A novel \textit{COL1A1} nonsense mutation causing osteogenesis imperfecta in a Chinese family

Wei Liu,1,2,3 Feng Gu,4,5,6 Jian Ji,1,2,3 Duanyang Lu,1,2,3 Xiaorong Li,1,2,3 Xu Ma 4,5,6

(The first two authors contributed equally to this publication)

1Tianjin Medical University Eye Center, 2International Intraocular Implant Training Center, 3Tianjin Medical University, Tianjin, China; 4Department of Genetics, National Research Institute for Family Planning, 5Peking Union Medical College, 6Department of Reproductive Genetics, WHO Collaborative Center for Research in Human Reproduction, Beijing, China

Purpose: To identify the genetic defect of osteogenesis imperfecta (OI) type I in a large Chinese family of five generations.

Methods: Seventeen members in an OI type I family were recruited, and clinical examinations were performed. All members were genotyped with microsatellite markers at loci associated with OI. A two-point LOD score was calculated using the Linkage package. A mutation was detected by direct sequencing.

Results: All affected individuals in the family had fractured a bone more than once, and their sclerae were blue. Significant evidence of linkage was obtained at markers D17S1180 (LOD score [Z]=2.91, at recombination fraction [θ]=0.0) and D17S1319 (Z=2.2, at θ=0.0), respectively. Sequencing of the \textit{COL1A1} gene revealed a C>T transition in exon 36, which caused a substitution of Gln at codon 644 to a stop codon (Q644X). This mutation was not observed in unaffected or 100 normal unrelated individuals.

Conclusions: This study is the first report that OI is associated with the mutation Q644X of \textit{COL1A1}.

Osteogenesis imperfecta (OI) is an autosomal-dominant connective tissue disorder characterized by brittle bones together with associated features such as short stature, dentinogenesis imperfecta, hyperlaxity of ligaments and skin, blue sclera, and hearing loss [1]. The ocular manifestations of OI are not usually sight threatening and typically consist of blue sclerae. Other ocular manifestations that may occur include perinuclear or cortical cataract, megalocornea, microcornea, keratoconus, nystagmus, glaucoma, ptosis, etc. The minimum prevalence of OI is estimated to be 1/10,000-1/30,000 [1,2]. Traditionally, it is usually classified into four types depending on phenotype and severity [3,4]. Type I is the most common and mildest deforming. Type II is the most severe and is lethal in the perinatal period. Type III is severely deforming, and fractures are often present at birth. Type IV is moderately deforming; its severity is between Type I and Type III.

More than 250 mutations in one of the two genes, \textit{COL1A1} and \textit{COL1A2}, which encode the proα1 and proα2 chains of collagen I, respectively, have been found in unrelated probands with the disease. Most of these mutations are single nucleotide changes, mostly resulting in a substitution for a glycine residue and disrupting the continuous Gly-X-Y sequence of the collagen triple helix. Others are in-frame, single exon splicing defects, and a few are deletions, duplications, or insertions [1].

To identify the genetic defect of OI type I in a large Chinese family of five generations, we carried out linkage analysis and identified a \textit{COL1A1} nonsense mutation in a Chinese OI type I family. In exon 36 of \textit{COL1A1} gene, a C to T transition at position 2,464 resulted in a glutamine (Gln) to stop codon substitution at codon 644 (Q644X). This mutation (Q644X) appeared to be novel, which is neither reported in the literature nor registered in the database of human type I and type III collagen mutations. We also found that the central corneal thickness (CCT) of the OI patients were much lower than normal.

METHODS

\textit{DNA specimens:} The protocol for the study was approved by the Ethics Committee of our institute. Informed consent was obtained from all family members participating in this study. The family comprised 22 affected individuals from a five generation pedigree, originating from the Hebei province, China. The study consisted of 17 members, including 8 affected individuals, 6 unaffected individuals, and 3 normal controls (Figure 1).

\textit{Clinical evaluations:} The past history was investigated and the systems review was performed. The detailed ocular examinations of the proband were performed and some parameters were measured including vision, IOP, CCT, ocular axis length, and corneal curvature.

\textit{Genotyping:} Exclusion analysis was performed by allele sharing of the microsatellite markers, which were linked with the known OI loci. Genotyping was performed using microsatellite markers D17S1293, D17S1180, D17S1319, and
D17S788 at 17q 11.2-22. The oligonucleotide primer sequences were taken from NCBI and GDB.

Linkage analysis: A two-point LOD score (Z) was calculated using the MLINK subprogram of the Linkage package (version 5.1). The mode of inheritance was considered to be autosomal dominant with 95% penetrance. The gene frequency was set at 1/15,000. As the allelic frequencies of the polymorphic markers are unknown in the Chinese population, they were considered to be equally distributed.

DNA sequencing: COL1A1 (NM_000088) was screened for mutations by direct sequencing. PCR products of the 51 exons and flanking intron sequences of COL1A1 were sequenced on an ABI-3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Oligonucleotide primers and conditions for amplification are available upon request.

Denaturing HPLC: To screen for the mutation identified in the patients, on remaining patients, family members, and 100 normal control subjects in exon 36 of the COL1A1 gene, Denaturing (D)HPLC was involved by using a commercial system (Wave DHPLC; Transgenomic, San Jose, CA). The sequence for DHPLC was: 5’-CCT CCA TTA CTG CTC CTC CCC-3’ for the forward primer, and 5’-GAG GGC CAA GCC ACT CAC AA-3’ for the reverse primer. The touchdown PCR profile was used, 95 °C during 5 min, then 10 cycles of denaturation at 95 °C (30 s), primer annealing at 63 °C (30 s) with the temperature decreasing 0.8 °C at each cycle, polymerization for 30 s at 72 °C; followed by 35 cycles with the same profile for denaturation and polymerization but with a constant annealing temperature of 55 °C. Polymerization was completed by an incubation of 10 min at 72 °C. DHPLC was performed as follows: initial concentration at 51% of buffer A (0.1 M triethylammonium acetate-TEAA; Transgenomic), and 49% of buffer B (0.1 M TEAA containing 25% acetonitrile; Transgenomic) at 64.7 °C.

RESULTS

Clinical data: All affected individuals in the family had fractured their long bones several times from minor trauma and their sclerae have been blue since birth. The proband (V:3) was a 14-year-old boy with blue sclerae (Figure 2). His uncorrected visual acuity was OD, 20/10; OS, 20/10. His intraocular pressures (Goldmann tonometry) were OD, 14.7 mmHg; OS, 18.3 mmHg. His corneal curvature measurements were OD, K1 43.3, K2 43.6; OS, K1 43.0, K2 44.0. The ocular axis

Figure 1. The osteogenesis imperfecta pedigree and haplotype analysis. Pedigree and haplotype analysis of the OI family showing segregation of four microsatellite markers on chromosome 17. Squares symbolize males, and circles represent females. Black and white lines denote affected and unaffected status, respectively.
lengths were normal: OD, 24.03 mm; OS, 24.23 mm. His CCT were low: OD, 434 mm; OS, 441 mm. No systemic or other ocular anomalies were observed in the patients.

**Linkage and haplotype analysis:** Allele-sharing analysis confirmed the linkage of the disease in the family with COL1A1 and no linkage with COL1A2 (data not shown). Haplotype analysis showed that all the affected individuals shared a common haplotype with markers D17S1293, D17S1180, D17S1319, and D17S788 at 17q11.2-22 (Figure 1). Significant evidence of linkage was observed with microsatellite markers D17S1180 (LOD score [Z]=2.91, at recombination fraction [θ]=0.0) and D17S1319 (Z=2.20, at θ=0.0), respectively (Table 1).

**Mutation detection of COL1A1:** Direct cycle sequencing of the amplified fragments of COL1A1 in two affected individuals identified a single base alteration of C2464T (Figure 3) in exon 36 of COL1A1, which resulted in a substitution of Gln at codon 644 to a stop codon (Q644X). Denaturing HPLC analysis confirmed this mutation, which cosegregated with all affected individuals in the family, and was not observed in any of the unaffected family members or 100 normal controls. The remainder of the coding sequence did not show any change.

**DISCUSSION**

OI is an autosomal-dominant disorder of connective tissue associated with defects in the structure or synthesis of type I collagen [5,6]. OI is clinically characterized by increased bone fragility with a propensity to fracture, often resulting in skeletal deformity. Typical manifestations also include hearing loss, dental abnormalities, and blue sclerae.

Although the spectrum of clinical features is wide, and an OI patient with Rieger’s anomaly has been reported [7], the typical ocular finding is blue sclera. The sclerae of OI can vary in color from normal to a slightly bluish or slate color to a bright blue [8]. The blue sclerae may result from thinning of the abnormal sclera [9], the uniformity of the scleral collagen fibers, the reduced number of collagen bundles, and the increased mucoid components within the sclera [10]. All of these alterations in the ultrastructure of the sclera may, in essence, allow it to behave more like corneal tissue, transmitting the light reflected from the underlying uveal pigment and giving it the bluish color observed clinically. Histopathologic and electron-microscopic examination of an eye from an OI patient revealed that the sclera was moderately thinned especially at the equator; the Bowman’s layer in the cornea was absent; and the collagen fibers of the cornea and the sclera were narrower than in the control [10].

**TABLE 1. TWO-POINT LOD SCORES FOR LINKAGE BETWEEN THE OI LOCUS AND 17Q11.2-22 MARKERS**

| Marker       | 0   | 0.1 | 0.2 | 0.3 | 0.4 | Zmax |
|--------------|-----|-----|-----|-----|-----|------|
| D17S1293     | 2.04| 1.76| 1.32| 0.78| 0.26| 2.04 |
| D17S1180     | 2.91| 2.23| 1.52| 0.80| 0.20| 2.91 |
| D17S1319     | 2.20| 1.62| 1.06| 0.57| 0.20| 2.20 |
| D17S788      | 0.37| 0.27| 0.17| 0.08| 0.02| 0.37 |

Two-point LOD scores of 2.91 at θ=0.0 with marker D17S1180, 2.20 at θ=0.0 with marker D17S1319, and 2.04 at θ=0.0 with marker D17S1293 were obtained.

![Figure 2](image1.png)

**Figure 2.** The blue sclera of affected individuals. The proband (V:3) was a 14-year-old boy, whose sclerae were blue. The thinning of the sclera makes it possible to transmit the light reflected from the underlying uveal pigment and gives it the bluish color observed clinically. His uncorrected visual acuity was OD, 20/10; OS, 20/10. His intraocular pressures (Goldmann tonometry) were OD, 14.7 mmHg; OS, 18.3 mmHg. His keratometry evaluation revealed the following: OD, K1 43.3, K2 43.6; OS, K1 43.0, K2 44.0. The ocular axis lengths were normal: OD, 24.03 mm; OS, 24.23 mm. His CCT were low: OD, 434 mm; OS, 441 mm. The other ocular examinations including the anterior chamber and the fundus had no positive findings.

![Figure 3](image2.png)

**Figure 3.** DNA sequence chromatograms of the unaffected and affected members in the family. A single transition was observed at position 2464 (C>T) of COL1A1, causing a substitution of Gln to a stop codon at codon 644 (Q644X). This resulted in a truncated protein.
As observed in our study, the CCT of OI patients is often low while the vision is usually not affected. Moreover, both CCT and ocular rigidity are negatively correlated with the presence of blue sclerae [11]. Therefore, for such patients, an ophthalmologist must be aware of an artificially low intraocular pressure (IOP) measurement resulting from the low CCT and the decreased ocular rigidity. Furthermore, when considering a keratorefractive treatment, the clinician should evaluate carefully the CCT to avoid unexpected results or complications [11]. In our study, the IOP of the left eye of the proband was 18.3 mmHg, and it may be harmful to such a low CCT. It was suggested to the proband that he come back to the hospital for eye examinations every six months.

The cornea and the sclerae are known to be thinner in OI patients due to the defective collagen synthesis. The eyeball may distend because of the IOP. We expected longer ocular anteroposterior diameter in such patients, and we measured this parameter. However, the ocular axis lengths of our 14-year-old proband were within the normal range. We speculate that the ocular axis length of OI patients may be in correlation with their age. As the patient ages, the “cumulative effect” of the persistent intraocular pressure on the relatively thin and weak eyeball may lengthen the ocular axis.

Since 1980s, when the mutations in COL1A1/COL1A2 were first described, over 250 mutations in these two genes have been found in OI patients with various phenotype from diverse racial and ethnic groups (database of human type I and type III collagen mutations and the Gene Mutation Database). The vast majority are missense point mutations that convert codons for the obligate glycines in the triple helix of type I collagen to codons for amino acids with bulkier side chains [12-14]. The helical domain of type I collagen is composed of uninterrupted repeats of the sequence Gly-X-Y, in which Gly is glycine, and X and Y could be any residue but cysteine or tryptophan. The presence of glycine at every third residue is crucial to helix formation because its small side chain can be accommodated in the spatial constraints of the interior of the helical trimer [15]. Therefore, the substitution of a glycine residue by an amino acid with a bulky, polar, or charged side chain will distort the conformation of the triple helix and perturb normal collagen assembly in the cell, secretion from the cell, or fibril assembly in the extracellular spaces, and finally, often lead to a severe OI phenotype (type II) [13,16].

Although several, apparently more common, mutations have been reported in more than one unrelated individual or family with a similar OI phenotype [12,14,15,17,18], the relationships between the phenotype and genotype are not clear yet. In the vast majority of cases, the mutations are “private” in that they have only been reported in one person or family [19]. In spite of this, we can still obtain some rough correlation between the phenotype and genotype, such as the more NH2-terminal a serine substitution is located, the more severe the phenotype tends to be [20]. Glycine substitutions at the COOH-terminal half of the protein result in a more severe phenotype, while mutations at the NH2-terminal half tend to manifest in a less severe phenotype [21]. The missense point mutation occurring at glycine codon tends to result in a lethal phenotype, while the mutation creating a premature stop codon often leads to a mild, type I phenotype [13] etc. In conclusion, the severity of the phenotype is strongly associated with the specific site of the mutation.

The mutation analysis of OI is a labor intensive, expensive process due to the “private” nature of offending mutations and their nonclustered distribution in considerably sized genes [22]. Therefore, in our study, members of the family were genotyped first with microsatellite markers at the two known loci of OI. Haplotype analysis showed that the affected individuals in this family shared a common haplotype with four markers, within the region of 17q11.2-22, where the COLIA1 gene is located. After screening COLIA1 for mutations by direct sequencing, we identified a C>T transition in exon 36 of the gene. The transition C2464T located in exon 36 was predicted to cause a substitution of Gln to a stop codon at codon 644 (Q644X). DHPLC was used as a rapid, inexpensive method to confirm the mutation. The results showed that this mutation cosegregated with all affected individuals and was not observed in unaffected family members or 100 normal unrelated individuals.

The nonsense mutation in our study, Q644X, could cause one α1(I) allele to be functionally void. The transcription products of such a COL1A1 gene are usually unstable and subjected to degradation via nonsense-mediated mRNA decay pathways (NMD), ultimately producing a truncated protein. The NMD is a universal phenomenon and describes the degradation of mRNA transcripts that contain premature termination signals [23]. For the COLIA1 gene, NMD is a nuclear phenomenon. It targets mRNA rather than hnRNA for degradation [24]. The recognition and decay of nonsense containing COL1A1 mRNA occurs either during or after splicing but prior to cytoplasmic translation [24]. Regardless of the position of the mutation within the gene or its relative position within an exon, the steady-state amounts of mRNA from the mutant allele can be reduced markedly by NMD in both cellular and nuclear RNA extracts of cells from affected individuals [23]. Bioinformatic analyses also showed that Q644 is located in a highly conserved region (data not shown). As a result, a reduced amount of structurally normal collagen type I chains are expressed by fibroblasts of affected individuals, thus leading to OI type I [13-15,19,25]. This study confirms that the decreased expression of proc1(I) chains resulting from premature-termination codons or RNA-splicing defects in the COLIA1 gene often leads to the mildest OI.

To our knowledge, 13 nonsense mutations have been described at the collagen domain of COL1A1: R42X [13], R75X [22], R183X [13,14,22], R237X [18,22], R294X [26], R420X [27], E500X [14], R519X [13], R704X [28], Q779X [22], R848X [22,28], R963X [12,29], and Q644X (in our study). Two have been found at the COOH-terminal domain: W1269X [26], W1325X [28]. All of the aforementioned nonsense mutations predictably led to OI type I except W1269X, which resulted in OI type IV. The majority of these converted CGA, which encodes arginine to TGA and creates a premature stop codon. Our study is the second reported nonsense mutation converting a glutamine codon to a premature stop codon. These
results suggest that close attention should be paid to arginine codons when screening the \textit{COL1A1} for mutations.

Until our report, there were seven point mutations in exon 36 of \textit{COL1A1} that were registered in the Human Type I and Type III Collagen Mutation Database. All of these mutations were missense mutations that converted a glycine codon to a serine, asparagine, or an arginine codon. Most of these mutations resulted in the severe \textit{OI} type II. The Q644X in our study is the first nonsense mutation observed to occur in exon 36, and it also indicates that the coding sequence in exon 36 is essential for \textit{COL1A1}’s function.

\section*{ACKNOWLEDGEMENTS}

The authors thank the family for participation in this project. We also thank Dr. Hua Liu, Dr. Wenjiang Ma, and Dr. Dan Hu for sample collection, Dr. Chen Qi for clinical examination, and Dr. Xiaolin Hao and Miss Meng Zhang for excellent technical assistance. This work is partly supported by the National “973” Basic Research Funding Scheme of China (grant number 2001CB5103) and National Infrastructure Program of Chinese Genetic Resources (2004DKA30490).

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