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Mutation-in-Brief

A novel *COL1A1* deletion/insertion pathogenic variant in a patient with osteogenesis imperfecta

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Highlights

- Delins are a relatively rare type of variant in *COL1A1* in patients with OI.
- Our novel delins variant leads to a mild OI phenotype.
- A delins variant should be considered in the genetic analysis of OI.

Key words: *COL1A1*, deletion/insertion (delins), osteogenesis imperfecta

Introduction

Osteogenesis imperfecta (OI) is a rare genetic connective tissue disease that is characterized by bone fragility and deformity. The prevalence of OI is approximately 1 in 15,000–20,000 births. Patients may present with extraskeletal features including dentinogenesis imperfecta, blue or gray sclerae, hearing loss, and ligamentous laxity. The phenotypic spectrum of OI ranges from mild to lethal. The classification proposed by Sillence (1979) identifies four OI subtypes according to clinical severity (1). Many new genes associated with OI have been identified. To date, approximately 24 causative genes of OI have been identified, affecting collagen folding, posttranslational modifications and processing, bone mineralization, and osteoblast differentiation. In approximately 85–90% of patients, OI is caused by autosomal dominant pathogenic variants in the *COL1A1* and *COL1A2* genes encoding type I collagen, which affect the quantity or structure of collagen. *COL1A1* and *COL1A2* encode the α1(I) and α2(I) chains of type I collagen, the most abundant proteins in bone, skin, and tendon extracellular matrices (2, 3). Frameshift variants of *COL1A1* often result in nonsense-mediated decay (NMD) and haploinsufficiency of α1(I) chains of type I collagen (4, 5). The novel deletion/insertion (delins) pathogenic variant c.3578-3654delins TCATCAGCC in *COL1A1*, resulting in a frameshift (p. Ser1193Ilefs*5), was identified in a patient with OI type I. Herein, we report the results of the clinical and genetic analyses of a patient with this novel delins variant.

Case Report

An 11-yr-old girl with a suspected OI was referred to our hospital for examination and treatment. She was delivered after 38 wk of gestation with a birth weight of 2,496 g (–1.10 SDS), length of 46.5 cm (–1.16 SDS), and

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head circumference of 32.7 cm (–0.37 SDS). She had no skeletal deformities at birth and normal psychomotor development. Low-trauma fractures occurred six times in both wrists, the left forearm, the left fibula and tibia, the right fourth finger, and the right elbow from the age of 5 yr. At the age of 11 yr, she had a normal height of 140.5 cm (–0.73 SDS) and weight of 37.7 kg (+0.01 SDS). She had blue sclerae and an impacted tooth on orthodontic therapy without bone deformity or dentinogenesis imperfecta. Serum concentrations of calcium, phosphate, alkaline phosphatase, and parathyroid hormone were within normal age-specific ranges; however, the serum level of 25-hydroxyvitamin D was 6 ng/mL, indicating vitamin D deficiency. The bone mineral density (BMD) value was 0.52 g/cm^2 (L1–L4,–1.75 SDS) at the lumbar spine and total body less head BMD value was 0.654 g/cm^2 (–1.48 SDS). X-ray films revealed a prominent lumbar osseous endplate and slight depression of the lower lumbar vertebrae. We clinically diagnosed the patient with OI. Pamidronate was intravenously administered. Both the mother and maternal uncle had frequent bone fractures during childhood and blue sclerae. The maternal grandfather also had blue sclerae. Both her father and younger brothers were healthy. A family tree is shown in **Fig. 1A**.

Mutation Analysis

The present study was approved by the Ethics Committee of the Faculty of Medicine, Osaka University (approval no. 688), and informed consent was obtained from the patient's parents. Genomic DNA was extracted from a blood sample. Whole-exome sequencing (WES) was performed by Macrogen (https://www.macrogen-japan.co.jp/) using an Illumina platform with 151-bp paired-end reads. Exome capture and library preparation were conducted using Agilent SureSelect V6 post (Agilent Technologies, Santa Clara, CA, USA). Trimmed reads were mapped to the reference human genome (GRCh37) using the Burrows-Wheeler Aligner. Picard to mark duplicates and the Genome Analysis Toolkit (GATK) to improve alignment accuracy were used. Variants were called using the GATK Haplotype Caller and ANNOVAR. The results revealed four deletion sites with 68 bases and three remaining sites with 10 bases within exon 48 of the *COL1A1* gene (**Fig. 1B**). PCR was performed for validation and segregation analyses using the following primers: 5′-GCTGGTCCTGTTGTATGTAGC-3′ (forward) and 5′-CCAGCACCATATGGTAGGGGCACAT-3′ (reverse). After PCR and agarose gel electrophoresis, a wild-type DNA band with an expected size of 476 bp and an additional approximately 400-bp band, which was likely derived from the variant allele, were detected (**Fig. 1C**). The smaller DNA band was extracted and purified from agarose gel using a gel extraction kit (QIAGEN) and then sequenced using the same primers. In comparison with the *COL1A1* reference sequence (NM_000088.4), a variant replacing 77 nucleotides with 9 nucleotides was detected (**Fig. 1D**). These results are consistent with the findings of WES. Based on the data obtained, the patient harbored the heterozygous delins variant c.3578-3654delinsTCATCAGCC in exon 48 of the *COL1A1* gene. This variant resulted in a frameshift and early termination of mRNA translation (p. Ser1193Ilefs*5). Sanger sequencing revealed that the mother had the same genotype, whereas the father did not harbor this variant.

Discussion

Herein, we demonstrate that a novel heterozygous delins variant in the *COL1A1* gene (c.3578-3654delinsTCATCAGCC) leads to mild OI. The phenotype of the patient was classified as OI type I, including recurrent fractures without bone deformity, low BMD, slight changes in the radiographs of the vertebrae, and blue sclerae. The reduction in lumbar BMD by –1.75 SDS was not striking, although the patient had frequent fractures. Slight concaves of the endplates of the spine might lead to an increase in lumbar BMD. In OI, high bone matrix mineralization at the tissue level, increased bone turnover, and microarchitectural changes result in decreased bone quality (6). Additionally, vitamin D deficiency may have contributed to the bone fragility of the patient. Thus, a combination of low BMD and reduced bone quality is thought to lead to frequent fractures.

The *COL1A1* gene encompasses a 17,554-base region on chromosome 17q21.33 and encodes type I collagen. Type I collagen, an interstitial fibrillar collagen, is a heterotrimer that contains two α1(I) chains and one α2(I) chain. The central triple helical domain is formed by 338 repeats of a Gly Xaa-Yaa triplet, and glycine is essential for the triple helical formation (3). In total, 1381 variants in the *COL1A1* gene have been reported in a database (https://databases.lovd.nl/shared/genes/COL1A1), including 999 substitutions (72.3%), 273 deletions (19.8%), 70 duplications (5.1%), 19 delins (1.4%), and 14 insertions (1.0%). Glycine substitution in the helical domain is most common, and delins are rare pathogenic variants of OI.

The variant detected in the exon at position 3578 of the *COL1A1* gene leads to the substitution of serine with isoleucine at residue 1193. By inserting TCATCAGCC at residue 1193, a premature termination codon (PTC) was generated five codons downstream of the insertion site. This PTC location indicated that mutant COL1A1 transcripts were recognized by the NMD system (4). Therefore, the variant is predicted to introduce a stop codon with subsequent NMD at the mRNA level, leading to haploinsufficiency. Haploinsufficiency of *COL1A1* reduces the production of structurally normal collagen, resulting in mild OI (5). This is consistent with our patient, who was classified as OI type I. According to a number of databases, including the Global Variome shared LOVD (https://databases.lovd.nl/shared/genes/COL1A1), the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), this variant is novel, and 37

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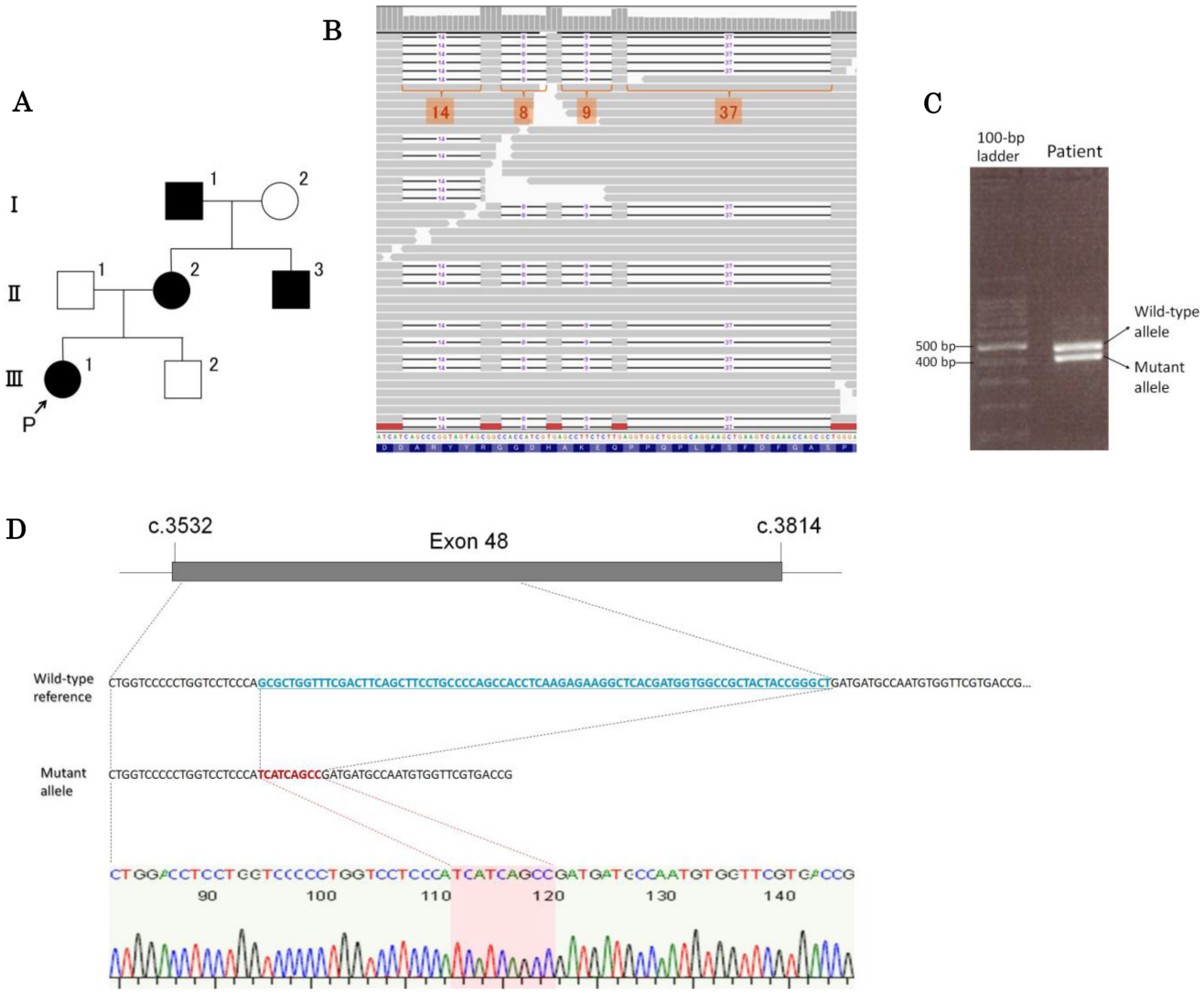

Fig. 1. A: Family pedigree. Squares represent males and circles represent females. Black symbols indicate the clinically affected individuals. Patient (III-1) is indicated by a black arrow. B: WES data from the Integrative Genome Viewer (IGV) (https://software.broadinstitute.org/software/igv/) show four heterozygous deletion sites (14, 8, 9, and 37 bases in each deletion located between Chr17:48264160-48264173, 48264178-48264185, 48264189-48264197, and 48264201-48264237, respectively) within exon 48 of the *COL1A1* gene. Deletion is indicated by a black line in a white square. The base-pair size of the deletion is labeled with a purple number at the center of the line and a larger orange number. C: PCR shows a wild-type band with an expected size of 476 bp and an approximately 400-bp band, which was likely derived from the mutant allele. The 100-bp ladder is shown on the left side. D: Sequencing chromatogram of the delins variant, replacing 77 nucleotides with 9 nucleotides (c.3578-3654delinsTCATCAGCC).

delins pathogenic variants have been identified to date, indicating that delins variants in OI are rare. These 37 variants resulted in 22 frameshifts, 9 substitutions of amino acids (5 glycine substitutions), and 6 splice-site variants. All frameshift variants are expected to undergo NMD and result in a mild or moderate phenotype, and amino acid substitutions or splice-site variants are predicted to lead to mild-to-severe outcomes.

In conclusion, we identified a novel delins pathogenic variant (c.3578-3654delinsTCATCAGCC) in exon 48 of the *COL1A1* gene. The genetic diagnosis was consistent with the phenotype of the patient. Delins are a relatively rare type of OI variant that need to be considered in genetic analyses.

Conflict of interests: Takuo Kubota received a scholarship donation from Teijin Pharma. Keiichi Ozono received honoraria from Kyowa Kirin, Alexion, and Novo Nordisk Pharma. The other authors have nothing to declare.

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