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 arthrocchlasia 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...
group primarily showed joint hypermobility and skin hyperextensibility, though various clinical and molecular overlaps between OI, COL1-related EDS, and C1ROD as well as intransal familial 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

## 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 intestines; 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 procollogen 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 (Cabrál 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 procollogen 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 varus 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 sclerae) 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
Clinical and molecular findings of 23 patients in this study

| Family | 1 | 2 | 3 | #4 | 4 | 5 | #6 | 6 |
|--------|---|---|---|----|---|---|----|---|
| Patient | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | #9 |
| Clinical suspicion | OI | OI | OI | OI | OI | OI | OI | OI | OI |
| Final diagnosis | OI type I | OI type I | OI type I | OI type I | OI | OI type I | OI type I | OI type I | OI type I |

### General

|  | Sex | Age (years) | Height (cm/SD) | Outcome |
|---|-----|-------------|----------------|---------|
| 1 | F | 67 | 143/−0.6 | Deceased |
| 2 | F | 41 | 160.8/0.4 | Alive |
| 3 | M | 55 | 154.5/−0.5 | Alive |
| #4 | F | 26 | 173.5/0.4 | Alive |
| #5 | F | 47 | 138.6/−3.7 | Alive |
| #6 | F | 6 | 111.4/−0.6 | Alive |
| #7 | F | 45 | 157.6/−0.3 | Alive |
| #8 | F | 18 | 157.5/0.0 | Alive |
| #9 | M | 27 | 152.8/−3.2 | Alive |

### Skeletal

|  | Multiple bone fractures | Frequency of fractures | Position of fractures | Dental abnormality | Joint hypermobility | Beighton score | Recurrent joint dislocation | Congenital hip dislocation | Joint contracture | Long bone deformity | Spinal deformity | Congenital clubfoot | Flat feet | Ruptures of tendon, ligament, or muscle | Intramuscular bleeding | Joint pain | DXA(vertebral: g/cm²) | T-score | Z-score |
|---|-------------------------|------------------------|-----------------------|-------------------|-------------------|--------------|---------------------------|--------------------------|----------------|----------------|----------------|----------------|----------|-----------------------------|----------------|-----------|-----------------|--------|--------|
| 1 | +                       | 8                      | LB, PT, FM, PB, FB   | −                 | NA                | 6            | +                         | −                        | −              | −              | +              | −              | NA       | −                           | NA            | NA        | −               | −      | −      |
| 2 | +                       | 10                     | PH, EL, PF           | −                 | +                 | 2            | −                         | −                        | −              | −              | −              | −              | +        | −                           | +             | +         | +               | +      | +      |
| 3 | +                       | 7                      | FM, TB, SC, EL, PF  | +                 | +                 | 6            | +                         | −                        | −              | −              | −              | −              | −        | −                           | −             | NA        | −               | −      | −      |
| #4 | +                       | 6                       | AK, EL, WR           | −                 | −                 | 2            | +                         | −                        | −              | −              | −              | +              | −        | −                           | −             | −         | −               | −      | −      |
| #5 | +                       | 6                       | FM, PH, PF, EL, PT  | −                 | −                 | 4            | +                         | −                        | −              | −              | −              | +              | −        | −                           | −             | NA        | −               | −      | −      |
| #6 | +                       | 2                       | SC, PF               | −                 | +                 | 2            | +                         | −                        | +              | +              | +              | +              | +        | −                           | +             | −         | −               | −      | −      |
| #7 | +                       | 6                       | SK, HM, EL, PH, KN  | +                 | −                 | 6            | +                         | −                        | −              | −              | −              | −              | −        | −                           | −             | −         | −               | −      | −      |
| #8 | +                       | 5                       | CX, HM, PT           | −                 | −                 | 6            | +                         | −                        | −              | −              | −              | −              | −        | −                           | −             | −         | −               | −      | −      |

(Continues)
| Family | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | #9 |
|--------|----|----|----|----|----|----|----|----|----|
| Skin   | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Hyperextensibility | - | - | - | - | - | - | - | - | - |
| Fragility | - | - | - | - | - | - | - | - | - |
| Atrophic scars | - | - | - | - | - | - | - | - | - |
| Translucency | - | - | - | - | - | - | - | - | - |
| Soft, doughy skin | - | - | - | - | - | - | - | - | - |
| Hypertrophic papules | - | - | - | - | - | - | - | - | - |
| Easy bruising | - | - | - | - | - | - | - | - | - |
| Eye and ear | - | - | - | - | - | - | - | - | - |
| Blue sclerae | - | - | - | - | - | - | - | - | - |
| Retinal detachment | - | - | - | - | - | - | - | - | - |
| Retractive errors | - | - | - | - | - | - | - | - | - |
| Hearing impairment | - | - | - | - | - | - | - | - | - |
| Cardiovascular | - | - | - | - | - | - | - | - | - |
| Ascending aorta | - | - | - | - | - | - | - | - | - |
| Aortic dissection | - | - | - | - | - | - | - | - | - |
| Cardiac valve disorder | - | - | - | - | - | - | - | - | - |
| Renal disease | - | - | - | - | - | - | - | - | - |
| Hypertension | - | - | - | - | - | - | - | - | - |
| Others | - | - | - | - | - | - | - | - | - |
| Variant | - | - | - | - | - | - | - | - | - |
| COL1A1(PV: OI) | c.779G > A | p.Gly260Asp | Exon 11 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.572G > T | p.Gly191Val | Exon 7 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.815A > G | p.Glu272Asp | Exon 19 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1116G > A | p.Glu372Lys | Exon 25 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1422C > T | p.Arg474* | Exon 25 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1679del | p.Glu560Valfs*20 | Exon 25 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.2829+1G > A | p.Glu943Lys | Exon 25 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1243C > T | p.Arg415* | Exon 25 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.769G > A | p.Glu257Arg | Exon 19 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1579delIle | p.Glu527Valfs*20 | Exon 19 | - | - | - | - | - | - |
| COL1A1(PV: OI, C1ROD) | c.1679del | p.Glu560Valfs*20 | Exon 25 | - | - | - | - | - | - |

| Family | #10 | #11 | #12 | #13 | #14 | #15 | #16 | #17 |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| Clinical suspicion | Mother | Daughter | Elder brother | Younger brother | Father | Daughter | Father | Daughter |
| Final diagnosis | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I |

| Family | #10 | #11 | #12 | #13 | #14 | #15 | #16 | #17 |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| Clinical suspicion | Mother | Daughter | Elder brother | Younger brother | Father | Daughter | Father | Daughter |
| Final diagnosis | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I | OI type I |
| Family | 7 | 8 | 9 | 10 | 11 |
|--------|---|---|---|----|----|
| Patient | #10 | #11 | #12 | #13 | #14 | #15 | #16 | #17 |
|        | Mother | Daughter | 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 | Alive | Alive | Alive | Alive | Alive | Alive | Alive | Alive |
| Skeletal | | | | | | | | |
| Multiple bone fractures | + | – | + | + | + | + | + | – |
| Frequency of fractures | 5 | – | 12 | 5 | 2 | 5 | – | – |
| Position of fractures | RD, UL, FB | – | FM | TB, FB, EL, RD | TB, RD, UL, CB, PF | TB, HM | FM, PF | – |
| Dental abnormality | + | – | + | – | – | – | + | + |
| Joint hypermobility | – | – | + | + | – | – | – | – |
| Beighton score | 7 | 7 | – | – | 4 | 4 | – | – |
| Recurrent joint dislocation | – | – | – | – | – | – | – | – |
| Congenital hip dislocation | – | – | – | – | – | – | – | – |
| Joint contracture | – | – | + | – | – | – | – | – |
| Long bone deformity | – | + | + | – | – | – | – | – |
| Spinal deformity | – | – | + | – | – | – | – | + |
| Congenital clubfoot | – | – | – | – | – | – | – | – |
| Flat feet | – | – | + | – | – | – | – | – |
| Ruptures of tendon, ligament, or muscle | – | – | – | – | – | – | – | – |
| Intramuscular bleeding | – | – | – | – | – | – | – | – |
| Joint pain | – | – | NA | – | – | – | – | – |
| DXA(vertebral: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 | | | | | | | | |
| Hyperextensibility | – | – | – | + | – | – | – | – |
| Fragility | – | – | – | – | – | – | – | – |
| Atrophic scars | – | – | – | – | – | – | – | – |
| Translucency | – | – | – | – | – | – | – | – |
| Soft doughy skin | – | – | – | + | – | – | – | – |
| Piezogenic papules | – | – | NA | NA | – | – | + | – |
| Easy bruising | + | – | – | – | – | – | – | – |

(Continues)
| Family | 7   | 8   | 9   | 10  | 11  |
|--------|-----|-----|-----|-----|-----|
| Patient | #10 | #11 | #12 | #13 | #14 | #15 | #16 | #17 |
|        | Mother | Daughter | Elder brother | Younger brother | Father | Daughter |
| Eye and ear |     |       |       |       |       |       |     |     |
| Blue sclerae | + | + | + | + | + | + | + | + |
| Retinal detachment | – | – | NA | – | – | – | – | – |
| Refractive errors | – | – | Myopia | – | – | – | Myopia | NA |
| Hearing impairment | – | – | – | – | – | – | NA | – |
| Cardiovascular |     |       |       |       |       |       |     |     |
| Vascular dissection | – | – | – | NA | – | – | – | NA |
| Arterial aneurysm | – | – | – | NA | – | – | – | NA |
| Cardiac valve disorder | – | – | – | NA | – | – | – | NA |
| Renal disease | – | – | NA | NA | – | – | Rt. renal cyst | NA |
| Hypertension | – | – | – | – | – | – | NA | – |
| Others |     |       |       |       |       |       |     |     |
| Variant |     |       |       |       |       |       |     |     |
| COL1A1(UV) | c.559del | COL1A2(UV) | c.1963G > C | COL1A2(PV: OI) | c.693 + 1G > A | COL1A2(PV: OI) | c.2314G > A | COL1A1(UV) | c.2362G > A |
| COL1A2(UV) | p.Arg187Valfs'78 | p.Gly655Arg | Exon 7 | Exon 32 | Intron 14 | Exon 38 | p.Gly772Ser | Exon 34 |
| COL1A2(PV: OI) | | | | | | | p.Gly788Ser | |
| COL1A1(UV) | | | | | | | | |
| Family | 12  | 13  | 14  | 15  |
| Patient | #18 | #19 | #20 | #23 |
|        | Father | Daughter | Son | #21 | #22 | #23 |
| Clinical suspicion | OI | aEDS | aEDS | cEDS | vEDS | vEDS or OI |
| Final 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 | Alive | Alive | Alive | Alive | Alive | Alive |
### TABLE 1 (Continued)

| Family | 12 | 13 | 14 | 15 |
|--------|----|----|----|----|
|        | #18 | #19 | #20 |    | #21 | #22 | #23 |
| Patient | Father | Daughter | Son |    |    |    |    |

**Skeletal**
- Multiple bone fractures
  - #18: +
  - #19: -
  - #20: -
  - #21: -
  - #22: +
  - #23: -
- Frequency of fractures
  - #18: 6
  - #19: -
  - #20: -
  - #21: 2
  - #22: 1
- Position of fractures
  - #18: NA
  - #19: -
  - #20: -
  - #21: EL
  - #22: TB
- Dental abnormality
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
- Joint hypermobility
  - #18: +
  - #19: +
  - #20: +
  - #21: +
  - #22: +
- Beighton score
  - #18: 9
  - #19: 9
  - #20: 8
  - #21: 6
  - #22: NA
  - #23: NA
- Recurrent joint dislocation
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
- Congenital hip dislocation
  - #18: -
  - #19: +
  - #20: -
  - #21: -
  - #22: -
  - #23: -
- Joint contracture
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
- Long bone deformity
  - #18: -
  - #19: -
  - #20: -
  - #21: +
  - #22: NA
- Spinal deformity
  - #18: -
  - #19: +
  - #20: NA
  - #21: -
  - #22: -
- Congenital clubfoot
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
  - #23: +
- Flat feet
  - #18: +
  - #19: +
  - #20: +
  - #21: -
  - #22: -
  - #23: -
- Ruptures of tendon, ligament, or muscle
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
  - #23: -
- Intramuscular bleeding
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
  - #23: -
- Joint pain
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: -
  - #23: -
- DXA (R: V: g/cm2)
  - #18: 1.048
  - #19: NA
  - #20: NA
  - #21: NA
  - #22: 0.787
  - #23: 0.623
- T-score
  - #18: -0.5
  - #19: NA
  - #20: NA
  - #21: -0.8
  - #22: -3.5
  - #23: -0.8
- Z-score
  - #18: -0.5
  - #19: NA
  - #20: NA

**Skin**
- Hyperextensibility
  - #18: +
  - #19: +
  - #20: +
  - #21: -
  - #22: +
  - #23: -
- Fragility
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: +
  - #23: +
- Atrophic scars
  - #18: -
  - #19: -
  - #20: -
  - #21: +
  - #22: -
  - #23: -
- Translucency
  - #18: -
  - #19: -
  - #20: -
  - #21: -
  - #22: +
  - #23: +
- Soft doughy skin
  - #18: -
  - #19: +
  - #20: +
  - #21: +
  - #22: +
  - #23: +
- Piezogenic papules
  - #18: NA
  - #19: +
  - #20: +
  - #21: -
  - #22: NA
  - #23: NA
- Easy bruising
  - #18: +
  - #19: +
  - #20: +
  - #21: +
  - #22: -
  - #23: +

(Continues)
**TABLE 1** (Continued)

| Family | 12 | 13 | 14 | 15 |
|--------|----|----|----|----|
|        | #18 | #19 | #20 | #21 | #22 | #23 |
| Patient| Father | Daughter | Son | #21 | #22 | #23 |
| Eye and ear | | | | | | |
| Blue sclerae | + | + | + | – | + | + |
| Retinal detachment | – | – | NA | – | – | – |
| Refractive errors | Myopia | Myopia, astigmatism | NA | – | – | – |
| Hearing impairment | NA | – | – | – | NA | |
| Cardiovascular | | | | | | |
| Vascular dissection | – | – | – | – | Bil. ICA | Bil. VA |
| Arterial aneurysm | – | – | – | – | – | AAA |
| Cardiac valve disorder | NA | – | – | – | – | Lt. renal AVM |
| Renal disease | NA | NA | NA | NA | – | – |
| Hypertension | NA | NA | NA | NA | – | – |
| Others | | | | | | |

| Variant | \(\text{COL1A2(PV: OI, C1ROD)}\) | \(\text{COL1A1(PV: cEDS)}\) | \(\text{COL1A1(PV: OI)}\) | \(\text{COL1A1(UV)}\) |
|---------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Col. | c.432 + 4_432 + 7del | c.934C > T | c.658C > T | c.571G > T |
| Intron | 9 | 14 | 9 | 7 |
| Protein change | p.Arg312Cys | p.Arg220* | p.Gly191Cys | |

Abbreviations: +, present; –, absent; AAA, abdominal aortic aneurysm; AK, ankle; AR, aortic valve regurgitation; AVM, arteriovenous malformation; Bil., bilateral; C1ROD, COL1-related overlap disorder; CB, clavicle bone; CX, coxal bone; EL, elbow; F, female; FB, fibula; FM, femur; HM, humerus; ICA, inner carotid artery; KN, knee; LB, lumbar spine; Lt., left; M, male; MCA, middle cerebral artery; MR, mitral valve regurgitation; NA, not available; PB, pubis; PF, phalanx of foot; PH, phalanx of hand; PKD, polycystic kidney disease; PT, patella; PV, previously published variant; RD, radius; Rt., right; SAH, subarachnoidal hemorrhage; SC, scapula; SK, skull; TB, tibia; TR, tricuspid valve regurgitation; UL, ulna; URA, unilateral renal aplasia; UV, unpublished variant; VA, vertebral artery; WR, wrist; YAM, young adult 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.Gly191Cys” 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.
(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 on this lesion give rise to the conformational change of the pro-collagen 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...
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 incidental. 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

**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.
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. Shūjiro Hayashi, Shinichirō 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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SUPPORTING INFORMATION

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

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