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ORIGINAL ARTICLE

Genetic analysis in Japanese patients with osteogenesis imperfecta: Genotype and phenotype spectra in 96 probands

Yousuke Higuchi[1] | **Kosei Hasegawa**[2] | **Natsuko Futagawa**[1,2] | **Miho Yamashita**[3] | **Hiroyuki Tanaka**[4] | **Hirokazu Tsukahara**[1]

[1]Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

[2]Department of Pediatrics, Okayama University Hospital, Okayama, Japan

[3]Faculty of Human Life Sciences, Notre Dame Seishin University, Okayama, Japan

[4]Department of Pediatrics, Okayama Saiseikai General Hospital, Okayama, Japan

Correspondence
Kosei Hasegawa, Department of Pediatrics, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan.
Email: haseyan@md.okayama-u.ac.jp

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Abstract

Background: Osteogenesis imperfecta (OI) is a rare connective-tissue disorder characterized by bone fragility. Approximately 90% of all OI cases are caused by variants in COL1A1 or COL1A2. Additionally, IFITM5 variants are responsible for the unique OI type 5. We previously analyzed COL1A1/2 variants in 22 Japanese families with OI through denaturing high-performance liquid chromatography screening, but our detection rate was low (41%).

Methods: To expand the genotype-phenotype correlations, we performed a genetic analysis of COL1A1/2 and IFITM5 in 96 non-consanguineous Japanese OI probands by Sanger sequencing.

Results: Of these individuals, 54, 41, and 1 had type 1 (mild), type 2–4 (moderate-to-severe), and type 5 phenotypes, respectively. In the mild group, COL1A1 nonsense and splice-site variants were prevalent (n = 30 and 20, respectively), but there were also COL1A1 and COL1A2 triple-helical glycine substitutions (n = 2 and 1, respectively). In the moderate-to-severe group, although COL1A1 and COL1A2 glycine substitutions were common (n = 14 and 18, respectively), other variants were also detected. The single case of type 5 had the characteristic c.-14C>T variant in IFITM5.

Conclusion: These results increase our previous detection rate for COL1A1/2 variants to 99% and provide insight into the genotype-phenotype correlations in OI.

KEYWORDS

COL1A1, *COL1A2*, *IFITM5*, Osteogenesis imperfecta, variant

1 | INTRODUCTION

Osteogenesis imperfecta (OI) is a hereditary connective-tissue disease with an incidence of 1:10,000–20,000 births. It is characterized by various degrees of bone fragility and fractures, blue sclera, dentinogenesis imperfecta, and onset of hearing loss in young adulthood (Marini et al., 2017). The spectrum of clinical severity ranges from mild to severe, and the four classical categories proposed by Sillence et al., (1979) have recently been re-categorized into five (Mortier et al., 2019; Van Dijk & Sillence, 2014). OI type 1 is the mildest form characterized by straight limbs (non-deforming).

Type 2 is perinatally lethal, and is characterized by the presence of intrauterine fractures (perinatally lethal). Type 3 is the most severe form in which patients survive the neonatal period. It presents with multiple fractures and progressive skeletal deformities (progressively deforming). Patients with type 4 OI show a severity intermediate between those of type 1 and 3 patients. They present with varying degrees of bone deformity (moderately deforming). Type 5 is characterized by moderate-to-severe bone fragility. It is unique in the sense that it presents with ossification of the interosseous membrane, radial head dislocation, subphyseal metaphyseal radiodense bands, and hyperplastic callus formation (Glorieux et al., 2000).

Type I collagen is a fundamental component of the extracellular matrices of the bone, skin, tendon, and other connective-tissue types. It consists of two α1 (I) and one α2 (I) polypeptide chains encoded by *COL1A1* and *COL1A2*, respectively. Each chain has triple-helical domains composed of Gly-X-Y tripeptide repeats and is assembled into a heterotrimer. The helical domain is flanked by globular *N*- and *C*-terminal propeptides. The assembly proceeds from the *C*-terminal toward the *N*-terminal. Type I procollagen is secreted from the rough endoplasmic reticulum (ER), and then the *C*- and *N*-terminal propeptides are cleaved off. Most genetic defects affecting bone metabolism cause the recessive forms of OI: (i) posttranslational modification (*CRTAP*, *P3H1*, and *PPIB*), (ii) processing and crosslinking (*SERPINH1*, *FKBP10*, *PLOD2*, and *BMP1*), (iii) osteoblast differentiation and function (*SP7*, *TMEM38B*, *WNT1*, *CREB3L1*, *SPARC*, *SEC24D*, and *MBTPS2*), or (iv) bone mineralization and resorption (*PLS3* and *SERPINF1*) (Marini et al., 2017; Etich et al., 2020). Nevertheless, dominant *COL1A1* and *COL1A2* changes occur in ~90% of OI patients (Marini et al., 2017). Reduced levels of wildtype collagens, as in *COL1A1* haploinsufficiency, cause a mild OI phenotype. In contrast, structural abnormalities develop mainly in the more severe forms of OI. Substitution of glycine especially with serine within the Gly-X-Y tripeptide repeat region is the most common triple-helical assembly disruption in type I procollagen (Lindahl et al., 2015; Marini et al., 2007; Rauch et al., 2010). OI type 5 is caused by the heterozygous variant c.-14C>T in the 5′-UTR of *IFITM5*. This gene encodes interferon-induced transmembrane protein-5, which is involved in early bone mineralization (Cho et al., 2012; Semler et al., 2012). Several studies have reported that this particular variant is the third most common change found in OI patients (Bardai et al., 2016; Liu et al., 2017; Patel et al., 2015).

OI is mainly caused by variants in type I collagen; however, the genetic analysis of OI is laborious and time-consuming because type I collagen genes are large (*COL1A1* and *COL1A2* have 51 and 52 exons within 18 and 38 kb, respectively) and have no apparent variant hotspots. Limited published data are available on the molecular diagnosis of OI in Japan (Kanno et al., 2018; Ohata et al., 2019). We previously reported six *COL1A1* and three *COL1A2* variants in 22 families with OI through Sanger sequencing and heteroduplex screening using denaturing high-performance liquid chromatography (DHPLC) (Kataoka et al., 2007). Nevertheless, the detection rate was low (41%) and changes in *IFITM5* were not analyzed. In the present study, we investigated the genotype and phenotype spectra of a larger group of Japanese patients with OI.

2 | METHODS

2.1 | Editorial policies and ethical considerations

All the procedures in this study were performed in accordance with the 1964 Helsinki Declaration and 2003 Japanese Ethical Guidelines for Clinical Research, as well as their later amendments. This study was approved by the Ethics Committee of Okayama University Hospital (No. 1701-038). Written informed consent for the genetic analyses and publication was obtained from the probands and/or their legal guardians.

2.2 | Subjects

Whole-blood or DNA samples were obtained between March 2010 and January 2020 from non-consanguineous Japanese OI probands in our facility and 41 other nationwide facilities for the genetic analysis of *COL1A1*, *COL1A2*, and *IFITM5*. Patients were clinically diagnosed based on bone fragility, recurrent fractures, and bone deformity with or without family history by their attending physicians, and the diagnoses were afterward confirmed by one of the authors with sufficient experience (KH or HTa). Clinical information, including age, sex, family history, Sillence classification, extraskeletal features (blue sclera, dentinogenesis imperfecta (DI), and hearing loss), and radiographs, was also obtained. Subjects were classified into three age-based categories namely, child (0–12 years), adolescent (13–18 years), and adult (19 years and above). Where available, baseline bone mineral density (BMD) (before bisphosphonate treatment) and Z-score of the lumbar spine evaluated through dual-energy X-ray absorptiometry were also collected. OI types 1–4 were re-classified as “mild OI (OI type 1)” and “moderate-to-severe OI (OI types 2–4)” because OI classification depends on the amount of clinical information available and is somewhat subjective (Bardai et al., 2016). The number and frequency of fractures are likely to be affected by the patient's age, treatment method (i.e., modified by bisphosphonate therapy), and also depends on the patient's or their family's recollection, especially in

elder patients. Categories were determined according to the presence (moderate-to-severe OI) or absence (mild OI) of any deformity in the lower extremities as a simpler severity indicator (Van Dijk & Sillence, 2014). Bone fragility was defined as 1) prenatal bowing, shortening, or fractures of long bones: 2) minor postnatal trauma or unexplained bone fractures.

2.3 | Sanger sequencing

Genomic DNA was extracted from blood samples with a genomic DNA Extraction Kit (Qiagen) by following the manufacturer's instructions. Previously, PCR primers corresponding to all the exons or >30-bp regions flanking the exon-intron junctions of *COL1A1* and *COL1A2* have been used (Kataoka et al., 2007; Körkkö et al., 1998). From these previously tested primers, 16 and 22 primer pairs, spanning multiple exons, were selected for *COL1A1* and *COL1A2*, respectively. Primers for *IFITM5* were designed. The primer sets and PCR conditions are shown in Data S1. Amplicons were analyzed on a 3% agarose gel and purified using a QIAquick Purification Kit (Qiagen). Sequencing was performed in a BigDye Terminator v. 3.1 Cycle Sequencing Kit and the ABI Prism 310 Genetic Analyzer (Applied Biosystems). Raw sequencing data are available upon request. First, *IFITM5* was sequenced in individuals clinically suspected of being OI type 5. *COL1A1* and *COL1A2* were then sequenced (exons 1–51 and 1–52, respectively). Sequence reads were aligned with reference sequences from GenBank (*COL1A1*: NG_007400.1; *COL1A2*: NG_007405.1; and *IFITM5*: NG_032892.1). Nucleotides were numbered according to the cDNA references (*COL1A1*: NM_000088.3; *COL1A2*: NM_000089.3; and *IFITM5*: NM_001025295.3). Amino acid residues were numbered according to the protein references (COL1A1: NP_000079.2; COL1A2: NP_000080.2; and IFITM5: NP_001020466.1). Non-synonymous or splice-site variants were considered putative mutations if they were not listed in the Single Nucleotide Polymorphism (SNP) database or 1,000 Genomes Project. Variant pathogenicity was analyzed with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/index.php), Mutation Taster (http://www.mutationtaster.org/), and Human Splicing Finder (http://www.umd.be/HSF3/HSF.shtml) (Adzhubei et al., 2010; Choi et al., 2012; Schwarz, Rödelsperger, Schuelke, & Seelow, 2010; Desmet et al., 2009). All the variants were classified according to the ACMG variant interpretation guideline (Richards et al., 2015). Allele frequencies of novel missense variants were examined in the genome aggregation database (gnomAD, https://gnomad.broadinstitute.org/). The novelty was defined according to the Osteogenesis Imperfecta Variant Database (http://www.le.ac.uk/ge/collagen/), and any causal variant was searched from *COL1A1* exon 1 and progress through *COL1A2* exon 52. If the variant had previously been reported and corresponded to the phenotypes, the remaining exons were not sequenced. The proband variant data were deposited in the Global Variome shared LOVD database (https://databases.lovd.nl/). A segregation analysis of the proband relatives was optionally conducted.

2.4 | Statistical analysis

Data are presented as median and range. Either, a Pearson's chi-square test or a Fisher's exact test was applied to categorical data and the Mann–Whitney *U* test was used on continuous variables. $p < 0.05$ was considered a significant difference (Wasserstein et al., 2019). All the analyses were run in Stata v. 14.2 (StataCorp).

3 | RESULTS

3.1 | Clinical characteristics

The present study included 96 probands (52 males and 44 females) from 96 unrelated families. Of these, 44 had a family history of OI. The median age at the time of analysis was 4.0 years (range, 0–52 years). Fifty-four (56.3%) were classified as the mild OI group, 41 (42.7%) were in the moderate-to-severe OI group, and1(1.0%) with the ossification of the interosseous membrane and radial head dislocation was categorized as OI type 5. Eighteen in the mild group and 20 in

TABLE 1 Clinical characteristics of the study population

	Mild OI (n = 54)	Moderate-to-severe OI (n = 41)	OI type 5 (n = 1)
Sex (male/female)	32/22	20/21	0/1
Age (years, median (range))	4.0 (0–52)	2.0 (0–50)	26
Child (0–12)	44	27	0
Adolescent (13–18)	2	7	0
Adult (19 and above)	8	7	1
Blue sclera	46/47	20/28	0/1
Dentinogenesis imperfecta	9/29	9/15	0/1
Hearing loss	4/22	0/4	0/1
Family history	31/54	12/41	1/1

OI, osteogenesis imperfecta.

Results represent number of positives/total.

TABLE 2 Summary of the phenotype spectra

Group	Gene	Patient ID	Age	Sex	Bluesclera	DI	Deafness	Lower extremity deformities	Reccurent fractures	Bone fragility	Number of affected family members who had the identical variant	Number of affected family members who were not sequenced
Mild	COL1A1	1	52	F	−	N/A	N/A	−	+	N/A		1
		2	22	M	+	+	+	−	+	+		1
		3	8	F	+	+	N/A	−	+	+		1
		4	1	M	N/A	N/A	N/A	−	+	N/A		
		5	4	F	+	−	N/A	−	+	+	1	
		6	1	F	+	N/A	N/A	−	+	+	1	
		7	28	F	+	N/A	N/A	−	+	N/A		
		8	0	F	+	N/A	N/A	−	+	+		
		9	1	M	+	N/A	N/A	−	−	−		
		10	0	M	+	N/A	N/A	−	+	+		
		11	1	F	+	N/A	N/A	−	−	−		1
		12	28	M	+	+	−	−	N/A	N/A		1
		13	7	M	+	−	−	−	N/A	N/A	1	
		14	11	M	+	−	+	−	+	+		
		15	38	F	+	+	+	−	+	+		
		16	0	F	+	−	−	−	−	−	2	
		17	7	F	+	−	−	−	+	+		
		18	0	F	N/A	N/A	N/A	−	N/A	N/A	1	
		19	8	M	+	N/A	N/A	−	+	+		
		20	0	M	+	−	N/A	−	−	+		
		21	0	M	+	N/A	N/A	−	−	−	1	
		22	12	M	+	−	−	−	+	+		
		23	4	F	N/A	N/A	N/A	−	+	+		
		24	11	F	+	N/A	−	−	+	+	1	
		25	0	M	+	N/A	N/A	−	−	+	1	
		26	3	M	+	+	N/A	−	−	+	1	
		27	40	F	N/A	N/A	N/A	−	N/A	N/A		
		28	10	M	+	+	N/A	−	+	+	1	
		29	0	M	+	N/A	N/A	−	−	+		1

(Continues)

TABLE 2 Continued

Group	Gene	Patient ID	Age	Sex	Bluesclera	DI	Deafness	Lower extremity deformities	Reccurent fractures	Bone fragility	Number of affected family members who had the identical variant	Number of affected family members who were not sequenced
		30	18	M	+	−	−	−	+	N/A	1	
		31	4	M	+	N/A	N/A	−	−	−	2	
		32	0	F	N/A	N/A	N/A	−	−	−		1
		33	28	M	+	−	−	−	+	+	2	
		34	3	M	+	−	N/A	−	+	+	1	
		35	24	F	+	−	+	−	N/A	N/A		
		36	2	M	+	−	−	−	+	+	4	
		37	11	M	+	−	−	−	+	+		
		38	4	M	+	N/A	N/A	−	+	+		
		39	9	M	+	−	−	−	N/A	N/A		
		40	2	M	+	+	N/A	−	+	+	2	
		41	12	M	+	−	N/A	−	+	N/A	1	
		42	0	F	+	N/A	N/A	−	−	+	1	
		43	1	F	N/A	N/A	N/A	−	N/A	N/A	1	
		44	3	F	+	+	−	−	+	+	2	1
		45	11	M	+	+	−	−	+	+		
		46	1	F	+	−	−	−	+	+		1
		47	0	M	+	N/A	N/A	−	+	+		1
		48	3	M	+	−	−	−	+	−		
		49	12	M	N/A	N/A	N/A	−	+	+		
		50	10	M	+	−	−	−	N/A	N/A		
		51	5	F	+	−	N/A	−	+	+		
		52	14	M	+	N/A	N/A	−	+	+		
	COL1A2	53	0	F	+	N/A	−	−	+	+	2	
		54	8	M	+	−	−	−	+	+		
Moderate to severe	COL1A1	1	14	M	+	−	N/A	+	+	+		
		2	0	F	N/A	N/A	N/A	+	+	+		
		3	0	F	+	N/A	N/A	+	+	+		

Continues

TABLE 2 Continued

Group	Gene	Patient ID	Age	Sex	Blue sclera	DI	Deafness	Lower extremity deformities	Reccurent fractures	Bone fragility	Number of affected family members who had the identical variant	Number of affected family members who were not sequenced
		4	36	F	N/A	N/A	N/A	+	+	+		
		5	4	M	+	+	N/A	+	+	+		
		6	1	M	+	N/A	N/A	+	+	+		
		7	33	F	+	+	−	+	+	+		
		8	0	M	+	N/A	N/A	+	+	+		
		9	0	M	N/A	N/A	N/A	+	N/A	N/A	1	
		10	15	F	−	+	N/A	+	+	+		
		11	0	M	+	N/A	N/A	+	+	+	1	
		12	0	F	−	N/A	N/A	+	−	+	1	
		13	2	F	N/A	N/A	N/A	+	+	+		
		14	0	M	N/A	N/A	N/A	+	+	+		
		15	30	F	+	+	N/A	+	+	+		
		16	16	M	+	−	N/A	+	+	+	1	
		17	28	F	+	+	N/A	+	+	+		
		18	11	M	−	−	−	+	+	+		
		19	17	F	+	+	N/A	+	+	+		
		20	6	M	−	−	−	+	+	+		
		21	0	F	−	N/A	N/A	+	+	+	1	
	COL1A2	22	7	M	+	N/A	N/A	+	+	+	1	
		23	33	M	+	+	N/A	+	+	+	1	
		24	0	M	+	N/A	N/A	+	+	+		
		25	0	M	N/A	N/A	N/A	+	+	+		
		26	1	M	+	N/A	N/A	+	+	+		1
		27	13	M	+	N/A	N/A	+	+	+		
		28	0	F	−	N/A	N/A	+	+	+		
		29	0	F	N/A	N/A	N/A	+	+	+		
		30	13	M	N/A	N/A	N/A	+	N/A	N/A		
		31	50	F	N/A	N/A	N/A	+	+	+		
		32	0	M	N/A	N/A	N/A	+	+	+	1	

Continues

TABLE 2 Continued

Group	Gene	Patient ID	Age	Sex	Blueclera	DI	Deafness	Lower extremity deformities	Recurrent fractures	Bone fragility	Number of affected family members who had the identical variant	Number of affected family members who were not sequenced
		33	3	F	–	N/A	N/A	+	–	+	1	
		34	1	F	N/A	N/A	N/A	+	+	+		
		35	7	M	–	N/A	N/A	+	+	+	1	
		36	13	F	+	–	N/A	+	+	+		1
		37	0	F	+	–	–	+	+	+		
		38	0	F	N/A	N/A	N/A	+	+	+		
		39	42	F	+	+	N/A	+	+	+		
		40	0	M	N/A	N/A	N/A	+	+	+		
		41	1	F	+	+	N/A	+	+	+		
OI type 5	IFITM5	1	26	F	–	–	–	–	+	+	1	

Abbreviations: DI, dentinogenesis imperfecta; N/A, not available.

the moderate-to-severe group were aged ≤1 years. All infantile patients without extra-skeletal manifestation and family history showed moderate-to-severe OI. The scleral color was reported in 76 cases. Nearly all of the mild OI patients had blue sclera, compared with only 71.4% of the moderate-to-severe OI patients ($p = 0.001$). Dental status was reported for 45 cases, and nine from each group presented with DI ($p = 0.064$). Hearing status was reported for 27 cases. Four comparatively older cases (11 y, 22 y, 24 y, and 38 y) in the mild OI group had a hearing impairment. The BMD Z-scores of the lumbar spine in bisphosphonate-naïve were lower in the moderate-to-severe OI group (n = 5) than in the mild OI group (n = 17) ($p = 0.001$) (Figure S1) (Tables 1 and 2).

3.2 | Genotype-phenotype correlation

All but one of the probands had a pathogenic or likely pathogenic variant. There were 60, 21, and 1 variants of *COL1A1*, *COL1A2*, and *IFITM5* identified, respectively (Table 3). Thirty-six novel variants were discovered. Ten variants were detected two or three times. All the probands and affected family members with the same variant had similar OI severities. Nearly all the cases with *COL1A1* splice-site or nonsense variants (97.8%) had blue sclera compared with 78.9% of the cases harboring *COL1A1* or *COL1A2* glycine substitutions. DI was present in 40.0% of the splice-site or nonsense variants, whereas 50.0% of the cases with the missense variants presented with DI. All three cases with *COL1A1* C-terminal propeptide missense variants lacked blue sclera. The OI type 5 proband had the variant c.-14C>T in *IFITM5*. A segregation analysis was performed on 32 families (21 in the mild OI group, 10 in the moderate-to-severe OI group, and 1 in the OI type 5 group), and the identified variants were validated. Most of the affected family members were patients' parent, but some offspring, siblings, grandparents, or cousins were also affected.

In the mild OI group, *COL1A1* nonsense and splice-site variants were found in 30 and 20 probands, respectively. Triple-helical domain glycine substitutions were detected in three probands. We identified a novel *de novo* noncanonical *COL1A1* splice-site variant c.696+4delA (IVS9 +4delA) (Patients ID 4) as likely pathogenic. The patient had no other variant in *COL1A1*, *COL1A2*, or *IFITM5*, and unaffected parents did not carry this variant. The *COL1A2* C-terminal propeptide variant c.3883T>C (p.Ser1295Pro) was classified as a variant of uncertain significance (VUS). It has previously been reported as benign/likely benign (rs757449082), and had a very low allele frequency in East Asian populations (0.0006). In the presented study, variants causing mild OI were distributed throughout *COL1A1* (Figure 1).

In the moderate-to-severe OI group, *COL1A1* splice-site variants were identified in three cases (c.697-1G>A

TABLE 3 Summary of the genotype spectra

Group	Gene	Patient ID	Genotype		Exon(IVS) number	Novelty[a]	ACMG criteria	Class	PolyPhen−2		PROVEAN		Mutation Taster	Human splicing finder	
Mild	COL1A1	1	p.Cys70Ter	c.210C>A	2	†	PVS1 PS3	Pathogenic					1	Disease causing	
		2	p.Cys70Ter	c.210C>A	2	†	PVS1 PS3	Pathogenic					1	Disease causing	
		3		c.334-9A>G	3		PS1 PM2	Likely pathogenic					0.999	Polymorphism	Most probably affecting splicing.
		4	p.Gly130TrpfsTer39	c.386dup	5		PVS 1PS3	Pathogenic					1	Disease causing	
		5	p.Gly130TrpfsTer39	c.386dup	5		PVS 1PS3	Pathogenic					1	Disease causing	
		6	p.Gly130TrpfsTer39	c.386dup	5		PVS 1PS3	Pathogenic					1	Disease causing	
		7	p.Gly145AspfsTer120	c.432del	5		PVS 1PS3	Pathogenic					1	Disease causing	
		8		c.642+1G>C	8	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		9	p.Gly224Ser	c.670G>A	9	†	PS1 PS3 PP1	Pathogenic	0.999	Probably damaging	−4.788	Deleterious	0.999	Disease causing	
		10		c.696+4del	9	†	PS2 PM2 PP3	Likely pathogenic					1	Disease causing	Most probably affecting splicing.
		11	p.Glu234LysfsTer31	c.700del	10		PVS1 PS3	Pathogenic					1	Disease causing	
		12		c.750+2 T>A	10		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		13	p.Gln300Ter	c.898C>T	13	†	PVS1 PS3	Pathogenic					1	Disease causing	
		14	p.Ala340LysfsTer201	c.1017del	16	†	PVS1 PS3	Pathogenic					1	Disease causing	
		15	p.Gly377AlafsTer164	c.1128del	17		PVS1 PS3	Pathogenic					1	Disease causing	
		16	p.Arg415Ter	c.1243C>T	19		PVS1 PS3	Pathogenic					1	Disease causing	
		17	p.Gly424AlafsTer117	c.1269del	19		PVS1 PS3	Pathogenic					1	Disease causing	
		18		c.1299+1G>A	19		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		19		c.1299+1G>A	19		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		20		c.1299+1G>A	19		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		21		c.1299+1G>T	19	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		22		c.1669-1G>A	24	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.

(Continues)

TABLE 3 Continued

Group	Gene	Patient ID	Genotype		Exon(IVS) number	Novelty[a]	ACMG criteria	Class	PolyPhen−2		PROVEAN		Mutation Taster	Human splicing finder	
		23	p.Pro569GlnfsTer12	c.1703del	25	†	PVS1 PS3	Pathogenic					1	Disease causing	
		24	p.Arg598Ter	c.1792C>T	26		PVS1 PS3	Pathogenic					1	Disease causing	
		25	p.Arg598Ter	c.1792C>T	26		PVS1 PS3	Pathogenic					1	Disease causing	
		26	p.Arg598Ter	c.1792C>T	26		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		27		c.1821+1G>A	26		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		28		c.1822-1G>A	26		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		29		c.1876-1G>A	27	†	PVS1 PS3	Pathogenic					1	Disease causing	
		30	p.Pro652GlnfsTer114	c.1955del	29	†	PVS1 PS3	Pathogenic					1	Disease causing	
		31	p.Gly671AlafsTer95	c.2010del	30		PVS1 PS3	Pathogenic					1	Disease causing	
		32	p.Arg697Ter	c.2089C>T	31		PVS1 PS3	Pathogenic					1	Disease causing	
		33	p.Gly725AlafsTer41	c.2172del	32	†	PVS1 PS3	Pathogenic					1	Disease causing	
		34	p.Gln822Ter	c.2464C>T	36		PVS1 PS3	Pathogenic					1	Disease causing	
		35	p.Lys826ArgfsTer5	c.2477_2478del	36	†	PVS1 PS3	Pathogenic					1	Disease causing	
		36		c.2614-1G>T	37	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		37	p.Arg882Ter	c.2644C>T	38		PVS1 PS3	Pathogenic					1	Disease causing	
		38		c.2668-1G>C	38	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		39	p.Gly971ArgfsTer138	c.2910_2911insAG	40	†	PVS1 PS3	Pathogenic					1	Disease causing	
		40		c.3045+2 T>A	41	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		41		c.3045+2 T>A	41	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		42		c.3045+2 T>A	41	†	PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.
		43	p.Gly1022Ala	c.3065G>C	42		PS1 PS3	Pathogenic	0.998	Probably damaging	−5.117	Deleterious	0.999	Disease causing	
		44		c.3207+1G>A	43		PVS1 PS3	Pathogenic					1	Disease causing	Most probably affecting splicing.

Continues

TABLE 3 Continued

Group	Gene	Patient ID	Genotype		Exon(IVS) number	Novelty[a]	ACMG criteria	Class	PolyPhen−2		PROVEAN		Mutation Taster	Human splicing finder
		45		c.3207+1_3207+2del	43		PVS1 PS3	Pathogenic					1	Disease causing Most probably affecting splicing.
		46		c.3262-1G>C	44	†	PVS1 PS3	pathogenic					1	Disease causing most probably affecting splicing.
		47	p.Ile1107SerfsTer19	c.3318_3325del	45	†	PVS1 PS3	Pathogenic					1	Disease causing
		48	p.Gly1121AlafsTer118	c.3360del	45		PVS1 PS3	Pathogenic					1	Disease causing
		49	p.Arg1141Ter	c.3421C>T	46		PVS1 PS3	Pathogenic					1	Disease causing
		50	p.Arg1141Ter	c.3421C>T	46		PVS1 PS3	Pathogenic					1	Disease causing
		51	p.Gln1240Ter	c.3718C>T	48		PVS1 PS3	Pathogenic					1	Disease causing
		52	p.Tyr1301ThrfsTer30	c.3900del	49	†	PVS1 PS3	Pathogenic	1	Probably damaging	−3.622	Deleterious	0.999	Disease causing
	COL1A2	53	p.Gly334Ser	c.1000G>A	19		PS1 PS3 PP1	Pathogenic	1	Probably damaging	−4.798	Deleterious	0.999	Disease causing
		54	p.Ser1295Pro	c.3883T>C	51	†	PM2 PM6 PP3	VUS	1	Probably damaging	−3.622	Deleterious	1	Disease causing
Moderate to severe	COL1A1	1		c.697-1G>A	9	†	PVS1 PS3	Pathogenic					1	Disease causing Most probably affecting splicing.
		2	p.Gly257Arg	c.769G>A	11		PS1 PS3	Pathogenic	1	Probably damaging	−6.363	Deleterious	0.999	Disease causing
		3	p.Gly299Asp	c.896G>A	13		PS1 PS3	Pathogenic	0.999	Probably damaging	−5.747	Deleterious	0.999	Disease causing
		4	p.Gly365Ser	c.1093G>A	17	†	PS1 PS3	Pathogenic	0.999	Probably damaging	−4.349	Deleterious	0.999	Disease causing
		5	p.Ala408del	c.1222_1224del	19	†	PM2 PM4 PM6 PP3	Likely pathogenic			−9.166	Deleterious	0.999	Disease causing
		6	p.Gly452Ser	c.1354G>A	21	†	PS3 PM2 PM6 PP3 PP4	Likely pathogenic	0.999	Probably damaging	−4.475	Deleterious	0.999	Disease causing
		7	p.Gly530Ser	c.1588G>A	23		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−4.755	Deleterious	0.999	Disease causing
		8		c.1668+1G>A	24	†	PVS1 PS3 PM6	Pathogenic					1	Disease causing Most probably affecting splicing.
		9	p.Gly560Ser	c.1678G>A	25		PS1 PS3	Pathogenic	1	Probably damaging	−4.798	Deleterious	0.999	Disease causing
		10	p.Gly767Ser	c.2299G>A	33/34		PS1 PS3 PM6	Pathogenic	0.999	Probably damaging	−5.140	Deleterious	0.999	Disease causing
		11	p.Gly821Ser	c.2461G>A	36		PS1 PS3	Pathogenic	1	Probably damaging	−5.178	Deleterious	0.999	Disease causing
		12	p.Gly821Ser	c.2461G>A	36		PS1 PS3	Pathogenic	1	Probably damaging	−5.178	Deleterious	0.999	Disease causing

Continues

TABLE 3 Continued

Group	Gene	Patient ID	Genotype		Exon(IVS) number	Novelty[a]	ACMG criteria	Class	PolyPhen−2		PROVEAN		Mutation Taster	Human splicing finder
		13	p.Gly866Ser	c.2596G>A	37		PS1 PS3	Pathogenic	1	Probably damaging	−5.212	Deleterious	0.999	Disease causing
		14	p.Gly1040Ser	c.3118G>A	43		PS1 PS3	Pathogenic	1	Probably damaging	−5.208	Deleterious	0.999	Disease causing
		15	p.Gly1040Ser	c.3118G>A	43		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−5.208	Deleterious	0.999	Disease causing
		16	p.Gly1052Cys	c.3154G>T	43	†	PS3 PM2 PP3 PP4	Pathogenic	1	Probably damaging	−8.164	Deleterious	0.999	Disease causing
		17	p.Gly1076Ser	c.3226G>A	44		PS1 PS3	Pathogenic	1	Probably damaging	−5.759	Deleterious	0.999	Disease causing
		18	p.Ala1375Thr	c.4123G>A	50	†	PM2 PM6 PP3 PP4	Likely pathogenic	0.997	Probably damaging	−3.610	Deleterious	0.999	Disease causing
		19		c.4248+1G>A	50	†	PVS1 PS3	Pathogenic					1	Disease causing Most probably affecting splicing.
		20	p.Ala1443Val	c.4328C>T	51	†	PM2 PM6 PP3 PP4	Likely pathogenic	0.997	Probably damaging	−3.781	Deleterious	0.999	Disease causing
		21	p.Ala1443Val	c.4328C>T	51	†	PM2 PM6 PP3 PP4	Likely pathogenic	0.997	Probably damaging	−3.781	Deleterious	0.999	Disease causing
	COL1A2	22	p.Gly238Val	c.713G>T	15	†	PS3 PM2 PP3 PP4	Likely pathogenic	1	Probably damaging	−7.145	Deleterious	0.999	Disease causing
		23		c.1036-2A>C	19		PVS1 PS3	Pathogenic					1	Disease causing Most probably affecting splicing.
		24	p.Gly358Ser	c.1072G>A	20		PS1 PS3	Pathogenic	1	Probably damaging	−5.059	Deleterious	0.999	Disease causing
		25	p.Gly460Ser	c.1378G>A	24		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−5.119	Deleterious	0.999	Disease causing
		26	p.Gly496Ser	c.1486G>A	25	†	PS1 PS3	Pathogenic	1	Probably damaging	−5.420	Deleterious	0.999	Disease causing
		27	p.Gly529Asp	c.1586G>A	27		PS1 PS3	Pathogenic	1	Probably damaging	−6.258	Deleterious	0.999	Disease causing
		28	p.586insAla	c.1755_1756insGCT	30	†	PM2 PM4 PM6 PP3	Likely pathogenic			−7.425	Deleterious	0.874	Disease causing
		29	p.Gly601Val	c.1802G>T	31		PS1 PS3	Pathogenic	1	Probably damaging	−7.736	Deleterious	0.999	Disease causing
		30	p.Gly700Arg	c.2098G>C	35		PS1 PS3	Pathogenic	1	Probably damaging	−5.389	Deleterious	0.999	Disease causing
		31	p.Gly703Val	c.2108G>T	35		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−8.124	Deleterious	0.999	Disease causing
		32	p.Gly739Arg	c.2215G>A	37		PS1 PS3	Pathogenic	1	Probably damaging	−5.393	Deleterious	0.999	Disease causing
		33	p.Gly772Ser	c.2314G>A	38		PS1 PS3	Pathogenic	0.769	Possibly damaging	−5.427	Deleterious	0.999	Disease causing

Continues

TABLE 3 Continued

Group	Gene	Patient ID	Genotype		Exon(IVS) number	Novelty[a]	ACMG criteria	Class	PolyPhen−2		PROVEAN		Mutation Taster	Human splicing finder
		34	p.Gly892Asp	c.2675G>A	42		PS1 PS3	Pathogenic	1	Probably damaging	−5.979	Deleterious	0.999	Disease causing
		35	p.Gly931Arg	c.2791G>A	43		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−5.342	Deleterious	0.999	Disease causing
		36	p.Gly1012Ser	c.3034G>A	46		PS1 PS3	Pathogenic	1	Probably damaging	−5.359	Deleterious	0.999	Disease causing
		37	p.Gly1012Ser	c.3034G>A	46		PS1 PS3	Pathogenic	1	Probably damaging	−5.359	Deleterious	0.999	Disease causing
		38	p.Gly1012Cys	c.3034G>T	46	†	PS3 PM2 PM5 PM6 PP3 PP4	Pathogenic	1	Probably damaging	−7.910	Deleterious	0.999	Disease causing
		39	p.Gly1087Val	c.3260G>T	48	†	PS1 PS3	Pathogenic	1	Probably damaging	−8.054	Deleterious	0.999	Disease causing
		40	p.Gly1093Arg	c.3277G>C	49	†	PS1 PS3	Pathogenic	1	Probably damaging	−7.162	Deleterious	0.999	Disease causing
		41	p.Gly1102Val	c.3305G>T	49		PS1 PS3 PM6	Pathogenic	1	Probably damaging	−7.409	Deleterious	0.999	Disease causing
OI type 5	IFITM5	1		c. −14C>T										

Abbreviations: ACMG, The American College of Medical Genetics and Genomics; N/A, not available; VUS, variants of uncertain significance.

[a]Novel variant unreported in the collagen type I variant database (http://www.le.ac.uk/ge/collagen/) is marked with a dagger (†).

(IVS9 −1G>A), c.1668+1G>A (IVS24 +1G>A), and c.4248+1G>A (IVS50 +1G>A). Triple-helical domain glycine substitutions were found in 32 cases. *COL1A1* C-terminal propeptide missense variants were present in three cases, and in-frame insertion or deletion within the triple-helical domains was found in two. The *COL1A1* C-terminal propeptide variant c.4328C>T (p.Ala1443Val) has previously been deposited as a VUS(rs1131692326). Its allele frequency has not been reported, but it has recently been cited as the causative variant for OI type 3 (Li, et al., 2019). Glycine-to-serine substitutions in the triple-helical domain occurred at the highest frequency (*COL1A1*, n = 11; *COL1A2*, n = 6). Substitutions to branched or charged amino acids (valine, aspartic acid, and arginine) were found at the *N*-terminal portion of *COL1A1* (n = 2), whereas such variants were scattered throughout *COL1A2* (n = 12). Taken as a whole, the variants causing moderate-to-severe OI were skewed toward the C-terminal of *COL1A1* and *COL1A2* (Figure 1).

4 | DISCUSSION

The present study showed genotypic and phenotypic correlations among non-consanguineous Japanese OI patients. To the best of our knowledge, our study population constitutes one of the largest Japanese OI populations assessed to date. Sanger sequencing identified type I collagen variants in 99% of the individuals tested. The detection rate for type I collagen mutation ranges from 60% to 100% in various OI populations (Ho Duy et al., 2016; Lin et al., 2015; Lindahl et al., 2015; Mohd Nawawi et al., 2018). Bardai et al. have reported 598 individuals with a typical OI phenotype (mild, moderate-to-severe, and type 5 in 43%, 52%, and 5%, respectively), and all the patients that were classified as mild OI had *COL1A1* or *COL1A2* variants, whereas only 85% did in the moderate-to-severe OI group (Bardai et al., 2016). Recessive OI usually manifests the more severe phenotype. Therefore, the detection rate may be affected by the ethnicity and consanguinity of the study population, and the disease severity (Fernandes et al., 2020; Mrosk et al., 2018). Ohata et al., (2019) have conducted comprehensive genetic analyses through targeted next-generation sequencing in 53 Japanese OI patients but only detected variants in *COL1A1*, *COL1A2*, and *IFITM5*. In fact, autosomal recessive diseases are very rare in Japan (e.g., phenylketonuria has a rate of 1:125,000, and cystic fibrosis a rate of 1:350,000), so recessive OI patients are scarcely reported (Aoki & Wada, 1988; Takeyari et al., 2018; Yamashiro et al., 1997). We believe that Sanger sequencing may still be applicable in the genophenotypic analysis of non-consanguineous OI populations (Mrosk et al., 2018). Moreover, our detection rate in the present study was superior to that of the previous one (Kataoka et al., 2007) because, in the present study, OI diagnosis was made by a clinician

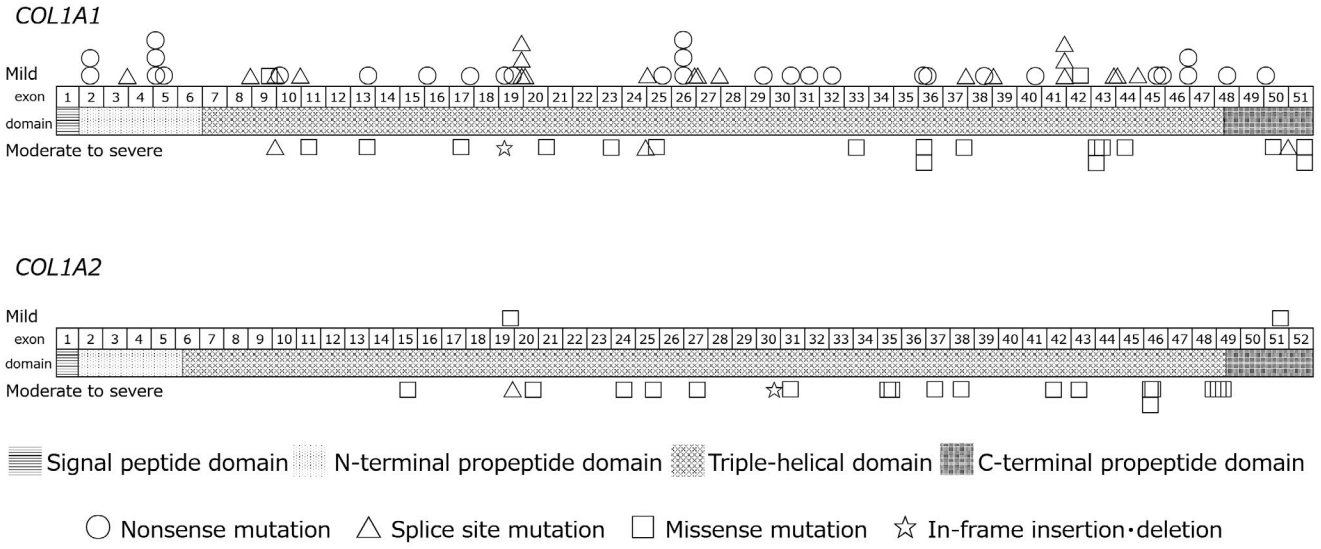

FIGURE 1 Variant spectra of *COL1A1* and *COL1A2*. Symbols above the box represent the variants in the mild OI group. Symbols below the box represent the variants in the moderate-to-severe OI group. Aligned symbols represent the same variant

familiar with OI, and DHPLC screening was omitted in case of false negatives (Phadke, 2015; Stephen et al., 2014). One proband presenting with the specific features of OI type 5 harbored the characteristic c.-14C>T variant in *IFITM5*. Whereas other *IFITM5* variants have been reported in some OI patients, a distinct phenotype of OI type 5 is exclusively observed in patients with this variant (Mohd Nawawi et al., 2018; Rodriguez Celin et al., 2018).

Blue sclera occurred more frequently in the mild OI group than the moderate-to-severe group. There was no significant difference between the two groups in terms of DI incidence. *COL1A1* splice-site or nonsense variants were the most closely correlated with the blue sclera, whereas there was no association between this genotype and DI. The latter results could be explained by the relative lack of information on the dental status of the test population because its members were comparatively young. Approximately 90% of the OI type 1 probands had blue sclera, and ~70% of the OI type 3 or 4 probands manifested this trait; whereas ~10% of OI type 1 had DI and ~60% of OI type 3 and 4 cases presented with the dental aberrations (Bardai et al., 2016; Lindahl et al., 2015; Patel et al., 2015). Hearing loss usually develops between the second and fourth decades of OI patients. Nevertheless, the relationship between this symptom and the molecular data has not been fully elucidated (Swinnen et al., 2011; Hald et al., 2018). In the present study, four individuals presented with hearing impairment in adolescence and young adulthood, but the number of cases was insufficient for the assessment of genotypic associations.

Glycine is the only residue small enough to fit into the sterically restricted space at the helical center. Most of the glycine substitutions produced moderate-to-severe phenotypes, but some resulted in the mild form. Our results corroborate those previously reported (Lindahl et al., 2015; Rauch et al., 2010; Zhytnik et al., 2017). Lindahl et al. have reported that 32% of the type 1 OI cases are caused by qualitative variants in *COL1A1* and *COL1A2*. Phenotypes resulting from *COL1A1* missense variants may be more severe than those caused by *COL1A2* variants because 75% of the procollagen molecules in the heterozygotes of *COL1A1* mutants are expected to have ≥1 mutant pro-α1(I) chain. In contrast, in the case of *COL1A2* variants, only 50% of the molecules presumably contain the mutant pro-α2(I) chain (Brodsky & Persikov, 2014). Glycine substitutions to branched or charged amino acids are highly disruptive to helix stability and are mainly confined to the region near the *N*-terminal of *COL1A1*. On the other hand, they are observed throughout *COL1A2* (Marini et al., 2007; Rauch et al., 2010). These characteristics are consistent with our findings. In contrast, substitutions by small amino acids (cysteine, alanine, and serine) produce variable outcomes. For example, the variant c.3505G>A (p.Gly1169Ser) in *COL1A1* exon 47 is reported seventeen times in the OI variant database, and four of these are described as OI type 1. Familial cases of this variant present with significant phenotype diversity, ranging from mild to severe (Liu et al., 2016). A recent study has reported that familial cases with haploinsufficiency have lower phenotypic variability, whereas those with structural abnormality are more likely to show higher phenotypic variability (Zhytnik et al., 2020). Our familial cases also showed a similar tendency; this may reflect the fact that the majority of patients exhibited a mild phenotype. The effects of genetic, epigenetic, environmental, or other unidentified factors on the same change and the corresponding variability in its clinical severity remain unknown. Several dozens in-frame insertions or deletions in the Gly-X-Y tripeptide repeat region are reported

in the OI variant database, most of which are tripeptide or any multiple of three amino acids. Duplication or deletion of one or two Gly-X-Y triplets shift the register of collagen chains and prevent the incorporation of the mutant chains into the collagen trimer, leading to lethal or severe OI (Cabral et al., 2003). All but one of single or double amino acid insertion or deletion also show moderate-to-severe phenotypes, similar to our findings.

Splice-site variants that cause exon skipping within the triple-helical domain can maintain the reading frame and lead to shifted alignment, impaired triple helix structures, and severe phenotypes (Marini et al., 2007). Conversely, variants resulting from intron retention or cryptic-site activation may cause premature termination codons and produce milder phenotypes. In a study on *COL1A1* splice variants, all individuals with IVS(X)-1G>A acceptor site transitions showed the OI type 1 phenotype (Schleit et al., 2015). Guanine is the first nucleotide in most *COL1A1* exons and particularly in the triple-helical domains. This transition creates a new splice acceptor 1 nt downstream and produces a frameshift and a mild OI phenotype (Byers, 2014). This hypothesis may plausibly predict the effects of splice–acceptor-site variants. However, we found the *COL1A1* splice-site variant c.697-1G>A (IVS9 −1G>A) in an individual with bone deformities. Furthermore, certain IVS(X)-1G>A acceptor-site variants are described as type 4 in the OI-variant database. On the other hand, splice–donor-site variants may result in different, less predictable, OI severities as well.

The *C*-terminal propeptide domains of procollagen are essential for the folding of the three proα(I)-chains and their secretion from the rough ER (Barnes et al., 2019; Doan et al., 2020). The crystalline structure of the *C*-terminal propeptide trimer resembles a flower and has a stalk, base, and petal region. It harbors a conserved amino acid sequence (Malfait et al., 2014; Sharma et al., 2017). Symoens et al. have reported 30 unique *C*-terminal propeptide defects (Symoens et al., 2014). The missense variants in *COL1A1* may induce a more severe OI phenotype than those in *COL1A2*, which would be consistent with the results of our study. The *COL1A2* c.3883C>T (p.Ser1295Pro, rs757449082) variant was classified as VUS, which has two interpretations (either likely benign with osteogenesis imperfecta or benign with Ehlers-Danlos Syndrome Arthrochalasia Type 2). However, we think that this variant could cause a mild OI phenotype. Furthermore, all three probands with this type of variant (moderate-to-severe group, Patients IDs 18, 20, and 21) had normal sclera. When other study results are also considered, only 6/19 (32%) of such patients present with the blue sclera (Barnes et al., 2019; Li, Mao, et al., 2019; Lu et al., 2014; Symoens et al., 2014). Although the bluish color of the sclera may fade with age in moderate-to-severe OI patients (Sillence et al., 1993), there may be a correlation between *COL1A1* C-terminal propeptide missense variants and normal sclera.

The present study had several limitations. First, most of the *COL1A1* and *COL1A2* variants in this study population were probably skewed toward the mild phenotype. Second, our method may have overlooked monoallelic, biallelic, or digenic causative variants; several studies have reported patients with double pathogenic variants (Ackermann & Levine, 2017; Ho Duy et al., 2016; Mohd Nawawi et al., 2018; Mrosk et al., 2018; Takagi et al., 2015). Third, we did not perform functional analyses on the proteins, mRNAs, or cDNAs isolated from cultured fibroblasts. Such assays detect quantitative and/or qualitative defects and may help elucidate variants within the clinical spectrum (Li et al., 2019; Van Dijk et al., 2012).

The present study described the variant spectra of *COL1A*, *COL1A2*, and *IFITM5* among 96 OI probands, thereby elucidating the genotypic and phenotypic correlations in OI. This sample population constitutes one of the largest ones analyzed in Japan, so that our detection rate for type I collagen variants have improved relative to that of our previous report. Consequently, we found that C-terminal propeptide missense variants of the α1 (I) chain may be related to the normal sclera.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

KH and HTa designed this study. YH, KH, and MY performed the sequencing. YH, KH, and NF analyzed the data. YH drafted the manuscript. KH and NF helped draft the manuscript. HTa and HTs reviewed and finalized the manuscript. All the authors approved the final manuscript as submitted and agreed to be accountable for all the aspects of the work.

ORCID

Yousuke Higuchi https://orcid.org/0000-0001-5253-6569
Kosei Hasegawa https://orcid.org/0000-0002-1751-6527

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SUPPORTING INFORMATION

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

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