

A novel frameshift deletion in the *COL1A1* gene identified in a Chinese family with osteogenesis imperfecta

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ABSTRACT. Osteogenesis imperfecta (OI) is a genetically heterogeneous group of disorders, characterized by abnormal bone fragility, blue sclera, deafness, joint laxity, and soft-tissue dysplasia. The purpose of this study was to elucidate the genetic or molecular basis for OI type IA in a Chinese family. We evaluated the members of a family, in which six individuals are affected with increased bone fragility and blue sclera. Results of exome sequencing revealed a

novel 1-bp deletion (c.2329delG, p.A777fs) in exon 33 of the *COL1A1* gene in two affected individuals, but not in a control family member without OI. The variation co-segregated with the disease in all the OI patients but not in the unaffected family members. The mutation caused a frameshift alteration after codon 777, leading to premature termination of the COL1A1 protein. Thus, our findings identified a novel frameshift deletion c.2329delG (p.A777fs) in the *COL1A1* gene, which is associated with OI type IA in a Chinese family.

Key words: Osteogenesis imperfecta; Collagen; *COL1A1* gene; Frameshift deletion

INTRODUCTION

Osteogenesis imperfecta (OI), also known as fragilitas ossium, idiopathic osteopsathyrosis, or periosteal dysplasia, is a genetically heterogeneous group of disorders. It is characterized by abnormal bone fragility, blue sclera, deafness, joint laxity, and soft-tissue dysplasia (Sillence et al., 1979; Sillence, 1988). As a rare congenital systemic disease, its estimated prevalence ranges between 1/10,000 and 1/20,000 (Forin, 2007). The severity of skeletal and extra-skeletal manifestations varies widely. Based on the clinical phenotype and disease severity, the Sillence classification differentiates four types (I-IV) of OI (Sillence et al., 1979; Sillence, 1988). Type I is a mild form of the disease with characteristic blue sclera; type II (perinatal lethal) and type III (progressive deformation) are subgroups with more severe phenotypes, while OI type IV demonstrates only a moderate phenotype with a normal appearance of the sclera. Recently, new types of OI (V-XII) with unique clinical and histological features have been added to the spectrum of OI (Glorieux et al., 2000, 2002; Ward et al., 2002; Forlino et al., 2011).

In approximately 95% of all patients with OI, the disease is caused by a dominant mutation in the gene encoding collagen I, which is the most abundant protein in connective tissues (Sykes, 1993; Rauch and Glorieux, 2004; Lindahl et al., 2008). Collagen I is comprised of two alpha 1(I) chains and one alpha 2(I) chain, encoded by the *COL1A1* and *COL1A2* genes, respectively (Dickson et al., 1984; Lindahl et al., 2008). The inheritance mode of OI types I-IV is autosomal dominant. The majority of patients have heterozygous mutations in *COL1A1* and/or *COL1A2*, with more than 60% mutations found in the *COL1A1* gene (Dickson et al., 1984; Cohn et al., 1986). In the Leiden Open Variation Database (a web-based open-source database), 1377 variants of *COL1A1* were reported as of March 2014 (https://oi.gene.le.ac.uk/home.php?select_db=COL1A1). Some of them have been shown to be associated with OI. However, this is the first time, to the best of our knowledge, that a frameshift deletion (p.A777fs) in the *COL1A1* gene has been identified to be associated with a family with OI type IA.

MATERIAL AND METHODS

Four generations in a Chinese family with OI type IA from the Province of Sichuan in southwestern China underwent clinical and molecular genetic examination (Figure 1). The Review Board of Shenzhen Eye Hospital, Jinan University, approved this study. A written informed consent was obtained from each of the participants.

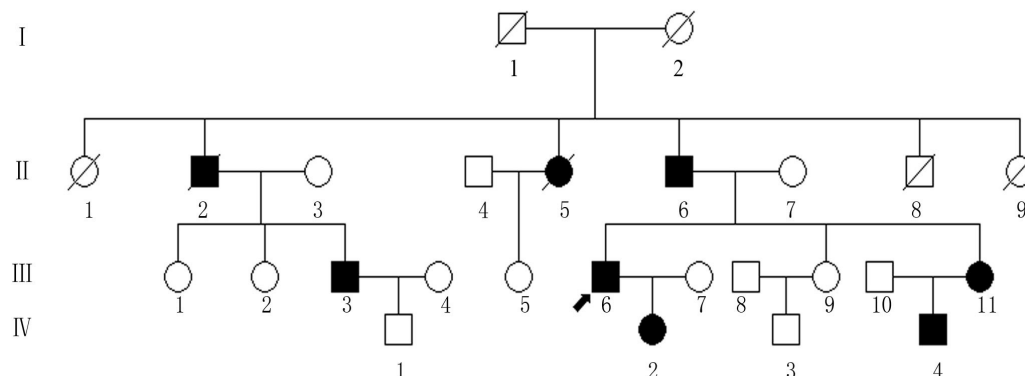


Figure 1. Pedigree of the family with osteogenesis imperfecta (Type I). Solid symbols: affected individuals; open symbols: unaffected individuals; arrow: proband; slash: deceased person.

Peripheral blood was collected for DNA analysis from twelve family members. Genomic DNA was extracted from 200 μ L peripheral venous blood using the QiAmp Blood DNA mini Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. The DNA samples were stored at -20°C until analysis. DNA integrity was evaluated by 1% agarose gel electrophoresis. Exome sequencing was performed for two patients (III:6 and IV:4) and one unaffected family member (III:9) as a control (Figure 1). After extracting 4 μ g human genomic DNA from the peripheral venous blood samples, the IlluminaTruSeqExome Enrichment Kit (62 Mb) (Illumina, San Diego, CA, USA) was used to collect the protein coding regions of the DNA. The kit included >340,000 95 mer probes, each constructed against the human National Center for Biotechnology Information (NCBI) Build37/hg19 reference genome. The probe set was designed to enrich >200,000 exons, spanning 20,794 genes of interest. The sequencing reads were aligned to the human reference genome (NCBI Build 36.3). Identified variants were filtered against the single nucleotide polymorphism database (dbSNP 129, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), and the 1000 genome project (www.1000genomes.org/, consisting of 1094 individuals from the 20101123 sequence and alignment release of the 1000 genomes project) and HapMap 8 (<http://hapmap.ncbi.nlm.nih.gov/>) databases. As the variant in the disease-causing gene COL1A1 was identified that shared by two affected individuals, Sanger sequencing was applied to determine whether this variant co-segregated with the disease phenotype in this family. Primers flanking the coding exons 33-35 of COL1A1 were designed based on the genomic sequences of the human genome (Build36.3/hg18) and synthesized (BGI-Guangzhou Co., Shenzhen, Guangdong, China). Forward primer: CTCTCAGGAAACCCAGACACA; Reverse primer: TTGTCCTCATTCCGTCCTC. PCRs were performed using the following program: initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 40s, and final extension at 72°C for 5 min. Sequencing data were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

RESULTS

All affected individuals in the family (II:6, III:3, III:6, III:11, IV:2, and IV:4) showed

an increased bone fragility and blue sclera, and were without dentinogenesis imperfecta. The proband (III:6, Figure 1), a 53-year old male, presented with a history of multiple bone fractures, blue sclera, hearing loss, loose joints, and slight abnormal spinal curvature, but without dentinogenesis imperfecta. He had two non-traumatic fractures including a fracture of his left ulna at the age of six years. His 25-year old daughter (IV:2) presented with an increased bone fragility, blue sclera, and otosclerosis, but without hearing loss or abnormal teeth. She had experienced three pathological fractures without trauma at the age of one, three, and ten years. The proband's father (II:6), sister (III:11, Figure 2), and the additional family members tested (III:3 and IV:4) had similar clinical manifestations with increased bone fragility, blue sclera, and healthy teeth. None of the other family members showed any symptoms of an increased bone fragility or blue sclera.

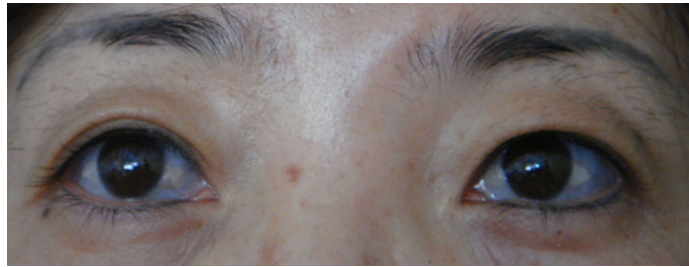


Figure 2. Blue sclera of the patient with osteogenesis imperfecta (III:11).

After being filtered against several public databases and removal of all previously reported variants, 110 single nucleotide variants were identified in the two patients (III:6, IV:4) but not in the control (III:9). The *COL1A1* gene, recognized as the most common candidate gene of OI, was in the list of the remaining genes. Sanger sequencing revealed that one variant (c.2329delG) of *COL1A1* co-segregated with the phenotype in the family (Figure 3A and B). This variant was a novel heterozygous 1-bp deletion, and was identified in exon 33 of the *COL1A1* gene in all patients. The mutation (p.A777fs) caused a frameshift alteration after codon 777 and resulted in a truncated protein at codon 1107.

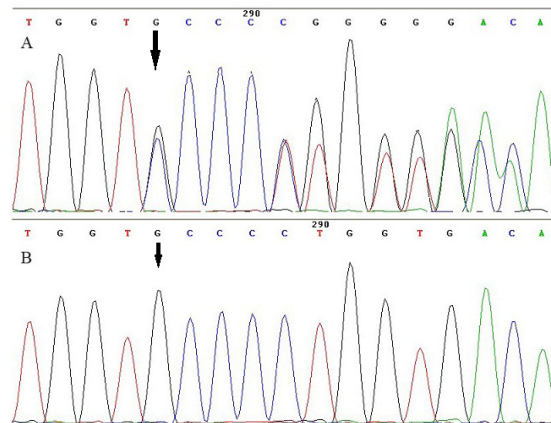


Figure 3. *COL1A1* Sequence results from the affected subject (III:6, c.2329delG) (A) and the control (III:8, wild type) (B). Arrows indicate the nucleotide changes.

DISCUSSION

In this Chinese pedigree, all family members affected with OI type IA showed a novel frameshift deletion in the *COL1A1* gene. All the patients revealed an abnormally high bone fragility and blue sclera without dentinogenesis imperfecta. In addition, a frameshift deletion in the *COL1A1* gene was identified in all affected individuals, so that both phenotype and genotype showed a pedigree typical for OI type IA.

Type I is the most common and the mildest form of OI, and is characterized by blue sclera, increased bone fragility, minor skeletal deformities, and autosomal dominant inheritance (Sillence et al., 1979; Sillence, 1988). The occurrence of dentinogenesis imperfecta is used to classify patients into OI type IA (without dentinogenesis imperfecta) or OI type IB (with dentinogenesis imperfecta) (Sillence et al., 1979; Paterson et al., 1983; Sillence, 1988). It has remained unclear whether the phenotypes of OI types IA and IB are resultant from different types of collagen mutations. Although the *COL1A1* gene is the predominant locus for OI (Dickson et al., 1984; Cohn et al., 1986), previous studies have suggested that *COL1A2* mutations might be a major cause of OI type IB (Mundlos et al., 1996). The 100% penetrance for blue sclera and the age-dependency of the penetrance of hypacusia might explain why patient III:6 presented with hearing loss in this pedigree, while the other younger patients had no hearing impairment (Garretsen and Cremers, 1991).

Patients suffering from OI are born with symptoms due to defective connective tissues usually because of a deficiency of collagen, or because the gene is a prenatal lethal factor (Sykes, 1993; Rauch and Glorieux, 2004). Previous studies have shown that such collagen deficiency resulted from an amino acid substitution of glycine to bulkier amino acids in the collagen triple helix structure. The larger amino acid side-chains cause a steric hindrance that creates a bulge in the collagen complex, which in turn influences both the molecular mechanics as well as the interaction between molecules (Gautieri et al., 2009). As a result, the tissue may respond by hydrolyzing the abnormal collagen structure. If the abnormal collagen is not hydrolyzed, the molecular interaction between the collagen fibrils and the hydroxyapatite crystals involved in bone formation is altered, which leads to brittleness.

The *COL1A1* gene is located on chromosome 17q21.33 and consists of 51 exons; it encodes the collagen alpha-1(I) chain with 1464 amino acid residues. The main structure of the collagen chain is a triple helix repeat and the sequence consists predominantly of repeats of the G-X-Y triplet, with the polypeptide chains forming a triple helix. The first position of the repeat is glycine, while the second and third position can be any residue but frequently are proline or hydroxyproline. Mutations of *COL1A1* might affect the collagen biosynthesis pathway and influence the extracellular matrix-receptor interaction (Gajko-Galicko, 2002). Therefore, *COL1A1* might play a significant role in the pathogenesis of OI.

The c.2329delG mutation in exon 33 of the *COL1A1* gene causes a frameshift alteration after codon 777 (alanine), leading to a premature termination codon at codon 1107. The encoded truncated protein is thus likely to be unstable and degraded by the nonsense-mediated mRNA decay mechanism. Alternatively, structurally altered chains might disturb the formation of the collagen triple helix, thus secondarily affecting the function of the normal chains. In conclusion, this study identified a novel frameshift deletion (c.2329delG; p.A777fs) in the *COL1A1* gene in a Chinese family with OI type IA.

Conflict of interest

The authors declare no conflict of interest.

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