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A novel *COL1A1* variant in a family with clinical features of hypermobile Ehlers-Danlos syndrome that proved to be a *COL1*-related overlap disorder

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Abstract

COL1-related overlap disorder is a condition, which is not yet considered as part of the 2017 EDS classification. However, it should be investigated as an alternative diagnosis for any patient with hypermobile EDS. This could allow providing appropriate genetic counseling.

KEYWORDS

COL1A1, connective tissue, Ehlers-Danlos Syndrome, hypermobile EDS, joint laxity

1 | INTRODUCTION

We reported a novel *COL1A1* variant, p.(Arg312Leu), at a residue previously involved in classical and vascular EDS.

The three patients affected, belonging to the same family, presented manifestations consistent with the hypermobile EDS and no major cardiovascular complications. We ultimately classified them as *COL1*-related overlap disorder.

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TABLE 1 Comparison of demographic, clinical, and genetic features of the three affected individuals of this family

	Patient II.3	Patient III.2	Patient II.2
Sex/Age	F/38y	F/9y	F/37y
Height /Weight	161cm/53kgs	130cm/24kgs	161cm/61kgs
Arm span-to-height ratio >1.05 ^a	-	-	-
Morphotype	Normal	Normal	Normal
Age at 1st symptoms	6-10y	3y	2y
<i>COL1A1</i> c.935G>T p.(Arg312Leu)	+	+	+
Beighton score (GJH ^{a,b})	6/9	7/9	7/9
Arachnodactyly ^a	+	-	-
Swan-Neck deformity	+	+	+
Feet deformity	flatfeet ^b	flatfeet ^b	-
Limbs deformities	Elbow recurvatum Genu recurvatum	Elbow and genu recurvatum Genu varum, Coxa Valga Femoral anteversion	Genu recurvatum Genu varum
Pectus deformity	-	-	-
Congenital hip dislocation	-	+	-
Chronic pain for ≥3 months ^a	+	+	+
Localization	H, S, W, T	H, Fi, W, S, K	B, W, K, H, Fe, S
Recurrent joint dislocations ^a	W, S	Fi, A, Bilateral fibula	S, K
Joint instability ^a	Fi, W	A, S	A, Fi
Fractures	Left elbow (x2)	Epitrochlea, Growth plate (W)	Separated growth plate (E)
Contortionism	+	+	+
Proprioceptive disorders/falls	+/+	+/+	+/+
Scoliosis	-	+	-
Osteodensitometry	Mild osteopenia Vertebral T-score: +0.4 Femoral T-score: -1.4	Mild osteopenia Vertebral T-score = -0.7 Vertebral Z-score = -1.2	Normal Vertebral T-score: -0.6 < T < 0.6 Femoral T-score: -0.1
Orthopedic surgery	Elbow surgeries	Femoral osteotomy Screwing of the fibula	Discal hernia

(Continues)

TABLE 1 (Continued)

	Patient II.3	Patient III.2	Patient II.2
Cutaneous involvement	Soft, Velvety skin ^{a,b}	+	-
	Skin hyperextensibility ^{a,b}	+++	+
	Thin, translucent skin	+	+
	Unexplained striae ^a	+(K)	+(K)
	Atrophic scarring ^a	-	-
	Poor wound healing	Large scars	-
	Easy bruising	+	+
	Piezogenic papules ^a	+	+
	Light blue sclerae ^b	+	+
	Ophthalmology symptoms	Visual fatigue	Strabismus, Hyperopia
Eye, dental, and ORL aspects	Dental fragility	Tooth enamel defects	Gingivitis
	Dental crowding, high or narrow palate ^a	Tooth mobility	-
	Hearing symptoms	+	-
	Mitral valve prolapse ^a	HL, ear infection	HL, HA, ear infection, tinnitus
	Aortic root dilatation ^a	-	-
	Cardiovascular symptoms	-	PoTS
	Vascular abnormalities on medical imagery	Thin mitral valve	Aortic microfistula (between ascending aorta and right pulmonary artery)
	Abdominal hernia ^a	Mild mitral insufficiency	Thoracic aortic dilation (ductus diverticulum or post-traumatic pseudoaneurysm)
	Gastrointestinal symptoms	-	-
	Pelvic floor, prolapse(R or U) ^a	GERD, SC, CP, AP, D	GERD, SC, D, AP
Digestive and urogenital aspect	Urinary problems	Mild incontinence	Dysuria
	Abnormal fatigue	+	+
	Bleeding tendency	GG, EP	GG
	Neuropsychology disorders	Memory disorders	Attention
	Others	Headaches	Headaches
		Sleep disorders	Sleep disorders
			Depressive
			Urine retention (in the past)
			GERD, constipation
			PoTS
		Thoracic aortic dilation (ductus diverticulum or post-traumatic pseudoaneurysm)	

Abbreviations: + and -, indicate the presence or absence of a clinical feature, respectively; AP, abdominal pain; B, Back; cm, centimeters; CP, colopathy; D, diarrhea; E, elbow; EP, epistaxis; F, female; Fe, feet; Fi, fingers; GERD, gastroesophageal reflux disease; GG, gingivorrhagia; GJH, generalized joint hypermobility; H, hands; HA, hyperacusis; HL, Hearing loss; K, knees; Kgs, kilograms; L, Lumbar; LBT, longer bleeding time; M, male; MG, menorrhagia; PoTS, Postural tachycardia syndrome; R, rectal; S, shoulders; SC, severe constipation; T, thumbs; U, uterine; VP, vesosphincteric problems; W, wrists; Y, years.

^aDiagnostic criteria of hEDS according the 2017 Classification of EDS.

^bDiagnostic criteria for CIROD.

Ehlers-Danlos Syndromes (EDSs) are a heterogeneous group of rare heritable connective tissue disorders (HCTD) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility.¹ The 2017 international classification identifies thirteen subtypes of EDS based on clinical criteria and pathogenic variants in 19 genes.¹ Later, a 14th subtype of EDS caused by variants in the *AEBP1* gene has been identified.^{2,3} Classification of EDS can be challenging for clinicians, due to the phenotypic variability and the clinical overlap between EDS subtypes as well as with other HCTD. Causative genes have been identified in all EDS subtypes, except for the hypermobile EDS (hEDS) (OMIM #130020).^{1,4} Two candidate genes are currently under investigation: *TNXB* haploinsufficiency could explain up to 5% of hEDS⁵ and a variant in the *LZTS1* gene has been found in four families.⁶ Hypermobile EDS is the most frequent type of EDS, gathering more than 80% of patients. It has an autosomal dominant mode of inheritance.⁴ A checklist for hEDS diagnostic criteria helps differentiating hEDS from other EDS subtypes and other HTCD as no genetic etiology has been found yet (<https://www.ehlers-danlos.com/heds-diagnostic-checklist/>).

Pathogenic variants in *COL1A1* encoding the pro- α 1 chain of type I procollagen lead to osteogenesis imperfecta (OI), arthrochalasia EDS, some classical EDS (cEDS), and vascular EDS (vEDS) forms. Arthrochalasia EDS (aEDS) is due to heterozygous mutations in either *COL1A1* or *COL1A2*. Numerous altering splicing sites variants have been reported including among them mutation leading to skipping of exon 6.^{7,8} In the triple helical domain, a deleterious missense variant c.934C>T, p.(Arg312Cys) has been described in patients with either vascular or classical EDS prone to phenotypes with vascular complications.^{9–13} Missense variants affecting glycine residues located in the triple helical domain or in the procollagen N-proteinase have been described in most patients with OI and OI/EDS overlap syndrome now called “*COL1*-related overlap disorder” (C1ROD).^{14–16}

OI is a HCTD mainly characterized by bone fragility, blue sclerae, and growth, teeth, and hearing impairments.¹⁷ Patients with C1ROD display a phenotype that combines EDS features, with or without signs of mild-moderate OI, and they carry variants in *COL1A1* and *COL1A2*. For C1ROD diagnosis, patients should harbor a set of minimal criteria. The underlying idea of C1ROD classification is to offer molecular testing, as these patients would be otherwise described as having a hEDS.¹⁵

Herein, we report a two-generation family initially thought to be affected with hEDS. We evidenced a novel heterozygous mutation in exon 14 of *COL1A1*, c.935G>T, predicting a novel p.(Arg312Leu) deleterious missense variant. A previously described missense at this very same residue, p.(Arg312Cys), was reported in patients affected with cEDS and vEDS.^{9,10,12,18} Here, the patients presented with a combination of hEDS features and blue sclerae but no major vascular

complication. This report expands the genotype and phenotype spectrum of C1ROD and highlights the importance of genetic testing for hEDS patients.

2 | CLINICAL REPORT

The proband (II.3) was first seen at the French Reference Center for nonvascular EDS, for a suspicion of hEDS, at the age of 38. She was born at term from nonconsanguineous French-Portuguese parents. Her mother (I.3) was healthy. Her father (I.2) died in a motorcycle accident at the age of 25. She had two healthy siblings (II.4 and II.5). She had a normal psychomotor development. First sprains occurred around the age of 8. Recurrent dislocations, instability, falls, chronic fatigue, joint pain, easy bruising, and gingivorrhagia occurred throughout childhood and adulthood. She had been diagnosed for a hEDS since she fulfilled the 2017 diagnostic criteria (Table 1).¹ After her evaluation, we performed clinical evaluation of her children (III.1 and III.2) and two cousins (II.1 and II.2) who also presented symptoms (Figure 1A). Individuals I.1, II.2, and III.2 were previously diagnosed with EDS by other care providers.

The proband's daughter (III.2) and her cousin (II.2) also met the 2017 diagnostic criteria for hEDS classification (Table 1).¹ Their clinical and molecular data are shown in Table 1. All hEDS-affected members had joint hypermobility, recurrent small joint instabilities and sprains, chronic joint pain since childhood, proprioceptive disorders and falls, chronic fatigue, genu and/or elbow recurvatum, easy bruising, blue sclerae, gingivorrhagia, and gastrointestinal disorders. Skin abnormalities varied among the affected members. Particularly, cutaneous examination of patient II.3 showed a velvety and very hyperextensible skin (at least 3 cm), large scars, large and easy bruising, and unexplained striae and no atrophic scars (Figure 1B).

Patients II.2, II.3, and III.2 underwent bone and cardiovascular explorations. Patient II.3 showed a thin mitral valve with a mild mitral insufficiency, patient II.2 had a small thoracic aorta dilatation (0.28 x 0.26 cm) located at the junction of the aortic arch and descending aorta which could appear to be either a ductus diverticulum or a post-traumatic pseudoaneurysm, and patient III.3 had a microfistula located between the ascending aorta and right pulmonary artery. No other vascular abnormality was detected. Bone density scan revealed a mild osteopenia in patients II.3 and III.2. Patients II.1 and III.1 have finally not been diagnosed for an EDS.

2.1 | Molecular findings

The presence of a significant cutaneous involvement in the proband (II.3) led us to perform genetic testing to exclude

cEDS or classical-like EDS (cIEDS). Next-generation sequencing (NGS) of the proband's DNA revealed no mutation neither in *COL5A1*, *COL5A2*, *TNXB*, *AEBP1* genes, nor in any other tested gene (excluding alternative genetic diagnoses of HTCD), but in *COL1A1* (Supporting information 1: The list of genes included in the panel is available online). The proband (II.3) harbored a novel heterozygous variant c.935G>T in exon 14 of the *COL1A1* gene predicting the p.(Arg312Leu) missense variation (Figure 1C). The two other affected patients (II.2 and III.2) carried the same variant while the unaffected relatives (I.3, II.1, and III.1) did not. It was rated "pathogenic" according to ACMG criteria following in silico prediction tools analysis (<https://varsome.com> accessed on 12 15th 2020).¹⁹ The background criteria attribution by Varsome include the following: PM1, PM2, PM5, PP2, and PP3. Further criteria after clinical examination and segregation study allowed using PP1 and PP4 criteria, respectively. Finally, as the variant affects a residue for which a different amino acid change is definitively pathogenic, the criterion PS1 was also considered. On these criteria, the variant was rated "pathogenic." This variant, recorded in dbSNP (rs930476771), is not reported in the ClinVar, HGMD Pro, and LOVD databases. Its allelic frequency is 1/ 242 136 in GnomAD.

2.2 | Immunolabeling of COLI deposition by dermal fibroblasts in culture

We investigated the kinetics of collagen I secretion and deposition in the extracellular matrix in dermal fibroblast cultures from patient II.3 and a control individual by immunostaining PFA-fixed cells with a recombinant antibody against $\alpha 1(I)$ (ab138492, Abcam) 4, 7, 11, and 13 days post-seeding. As shown in Figure 2A, the p.(Arg312Leu) variant does not appear to significantly hamper collagen I secretion. Accordingly, we did not detect increased intracellular retention. COLI secretion kinetics was similar in both fibroblast cultures studied with COLI fibrils detectable by immunostaining 7 days postseeding. Of note, co-staining with a monoclonal antibody against fibronectin (HFN7.1, DSHB) revealed a possibly delayed secretion of fibronectin in the ECM at 4 days postseeding (Figure 2B).

2.3 | Rectification of the diagnosis

Considering the result of the genetic testing and in the light of the study published by Morlino and collaborators in 2019, we reconsidered the diagnosis in this family. The diagnosis of C1ROD relies on minor or major criteria.¹⁵ The patients of this study had at least the following three or four major criteria: blue sclerae (II.2, II.3, and III.2), flatfeet without hindfoot

deformity (II.3 and III.2), generalized joint hypermobility (II.2, II.3, and III.2), significantly hyperextensible or soft and doughy skin (II.2, II.3, and III.2) (Table 1). Therefore, their diagnoses have been retrospectively reevaluated for C1ROD.

3 | DISCUSSION

The affected patients presented here were initially diagnosed for the hypermobile EDS (hEDS). Although hEDS patients are generally not offered genetic testing because of the absence of candidate genes, the presence of a significant cutaneous involvement in the proband prompted us to perform genetic testing to exclude cEDS, cIEDS, or other HTCD. This decision allowed us to identify a novel pathogenic *COL1A1* variant p.(Arg312Leu) and rectify the diagnosis for a C1ROD. Importantly, it gave us the opportunity to provide appropriate genetic counseling.

The presence of the variant in affected patients (II.2, II.3, and III.2) but not in healthy relatives (I.3, II.1, III.1) corroborated the co-segregation of the variant with the disease. These observations suggest that the collagen I variant may be responsible for the phenotype. The affected arginine is located in the triple helical domain of the protein characterized by the repetition of the Gly-X-Y amino acid sequence implicated in the trimer formation. Structure modeling predicts a salt bridge by direct contact between the two oppositely charged Glu309 and Arg312 residues (Supporting information 2A available online). This interaction between two adjacent intrachain X residues has been described as a likely event by Persikov et al.²⁰ Collagens have a high proportion of charged amino acids. In fact, Arg, Lys, Glu, and Asp constitute 15%-20% of the sequence of the proteins in this family. Charged amino acids may play a dual role in collagen stabilization and folding, first at the level of triple helical assembly and second during fibril formation.²¹ It is of note that at the residue, the previously reported p.(Arg312Cys) (Figure 1C) generated an aberrant disulfide bonding resulting in intracellular retention.¹² Replacement of an arginine by a leucine residue does not generate aberrant disulfide bonding. Accordingly, immunostaining of cultured fibroblasts from patient II.2 at different time points revealed that the kinetics of COLI fibrils secretion in the ECM does not seem significantly hampered (Figure 2). One limitation of this experimental procedure is that it does not address directly processing of pN-collagen to collagen, as reported for the p.(Arg312Cys).¹² Thus, additional investigations are needed.

Synthetic models showed that the side-chain conformation of the charged aspartic acid and lysine residues, which form a network of ionic hydrogen bonds spiraling along the helical axis and contribute to the stability of homotrimeric triple helices.²¹ Although replacing Lys for Arg is less favorable, it can still contribute to the triple helix stability.

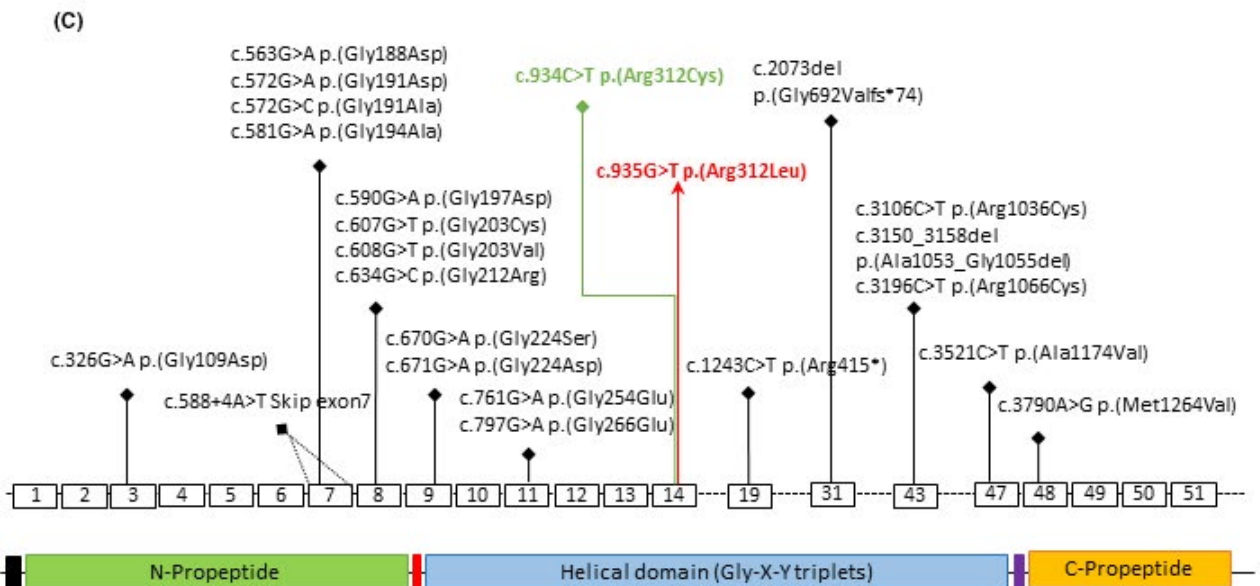
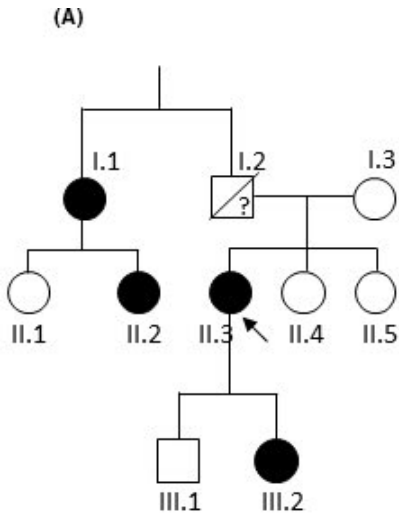


FIGURE 1 Family pedigree, clinical presentation of the patients, and genomic structure of the *COL1A1* gene. (A) Family pedigree. Circles indicate females, and squares indicate males. Symbols in black indicate individuals affected with a hEDS. Individual I.2 died in a motorcycle accident at age 25. His medical history is unknown. Individuals I.3, II.4, II.5, and III.1 are healthy. I.1 has not been reevaluated by the referral center. (B) Clinical presentation of the patients. Laxity of the thumb (a), swan-neck deformity of the fingers (b), laxity of the ankle (c, d) and bruising (d, arrow), piezogenic papules of the heel and mildly flat feet (e), hyperextensible skin (f, g), large scars (h), high and narrow palate (i), hyperlaxity of the fifth and third fingers (j, k), and blue sclerae (l). (C) Genomic structure of the *COL1A1* gene including exons (white rectangles) and introns (black horizontal line). Adapted from Morlino et al, 2019. Previously identified variants associated with CIROD are noted in black.¹⁵ The variant identified in patients II.2, II.3, and III.2 is indicated in red. The previously identified variant associated with *COL1A1*-cEDS is noted in green for the missense c.934C>T p.(Arg312Cys).^{12,18} The $\alpha 1(I)$ collagen chain is characterized by a signal peptide (black box), procollagen N- and C- proteinase cleavage sites (little red and purple boxes), N- and C-propeptides (green and orange boxes), a large helical domain composed of Gly-X-Y triplets (blue box)

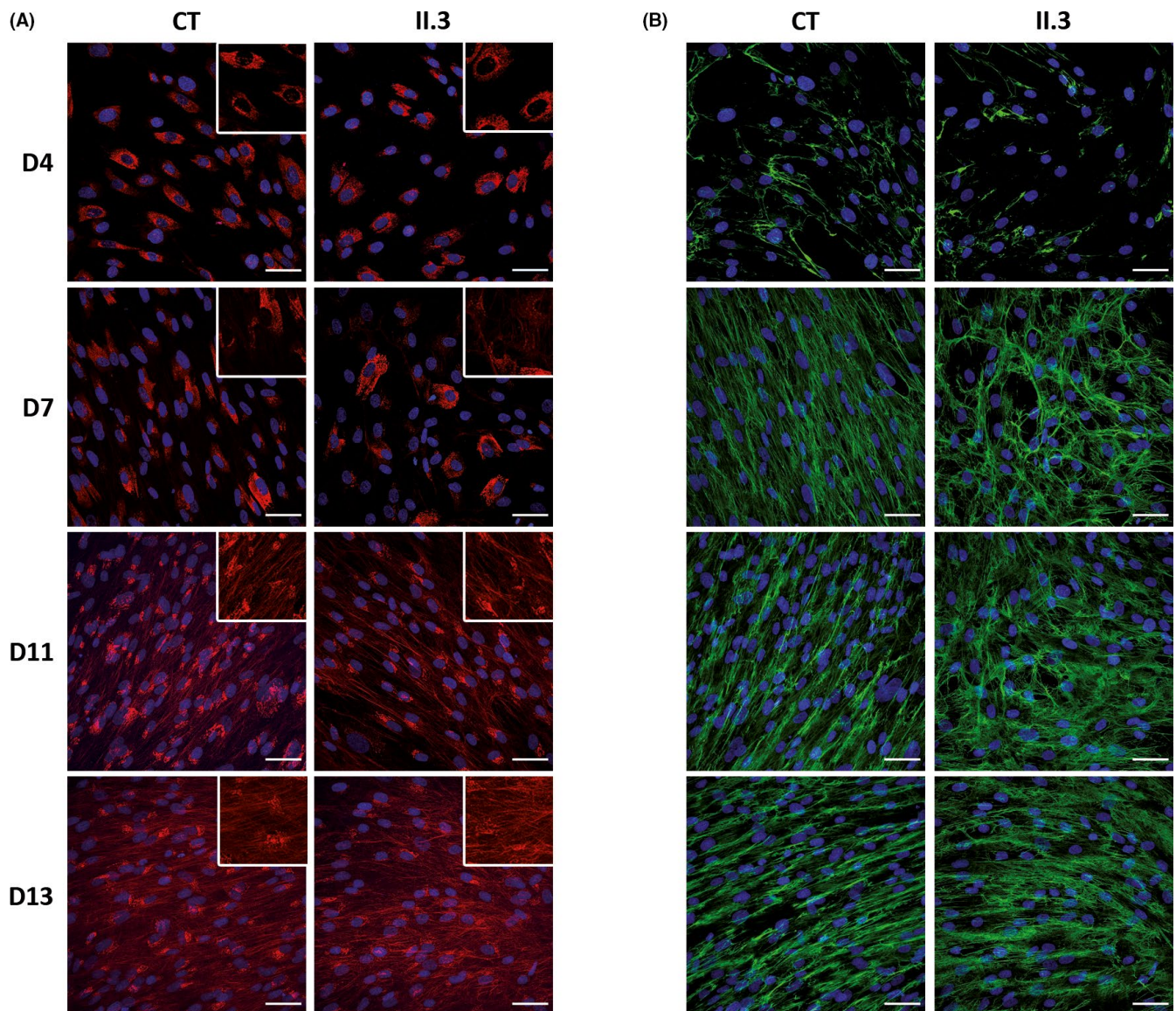


FIGURE 2 Collagen I and fibronectin secretion by dermal fibroblasts in culture visualized by confocal imaging. (A) COL1 (red) was detected with a recombinant antibody (ab138492, Abcam) on PFA-fixed cells from patient II.3 (II.3) and a control individual (CT). Inserts are 200X zooms. (B) Co-immunolabeling with a monoclonal antibody (HFN7.1, DSHB) against fibronectin (green). Nuclei are identified by DAPI staining (blue). Scale bars: 50µm

Because of its complex hierarchical self-assembly and the scale of the resulting supramolecular structures, information at atomic resolution for collagenous proteins is scarce.^{21–24}

Because of the absence of structural data at the atomic level on the segment studied here, the mechanism for the collagen defect in the Arg312Leu variant remains speculative. The

Gly-Arg-Hyp replacement by Gly-Leu-Hyp (hyp referring to hydroxyproline) may not affect thermal stability per se (http://rwjms.umdnj.edu/lab/collagen_research/assets/level_1_2/calculator.htm). In contrast, assuming an arbitrary register of the two $\alpha 1$ chain and the $\alpha 2$ chain starting at residues 111 and 91, respectively, the mutated triplet would precede a zone of decreased thermal stability (Supporting information 2B available online). Therefore, suppression of the suggested intrachain salt bridge between Glu309 and Arg312 might further increase the consequence of the locally unfavorable helical structure. Indeed, the influence of local unwinding has previously been demonstrated.^{25,26} As indicated for the p.(Arg312Cys) deleterious variant,¹² residue 312 is located within the first major ligand-binding region delineated by Di Lullo and collaborators.²⁷ Therefore, the p.(Arg312Leu) may disrupt COL1 fibrils-ligands interactions, notably integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$, and interleukin-2. Additional studies are warranted to experimentally investigate these interactions.

This novel variation in *COL1A1* completes the previous genetic data described in the study of Morlino and its collaborators: twenty-one different pathogenic variants in *COL1A1* were previously associated with C1ROD (Figure 1C). Most of them were heterozygous missense variants, especially glycine substitutions, located within or near the procollagen N-proteinase cleavage site,¹⁵ that affect the processing of the N-propeptide, resulting in a defect of collagen fibrillogenesis.¹⁴⁻¹⁶

In our report, the p.(Arg312Leu) variation is associated with heterogeneous phenotypes combining major manifestations of hEDS. The number of affected patients of our family was too limited to accurately describe a phenotypic pattern, but it is interesting to note that the most common EDS features included: generalized joint hypermobility (3/3), recurrent joint instability, and/or dislocations (3/3), swan-neck deformity of the fingers (3/3), chronic pain and fatigue (3/3), easy bruising (3/3), gingivorrhagia (3/3), gastrointestinal symptoms, and urinary symptoms (3/3), mild flatfeet (2/3), hyperextensible skin (2/3), and velvety skin (2/3). OI features included light blue sclerae (3/3), mild hearing loss (2/3), and mild osteopenia (2/3).

Cardiovascular involvement has been reported for another variant at the same position, p.(Arg312Cys) in vEDS and cEDS. It is associated with potential vascular fragility, ranging from the absence of vascular complication to arterial ruptures in cEDS-affected patients.^{9,10,12,13} Interestingly, cardiovascular explorations of our patients showed no major vascular complication except a small thoracic aorta dilatation in patient II.3, but which could also be a ductus diverticulum or a post-traumatic aortic pseudoaneurysm since the patient had a car accident in the past. An annual vascular follow-up will be organized to monitor the evolution of this abnormality. However, we have no clue to link this to incomplete penetrance in a small number of affected patients, to the

respective role of Leucine or Cysteine at residue 312, or to any modulating gene. In any case, considering the cardiovascular heterogeneity associated to the variant p.(Arg312Cys), we cannot exclude a potential vascular risk for p.(Arg312Leu) patients. Therefore, we still recommend extensive cardiovascular follow-up in such patients, at least until the absence of cardiovascular risks can be reliably confirmed by further observations.

Our data support the recently defined phenotype of C1ROD and expand the mutational spectrum of *COL1A1* mutations. Further reports will help us to define more precisely the phenotype pattern. Moreover, our findings highlight the importance of considering genetic testing to hEDS patients, especially when they fulfill C1ROD criteria. This should allow providing proper genetic counseling and adapting clinical management.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

FM gathered the data and drafted the study. FM, DMP, MeC, AV, MiC, and BK wrote, reviewed, and/or revised the manuscript. GF, BK, and MiC performed clinical examinations of patients. GF and BK performed the skin biopsy. MN and JV performed NGS analysis. MeC and DMP interpreted the NGS results. DMP also performed collagen structural analysis. AV and CG performed immunostaining analysis. MA was the radiologist. CR is the head of the radiology department. RP was involved in the interpretation of genetic data. BK conceived and supervised the study.

EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS

Data contained were obtained through routine clinical care and for a diagnosis purpose. They are not considered as research at our institution; thus, ethics approval was not obtained. We informed the patients about the process of publishing a case report, and signed informed consents were obtained for molecular studies and for publication of photographs. Patients'

data are collected in a database, which has been approved by the National Liberty Commission (CNIL Commission Nationale Informatique et Libertés).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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