Three Patient Kindred with a Novel Phenotype of Osteogenesis Imperfecta due to a *COL1A1* Variant

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What is already known on this topic?

Osteogenesis imperfecta (OI) is a heterogeneous connective tissue disorder characterized by reduced bone mass and increased bone fragility. Peak fracture rates in OI occur during the toddler and adolescent years, decline during adulthood, and increase again after age 55 years.

What this study adds?

We describe a kindred including three members who presented with a unique phenotype of OI, presumably due to a proline-to-leucine missense variant in the *COL1A1* gene. All three members had a pattern of prenatal bone deformities, followed by multiple, nontraumatic long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter. To our knowledge, a clinical phenotype of OI characterized by cessation of nontraumatic fractures after the first two years of life has not been described previously.

Abstract

Osteogenesis imperfecta (OI) is characterized by fractures and progressive bone deformities. Fracture rates peak during the toddler and adolescent years and decline during adulthood but do not stop entirely. We describe a kindred, the affected members of which were the mother and two sons, who presented with an apparently unique phenotype of OI. Our patients demonstrated a pattern of prenatal bone deformities followed by multiple, nontraumatic long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter. No extra-skeletal manifestations have been noted to date. The mother did not receive bisphosphonate therapy but had no nontraumatic fractures after the age of five months. Intravenous bisphosphonate therapy was started for both sons within two months of birth, with the most recent infusions at age 18 months and 28 months in Patients 2 and 3, respectively. Two patients harbored a variant of uncertain significance in the *COL1A1* gene. This heterozygous variant, c.3548C > T; p.(Pro1183Leu), is listed in the OI Variant Database as affecting only one other individual with osteopenia. We describe three family members with a unique presenting phenotype of OI, characterized by cessation of nontraumatic fractures after the first two years of life.
Keywords: Fragility fractures, collagen, child, bisphosphonates, bone density

Introduction

Osteogenesis imperfecta (OI) is a heterogeneous connective tissue disorder characterized by reduced bone mass and increased bone fragility (1,2). The disorder is primarily caused by variants in the genes involved in the synthesis or post-transcription modification of type 1 collagen (3). Type

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1 procollagen is composed of two proα1(I) and one proα2(I) chains that are encoded by the *COL1A1* (OMIM #120150) and *COL1A2* (OMIM #120160) genes, respectively (2). The triple-stranded procollagen molecule is assembled and then secreted into the extracellular space, where the propeptides are enzymatically cleaved to form mature collagen (4). Although there are numerous other OI-related genes, most cases of OI (85-90%) are caused by variants in the *COL1A1* and *COL1A2* genes.

Typically, point variants affecting glycine residues in the triple-helix structure of type 1 collagen have been predicted to disrupt protein folding, resulting in OI (5). The substitution of leucine for proline (Pro) in exon 49 of the *COL1A1* gene is listed in the OI Variant Database but to date, it has been associated with only one individual affected by osteopenia and it has been designated as a variant of unknown pathogenicity (6).

The severity of OI varies from mild, to progressively deforming, to perinatally lethal (7). Peak fracture rates in OI occur during the toddler and adolescent years, decline during adulthood, and increase again after age 55 years (8). However, to our knowledge, a clinical phenotype of OI characterized by cessation of nontraumatic fractures after the first two years of life has not been described previously.

We describe a kindred of three members who presented with a unique phenotype of OI, presumably due to a Pro-to-leucine missense variant in the *COL1A1* gene. All three members had a pattern of prenatal bone deformities followed by multiple, nontraumatic, long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter.

Case Reports

We describe a nonconsanguineous family of European descent, in which three members were affected by OI (Table 1, Figure 1). Informed verbal consent was obtained from the mother.

Patient 1: Mother

The mother received the diagnosis of OI when she was 23 years old, after the birth of her second child (Patient 2), whose prenatal ultrasound suggested OI. The mother had nine nontraumatic long bone fractures, primarily femoral, before the age of five months. Her prenatal ultrasound findings were unknown. She did not receive bisphosphonate treatment but did not have any other nontraumatic fractures after five months of age. She had normal sclerae, dentition, hearing, echocardiogram and stature (159.8 cm, 25th percentile) (9). She had no spinal or cranial deformities,

no joint hypermobility and did not bruise easily. We were unable to obtain data regarding her bone turnover markers and bone mineral density (BMD). She had an unaffected 17-year-old daughter. As of manuscript writing, Patient 1 was 39 years old.

Patient 2: Older Sibling

Patient 2 was the second child of Patient 1 and an unaffected father. The father's height was 188 cm (94th percentile) (9). At five months gestation, a prenatal ultrasound showed evidence of bowing of both femurs. Patient 2 was born at 38 weeks gestation via caesarean delivery without respiratory distress at birth. His birth weight was 4.28 kg (90th percentile), length was 52 cm (50th percentile) and head circumference was 38 cm (90th percentile) (9). At age three days, during circumcision, he had a right femur fracture (Figure 1). Subsequently, he had fractures of bilateral humeri and femurs at six days of life and sustained a fracture of the left femur at age four weeks. There was minimal or no trauma associated with any of these fractures.

A diagnosis of OI was considered and pamidronate infusions were initiated (0.75 mg/kg per day on two consecutive days, every two months with a total of eight infusions) from the age of six weeks through 18 months at another center. He tolerated the infusions well. Age-related developmental milestones were normal. At age three months, he was at the 95th percentile for length and 97th percentile for weight. His sclerae, hearing and dentition were normal. He had no spinal or cranial deformities, no joint hypermobility, and did not bruise easily. At age 14 months, his lumbar spine BMD was 0.51 g/cm^2 and left hip BMD was 0.57 g/cm^2 (reference range not available for this age group) (10). He had normal serum calcium (10.6 mg/dL; reference range 9-11 mg/dL) and total alkaline phosphatase (274 U/L; reference range 150-420 U/L).

Given the paucity of fractures (no fractures since age four weeks, despite being very active), pamidronate therapy was discontinued at age 18 months. As of manuscript writing, he was 17 years old, had normal ambulation and no deformities. A follow-up BMD was not available for him. His height was at the 75th percentile and weight was at the 95th percentile. His corrected midparental height was at the 65th percentile.

Patient 3: Younger Sibling

Patient 3 was born at 39 weeks gestation to Patient 1 and a different father. The father's height was 170 cm (17th percentile) (9). At 26 weeks gestation, an ultrasound showed bowing of both femurs. Patient 3 was born via caesarean delivery, without respiratory distress at birth.

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Table 1. Clinical characteristics of kindred cases

	Patient 1 Mother	Patient 2 Older sibling	Patient 3 Younger sibling
Current age	39 y	17 y	36 m
Current height (percentile)	25	75	27
Fractures, age	R femur, < 5 m L femur, < 5 m	R femur, 3 d R humerus, 6 d L humerus, 6 d R femur, 6 d L femur, 6 d L femur, 4 w	L 9th rib, 1 d R 8-10th rib, 2 w R tibia, 5 w R femur, 5 w L femur, 5 w R femur, 6 w L femur, 6 w L tibia, 15 w L5 compression, 21 m
Total fractures, n	9	6	11
BMD (g/cm²), age	N/A	14 m Lumbar spine: 0.51 L hip: 0.57	**12 m** Lumbar spine: 0.44 TBLH: 0.34 **28 m** Lumbar spine: 0.54 TBLH: 0.46
Bisphosphonate treatment	No	Yes Pamidronate	Yes Zoledronic acid
Total infusions, n	-	8	6
Age at last infusion	-	18 m	28 m
Sclerae	Normal	Normal	Normal
Dentition	Normal	Normal	Normal
Hearing	Normal	Normal	Normal
Echocardiogram	Normal	N/A	N/A
Spinal deformity	None	None	None
Extremity deformity	None	None	None
Joint mobility	Normal	Normal	Normal
Easy bruising	None	None	None
Genotype	c.3548C > T; p.(Pro1183Leu) Heterozygous	c.3548C > T; p.(Pro1183Leu) Heterozygous	N/A

y: years; m: months, d: days, w: weeks, R: right, L: left, L5: 5th lumbar vertebrae, BMD: bone mineral density, N/A: not available, TBLH: total body less head

His birth weight was 3.8 kg (75th percentile), length was 48 cm (25th percentile) and head circumference was 36.5 cm (65th percentile). Radiographic studies on the first day of life confirmed left posterior ninth rib fracture and femoral bowing bilaterally (Figure 2). Wormian bones were noted along the lambdoid sutures. He had nontraumatic fractures of the eighth through tenth ribs on the right side at two weeks, right tibia and bilateral femur fractures at five weeks, bilateral femur fractures at six weeks (distinct locations each time) and left tibia fracture at 15 weeks.

Physical examination showed that he had white sclerae and no dysmorphic features. His hearing screen was normal.

Serum ionized calcium (5.89 mg/dL; reference range 3.9-6.0 mg/dL), phosphorus (6.3 mg/dL; reference range 4.5-9.0 mg/dL), total alkaline phosphatase (250 U/L; reference range 150-420 U/L) and 25-hydroxy-vitamin D (37 ng/mL; reference range ≥20 ng/mL) concentrations were normal. Zoledronic acid infusions were initiated every three months from age three weeks to nine months (0.0125 mg/kg for the first dose, 0.025 mg/kg for the next three doses, each infused over 60 minutes). The fracture at age 15 weeks occurred after he had received two infusions of zoledronic acid. Given the paucity of further symptoms of OI and the fact that his mother and older sibling had no fractures after

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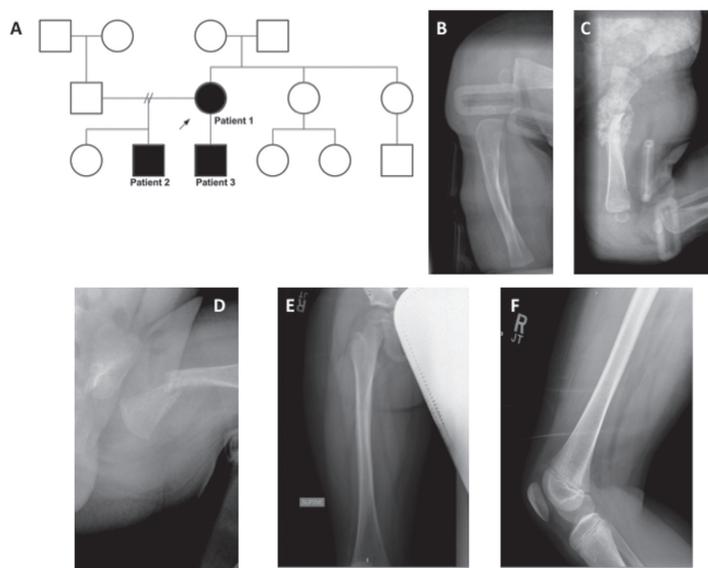
Figure 1. (A) Pedigree chart. The index case is indicated by the arrow. (B-F) Radiographs of Patient 2. (B) Bowing of midright tibial shaft at age three weeks, lateral view. (C) Abundant callus formation around a healing midshaft right femoral fracture at age three weeks, lateral view. (D) Acute proximal-shaft left femoral fracture at age four weeks, anteroposterior view. (E) Right femur anteroposterior view at age 10 years. (F) Right femur lateral view at age 10 years
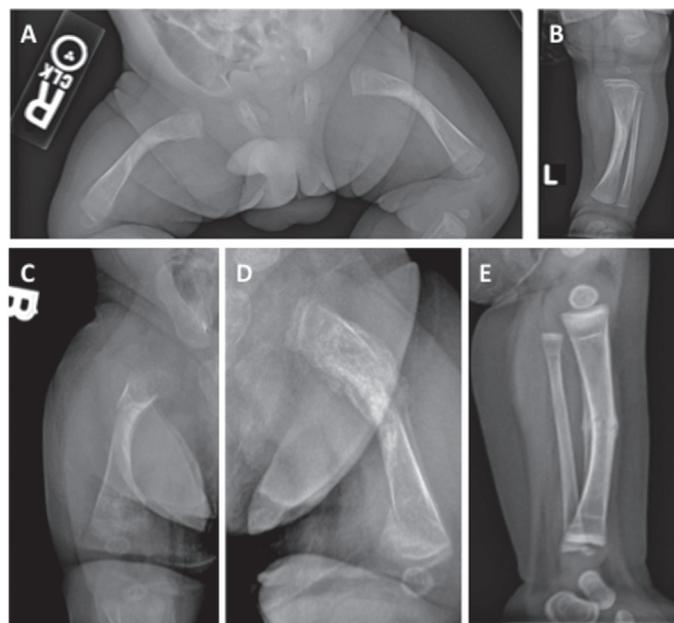
Figure 2. Radiographs of Patient 3. (A) Proximal femoral shaft bowing, right greater than the left, at age two weeks, anteroposterior view. Note the mild diffuse osteopenia. (B) Left tibial midshaft bowing at age two weeks, anteroposterior view. (C) Proximal right femoral fracture at age six weeks, lateral view. (D) Periosteal reaction and callus formation around a healing proximal left femoral fracture sustained at age six weeks, lateral view. (E) Transverse midshaft fracture of left tibia at age 15 weeks, lateral view

the first few months of life, bisphosphonate therapy was discontinued when he was nine months old.

At age 21 months, he was noted to have a new mild compression of the anterior-superior endplate of the 5th lumbar vertebral body (L5). He also had a mildly displaced oblique fracture of left distal tibial metaphysis due to moderate trauma (his leg was caught on the edge of a slide while sitting on his father's lap) at age 25 months. He was treated with two additional infusions of zoledronic acid at 21 months and 28 months (a total of six infusions). He has not sustained additional low-trauma fractures since. The mild L5 vertebral body deformity was less apparent on subsequent imaging seven months later.

He achieved normal developmental milestones, including appropriate dentition for age. Baseline BMD was obtained at 12 months (lumbar spine 0.44 g/cm^2; total body excluding head 0.34 g/cm^2; reference range not available for this age group) (10). A follow-up BMD at age 28 months after six zoledronic acid infusions revealed an improvement of 23.7% in spinal bone density (0.54 g/cm^2) and 36.5% in total body, excluding head, bone density (0.46 g/cm^2), compared to baseline. As of manuscript writing, he was 36 months old and at 27th percentile for length and 81st percentile for weight. His corrected midparental height was at the 20th percentile.

DNA Sequencing and *in silico* Analysis

Clinical information was collected from the patients and abstracted from the medical records. Conformation-sensitive gel electrophoresis (CSGE) was performed by Matrix DNA Diagnosis (New Orleans, LA, USA). The variant was described using the Human Genome Variation Society nomenclature (11). The pathogenicity of the identified variant was analyzed using the *in silico* prediction software, Alamut Visual (missense predictors: Align GVGD v.2007, SIFT v.6.20 and PolyPhen-2 HumVar; splicing algorithms: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and HSF) (12).

Results

Identification of the *COL1A1* Variant

Given the concern for OI in Patient 2, CSGE was performed for the *COL1A1*- and *COL1A2*- coding exons. The CSGE analysis identified a heterozygous missense alteration in exon 49 of the *COL1A1* gene resulting in a C to T nucleotide substitution that converted a Pro (CCT) to a leucine (CTT), [NM_000088.3:c.3548C > T; p.(Pro1183Leu), (RefSeqGene NG_007400.1:g.19734C > T)].

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The patient's mother was heterozygous for the same variant and no variations were present in the father. The variant was classified as 'variant of uncertain significance'. *In silico* analysis was equivocal for potential pathogenicity of this variant (Align GVGD - Class C65 deleterious; SIFT - deleterious; PolyPhen-2 - benign). No significant changes were noted in canonical splice sites.

Discussion

We describe a kindred with a unique presenting phenotype of OI. The OI was presumably attributable to a missense variant c.3548C > T; p.(Pro1183Leu) that replaced Pro with leucine in the Y position of the glycine-Xaa-Yaa sequence of the proα1(I) chain (exon 49 of *COL1A1*). The same variant has been reported previously in only one individual [c.3548C > T; p.(Pro1183Leu)] (6). The referral reason for testing that individual was reported as symptoms of OI and a strong family history of recurrent femoral fractures and osteopenia. The variant was designated to be of unknown pathogenicity, and further family studies to clarify pathogenicity were requested. As of manuscript writing, that patient was 49 years old, and updates on the patient and results from family studies could not be obtained (personal communication, Dr. Meena Balasubramanian, 2018). As more individuals with this specific variant are identified, the clinical phenotype of this form of OI will become clearer.

Lim et al (13) described a patient with Ser40Trp variant in *IFITM5* gene, who presented with multiple fractures in the prenatal period. She remained fracture free after birth with normal BMD. She had blue sclerae, progressive lower limb deformities during childhood and severe short stature (standard deviation -3.5). Her mother, who did not have a history of fractures, was noted to have somatogonadal mosaicism for this variant and became pregnant with a second child with multiple prenatal fractures, found to have the same variant. This is in contrast with our patients who had normal sclerae, absence of postnatal limb deformities, normal stature and a variant in the *COL1A1* gene, although with similar phenotypic bone deformity pattern.

Type 1 procollagen helical domains contain a repetitive glycine-Xaa-Yaa sequence, where Xaa and Yaa are often the amino acids Pro or hydroxyproline (Hyp) (14,15). Some evidence suggests that Pro and Hyp are responsible for the thermal stability of the triple-helix structure (16). Bryan et al (17) hypothesized that a missense variant in the highly stable region of the collagen molecule, such as Gly-Pro-Hyp, will lead to a greater disruption and more severe clinical consequences than the same missense variant in a less stable region. Collagen chains with these abnormal molecules are overmodified and secreted inefficiently, which disrupts helix stability (18).

Various factors have been proposed to explain how the substitution of one amino acid for another affects collagen processing and the phenotype of OI, including the coordinates of the variant and its position relative to the C-terminus of the propeptide (5,14,19). The proximity of a variant to the C-terminus of the molecule is important because assembly of the collagen triple-helix chain begins at the C-terminal end and propagates toward the N-terminal end (20). Our kindred harbored a variant close to the C-terminus, at amino acid position 1183 on exon 49 of the *COL1A1* gene. Exon 49 is 283 base pairs long (cDNA base numbers 3532-3814) and encodes the last 15 amino acids of the triple-helix region (amino acids 1178-1192), the entire C-telopeptide (amino acids 1193-1218) and part of the C-propeptide domain (amino acids 1219-1271). A small number of point variants in the C-terminal propeptide have been identified as causing varying clinical phenotypes of OI, ranging from mild to lethal and high bone-mass OI (18,19,21). Although missense variants in the C-propeptide are known to impair or prevent proα chain assembly in the endoplasmic reticulum (19), the outcome of each substitution may differ, depending on the specific gene transcript.

For patients with milder forms of OI, the incidence of fracture declines with age but does not stop entirely (8). Interestingly, cessation of OI associated fractures after the first two years of life, as seen in our patients, has not been described in the literature in association with *COL1A1* variants. The pathophysiology resulting in this phenotype remains uncertain. There is evidence that posttranslational modification of procollagen is temporally regulated, and this regulation may be crucial for its folding, secretion, and extracellular matrix assembly (22). We speculate that a sequential improvement in these processes during the first two years of life in our patients resulted in paucity of fractures thereafter.

Finally, the long-term benefit of intravenous bisphosphonate therapy in our patients remains unclear, particularly because Patient 1 did not receive this therapy and did not sustain any nontraumatic fractures after five months of age. Patient 2 received his last infusion of pamidronate at age 18 months. At age 17 years, he was of normal adult height and remained fracture free, despite an unrestricted, active lifestyle. We speculate that prolonged bisphosphonate therapy may not provide additional benefit to those with this form of OI, although our sample size and duration of follow-up is too small to draw a definitive conclusion.

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Our case series has certain other limitations including lack of genetic testing for variants in other OI-related genes such as *IFITM5*, which have been associated with a phenotype similar to that seen in our patients. Patient 1 and Patient 2 underwent genetic testing almost 17 years ago. To our knowledge, at that point in time, typical OI genetic testing included primarily *COL1A1* and *COL1A2* genes. Currently, we do not have the funding available to update genetic testing for Patient 1 and Patient 2, or send genetic testing for Patient 3 for research purposes. It would have entailed a huge economic burden on the family and the genetic testing results were unlikely to influence clinical decision making.

Conclusion

We describe a three-patient kindred with a unique phenotype of OI, presumably due to a variant in the *COL1A1* gene. The OI phenotype of affected individuals included a pattern of mild-moderate bone deformities prenatally and multiple nontraumatic fractures limited to the first two years of life. We speculate that the Pro-to-leucine substitution in close proximity to the C-propeptide domain might have influenced folding of the triple helix or helix stability. Given the small number of patients in our kindred, the full range of phenotypes associated with this variant remains to be established. Whether antiresorptive therapy is beneficial later in life also remains unclear.

Ethics

Informed Consent: Informed verbal consent was obtained from the mother.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Concept: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Design: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Data Collection or Processing: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Analysis or Interpretation: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Literature Search: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben, Writing: Nidhi Gupta, Seth W. Gregory, David R. Deyle, Peter J. Tebben.

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