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ORIGINAL ARTICLE

Clinical and molecular features of patients with *COL1*-related disorders: Implications for the wider spectrum and the risk of vascular complications

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Abstract

Abnormalities in type I procollagen genes (*COL1A1* and *COL1A2*) are responsible for hereditary connective tissue disorders including osteogenesis imperfecta (OI), specific types of Ehlers-Danlos syndrome (EDS), and *COL1*-related overlapping disorder (C1ROD). C1ROD is a recently proposed disorder characterized by predominant EDS symptoms of joint and skin laxity and mild OI symptoms of bone fragility and blue sclera. Patients with C1ROD do not carry specific variants for COL1-related EDS, including classical, vascular, cardiac-valvular, and arthrochalasia types. We describe clinical and molecular findings of 23 Japanese patients with pathogenic or likely pathogenic variants of *COL1A1* or *COL1A2*, who had either OI-like or EDS-like phenotypes. The final diagnoses were OI in 17 patients, classical EDS in one, and C1ROD in five. The OI group predominantly experienced recurrent bone fractures, and the EDS

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group primarily showed joint hypermobility and skin hyperextensibility, though various clinical and molecular overlaps between OI, *COL1*-related EDS, and C1ROD as well as intrafamilial phenotypic variabilities were present. Notably, life-threatening vascular complications (vascular dissections, arterial aneurysms, subarachnoidal hemorrhages) occurred in seven patients (41% of those aged >20 years) with OI or C1ROD. Careful lifelong surveillance and intervention regarding bone and vascular fragility could be required.

KEYWORDS
COL1A1, *COL1A2*, *COL1*-related overlap disorder, Ehlers-Danlos syndrome, osteogenesis imperfecta

1 | INTRODUCTION

Pathogenic variants in genes encoding two alpha chains of type I collagen (*COL1A1* or *COL1A2*) are associated with several hereditary connective tissue disorders (HCTD), including autosomal dominant osteogenesis imperfecta (OI) type I – IV (MIM #166200, 166210, 259420, 166220) (Lim et al., 2017; Marini et al., 2017), postmenopausal osteoporosis (MIM #166710) (Grant et al., 1996), Caffey disease (MIM #114000) (Gensure et al., 2005), and four types of Ehlers-Danlos syndrome (EDS): classical type (cEDS, MIM #130000), cardiac valvular type (cvEDS, MIM #225320), a subset of vascular type (vEDS, MIM #130050), and arthrochalasia type (aEDS, MIM #130060, 617821) (Brady et al., 2017; Malfait et al., 2017).

OI is characterized by bone fragility with multiple bone fractures, skeletal deformity, growth impairment, blue sclera, dentinogenesis imperfecta, late-onset hearing loss, and normal intellectual development (Forlino & Marini, 2016). The clinical phenotypes of OI are highly variable, ranging from prenatally lethal with extremely severe bone deformities and multiple bone fractures to relatively asymptomatic with low bone mass and susceptibility to fractures. Approximately 85%–90% of OI cases show autosomal dominant inheritance caused by heterozygous pathogenic variants in *COL1A1* or *COL1A2* (Lim et al., 2017).

EDS is characterized by generalized joint hypermobility (GJH), skin hyperextensibility, and tissue fragility. cEDS is characterized by three major symptoms: joint hypermobility, skin hyperextensibility, and delayed wound healing. The majority of cEDS cases are caused by heterozygous pathogenic variants in *COL5A1* or *COL5A2*, while a specific variant in *COL1A1* (p.Arg312Cys) gives rise to cEDS with vascular fragility including rupture and dissection of medium-sized arteries (Adham et al., 2020). vEDS is characterized by thin and translucent skin and tissue fragility of arteries and intestine; serious complications including vascular rupture and dissection as well as intestinal and uterine rupture occur in about 70% of patients. The main cause of vEDS is the presence of heterozygous pathogenic variants in a gene encoding type III procollagen (*COL3A1*); however, heterozygous pathogenic variants in *COL1A1* (p.Arg312Cys; p.Arg574Cys; p.Arg1093Cys) are also associated with vEDS. aEDS is caused by heterozygous pathogenic variants that give rise to partial or complete loss of exon 6 in *COL1A1* or *COL1A2*, leading to the inhibition of procollagen N-terminal site cleavage and disruption of collagen fibril assembly and cross-linking. Symptoms of aEDS include severe congenital GJH and bilateral hip dislocation, joint subluxation and dislocation, and hyperelastic or redundant skin. In addition to three major symptoms of cEDS, cvEDS is characterized by severe cardiac-valvular defects, including aortic valve regurgitation, mitral valve prolapses, and subsequent left ventricular hypertrophy. Homozygous or compound heterozygous pathogenic variants in *COL1A2* were found to be causal for cvEDS, which leads to the complete absence of pro-alpha-2 collagen chains and the generation of pro-alpha-1 homotrimers.

Recently, patients with mixed phenotypes of both OI and EDS have been found to harbor heterozygous pathogenic variants in *COL1A1* or *COL1A2* (Cabral et al., 2005; Malfait et al., 2013). In the series by Malfait et al. (2013), EDS-related symptoms were more prominent than OI-related symptoms in these patients. All variants were located near the N-terminal helical region of the type I collagen alpha-1 or alpha-2 chain and were postulated to interfere with the cleavage of the procollagen N-propeptide, which did not correspond to the variants of the other known *COL1*-related types of EDS (cEDS, vEDS, aEDS, and cvEDS). Therefore, Malfait et al. (2013) proposed to name the condition "OI/EDS overlap syndrome." Morlino et al. (2020) described another cohort of patients with similar features and suggested the term "*COL1*-related overlap disorder (C1ROD)," considering a wide spectrum of bridging phenotypes between OI and EDS. Major criteria for molecular testing were proposed as follows: (1) blue sclera, (2) flat feet with valgus deformity of the hindfoot, (3) generalized joint hypermobility according to the age, and (4) significantly soft and doughy, and/or hyperextensible skin. Although many of the variants found in C1ROD were located in the N-terminal helical region, several variants were located outside the region. Additional patients with C1ROD have been described (Budsamongkol et al., 2019; Foi et al., 2021; Gnoli et al., 2021).

The delineation of a wide clinical spectrum of C1ROD is insufficient because previously reported cohorts have recruited either

patients with OI-like phenotypes or those with EDS-like phenotypes. Here, we report detailed and comprehensive clinical and molecular features of patients who met the criteria of C1ROD from a unique cohort, including both patients with OI-like phenotypes and those with EDS-like phenotypes. In addition, it is noteworthy that life-threatening vascular events seemed to be associated with the condition.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

Blood samples and clinical information were collected after obtaining written informed consent from patients and/or their parents. This study was approved by the Ethics Committee at Shinshu University School of Medicine (Matsumoto, Japan) (#628, #4171).

2.2 | Patient enrollment

Japanese patients who were clinically suspected to be patients with OI or EDS and were found to have pathogenic or likely pathogenic variants in *COL1A1* or *COL1A2* through our genetic investigation were enrolled. Clinical suspicion of OI was made based on the occurrence of recurrent bone fractures and/or family history of OI, and that of EDS was made based on the presence of joint hypermobility (indicated as Beighton score > 4 points) and skin hyperextensibility with or without bone fragility.

2.3 | Genetic investigation

Genomic DNA was extracted from peripheral blood leukocytes of the patients using Gentra Puregene Blood Kit or QIAamp DNA Blood Mini Kit on QIAcube (Qiagen, Hilden, Germany). Next-generation sequencing was performed on an Ion PGM™, Ion PGM™ Dx, or Ion GeneStudio™ S5 (Thermo Fisher Scientific, Waltham, MA, USA), using Ion AmpliSeq™ custom panels designed with Ion AmpliSeq™ Designer (https://www.ampliseq.com/) for 17 genes (version 1), 54 genes (version 2), 52 genes (version 3), 71 genes (version 4), and 52 genes (version 5) associated with HCTD, including *COL1A1* and *COL1A2* (Table S1). The sequencing data were mapped to human genome hg19 using Torrent Suite™ software (Thermo Fisher Scientific), and single-nucleotide variants and small insertions/deletions were detected from the mapped data using the Torrent Suite™ plug-in. The variants were annotated using SnpEff (http://pcingola.github.io/SnpEff/) (Cingolani et al., 2012). The candidate variant was confirmed by Sanger sequencing on an ABI 3130xl Genetic Analyzer using a BigDye™ Direct Cycle Sequencing Kit using M13 tailed primers and BigDye™ XTerminator Purification Kit (Thermo Fisher Scientific).

3 | RESULTS

3.1 | Clinical findings

Detailed and comprehensive clinical and molecular findings of the patients are shown in Table 1. Twenty-three patients from 15 families aged 3–67 years were described: Eight were males (35%), and 15 were females (65%). Initial clinical suspicion was OI in 18 patients (#1 – #18; 78%) predominantly showing bone fragility and EDS in the remaining five patients (22%), who presented joint hypermobility and/or skin hyperextensibility: aEDS (patients #19, #20), cEDS (patient #21), vEDS (patient #22), and vEDS or OI (patient #23).

In family #12, patient #19 was first clinically suspected to have aEDS based on two major criteria (congenital bilateral hip dislocation and skin hyperextensibility) plus GJH (without multiple dislocation/subluxation) and three minor criteria (kyphoscoliosis, atrophic scars, and easy bleeding). Later, her younger brother, patient #20, was also suspected of having aEDS based on symptoms similar to patient #19 including skin hyperextensibility, GJH (without multiple dislocation/subluxation), atrophic scars, and easy bleeding though he did not have congenital hip dislocation. However, her father, patient #18, was suspected of having OI type I because his main medical condition included multiple bone fractures. He had blue sclerae while also showing GJH and skin hyperextensibility.

Patient #21 was suspected of having cEDS based on two major criteria (skin hyperextensibility and atrophic scars, GJH) plus two minor criteria (easy bleeding and soft and doughy texture). Patient #22 was suspected of having vEDS based on an episode of internal carotid artery dissection and skin translucency. Though patient #23 was suspected of having vEDS based on an episode of vertebral artery dissection and congenital clubfeet, she also presented OI-related symptoms (significant reduction of bone mineral density with an episode of bone fracture and blue sclera) and joint and skin features (joint hypermobility, easy bleeding, and soft and doughy texture) not typical for vEDS.

The final diagnosis was classical OI in 17 patients (#1 – #17; 74%), classified into "the OI group." The final diagnoses were C1ROD in five patients (#18, #19, #20, #22, and #23; 22%) and *COL1*-related cEDS in one patient (#21; 4%), both classified into "the EDS group." All five patients with C1ROD met the criteria for submitting molecular testing to the diagnosis, as proposed by Morlino et al. (2020). Thirteen patients in the OI group were subclassified as having OI type I without long bone deformity and with blue sclerae. The remaining four patients (#5, #9, #11, and #12) were considered unclassified because they had both long bone deformities (also with short stature <2.0 *SD* in adult cases) and blue sclerae. The median height *SD* score was −1.2 in both groups, with the heights of five patients including one in the EDS group, below −2.0 *SD*. Only one patient in the OI group (#1) deceased due to vascular complications as mentioned below.

TABLE 1 C inica and mo ecu ar findings of 23 patients in this study

Family	1	2	3		4		5		6
			#3	#4	#5	#6	#7	#8	
Patient	#1	#2	Mother	Son	Mother	Daughter	Mother	Daughter	#9
C inica suspicion	O	O	O	O	O	O	O	O	O
Fina diagnosis	O type	O type	O type	O type	O unc assified	O type	O type	O type	O unc assified
General									
Sex	F	F	F	M	F	F	F	F	M
Age (years)	67	41	55	26	47	6	45	18	27
Height (cm/SD)	143/−0.6	160.8/+0.4	154.5/−0.5	173.5/+0.4	138.6/−3.7	111.4/−0.6	156.6/−0.3	157.5/0.0	152.8/−3.2
Outcome	Deceased	A ive	A ive	A ive	A ive	A ive	A ive	A ive	A ive
Skeletal									
Mu tip e bone fractures	+	+	+	+	+	+	+	+	+
Frequency of fractures	8	10	7	6	>50	2	2	6	5
Position of fractures	LB, PT, FM, PB, FB	PH, EL, PF	FM, TB, SC, EL, PF	AK, EL, WR	FM, PH, PF, EL, PT	FM	SC, PF	SK, HM, EL, PH, KN	CX, HM, PT
Denta abnorma ity	−	−	+	−	−	−	NA	−	−
Joint hypermobi ity	NA	+	−	−	−	−	−	+	−
Beighton score		6		2		4	2	8	
Recurrent joint dis ocation	+	−	−	+	−	−	−	+	+
Congenita hip dis ocation	−	−	−	−	−	−	−	−	NA
Joint contracture	−	−	−	−	+	−	−	−	NA
Long bone deformity	−	−	−	−	+	−	−	−	+
Spina deformity	+	+	−	+	−	−	−	+	+
Congenita c ubfoot	−	−	−	−	−	−	−	−	NA
F at feet	NA	+	+	−	+	+	+	+	+
Ruptures of tendon, igament, or musc e	−	−	−	−	−	−	+	+	+
ntramuscu ar b eeding	NA	−	−	−	−	−	−	−	−
Joint pain	NA	NA	−	−	NA	NA	NA	+	NA
DXA(vertebra :g/cm2)	0.548	1.132	0.743	0.961	0.547	0.432	0.645	1.145	0.936
T-score	−4.8	−0.2	−2.9	−1.0	−4.8	−	82 (%YAM)	−	−2.1
Z-score	−3.4	−0.2	−2.2	−1.0	−4.7	−		0	−2.1

TABLE 1 (Continued)

Family	1	2	3		4		5		6
			#3	#4	#5	#6	#7	#8	
Patient	#1	#2	Mother	Son	Mother	Daughter	Mother	Daughter	#9
Skin									
Hyperextensibi ity	NA	NA	–	–	–	–	+	–	–
Fragi ity	NA	NA	–	–	+	+	+	+	–
Atrophic scars	NA	NA	–	–	–	–	–	–	–
Trans ucency	NA	+	–	–	NA	NA	+	–	–
Soft doughy skin	NA	NA	+	–	+	+	NA	NA	–
Piezogenic papu es	NA	NA	NA	NA	–	–	+	–	NA
Easy bruising	NA	NA	–	–	–	–	–	–	+
Eye and ear									
B ue sc erae	+	+	+	+	+	+	+	+	+
Retina detachment	NA	–	–	–	–	–	–	–	–
Refractive errors	NA	Myopia	Myopia	Myopia	–	–	Myopia	Myopia	+
Hearing impairment	+	+	+	–	+	–	+	+	–
Cardiovascular									
Vascu ar dissection	Ascending aorta	–	–	–	–	NA	–	NA	–
Arteria aneurysm	Lt. MCA	Bi . CA	Rt. CA	–	–	NA	Rt. MCA	NA	–
Cardiac va ve disorder	AR	–	–	NA	–	NA	–	NA	MR, TR
Rena disease	PKD	URA	NA	NA	NA	NA	NA	NA	–
Hypertension	+	–	–	NA	–	–	+	–	NA
Others	SAH due to MCA rupture	SAH due to CA rupture	SAH due to CA rupture						Coronary-pu monary artery fistu a
Variant									
	COL1A1(PV: O)	*COL1A1*(PV: O)	*COL1A1*(PV: O , C1ROD)		*COL1A1*(PV: O)		*COL1A1*(UV)		*COL1A1*(UV)
	c.779G > A	c.2829 + 1G > A	c.1243C > T		c.769G > A		c.1679de		c.572G > T
	p.G y260Asp		p.Arg415*		p.G y257Arg		p.G y560Va fs*20		p.G y191Va
	Exon 11	ntron 39	Exon 19		Exon 11		Exon 25		Exon 7

Family	7		8	9	10		11	
	#10	#11			#14	#15	#16	#17
Patient	Mother	Daughter	#12	#13	Elder brother	Younger brother	Father	Daughter
C inica suspicion	O	O	O	O	O	O	O	O
Fina diagnosis	O type	O unc assified	O unc assified	O type	O type	O type	O type	O type

(Continues)

TABLE 1 (Continued)

Family	7		8	9	10		11	
	#10	#11			#14	#15	#16	#17
Patient	Mother	Daughter	#12	#13	Elder brother	Younger brother	Father	Daughter
General								
Sex	F	F	F	F	M	F	M	F
Age (years)	37	3	26	14	16	9	41	8
Height (cm/SD)	151/−1.2	86.3/−1.7	133.9/−4.3	148.4/−1.5	150.3/−3.3	122/−1.8	168.5/−0.5	124.8/−1.8
Outcome	A ive	A ive	A ive	A ive	A ive	A ive	A ive	A ive
Skeletal								
Mu tip e bone fractures	+	−	+	+	+	+	+	−
Frequency of fractures	5	−	12	5	5	2	5	−
Position of fractures	RD, UL, FB	−	FM	TB, FB, EL, RD	TB, RD, UL, CB, PF	TB, HM	FM, PF	−
Denta abnorma ity	+	−	+	−	−	−	+	+
Joint hypermobi ity	−	−	+	+	−	−	−	−
Beighton score			7	7			4	4
Recurrent joint dis ocation	−	−	−	−	−	−	−	−
Congenita hip dis ocation	−	−	−	−	−	−	−	−
Joint contracture	−	−	+	−	−	−	−	−
Long bone deformity	−	+	+	−	−	−	−	−
Spina deformity	−	−	+	−	−	−	−	+
Congenita c ubfoot	−	−	−	−	−	−	−	−
F at feet	−	−	+	+	−	−	−	−
Ruptures of tendon, igament, or musc e	−	−	−	−	−	−	−	−
ntramuscu ar b eeding	−	−	−	−	−	−	−	−
Joint pain	−	−	NA	−	−	−	−	−
DXA(vertebra :g/cm2)	NA	NA	0.886	0.864	0.837	0.716	0.981	0.55
T-score			−1.9	80 (%YAM)	−	−	−0.9	−
Z-score			−1.5	−1.7	0	0	−0.9	−1.5
Skin								
Hyperextensibi ity	−	−	−	+	−	−	−	−
Fragi ity	−	−	−	−	−	−	−	−
Atrophic scars	−	−	−	−	−	−	−	−
Trans ucency	−	−	−	−	−	−	−	−
Soft doughy skin	−	−	−	+	−	−	−	−
Piezogenic papu es	−	−	NA	NA	−	−	+	−
Easy bruising	+	−	−	−	−	−	−	−

TABLE 1 (Continued)

Family	7		8	9	10		11	
	#10	#11			#14	#15	#16	#17
Patient	Mother	Daughter	#12	#13	Elder brother	Younger brother	Father	Daughter
Eye and ear								
B ue sc erae	+	+	+	+	+	+	+	+
Retina detachment	–	–	NA	–	–	–	–	–
Refractive errors	–	–	Myopia	–	–	–	Myopia	NA
Hearing impairment	–	–	–	–	–	–	NA	–
Cardiovascular								
Vascu ar dissection	–	–	–	NA	–	–	–	NA
Arteria aneurysm	–	–	–	NA	–	–	–	NA
Cardiac va ve disorder	–	–	–	NA	–	–	–	NA
Rena disease	–	–	NA	NA	–	–	Rt. rena cyst	NA
Hypertension	–	–	–	–	–	–	–	NA
Others								
Variant								
	COL1A1(UV)		*COL1A2*(UV)	*COL1A2*(PV: O)	*COL1A2*(PV: O)		*COL1A1*(PV: O)	
	c.559de		c.1963G > C	c.693 + 1G > A	c.2314G > A		c.2362G > A	
	p.Arg187Va fs*78		p.G y655Arg		p.G y772Ser		p.G y788Ser	
	Exon 7		Exon 32	ntron 14	Exon 38		Exon 34	

Family	12			13	14	15
	#18	#19	#20			
Patient	Father	Daughter	Son	#21	#22	#23
C inica suspicion	O	aEDS	aEDS	cEDS	vEDS	vEDS or O
Fina diagnosis	C1ROD	C1ROD	C1ROD	cEDS	C1ROD	C1ROD
General						
Sex	M	F	M	M	M	F
Age (years)	34	4	2	18	47	45
Height (cm/SD)	163.2/−1.5	99.7/−0.9	85.2/0.0	143.8/−1.5	173/−0.1	135.5/−4.0
Outcome	A ive	A ive	A ive	A ive	A ive	A ive

(Continues)

TABLE 1 (Continued)

Family	12			13	14	15
	#18	#19	#20			
Patient	Father	Daughter	Son	#21	#22	#23
Skeletal						
Mu tip e bone fractures	+	−	−	−	+	−
Frequency of fractures	6	−	−	−	2	1
Position of fractures	NA	−	−	−	EL	TB
Denta abnorma ity	−	−	−	−	−	−
Joint hypermobi ity	+	+	+	+	+	+
Beighton score	9	9	8	6	NA	NA
Recurrent joint dis ocation	−	−	−	−	−	+
Congenita hip dis ocation	−	+	−	−	−	−
Joint contracture	−	−	−	−	−	−
Long bone deformity	−	−	−	−	+	NA
Spina deformity	−	+	NA	−	−	−
Congenita c ubfoot	−	−	−	−	−	+
F at feet	+	+	+	−	−	−
Ruptures of tendon, igament, or musc e	−	−	−	−	−	−
ntramuscu ar b eeding	−	−	−	−	−	−
Joint pain	−	−	−	−	−	−
DXA(vertebra :g/cm2)	1.048	NA	NA	NA	0.787	0.623
T-score	−0.5				−0.8	−3.5
Z-score	−0.5				−0.8	
Skin						
Hyperextensibi ity	+	+	+	−	+	−
Fragi ity	−	−	−	−	+	+
Atrophic scars	−	−	−	+	−	−
Trans ucency	−	−	−	−	+	+
Soft doughy skin	−	+	+	+	+	+
Piezogenic papu es	NA	+	+	−	NA	NA
Easy bruising	+	+	+	+	−	+

TABLE 1 (Continued)

Family	12			13	14	15
	#18	#19	#20			
Patient	Father	Daughter	Son	#21	#22	#23
Eye and ear						
B ue sc erae	+	+	+	−	+	+
Retina detachment	−	−	NA	−	−	−
Refractive errors	Myopia	Myopia, astigmatism	NA	−	−	−
Hearing impairment	NA	−	−	−	−	NA
Cardiovascular						
Vascu ar dissection	−	−	−	−	Bi . CA	Bi . VA
Arteria aneurysm	−	−	−	−	−	AAA
Cardiac va ve disorder	NA	−	−	−	−	−
Rena disease	NA	−	−	−	−	Lt. rena AVM
Hypertension	NA	NA	NA	−	−	NA
Others					Cerebra infarction	Latera medu ary infarction
Variant						
	COL1A2(PV: O , C1ROD)			*COL1A1*(PV: cEDS)	*COL1A1*(PV: O)	*COL1A1*(UV)
	c.432 + 4_432 + 7de			c.934C > T	c.658C > T	c.571G > T
				p.Arg312Cys	p.Arg220*	p.G y191Cys
	ntron 9			ntron 14	Exon 9	Exon 7

Abbreviations: +, present −, absent AAA, abdomina aortic aneurysm AK, ank e AR, aortic va ve regurgitation AVM, arteriovenous ma formation Bi ., bi atera C1ROD, COL1-re ated over ap disorder CB, c avic e bone CX, coxa bone EL, e bow F, fema e FB, fibu a FM, femur HM, humerus CA, inner carotid artery KN, knee LB, umbar spine Lt., eft M, ma e MCA, midd e cerebra artery MR, mitra va ve regurgitation NA, not avai ab e PB, pubis PF, pha anx of foot PH, pha anx of hand PKD, po ycystic kidney disease PT, pate a PV, previous y pub ished variant RD, radius Rt., right SAH, subarachnoida hemorrhage SC, scapu a SK, sku TB, tibia TR, tricuspid va ve regurgitation UL, u na URA, uni atera rena ap asia UV, unpub ished variant VA, vertebra artery WR, wrist YAM, young adu t mean.

3.2 | Molecular findings

Fifteen pathogenic or likely pathogenic variants were identified: 11 variants were in *COL1A1* (NM 000088.3) (Figure 1a), and the remaining four were in *COL1A2* (NM 000089.3) (Figure 1b). All variants were located within the triple helical domain of *COL1A1* or *COL1A2*.

Among the *COL1A1* variants, six were missense variants, four were nonsense or frameshift variants that resulted in premature stop codons, and one was a splice-site variant. The final diagnoses of relevant patients were as follows: C1ROD in patient #22 with a nonsense variant (p.Arg220*) and patient #23 with a missense variant (p.Gly191Cys); cEDS in patient #21 with a missense variant (p.Arg312Cys) and OI in the remaining 13 patients (eight families) with four missense variants (p.Gly191Val, p.Gly257Arg, p.Gly260Asp, and p.Gly788Ser), three nonsense or frameshift variants (p.Arg187Valfs*78, p.Arg415*, and p.Gly560Valfs*20), or one splice-site variant (c.2829 + 1G > A). Two missense variants (p.Gly191Cys and p.Gly191Val) and two frameshift variants (p.Arg187Valfs*78 and p.Gly560Valfs*20) were novel. According to the 2015 American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines (Richards et al., 2015), "p.Gly560Valfs*20" was classified as pathogenic and "p.Gly191Cys," "p.Gly191Val," and "p.Arg187Valfs*78" were classified as likely pathogenic. "p.Arg220*" associated with C1ROD (patient #22) was previously reported in a patient with OI type I (Körkkö et al., 1998), and "p.Arg415*" associated with OI (patient #3, #4) was previously reported in patients with OI type I or C1ROD (Morlino et al., 2020; Willing et al., 1996).

Among the *COL1A2* variants, two were missense variants (p.Gly655Arg and p.Gly772Ser), one was a splice-site variant (c.693 + 1G > A), and the remaining was a small deletion (c.432 + 4 432 + 7del). "p.Gly655Arg" was a novel variant, classified as pathogenic, according to the ACMG/AMP guidelines (Richards et al., 2015), and the others were reported previously. The final diagnoses of relevant patients were OI in patients with "p.Gly655Arg," "p.Gly772Ser," or "c.693 + 1G > A." A small deletion variant (c.432 + 4 432 + 7del) was found in a family with a final diagnosis as C1ROD, including a father suspected of having OI (patient #18) and two children suspected of having aEDS (patients #19, #20). The variant was previously reported in patients with OI or C1ROD (Malfait et al., 2013; Marini et al., 2007).

3.3 | Vascular complications

A total of ten vascular complications, including arterial dissections, vascular aneurysms, and/or arterial–arterial/arterial–venous fistulas, were identified in seven patients, 41% of those aged >20 years: five patients with the final diagnosis as OI type I (patients #1, #2, #3, #7, and #9) and two patients with C1ROD(patients #22, #23). All the seven patients had pathogenic or likely pathogenic variants in *COL1A1*: Three were missense variants (patient #1, p.Gly260Asp; patient #9, p.Gly191Val; and patient #23, p.Gly191Cys), three were nonsense/frameshift variants (patient #3, p.Arg415*; patient #7, p.Gly560Valfs*20; and patient #22, p.Arg220*), and one was a splice-site variant (patient #2, c.2829 + 1G > A).

Vascular complications included a left middle cerebral artery aneurysm (Figure 2a), resulting in a left temporal and occipital lobe subarachnoid hemorrhage (SAH) (Figure 2b), and an acute aortic dissection (Figure 2c–e) in patient #1; SAH due to a ruptured internal carotid artery aneurysm in patients #2 and #3; an unruptured aneurysm of the right middle cerebral artery (Figure 2f) in patient #7; a coronary to pulmonary artery fistula in patient #9; bilateral internal carotid artery dissections (Figure 2g–j) in patient #22; and bilateral vertebral artery dissections, two saccular abdominal aortic aneurysms, and an aneurysmal-type arteriovenous malformation in the left kidney in patient #23. Detailed clinical courses of patients #1, #2, #3, #7, #22, and #23 are provided in the Supplemental Information.

3.4 | Histopathological investigation

Transmission electron microscopy (TEM)-based histopathological evaluation of the skin specimens was performed on patients #22 (Figure 3a) and #23 (Figure 3b). Abnormal cauliflower-like collagen fibrils with irregular margins were occasionally observed in both patients, similar to previous reports regarding C1ROD (Cabral et al., 2007; Malfait et al., 2013; Morlino et al., 2020).

4 | DISCUSSION

We have presented clinical and molecular features of 23 patients from 15 families with pathogenic or likely pathogenic variants in *COL1A1* or *COL1A2*, recruited based either on OI-like or EDS-like phenotypes. Whereas the initial clinical suspicion was OI, aEDS, cEDS, or vEDS, the final diagnoses were OI (type I or unclassified), *COL1*-related cEDS, and C1ROD according to clinical and molecular findings. Phenotypic and molecular overlaps among patients in the OI group (patients #1 – #17) and those in the EDS group (patients #18 – #23), as well as intra-familiar phenotypic variabilities, were noted. Life-threatening vascular complications including arterial dissections and aneurysms occurred in both groups.

Patients in the OI group tended to show similar skeletal features to those in the EDS group besides recurrent bone fractures. Patients in the EDS group tended to show markedly higher frequencies in skin hyperextensibility, soft and doughy skin, and piezogenic papules than those in the OI group. Intrafamilial phenotypic variabilities included a presumably age-dependent severity in a family in the OI group (family #4) and an age-independent one in a family in the EDS group (family #12). In addition, patient #19 had a bilateral hip dislocation, a characteristic finding of aEDS, which suggests a phenotypical overlap between C1ROD and aEDS, as described by Morlino et al. (2020).

Regarding the variants identified both in the current study and in previous publications, six were found only in patients with OI

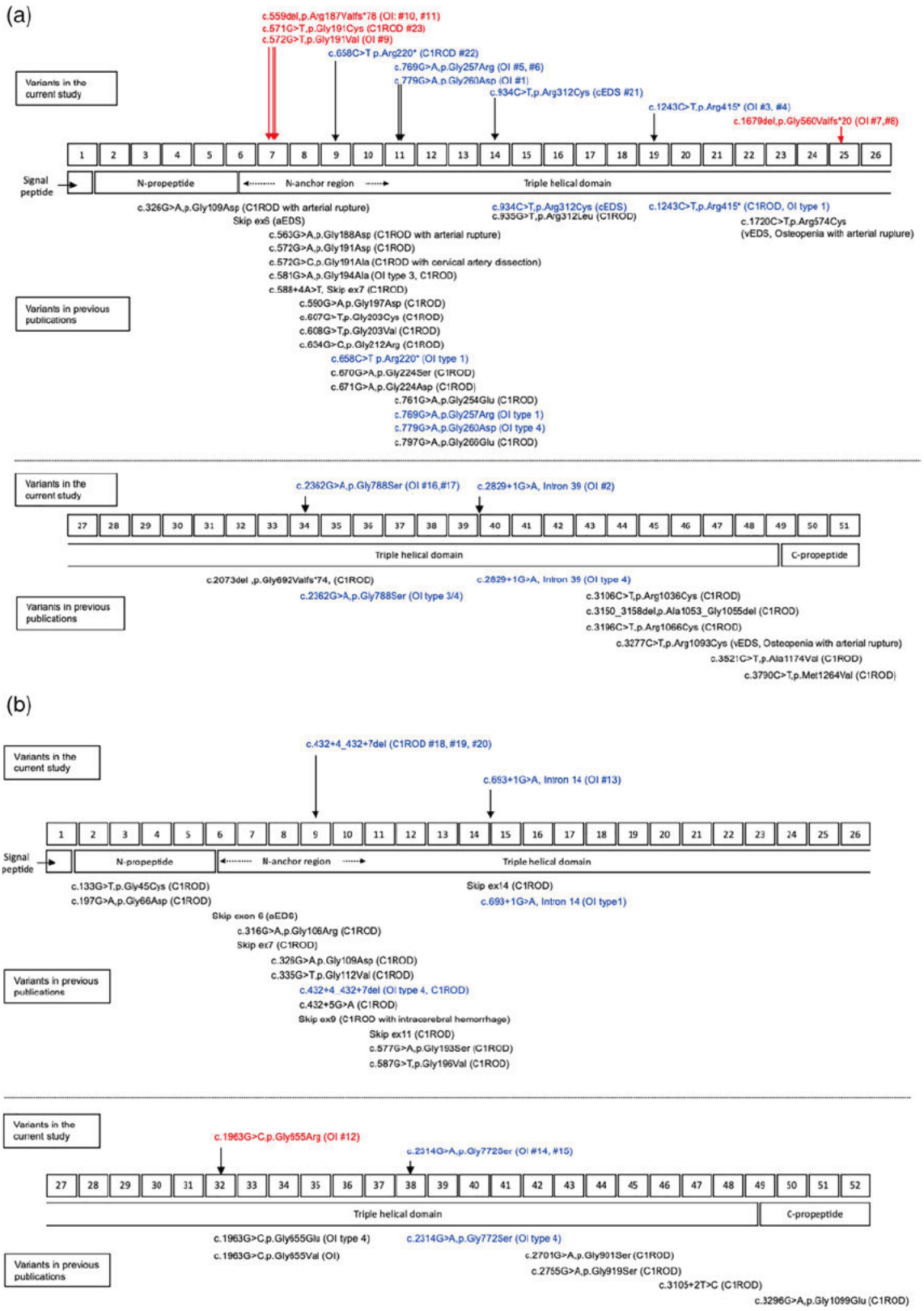

FIGURE 1 Schematic structure, location of domain organizations, and distribution of the mutations in *COL1A1* (a) and *COL1A2* (b). The numbers in the upper center rectangles indicate the exon numbers, and the rectangles in the lower center indicate the domain organizations of the proteins. Variants found in the current study are shown above the exon rectangles; unpublished variants are displayed in red, and previously published variants are displayed in blue. Variants in previous publications are shown below the domain rectangles. Variants both identified in the current study and previous publications are displayed in blue. aEDS, Ehlers-Danlos syndrome arthrochalasia type; C1ROD, COL1-related overlap disorder; OI, osteogenesis imperfecta; cEDS Ehlers-Danlos syndrome classical type; vEDS, Ehlers-Danlos syndrome vascular type

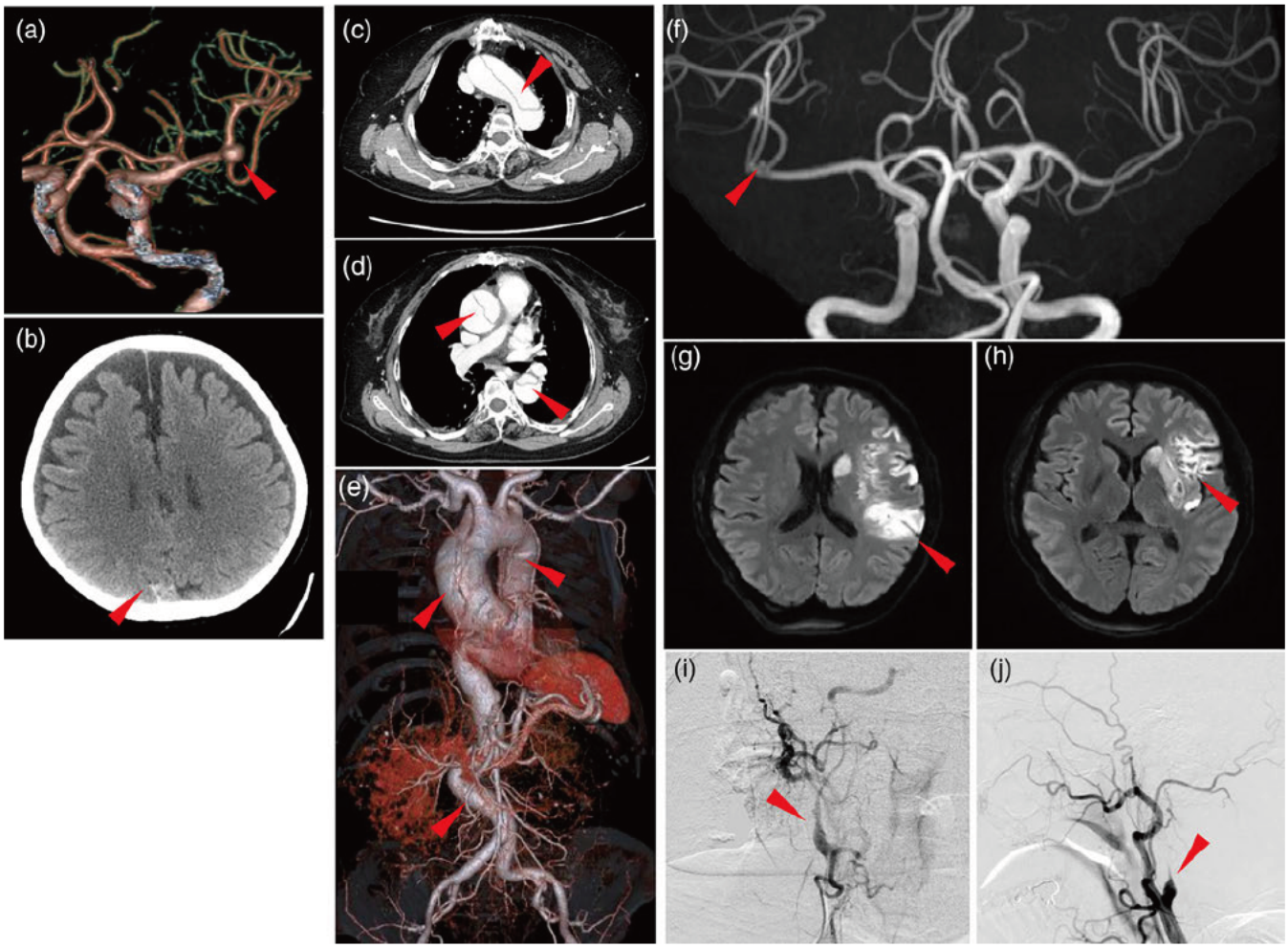

FIGURE 2 Radiographic findings of three patients with vascular complications. (a–e, patient #1) A brain enhanced computed tomography (CT)-based angiography (a) shows a left inner carotid artery aneurysm (arrowhead). A brain CT reveals an occipital subarachnoid hemorrhage (b). Chest CT images (c, d) and chest to abdominal CT-based angiography (e) show an extensive aortic dissection from the ascending aorta, aortic arch, and descending thoracic to abdominal aorta (arrowheads). (f, patient #7) A magnetic resonance imaging (MR)-based angiography shows a right inner carotid artery aneurysm of 2 mm in diameter (f). (g–j, patient #23) A diffusion-weighed brain MR imaging (g, h) shows a high intensity area in the left middle cerebral artery region. A digital subtraction angiography shows stenotic lesions (arrowheads) of the right (i) and left (j) inner carotid arteries

(*COL1A1*: p.Gly257Arg, p.Gly260Asp, p.Gly788Ser, c.2829 + 1G > A, *COL1A2*: c.693 + 1G > A, and p.Gly772Ser), three in both OI and C1ROD (*COL1A1*: p.Arg220*, p.Arg415*, and *COL1A2*: c.432 +– 4 432 + 7del), and one in cEDS (*COL1A1*: p.Arg312Cys). Pathogenic or likely pathogenic variants of patients with C1ROD included a missense (p.Gly191Cys) (patient #23) and a nonsense (p.Arg220*) (patient #22) variants in *COL1A1* and a small deletion variant (c.432 +– 4 432 + 7del) in *COL1A2* (patients #18, #19, #20), all of which were located within the first 85 N-terminal residues of the type I collagen helical domain. This region functions as the N-terminal anchor for the stabilization and adequate folding of the collagen triple helix. Pathogenic variants in this lesion give rise to the conformational change of the procollagen N-proteinase cleavage site and inhibition of normal N-propeptide processing, leading to the decreased collagen fibril diameter and strength, ultimately resulting in the development of both OI and EDS overlap phenotypes (Cabral et al., 2005; Makareeva et al., 2006; Malfait et al., 2013). According to the current study and previous reports, most of the variants located in the N-terminal propeptide or near the N-terminal helical region (upstream of exon 14) were glycine substitution missense or exon skipping variants except for a nonsense (p.Arg220*) and arginine substitution missense variants. On the other hand, variants downstream of exon 14 included glycine and nonglycine substitution missense, nonsense, frameshift, and in-frame deletion variants (Budsamongkol et al., 2019; Foi et al., 2021; Gnoli et al., 2021; Morlino et al., 2020). In view of these findings, a genotype–phenotype correlation underlying C1ROD seemed weaker than that in *COL1*-related EDS. Multiple intralocus, extralocus, or epigenetic factors as well as nongenetic modifiers might contribute to the prominent EDS phenotype as the key features of C1ROD.

In the current study, four patients in the OI group and two in the EDS group suffered significant vascular complications (vascular

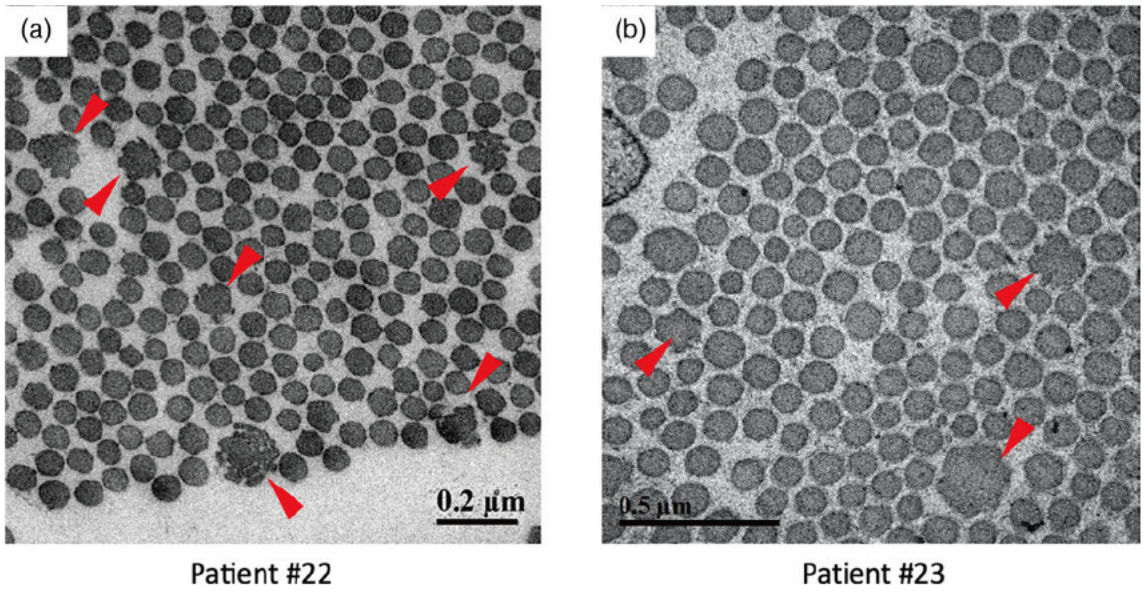

FIGURE 3 Transmission electron microscopic findings of skin specimens from patient #22 (a) and #23 (b). Occasional abnormal cauliflower-like collagen fibrils with irregular margins (arrowheads) are noted in both images

dissection, arterial aneurysm, and subarachnoidal hemorrhage). Especially three patients with OI (patients #1, #2, and #3) developed SAH, and one (patient #1) developed aortic dissection leading to death. Vascular complications have been described as a rare but serious life-threatening event in *COL1*-related OI (Balasubramanian et al., 2019; Gaberel et al., 2016) and C1ROD (Feshchenko et al., 1998; Malfait et al., 2013; Mayer et al., 1996). Type I collagen, highly expressed in the myocardium, heart valves, chorda tendinea, and arterial walls, plays key role in maintaining the structural integrity and tensile strength of arterial walls (Folkestad et al., 2016; Vouyouka et al., 2001). Mimata et al. (1997) reported that type I and type III collagens are diffusely and homogeneously distributed in the luminal and abluminal layers in the cerebral aneurysmal wall. Etminan et al. (2014) revealed that structural remodeling of type I collagen is accelerated in a cerebral aneurysm, which contributes to the formation and progression of cerebral aneurysm. Moreover, McNeeley et al. (2012) revealed the histological change of cystic medial degeneration in the dissected aortic wall tissue of a patient with OI. Therefore, both *COL1*-related OI and C1ROD, caused by defects in type I collagen biosynthesis, are likely to develop serious vascular complications. It remains challenging to explain the underlying pathology of the differences between the phenotypic severity and the prevalence of life-threatening vascular complications; however, two independent contributing factors have been reported to affect the formation of cerebral aneurysms. Yoneyama et al. (2004) described that *COL1A2* rs42524 single-nucleotide polymorphism (SNP) in the triple-helical domain was strongly related to the occurrence of cerebral aneurysms in a Japanese cohort; however, the SNP was not found in the current cohort. Moreover, Perrone et al. (2015) suggested autosomal dominant polycystic kidney disease (ADPKD) to be associated with an increased risk of cerebral aneurysm and aneurysmal SAH. The formation of cerebral aneurysms in patient #1 may have been derived and accelerated not only by a vascular fragility associated with the defect of type I collagen but also by ADPKD. An unruptured middle cerebral artery aneurysm detected in patient #7 could have been coincidental. In the general adult population cohort, the prevalence of unruptured intracranial aneurysms was 0.5%–3%, with a male-to-female ratio of 1:3 (Brown & Broderick, 2014). In contrast, in the current cohort, four of 13 adult patients (31%) were complicated by intracranial aneurysms. It is difficult to prove whether aneurysms developed due to the presence of the *COL1A1* frameshift variant; however, given the high prevalence of the intracranial aneurysm in the current small cohort, we consider the occurrence of vascular events to be related to the pathogenic variants of *COL1A1* or *COL1A2*.

There is no consensus regarding the use of beta-blockers for the treatment or prevention of vascular complications in patients with *COL1*-related OI or C1ROD. However, the efficacy of beta-blockers has been recognized in the management of vascular lesions in patients with other HCTDs including Marfan syndrome, Loeys-Diez syndrome, and vEDS (Baderkhan et al., 2021; MacCarrick et al., 2014; Shores et al., 1994). Beta-blockers prevent hypertension and pulsatile aortic wall stress (Goldfinger et al., 2014), which could contribute to reducing the progression of arterial aneurysm and dissection in patients with HCTDs. Therefore, beta-blockers might be a reasonable therapeutic or prophylactic option for vascular complications in patients with *COL1*-related OI or C1ROD.

Several limitations exist for this study. First, the number of patients, especially in the EDS group, was small. Statistical comparison between the OI and EDS groups was considered difficult. However, the tendency of clinical symptoms could be recognized, such as high frequencies of joint hypermobility and skin

hyperextensibility and a considerable frequency of vascular complications. Second, the collection of clinical symptoms and events could be incomplete, especially regarding possible age-dependent events in younger patients. Children (patients #6, #11, #17, #19, and #20) of affected parents with recurrent bone fractures could experience fractures during adulthood. Still, other factors (e.g., optimization of life-styles, pharmacological intervention) could have some effects on the occurrence of such events. In addition, patient #21, aged 18 years, with a recurrent variant "p.Arg312Cys" for cEDS susceptible to severe vascular involvement, could develop vascular complications in his adulthood. Further clinical and molecular investigations, including larger patient numbers from variable recruitment like the current study as well as longitudinal data collection, would be required to clarify the comprehensive picture of *COL1*-related disorders.

In conclusion, the current cohort included additional patients with C1ROD, and various clinical and molecular overlaps between OI and C1ROD as well as intra-familial phenotypic variabilities were present. Notably, life-threatening vascular complications (vascular dissections, arterial aneurysms, and subarachnoidal hemorrhages) occurred in seven patients (41% of those aged >20 years), independently from the background HCTD-related phenotypes. Careful lifelong surveillance and intervention could be required.

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee at Shinshu University School of Medicine (Matsumoto, Japan) (#628, #4171).

INFORMED CONSENT STATEMENT

Written informed consent was obtained from all patients or their guardians.

AUTHOR CONTRIBUTIONS

Ryojun Takeda and Tomoki Kosho designed the study. Tomomi Yamaguchi performed molecular investigation and interpreted the data with Tomoki Kosho. Shujiro Hayashi, Shinichirou Sano, Hiroshi Kawame, Sachiko Kanki, Hidekane Yoshimura and Yukio Nakamura collected clinical data. Ryojun Takeda combined clinical and molecular data and wrote the draft of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

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

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REFERENCES

Adham, S., Dupuis-Girod, S., Charpentier, E., Mazzella, J. M., Jeunemaitre, X., & Legrand, A. (2020). Classical Ehlers-Danlos syndrome with a propensity to arterial events: A report on a French family with a COL1A1 p.(Arg312Cys) variant. *Clinical Genetics*, *97*, 357 361. https://doi.org/10.1111/cge.13643

Baderkhan, H., Wanhainen, A., Stenborg, A., Stattin, E. L., & Björck, M. (2021). Celiprolol treatment in patient with vascular Ehlers-Danlos syndrome. *European Journal of Vascular and Endovascular Surgery*, *61*, 326 331. https://doi.org/10.1016/j.ejvs.2020.10.020

Balasubramanian, M., Verschueren, A., Kleevens, S., Luyckx, I., Perik, M., Schirwani, S., Mortier, G., Morisaki, H., Rodrigus, I., Laer, L. V., Verstraeten, A., & Loeys, B. (2019). Aortic aneurysm/dissection and osteogenesis imperfecta: Four new families and review of the literature. *Bone*, *121*, 191 195. doi:10.1016/j.bone.2019.01.022

Brady, A. F., Demirdas, S., Fournel-Gigleux, S., Ghali, N., Giunta, C., Kapferer-Seebacher, I., Kosho, T., Mendoza-Londono, R., Pope, M. F., Rohrbach, M., Van Damme, T., Vandersteen, A., van Mourik, C., Voermans, N., Zschocke, J., & Malfait, F. (2017). The Ehlers-Danlos syndromes, rare types. *American Journal of Medical Genetics part C Seminars in Medical Genetics*, *175*, 70 115. https://doi.org/10.1002/ajmg.c.31550

Brown, R. D., & Broderick, J. P. (2014). Unruptured intracranial aneurysms: Epidemiology, natural history, management option, and familial screening. *Lancet Neurology*, *13*, 393 404. https://doi.org/10.1016/S1474-4422(14)70015-8

Budsamongkol, T., Intarak, N., Theerapanon, T., Yodsanga, S., Porntaveetus, T., & Shotelersuk, V. (2019). A novel mutation in COL1A2 leads to osteogenesis imperfecta/Ehlers-Danlos overlap syndrome with brachydactyly. *Genes & Diseases*, *6*, 138 146. https://doi.org/10.1016/j.gendis.2019.03.001

Cabral, W. A., Makareeva, E., Colige, A., Letocha, A. D., Ty, J. M., Yeowell, H. N., Pals, G., Leikin, S., & Marini, J. C. (2005). Mutations near amino end of alpha1(I) collagen cause combined osteogenesis imperfecta/Ehlers-Danlos syndrome by interference with N-propeptide processing. *Journal of Biological Chemistry*, *280*, 19259 19269. https://doi.org/10.1074/jbc.M414698200

Cabral, W. A., Makareeva, E., Letocha, A. D., Scribanu, N., Fertala, A., Steplewski, A., Keene, D. R., Persikow, A. V., Leikin, S., & Marini, J. C. (2007). Y-position cysteine substitution in type I collagen (alpha1 (I) R888C/p.R1066C) is associated with osteogenesis imperfecta/Ehlers-Danlos syndrome phenotype. *Human Mutation*, *28*, 396 405. https://doi.org/10.1012/humu.20456

Cingolani, P., Platts, A., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly*, *6*, 80 92. https://doi.org/10.4161/fly.19695

Etminan, N., Dreier, R., Bucchholz, B. A., Beseoglu, K., Bruckner, P., Matzenauer, C., Torner, J. C., Brown, R. D., Steiger, H. J., Hänggi, D., & Macdonald, R. L. (2014). Age of collagen in intracranial saccular aneurysms. *Stroke*, *45*, 1757 1763. https://doi.org/10.1161/STROKEAHA.114.005461

Feshchenko, S., Brinckmann, J., Lehmann, H. W., Koch, H. G., Muller, P. K., & Kugler, S. (1998). Identification of a new heterozygous point mutation in the COL1A2 gene leading to skipping of exon 9 in a patient with joint laxity, hyperextensibility of skin and blue sclera. Mutations in brief no.166. Online. *Human Mutation*, *12*, 138. https://doi.org/10.1002/(SICI)1098-1004(1998)12:2<138:AID-HUMU17>3.0.CO;2-D

Foi, M., De Mazancourt, P., Metay, C., Carlier, R., Allamand, V., Gartioux, C., Gillas, F., Miri, N., Jobic, V., Mekki, A., Richard, P., Michot, C., & Benistan, K. (2021). A novel COL1A1 variant in a family with clinical features of hypermobile Ehlers-Danlos syndrome that proved to be a COL1-related overlap disorder. *Clinical Case Reports*, *9*, e04128. doi:10.1002/ccr3.4128

Folkestad, L., Hald, J. D., Gram, J., Langdahl, B. L., Hermann, A. P., Diederichsen, A. C., Abrahamsen, B., & Brixen, K. (2016). Cardiovascular disease in patients with osteogenesis imperfecta A nationwide, register-based cohort study. *International Journal of Cardiology*, *225*, 250 257. https://doi.org/10.1016/j.ijcard.2016.09.107

Forlino, A., & Marini, J. C. (2016). Osteogenesis imperfecta. *The Lancet*, *387*, 1657 1671. https://doi.org/10.1016/S0140-6736(15)00728-X

Gaberel, T., Rochey, A., di Palma, C., Lucas, F., Touze, E., & Emery, E. (2016). Ruptured intracranial aneurysm in patients with osteogenesis imperfecta: 2 familial cases and a systematic review of the literature. *Neurochirurgine*, *62*, 317 320. https://doi.org/10.1016/j.neuchi.2016.07.004

Gensure, R. C., Mäkitie, O., Barclay, C., Chan, C., Depalma, S. R., Bastepe, M., Abuzahra, H., Couper, R., Mundlos, S., Sillence, D., Ala kokko, L., Seidman, J. G., Cole, W. G., & Jüppner, H. (2005). A novel COL1A1 mutation in infantile cortical hyperostosis (Caffey disease) expands the spectrum of collagen-related disorders. *Journal of Clinical Investigation*, *115*, 1250 1257. https://doi.org/10.1172/JCI22760

Gnoli, M., Brizola, E., Tremosini, M., Pedrini, E., Maioli, M., Mosca, M., Bassotti, A., Castronovo, P., Giunta, C., & Sangiorgi, L. (2021). COL1-related disorders: Case report and review of overlapping syndromes. *Frontiers in Genetics*, *12*(640), 558. https://doi.org/10.3389/fgene.2021.640558

Goldfinger, J. Z., Halperin, J. L., Marin, M. L., Stewart, A. S., Eagle, K. A., & Fuster, V. (2014). Thoracic aortic aneurysm and dissection. *Journal of the American College of Cardiology*, *64*, 1725 1739. https://doi.org/10.1016/j.jacc.2014.08.025

Grant, S. F., Reid, D. M., Blake, G., Herd, R., Fogelman, I., & Ralston, S. H. (1996). Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nature Genetics.*, *14*, 203 205. https://doi.org/10.1038/ng1096-203

Körkkö, J., Ala-Kokko, L., De Paepe, A., Nuytinck, L., Earley, J., & Prockop, D. J. (1998). Analysis of the COL1A1 and COL1A2 genes by PCR amplification and scanning by conformation-sensitive gel electrophoresis identifies only COL1A1 mutations in 15 patients with osteogenesis imperfecta type I: Identification of common sequences of null-allele mutations. *American Journal of Human Genetics*, *62*, 98 110. https://doi.org/10.1086/301689

Lim, J., Grafe, I., Alexander, S., & Lee, B. (2017). Genetic causes and mechanisms of Osteogenesis Imperfecta. *Bone*, *102*, 40 49. https://doi.org/10.1016/j.bone.2017.02.004

MacCarrick, G., Blank, J. H., 3rd., Bowdin, S., El-Hamamsy, I., Frischmeyer-Guerrerio, P. A., Guerrerio, A. L., Sponseller, P. D., Loeys, B., & Dietz, H. C., 3rd. (2014). Loeys-Dietz syndrome: A primer for diagnosis and management. *Genetics in Medicine*, *16*, 576 587. https://doi.org/10.1038/gim.2014.11

Makareeva, E., Cabral, W. A., Marini, J. C., & Leikin, S. (2006). Molecular mechanism of alpha 1(I)-osteogenesis imperfecta/Ehlers-Danlos syndrome: Unfolding of an N-anchor domain at the N-terminal end of the type I collagen triple helix. *Journal of Biological Chemistry*, *281*, 6463 6470. https://doi.org/10.1074/jbc.M511830200

Malfait, F., Francomano, C., Byers, P., Belmont, J., Berglund, B., Black, J., Bloom, L., Bowen, J. M., Brady, A. F., Burrows, N. P., Castori, M., Cohen, H., Colombi, M., Demirdas, S., De Backer, J., De Paepe, A., Fournel-Gigleux, S., Frank, M., Ghali, N., … Tinkle, B. (2017). The 2017 international classification of the Ehlers-Danlos syndromes. *American Journal of Medical Genetics part C Seminars in Medical Genetics*, *175*, 8 26. https://doi.org/10.1002/ajmg.c.31552

Malfait, F., Symoens, S., Goemans, N., Gyftodimou, Y., Holmberg, E., Lopez-Gonzalez, V., Mortier, G., Nampoothiri, S., & De Paepe, A. (2013). Helical mutations in type I collagen that affect the processing of the amno-propeptide result in an Osteogenesis Imperfecta/Ehlers-Danlos syndrome overlap syndrome. *Orphanet Journal of Rare Disease*, *8*, 78. https://doi.org/10.1186/1750-1172-8-78

Marini, J. C., Forlino, A., Bächinger, H. P., Bishop, N. J., Byers, P. H., Paepe, A., Fassier, F., Fratzl-Zelman, N., Kozloff, K. M., Krakow, D., Montpetit, K., & Semler, O. (2017). Osteogenesis Imperfecta. *Nature Reviews Disease Primers*, *3*(17), 052. https://doi.org/10.1038/nrdp.2017.52

Marini, J. C., Forlino, A., Cabral, W. A., Barnes, A. M., San Antonio, J. D., Milgrom, S., Hyland, J. C., Körkkö, J., Prockop, D. J., de Paepe, A., Coucke, P., Symoens, S., Glorieux, F. H., Roughley, P. J., Lund, A. M., Kuurila-Svahn, K., Hartikka, H., Cohn, D. H., Krakow, D., Mottes, M., Schwarze, U., Chen, D., Yang, K., Kuslich, C., Troendle, J., Dalgleish, R., & Byers, P. H. (2007). Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: Regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. *Human Mutation*, *28*, 209 221. https://doi.org/10.1002/humu.20429.

Mayer, S. A., Rubin, B. S., Starman, B. J., & Byers, P. H. (1996). Spontaneous multivessel cervical artery dissection in a patient with a substitution of alanine for glycine (G13A) in the alpha 1(I) chain of type I collagen. *Neurology*, *47*, 552 556. https://doi.org/10.1212/wnl.47.2.552

Mcneeley, M. F., Dontchos, B. N., Laflamme, M. A., Hubka, M., & Sadro, C. T. (2012). Aortic dissection in osteogenesis imperfecta: Case report and review of the literature. *Emergency Radiology*, *19*, 553 556. doi:10.1007/s10140-012-1044-1

Mimata, C., Kitaoka, M., Nagahiro, S., Iyama, K., Hori, H., Yoshioka, H., & Ushio, Y. (1997). Differential distribution and expressions of collagens in the cerebral aneurysmal wall. *Acta Neuropathologica*, *94*, 197 206. doi:10.1007/s004010050694

Morlino, S., Micale, L., Ritelli, M., Rohrbach, M., Zoppi, N., Vandersteen, A., Mackay, S., Agolini, E., Cocciadiferro, D., Sasaki, E., Madeo, A., Ferraris, A., Reardon, W., Di Rocco, M., Novelli, A., Grammatico, P.,

Marfait, F., Mazza, T., Hakim, A., … Castori, M. (2020). COL1-related overlap disorder: A novel connective tissue disorder incorporating the osteogenesis imperfecta/Ehlers-Danlos syndrome overlap. *Clinical Genetics*, *97*, 396 406. https://doi.org/10.1111/cge.13683

Perrone, R. D., Malek, A. M., & Watnick, T. (2015). Vascular complications in autosomal dominant polycystic kidney disease. *Nature Reviews Nephrology*, *11*, 589 598. https://doi.org/10.1038/nrneph.2015.128

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine: official journal of the American College of Medical Genetics*, *17*, 405 424. https://doi.org/10.1038/gim.2015.30

Shores, J., Berger, K. R., Murphy, E. A., & Pyeritz, R. E. (1994). Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. *New England Journal of Medicine*, *330*, 1335 1341. https://doi.org/10.1056/NEJM199405123301902

Vouyouka, A. G., Pfeiffer, B. J., Liem, T. K., Taylor, T. K., Mudaliar, J., & Phillips, C. L. (2001). The role of type I collagen in aortic wall strength with a homotrimeric [α1(I)]3 collagen mouse model. *Journal of Vascular Surgery*, *33*, 1263 1270. https://doi.org/10.1067/mva.2001.113579

Willing, M. C., Deschenes, S. P., Slayton, R. L., & Roberts, E. J. (1996). Premature chain termination is a unifying mechanism for COL1A1 null alleles in osteogenesis imperfecta type I cell strains. *American Journal of Human Genetics*, *59*, 799 809.

Yoneyama, T., Kasuya, H., Onda, H., Akagawa, H., Hashiguchi, K., Nakajima, T., & Inoue, I. (2004). Collagen type 1 alpha2 (COL1A2) is the susceptible gene for intracranial aneurysms. *Stroke*, *35*, 443 448. https://doi.org/10.1161/01.STR.0000110788.45858.DC

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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