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. The 2017 classification consists of 13 types of EDS based on clinical manifestations and genetic abnormalities in 19 different genes. Main common characteristics are generalized joint hypermobility (GJH), skin hyperextensibility, and tissue fragility. Scoliosis is another common manifestation in EDS patients. 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. 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) and type II collagen (COL2A1) can be found. About type V collagen defects, a recent study showed that 83.5% were located in COL5A1 and 16.5% in COL5A2. Inheritance is autosomal dominant. The major criteria for cEDS consist of skin hyperextensibility, atrophic scarring, and GJH.

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 automat). 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 materials). 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), FKB14 (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), EZTS1 (NM_021020), FLNA (NM_001456), COL4A1 (NM_000393), 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), FN1B (NM_001457), CANT1 (NM_138793), SLC2A10 (NM_030777), ABC6 (NM_001171), GCCX (NM_000821), ENPP1 (NM_006208), AEBP1 (NM_001129), SKI (NM_003036); TGFB2 (NM_002328); TGFB3 (NM_003239); TGFBRI (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 Thermoscientific, 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.
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. 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 translucid 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. 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 isthmic 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. 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 Angstrom 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) showed that there was no room for glutamic acid to fit into the core of the triple helix instead of the glycine (Figure 2).
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.

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

ACKNOWLEDGMENTS
The authors gratefully thank the patient and her family for their contribution. Authors also thank the patient’s organization UNSED for its donation that contributed to pay the publication charges.

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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### 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 |
|--------------------------|------------------|---------|
| Significant skin hyperextensibility and atrophic scarring | Major | No |
| Generalized joint hypermobility | Major | Yes |
| Easy bruising | Minor | Yes |
| Soft, doughy skin | Minor | Yes |
| Subcutaneous spheroids | 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 | Minor | Yes (pain) |
| (sprains, dislocation/subluxation, pain, pes planus) | | |
| Family history at first degree relative | Minor | No |

*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.*

### Table 2
Clinical scoring of the proband for the diagnosis of classical and kyphoscoliotic type of Ehlers–Danlos syndrome (EDS)

| Clinical characteristics | Type of criteria | Proband |
|--------------------------|------------------|---------|
| Congenital muscle hypotonia | Major | Yes |
| Congenital or early-onset kyphoscoliosis (progressive or non-progressive) | Major | Yes |
| Generalized joint hypermobility | Major | Yes |
| Skin hyperextensibility | Minor | No |
| Easy bruising | 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.*
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**How to cite this article:** Foy M, de Mazancourt P, Bremond Gignac D, et al. Classical Ehlers–Danlos syndrome with severe kyphoscoliosis due to a novel pathogenic variant of *COL5A2*. *Clin Case Rep*. 2022;10:e06338. doi: 10.1002/ccr3.6338