Clinical Vignette

Ehlers-Danlos Syndrome with Glycosaminoglycan Abnormalities: A Report of the Rare Musculocontractural and Spondylodysplastic Subtypes

Roopadarshini B[1], Neelam Saini[1], Ashwin Dalal[2], Shagun Aggarwal[1,2]

1 Department of Medical Genetics, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.

2 Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, Telangana, India.

Correspondence to: Dr. Shagun Aggarwal *Email:* shagun.genetics@gmail.com

Abstract

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of genetic connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and atrophic scarring. The majority of cases are caused by mutations in the collagen-encoding or collagen-modifying genes. A rarer subset of EDS with atypical presentation is caused by an abnormality in glycosaminoglycan synthesis- the spondylodysplastic type of EDS related to mutations in the genes *B4GALT7, B3GALT6 or SLC39A13*, and the musculocontractural EDS caused by biallelic pathogenic variants in *CHST14* or *DSE* genes. We report two cases of EDS related to *CHST14* and *B3GALT6* gene variants and discuss the unique features of these rarer subtypes.

Keywords: Ehlers-Danlos, *B3GALT6* gene, *CHST14* gene, Spondylodysplastic EDS, Musculocontractural EDS

Introduction

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of genetic connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and atrophic scarring caused primarily by mutations in the collagen-encoding genes or genes encoding collagen-modifying enzymes. The 2017 International Classification of the Ehlers–Danlos Syndromes describes 13 types based on characteristic phenotypic manifestations (Malfait et al., 2017). The tenth subtype of EDS as per this nosology is caused by variations in genes coding for the enzymes responsible for adding sugar moieties to the linker region of proteoglycans and are also classified as "linkeropathies" (Caraffi et al., 2019). This subtype of EDS is called the spondylodysplastic EDS, previously classified as the progeroid EDS. Mutations in the genes *B4GALT7* and *B3GALT6* (coding for galactosyltransferase I and II respectively) and *SLC39A13* are described to be causative. Specific clinical diagnostic criteria have been defined, of which the major criteria comprise progressive short stature, hypotonia, and bowing of limbs. The eleventh described subtype called the musculocontractural Ehlers–Danlos Syndrome (MC-EDS) is caused by biallelic pathogenic variants in the gene for carbohydrate sulfotransferase 14/ dermatan 4-*O*-sulfotransferase 1 (*CHST14*/*D4ST1*) (MIM#601776), or the gene for dermatan sulfate epimerase (*DSE*) (MIM#615539) (Kosho et al., 2016; Brady et al., 2017). Major criteria for this subtype are multiple congenital joint contractures, characteristic facies, and prominent skin findings. Both the above types of EDS have common molecular abnormalities involving glycosaminoglycans which play an important role in the formation of collagen fibrils in the connective tissue. We describe one case each of musculocontractural EDS type 1 (*CHST14* associated) and spondylodysplastic EDS type 2 (*B3GALT6* associated), further reiterating the phenotypic spectrum of these conditions.

Patient description:

Patient 1: A 2-year-8-month-old female child, fifth born to third-degree consanguineous parents, was brought to the genetics clinic with bilateral clubfoot deformity since birth and motor developmental delay. She was born at term

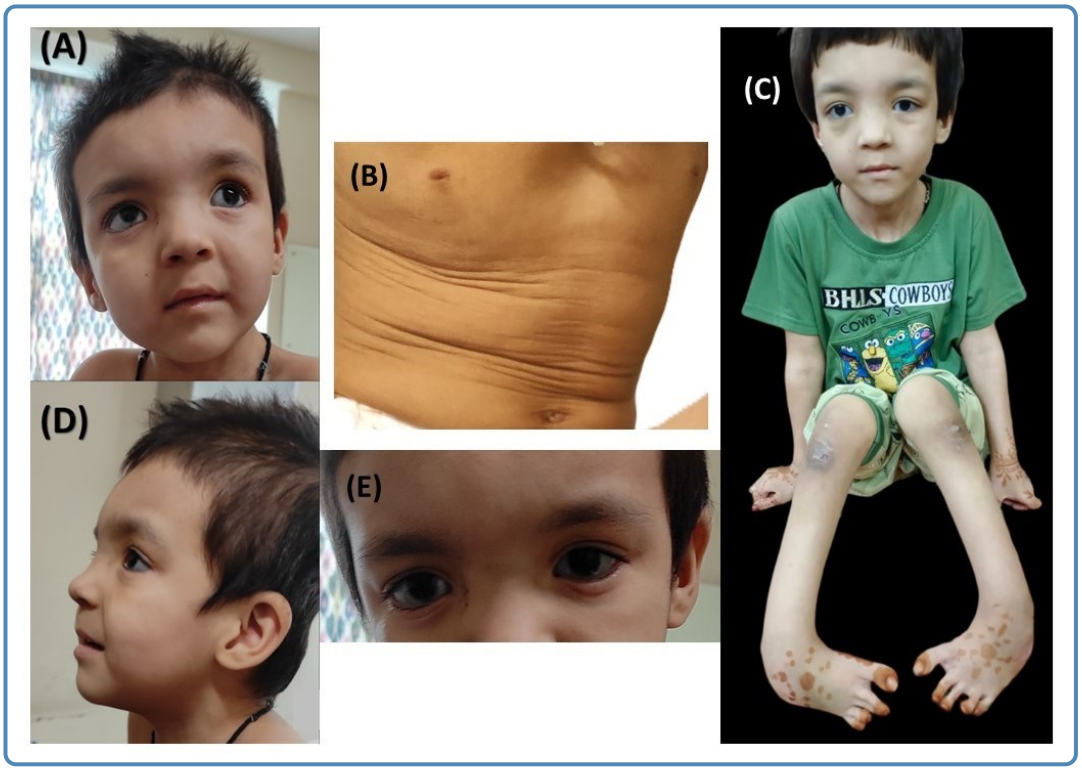

Figure 1 Child with EDS musculocontractural type 1. (A) Hypertelorism, epicanthal folds, down slanting palpable fissures, sagging skin on cheeks. (B) Excessive wrinkling & sagging of skin over the chest and abdomen with pectus excavatum. (C) Progeroid appearance, bilateral club feet, increased gap between 1st and 2nd toes, spatulate toes, callosity with scarring on the anterior aspect of the knee (D) Brachycephalic skull, low-set ears (E) Blue sclerae

with a birth weight of 3.5 kg. A significant motor delay was evident, and she had not achieved independent ambulation. Craniofacial dysmorphism was appreciated in the form of a brachycephalic skull, tall forehead, hypertelorism, down slanting palpebral fissure, epicanthal folds, blue sclera, broad nasal bridge, and long, smooth philtrum (**Figures 1A, 1D & 1E**). On head-to-toe examination, widely spaced nipples with sagging skin, increased skin fold over the trunk and abdomen, pectus excavatum (**Figure 1B**), and bilateral talipes equinovarus deformity were seen (**Figure 1C**). The skin had a soft doughy feel, was hyperextensible, and had excessive wrinkles. Shallow palmar creases were seen. Significant joint laxity and hypotonia were appreciated. The Z-scores for head circumference (-3.32), weight (-4.15), and height (-3.31) indicated significant failure to thrive. Other systemic examinations were normal. Her intellect and vision were normal. A provisional clinical diagnosis of cutis laxa or Ehlers-Danlos syndrome was made.

A skeletal survey showed scoliosis of the thoracolumbar spine and developmental dysplasia of bilateral hips. Hearing evaluation revealed bilateral profound hearing loss. Magnetic resonance imaging (MRI) of the brain showed T1 hypointensity and T2 hyperintensities in the subcortical region, involving the corpus callosum and corona radiata of the bilateral frontal regions (**Figure 3A**). 2D echocardiography of the heart and ophthalmological evaluation were within normal limits. Whole exome sequencing from peripheral leucocyte DNA revealed a homozygous missense variant in the *CHST14* gene (NM_130468.4) c.652C>A; p.Arg218Ser. Computational analysis with online tools such as Frankin by Genoox (`https://franklin.genoox.com/analysis-tool/join-cta`) and VarSome (`https://varsome.com/`) showed it was a likely pathogenic variant [criteria PP3 + PM2 + PP5 as per variant classification guidelines of the American College of Medical Genetics and Genomics & Association for Molecular Pathology (ACMG/AMP)]

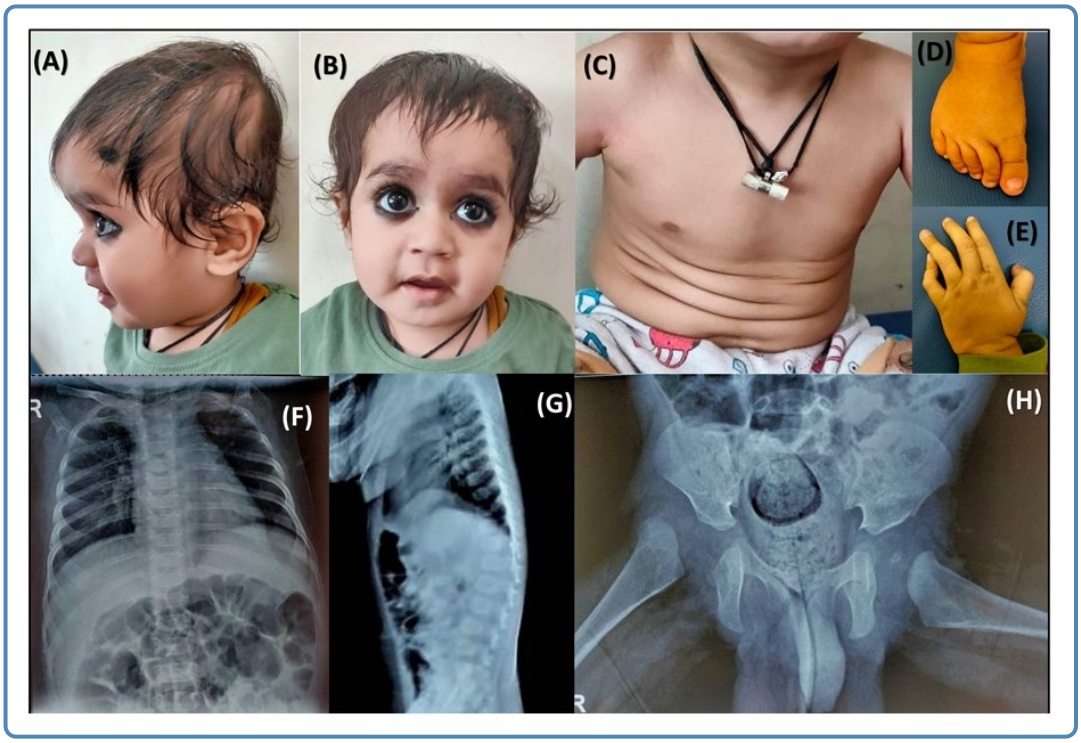

Figure 2 Child with EDS spondylodysplastic type 2. (A) Frontal bossing, deep-set eyes, long prominent philtrum, low-set prominent ears, sparse scalp hair. (B) Prominent eyes with mild down slant, and sagging cheeks. (C) Increased skin folds over the chest and abdomen. (D) & (E) Spatulate toes with radial deviation, long tapering fingers with ulnar deviation of third to fifth digit, and flexion contractures of proximal interphalangeal joints. (F) & (G) X-ray chest and spine showing exaggerated thoracic kyphosis with lumbar lordosis, unremarkable ribs, and vertebral bodies (H) X-ray pelvis showing bilateral hip dislocation.

and was consistent with the clinical phenotype. The patient was diagnosed to be affected with Ehlers-Danlos syndrome, MC-EDS type 1.

Patient 2: An 8-month-old boy, second born to a non-consanguineous couple, was brought with a history of motor developmental delay and developmental dysplasia of the hip (DDH). He was born at term with a weight of 3.5 kg. A plaster cast was applied for DDH at 4 months of age. On examination, he had craniofacial dysmorphisms such as frontal prominence, plagiocephaly, midface hypoplasia, deep-set prominent eyes with mild down slant, and long philtrum (**Figure 2A & 2B**). Sparse scalp hair (**Figure 2A**), and increased skin folds over the chest and abdomen were observed (**Figure 2C**). Increased joint laxity especially of hand and feet with a Beighton score of 6/9, and soft skin with increased palmar creases was appreciated. Spatulate toes with radial deviation, broad first toe, long tapering fingers with ulnar deviation of third to fifth digits, and flexion contractures of proximal interphalangeal joints were noted (**Figures 2D & 2E**). Hearing, vision, and cognition were normal. Length (67 cm) was on the 5th centile, head circumference (44 cm) on the 33rd centile, and weight (7 kg) on the 3rd centile. Central nervous system examination revealed hypotonia with diminished deep tendon reflexes. The pelvic radiograph showed bilateral hip dislocation (**Figure 2H**).

2D echocardiography detected a 3mm patent foramen ovale. Mixed hearing loss (unilateral) was present, and eye evaluation was normal. Radiographs showed generalized osteopenia with thin cortices, wavy long bones and ribs, thoracic kyphosis, and exaggerated lumbar lordosis (**Figures F&G**). A provisional diagnosis of Ehlers-Danlos syndrome or Larsen syndrome was made. Whole exome sequencing from leukocyte DNA revealed two variants in the *B3GALT6*

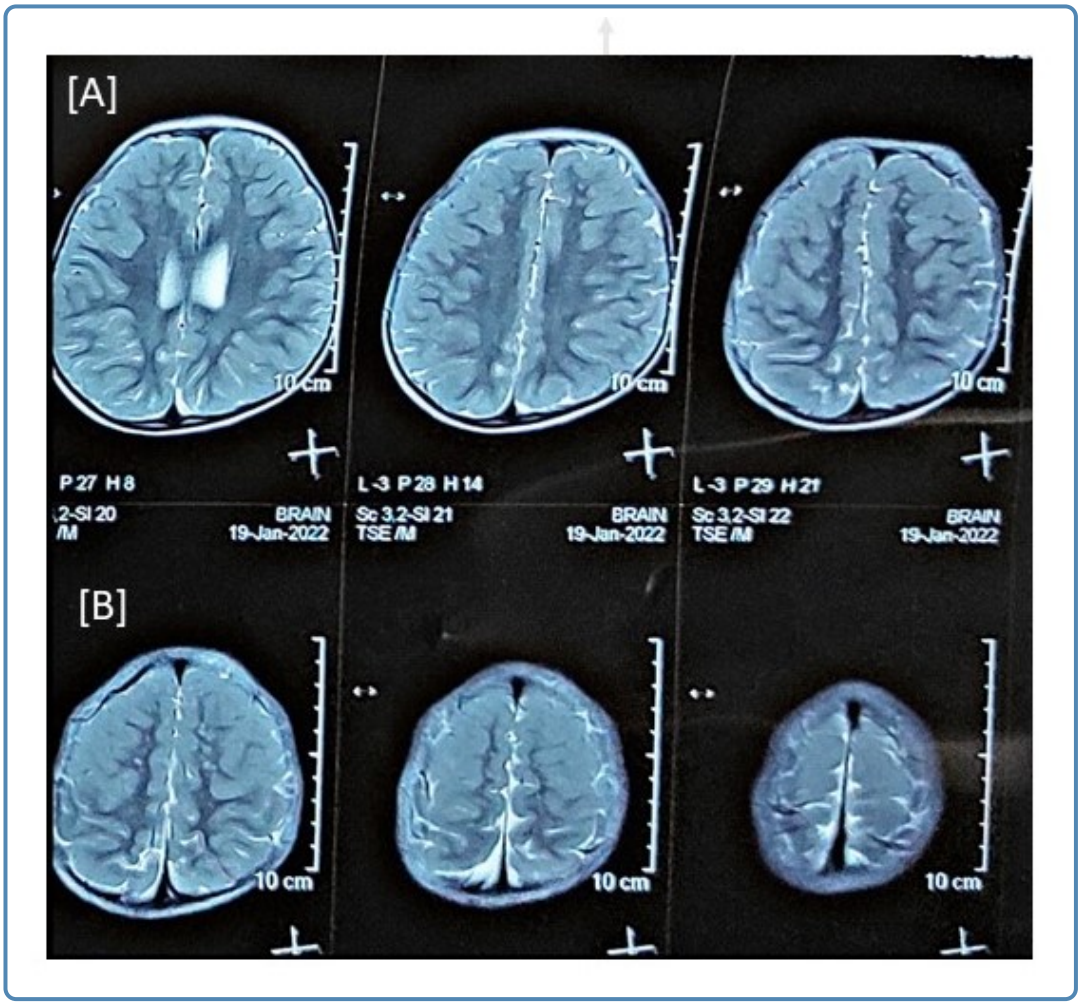

Figure 3 MRI images showing white matter changes in the child with MC-EDS [A] T2 hyperintensities in subcortical regions of bilateral frontal lobes [B] T2 hyperintensities in bilateral centrum semiovale.

gene, (NM_080605.4) c.545A>G; p.Tyr182Lys, a previously reported likely pathogenic variant (criteria PP3 + PM2 + PP2 + PP5 as per ACMG/ AMP guidelines) (Van Damme et al., 2018); and c.749C>T; p. Ala250Val, a novel variant of uncertain significance (criteria PM2 + PM1 + PP2 + PP3 as ACMG/ AMP guidelines). Both variants are not present in publicly available population databases (dbSNP - http://www.ncbi.nlm.nih.gov/SNP; 1000 Genomes - https://www.internationalgenome.org/; gnomAD - https://gnomad.broadinstitute.org/) *in-silico* prediction programs like SIFT (https://sift.bii.a-star.edu.sg/); PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/); MutationTaster2 (https://www.mutationtaster.org/); and CADD (https://cadd.bihealth.org/) predicted both these variants to be deleterious. Parental segregation analysis revealed each of the variants to be present in a heterozygous state in one of the parents. The clinical presentation and the molecular report indicated that the patient was affected with Spondylodysplastic Ehlers-Danlos syndrome type 2 (EDSSPD2).

Discussion

MC-EDS was reported to be caused by biallelic variants in the *CHST14* gene by Dündar et al. (2009). At present, 66 patients with 48 families are reported in the literature (Minatogawa et

al., 2022). Syx et al. (2015), described two patients from the same family, with homozygous c.652C>A variants in the *CHST14* gene, similar to our case. These patients presented with severe bilateral talipes equinovarus, dislocation of the hip but no adducted thumbs. Speech and motor development were delayed, in contrast to our case report where only motor developmental delay was evident. Very few Indian cases are reported (Lautrup et al., 2020). The clinical phenotype of our patient is consistent with the known phenotypic spectrum of this condition. However, our patient does not report any significant morbidity in the form of subcutaneous hematomas, recurrent joint dislocations, or systemic complications. This indicates possibly a mild phenotypic presentation or these findings could evolve. An unusual finding was the white matter changes in brain imaging. Previous reports have described periventricular heterotopias, ventricular enlargement/ asymmetry, absence of the left septum pellucidum, short corpus callosum, and cerebellar hypoplasia (Minatogawa et al., 2022). Mice models by Li et al. (2019) showed that Chst14/D4st1 deficiency resulted in impaired spatial learning, memory, and long-term potentiation. Chondroitin and dermatan sulfate are extracellular components of the central nervous system (CNS) and interact with growth factors and neurotrophic factors influencing neuronal migration, axon guidance, neurite outgrowth, and synaptic plasticity. The CNS findings in our case were likely a part of the EDS phenotype, and follow-up has been advised.

Mutations in *B3GALT6* were first described by Nakajima et al. (2013). Subsequently, Damme et al. (2018), described 12 cases of biallelic *B3GALT6* gene mutations causing EDSSPD. In recent literature, a heterozygous variant in *B3GALT6* co-segregated with clinical features such as elbow contracture, scoliosis, and facial dysmorphism (Shen et al., 2022). All patients with homozygous variants had features of both EDS and spondylo-meta-epiphyseal dysplasia. Some of them had complications like aortic dilatation/ aneurysm, cervical spine instability, and respiratory insufficiency. *B3GALT6* mutation causes complete loss of galactosyltransferase activity leading to deficient GAG synthesis and disrupted collagen organization. A patient with the variant (c.545A>G) in a homozygous state was described in a 3-year-old Iranian boy born of a consanguineous couple. Facial features were similar in both cases like frontal bossing, midface hypoplasia, downward slanting eyes, and long prominent philtrum. As of today, disease-causing variations in the *B3GALT6* gene have been reported in around 50 patients. The facial dysmorphic features, skin findings, and joint abnormalities in the previously reported patients are similar to the findings in our case. Previous studies have shown a significant burden of skeletal abnormalities including spinal deformities, fractures, and short stature, which were not present in our patient. These may be age-dependent features. Regular follow-up and serial radiographs are planned for the patient to look for these evolving findings. **Tables 1 & 2** depict the diagnostic major and minor criteria for these rare subtypes of EDS as per the nosology along with the features as seen in these two cases. Although hearing loss is not included in the major or minor criteria, both of our patients presented with sensorineural hearing loss, and similar cases have been reported in literature associated with both these subtypes of EDS.

These two patients reiterate the phenotypic presentation of two relatively rarer subtypes of EDS syndrome which involve abnormalities of the glycosaminoglycans (GAGs). GAGs are important components of connective tissue and help in the formation of the collagen fibril network. Disorders of their synthesis and processing result in complex forms of EDS which present with a more complicated clinical phenotype as compared to the EDS resulting from collagen mutations. This phenotype includes significant skeletal abnormality as seen in *B3GALT6*-associated EDSSPD, joint contractures as seen in *CHST14*-associated MC-EDS, as well as more widespread systemic involvement in the form of structural cardiac defects, brain abnormalities, ophthalmic, and hearing abnormalities, etc. This indicates the ubiquitous role of GAGs during embryonic development as well as a role in connective tissue integrity throughout the lifespan of the individual.

Conclusion

EDS resulting from glycosaminoglycan abnormalities are rarer, show severe phenotypes with multi-systemic involvement, and overlap with other conditions like skeletal dysplasia and arthrogryposis. Deep phenotyping helps in the computational analysis of exome sequencing data sets, enabling timely and accurate molecular diagnosis. This is crucial for adequate managem-

TABLE 1	MUSCULOCONTRACTURAL EDS	PREVALENCE OF FINDING	PATIENT 1
MAJOR CRITERIA	1. Congenital multiple contractures, characteristic adduction-flexion contractures and/or talipes equinovarus (clubfoot)	98%, 95%	+
	2. Characteristic craniofacial features, evident at birth/early infancy	Hypertelorism 92% Down-slanting palpebral fissures 95% Smallmouth 88% Blue sclerae 86% slender face 83% Long philtrum 80% Low-set ears 71% Thin upper lip 65% Brachycephaly 54% Midface hypoplasia 58%	+ + + + + + + + + +
	3. Cutaneous features- skin hyperextensibility, easy bruisability, skin fragility with atrophic scars increased palmar wrinkling	100%	+
MINOR CRITERIA	1. Recurrent/chronic dislocations	90%	
	2. Pectus deformities (flat, excavated)	84%	+
	3. Spinal deformities (scoliosis, kyphoscoliosis)	87%	+
	4. Peculiar fingers (tapering, slender, cylindrical)	87%	+
	5. Progressive talipes deformities (valgus, planus, cavum)	98%	+
	6. Large subcutaneous hematomas	81%	
	7. Chronic constipation	85%	
	8. Colonic diverticula	35%	
	9. Pneumothorax	10%	
	10. Nephrolithiasis	29%	
	11. Hydronephrosis	51%	
	12. Cryptorchidism in males	88%	
	13. Strabismus	66%	+
	14. Refractory errors	93%	
	15. Glaucoma/elevated IOP	49%	
	MINIMUM CRITERIA FOR CLINICAL DIAGNOSIS	1. At birth/early childhood- major criteria 1 and 2 2. In adolescence and adulthood- major criteria 1 and 3	3 major and 5 minor criteria

TABLE 2	SPONDYLODYSPLASTIC EDS	PREVALENCE OF FINDING	PATIENT 2
MAJOR CRITERIA	1. Short stature (progressive in childhood)	99%	+
	2. Muscle hypotonia	65%	+
	3. Bowing of limbs	80%	
MINOR CRITERIA	1. Skin hyperextensibility, soft doughy skin, thin translucent skin	85%	+
	2. Pes planus	75%	
	3. Delayed motor development		+
	4. Osteopenia	85%	+
	5. Delayed cognitive development	50%	
GENE-SPECIFIC MINOR CRITERIA (*B3GALT6*)	1. Kyphoscoliosis (congenital, early onset, progressive)	90%	
	2. Joint hypermobility	92%	+
	3. Joint contractures (congenital or progressive) especially hands	60%	+
	4. Peculiar fingers (slender, tapered, arachnodactyly, spatulate, with broad distal phalanges	80%	+
	5. Talipes equinovarus	62%	
	6. Characteristic craniofacial features	Plagiocephaly, prominent forehead 90% Asymmetric face & flat midface 75% Straight and fine hair 45% Sparse eyelashes Blue sclerae 70% Proptosis 60% Down-slanting palpebral fissures, High and narrow palate, malocclusion, low-set ears	 + + + + + +
	7. Tooth discoloration, dysplastic teeth	75%	
CHARACTERISTIC RADIOGRAPHIC FINDINGS	1. Osteoporosis with multiple spontaneous fractures		
	2. Ascending aorta aneurysm		
	3. Lung hypoplasia, restrictive lung disease		
CLINICAL DIAGNOSIS	1 and 2 of the major criteria plus characteristic radiographic abnormalities and at least 3 minor criteria		2 major and 7 minor criteria

ent, follow-up, and prevention of complications, to predict recurrence risk in the family, and for timely prenatal invasive testing. Quality of life can be improved with proper rehabilitation. Diligent follow-up of cases helps in preventing life-threatening complications or early death/ disability due to ruptured or dissecting aneurysm, massive hematomas, poor wound healing/infection, retinal detachment, fractures, joint dislocation followed by vascular or neural compromise.

Conflict of Interests: None

Declaration: Informed consent was taken for clinical photographs from the guardian.

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