Osteogenesis Imperfecta Type I Caused by a Novel Mutation in the Start Codon of the COL1A1 Gene in a Korean Family

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Abstract. Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by susceptibility to bone fractures ranging in severity from perinatal death to a subtle increase in fracture frequency. We report the case of a patient who appeared healthy at birth and did not experience any fractures until 12 months of age. We observed blue sclera, frequent fractures without commensurate trauma, nearly normal stature, the absence of dentinogenesis imperfecta, no bony deformity, and no limitation of mobility in the patient – all characteristics suggestive of OI Type I. The patient’s mother also had blue sclera and a history of frequent fracture episodes until the age of 15 years. A novel COL1A1 missense mutation (c.2T>G) disrupting the start codon of the gene (ATG to AGG (Met1Arg)) was found in the patient and his mother.

Keywords: osteogenesis imperfecta, osteogenesis imperfecta Type I, COL1A1, Met1Arg

Introduction

Osteogenesis imperfecta (OI) comprises a heterogeneous group of disorders characterized by susceptibility to bone fractures that range in severity from perinatal lethality to a subtle increase in fracture frequency. The reported incidence of OI varies from approximately 1/100,000 to 1/25,000 [1,2]. Almost all patients have presumed or proven defects in Type I (pro)collagen biosynthesis [3]. In 1979, David Sillence developed the following four-Type classification that is still in use for classifying clinical/radiological features: OI Types I (mild OI with bone fragility and blue sclera), II (perinatal lethal), III (progressive deforming), and IV (normal sclera and mild deformity) [4,5]. OI Type I is the most common and is usually mild and non-deforming. Dominant mutations of COL1A1 or COL1A2, which encode collagen Type I alpha chains, appear to cause the majority of OI types.

Type I collagen is the major structural protein of the bone, skin, and other tissues, and consists of two alpha-1 chains and one alpha-2 chain [6]. Each chain contains 338 units of uninterrupted repeats of the Gly-X-Y triplet. The conserved glycine residue at every third position of triple-helical domains is essential for the alpha chains to intertwine correctly and for the integrity of the protein, whereas the X and Y may be any amino acid residue [7,8]. Patients with a mild phenotype often have a strong family history of OI, although sporadic cases have been reported. In contrast, severe phenotypes are usually de novo and are most commonly associated with glycine substitutions in the helical domain of the collagen molecule, which are predicted to disrupt protein folding and structure. Frameshift and nonsense mutations, which usually result in reduced synthesis of normal collagen, bring about a more predictable and milder phenotypic outcome.

A 3-year-old boy was suspected of having OI Type I due to the following clinical evidence: spontaneous fracture without trauma event, blue sclera, and family history. In the present study, we describe the clinical and radiological findings of one Korean family with a novel mutation in the start codon of COL1A1 (c.2T>G).
Case Description

The patient was born after 39 weeks’ gestation by cesarean section. His birth height was 46 cm (10–25th percentile) and his birth weight was 2,640 g (10–25th percentile). No limb deformities or other abnormalities, including joint hyperlaxity or skin hyperelasticity, were noted at birth. The patient was born from non-consanguineous parents. He was able to sit independently at 9 months and walk independently at 12 months.

The patient first visited a hospital at 12 months of age (2010-11-13) due to a spontaneous fracture in the right femur midshaft (Figure 1A). At that time, his height was 78.6 cm (50–75th percentile) and his weight was 9.5 kg (25–50th percentile). The data from biological tests (i.e., routine blood cell count, blood and urinary levels of calcium, phosphate, creatinine, serum alkaline phosphatase, 25-hydroxy vitamin D, parathyroid hormone, and urine analysis) appeared within normal ranges, while the serum level of the bone resorption marker Type I collagen telopeptide was slightly above normal values. The fracture of the midshaft in the right femur was in a healing state when the patient was aged 15 months (Figure 1B). After the first fracture, the patient frequently experienced non-traumatic fractures. No radiographic signs of rickets were observed. The history of frequent fractures without apparent injury suggested bone fragility; blue sclera and the radiological features described below led to a diagnosis of OI.

Taking the patient’s history, we came to know that his mother had had about 10 episodes of nontraumatic fracture of various bones, including the left humerus, right ankle, and most phalanges up to the age of 15 years. However, she had not experienced any fracture for 20 years, up to the age of 35 years. The mother’s parents did not have history of fractures or blue sclera. She had not received therapy with pamidronate. Her height and weight were 158 cm, and 65 kg, respectively. Like the patient, she had blue sclera. Her visual acuity was normal, and she did not show hearing impairment, joint movement limitations, bone deformity, or laboratory abnormalities. She was suspected of having OI because the proband was suspected to have OI. The proband’s father and his younger brother were healthy and had white sclera. Based on these findings, including frequent fractures, family history, blue sclera, and no bony deformities, we classified the present case as OI Type I.

In radiological findings from when the patient was aged 2 years 2 months, no visible signs of fractures were seen; however, the height of spine at T11 and L2 were mildly decreased (Figure 1C, 1D, and 1E). These findings were

Figure 1. A. Right femur radiogram taken at the age of 12 months shows a fracture of the midshaft in the right femur. B. Right femur radiogram taken at the age of 15 months shows healing state of the fracture of the midshaft in the right femur. C. Radiographs of the right and left leg when the patient was 3 years 2 months old show bilateral coxa valga and mild osteoporotic features. An old fracture line at the midshaft of right femur is evident. D&E. Anterior-posterior and lateral views of the spine of the patient at the age of 3 years 2 months shows generalized osteopenia and mildly decreased height of the spine at T11 and L2, which might be sequelae of previous fractures. Neither scoliosis nor kyphosis is seen.

Novel mutation in the start codon of COL1A1, causing OI Type I
considered sequelae of previous fractures. In addition, generalized osteopenia was seen. There were no Wormian (intrasutural) bones in the skull. Among the five fractures in his lifetime until the age of 3 years, no case of fractures led to surgical intervention or bony deformity. In radiograms taken when the patient was aged 3 years 2 months, the forearm showed no sign of calcification of the interosseous membrane or bilateral dislocation of the radial heads.

In order to identify the genetic cause in this patient with OI Type I, blood samples were collected from the patient and his parents after informed consent had been obtained. This study was approved by the Institute Review Board Committee at the Samsung Medical Center.

**Mutations in COL1A1.** COL1A1 exons and their flanking introns were amplified using primer sets designed by the authors (available upon request). PCR was performed with a thermal cycler (Veri, Applied Biosystems, Foster City, CA) as follows: 32 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. After treatment of the amplicon (5 µL) with 10 U shrimp alkaline phosphatase and 2 U exonuclease I (USB Corp., Cleveland, OH), direct sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) on the ABI Prism 3730xl genetic analyzer (Applied Biosystems). To describe sequence variations, we followed the guidelines of the Human Genome Nomenclature Committee (HGVS); so “A” of the ATG translation start site was numbered +1 for the DNA sequence and the first methionine was numbered +1 for the protein sequence.

Direct sequencing analysis of the COL1A1 gene identified one novel heterozygous variation, c.2T>G, in the patient; his mother had the same variation (Figure 2). Neither the father nor the younger brother of the patient had the variation. These variants were not found in 98 control chromosomes. Methionine is well conserved among different mammalian species (Figure 3).

The variation was analyzed by in silico analysis as the likely cause of the disorder. The c.2T>G variation showed a “possibly damaging change” in the PolyPhen prediction of the functional effect of human non-synonymous single nucleotide polymorphisms (nsSNPs) (http://genetics.bwh.harvard.edu/pph2/). The c.2T<G was also predicted to be “not tolerated” on in silico prediction using SIFT (http://sift.jcvi.org/www/SIFTing_databases.html).

**Discussion**

This is the first delineation of a heterozygous mutation in the start codon of COL1A1 causing OI Type I. Our patient appeared healthy at birth and did not suffer any fracture until 12 months of age. Blue sclera, frequent fractures without commensurate trauma, nearly normal stature, the absence of dentinogenesis imperfecta, no bony deformity, and no limitation of mobility were characteristics...
Novel mutation in the start codon of COL1A1, causing OI Type I. A skeletal survey of our patient showed general osteoporosis and sequelae of previous fractures, also characteristic of OI Type I. Our patient had experienced five fractures over his three years of life, and no episodes of fractures required open reduction. No progressive limb or spine deformities had been evident at his recent evaluation at the age of 3 years. The patient’s mother also had blue sclera and history of frequent fracture episodes until the age of 15 years. This family is consistent with a dominant pattern of inheritance of OI Type I.

In infants or children who present with unexplained or multiple fractures, the differential diagnosis includes the infant or child having an inherent predisposition to skeletal fractures [9,10], the most common of which is OI [11]. In one study of OI diagnosed at an age below 18 years, the corresponding NCBI 36 human genome and the position of the first amino acid (M, methionine; from UCSC genome browser). Methionine is conserved among different mammalian species.
years [12], a family history of OI was present in 73.9% of OI Type I cases, and age at diagnosis was between birth and 12 months in 43.5% of OI Type I cases. Fracture number in infants diagnosed after birth and by 12 months was just under one. The main diagnostic features present at diagnosis of OI Type I were family history, blue sclera, and osteopenia.

OI usually results from structural or quantitative changes in Type I collagen proteins encoded by the COL1A1 and COL1A2 genes. The mutation profiles of these genes are not restricted to any specific region, but are scattered throughout the entire structural domains and show enormous diversity [13]. A large number of different COL1A1/ COL1A2 mutations have been found, and the functional and phenotypic consequences of many types of mutations vary widely [14]. Exceptions to this rule are nonsense or frameshift mutations in COL1A1, which are consistently associated with an OI Type I phenotype, the mildest form of OI [15]. At a molecular level, nonsense or frameshift mutations in COL1A1 usually trigger nonsense mediated mRNA decay and result in collagen Type I haploinsufficiency [16]. Only about half of the normal amount of collagen Type I protein is produced, although the structure of the produced collagen Type I protein is thought to be normal. These functional consequences of the mutation are largely independent of where in the gene the mutation occurs. Therefore, haploinsufficiency mutations are thought to have a rather homogeneous downstream effect.

One missense mutation was identified in our patient with OI Type I, Met1Arg (c.2T>G), which resulted in a substitution of the initiating amino acid methionine. This mutation was detected in the patient and his mother, who also had OI Type I features, but not in his father, brother, or control samples. The mutation was a T-to-G nucleotide transition in the start codon of COL1A1, which changed the codon from ATG (methionine) to AGG (arginine) (ATG >AGG, 1M>R). The start codon mutation could lead to the absence of protein translation in the ribosome, and therefore a lack of COL1A1 synthesis resulting in collagen Type I haploinsufficiency.

The mutation affecting the ATG start codon has not been reported previously in the COL1A1 gene, and these kinds of mutations are rare. In mammals, the ATG (AUG in RNA) start codon is typical for ribosomal translation and it is recognized most efficiently when it is located in a favorable nucleotide context known as the Kozak consensus sequence (in particular, a purine at position _3 and G at position þ4, RccAUGG (R¼purine)) [17]. The augmenting effect of G at position þ4, however, is diminished when it is combined with U at position þ5 [17]. Very rarely in higher organisms, non-AUG codons may also initiate translation, but this does not include AGG or AAG [18]. The mutation c.2T>G produced an AGG codon, which is not suitable to initiate translation and therefore explains the functional consequence of this mutation. It remains to be seen whether loss of the initiating methionine would lead to the use of a later methionine for initiation. It is rarely possible that when the consensus is weak or disrupted, the ribosomal translation starts from a secondary downstream AUG codon, a process called leaky scanning. Moreover, the downstream AUG is more than 540 nucleotides away in the COL1A1 gene. Even if translation was initiated from this codon, the translated protein would result in an incomplete or truncated protein that would lack the N-terminal leading signal peptide.

OI is a worldwide hereditary metabolic bone disorder, and currently there are no effective therapies for the affected individuals [19]. Thus, the genetic diagnostic technique may be important for providing families with accurate information and appropriate counseling regarding the health of the fetus. In summary, we reported a novel mutation in the start codon of COL1A1 disrupting the start codon of the gene (ATG to AGG (Met1Arg)) in a Korean family with OI Type I. Identification of this mutation will expand understanding and significance of the start codon of COL1A1 gene in the pathogenesis of OI Type I.
References

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