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Abstract

Glycosaminoglycans (GAGs) such as dermatan sulfate (DS), chondroitin sulfate (CS), and heparan sulfate are side chains composed of repeating disaccharides bound to core proteins to form proteoglycans (PGs). Biosynthesis of CS and DS is shown in Fig. 156.1. It is initiated by the synthesis of a tetrasaccharide linker region, glucuronic acidb1-3galactoseb1-3galactoseb1-4xyloseb1-O- (GlcA-Gal-Gal-Xyl-), onto serine residues of specific core proteins of PGs, by b-xylosyltransferase, b1,4-galactosyltransferase-I, b1,3-galactosyltransferase-II, and b1,3-glucuronosyltransferase-I, respectively. Subsequently, a repeating disaccharide region [N-acetyl-D-galactosamine(GalNAc)-GlcA]$_n$ of chondroitin is elongated by the actions of N-acetyl-D-galactosaminyltransferase-I, N-acetyl-D-galactosaminyltransferase-II, and CS-glucuronyltransferase-II encoded by chondroitin synthase -1, -2, and -3 and chondroitin polymerizing factor. CS chains are matured by modifications by chondroitin 4-O-sulfotransferase, chondroitin 6-O-sulfotransferase, and uronyl 2-O- sulfotransferase (UST). A disaccharide repeating region of dermatan is synthesized through epimerization of a carboxyl group at C5 from GlcA to L-iduronic acid (IdoA) by dermatan sulfate epimerase (DSE). A mature DS chain is synthesized through sulfation by dermatan 4-O-sulfotransferase (D4ST), dermatan 6-O-sulfotransferase (D6ST), and UST.

Carbohydrate (N-Acetylgalactosamine 4-0) Sulfotransferase 14 (CHST14)

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Tomoki Kosho, Shuji Mizumoto, and Kazuyuki Sugahara

Contents

Introduction

Glycosaminoglycans (GAGs) such as dermatan sulfate (DS), chondroitin sulfate (CS), and heparan sulfate are side chains composed of repeating disaccharides bound to core proteins to form proteoglycans (PGs). Biosynthesis of CS and DS is shown in Fig. 156.1. It is initiated by the synthesis of a tetrasaccharide linker region, glucuronic acidb1-3galactoseb1-3galactoseb1-4xyloseb1-O- (GlcA-Gal-Gal-Xyl-), onto serine residues of specific core proteins of PGs, by b-xylosyltransferase,
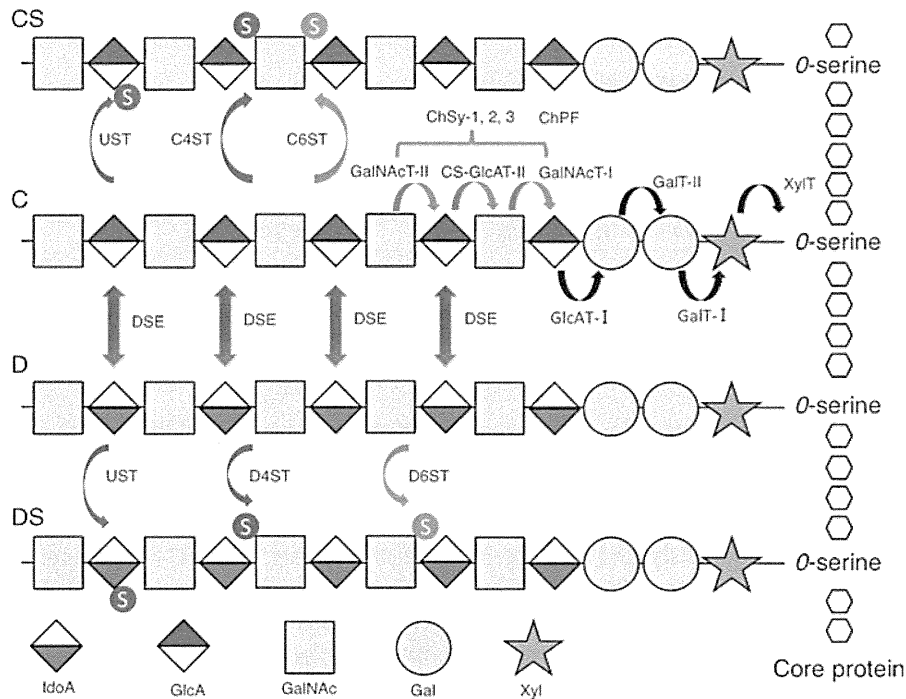
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Fig. 156.1 Biosynthesis of DS and CS (Kosho in press). *C* chondroitin, *CS* chondroitin sulfate, *D* dermatan, *DS* dermatan sulfate, *Ser* serine, *Xyl* D-xylose, *Gal* D-galactose, *GlcA* D-glucuronic acid, *GalNAc* N-acetyl-D-galactosamine, *IdoA* L-iduronic acid, *XylT* xylosyltransferase, *GalT-I* galactosyltransferase-I, *GalT-II* galactosyltransferase-II, *GlcAT-I* glucuronyltransferase-I, *GalNAcT-I* N-acetyl-D-galactosaminyltransferase-I, *CS-GlcAT-II* CS-glucuronyltransferase-II, *GalNAcT-II* N-acetyl-D-galactosaminyltransferase-II, *ChSy* chondroitin synthase, *ChPF* chondroitin polymerizing factor, *C4ST* chondroitin 4-O-sulfotransferase, *C6ST* chondroitin 6-O-sulfotransferase, *UST* uronyl 2-O-sulfotransferase, *DSE* dermatan sulfate epimerase, *D4ST* dermatan 4-O-sulfotransferase, and *D6ST* dermatan 6-O-sulfotransferase

26 b1,4-galactosyltransferase-I, b1,3-galactosyltransferase-II, and b1,3-glucuronosyl-
27 transferase-I, respectively. Subsequently, a repeating disaccharide region [N-acetyl-
28 D-galactosamine(GalNAc)-GlcA]$_n$ of chondroitin is elongated by the actions of
29 N-acetyl-D-galactosaminyltransferase-I, N-acetyl-D-galactosaminyltransferase-II,
30 and CS-glucuronyltransferase-II encoded by chondroitin synthase -1, -2, and -3
31 and chondroitin polymerizing factor. CS chains are matured by modifications
32 by chondroitin 4-O-sulfotransferase, chondroitin 6-O-sulfotransferase, and uronyl
33 2-O- sulfotransferase (UST). A disaccharide repeating region of dermatan is
34 synthesized through epimerization of a carboxyl group at C5 from GlcA to
35 L-iduronic acid (IdoA) by dermatan sulfate epimerase (DSE). A mature DS chain
36 is synthesized through sulfation by dermatan 4-O-sulfotransferase (D4ST),
37 dermatan 6-O-sulfotransferase (D6ST), and UST.

Au1

Databanks

IUBMB enzyme nomenclature: E.C.2.8.2.35

Carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 14 (CHST14)

Species	Gene symbol	GenBank accession number	UniProt ID	PDB accession number
Mus musculus	Chst14	NM_028117.3	Q80V53	N/A
Homo sapiens	CHST14	NM_130468	Q8NCH0	N/A

Name and History

The CHST14 gene encodes dermatan 4-O-sulfotransferase-1 (D4ST1), which cat-alyzes the 4-O-sulfation of GalNAc residues in DS. Evers et al. (2001) cloned cDNA of CHST14, based on its homology to CHST10 coding for human natural killer-1 sulfotransferase. Evers et al. (2001) showed mRNA of CHST14 to be expressed ubiquitously and the protein to transfer sulfate to the C-4 hydroxyl of GalNAc in the sequence IdoA-GalNAc immediately after epimerization of GlcA to IdoA and designated the enzyme as D4ST1. Mikami et al. (2003), who had identified CHST14/D4ST1 independently by public database search, reported further characterization of the enzyme specificities that partially desulfated DS also served as an excellent acceptor, while nearly exhaustively desulfated DS had been shown to be an acceptor (Evers et al. 2001). In 2009–2010, human CHST14/D4ST1 deficiency was identified as a clinically recognizable syndrome and designated as "D4ST1-deficient Ehlers–Danlos syndrome (DD-EDS)."

Structure

The CHST14 gene, localized at 15q14, is a single exon gene with an open reading frame (ORF) of 1,131 base pairs (Evers et al. 2001). Human CHST14/D4ST1, consisting of 376 amino acids with an estimated molecular mass of 43 kDa, is a type II membrane protein with an N-terminal transmembrane region, binding sites for 3′-phosphoadenosine-5′-phosphosulfate (PAPS), and two potential N-glycosylation sites (Evers et al. 2001).

Enzyme Activity Assay and Substrate Specificity

This enzyme catalyzes transfer of sulfate to C4 position of GalNAc residues of dermatan. The standard reaction mixture (60 μl) includes 10 μl of the enzyme sources, 50 mM imidazole-HCl, pH 6.8, 2 mM dithiothreitol, 10 μM [^{35}S]PAPS (~1 or 3 × 10^5 dpm), and desulfated DS as an acceptor (10 nmol as disaccharide) (Mikami et al. 2003). The reaction mixtures are incubated at 37 °C for 1 h and

67 subjected to gel filtration using a syringe column packed with Sephadex G-25
68 (superfine) (Mikami et al. 2003). [^{35}S]Sulfate incorporation into polysaccharides
69 is quantified by determination of the radioactivity in the flow-through fractions by
70 liquid scintillation counting (Mikami et al. 2003).
71   Both nearly exhaustively desulfated DS and partially desulfated DS serve as
72 excellent substrates for the enzyme (Evers et al. 2001; Mikami et al. 2003).

73 Preparation

74 Cell lysates are prepared with 200 µl of the M-PER$^{®}$ mammalian protein extraction
75 reagent (Thermo Fisher Scientific Inc., Waltham, MA). A DNA fragment, which
76 encodes the human CHST14/D4ST1 protein lacking the first N-terminal 62 amino
77 acids including the predicted transmembrane region, was subcloned into the BamHI
78 site of the expression vector p3XFLAG-CMV-8 (Sigma), resulting in the fusion of
79 CHST14 to the preprotrypsin leader sequence and the 3XFLAG tag sequence at the
80 N-terminus present in the vector. The expression plasmid was transfected
81 into COS-7 cells using FuGENE6 transfection reagent (Roche Diagnostics, Basel,
82 Switzerland). After 3 days the culture medium was incubated with the anti-FLAG
83 affinity resin (Sigma or Wako, Osaka, Japan), which was washed with 25 mM Tris,
84 pH 7.4/150 mM NaCl/0.05 % Tween-20, and then analyzed by SDS–PAGE
85 followed by western analysis using anti-FLAG monoclonal antibody conjugated
86 with horseradish peroxidase (Sigma or Wako) (Mikami et al. 2003; van Roij
87 et al. 2008).

88 Biological Aspects

89 Clinical features of human CHST14/D4ST1 deficiency suggest that CHST14/
90 D4ST1 and DS would play a crucial role in fetal development and maintenance
91 of connective tissues in multiple organs/tissues. Pathophysiological evidence
92 revealed in human CHST14/D4ST1 deficiency indicates the substantial role of
93 CHST14/D4ST1 to regulate CS/DS disaccharide composition of a GAG chain of
94 decorin (and probably other DS-PGs), the GAG chains of which would exhibit
95 various biological effects such as appropriate assembly of collagen fibrils mediated
96 by decorin. Ubiquitous expression of CHST14 would also suggest multisystem
97 effects of the enzyme.

98 Knockout and Transgenic Mice

99 Knockout mice were generated by homologous recombination and targeting of the
100 only coding exon (exon 1) of the Chst14 gene (Tang et al. 2010; Bian et al. 2011).
101 Phenotypic analysis of the F2 mice showed that the mutation affected bone
102 metabolism, cardiology, neurology, ophthalmology, metabolism, and growth

103 (Tang et al. 2010). Chst14/D4st1-deficient mice had decreased neurogenesis
104 and diminished proliferation of neural stem cells (NSCs) accompanied by
105 increased expression of glutamate–aspartate transporter (GLAST) and epidermal
106 growth factor (EGF) in comparison with wild-type controls as well as Chst11/
107 C4st1-deficient mice (Bian et al. 2011). There is no report regarding *Chst14*
108 transgenic mice.

109 Human Disease

110 Recessive loss-of-function mutations in the *CHST14* gene were identified in
111 patients with three independently reported conditions: a rare type arthrogryposis
112 syndrome, "adducted thumb–clubfoot syndrome (ATCS)" (Dündar et al. 2009);
113 a specific type of Ehlers–Danlos syndrome (EDS), "EDS, Kosho type" (Kosho et al.
114 2010; Miyake et al. 2010); and a subset of kyphoscoliosis-type EDS without lysyl
115 hydroxylase deficiency, "musculocontractural EDS") (Malfait et al. 2010). These
116 conditions were concluded to represent a single clinical entity, a new form of
117 EDS coined as "D4ST1-deficient EDS (DD-EDS)" (Kosho et al. 2011; Shimizu
118 et al. 2011). To date, 26 patients with DD-EDS have been reported (Table 156.1)
119 (Kosho in press).
120 Clinical manifestations are summarized in Table 156.2, characterized by pro-
121 gressive multisystem fragility-related manifestations (skin hyperextensibility and
122 fragility, progressive spinal and foot deformities, large subcutaneous hematoma)
123 and various malformations (facial features, congenital eye/heart/gastrointestinal
124 defects, congenital multiple contractures) (Kosho et al. 2011; Shimizu et al. 2011).
125 Characteristic craniofacial features including large fontanelle, hypertelorism,
126 short and downslanting palpebral fissures, blue sclerae, short nose with hypoplastic
127 columella, low-set and rotated ears, high palate, long philtrum, thin upper lip
128 vermilion, small mouth, and micro-retrognathia are noted at birth to early childhood
129 (Fig. 156.2a, b). Slender and asymmetrical facial shapes with protruding jaws are
130 noted from school age (Fig. 156.2c) (Kosho et al. 2005, 2010, 2011; Shimizu et al.
131 2011; Kosho in press).
132 Congenital multiple contractures, such as adduction–flexion contractures of
133 thumbs and talipes equinovarus, were cardinal features (Fig. 156.2d, g). Peculiar
134 finger shape, described as "tapering," "slender," and "cylindrical," is also noted
135 (Fig. 156.2e, f). Talipes deformities (planus, valgus) (Fig. 156.2h) and spinal
136 deformities (scoliosis, kyphoscoliosis) with tall vertebral bodies and decreased
137 physiological curvature (Fig. 156.2j, k) develop. Marfanoid habitus, recurrent
138 joint dislocations, and pectus deformities (flat and thin, excavatum, carinatum)
139 are also evident (Kosho et al. 2005, 2010, 2011; Shimizu et al. 2011; Kosho
140 in press).
141 Cutaneous features include hyperextensibility to redundancy, bruisability,
142 fragility leading to atrophic scars, acrogeria-like fine palmar creases or wrinkles
143 (Fig. 156.2e, f), hyperalgesia to pressure, and recurrent subcutaneous infections
144 with fistula formation (Kosho et al., 2005, 2010; Shimizu et al., 2011).

Au2

Au3

t2.1   **Table 156.1** Reported patients with DD-EDS (Kosho in press)

t2.2 Patient	Family	Origin	CHST14 mutations	Sex	Age at initial publication	References
t2.3 1	1	Turkish	V49X homo	F	3.5 y	Dündar et al. 1997
t2.4 2	1	Turkish	V49X homo	M	1.5 y	Dündar et al. 1997
t2.5 3	1	Turkish	V49X homo	F	6 y	Dündar et al. 1997
t2.6 4	2	Japanese	Y293C homo	M	4 y	Sonoda and Kouno 2000
t2.7 5	2	Japanese	Y293C homo	M	7 m	Sonoda and Kouno 2000
t2.8 6	3	Austrian	R213P homo	M	0 d[a]	Janecke et al. 2001
t2.9 7	3	Austrian	R213P homo	M	12 m	Janecke et al. 2001
t2.10 8	4	Turkish	[R135G;L137Q] homo	F	1–4 m[a]	Dündar et al. 2001
t2.11 9	4	Turkish	[R135G;L137Q] homo	M	1–4 m[a]	Dündar et al. 2001
t2.12 10	4	Turkish	[R135G;L137Q] homo	M	1–4 m[a]	Dündar et al. 2001
t2.13 11	4	Turkish	[R135G;L137Q] homo	M	3 m	Dündar et al. 2001
t2.14 12	5	Japanese	P281L/Y293C	F	11 y	Kosho et al. 2005
t2.15 13	6	Japanese	P281L homo	F	14 y	Kosho et al. 2005
t2.16 14	7	Japanese	P281L homo	M	32 y	Kosho et al. 2010
t2.17 15	8	Japanese	K69X/P281L	M	32 y	Kosho et al. 2010
t2.18 16	9	Japanese	P281L/C289S	F	20 y	Kosho et al. 2010
t2.19 17	10	Japanese	P281L/Y293C	F	4 y	Kosho et al. 2010
t2.20 18	11	Turkish	V49X homo	F	22 y	Malfait et al. 2010
t2.21 19	11	Turkish	V49X homo	F	21 y	Malfait et al. 2010
t2.22 20	12	Indian	E334Gfs*107 homo	F	12 y	Malfait et al. 2010
t2.23 21	13	Japanese	P281L/Y293C	M	2 y	Shimizu et al. 2011
t2.24 22	14	Japanese	F209S/P281L	M	6 y	Shimizu et al. 2011
t2.25 23	15	Dutch	V48X homo	F	20 y	Voermans et al. 2012
t2.26 24	16	Afghani	R274P homo	F	11 y	Mendoza-Londono et al. 2012
t2.27 25	16	Afghani	R274P homo	F	0 y	Mendoza-Londono et al. 2012
t2.28 26	17	Miccosukee	G228Lfs*13	F	16 y	Winters et al. 2012

t2.29   *Homo* homozygous mutation, / compound heterozygous mutation, *F* female, *M* male, *y* years old, *m* months old, *d* day

[a]Dead at the time of publication

[b]Also reported in a paper by Yasui et al. (2003)

Table 156.2 Clinical manifestations in DD-EDS (Kosho et al. 2011). *ASD* atrial septal defect, *MVP* mitral valve prolapse, *MR* mitral valve regurgitation, *AR* aortic valve regurgitation, *ARD* aortic root dilation

Craniofacial	
	Large fontanelle (early childhood)
	Hypertelorism
	Short and downslanting palpebral fissures
	Blue sclerae
	Short nose with hypoplastic columella
	Ear deformities (prominent, posteriorly rotated, low set)
	Palatal abnormalities (high, cleft)
	Long philtrum and thin upper lip
	Small mouth/micro-retrognathia (infancy)
	Slender face with protruding jaw (from school age)
	Asymmetric face (from school age)
Skeletal	
	Marfanoid habitus/slender build
	Congenital multiple contractures (fingers, wrists, hips, feet)
	Recurrent/chronic joint dislocations
	Pectus deformities (flat, excavated)
	Spinal deformities (scoliosis, kyphoscoliosis)
	Peculiar fingers (tapering, slender, cylindrical)
	Progressive talipes deformities (valgus, planus, cavum)
Cutaneous	
	Hyperextensibility/redundancy
	Bruisability
	Fragility/atrophic scars
	Fine/acrogeria-like palmar creases
	Hyperalgesia to pressure
	Recurrent subcutaneous infections/fistula
Cardiovascular	
	Congenital heart defects (ASD)
	Valve abnormalities (MVP, MR, AR, ARD)
	Large subcutaneous hematomas
Gastrointestinal	
	Constipation
	Diverticula perforation
Respiratory	
	(Hemo)pneumothorax

(continued)

t3.38 **Table 156.2** (continued)

t3.39 *Urogenital*	
t3.40	Nephrolithiasis/cystolithiasis
t3.41	Hydronephrosis
t3.42	Dilated/atonic bladder
t3.43	Inguinal hernia
t3.44	Cryptorchidism
t3.45	Poor breast development
t3.46 *Ocular*	
t3.47	Strabismus
t3.48	Refractive errors (myopia, astigmatism)
t3.49	Glaucoma/elevated intraocular pressure
t3.50	Microcornea/microphthalmia
t3.51	Retinal detachment
t3.52 *Hearing*	
t3.53	Hearing impairment
t3.54 *Neurological*	
t3.55	Ventricular enlargement/asymmetry
t3.56 *Development*	
t3.57	Hypotonia/gross motor delay

145   The most serious complication is recurrent large subcutaneous hematoma, which
146 sometimes progresses acutely and massively to be treated intensively (admission,
147 blood transfusion, surgical drainage) and is supposed to be caused by the rupture of
148 subcutaneous arteries or veins (Fig. 156.2i) (Kosho et al. 2005, 2010, 2011;
149 Shimizu et al. 2011; Kosho in press).

150   Sulfotransferase activity toward dermatan in the affected skin fibroblasts was
151 significantly decreased to 6.7 % in a patient with a compound heterozygous mutation
152 "P281L/Y293C" (Patient 12, in Table 156.1) and to 14.5 % in a patient with a homo-
153 zygous mutation "P281L" (Patient 14), compared with each age- and sex-matched
154 control (Miyake et al. 2010) (Fig. 156.3a(a)). Disaccharide composition analysis of
155 CS/DS chains isolated from the affected skin fibroblasts (Patient 12, 14) showed a
156 negligible amount of DS and excess CS, which was suggested to result from impaired
157 4-O-sulfation lock due to D4ST1 deficiency followed by back epimerization from IdoA
158 to GlcA (Dündar et al. 2009; Miyake et al. 2010) (Fig. 156.3a(b)). A major DS-PG in
159 the skin, decorin, was also investigated, which consists of a core protein and a single
160 GAG chain that plays an important role in assembly of collagen fibrils possibly through
161 electrostatic interaction between decorin DS chains and adjacent collagen fibrils
162 (Nomura 2006). GAG chains of decorin from the affected skin fibroblasts contained
163 exclusively CS and no DS disaccharides, while those from the controls contained
164 mainly DS disaccharides (approximately 95 %) (Miyake et al. 2010) (Fig. 156.3a(c)).

165   Light microscopy of hematoxylin- and eosin-stained affected skin specimens
166 showed that fine collagen fibers were present predominantly in the reticular to
167 papillary dermis with marked reduction of normally thick collagen bundles (Miyake
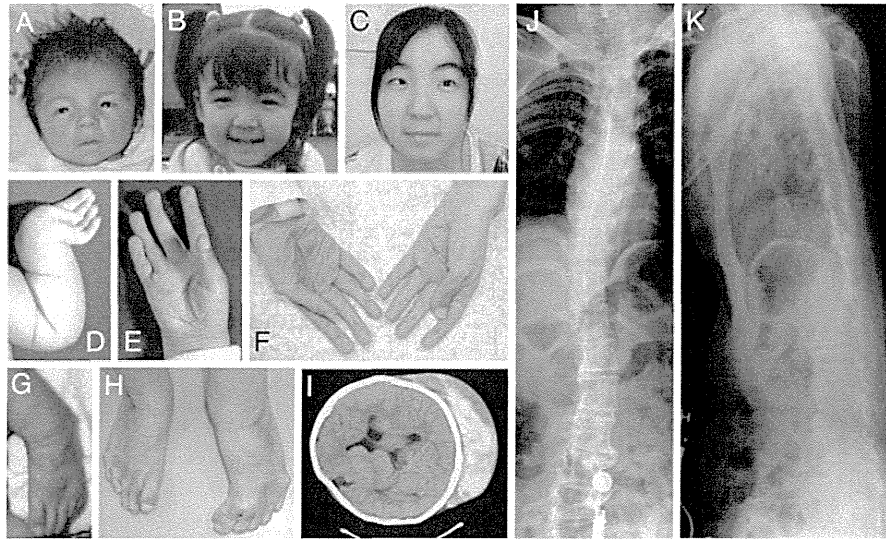
Fig. 156.2 Clinical photographs of patients with DD-EDS (Kosho et al. 2005; Kosho et al. 2010). Patient 12 at age 23 days (**a**), 3 years (**b**), 6 years (**i**), and 16 years (**c**). Patient 13 at age 3 months (**d**), 5 years (**e**), and 28 years (**f, j, k**). Patient 14 in the neonatal period (**g**) and at age 28 years (**h**). Patient number is according to Table 156.1

168 et al. 2010) (Fig. 156.3b(a, b)). Electron microscopy showed that collagen fibrils in
169 affected skin specimens were dispersed in the reticular dermis, compared with the
170 regularly and tightly assembled ones observed in the control's, whereas each collagen
171 fibril in affected skin specimens was smooth and round, not varying in size and shape,
172 similar to each collagen fibril of the control's (Miyake et al. 2010) (Fig. 156.3b(c, d)).
173   These glycobiological and pathological findings suggested skin fragility in
174 this disorder to be caused by impaired assembly of collagen fibrils resulting from
175 the replacement of a DS with a CS chain of decorin (Miyake et al. 2010);
176 (Kosho 2011) (Fig. 156.3c(a, b)). The disorder represents the first human disorder
177 that emphasizes the role of CHST14/D4ST1 and DS to play in human development
178 and the maintenance of the extracellular matrices (Zhang et al. 2010).

179 Future Perspectives

180 Detailed evaluation of knockout mice and patients with DD-EDS would contribute
181 to delineate multisystem roles of CHST14/D4ST1 and DS. Pathological investiga-
182 tion of various organs/tissues would address the question whether involvement of
183 other organs/tissues might result from impaired assembly of collagen fibrils medi-
184 ated by decorin. Glycobiological investigation focusing on various DS-PGs would
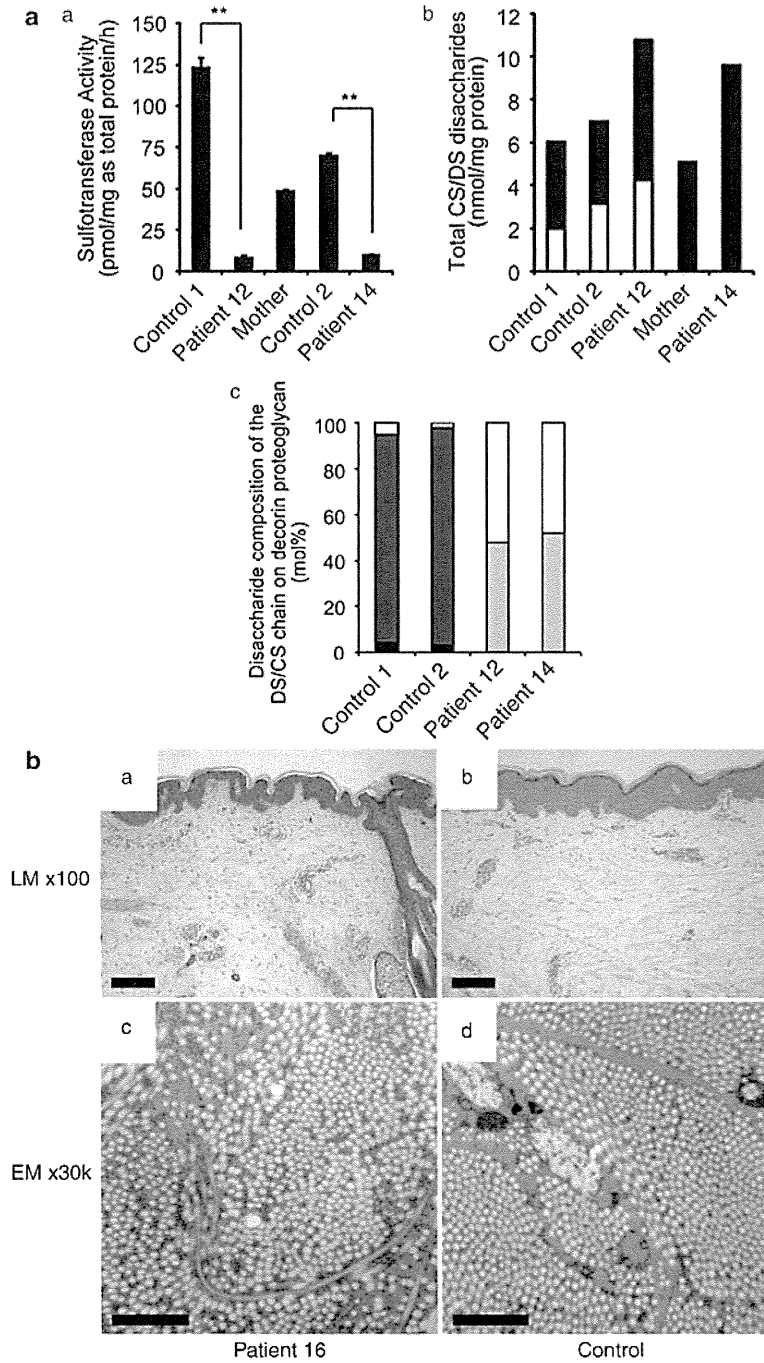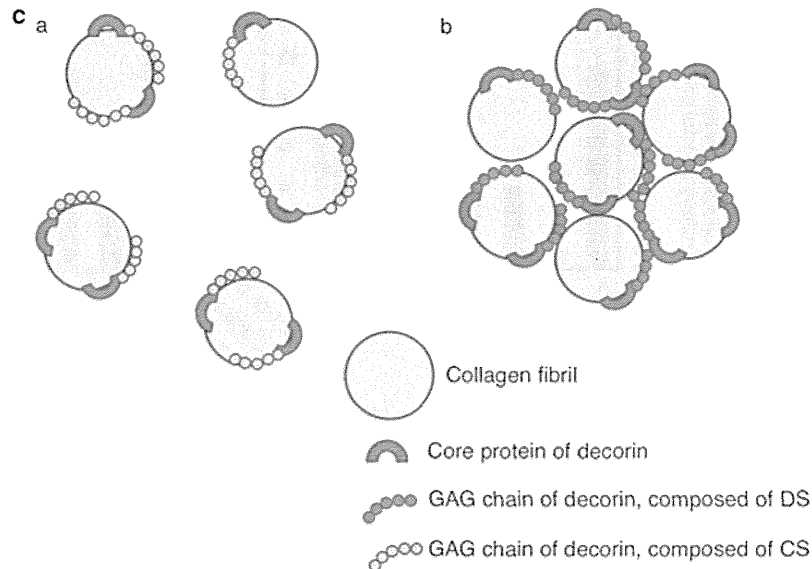185 uncover the contribution of DS-PGs in addition to decorin.

Fig. 156.3 (continued)

Fig. 156.3 (a) Glycobiological studies (Miyake et al. 2010). Control 1 is Patient 12's age- and sex-matched control. Mother is Patient 12's. Control 2 is Patient 14's age- and sex-matched control. Patient number is according to Table 156.1. (a) Sulfotransferase activity of skin fibroblasts. (b) The total amounts of CS and DS derived from skin fibroblasts. The total disaccharide contents of CS and DS are shown in a *black box* and a *white box*, respectively. (c) Proportion of the disaccharide units in the CS/DS hybrid chains in decorin secreted by the fibroblasts. A *white box* and a *light gray box* indicate GlcUA-GalNAc (4S) and GlcUA-GalNAc (6S), respectively, both composing CS. A *dark gray box* and a *black box* indicate IdoUA-GalNAc(4S) and IdoUA-GalNAc (6S), respectively, both composing DS. (b). Pathological studies (Miyake et al. 2010). Light microscopy (LM) of a hematoxylin- and eosin-stained skin specimen of Patient 16 in Table 156.1 (a) and that of her age- and sex-matched control. (b) Scale bars indicate 500 μm. Electron microscopy (EM) of a skin specimen of Patient 16 (c) and that of the control (d). Scale bars indicate 1 μm. (c) Schema of binding model of decorin to collagen fibrils (Nomura 2006). Putative spatial relationship between collagen fibrils and decorin in skin specimens of patients with DD-EDS (a) and normal control subjects (b) (Kosho 2011)

Cross-References

Further Reading

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Mikami et al. (2003): Further characterization of enzyme specificities.

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Miyake et al. (2010): Further characterization of pathophysiology of CHST14/D4ST1 deficiency especially showing impaired assembly of collagen fibrils resulting from loss of DS in the GAG chain of decorin.

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Discovery and Delineation of Dermatan 4-*O*-Sulfotransferase-1 (D4ST1)-Deficient Ehlers-Danlos Syndrome

Tomoki Kosho

Additional information is available at the end of the chapter

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1. Introduction

The Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders affecting as many as 1 in 5000 individuals, characterized by joint and skin laxity, and tissue fragility [1]. The fundamental mechanisms of EDS are known to consist of dominant-negative effects or haploinsufficiency of mutant procollagen α-chains and deficiency of collagen-processing-enzymes [2]. In a revised nosology established in the nomenclature conference held in June 1997 at Villefranche-sur-Mer, France, Beighton et al. [3] classified EDS into six major types (Table 1): classical type (OMIM#130000), hypermobility type (OMIM#130020), vascular type (OMIM#130050), kyphoscoliosis type (OMIM#225400), arthrochalasia type (OMIM#130060), and dermatosparaxis type (OMIM#225410). Additional minor variants of EDS have been identified with molecular and biochemical abnormalities: dermatan 4-*O*-sulfotransferase-1 (D4ST1)-deficient type/musculocontractural type (OMIM#601776), Brittle cornea syndrome (OMIM#229200), EDS-like syndrome due to tenascin-XB deficiency (OMIM#606408), EDS with progressive kyphoscoliosis, myopathy, and hearing loss (OMIM#614557); the spondylocheiro dysplastic form (OMIM#612350), cardiac valvular form (OMIM#225320), and progeroid form (OMIM#130070) [4] (Table 1). This chapter focuses on a recent breakthrough in EDS: discovery and delineation of D4ST1-deficient EDS (DD-EDS).

2. History of D4ST1-deficient EDS

DD-EDS, caused by loss-of-function mutations in the carbohydrate sulfotransferase 14 (*CHST14*) gene coding D4ST1, has been identified independently as a rare type of arthrogryposis syndrome, "adducted thumb–clubfoot syndrome (ATCS)" [5]; as a specific

form of EDS, "EDS, Kosho Type" (EDSKT) [6]; and as a subset of kyphoscoliosis type EDS without evidence of lysyl hydroxylase deficiency, "Musculocontractural EDS" (MCEDS) [7].

	Prevalence [s]	Inheritance	Causative gene(s)
Major types			
Classical type	1/20,000	AD	COL5A1, COL5A2
Hypermobility type	1/5,000-20,000	AD	TNXB[a]
Vascular type	1/50,000-250,000	AD	COL3A1
Kyphoscoliosis type	1/100,000	AR	PLOD
Arthrochalacia type	30	AD	COL1A1*, COL1A2*
Dermatosparaxis type	8	AR	ADAMTS-2
Other variants			
D4ST1-deficient type	26	AR	CHST14
Brittle cornea syndrome	11	AR	ZNF469
EDS-like syndrome due to tenascin-XB deficiency	10	AR	TNXB
EDS with progressive kyphoscoliosis myopathy, and hearing loss	7	AR	FKBP14
Spondylocheiro dysplastic form	8	AR	SLC39A13
Cardiac valvular form	4	AR	COL1A2
Progeroid form	3	AR	B4GALT7

[s], a fraction number represents the prevalence such as "one affected person in 20,000 individuals" for "1/20,000" and an integral number represents the sum of previously reported patients; AD, autosomal dominant; AR, autosomal recessive; COL5A1 or COL5A2, α1(V) or α2(V) procollagen; TNXB, tenascin-X; [a], in a small subset of cases; COL3A1, α1(III) procollagen; PLOD, lysyl hydroxylase; COL1A1 or COL1A2, α1(I) or α2(I) procollagen; *, splice-site mutations of the genes; ADAMTS2, procollagen I N-proteinase; CHST14, carbohydrate sulfotransferase 14; ZNF469, zinc finger protein 469; FKBP14, FK506-binding protein 14; SLC39A13, a membrane-bound zinc transporter; B4GALT7; xylosylprotein 4-beta-galactosyltransferase

Table 1. Classification of Ehlers-Danlos Syndromes

2.1. Adducted thumb–Clubfoot syndrome

The original report of ATCS was written by Dündar et al. [8] from Erciyes University, Turkey, presenting two cousins, a boy aged 3.5 years and a girl aged 1.5 years, from a consanguineous Turkish family. In common, they had moderate to severe psychomotor developmental delay, ocular anterior chamber abnormality, facial characteristics, generalized joint laxity, arachnodactyly, camptodactyly, and distal arthrogryposis with adducted thumbs and clubfeet. They reported another patient with ATCS, a boy aged 3 months, from a consanguineous Turkish family including three affected siblings who died of unknown etiology between the ages of 1 and 4 months [9]. The patient also had bilateral nephrolithiasis, a unilateral inguinal hernia, and bilateral cryptorchidism. The authors

suggested that two brothers, aged 22 months and 7 months, from a Japanese consanguineous family reported by Sonoda and Kouno [10] would also fit the diagnosis of ATCS. The brothers had multiple distal arthrogryposis, characteristic facial features, cleft palates, short stature, hydronephrosis, cryptorchidism, and normal intelligence. Dündar et al. [9] also showed follow-up observations of the original patients: the intelligence quotient (IQ) was roughly 90 in one subject at age 7 years and 2 months and the other died of unknown cause at 5 years of age. Janecke et al. [11] from Innsbruck Medical University, Austria, reported two brothers with ATCS from a consanguineous Austrian family, one of whom died shortly after birth because of respiratory failure. The authors concluded that all these patients represented a new type of arthrogryposis with central nervous system involvement, congenital heart defects, urogenital defects, myopathy, connective tissue involvement (generalized joint laxity), and normal or subnormal mental development. In 2009, Dündar et al. reported that *CHST14* was the causal gene for ATCS through homozygosity mapping using samples from four previously published consanguineous families. The authors mentioned some follow-up clinical findings including generalized joint laxity, delayed wound healing, ecchymoses, hematomas, and osteopenia/osteoporosis; and categorized ATCS as a generalized connective tissue disorder [5].

2.2. EDS, Kosho type

We encountered the first patient with a specific type of EDS in 2000 and the second with parental consanguinity in 2003. They were Japanese girls with strikingly similar symptoms: characteristic craniofacial features; skeletal features including multiple congenital contractures, malfanoid habitus, pectus excavatum, generalized joint laxity, recurrent dislocations, and progressive talipes and spinal deformity; skin hyperextensibility, bruisability, and fragility with atrophic scars; recurrent hematomas; and hypotonia with mild motor developmental delay [12]. These symptoms overlapped those in the kyphoscoliosis type EDS (previously known as EDS type VI), which is typically associated with deficiency of lysyl hydroxylase (EDS type VIA) [13]. A rare condition with the clinical phenotype of the kyphoscoliosis type EDS but with normal lysyl hydroxylase activity were reported and named as EDS type VIB [13]. Therefore, we tentatively proposed that the two patients represented a clinically recognizable subgroup of EDS type VIB [12]. Through their long-term clinical evaluation as well as four additional unrelated Japanese patients including one with parental consanguinity and another reported by Yasui et al. [14], we concluded that they—four female patients and two male patients aged 4–32 years, represented a new clinically recognized type of EDS with distinct craniofacial characteristics, multiple congenital contractures, progressive joint and skin laxity, and multisystem fragility-related manifestations [15]. The disorder has been registered as EDS Kosho Type (EDSKT) in the London Dysmorphology Database (http://www.lmdatabases.com/index.html) and in POSSUM (http://www.possum.net.au/). In 2009, we identified *CHST14* as causal for the disorder through homozygosity mapping using samples from two consanguineous families and all the other patients were also found to have compound heterozygous *CHST14* mutations [6].

2.3. Musculocontractural EDS

Malfait et al. [7] from Ghent University, Belgium have found mutations in CHST14 through homozygosity mapping of two Turkish sisters and an Indian girl both presenting clinically with EDS VIB and with parental consanguinity. They had distinct craniofacial features, joint contractures, and wrinkled palms in addition to common features of kyphoscoliosis type EDS including kyphoscoliosis, muscular hypotonia, hyperextensible, thin, and bruisable skin, atrophic scarring, joint hypermobility, and variable ocular involvement. Malfait et al. [7] concluded that their series and ATCS, as well as EDSKT, formed a phenotypic continuum based on their clinical observations and identification of an identical mutation in both conditions; and proposed to coin the disorder as "musculocontractural EDS" (MCEDS).

3. Pathophysiology of D4ST1-deficient EDS

3.1. Glycobiological abnormalities in D4ST1-deficient EDS

D4ST1 is a regulatory enzyme in the glycosaminoglycan (GAG) biosynthesis that transfers active sulfate to position 4 of the N-acetyl-D-galactosamine residues of dermatan sulfate (DS) (Fig. 1) [16, 17]. DS, together with chondroitin sulfate (CS) and heparan sulfate, constitutes GAG chains of proteoglycans and is implicated in cardiovascular disease, tumorigenesis, infection, wound repair, and fibrosis via DS-containing proteoglycans such as decorin and biglycan [18].

Sulfotransferase activity toward dermatan in the skin fibroblasts derived from the patients was significantly decreased to 6.7% (patient 1 with a compound heterozygous mutation: P281L/Y293C) and 14.5% (patient 3 with a homozygous mutation: P281L) of each age- and sex-matched control) (Fig. 2A). Disaccharide composition analysis of CS/DS chains isolated from the skin fibroblasts showed a negligible amount of DS and a slight excess of CS (Fig. 2B). Subsequently, we focused on a major DS proteoglycan in the skin, decorin, consisting of core protein and one GAG chain and playing an important role in assembly of collagen fibrils (Nomura, 2006). No DS disaccharides were detected in the GAG chains of decorin from the patients, whereas the GAG chains of decorin from the controls were mainly composed of DS disaccharides (approximately 95%) (Fig. 2C) [6].

3.2. Pathological abnormalities in D4ST1-deficient EDS

Hematoxylin and eosin (H&E)-stained light microscopy on patients' skin specimens showed that fine collagen fibers were present predominantly in the reticular to papillary dermis with marked reduction of normally thick collagen bundles (Fig. 3a, b). Electron microscopy showed that collagen fibrils were dispersed in the reticular dermis, compared with the regularly and tightly assembled ones observed in the control; whereas each collagen fibril was smooth and round, not varying in size and shape, similar to each fibril of the control (Fig. 3c, d) [6].

Patient	Family	Origin	*CHST14* mutations	Sex	Age at initial publication	References
1	1	Turkish	V49X homo	F	3.5y	[8]
2				M	1.5y	
3				F	6y	
4	2	Japanese	Y293C homo	M	4y	[10]
5				M	7m	
6	3	Austrian	R213P homo	M	0d†	[11]
7				M	12m	
8	4	Turkish	[R135G;L137Q] homo	F	1–4m†	[9]
9				M	1–4m†	
10				M	1–4m†	
11				M	3m	
12	5	Japanese	P281L/Y293C	F	11y	[12]
13	6	Japanese	P281L homo	F	14y	[12]
14	7	Japanese	P281L homo	M	32y	[15]
15	8	Japanese	K69X/P281L	M	32y	[14,15]
16	9	Japanese	P281L/C289S	F	20y	[15]
17	10	Japanese	P281L/Y293C	F	4y	[15]
18	11	Turkish	V49X homo	F	22y	[7]
19				F	21y	
20	12	Indian	E334Gfs*107 homo	F	12y	[7]
21	13	Japanese	P281L/Y293C	M	2y	[21]
22	14	Japanese	F209S/P281L	M	6y	[21]
23	15	Dutch	V48X homo	F	20y	[23]
24	16	Afghani	R274P homo	F	11y	[24]
25				F	0y	
26	17	Miccosukee	G228Lfs*13	F	16y	[25]

homo, homozygous mutation; /, compound heterozygous mutation; F, female; M, male; y, years old; m, months old; †, dead at the time of publication

Table 2. Reported patients with D4ST1-deficient EDS