between APS and ATP [22, 23]. ATP sulfurylase and APS kinase are encoded by the respective genes in bacteria, fungi, yeast, and plants [21]. On the other hand, both enzymes are fused in animals, resulting in a polypeptide designated PAPS synthase (PAPSS), which is a bifunctional enzyme composed of the N-terminal APS kinase domain and C-terminal ATP sulfurylase domain [21]. Following the formation of PAPS in the cytosol, PAPS is translocated into the Golgi by PAPS transporters [24].

3. Biosynthesis of GAG Chains

3.1. GAG-Protein Linkage Region. CS, DS, HS, and heparin chains are attached to serine residues in core proteins through the common GAG-protein linkage region tetrasaccharide, GlcUAβ1-3galactoseβ1-4galactoseβ1-xyloseβ1-O- (GlcUA-Gal-Gal-Xyl-O-) (Figure 2) [1, 5]. The transfer of a Xyl residue from uridine diphosphate (UDP)-Xyl to specific serine residues in the newly synthesized core proteins of PGs in the endoplasmic reticulum and cis–Golgi compartments is initiated by β-xylosyltransferase (XylT) (Figure 2 and Table 2) [25, 26]. β1,4-Galactosyltransferase-I (GalT-I), which is encoded by B4GALT7, then transfers a Gal residue from UDP-Gal to the Xyl-O-serine in the core proteins [27, 28]. β1,3-Galactosyltransferase-II (GalT-II), which is encoded by B3GALT6, transfers another Gal residue from UDP-Gal to the Gal-Xyl-O-serine [29]. Finally, β1,3-glucuronosyltransferase-I (Glcat-I), which is encoded by B3GAT3, transfers a GlcUA residue from UDP-GlcUA to the Gal-Gal-Xyl-O-serine (Figure 2 and Table 2) [30]. These enzymes may form a multienzyme complex such as the so-called GAGsome for GAG synthesizing enzymes for the construction of the linkage region [31, 32].

Several modifications including the 2-O-phosphorylation of the Xyl residue as well as sulfation at the C-6 position of the first Gal and at C-4 or C-6 of the second Gal residue have been reported [5]. GAG-Xyl kinase, encoded by FAM20B, Xyl phosphatase, encoded by ACPL2, and Gal-6-O-sulfotransferase, encoded by CHST3 (C6STI), have so far been identified (Table 2) [33–35]. These modifications affect the glycosyltransferase reactions of GalT-I and Glcat-I in vitro and may regulate the formation of GAG chains [36, 37].

3.2. Repeating Disaccharide Region of CS and DS. Chain polymerization of the repeating disaccharide region in CS and DS is initiated by β which is encoded by B3GALT6 and may form a multienzyme complex such as the so-called GAGsome for GAG synthesizing enzymes for the construction of the linkage region [31, 32].

The sulfation of GAGs is required for the exertion of their physiological functions. Sulfotransferases catalyze the transfer of sulfate from the donor substrate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to the corresponding acceptor substrates [21]. PAPS is synthesized from ATP and inorganic sulfate in the cytosol, and the reaction takes place in two sequential steps [21–23]. ATP sulfurylase first catalyzes the reaction between ATP and inorganic sulfate to form the biosynthetic intermediate, adenosine 5′-phosphosulfate (APS) [22, 23]. The formation of the active sulfate, PAPS, is then catalyzed by APS kinase, which involves a reaction between APS and ATP [22, 23]. ATP sulfurylase and APS kinase are encoded by the respective genes in bacteria, fungi, yeast, and plants [21]. On the other hand, both enzymes are fused in animals, resulting in a polypeptide designated PAPS synthase (PAPSS), which is a bifunctional enzyme composed of the N-terminal APS kinase domain and C-terminal ATP sulfurylase domain [21]. Following the formation of PAPS in the cytosol, PAPS is translocated into the Golgi by PAPS transporters [24].
| Transporters and enzymes | Coding genes (synonym) | Chromosomal location | mRNA accession number | MIM number | Human genetic disorders | Clinical features | References for the human diseases | References for the knockout mice |
|--------------------------|------------------------|----------------------|-----------------------|------------|------------------------|------------------|----------------------------------|----------------------------------|
| Solute carrier family 26 member A2 (diastrophic dysplasia sulfate transporter) | SLC26A2 (DTDST) | 5q31–q34 | NM_000112 | 600972 256050 222600 226900 | Achondrogenesis type IB Atelosteogenesis type II Diastrophic dysplasia Multiple epiphyseal dysplasia autosomal recessive type | Lethal chondrodysplasia with severe under-development of skeleton, extreme micromelia, death before or immediately after birth. Epiphyseal dysplasia and early onset osteoarthritis. | [38–40] | [41] |
| Solute carrier family 35 member D1 (UDP-GlcUA/UDP-GalNAc dual transporter) | SLC35D1 (UGTrel7) | 1p32-p31 | NM_015139 | Schneckenbecken dysplasia | Neonatal lethal chondrodysplasia, platyspondyly with oval-shaped vertebral bodies, extremely short long bones with dumbbell-like appearance, and small ilia with snail-like appearance. | [42] | [42] |
| PAPS synthase 2 | PAPSS2 | 10q24 | NM_004670 NM_001015880 | 612847 | Spondyloepimetaphyseal dysplasia Pakistan type (PAPSS2 type) Hyperandrogenism Brachydactyia autosomal recessive type | Short stature, brachydactyly, kyphoscoliosis, and mild generalized brachydactyly. Androgen excess, premature pubarche, hyperandrogenic anovulation, low level of serum, dehydroepiandrosterone, short trunk, kyphosis, and scoliosis. | [43–48] | [22, 23, 49–53] |
| 3'-Phosphoadenosine 5'-phosphate 3'-phosphatase | IMPAD1 (PAPP) | 8q1.1 | NM_017813 | 614078 614080 | Chondrodysplasia with joint dislocations GPAPP type | Short stature, chondrodysplasia, with brachydactyly, congenital joint dislocations, cleft palate, and facial dysmorphism. | [54] | [55] |

MIM: mendelian inheritance in man.
Among the several transporters and biosynthetic enzymes involved in PAPS and UDP-sugars, some of the mutations that occur have been shown to cause human genetic disorders and are listed here.
Table 2: Biosynthetic enzymes of the GAG-linkage region tetrasaccharide.

| Enzymes (activity) | Coding genes (synonym) | Chromosomal location | mRNA access number | MIM number | Human genetic disorders | Clinical features                                                                 | References for the human diseases | References for the knockout mice |
|--------------------|-------------------------|----------------------|--------------------|------------|------------------------|--------------------------------------------------------------------------------|----------------------------------|---------------------------------|
| Xylosyltransferase (XylT) | XYLT1                   | 16p12.3              | NM_022166          | 608124     | Desbuquois dysplasia type 2, Short stature syndrome | Short stature, joint laxity, advanced carpal ossification, and hand anomalies.       | [56–58]                          | [59]                            |
|                     | XYLT2                   | 17q21.33             | NM_022167          | 608125     |                        | —                                                                               | —                                | [60]                            |
| β4-Galactosyltransferase-I (GalT-I) | B4GALT7               | 5q35.2-q35.3         | NM_007255          | 130070     | Ehlers-Danlos syndrome progeroid type 1 Larsen of Reunion Island syndrome | Developmental delay, aged appearance, short stature, craniofacial dysmorphism, and generalized osteopenia. Multiple dislocations, hyperlaxity, dwarfism, and distinctive facial features. | [61–69]                          | —                               |
| β3-Galactosyltransferase-II (GalT-II) | B3GALT6                | 1p36.33              | NM_080605          | 271640     | Ehlers-Danlos syndrome progeroid type 2 Spondyloepimetaphyseal dysplasia with joint laxity type 1 | Sparse hair, wrinkled skin, defective wound healing with atrophic scars, osteopenia, and radial head dislocation. Spatulate finger with short nail, hip dislocation, elbow contracture, clubfoot, and mild craniofacial dysmorphism including prominent eye, blue sclera, long upper lip, and small mandible with cleft palate. | [70–72]                          | —                               |
| β3-Glucuronyltransferase-I (GlcAT-I) | B3GAT3                 | 11q12.3              | NM_012200          | 245600     | Larsen-like syndrome B3GAT3 type Multiple joint dislocations, short stature, craniofacial dysmorphism, and congenital heart defects | Joint dislocations mainly affecting the elbow, congenital heart defects such as bicuspid aortic valve, aortic root dilatation. | [73, 74]                         | [75, 76]                        |
| Xylose 2-O-kinase | FAM20B (GXXK)           | 1q25                 | NM_014864          | 611063     |                        | —                                                                               | —                                | —                               |
| Xylose 2-O-phosphatase | ACPL2 (XYLP)           | 3q23                 | NM_152282          | —          |                        | —                                                                               | —                                | —                               |

—, not reported.

B4GALT7, xylosylprotein beta 1,4-galactosyltransferase 7; B3GALT6, beta 1,3-galactosyltransferase 6; B3GAT3, beta 1,3-glucuronyltransferase 3; FAM20B, Family with sequence similarity 20 member B; ACPL2, acid phosphatase-like 2.
Table 3: Biosynthetic enzymes of CS and DS chains.

| Enzymes (activity) | Coding genes (synonym) | Chromosomal location | mRNA accession number | MIM number | Human genetic disorders | Clinical features | References for the human diseases | References for the knockout mice |
|--------------------|-------------------------|----------------------|-----------------------|------------|-------------------------|------------------|----------------------------------|-------------------------------|
| Chondroitin synthase (GalNAcT-II, CS-GlcAT-II) | CHSY1 | 15q26.3 | NM_014918 | 605282 608183 | Tentoymy preaxial brachydactyly syndrome | Short stature, limb malformation, hearing loss. | [77–80] | [81] |
|                   | CHSY2 (CHSY3, CSS3) CHSY3 (CHPF2, CSGLCA-T) | 5q23.3 | NM_0175856 | 609963 | | | — | — |
|                   | CHSY2 (CHSY3, CSS3) CHSY3 (CHPF2, CSGLCA-T) | 7q36.1 | NM_019015 | 608037 | | | — | — |
| Chondroitin-polymerizing factor (GalNAcT-II, CS-GlcAT-II) | CHPF (CSS2) | 2q35 | NM_024536 | 610405 | | | — | [81, 82] |
| Chondroitin N-acetylgalactosaminyltransferase (GalNAcT-I, GalNAcT-II) | CSGALNACT1 | 8p12.13 | NM_018371 | — | Hereditary motor and sensory neuropathy | — | — | — |
| Chondroitin N-acetylgalactosaminyltransferase (GalNAcT-I, GalNAcT-II) | CSGALNACT2 | 10q11.21 | NM_018590 | — | Unknown type Bell's palsy | — | — | [83] |
| Dermatan sulfate epimerase | DSE | 6q22 | NM_013352 | 615539 605942 | Ehlers-Danlos syndrome | Characteristic facial features, congenital contractures of the thumbs and the feet, hypermobility of finger, elbow, and knee joints, atrophic scarring of the skin, and myopathy. Alternating episodes of depression and mania or hypomania, and congenital malformation of the diaphragm. | [87] | [88, 89] |
| Dermatan sulfate epimerase | DSEL | 18q22.1 | NM_032160 | 611125 | Bipolar disorder | — | — | — |
| Uranyl 2-O-sulfotransferase | UST | 6q25.1 | NM_005715 | 610752 | | | — | — |
| Chondroitin 4-O-sulfotransferase | CHST11 (CAST-1) | 12q | NM_018413 | 610128 | | | — | — |
| Chondroitin 4-O-sulfotransferase | CHST12 (CAST-2) | 12q | NM_018641 | 610129 | | | — | — |
| Chondroitin 4-O-sulfotransferase | CHST13 (CAST-3) | 3q21.3 | NM_152889 | 610124 | | | — | — |
| Enzymes (activity) | Coding genes (synonym) | Chromosomal location | mRNA accession number | MIM number | Human genetic disorders | Clinical features | References for the human diseases | References for the knockout mice |
|-------------------|------------------------|----------------------|-----------------------|------------|-------------------------|------------------|-------------------------------|-------------------------------|
| Dermatan 4-O-sulfotransferase | CHST14 (D4ST-I) | 15q15.1 | NM_130468 | 601776 608429 | Ehlers-Danlos syndrome musculocontractural type 1 Adducted thumb-clubfoot syndrome | Craniofacial dysmorphism, multiple contractures, progressive joint and skin laxities, multisystem fragility-related manifestations, contractures of thumbs and feet, defects of heart, kidney and intestine. | [97–106] | [96, 107] |
| Chondroitin 6-O-sulfotransferase | CHST3 (C6ST-I) | 10q22.1 | NM_004273 | 143095 608799 | Spondyloepiphyseal dysplasia with congenital joint dislocations Spondyloepiphyseal dysplasia Omani type Chondrodysplasia with multiple dislocations Humerspinal dysostosis Larsen syndrome autosomal recessive type Desbuquois syndrome | 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. | [108–113] | [114–116] |
| N-Acetylgalactosamine-4-sulfate-6-O-sulfotransferase | CHST15 (GalNAc4S-6ST) | 10q26 | NM_015892 | 608277 | — | — | — | [117] |

—: not reported.
CSS: chondroitin sulfate synthase; DSEL: dermatan sulfate epimerase-like; CHST: carbohydrate sulfotransferase.
| Enzymes (activity) | Coding genes (synonym) | Chromosomal location | mRNA accession number | MIM number | Human genetic disorders | Clinical features | References for the human diseases | References for the knockout mice |
|-------------------|------------------------|----------------------|-----------------------|------------|------------------------|------------------|-------------------------------|-------------------------------|
| Exostosin (GlcA and GlcNAc transferases) | EXT1 | 8q24.11 | NM_000127 | 133700 215300 608177 | Exostoses multiple type 1 | Chondrosarcoma | The formation of cartilage-capped tumors (exostoses) that develop from the growth plate of endochondral bones, especially of long bones. | [118] [119–135] |
| | EXT2 | 11p12-p11 | NM_000401 | 133701 608210 | Exostoses multiple type 2 | Same as above. | | [118] [136] |
| Exostosin-like 2 (GlcNAc transferase-I) | EXT2 | 1p21 | NM_00439 | 602411 | — | — | — | [137, 138] |
| Exostosin-like 1 (GlcNAc transferase-II) | EXT1 | 1p36.1 | NM_004455 | 607138 | — | — | — | — |
| Exostosin-like 3 (GlcNAc transferase I and II) | EXT3 | 8p21 | NM_004440 | 605744 | — | — | — | [139] |
| GlcNAc N-deacetylase and N-sulfotransferase | NDST1 | 5q33.1 | NM_001543 | 600858 | — | — | — | — |
| | NDST2 | 10q22 | NM_003635 | 603268 | — | — | — | — |
| | NDST3 | 4q26 | NM_004784 | 603950 | — | — | — | — |
| | NDST4 | 4q26 | NM_022569 | 65039 | — | — | — | — |
| HS GlcUA C5-epimerase | GLCE | 15q23 | NM_015554 | 61234 | — | — | — | [169–172] |
| HS 2-O-sulfotransferase | HS2ST1 | 1p22.3 | NM_012262 | 604844 | — | — | — | [153, 162, 173–179] |
| HS 6-O-sulfotransferase | HS6ST1 | 2q21 | NM_004807 | 614880 604846 | Hypogonadotropic hypogonadism 15 with or without anosmia | Lack of sexual maturation and low levels of circulating gonadotropins and testosterone. | | [180] [177, 178, 181, 182] |
| | HS6ST2 | 15q26.2 | NM_147174 | 300545 | — | — | — | [182, 183] |
| | HS6ST3 | 15q32.1 | NM_153456 | 609401 | — | — | — | — |
| HS 3-O-sulfotransferase | HS3ST1 | 4p16 | NM_005114 | 603244 | — | — | — | [184] |
| | HS3ST2 | 16p12 | NM_006403 | 604056 | — | — | — | — |
| | HS3ST3A | 17p12 | NM_006402 | 604057 | — | — | — | — |
| | HS3ST3B | 17p12 | NM_006404 | 604058 | — | — | — | — |
| | HS3ST4 | 16p11.2 | NM_006400 | 604059 | — | — | — | — |
| | HS3ST5 | 6q22.31 | NM_153612 | 609407 | — | — | — | — |
| | HS3ST6 | 16p13.3 | NM_001009966 | — | — | — | — | — |
| HS 6-O-endosulfatase | SULF1 | 8q13.2-q13.3 | NM_005170 | 61002 | — | — | — | [185–191] |
| | SULF2 | 20q12-q13.2 | NM_018837 | 61003 | — | — | — | [185–192] |

---: not reported.
and DS chains is initiated by the transfer of the first GalNAc from UDP-GalNAc to the GlcUA residue in the linkage region tetrasaccharide, GlcUA-Gal-Gal-Xyl-O-, by β1,4-N-acetylglactosaminyltransferase-I (GalNAcT-I) (Figure 2) [193–196]. Alternatively, the transfer of a GlcNAc residue from UDP-GlcNAc to the linkage region tetrasaccharide by α1,4-N-acetylgalactosaminyltransferase-I (GalNAcT-I) is known to result in the initiation of the repeating disaccharide region of HS and heparin chains (Figure 2) [197–201]. Six chondroitin synthase family members have been identified including chondroitin synthases (ChSy), chondroitin-polymerizing factor (ChPF), and CSGalNAcTs (Figure 2 and Table 3) [193–196, 202–208]. ChSy1 is composed of 802 amino acids and is a bifunctional glycosyltransferase that exhibits CS-GlcAT-II and GalNAcT-II activities, which are required for the biosynthesis of the repeating disaccharide region, -4GlcUAβ1-3Galβ1-3Galβ1-4Xylβ1-, by XylT, which transfers a Xyl residue from UDP-Xyl to the specific serine (Ser) residue(s) at the GAG attachment sites. The linkage tetrasaccharide is subsequently constructed by GalT-I, GalT-II, and GlcAT-I. These four enzymes are common to the biosynthesis of CS, DS, HS, and heparin. The first β1-4-linked GalNAc residue is then transferred to the GlcUA residue in the linkage region by GalNAcT-I, which initiates the assembly of the chondroitin backbone, thereby resulting in the formation of the repeating disaccharide region, [-3GalNAcβ1-4GlcUAβ1-]n, by CS-polymerase. Alternatively, the addition of α1-4-linked GlcNAc to the linkage region by GlcNAcT-I initiates the assembly of the repeating disaccharide region [-4GlcNAcα1-4GlcUAβ1-]n of HS and heparin by HS-polymerase. Following the formation of the chondroitin and heparan backbones, both precursor chains are modified by sulfation and epimerization (see Figure 3). Each enzyme, its coding gene, and the corresponding inheritable disorder are described under the respective sugar symbols from the top of each line. SEMDJL1, spondyloepimetaphyseal dysplasia with joint laxity type 1.
Figure 3: Modification pathways of CS, DS, HS, and heparin. After formation of the GAG backbones, including chondroitin and heparan, each sugar residue is modified by sulfation, which is catalyzed at various positions by sulfotransferases, as indicated in the figure. C4ST and C6ST transfer a sulfate group from PAPS to the C-4 or C-6 position of the GalNAc residues in the CS chain, which results in the formation of A-units and C-units, respectively. Further sulfations are catalyzed by GalNAc4S-6ST or UST, which is required for the formation of disulfated disaccharide units, E-units and D-units, respectively. DS-epimerase converts GlcUA into IdoUA by epimerizing the C-5 carboxy group in the chondroitin precursor, thereby resulting in the formation of the dermatan backbone. D4ST, which is distinct from C4ST, transfers a sulfate group from PAPS to the C-4 position of the GalNAc residues in dermatan to form the IA-units. The disulfated disaccharide units, IB and IE, are infrequently synthesized by UST and GalNAc4S-6ST, which are the same enzymes as those responsible for the biosynthesis of B and E units in CS chains. Following the synthesis of the backbone of HS or heparin by HS polymerases, the first modifications, N-deacetylation and N-sulfation, are catalyzed by NDST. Some GlcUA residues are then converted to IdoUA residues by GLCE. Thereafter, the hydroxy groups at the C-2 of IdoUA and C-3 of N-sulfated glucosamine and/or GlcNAc are sulfated by specific sulfotransferases. The 6-O-desulfation of the N-sulfated GlcN residue in the HS and heparin chains occurs by the action of SULF in order to modify the fine structure of HS for the regulation of interactions with various signaling molecules. C4ST, chondroitin 4-O-sulfotransferase; C6ST, chondroitin 6-O-sulfotransferase; D4ST, dermatan 4-O-sulfotransferase; DSE, dermatan sulfate C5-epimerase; GalNAc4S-6ST, GalNAc 4-sulfate 6-O-sulfotransferase; GLCE, heparan sulfate C5-epimerase; HS2ST, heparan sulfate 2-O-sulfotransferase; HS3ST, heparan sulfate 3-O-sulfotransferase; HS6ST, heparan sulfate 6-O-sulfotransferase; NDST, N-deacetylase/N-sulfotransferase; UST, uronyl 2-O-sulfotransferase; SULF, 6-O-desulfotransferase.

3.3. Repeating Disaccharide Region of HS and Heparin. Following the construction of the linkage region tetrasaccharide, GlcUA-β1-3Gal-β1-4Xyl-β1-O-serine, on the core protein, transfer of the GlcNAc residue from UDP-GlcNAc to the tetrasaccharide induces chain polymerization of the repeating disaccharide region of HS and heparin catalyzed by GlcNAcT-I [197–201] (Figure 2). After the addition of the first GlcNAc to the linkage region, the growing pentasaccharide is further elongated by alternate additions of GlcUA and GlcNAc from UDP-GlcUA and UDP-GlcNAc by HS-β1,4glucuronyltransferase-II (HS-GlcAT-II) and α1,4-N-acetylglucosaminyltransferase-II (GlcNAcT-II), respectively (Figure 2). Exostosin 1 (EXT1) as well as 2 (EXT2) both exhibit HS-GlcAT-II and GlcNAcT-II activities [199, 224–226] (Table 4). Furthermore, the heterodimeric complex of EXT1 and EXT2 exhibits HS polymerase activity on a linkage region tetrasaccharide acceptor in vitro, which results in the biosynthesis of HS and heparin polysaccharides [227, 228]. Three homologous genes to the EXT have been identified [6, 14, 229]. EXTL1 and EXTL2 exhibit GlcNAcT-II and GlcNAcT-I activities, respectively, whereas EXTL3 has not only GlcNAcT-I, but also GlcNAcT-II activities (Figure 2 and Table 4) [200, 201].
After the formation of the repeating disaccharide backbone of HS chains by EXTs and EXTLs, GlcNAc residues are converted into GlcN residues by GlcNAc-N-deacetylase (Figure 3) [6, 14, 198]. A sulfate group is subsequently transferred from PAPS to the GlcN by GlcN-N-sulfotransferase [6, 14, 198]. Both enzymes are encoded by a single gene, GlcNAc N-deacetylase/N-sulfotransferase (Figure 3 and Table 4) [230–233]. The interconversion of GlcUA to IdoUA in HS and heparin is catalyzed by HS 2-O-sulfotransferase, HS 3-O-sulfotransferase, and HS 6-O-sulfotransferase, respectively (Figure 3 and Table 4) [237–244]. The desulfation of 6-O-sulfated GlcNS residues in HS chains by HS 6-O-endosulfatase modifies the fine structure of HS in order to regulate various biological events including cell signaling, tumor growth, and angiogenesis (Figure 3 and Table 4) [245–247].

4. Knockout and Transgenic Mice of GAG Biosynthetic Enzymes

4.1. Xylt1. A recessive dwarf mouse mutant (pug) obtained from an N-ethyl-N-nitrosourea mutagenesis screen was attributed to a missense mutation in Xylt1, which resulted in the substitution of an amino acid (p.Trp932Arg) [59]. XylT activity in the pug mutant was markedly reduced in vitro, which resulted in a decrease in the amount of GAGs in cartilage. Furthermore, early ossification was reported in this mutant, which resulted in a shorter body length than that of a wild-type embryo. These phenotypes may be caused by an upregulation of Indian hedgehog signaling but not MAPK signaling due to lack of GAGs [59].

4.2. B3gat3 (GlcAT-I). Mice deficient in GlcAT-I synthesize a smaller CS and HS chain in their blastocysts than that of the heterozygous mice [75]. In addition, these mice exhibit an embryonic lethality before the 8-cell stage due to the failure of cytokinesis, which has been attributed to a deficiency in CS, but not HS based on the findings reported in embryos treated with chondroitinase and heparinase [76]. Moreover, interaction of CS with E-cadherin, which regulates the differentiation of embryonic stem cells, may control Rho signaling pathway [76]. These findings indicated that CS, but not HS, is involved in regulating cell division in mammals.

4.3. Csgalnact1 and Csgalnact2. CSGalNActI-null mice have been shown to produce a smaller amount as well as a shorter length of CS chains than the wild-type [84, 85]. These mice also have shorter limbs and axial skeleton and a thinner growth plate in cartilage than wild-type mice, which results in a slightly shorter body length and smaller body weight [84, 85]. It is likely that the reduction in CS may affect normal chondrogenesis and formation of type II collagen fibers [84]. These findings suggest that CSGalNActI is essential for the differentiation and maturation of cartilage.

A deficiency in CSGalNActI, but not CSGalNAct2, has been shown to promote axonal regeneration following spinal cord injury [86]. CS-PGs function as barrier-forming molecules during axonal regeneration after damage to the nervous system [10]. Thus, the down- and upregulation of CS and HS biosynthesis, respectively, in the scars of CSGalNActT1−/− mice led to better recovery from injuries in the nervous system than the wild type.

4.4. Chsyl. Chsyl-deficient mice are viable but exhibit chondrodysplasia, progression of the bifurcation of digits, delayed endochondral ossification, and reduced bone density [81]. Furthermore, a decrease in 4-O-sulfation and increase in 6-O-sulfation as well as desulfation of the GalNAc residues of CS have been reported in the cartilage of Chsyl−/− mice. The signaling of hedgehog but not of FGF, bone morphogenetic protein, or transforming growth factor-β altered in primary chondrocytes from Chsyl-deficient mice [81], which suggests that CS-PGs and hedgehog protein may coordinate to regulate skeletal development and digit patterning.

4.5. Chpf. Mice deficient in Chpf, also known as chondroitin sulfate synthase-2 (CSS2), are fertile and viable and exhibit no obvious abnormalities including osteoarthritis and cartilage development [82]. These findings are consistent with the study by Wilson et al. [81].

4.6. Dse and Dsel. The body weight of Dse−/− mutant mice, which have fewer IdoUA residues in the skin, is ~30% smaller than that of the wild type [88, 89]. Although no significant differences were observed in the content of collagen between Dse−/− and the wild type, the ultrastructure of collagen fibrils in the dermis and hypodermis was thicker in the deficient mice than in the wild type, and a decline in their mechanical strength was also noted in the deficient mice. On the other hand, no morphological or histological abnormalities have been reported in mice targeted with the disruption of DS epimerase-2 encoded by Dsel [93]. In addition, 4-O-sulfation of the DS chain was decreased in the brain of Dse−/− mice, whereas the adult Dse−/− brain had normal structures in the extracellular matrix. The function of Dse2 appears to be compensated by Dsel [93].

4.7. Chst3 (C6st1). The number of 6-O-sulfated disaccharide units including the C-unit (GlcUA–GalNAc6-O-sulfate) and D-unit (GlcUA2-O-sulfate–GalNAc6-O-sulfate) was shown to be markedly reduced in the spleens and brains of C6st1-deficient mice, and the number of naïve T lymphocytes was also decreased in the spleen [114]. However, brain development in C6st1−/− mice is normal in spite of a decrease in D-units in the CS chains of the null mice. CS-PGs are newly synthesized in the central nervous system following injury, and this inhibits axonal regeneration [10, 248]. Furthermore, upregulation of the expression of C6st1 and 6-O-sulfated CS-PGs has been demonstrated in glial scars after a cortical injury [249]. C6st1−/− mice had
fewer or a similar number of regenerative axons after axotomy to the wild type [115].

An increase in chondroitin 6-O-sulfation was observed in the developing brains of C4st1-transgenic mice and affected the formation of the perineuronal nets and cortical plasticity [116], which are specialized structures of the dense organized matrix, which are composed of CS-PGs, hyaluronan, tenascins, and link proteins and regulate neuronal plasticity and neuroprotection [250]. Chondroitin 6-O-sulfate may regulate the maturation of parvalbumin-expressing interneurons through the incorporation of Otx2 [116], which regulates ocular dominance plasticity.

4.8. Chst11 (C4st1). The C4st1 gene was identified as a target gene of bone morphogenetic protein signaling using gene trap experiments [94]. C4st1-mutant mice exhibit severe dwarfism and die within six hours of birth due to respiratory failure [95]. Moreover, severe chondrodysplasia with abnormalities in the cartilage growth plate and chondrocyte columns, marked reductions in GAG content and 4-O-sulfated CS, the downregulation of bone morphogenetic protein signaling, and the upregulation of transforming growth factor-β have been observed in these mice. These findings indicated that C4ST1 and the 4-O-sulfation of CS chains were essential for the signaling pathways of bone morphogenetic protein and transforming growth factor-β as well as cartilage morphogenesis.

4.9. Chst14 (D4st1). D4st1−/− mice have a smaller body weight, a kinked tail, and more fragile skin and are less fertile than the wild type [107]. In addition, axonal regrowth is initially facilitated in D4st1−/− mice following nerve transection.

Furthermore, the impaired proliferation of neural stem cells, reduced neurogenesis, and an altered subpopulations of radial glial cells have been reported in D4st1-deficient mice [96]. The epitope structure recognized by the monoclonal anti-CS antibody 473HD, which contains the D-unit (GlcUA-2-O-sulfate-GalNAc-6-O-sulfate) and iA-unit (IdoUA-GalNAc4-O-sulfate) in the CS-DS hybrid chains on PGs, such as phosphacan, is required for the formation of neurospheres and as a marker for radial glial cells [251]. Expression of the 473HD epitope was shown to be decreased in the neural stem cells of D4st1−/− mice, and this resulted in the altered formation of neurospheres [96]. These findings indicated that DS chains and/or D4ST1 are essential for the proliferation and differentiation of neural stem cells.

4.10. Chst15 (Galnac4s-6st). Galnac4s-6st-null mice are viable and fertile and completely defective in the E-unit, GlcUA-GalNAc(4-,6-O-disulfates), in both CS and DS chains [117]. The activities of carboxypeptidase A and trypsinase from bone marrow-derived mast cells in Galnac4s-6st−/− were lower than those in the wild type, which suggested that the E-unit-containing CS chain or CS-PGs may be involved in the retention of these proteases in the granules of mast cells.

4.11. Ext1 and Ext2. Gene knockout mice produced by the targeted disruption of the gene encoding Ext1 and Ext2 died by embryonic day 8.5–14.5 due to defects in the formation of the mesoderm and a failure in egg cylinder elongation [119–121, 136]. The GlcUA and GlcNAc transferase activities are decreased and HS chains are shorter in mice carrying a hypomorphic mutation in EXT1 generated by gene trapping, which affect the signaling pathways of Indian hedgehog and parathyroid hormone-related peptide [120, 121]. Thus, it is difficult to analyze the in vivo functions of HS chains using conventional knockout mice. A growing number of conditional knockout mice produced by targeted disruption of the gene encoding HS biosynthetic enzymes has provided an insight into the physiological functions of HS and HS-PGs [14]. For example, pluripotent embryonic stem cells in which Ext1 was disrupted fail to differentiate into neural precursor cells and mesoderm cells due to the enhancement of Fgf signaling and retention of the high expression of Nanog [122, 123]. Conditional Ext1-knockout mice selectively disrupted in the nervous system die within the first day of life and have defective olfactory bulbs, midbrain-hindbrain region, and axon guidance due to a disturbance in signaling pathways including Fgf8 and Netrin-1 [124–126]. Conditional Ext1-knockout mice specific for postnatal neurons exhibit a large number of autism-like phenotypes in spite of a normal morphology in the brain [127]. On the other hand, mice in which Ext1 was specifically disrupted for chondrocytes and the limb bud, Ext2 heterozygous mice, and compound Ext1+/−/Ext2−/− mice display severe skeletal defects with cartilage differentiation and chondrocyte maturation, and these defects resembled an autosomal dominant inherited genetic disorder, human hereditary multiple exostoses [128–132]. Disruption of the Ext1 gene in glomerular podocytes results in an abnormal morphology in these cells [133]. Furthermore, conditional knockout mice lacking Ext1 in the high endothelial venules and vascular endothelium cells show a decrease in lymphocyte homing to peripheral lymph nodes and a compromised contact hypersensitivity response [134, 135]. These findings suggest that HS and HS-PGs are essential for playing a role in their physiological functions in a tissue-specific manner.

4.12. Ext2 and Ext3. Mice deficient in Ext2 are viable and develop normally; however, they produce a larger amount of GAG chains [137, 138]. Liver regeneration was shown to be impaired in these knockout mice following liver injury induced by administration of CCl4 due to suppression of the response to hepatocyte growth factor [137]. Mice deficient in Ext3 are embryonically lethal, which is similar to mice lacking Ext1 or Ext2 [139]. In addition, selective inactivation of the Ext3 gene in pancreatic islet β-cells caused an abnormal morphology as well as a reduction in the proliferation of the islets, which resulted in defective insulin secretion [139]. However, it remains to be determined how HS, HS-PGs, or Ext3 is involved in insulin secretion.
4.13. Ndst1, 2, and 3. Functional analyses of HS and heparin using Ndst1-deficient mice have been performed in approximately 20 studies to date [140–164]. Representative studies have been reviewed in this chapter. Ndst1-deficient mice die after birth and have cerebral hypoplasia, axon guidance errors, defects in the eye and olfactory bulbs, insufficient milk production caused by a defect in lobuloalveolar expansion in the mammary gland, and morphological abnormalities in the podocytes [140–142, 145, 156, 157, 162, 164]. Ndst1 conditional knockout mice specific for the liver accumulated triglyceride-rich lipoproteins due to a reduction in the clearance of cholesterol-rich lipoprotein particles [148, 163]. Furthermore, mice with the endothelial-targeted deletion of Ndst1 exhibited suppressed experimental tumor growth and angiogenesis including microvascular density and branching of the surrounding tumors due to altered responses to Fgf2 and Vegf, which resulted in reduced Erk phosphorylation [147] and attenuated allergic airway inflammation [151].

Embryos from Ndst2-deficient mouse are viable and fertile, whereas their mast cells are unable to synthesize heparin, which leads to changes in morphology and severely reduced amounts of granule proteases [165–167]. These findings indicated that the storage of proteases in granules is controlled by heparin or heparin-PG, such as serglycin [165–167]. On the other hand, Ndst3-deficient mice develop normally and are fertile [168].

4.14. GlcC (HS GlcUA C5-epimerase). Mice with the targeted disruption of HS epimerase die immediately after birth and have agenesis of the kidney, a shorter body length, and lung defects [169, 170]. Furthermore, developmental abnormalities in the lymphoid organs, including the spleen, thymus, and lymph nodes, have been reported in the knockout mice [171, 172]. IdoUA-containing HS chains are critical for early morphogenesis of the thymus through binding with Fgf2, Fgf10, and bone morphogenetic protein 4 [171]. In addition, the interaction of HS with a proliferation inducing ligand, hepatocyte growth factor, and CXCL12α is required for B-cell maturation [172].

4.15. Hs2st. Gene trap mice lacking Hs2st die during the neonatal period and exhibit renal aplasia and defects in the eyes, skeleton, and retinal axon guidance [173–179]. In addition, the cell-specific disruption of Hs2st in the endothelial and myeloid cells enhanced the infiltration of neutrophils due to an increase in their binding to IL-8 and macrophage inflammatory protein-2 [162]. Mice with the specific disruption of Hs2st in the liver accumulate plasma triglycerides and the uptake of very-low-density lipoproteins is reduced, whereas mice with the specific disruption of Hs6st in the liver do not. These findings suggest that the clearance of plasma lipoproteins is dependent on the 2-O-sulfation of HS [153].

4.16. Hs3st1. HS3ST1+/− mice display normal development and anticoagulant activity [184]; however, it was previously demonstrated that the GlcN-3-O-sulfate structure was essential for the anticoagulant activity of heparin and HS [252]. Other HS3ST family members such as HS3ST2, HS3ST3a, HS3ST3b, HS3ST4, HS3ST5, and HS3ST6 may compensate for the loss of HS3ST1 [184].

4.17. Hs6st1 and Hs6st2. HS6ST1-null mice die during the late embryonic stage, are smaller than the wild type at birth, and have defective retinal axon guidance due to the disturbance of Slit-Robo signaling [177, 178, 181]. In contrast, HS6ST2-deficient mice develop normally [183]. However, serum levels of thyroid-stimulating hormone and the thyroid hormone, thyroxin, are higher and lower, respectively, in the deficient mice, which cause a reduction in energy metabolism with an increase in body weight [183]. The storage of mast cell proteases is altered in double knockout mice with HS6ST1−/−/HS6ST2−/− [182], and their embryonic fibroblasts are partially defective in FGF signaling [253].

4.18. Sulf1 and Sulf2 (HS 6-O-endosulfatase). Sulf1−/− mice exhibit no apparent abnormalities [185]. On the other hand, Sulf2−/− mice have a smaller body size and mass [185, 192]. Mice deficient in both Sulf1 and Sulf2 have multiple defects including skeletal and renal malformations, which result in neonatal lethality [186]. HS 6-O-sulfation and/or desulfation by Sulfs are known to be involved in the cartilage homeostasis mediated by bone morphogenetic protein and Fgf [187], dentinogenesis through Wnt signaling [188], neurite outgrowth mediated by glial cell line-derived neurotrophic factor [189], muscle regeneration [190], and brain development [191]. These findings indicate that the fine-tuning of 6-O-sulfation by Sulfs may control multiple functions of HS chains during morphogenesis.

5. Human Disorders Affecting the Skeleton and Skin due to the Disturbance of GAGs

5.1. PAPSS2. Spondyloepimetaphyseal dysplasia of Pakistani type, which is characterized by kyphoscoliosis, generalized brachydactyly, short and bowed lower limbs, and enlarged knee joints, is caused by mutations in PAPSS2: p.Ser438X and p.Arg329X [43, 44].

Patients with mutations in PAPSS2, resulting in the substitution of corresponding amino acids (pThr48Arg, p.Arg329X, and p.Ser475X), also have spondyloepiphyseal and premature pubarche, which are accompanied by a short stature, bone dysplasia, excess androgens, hyperandrogenic anovulation, and the loss of dehydroepiandrosterone sulfate [45]. Sulfotransferase 2A1 has been shown to transfer a sulfate group from PAPS to dehydroepiandrosterone (DHEA) in the adrenal glands and liver, resulting in the formation of DHEA-sulfate [254]. The inactivation of PAPSS2 inhibits not only the formation of PAPS but also the conversion of DHEA into DHEA-sulfate, which leads to the accumulation of DHEA in patients [45]. Excess DHEA is finally converted to testosterone through androsterone.

Autosomal recessive brachyolmia, which is a heterogeneous group of skeletal dysplasias and primarily affects the spine, is also caused by PAPSS2 mutations [46, 47]. Brachyolmia is characterized by a short stature due to a short
trunk, irregular endoaplates, a narrow intervertebral disc, calcification of cartilage in the ribs, a short femoral neck and metacarpals, and normal intelligence [46–48]. However, the excess amount of androgens cannot be detected in these patients. Furthermore, PAPS synthase activity was absent in the recombinant mutant enzymes, including p.Cys43Tyr, p.Leu76Gln, and p.Val540Asp [47].

5.2. XYLT1. Mutation in XYLT1 causes an autosomal recessive short stature syndrome characterized by alterations in the distribution of fat, intellectual disabilities, and skeletal abnormalities including a short stature and femoral neck, thickened ribs, plump long bones, and distinct facial features [56]. The homozygous mutation in XYLT1 gives rise to the substitution of the amino acid, p.Arg481Trp in the deduced catalytic domain, which results in decorin without a DS side chain in addition to mature decorin-PG with a DS chain from the fibroblasts of the patient [56]. In addition, the mutant XYLT1 is diffusely localized in the cytoplasm and partially in the Golgi in the fibroblasts of the patient. Desbuquois dysplasia type 2 is a multiple dislocation group of skeletal disorders that is characterized by a short stature, joint laxity, and advanced carpal ossification [57]. Five distinct XYLT1 mutations have been identified to date, including a missense substitution (p.Arg598Cys), nonsense mutation (p.Arg147X), truncated form mutation (p.Pro93AlafsX69), and two splice site mutations [58]. Furthermore, fibroblasts from the affected individuals synthesized a smaller amount of CS and/or DS than those from healthy controls [58].

5.3. B4GALT7 (GalT-I). Ehlers-Danlos syndrome is a heterogeneous group of heritable connective tissue disorders characterized by joint and skin laxity as well as tissue fragility. Six major types (classical, hypermobility, vascular, kyphoscoliosis, arthrochalasia, and dermatosparaxis types) and several minor types, including the progeroid type, are currently known [255]. Mutations in B4GALT7 encoding GalT-I cause Ehlers-Danlos syndrome-progeroid type 1, which is characterized by an aged appearance, hypermobile joints, loose yet elastic skin, hypotonic muscles, craniofacial dysmorphism, a short stature, developmental delays, generalized osteopenia, and defective wound healing [61–64]. Galactosyltransferase activity is reduced in the mutant enzymes, p.Arg277Gln, p.Ala186Asp, p.Leu206Pro, and p.Arg270Cys, which results in the lack of DS side chains on decorin and biglycan core proteins and also smaller CS and HS side chains on other PGs [64–68].

A homozygous mutation in B4GALT7 (p.Arg270Cys) causes a variant of Larsen syndrome in Reunion Island in the southern Indian Ocean, which is called Larsen of Reunion Island syndrome, and is characterized by distinctive facial features, multiple dislocations, dwarfism, and hyperlaxity [69].

5.4. B3GALT6 (GalT-II). Ehlers-Danlos syndrome-progeroid type 2 is caused by mutations in B3GALT6 encoding GalT-II [70, 71]. GalT-II activity by the mutant enzyme (p.Ser309Thr) is significantly decreased, leading to the loss of GAG chains on the core proteins of various PGs [70]. The autosomal-recessive disorder, spondyloepimetaphyseal dysplasia with joint laxity type 1, which is characterized by hip dislocation, elbow contracture, clubfeet, platyspondyly, hypoplastic ilia, kyphoscoliosis, metaphyseal flaring, and craniofacial dysmorphisms such as prominent eyes, blue sclera, a long upper lip, and small mandible with cleft palate, is also caused by mutations in B3GALT6 [70–72, 256]. Skeletal and connective abnormalities in both Ehlers-Danlos syndrome-progeroid type 2 and spondyloepimetaphyseal dysplasia with joint laxity type 1 overlap; however, these individuals have no common mutations among fifteen different mutations [70]. The GalT-II activities of the recombinant enzymes, p.Ser65Gly-, p.Pro67Leu-, p.Asp156Asn-, p.Arg232Cys-, and p.Cys300Ser-B3GALT6, were shown to be significantly lower than those of wild-type-B3GALT6 [70]. The mutation that affected the initiation codon, c.1A>G (p.Met?), for B3GALT6 resulted in a lower molecular weight of the recombinant protein than that of the wild-type protein with the deletion of 41 amino acids at the N-terminus, which indicated a shift in translation at the initiation codon at the second ATG [70]. Although wild-type B3GALT6 is expressed in the Golgi, the mutant enzyme (p.Met?) is localized in the nucleus and cytoplasm [70], indicating that the mutant protein may not be functional due to its cellular mislocalization.

5.5. B3GAT3 (GlcAT-I). A mutation (p.Arg277Gln) in the B3GAT3 gene encoding GlcAT-I is known to cause Larsen-like syndrome [73, 74], which is characterized by dislocations in the hip, knee, and elbow joints, equinovarus foot deformities, and craniofacial dysmorphisms such as a flattened midface, depressed nasal bridge, hypertelorism, and a prominent forehead [257, 258]. These patients mainly have elbow dislocations with congenital heart defects including a bicuspid aortic valve in addition to characteristic symptoms of Larsen-like syndrome [73]. The p.Arg277Gln mutation results in a marked reduction in GlcAT-I activity in the fibroblasts of these patients and the recombinant enzyme protein [73]. Mature decorin-PG, which is secreted by fibroblasts and has a single DS side chain, was observed in the fibroblasts of healthy controls [73]. On the other hand, fibroblasts from patients generate both a PG form of decorin and DS-free decorin [73]. Moreover, the number of CS and HS in the patients’ cells is also reduced.

5.6. CSGALNACT1. Neuropathies including Guillan-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, hereditary motor sensory neuropathy, and unknown etiologies are partially caused by mutations in CSGALNACT1 encoding GalNAcT-I and GalNacT-II [83]. The GalNacT-II activities of the recombinant enzymes, CSGalNacT-I-His234Arg and -Met509Arg, were shown to be markedly reduced [83], which indicated that affect in CS chains on PGs in the nervous system may lead to peripheral neuropathies.
5.7. CHSY1. Patients with mutations in CHSY1 have Temtamy preaxial brachydactyly syndrome, which is an autosomal recessive congenital syndrome characterized by facial dysmorphism, dental anomalies, brachydactyly, hyperphalangism, growth retardation, deafness, and delayed motor and mental developments [77, 78]. Their mutations result in the substitution of amino acids and truncation of the CHSY1 protein including p.Gly5AlafsX30, p.Gly19-Leu28del, p.Glu33SerfsX1, p.Gln69X, and p.Pro539Arg and a splice-site mutation [77–79]. A heterozygous mutation in CHSY1 (p.Phe362Ser) was recently identified in a patient with neuropathy [80].

5.8. CHST3 (C6ST1). Spondyloepiphyseal dysplasia Omání type, which is characterized by severe chondrodysplasia with major involvement of the spine, is caused by a loss-of-function mutation in C6ST1 [108–111]. Patients with the substitution of amino acid in C6ST1, p.Arg304Gln, have severe kyphoscoliosis, a short stature, mild brachydactyly, rhizomelia, fusion of the carpal bones, and osteoarthritsis in the elbow, wrist, and knee joints [108, 109]. Furthermore, additional clinical features, including deafness, metacarpal shortening, and aortic regurgitations due to ventricular septal, mitral, and/or tricuspid defects, have been reported in Turkish siblings who had different mutations in C6ST1 (p.Tyr141Met and p.Leu286Pro) [110, 111]. Mutant enzymes of the recombinant C6ST1 and enzymes from the patients’ fibroblasts had markedly reduced C6ST activity, which resulted in the loss of chondroitin 6-O-sulfate in the fibroblasts [109–111]. Moreover, chondrodysplasia with multiple dislocations, Desbuquois syndrome, autosomal recessive Larsen syndrome, and humero-spinal dysostosis have been attributed to distinct CHST3 mutations (p.Leu259Pro, p.Arg222Trp, p.Leu307Pro, p.Tyr201X, p.F206X, p.Glu327Lys, p.Gly563AlafsX30, and a mutation at the splice site) [112, 113]. Different pathological diagnoses may be caused by the relatively narrow clinical features and age-related descriptions of the same conditions [113].

5.9. CHST14 (D4ST1). Ehlers-Danlos syndrome musculocontractural type 1, which is characterized by progressive joint and skin laxity, multiple congenital contractures, progressive multi-system complications, and characteristic craniofacial features, is caused by mutations in CHST14 encoding D4ST1 (p.Val49X, p.Lys69X, p.Pro281Leu, p.Cys289Ser, p.Tyr293Cys, and p.Glu334GlyfsX107) [97–102]. A recent study described a case of Ehlers-Danlos syndrome musculocontractural type 1 (p.Val49X) in which muscle hypoplasia and weakness was observed, which resulted in myopathy based on laboratory findings such as muscle biopsy, ultrasound, and electromyography [103].

The recombinant mutants of D4ST1 (p.Pro281Leu, p.Cys289Ser, and p.Tyr293Cys) and fibroblasts from affected individuals have markedly reduced sulfotransferase activity [99]. Furthermore, a single DS side chain on decorin-PG from the fibroblasts of patients was found to be replaced by a CS chain, but not dermatan [99]. Immature decorin-PG results in the dispersion of collagen bundles in the dermal tissues of patients.

The autosomal recessive disorder, adducted thumb-clubfoot syndrome, which is characterized by an adducted thumb, clubfoot, craniofacial dysmorphism, arachnodactyly cryptorchidism, an atrial septal defect, kidney defects, cranial ventricular enlargement, and psychomotor retardation, is also caused by mutations in CHST14 (p.Val49X, p.Arg35Gly, p.Leu137Gln, p.Arg213Pro, and p.Tyr293Cys) [104–106]. The fibroblasts of these patients lack DS chains and have an excess amount of CS chains.

5.10. DSE. A mutation in DSE (p.Ser268Leu) has been shown to cause Ehlers-Danlos syndrome musculocontractural type 2 [87]. Clinical features including hypermobility of the finger, elbow, and knee joints, characteristic facial features, contracture of the thumbs and feet, and myopathy have been observed in these patients. Epimerase activity is markedly reduced not only in the recombinant mutant DSE (p.Ser268Leu) but also in the cell lysate from these patients [87]. In addition, a decrease in the biosynthesis of DS accompanied by an increase in that of CS has been reported in the fibroblasts of these patients. The deficiencies associated with DSE in addition to D4ST1 affect the biosynthesis of DS, which implies that both enzymes are essential for the development of skin and bone as well as the maintenance of their extracellular matrices.

6. Conclusions
The biological roles of CS, DS, and HS chains in vivo have been revealed by examining knockout mice in addition to nematodes, fruit flies, and zebrafish [4, 8, 12–14]. However, the mice deficient in glycosyltransferases or sulfotransferases involved in the biosynthesis of GAGs showed embryonic lethality or death shortly after the birth. These observations indicate that GAGs or PGs are essential for early development. Furthermore, studies using the conditional knockout mice have revealed the specific functions of GAGs in individual organs. Recent advances in the study of human genetic diseases in the bone and connective tissue have also clarified the biological significance of the GAG side chains of PGs [7, 14, 20]. The clinical manifestations in human disorders caused by deficiency in the biosynthetic enzymes of GAGs do not always agree with the phenotypes of the deficiency in the corresponding enzymes in knockout mice. This contradiction may be due to the residual enzymatic activity or GAGs in human patients. Although null mutant mice show severe phenotypes including embryonic lethality, human patients appear to show various symptoms depending on the degree of remaining activity of the enzymes. Further comprehensive approaches to the study of molecular pathogeneses involving CS, DS, and HS chains are required to facilitate the development of therapeutics and design of new drugs for these diseases.
Abbreviations

B3GALT6: Beta 1,3-galactosyltransferase 6  
B4GALT7: Beta 1,4-galactosyltransferase 7  
B3GAT3: Beta-1,3-glucurononyltransferase 3  
C4ST: Chondroitin 4-O-sulfotransferase  
C6ST: Chondroitin 6-O-sulfotransferase  
CHST: Carbohydrate sulfotransferase  
CHSY: Chondroitin synthase  
CS: Chondroitin sulfate  
CSGALNACT: Chondroitin sulfate N-acetylgalactosaminyltransferase  
D4ST: Dermatan 4-O-sulfotransferase  
DS: Dermatan sulfate  
DSE: Dermatan sulfate-glucuronyl C5-epimerase  
DSEL: Dermatan sulfate epimerase-like  
GAG: Glycosaminoglycan  
GalNAc: N-Acetyl-d-galactosamine  
GalNAc4S-6ST: N-Acetyl-d-galactosaminyl sulfate 6-O-sulfotransferase  
GlcUA: d-Glucuronic acid  
HS: Heparan sulfate  
IdoUA: l-Iduronic acid  
PAPS: 3’-Phosphoadenosine 5’-phosphosulfate  
PAPSS2: 3’-Phosphoadenosine 5’-phosphosulfate synthase 2  
PG: Proteoglycan.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas 2410501 (to Kazuyuki Sugahara) and 26110719 (to Shuhei Yamada) from The Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT); by a Grant-in-Aid for Scientific Research (C) 24590071 (to Shuhei Yamada); by a Grant-in-Aid for Young Scientists (B) 25860037 (to Shuji Mizumoto) 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 Shuji Mizumoto); by the Research Institute of Meijo University (Tenkai) (to Shuji Mizumoto); and by the Fugaku Trust for Medical Research (to Shuhei Yamada).

References

[1] L. Kjellén and U. Lindahl, “Proteoglycans: structures and interactions,” Annual Review of Biochemistry, vol. 60, pp. 443–475, 1991.  
[2] R. V. Iozzo, “Matrix proteoglycans: from molecular design to cellular function,” Annual Review of Biochemistry, vol. 67, pp. 609–652, 1998.  
[3] M. Bernfield, M. Götte, P. W. Park et al., “Functions of cell surface heparan sulfate proteoglycans,” Annual Review of Biochemistry, vol. 68, pp. 729–777, 1999.  
[4] N. Perrimon and M. Bernfield, “Specificities of heparan sulphate proteoglycans in developmental processes,” Nature, vol. 404, no. 6779, pp. 725–728, 2000.  
[5] K. Sugahara and H. Kitagawa, “Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans,” Current Opinion in Structural Biology, vol. 10, no. 5, pp. 518–527, 2000.  
[6] J. D. Esko and S. B. Selleck, “Order out of chaos: assembly of ligand binding sites in heparan sulfate,” Annual Review of Biochemistry, vol. 71, pp. 435–471, 2002.  
[7] S. Mizumoto and K. Sugahara, “Bone and skin disorders caused by a disturbance in the biosynthesis of chondroitin sulfate and dermatan sulfate,” in Extracellular Matrix: Pathobiology and Signaling, N. Karamanos, Ed., pp. 97–118, Walter de Gruyter, Berlin, Germany, 2012.  
[8] K. Sugahara, T. Mikami, T. Uyama, S. Mizuguchi, K. Nomura, and H. Kitagawa, “Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate,” Current Opinion in Structural Biology, vol. 13, no. 5, pp. 612–620, 2003.  
[9] T. Mikami and H. Kitagawa, “Biosynthesis and function of chondroitin sulfate,” Biochimica et Biophysica Acta, vol. 1830, no. 10, pp. 4719–4733, 2013.  
[10] T. Laabs, D. Carulli, H. M. Geller, and J. W. Fawcett, “Chondroitin sulfate proteoglycans in neural development and regeneration,” Current Opinion in Neurobiology, vol. 15, no. 1, pp. 116–120, 2005.  
[11] M. M. Fuster and J. D. Esko, “The sweet and sour of cancer: glycosaminoglycans as novel therapeutic targets,” Nature Reviews Cancer, vol. 5, no. 7, pp. 526–542, 2005.  
[12] U. Häcker, K. Nybakken, and N. Perrimon, “Heparan sulphate proteoglycans: the sweet side of development,” Nature Reviews Molecular Cell Biology, vol. 6, no. 7, pp. 530–541, 2005.  
[13] H. E. Bülow and O. Hobert, “The molecular diversity of glycosaminoglycans shapes animal development,” Annual Review of Cell and Developmental Biology, vol. 22, pp. 375–407, 2006.  
[14] J. R. Bishop, M. Schuksz, and J. D. Esko, “Heparan sulfate proteoglycans fine-tune mammalian physiology,” Nature, vol. 446, no. 7139, pp. 1030–1037, 2007.  
[15] K. Sugahara and T. Mikami, “Chondroitin/dermatan sulfate in the central nervous system,” Current Opinion in Structural Biology, vol. 17, no. 5, pp. 536–545, 2007.  
[16] S. Mizumoto and K. Sugahara, “Glycosaminoglycans are functional ligands for receptor for advanced glycation end-products in tumors,” FERS Journal, vol. 280, no. 10, pp. 2462–2470, 2013.  
[17] D. Xu and J. D. Esko, “Demystifying heparan sulfate-protein interactions,” Annual Review of Biochemistry, vol. 83, pp. 129–157, 2014.  
[18] J. Filmus and M. Capurro, “The role of glypicans in Hedgehog signaling,” Matrix Biology, vol. 35, pp. 248–252, 2014.  
[19] S. Mizumoto, T. Uyama, T. Mikami, H. Kitagawa, and K. Sugahara, “Biosynthetic pathways for differential expression of functional chondroitin sulfate and heparan sulfate,” in Handbook of Carbohydrate Engineering, K. J. Yarema, Ed., pp. 289–324, CRC Press, Taylor & Francis Group, Boca Raton, Fla, USA, 2005.  
[20] S. Mizumoto, S. Ikegawa, and K. Sugahara, “Human genetic disorders caused by mutations in genes encoding biosynthetic enzymes for sulfated glycosaminoglycans,” Journal of Biological Chemistry, vol. 288, no. 16, pp. 10953–10961, 2013.
[21] K. V. Venkatachalam, "Human 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase: biochemistry, molecular biology and genetic deficiency," *IUBMB Life*, vol. 55, no. 1, pp. I–II, 2003.

[22] K. Sugahara and N. B. Schwartz, "Defect in 3'-phosphoadenosine 5'-phosphosulfate formation in brachymorphic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 12, pp. 6615–6618, 1979.

[23] K. Kurima, M. L. Warman, S. Krishnan et al., "A member of a family of sulfate-activating enzymes causes murine brachymorphism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8681–8685, 1998.

[24] S. Kamiyama, T. Suda, R. Ueda et al., "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase II is involved in the biosynthesis of the glycosaminoglycan-protein linkage region of proteoglycans," *Journal of Biological Chemistry*, vol. 278, no. 28, pp. 25958–25963, 2003.

[25] C. Götting, J. Kuhn, R. Zahn, T. Brinkmann, and K. Kleesiek, "A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[26] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 8, pp. 5201–5206, 2000.

[27] R. Almeida, S. B. Levery, U. Mandel et al., "Cloning and expression of a proteoglycan UDP-galactose:β-xylene 1,4-galactosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[28] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[29] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[30] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[31] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[32] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[33] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[34] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[35] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[36] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[37] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[38] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[39] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.

[40] T. Okajima, K. Yoshida, T. Kondo, and K. Furukawa, "Molecular cloning and expression of human UDP-D-xylose:1,4-β-xylosyltransferase I. A seventh member of the human β4-galactosyltransferase gene family," *Journal of Biological Chemistry*, vol. 274, no. 37, pp. 26165–26171, 1999.
[48] M. Shohat, R. Lachman, H. E. Gruber, and D. L. Rimoin, “Brachyolmia: radiographic and genetic evidence of hetero-
geneity,” American Journal of Medical Genetics, vol. 33, no. 2, pp. 209–219, 1989.
[49] P. W. Lane and M. M. Dickie, “Three recessive mutations producing disproportionate dwarfining in mice: aehondroplasia, brachymorphic, and stubby,” Journal of Heredity, vol. 59, no. 5, pp. 300–308, 1968.
[50] R. W. Orkin, R. M. Pratt, and G. R. Martin, “Undersulfated chondroitin sulfate in the cartilage matrix of brachymorphic mice,” Developmental Biology, vol. 50, no. 1, pp. 82–94, 1976.
[51] K. Sugahara and N. B. Schwartz, “Defect in 3′-phosphoadeno-
sine 5′-phosphosulfate synthesis in brachymorphic mice. I. Characterization of the defect,” Archives of Biochemistry and Biophysics, vol. 214, no. 2, pp. 589–601, 1982.
[52] K. Sugahara and N. B. Schwartz, “Defect in 3′-phosphoadeno-
sine 5′-phosphosulfate synthesis in brachymorphic mice. II. Tissue distribution of the defect,” Archives of Biochemistry and Biophysics, vol. 214, no. 2, pp. 602–609, 1982.
[53] M. Cortes, A. T. Baria, and N. B. Schwartz, “Sulfation of chondroitin sulfate proteoglycans is necessary for proper Indian hedgehog signaling in the developing growth plate,” Development, vol. 136, no. 10, pp. 1697–1706, 2009.
[54] L. E. L. M. Vissers, E. Lausch, S. Unger et al., “Chondrodysplasia and abnormal joint development associated with mutations in IMPAD1, encoding the Golgi-resident nucleotide phosphatase, gPAPP,” American Journal of Human Genetics, vol. 88, no. 5, pp. 608–615, 2011.
[55] J. P. Frederick, A. T. Tafari, S.-M. Wu et al., “A role for a lithium-inhibited Golgi nucleotidase in skeletal development and sulfation,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 33, pp. 11605–11612, 2008.
[56] J. Schreml, B. Durmaz, O. Cogulu et al., “The missing “link”: an autosomal recessive short stature syndrome caused by a hypofunctional XYLT1 mutation,” Human Genetics, vol. 133, no. 1, pp. 29–39, 2014.
[57] L. Faivre, V. Cormier-Daire, A. M. Eliott et al., “Desbuquois dysplasia, a reevaluation with abnormal and “normal” hands: radiographic manifestations,” American Journal of Medical Genetics, vol. 124, no. 1, pp. 48–53, 2004.
[58] C. Bui, C. Huber, B. Tuysuz et al., “XYLT1 mutations in Desbuquois dysplasia type 2,” American Journal of Human Genetics, vol. 94, no. 3, pp. 405–414, 2014.
[59] E. K. Mis, K. F. Liem, Y. Kong, N. B. Schwartz, M. Domowicz, and S. D. Weatherbee, “Forward genetics defines Xyltl as a key, conserved regulator of early chondrocyte maturation and skeletal length,” Developmental Biology, vol. 385, no. 1, pp. 67–82, 2014.
[60] E. Condac, R. Silasi-Mansat, S. Kosanke et al., “Polycystic dis-
ease caused by deficiency in xylosyltransferase 2, an initiating enzyme of glycosaminoglycan biosynthesis,” Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 22, pp. 9416–9421, 2007.
[61] E. Quentin, A. Gladen, L. Roden, and H. Kresse, “A genetic defect in the biosynthesis of dermatan sulfate proteoglycan: galactosyltransferase I deficiency in fibroblasts from a patient with a progeroid syndrome,” Proceedings of the National Academy of Sciences of the United States of America, vol. 87, no. 4, pp. 1342–1346, 1990.
[62] T. Okajima, S. Fukumoto, K. Furukawa, T. Urano, and K. Furukawa, “Molecular basis for the progeroid variant of Ehlers-
Danlos syndrome. Identification and characterization of two mutations in galactosyltransferase I gene,” Journal of Biological Chemistry, vol. 274, no. 41, pp. 28841–28844, 1999.
[63] M. Faiyaz-Ul-Haque, S. H. E. Zaidi, M. Al-Ali et al., “A novel missense mutation in the galactosyltransferase-I (B4GALT7) gene in a family exhibiting facioskeletal anomalies and Ehlers-Danlos syndrome resembling the progeroid type,” American Journal of Medical Genetics, vol. 128, no. 1, pp. 39–45, 2004.
[64] D. G. Seidler, M. Faiyaz-Ul-Haque, U. Hansen et al., “Defective glycosylation of decorin and biglycan, altered collagen structure, and abnormal phenotype of the skin fibroblasts of an Ehlers-Danlos syndrome patient carrying the novel Arg270Cys substitution in galactosyltransferase I (β4Galt7) gene,” Journal of Molecular Medicine, vol. 84, no. 7, pp. 583–594, 2006.
[65] M. Götte and H. Kresse, “Defective glycosaminoglycan substitu-
tion of decorin in a patient with progeroid syndrome is a direct consequence of two point mutations in the galactosyl-
transferase I (β4Galt7) gene,” Biochemical Genetics, vol. 43, no. 1-2, pp. 65–77, 2005.
[66] M. Götte, D. Spillmann, G. W. Yip et al., “Changes in heparan sulfate are associated with delayed wound repair, altered cell migration, adhesion and contractility in the galactosyltrans-
ferase I (β4Galt7) deficient form of Ehlers-Danlos syndrome,” Human Molecular Genetics, vol. 17, no. 7, pp. 996–1009, 2008.
[67] C. Bui, I. Talhaoui, M. Chabel et al., “Molecular characterization of β1,4-galactosyltransferase 7 genetic mutations linked to the progeroid form of Ehlers-Danlos syndrome (EDS),” FEBS Letters, vol. 584, no. 18, pp. 3962–3968, 2010.
[68] S. Rahuel-Clermont, F. Daligault, M.-H. Piet et al., “Biochemical and thermodynamic characterization of mutated β1,4-
galactosyltransferase 7 involved in the progeroid form of the Ehlers-Danlos syndrome,” Biochemical Journal, vol. 432, no. 2, pp. 303–311, 2010.
[69] F. Cartault, P. Munier, M. L. Jacquemont et al., “Expanding the clinical spectrum of B4GALT7 deficiency: homozygous p.R270Cmutation with founder effect causes Larsenof Reunion Island syndrome,” European Journal of Human Genetics, 2014, In press.
[70] M. Nakajima, S. Mizumoto, N. Miyake et al., “Mutations in B3GALT6, which encodes a glycosaminoglycan linker region enzyme, cause a spectrum of skeletal and connective tissue disorders,” American Journal of Human Genetics, vol. 92, no. 6, pp. 927–934, 2013.
[71] F. Malfait, A. Kariminejad, T. Van Damme et al., “Defective initiation of glycosaminoglycan synthesis due to B3GALT6 mutations causes a pleiotropic Ehlers-Danlos-syndrome-like connective tissue disorder,” American Journal of Human Genetics, vol. 92, no. 6, pp. 935–945, 2013.
[72] A. A. Vorster, P. Beighton, and R. S. Ramesar, “Spondyloepi-
metaphyseal dysplasia with joint laxity (Beighton type); mutation analysis in 8 affected south african families,” Clinical Genetics, 2014, In press.
[73] S. Baasanjav, L. Al-Gazali, T. Hashiguchi et al., “Faulty initiation of proteoglycan synthesis causes cardiac and joint defects,” American Journal of Human Genetics, vol. 89, no. 1, pp. 15–27, 2011.
[74] J. E. von Oettingen, W. H. Tan, and A. Dauber, “Skeletal dys-
plasia, global developmental delay, and multiple congenital anomalies in a 5-year-old boy-report of the second family with B3GAT3 mutation and expansion of the phenotype,” American
Y. Watanabe, K. Takeuchi, S. Higa Onaga et al., "Chondroitin sulfate is indispensable for pluripotency and differentiation of mouse embryonic stem cells," Scientific Reports, vol. 4, article 3701, 2014.

T. Izumikawa, B. Sato, and H. Kitagawa, "Chondroitin sulfate synthase 1, a potential target of BMP signaling," American Journal of Human Genetics, vol. 87, no. 6, pp. 757–767, 2010.

Y. Li, K. Laue, S. Temtamy et al., "Tentamy preaxial brachydactyly syndrome is caused by loss-of-function mutations in chondroitin synthase 1, a potential target of BMP signaling," American Journal of Human Genetics, vol. 87, no. 6, pp. 757–767, 2010.

J. Tian, L. Ling, M. Shboul et al., "Loss of CHSY1, a secreted FRINGE enzyme, causes syndromic brachydactyly in humans via increased NOTCH signaling," American Journal of Human Genetics, vol. 87, no. 6, pp. 768–778, 2010.

G. Sher and M. Naeem, "A novel CHSY1 gene mutation underlies Tentamy preaxial brachydactyly syndrome in a Pakistani family," European Journal of Medical Genetics, vol. 57, no. 1, pp. 21–24, 2014.

T. Izumikawa, K. Saigo, J. Shimizu, S. Tsuji, S. Kusunoki, and H. Kitagawa, "A chondroitin synthase-1 (ChSy-1) missense mutation in a patient with neuropathy impairs the elongation of chondroitin sulfate chains initiated by chondroitin N-acetylgalactosaminyltransferase-1," Biochimica et Biophysica Acta, vol. 1830, no. 10, pp. 4806–4812, 2013.

D. G. Wilson, K. Phamluong, W. Y. Lin et al., "Chondroitin sulfate synthase 1 (Chsy1) is required for bone development and digit patterning," Developmental Biology, vol. 363, no. 2, pp. 413–425, 2012.

H. Ogawa, S. Hatano, N. Sugiura et al., "Chondroitin sulfate synthase-2 is necessary for chain extension of chondroitin sulfate but not critical for skeletal development," PLoS ONE, vol. 7, no. 8, Article ID e43806, 2012.

K. Saigo, T. Izumikawa, T. Koike, J. Shimizu, H. Kitagawa, and S. Kusunoki, "Chondroitin beta-1,4-N-acetylgalactosaminyltransferase-1 missense mutations are associated with neuropathies," Journal of Human Genetics, vol. 56, no. 2, pp. 143–146, 2011.

Y. Watanabe, K. Takeuchi, S. Higa Onaga et al., "Chondroitin sulfate N-acetylgalactosaminyltransferase-1 is required for normal cartilage development," Biochemical Journal, vol. 432, no. 1, pp. 47–55, 2010.

T. Sato, T. Kudo, Y. Ikehara et al., "Chondroitin sulfate N-acetylgalactosaminyltransferase 1 is necessary for normal endochondral ossification and aggrecan metabolism," Journal of Biological Chemistry, vol. 286, no. 7, pp. 5803–5812, 2011.

K. Takeuchi, N. Yoshioka, S. Higa Onaga et al., "Chondroitin sulphate N-acetylgalactosaminytransferase-1 inhibits recovery from neural injury," Nature Communications, vol. 4, article 2740, 2013.

T. Müller, S. Mizumoto, I. Suresh et al., "Loss of dermatan sulfate epimerase (DSE) function results in musculoskeletal Ehlers-Danlos syndrome," Human Molecular Genetics, vol. 22, no. 18, pp. 3761–3772, 2013.

M. Maccarana, S. Kalamajski, M. Kongsgaard, S. Peter Magnusson, A. Oldberg, and A. Malmström, "Dermatan sulfate epimerase 1-deficient mice have reduced content and changed distribution of iduronic acids in dermatan sulfate and an altered collagen structure in skin," Molecular and Cellular Biology, vol. 29, no. 20, pp. 5517–5528, 2009.

B. Bartolini, M. A. Thelin, L. Svensson et al., "Iduronic acid in chondroitin/dermatan sulfate affects directional migration of aortic smooth muscle cells," PLoS ONE, vol. 8, no. 7, Article ID e66704, 2013.

D. Goossens, S. Van Gestel, S. Claes et al., "A novel CpG-associated brain-expressed candidate gene for chromosome 18q-linked bipolar disorder," Molecular Psychiatry, vol. 8, no. 1, pp. 83–89, 2003.

H. Zayed, R. Chao, A. Moshefri et al., "A maternally inherited chromosome 18q22.1 deletion in a male with late-presenting diaphragmatic hernia and microphthalmia - Evaluation of DSEL as a candidate gene for the diaphragmatic defect," American Journal of Medical Genetics, Part A, vol. 152, no. 4, pp. 916–923, 2010.

J. Shi, J. B. Potash, J. A. Knowles et al., "Genome-wide association study of recurrent early-onset major depressive disorder," Molecular Psychiatry, vol. 16, no. 2, pp. 193–201, 2011.

B. Bartolini, M. A. Thelin, U. Rauch et al., "Mouse development is not obviously affected by the absence of dermatan sulfate epimerase 2 in spite of a modified brain dermatan sulfate composition," Glycobiology, vol. 22, no. 7, pp. 1007–1016, 2012.

M. Klüppel, K. A. Vallis, and J. L. Wrana, "A high-throughput induction gene trap approach defines C4ST as a target of BMP signaling," Mechanisms of Development, vol. 118, no. 1-2, pp. 77–89, 2002.

S. Bian, N. Akyüz, C. Bernreuther et al., "Dermatan sulfotransferase Chst14/d4st1, but not chondroitin sulfotransferase Chst11/C4st1, regulates proliferation and neurogenesis of neural progenitor cells," Journal of Cell Science, vol. 124, no. 23, pp. 4051–4063, 2011.

T. Kosho, J. Takahashi, H. Ohashi, G. Nishimura, H. Kato, and Y. Fukushima, "Ehlers-Danlos syndrome type VIB with characteristic facies, decreased curvatures of the spinal column, and joint contractures in two unrelated girls," American Journal of Medical Genetics, vol. 138, no. 3, pp. 282–287, 2005.

T. Kosho, N. Miyake, A. Hatamochi et al., "A new Ehlers-Danlos syndrome with craniofacial characteristics, multiple congenital contractures, progressive joint and skin laxity, and multisystem fragility-related manifestations," American Journal of Medical Genetics, Part A, vol. 152, no. 6, pp. 1333–1346, 2010.

M. Miyake, T. Kosho, S. Mizumoto et al., "Loss-of-function mutations of CHST14 in a new type of Ehlers-Danlos syndrome," Human Mutation, vol. 31, no. 8, pp. 966–974, 2010.

F. Malfait, D. Syx, P. Vlummen et al., "Musculocontractural Ehlers-Danlos Syndrome (former EDS type VIB) and adducted thumb clubfoot syndrome (ATCS) represent a single clinical entity caused by mutations in the dermatan 4-sulfotransferase 1 encoding CHST14 gene," Human Mutation, vol. 31, no. 11, pp. 1233–1239, 2010.

K. Shimizu, N. Okamoto, N. Miyake et al., "Delineation of dermatan 4-O-sulfotransferase 1 deficient Ehlers-Danlos syndrome: observation of two additional patients and comprehensive review of 20 reported patients," American Journal of Medical Genetics, Part A, vol. 155, no. 8, pp. 1949–1958, 2011.
[102] K. A. Winters, Z. Jiang, W. Xu et al., “Re-assigned diagnosis of D4ST1-deficient Ehlers-Danlos syndrome (adducted thumb-clubfoot syndrome) after initial diagnosis of Marden-Walker syndrome,” *American Journal of Medical Genetics A*, vol. 158, no. 11, pp. 2935–2940, 2012.

[103] N. C. Voermans, M. Kempers, M. Lammens et al., “Myopathy in a 20-year-old female patient with D4ST-1 deficient Ehlers-Danlos syndrome due to a homozygous CHST14 mutation,” *American Journal of Medical Genetics, Part A*, vol. 158, no. 4, pp. 850–855, 2012.

[104] M. Dündar, T. Müller, Q. Zhang et al., “Loss of Dermatan-4-Sulfotransferase 1 Function Results in Adducted Thumb-Clubfoot Syndrome,” *American Journal of Human Genetics*, vol. 85, no. 6, pp. 873–882, 2009.

[105] M. Dündar, F. Demiryilmaz, I. Demiryilmaz et al., “An autosomal recessive adducted thumb-club foot syndrome observed in Turkish cousins,” *Clinical Genetics*, vol. 51, no. 1, pp. 61–64, 1997.

[106] T. Sonoda and K. Kouno, “Two brothers with distal arthrogryposis, peculiar facial appearance, cleft palate, short stature, hydronephrosis, retentio testis, and normal intelligence: a new type of distal arthrogryposis?” *American Journal of Medical Genetics*, vol. 91, no. 4, pp. 280–285, 2000.

[107] N. Akyüz, S. Rost, A. Mehanna et al., “Dermatan 4-O-sulfotransferase 1ablation accelerates peripheral nerve regeneration,” *Experimental Neurology*, vol. 247, pp. 517–530, 2013.

[108] A. Rajab, J. Kunze, and S. Mundlos, “Spondyloepiphyseal dysplasia Omani type: a new recessive type of SED with progressive spinal involvement,” *American Journal of Medical Genetics*, vol. 126, no. 4, pp. 413–419, 2004.

[109] H. Thiele, M. Sakano, H. Kitagawa et al., “Loss of chondroitin 6-O-sulfotransferase-1 function results in severe human chondrodysplasia with progressive spinal involvement,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 27, pp. 10155–10160, 2004.

[110] M. H. H. Van Rooij, S. Mizumoto, S. Yamada et al., “Embryonic fibroblasts with a gene trap mutation in Ext1 produce short heparan sulfate chains,” *Journal of Biological Chemistry*, vol. 279, no. 31, pp. 32134–32141, 2004.

[111] M. Inatani, F. Irie, A. S. Plump, M. Tessier-Lavigne, and Y. Yamaguchi, “Mammalian brain morphogenesis and midline axon guidance require heparan sulfate,” *Science*, vol. 302, no. 5647, pp. 1044–1046, 2003.

[112] Y. Matsumoto, F. Irie, M. Inatani, M. Tessier-Lavigne, and Y. Yamaguchi, “Netrin-1/DCC signaling in commissural axon guidance requires cell-autonomous expression of heparan sulfate,” *Journal of Neuroscience*, vol. 27, no. 16, pp. 4342–4350, 2007.

[113] B. M. Zak, B. E. Crawford, and J. D. Esko, “Hereditary multiple exostoses and heparan sulfate polymerization,” *Biochimica et Biophysica Acta - General Subjects*, vol. 1573, no. 3, pp. 346–355, 2002.

[114] X. Lin, G. Wei, Z. Shi et al., “Disruption of gastrulation and heparan sulfate biosynthesis in EXT1-deficient mice,” *Developmental Biology*, vol. 224, no. 2, pp. 299–311, 2000.

[115] L. Koziel, M. Kunath, O. G. Kelly, and A. Vortkamp, “Ext-dependent heparan sulfate regulates the range of Ihh signaling during endochondral ossification,” *Developmental Cell*, vol. 6, no. 6, pp. 801–813, 2004.

[116] S. Miyata, Y. Komatsu, Y. Yoshimura, C. Taya, and H. Kitagawa, “Persistent cortical plasticity by upregulation of chondroitin 6-sulfation,” *Nature Neuroscience*, vol. 15, no. 3, pp. 414–422, 2012.

[117] S. Ohtake-Niimi, S. Kondo, T. Ito et al., “Mice deficient in N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase are unable to synthesize chondroitin/dermatan sulfate containing N-acetylgalactosamine 4,6-bissulfate residues and exhibit decreased protease activity in bone marrow-derived mast cells,” *Journal of Biological Chemistry*, vol. 285, no. 27, pp. 20793–20805, 2010.

[118] B. M. Zak, B. E. Crawford, and J. D. Esko, “Hereditary multiple exostoses and heparan sulfate polymerization,” *Biochimica et Biophysica Acta - General Subjects*, vol. 1573, no. 3, pp. 346–355, 2002.
luminal area and no change in blood pressure in conscious mice," *Journal of Cardiovascular Translational Research*, vol. 5, no. 3, pp. 274–279, 2012.

[161] K. T. Bush, B. E. Crawford, O. B. Garner, K. B. Nigam, J. D. Esko, and S. K. Nigam, "N-sulfation of heparan sulfate regulates early branching events in the developing mammary gland," *Journal of Biological Chemistry*, vol. 287, no. 50, pp. 42064–42070, 2012.

[162] J. Axellson, D. Xu, B. N. Kang et al., "Inactivation of heparan sulfate 2-O-sulfotransferase accentuates neutrophil infiltration during acute inflammation in mice," *Blood*, vol. 120, no. 8, pp. 1742–1751, 2012.

[163] E. M. Foley, P. L. S. M. Gordts, P. L. A. Groen, M. Foekens et al., "Altered expression of connexin 37 and 43 in malignant glioma cell lines is associated with reduced cell proliferation," *Neurosurgery*, vol. 65, no. 6, pp. 1522–1530, 2009.

[164] T. V. Karlson, V. V. Iversen, E. Forsberg, L. Kjellén, R. K. Reed, and E.-A. B. Gjerde, "Impaired lymphoid organ development in mice lacking the glucuronyl C5-epimerase gene," *Blood*, vol. 115, no. 13, pp. 2972–2980, 2010.

[165] D. M. Shah, J. M. Tucker, S. A. Rogers et al., "Hs2st-mediated kidney mesenchyme induction regulates early ureteric bud branching," *Developmental Biology*, vol. 339, no. 2, pp. 354–365, 2010.

[166] C. L. R. Merry, S. L. Bullock, D. C. Swan et al., "The Molecular Phenotype of Heparan Sulfate in the Hs2st-/- Mutant Mouse," *Journal of Biological Chemistry*, vol. 276, no. 38, pp. 35429–35434, 2001.

[167] D. McLaughlin, F. Karlsson, N. Tian et al., "Specific modification of heparan sulphate is required for normal cerebral cortical development," *Mechanisms of Development*, vol. 120, no. 12, pp. 1481–1488, 2003.

[168] T. Pratt, C. D. Conway, N. M. M.-L. Tian, D. J. Price, and J. O. Mason, "Heparan sulphation patterns generated by specific heparan sulphotransferase enzymes direct distinct aspects of retinal axon guidance at the optic chiasm," *Journal of Neuroscience*, vol. 26, no. 26, pp. 6911–6923, 2006.

[169] C. D. Conway, K. M. Howe, N. K. Nettleton, D. J. Price, O. Mason, and T. Pratt, "Heparan sulfate sugar modifications mediate the functions of Slits and other factors needed for mouse forebrain commissure development," *Journal of Neuroscience*, vol. 31, no. 6, pp. 1955–1970, 2011.

[170] C. D. Conway, D. J. Price, T. Pratt, and J. O. Mason, "Analysis of axon guidance defects at the optic chiasm in heparan sulphotransferase compound mutant mice," *Journal of Anatomy*, vol. 219, no. 6, pp. 734–742, 2011.

[171] J. Tornberg, G. P. Sykiotis, K. Keefe et al., "Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 28, pp. 11524–11529, 2011.

[172] H. Habuchi, N. Hagai, N. Sugaya, F. Atsumi, R. L. Stevens, and K. Kimata, "Mice deficient in heparan sulfate 6-O-sulfotransferase-1 exhibit defective heparan sulfate biosynthesis, abnormal placentaion, and late embryonic lethality," *Journal of Biological Chemistry*, vol. 282, no. 21, pp. 15578–15588, 2007.

[173] M. F. Anower-E-Khuda, H. Habuchi, N. Hagai, O. Habuchi, T. Yokochi, and K. Kimata, "Heparan sulfate 6-O-sulfotransferase isoform-dependent regulatory effects of heparin on the activities of various proteases in mast cells and the biosynthesis of 6-O-sulfated heparin," *Journal of Biological Chemistry*, vol. 288, no. 6, pp. 3705–3717, 2013.

[174] N. Hagai, H. Habuchi, N. Sugaya et al., "Involvement of heparan sulfate 6-O-sulfation in the regulation of energy metabolism and the alteration of thyroid hormone levels in male mice," *Glycobiology*, vol. 23, no. 8, pp. 980–992, 2013.

[175] M. HajMohammedi, K. Enjyoji, M. Princivalle et al., "Normal levels of anticoagulant heparan sulfate are not essential for normal hemostasis," *Journal of Clinical Investigation*, vol. 113, no. 7, pp. 989–999, 2003.

[176] W. C. Lamanna, R. L. Baldwin, M. Padwa et al., "Heparan sulfate 6-O-endosulfatases: discrete in vivo activities and functional co-operativity," *Biochemical Journal*, vol. 400, no. 1, pp. 63–73, 2006.

[177] C. R. Holst, H. Bou-Reslan, B. B. Gore et al., "Secreted sulfatases Sulf1 and Sulf2 have overlapping yet essential roles in mouse neonatal survival," *PLoS ONE*, vol. 2, no. 6, article e575, 2007.

[178] S. Otsuki, S. R. Hanson, S. Miyaki et al., "Extracellular sulfatases support cartilage homeostasis by regulating BMP and FGF signaling pathways," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 22, pp. 10202–10207, 2010.
S. Hayano, H. Kurosaka, T. Yanagita et al., “Roles of heparan sulfate sulfation in dentinogenesis,” Journal of Biological Chemistry, vol. 287, no. 15, pp. 12217–12229, 2012.

X. Ai, T. Kitazawa, A.-T. Do, M. Kusche-Gullberg, P. A. Labosky, and C. P. Emerson Jr., “SULF1 and SULF2 regulate heparan sulfate-mediated GDFN signaling for esophageal innervation,” Development, vol. 134, no. 18, pp. 3327–3338, 2007.

A. Langsdorf, A.-T. Do, M. Kusche-Gullberg, C. P. Emerson Jr., and X. Ai, “Suls are regulators of growth factor signaling for satellite cell differentiation and muscle regeneration,” Developmental Biology, vol. 311, no. 2, pp. 464–477, 2007.

I. Kalus, B. Salmen, C. Viebahn et al., “Differential involvement of the extracellular 6-O-endosulfatases Sulf1 and Sulf2 in brain development and neuronal and behavioural plasticity,” Journal of Cellular and Molecular Medicine, vol. 13, no. 11-12, pp. 4505–4521, 2009.

D. H. Lum, J. Tan, S. D. Rosen, and Z. Werb, “Gene trap disruption of the mouse heparan sulfate 6-O-endosulfatase gene, Sulf2,” Molecular and Cellular Biology, vol. 27, no. 2, pp. 678–688, 2007.

T. Uyama, H. Kitagawa, J.-I. Tamura, and K. Sugahara, “Molecular cloning and expression of human chondroitin N-acetylgalactosaminyltransferase. The key enzyme for chain initiation and elongation of chondroitin/dermatan sulfate on the protein linkage region tetrasaccharide shared by heparin/heparan sulfate,” Journal of Biological Chemistry, vol. 277, no. 11, pp. 8841–8846, 2002.

M. Gotoh, T. Sato, T. Akashima et al., “Enzymatic synthesis of chondroitin with a novel chondroitin sulfate N-acetylgalactosaminyltransferase that transfers N-acetylgalactosamine to glucuronic acid in initiation and elongation of chondroitin sulfate synthesis,” Journal of Biological Chemistry, vol. 277, no. 41, pp. 38189–38196, 2002.

T. Uyama, H. Kitagawa, J. Tanaka, J.-I. Tamura, T. Ogawa, and K. Sugahara, “Molecular cloning and expression of a second chondroitin N-acetylgalactosaminyltransferase involved in the initiation and elongation of chondroitin/dermatan sulfate,” Journal of Biological Chemistry, vol. 278, no. 5, pp. 3072–3078, 2003.

T. Sato, M. Gotoh, K. Kiyohara et al., “Differential roles of two N-acetylgalactosaminyltransferases, CSGalNAct-T-1, and a novel enzyme, CSGalNAct-T-2. Initiation and elongation in synthesis of chondroitin sulfate,” Journal of Biological Chemistry, vol. 278, no. 5, pp. 3063–3071, 2003.

J. D. Esko and L. Zhang, “Influence of core protein sequence on glycosaminoglycan assembly,” Current Opinion in Structural Biology, vol. 6, no. 5, pp. 663–670, 1996.

K. Sugahara and H. Kitagawa, “Heparin and heparan sulfate biosynthesis,” IUBMB Life, vol. 54, no. 4, pp. 163–175, 2002.

T. Lind, F. Tufaro, C. McCormick, U. Lindahl, and K. Lidholt, “The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate,” Journal of Biological Chemistry, vol. 273, no. 41, pp. 26265–26268, 1998.

H. Kitagawa, H. Shimakawa, and K. Sugahara, “The tumor suppressor EXT-like gene EXT2 encodes an α1, 4-N-acetylgalactosaminyltransferase that transfers N-acetylgalactosamine and N-acetylglucosamine to the common glycosaminoglycan-protein linkage region: the key enzyme for the chain initiation of heparan sulfate,” Journal of Biological Chemistry, vol. 274, no. 20, pp. 13933–13937, 1999.

B.-T. Kim, H. Kitagawa, J.-I. Tamura et al., “Human tumor suppressor EXT gene family members EXT1 and EXT3 encode α1,4-N-acetylgalactosaminyltransferases that likely are involved in heparan sulfate/heparin biosynthesis,” Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 13, pp. 7176–7181, 2001.

H. Kitagawa, T. Uyama, and K. Sugahara, “Molecular cloning and expression of a human chondroitin synthase,” Journal of Biological Chemistry, vol. 276, no. 42, pp. 38721–38726, 2001.

H. Kitagawa, T. Izumikawa, T. Uyama, and K. Sugahara, “Molecular cloning of a chondroitin polymerizing factor that cooperates with chondroitin synthase for chondroitin polymerization,” Journal of Biological Chemistry, vol. 278, no. 26, pp. 23666–23671, 2003.

T. Izumikawa, T. Uyama, Y. Okuura, K. Sugahara, and H. Kitagawa, “Involvement of chondroitin sulfate synthase-3 (chondroitin synthase-2) in chondroitin polymerization through its interaction with chondroitin synthase-1 or chondroitin-polymerizing factor,” Biochemical Journal, vol. 403, no. 3, pp. 545–552, 2007.

T. Izumikawa, T. Koike, S. Shiozawa, K. Sugahara, J.-I. Tamura, and H. Kitagawa, “Identification of chondroitin sulfate glucuronyltransferase as chondroitin synthase-3 involved in chondroitin polymerization: chondroitin polymerization is achieved by multiple enzyme complexes consisting of chondroitin synthase family members,” Journal of Biological Chemistry, vol. 283, no. 17, pp. 11396–11406, 2008.

M. Gotoh, T. Yada, T. Sato et al., “Molecular cloning and characterization of a novel chondroitin sulfate glucuronyltransferase that transfers glucuronic acid to N-acetylgalactosamine,” Journal of Biological Chemistry, vol. 277, no. 41, pp. 38179–38188, 2002.

T. Yada, M. Gotoh, T. Sato et al., “Chondroitin sulfate synthase-2: molecular cloning and characterization of a novel human glycosyltransferase homologous to chondroitin sulfate glucuronyltransferase, which has dual enzymatic activities,” Journal of Biological Chemistry, vol. 278, no. 32, pp. 30235–30247, 2003.

T. Yada, T. Sato, H. Kayeama et al., “Chondroitin sulfate synthase-3. Molecular cloning and characterization,” Journal of Biological Chemistry, vol. 278, no. 41, pp. 39711–39725, 2003.

M. Kobayashi, G. Sugumaran, J. Liu, N. W. Shworsak, J. E. Silbert, and R. D. Rosenberg, “Molecular cloning and characterization of a human uronyl 2-sulfotransferase that sulfates iduronyl and glucuronyl residues in dermatan/chondroitin sulfate,” Journal of Biological Chemistry, vol. 274, no. 15, pp. 10474–10480, 1999.

S. Yamauchi, S. Mitra, T. Matsubara et al., “Molecular cloning and expression of chondroitin 4-sulfotransferase,” Journal of Biological Chemistry, vol. 275, no. 12, pp. 8975–8981, 2000.

N. Hirao, H. Nakagawa, E. Ong, T. O. Akama, M. N. Fukuda, and M. Fukuda, “Molecular cloning and expression of two distinct human chondroitin 4-O-sulfotransferases that belong to the HNK-1 sulfotransferase gene family,” Journal of Biological Chemistry, vol. 275, no. 26, pp. 20188–20196, 2000.

H.-G. Kang, M. R. Evers, G. Xia, J. U. Baenziger, and M. Schachner, “Molecular cloning and characterization of chondroitin-4-O-sulfotransferase-3: a novel member of the HNK-1 family of sulfotransferases,” Journal of Biological Chemistry, vol. 277, no. 38, pp. 34766–34772, 2002.

M. Fukuda, K. Uchimura, K. Nakashima et al., “Molecular cloning and expression of chick chondrocyte chondroitin 6-sulfotransferase,” Journal of Biological Chemistry, vol. 270, no. 31, pp. 18575–18580, 1995.
M. Maccarana, B. Olander, J. Malmström et al., “Biosynthesis of dermatan sulfate—I. Formation of L-iduronic acid residues,” *Journal of Biological Chemistry*, vol. 250, no. 9, pp. 11560–11568, 2001.

A. Malmström, M. Maccarana, “Two dermatan sulfate epimerases form iduronic acid domains in dermatan sulfate,” *Journal of Biological Chemistry*, vol. 284, no. 15, pp. 9788–9795, 2009.

M. R. Evers, G. Xia, H.-G. Kang, M. Schachner, and J. U. Beneziger, “Molecular cloning and characterization of a dermatan-specific N-acetylgalactosamine 4-O-sulfotransferase,” *Journal of Biological Chemistry*, vol. 276, no. 39, pp. 36344–36353, 2001.

T. Mikami, S. Mizumoto, N. Kago, H. Kitagawa, and K. Sugahara, “Specificities of three distinct human chondroitin/dermatan N-acetylgalactosamine 4-O-sulfotransferases demonstrated using partially desulfated dermatan sulfate as an acceptor: implication of differential roles in dermatan sulfate biosynthesis,” *Journal of Biological Chemistry*, vol. 278, no. 38, pp. 36115–36127, 2003.

C. McCormick, Y. Leduc, D. Martindale et al., “The putative tumour suppressor EXT1 alters the expression of cell-surface heparan sulfate,” *Nature Genetics*, vol. 19, no. 2, pp. 158–161, 1998.

C. McCormick, G. Duncan, K. T. Goutsos, and F. Tufaro, “The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 2, pp. 668–673, 2000.

C. Senay, T. Lind, K. Muguruma et al., “The EXT1/EXT2 tumor suppressors: catalytic activities and role in heparan sulfate biosynthesis,” *EMBO Reports*, vol. 1, no. 3, pp. 282–286, 2000.

B.-T. Kim, H. Kitagawa, J. Tanaka, J.-I. Tamura, and K. Sugahara, “In Vitro Heparan Sulfate Polymerization: crucial roles of core protein moieties of primer substrates in addition to the EXT1-EXT2 interaction,” *Journal of Biological Chemistry*, vol. 278, no. 43, pp. 41618–41623, 2003.
[242] J. Liu, N. W. Shworak, P. Sinaï et al., “Expression of heparan sulfate D-glucosaminyl 3-O-sulfotransferase isoforms reveals novel substrate specificities,” Journal of Biological Chemistry, vol. 274, no. 8, pp. 5185–5192, 1999.

[243] G. Xia, J. Chen, V. Tiwari et al., “Heparan sulfate 3-O-sulfotransferase isoform 5 generates both an antithrombin-binding site and an entry receptor for herpes simplex virus, type 1,” Journal of Biological Chemistry, vol. 277, no. 40, pp. 37912–37919, 2002.

[244] D. Xu, V. Tiwari, G. Xia, C. Clement, D. Shukla, and J. Liu, “Characterization of heparan sulphate 3-O-sulphotransferase isoform 6 and its role in assisting the entry of herpes simplex virus type 1,” Biochemical Journal, vol. 385, no. 2, pp. 451–459, 2005.

[245] G. K. Dhoot, M. K. Gustafsson, X. Ai, W. Sun, D. M. Standiford, and J. Emerson C.P., “Regulation of Wnt signaling and embryo patterning by an extracellular sulfatase,” Science, vol. 293, no. 5535, pp. 1663–1666, 2001.

[246] T. Ohto, H. Uchida, H. Yamazaki, K. Keino-Masu, A. Matsui, and M. Masu, “Identification of a novel nonlysosomal sulphatase expressed in the floor plate, choroid plexus and cartilage,” Genes to Cells, vol. 7, no. 3, pp. 173–185, 2002.

[247] M. Morimoto-Tomita, K. Uchimura, Z. Werb, S. Hemmerich, and S. D. Rosen, “Cloning and characterization of two extracellular heparin-degrading endosulfatases in mice and humans,” Journal of Biological Chemistry, vol. 277, no. 51, pp. 49175–49185, 2002.

[248] E. J. Bradbury, L. D. F. Moon, R. J. Popat et al., “Chondroitinase ABC promotes functional recovery after spinal cord injury,” Nature, vol. 416, no. 6881, pp. 636–640, 2002.

[249] F. Properzi, D. Carulli, R. A. Asher et al., “Chondroitin 6-sulphate synthesis is up-regulated in injured CNS, induced by injury-related cytokines and enhanced in axon-growth inhibitory glia,” European Journal of Neuroscience, vol. 21, no. 2, pp. 378–390, 2005.

[250] D. Wang and J. Fawcett, “The perineuronal net and the control of CNS plasticity,” Cell and Tissue Research, vol. 349, no. 1, pp. 147–160, 2012.

[251] A. von Holst, S. Sirko, and A. Faissner, “The unique 473HD-chondroitinsulfate epitope is expressed by radial glia and involved in neural precursor cell proliferation,” Journal of Neuroscience, vol. 26, no. 15, pp. 4082–4094, 2006.

[252] U. Lindahl, G. Backstrom, L. Thunberg, and I. G. Leder, “Evidence for a 3-O-sulfated D-glucosamine residue in the antithrombin-binding sequence of heparin,” Proceedings of the National Academy of Sciences of the United States of America, vol. 77, no. 11 I, pp. 6551–6555, 1980.

[253] N. Sugaya, H. Habuchi, N. Nagai, S. Ashikari-Hada, and K. Kimata, “6-O-sulfation of heparan sulfate differentially regulates various fibroblast growth factor-dependent signalings in culture,” Journal of Biological Chemistry, vol. 283, no. 16, pp. 10366–10376, 2008.

[254] I. T. Cook, T. S. Leyh, S. A. Kadhubar, and C. N. Falany, “Structural rearrangement of SULT2A1: effects on dehydroepiandrosterone and raloxifene sulfation,” Hormone Molecular Biology and Clinical Investigation, vol. 1, no. 2, pp. 81–87, 2010.

[255] A. De Paepe and F. Malfait, “The Ehlers-Danlos syndrome, a disorder with many faces,” Clinical Genetics, vol. 82, no. 1, pp. 1–11, 2012.

[256] P. Beighton, G. Gericke, K. Kozlowski, and L. Grobler, “The manifestations and natural history of spondylo-epimetaphyseal dysplasia with joint laxity,” Clinical Genetics, vol. 26, no. 4, pp. 308–317, 1984.