Short Communication

Collagen Type I Alpha 1 Mutation Causes Osteogenesis Imperfecta from Mild to Perinatal Death in a Chinese Family

Hong-Yan Liu[1], Jia Huang[1], Dong Wu[1], Tao Li[1], Liang-Jie Guo[1], Qian-Nan Guo[1], Hong-Dan Wang[1], Rui-Li Wang[2], Yue Wang[3]

[1]Department of Medical Genetics Institute, People's Hospital of Zhenzhou University (Henan Provincial People's Hospital), Zhenzhou, Henan 450053, China
[2]Department of Ultrasonic Diagnosis, People's Hospital of Zhenzhou University (Henan Provincial People's Hospital), Zhengzhou, Henan 450003, China
[3]Department of Gynecology and Obstetrics, People's Hospital of Zhenzhou University (Henan Provincial People's Hospital), Zhengzhou, Henan 450003, China

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Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease or Lobstein syndrome, is characterized by blue or gray sclerae, variable short stature, dentinogenesis imperfecta, hearing loss, and recurrent fractures. Based on clinical, genetic, and radiological features, Sillence *et al*.[1] classified the OI into four subtypes including type I: Mild, common, with blue sclera; type II: Perinatal lethal form; type III: Severe and age-related progressive deformity, with normal sclera; and type IV: Moderate severity with normal sclera. Based on mutated genes and inheritance patterns, the four subtypes are further classified into 15 types of OI in Online Mendelian Inheritance in Man. More than 90% of the patients with OI have mutations in collagen type I alpha (*COL1A*) 1 and *COL1A2*.[2]

To date, little is known about the molecular genetic basis of OI in Chinese population. Here, we report a typical OI family in China, consisting of five generations and a total of 31 individuals. Among 16 OI patients, two were fetuses. Pedigree analysis showed the autosomal dominant transmission. By examining the coding sequences of *COL1A1* and *COL1A2*, we found that the gene mutation was associated with the clinical characterization of this family.

Methods

Participants

The family with OI came from western region of Henan Province, China. The proband IV8 was examined for OI at Medical Genetics Institute of Henan Provincial People's Hospital before pregnancy. Because of the proband's first child with OI, further genetic counseling identified a total of 16 family members with same clinical symptoms. After informed consent was received from all individuals by explaining the nature and possible consequences of the study, comprehensive clinical examination was conducted for all family members, including height, development of tooth, limbs and joint deformation situation, history of fracture, eyes sclera, hearing, spine, walking, and past medical history. The comprehensive clinical information and blood samples from individual IV3 were not available because of his noncooperation. A five-generation pedigree was drawn for all patients as shown in Figure 1.

The study adhered to the tenets of the *Declaration of Helsinki* and was approved by the Ethics Committee of Henan Provincial People's Hospital.

DNA extraction

Five milliliters of peripheral venous blood (ethylenediaminetetraacetic acid-K2 anticoagulant) were collected from ten members of this family, including seven patients (III1, III5, III7, III12, IV6, IV8, and V1) and three unaffected individuals (III2, III8, and IV5). Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures using Qiagen whole blood kit (QIAGEN, Germantown, MD, USA).

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Address for correspondence: Dr. Hong-Yan Liu, Department of Medical Genetics Institute, People's Hospital of Zhenzhou University (Henan Provincial People's Hospital), Zhenzhou, Hennan 450003, China
E-Mail: liuhongyanqhhy@126.com

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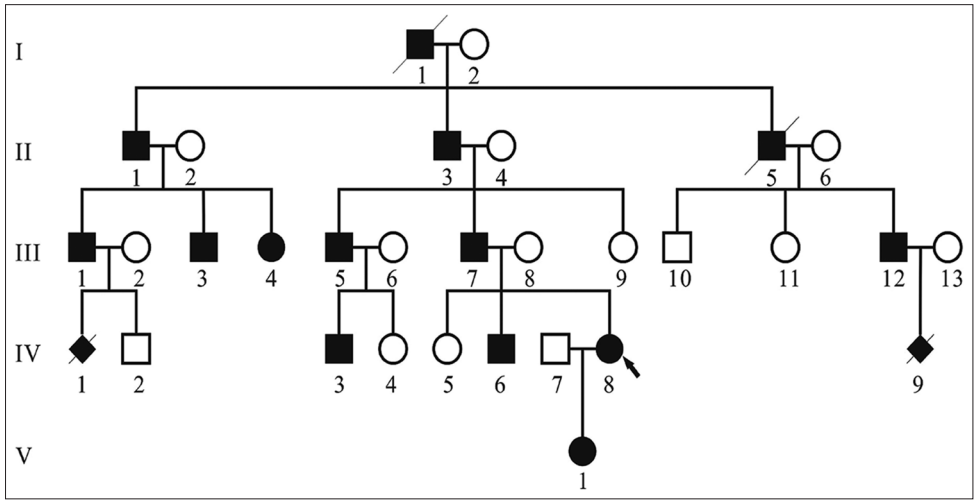

Figure 1: Pedigree of a Chinese family with the osteogenesis imperfecta. Black symbols indicate affected individuals, and white symbols indicate unaffected ones. IV8 (black arrow) indicates the proband.

Genetic analysis

First, the entire coding region and intron-exon boundaries of *COL1A1* of the proband IV8 were amplified by polymerase chain reaction (PCR). The primers were designed using the Oligo Primer Analysis Software Version 7 (Molecular Biology Insight, Inc., Cascade, CO, USA) and University of California, Santa Cruz (UCSC) *in silico* PCR was used to confirm its reliability. PCR amplification was performed in 25 μl PCR reactions for all the exons with conditions such as an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30–60 s, 35 cycles, followed by a final extension for 7 min at 72°C. PCR products were purified using QIA quick PCR purification kit (QIAGEN). Sequencing was performed using an ABI Big Dye Terminator Cycle Sequencing kit (v3.1) in an ABI 3730 Genetic Analyzer (Applied Bio Systems, Foster City, CA, USA). The sequencing results were compared with the human normal sequence from the UCSC 2013 human genome assembly. After identified a mutation in the proband, the affected exon 47 of *COL1A1* from the other individuals (affected individuals were III1, III5, III7, III12, IV6, and V1 and unaffected individuals were III2, III8, and IV5) was amplified and sequenced. In view of affected individual IV6 with severe symptoms, the *COL1A2* of the VI6 was amplified and sequenced. Restriction endonuclease BstUI was used to digest the PCR products from 180 unaffected normal cases to confirm the variation.

Results

Clinical examination

In this family, complete clinical information was available for 7 affected individuals. All patients showed clinical onset at ages from 1 to 2. The affected adult individuals were characterized with short stature ranging from 110 cm for IV6 to 151 cm for III5. Blue sclerae and recurrent fractures were common manifestations. Hearing impairment from the affected individuals was not found. Interestingly, the affected III5 with mild features only showed blue sclerae and dental shedding, but the affected IV6 with severe symptoms suffered from fractures on 8 separate occasions before puberty, severe limb deformity causing a walking limp, and severe scoliosis with the height of 110 cm. Another interesting symptom was that two affected individuals (IV6 and IV8 at ages 32 and 27 years, respectively.) retained deciduous teeth and did not have permanent teeth. Review of the family history revealed that the affected individuals (III1 and III12) had a history of stillbirth with OI fetus. Prenatal ultrasonography examination showed that the fetus was dead and the limb angulation deformities at 24 weeks' gestation. The younger affected individual V1 was born at full term with the birth weight on the low side of 2.3 kg, and bowlegs. Clearly, the clinical manifestations of the affected individuals in this family were highly heterogeneous. The main clinical features of all patients are summarized in Table 1.

Mutation analysis

By comparing the sequencing result to normal controls, we found G to A transition at the nucleotide position 3505 (exon 47) in *COL1A1* of the proband (IV8) and other six patients (III1, III5, III7, III12, IV6, and V1), but not in unaffected pedigree members (III2, III8, and IV5). This mutation resulted in glycine to serine substitution at amino acid position 1169 [Figure 2]. This missense mutation was not observed in either 180 unrelated normal controls or normal Chinese in 1000 genomes project. No mutation was found in *COL1A2* of affected individual IV6.

Discussion

In this study, we report a large Chinese family with OI. A novel missense mutation (c. 3505G> A, p.Gly1169Ser) in *COL1A1* was found in all affected individuals. This missense mutation caused by single base substitutions that convert a codon for a glycine to serine and is located in the triple-helical region of pro-α1 protein. Glycine, as the smallest amino acid, is the only residue that can occupy

Table 1: Clinical features of the eight patients of the Chinese family with osteogenesis imperfecta

Items	III1	III5	III7	III12	IV3	IV6	IV8	V1
Age (years)	43	60	67	42	29	32	27	4
Height (cm)	150	151	150	150	140	110	140	90
Dentinogenesis imperfect	+	−	+	−	NA	+	+	+
Retained deciduous teeth	−	−	−	−	NA	+	+	−
Opalescent teeth	−	−	−	−	NA	+	+	−
Dental shedding	+	+	+	−	NA	−	−	−
Fragility teeth	+	−	+	+	NA	+	+	−
Fractures/(times)	+/(NA)	−/(-)	+/(3)	+/(4 to 5)	+/(NA)	+/(8)	+/(2)	−/(-)
Joint laxity	+	−	+	−	NA	+	−	−
Joint dislocation	+	−	−	−	NA	+	−	−
Limb deformity/(body parts)	+/(right arm)	−/(-)	−/(-)	+/(bilateral arm, finger)	NA/ (NA)	+/(hip)	+/Knee joint	+/Bowlegs
Walking limp	−	−	−	+	+	+	−	−
Scoliosis	−	−	−	Slight	NA	Severe	−	−
Chest deformities	−	−	−	−	NA	+	−	−
Blue sclera	+	+	+	+	+	+	+	+
Deafness	−	−	−	−	NA	−	−	−
Other	Hypertension	−	−	−	NA	−	−	−

+ : Presence of trait; −: Absence of trait, NA: Not available.

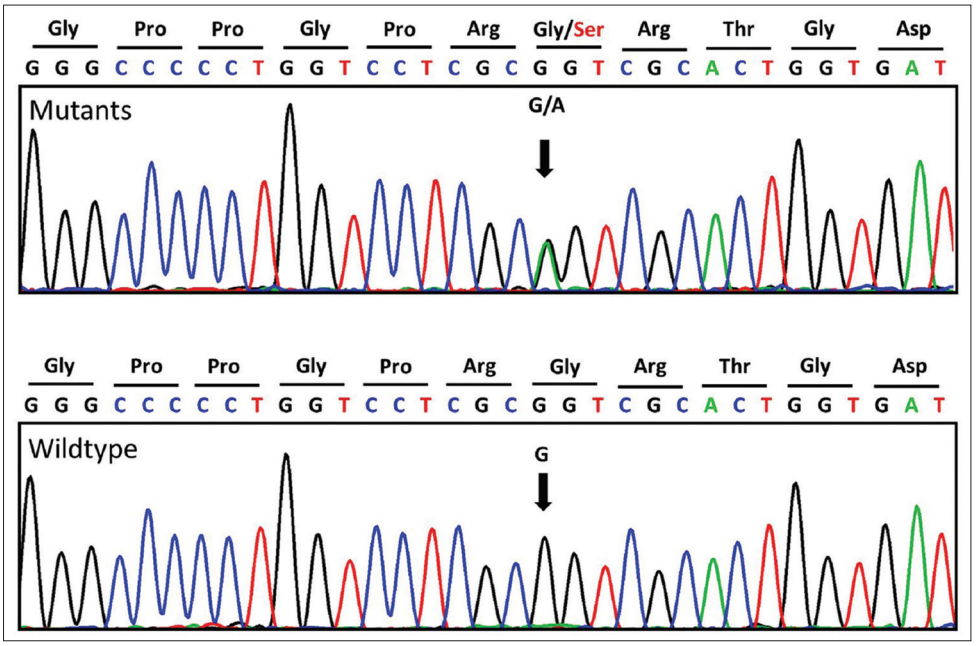

Figure 2: Sequencing results of the exon 47 in collagen type I alpha 1. Upper panel showed heterozygous mutation of c.3505G>A p.Gly1169Ser while lower panel showed wild-type alleles only.

the axial position of the triple helix. The triple-helical domain restricts every third residue to glycine, generating a repeating (Gly-X-Y) sequence pattern. Any change in a glycine residue appears to interfere with the formation of stable triple helix.

It is worth mentioning that the clinical manifestations of this mutation are variable in affected individuals of this family from mild (III5) to severe OI (patient IV6).However, significant phenotype variations within one family are very rare. A previous study reported that OI patient with the same mutation (p.Gly238Ser) in the *COL1A2* with one family patient showing mild phenotype,[3] and other family patients showing severe phenotype.[4] This phenomenon was also found in a Korean family with OI. The affected individual with the mutation (p.Val987_Pro989dup) in *COL1A2* showed phenotypic ranging from type I to IV.[5] So far, the reasons for the significant phenotypic variations in one family are not clear. Genetic factors such as additional genetic variants at or near the *COL1A2* may affect the gene expression or function. Nongenetic factors may also influence the phenotypic

characteristics. These factors may include nutritional and other environmental variations between different individuals.

In conclusion, we have identified a novel mutation c. 3505G > A (p.Gly1169Ser) in the *COL1A1* gene. Our finding provides additional evidence on the OI pathogenic mutation spectrum and extends our understanding on the heterogeneity of OI in Chinese population.

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Conflicts of interest

There are no conflicts of interest.

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