

CASE REPORT

Classical Ehlers–Danlos syndrome with severe kyphoscoliosis due to a novel pathogenic variant of *COL5A2*

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Abstract

We described a novel de novo missense variant of the gene encoding Collagen alpha-2(V) chain, associated with the classical Ehlers–Danlos syndrome (cEDS) (OMIM#130010), in a 14-year-old patient who presented with congenital and severe scoliosis, muscle hypotonia, ocular manifestations, and no atrophic scarring. This case expands the phenotypic spectrum of cEDS.

KEYWORDS

classical Ehlers–Danlos syndrome, *COL5A2*, kyphoscoliosis, molecular modeling, next generation sequencing

1 | INTRODUCTION

Ehlers–Danlos syndromes (EDS) are a heterogeneous group of hereditary connective tissue disorders (HTCD) caused by mutations in genes involved in the structure and/or biosynthesis of collagens and other extracellular

matrix proteins.¹ The 2017 classification consists of 13 types of EDS based on clinical manifestations and genetic abnormalities in 19 different genes.² Main common characteristics are generalized joint hypermobility (GJH), skin hyperextensibility, and tissue fragility. Scoliosis is another common manifestation in EDS patients.^{1,3,4} Especially,

severe kyphoscoliosis is a major criterion for the diagnosis of kyphoscoliotic EDS (kEDS) if associated with congenital muscle hypotonia and GJH. Kyphoscoliosis appears at birth or in infancy and is rapidly progressive. kEDS is due to pathogenic mutations in *PLOD1* or *FKBP14* genes.¹ Inheritance is autosomal recessive.

Classical Ehlers–Danlos syndrome (cEDS) is mainly caused by heterozygous mutations in *COL5A1* and *COL5A2* genes, encoding type V collagen. In rare cases, mutations in the gene encoding type I collagen (*COL1A1*) can be found. About type V collagen defects, a recent study showed that 83.5% were located in *COL5A1* and 16.5% in *COL5A2*.⁴ Inheritance is autosomal dominant. The major criteria for cEDS consist of skin hyperextensibility, atrophic scarring, and GJH.¹

Due to the phenotypic variability and overlapping clinical features, diagnosis between EDS subtypes can be challenging. Genetic testing using a panel of genes that includes all genes involved in EDS helps in the diagnostic confirmation.

Here, we present the case of a young female patient affected by a severe scoliosis requiring arthrodesis surgery and without atrophic scarring. She was initially thought to have a kEDS due to the presence of early-onset joint hyperlaxity, and severe and progressive kyphoscoliosis with hypotonia. Interestingly, molecular testing by next generation sequencing (NGS) performed at the age of 14 revealed an unreported *de novo* heterozygous variant c.3617G>A in *COL5A2*, confirming a cEDS instead of kEDS.

2 | METHODS

Patient guardians gave informed consent for genetic testing and publication. The proband DNA sample was analyzed by NGS (Nimblegen capture Seq Cap Ez Choice with a custom gene-panel and run on an Illumina miSeq automaton). The 55 genes panel was designed to study all EDS involved genes as well as the main HCTD overlapping phenotype genes (previously published in Foy et al., supplementary materials⁵). It included: *ADAMTS2* (NM_014244), *B3GALT6* (NM_080605), *B4GALT7* (NM_007255), *CHST14* (NM_130468), *COL1A1* (NM_000088), *COL1A2* (NM_000089), *COL3A1* (NM_000090), *COL5A1* (NM_000093), *COL5A2* (NM_000393), *DSE* (NM_001080976), *FKBP14* (NM_017946), *PLOD1* (NM_000302), *TNXB* (NM_019105), *COL12A1* (NM_004370), *C1R* (NM_001733), *C1S* (NM_001734), *SLC39A13* (NM_152264), *ZNF469* (NM_001127464), *PRDM5* (NM_018699), *LZTS1* (NM_021020), *FLNA* (NM_001456), *COL4A1* (NM_001845), *COL6A1* (NM_002474), *COL6A2* (NM_004369), *COL6A6* (NM_001102608), *ELN* (NM_000501), *FBLN5* (NM_006329), *FBN1* (NM_000138), *LOX* (NM_002317), *MYH11* (NM_002474), *RYR1*

(NM_000540), *SEPN1* (NM_020451, except exon 3), *SGCB* (NM_000232), *MYH7* (NM_000257), *TTN* (NM_001267550, only STOP codon and frameshift mutations are interpreted), *SMAD2* (NM_005901), *SMAD3* (NM_005902), *COL2A1* (NM_0018844), *COL11A1* (NM_001854), *COL11A2* (NM_080681), *COL9A1* (NM_001851), *COL9A2* (NM_001852), *COL9A3* (NM_001853), *MED12* (NM_005120), *FLNB* (NM_001457), *CANT1* (NM_138793), *SLC2A10* (NM_030777), *ABCC6* (NM_001171), *GGCX* (NM_000821), *ENPP1* (NM_006208), *AEBP1* (NM_001129), *SKI* (NM_003036), *TGFB2* (NM_003238), *TGFB3* (NM_003239), *TGFB1* (NM_004612), *TGFB2* (NM_003242).

NGS data were analyzed using Gensearch NGS software from Phenosystems. NGS confirmation and familial investigation were performed by dideoxysequencing of PCR-amplified *COL5A2* exon 50. The MasterMix PCR AmpliTaq Gold360 was from ThermoFisher Scientific, FastAP thermosensitive Alkaline Phosphatase was from ThermoFisher Scientific, and BigDye Terminator v1.1 cycle sequencing kit and formamide were from Applied Biosystems.

3 | RESULTS

3.1 | Clinical Report

A 9-year-old female patient presented to the referral center for EDS for a suspicion of kEDS due to the presence of a severe congenital scoliosis and joint hypermobility.

She was the first child of healthy unrelated parents, born at 38 weeks after a normal pregnancy. Birth parameters were normal. Congenital scoliosis and muscle hypotonia led to muscle biopsy, which ruled out a myopathy. Scoliosis has been treated with physiotherapy brace from the age of 18 months to slow down progression. She walked without support at 24 months. Cognitive development and schooling were normal, except holding a pen was difficult. She had joint hypermobility, back and knee chronic pain, fragile skin, and anal fissures. There was no history of sprains, fractures, or dislocations. Ocular follow-up revealed mild astigmatism and mild myopia with a moderate increased axial ocular length. Slit lamp examination detected a bilateral keratoglobus (Figure 1A). The characteristic pachymetry was extremely reduced. Guidelines were provided for parents, to avoid eye trauma or dangerous sport activity as the main risk resides in corneal perforation. The examination of the ocular surface found a tear film instability with a reduced break-up time. The wide-field fundus was normal. Echocardiography found a mitral valve prolapse. EDS was suspected at 5 years.

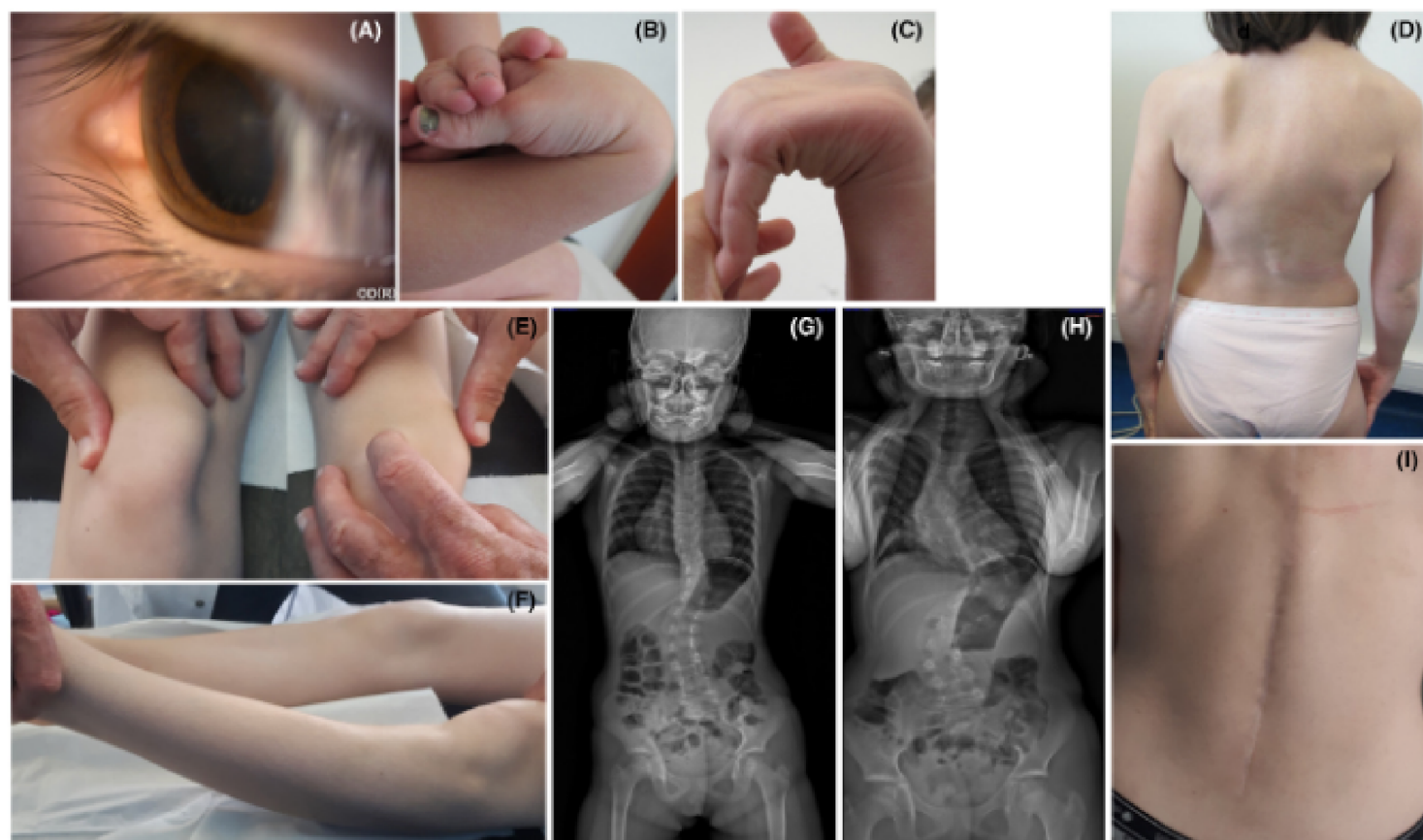


FIGURE 1 Clinical and radiological findings of the patient. (A) Corneal thinning with keratoglobus at slit lamp examination. The patient at age of 9 years presented joint hypermobility: (B) joint hypermobility of the thumb (Positive apposition of thumb on the forearm), (C) joint hypermobility of the wrists and fingers, (D) marked dorsal gibbosity, (E) hypermobility of patella, and (F) genu recurvatum, (G, H) 2D anteroposterior X-rays view at 6 and 12 years, respectively. (I) At the age of 14, she had a normal scar resulting of the arthrodesis correcting the spine.

On clinical examination at the age of 9 (length 123 cm (−2 DS) and weight 25 kg (−1.5 DS)), she had GJH with a Beighton score of 7/9.⁶ She had dorsal gibbosity, lumbar hyperlordosis, genu varum, genu recurvatum, pectus excavatum, and flexible flat feet (Figure 1B–F). She had very soft, velvety, and translucent skin with small ecchymosis, and normal scars. No atrophic scars, cutaneous hyperextensibility, subcutaneous spheroids, or molluscoid pseudotumours were noticed. Considering the severe and progressive kyphoscoliosis, hypotonia, and joint hypermobility, the diagnosis of kEDS was suspected.¹ Genetic testing was performed when available 5 years later.

She had a rheumatologic follow-up to control the evolution of the scoliosis and to adapt bracing and physiotherapy. At 14 years of age, she had spinal fusion surgery to correct the scoliosis (T3–L3) which progressed rapidly (Figure 1G,H). Scarring was normal (Figure 1I). A L5 unilateral isthmus lysis was observed. She had suffered from lower limbs chronic pain, recurrent knee dislocations, easy bruising, mild tricuspid valve regurgitation, and restrictive lung disease. Skin was moderately hyperextensible with piezogenic papules of the heels. Eyes were excavated.

3.2 | Molecular findings

NGS of the proband's DNA did not reveal mutations in *PLOD1* and *FKBP14*, the two genes involved in kEDS. It revealed a heterozygous c.3617G>A variant in *COL5A2* (NM_000393.5) predicting a p.Gly1206Glu missense variant in a triple helix domain. Analysis of the parents' DNA showed that it was *de novo*. It was classified as “pathogenic” according to varsome and ACMG criteria (PM2, PP3, and PS2). This variant was not found in gnomAD exomes.⁷ No additional variant met the ACMG “likely pathogenic” or “pathogenic” criteria in any other tested gene. No other variation was found in the genes panel.

It is known from other available collagen helical structures that Gly are stacked vertically on the center of the long axis of the molecule and that the 3 Å distance from the oxygen atom involved in interchain bonding stabilization is totally incompatible with a Glu residue lateral chain to fit in. 3D modeling (I-TASSER^{8,9}) showed that there was no room for glutamic acid to fit into the core of the triple helix instead of the glycine (Figure 2).

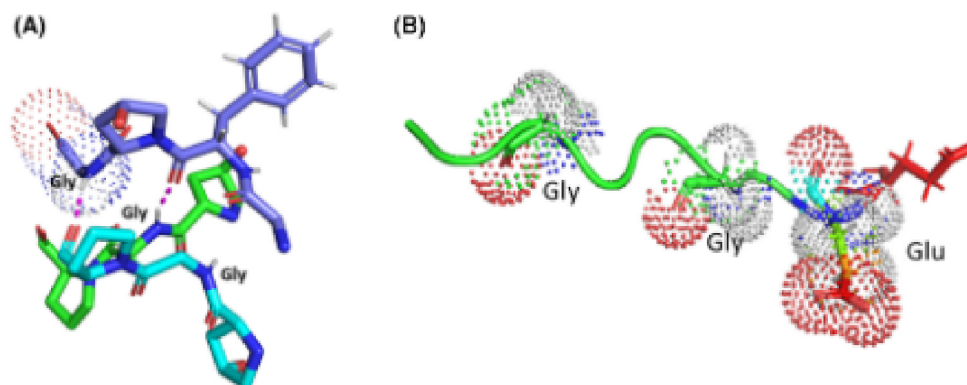


FIGURE 2 3D modeling of triple helix segments and thermodynamic stability profile of the relevant segment. (A) Representation of a triple helix stretch from 3D crystal data 4DMU (longitudinal axis). One Gly is represented as a dotted area, and the H bond involved is represented as a dotted line. (B) Longitudinal representation of the mutated area showing the volume occupied by the Glu residue. The dotted Glu residue is the site of the mutation reported here. Prediction of the mutated structure relies on I-TASSER prediction of 3D structure of residues 1201–1208 of the collagen $\alpha 2(V)$ chain preproprotein.^{8,9}

The 1206–229 peptide is at the very end of a putative triple helix organization. Thus, it is reasonable to speculate that replacement of a glycine for a glutamic acid is no longer consistent with a triple helix structure for the following 1206–229 sequence.

3.3 | Outcome and follow-up

Despite early-management, the kyphoscoliosis was complicated with a restrictive lung disease with a forced vital capacity evaluated at 51% at the age of 14. It led to the installation of a home ventilation for pulmonary rehabilitation. At the age of 15, a second arthrodesis was performed to correct the progression of the scoliosis with normal scarring again. She also benefited from cardiovascular and ophthalmologic follow-up. Myopia and corneal thinning remain approximately stable, and recommendations to avoid ocular injury were followed by the parents.¹⁰

4 | DISCUSSION

Medical history of the patient was not totally in line with the usual form of cEDS related to *COL5A2* or *COL5A1*, due to the lack of atrophic scarring.

Considering criteria for cEDS,¹ skin hyperextensibility and atrophic scarring are required for the diagnosis (major hallmarks), which the patient did not display during the clinical evaluation. The fragile skin and easy bruising were not sufficient to evoke a cEDS diagnosis (Table 1). In contrast, the patient fulfilled major criteria for the diagnosis of kEDS, which consist of congenital muscle hypotonia, congenital or early-onset kyphoscoliosis and GJH. She also displayed three minor criteria, which were easy bruisable skin,

pectus deformity, and myopia (Table 1).¹ The patient did not present the clinical criteria for another genetic diseases or HTCD such as cutis laxa or osteogenesis imperfecta.

Based on the clinical presentation at age 9, we would have expected mutations in *PLOD1* or *FKBP14* genes, had genetic testing been available. The development of panel of genes allowed to propose a NGS analysis including the genes involved in the 13 types of EDS and other connective tissue disorders, which identified a *de novo* heterozygous variant in *COL5A2* predicting a p.Gly1206Glu variant in a triple helix domain.

The presence of a severe scoliosis was unusual, and several explanations were possible: (i) the presence of another genetic event known to cause scoliosis, but whole-genome sequencing was not performed due to the absence of clinical call points for another genetic condition; (ii) a neuro-muscular pathology, but neurologic and muscular examinations were normal. Creatine kinase was normal (90.7 U/L); (iii) an additional idiopathic scoliosis could not be excluded. However, a high occurrence of mild-to-severe scoliosis in individuals with *COL5A2* variants compared to *COL5A1* variants was noticed in a cohort of 168 patients with cEDS,⁴ highlighting the importance of being aware of the possible presence of spinal deformity in cEDS.

This case expands the phenotypic spectrum of cEDS and illustrates the challenge to diagnose EDS subtypes and underlines the relevance of using a multigene panel for confirming the diagnosis.

AUTHOR CONTRIBUTIONS

MF has drafted and revised the manuscript. PDM has made substantial contributions in the acquisition, analysis, and interpretation of data, drafted and revised the work. DBG has made substantial contributions in the acquisition of data and has been involved in drafting the manuscript. FG, AM, and RC have made substantial contributions in

TABLE 1 Clinical scoring of the proband for the diagnosis of classical and kyphoscoliotic type of Ehlers–Danlos syndrome (EDS)

	Clinical characteristics	Type of criteria	Proband
Classical EDS OMIM_130000 and 130010	Significant skin hyperextensibility and atrophic scarring	Major	No
	GJH	Major	Yes
	Easy bruising	Minor	Yes
	Soft, doughy skin	Minor	Yes
	Subcutaneous spheroids/spherules	Minor	No
	Skin fragility (or traumatic splitting)	Minor	Yes
	Molluscoid pseudotumours	Minor	No
	Subcutaneous spheroids	Minor	No
	Hernia (or history of)	Minor	No
	Epicanthal folds	Minor	No
	Complications of joint hypermobility (sprains, dislocation/subluxation, pain, pes planus)	Minor	Yes (pain)
	Family history at first degree relative	Minor	No
Kyphoscoliotic EDS OMIM_225400 and 614557	Congenital muscle hypotonia	Major	Yes
	Congenital or early-onset kyphoscoliosis (progressive or non-progressive)	Major	Yes
	GJH with dislocations/subluxations	Major	Yes
	Skin hyperextensibility	Minor	No
	Easy bruisable skin	Minor	Yes
	Rupture/aneurysm of a medium-sized artery	Minor	No
	Osteopenia/osteoporosis	Minor	No
	Blue sclera	Minor	No
	Hernia (umbilical or inguinal)	Minor	No
	Pectus deformity	Minor	Yes
	Marfanoid habitus	Minor	No
	Talipes equinovarus	Minor	No
	Refractive errors (myopia, hypermetropia)	Minor	Yes

Note: Minimal clinical criteria suggestive for cEDS are the first major criterion plus either the second major criterion or at least three minor criteria. Minimal criteria suggestive for kEDS are 1 and 2 of the major criteria—congenital muscle hypotonia and congenital/early-onset kyphoscoliosis—plus either: major criterion 3, or three minor criteria (either general or gene-specific). Moreover, there are gene-specific minor criteria (four for *PLOD1* and four for *FKBP14*). Abbreviation: GJH, Generalized joint hypermobility.

the acquisition, analysis, and interpretation of data. KB designed, drafted, and revised the work.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CONSENT

Written informed consent for publication was obtained from the legal representatives of the patient for publication of this case report and accompanying images.

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