ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine
Open Access WILEY

Analysis of the clinical and genetic characteristics of a Chinese family with osteogenesis imperfecta type I

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Abstract

Background: Osteogenesis imperfecta type I (OI-I) is a rare genetic disorder characterized by skeletal deformity, bone fragility, blue sclerae, dentinogenesis imperfecta, and hearing loss. The current study aimed to confirm the clinical diagnosis and genetic cause of OI-I in a four-generation Chinese family.

Methods: Clinical investigation and pedigree analysis were conducted to characterize the phenotypic manifestations of a Chinese family with OI-I. Follow-up audiometry and imaging tests were used to evaluate the postoperative outcomes of stapes surgery in the proband with otosclerosis. Whole-exome sequencing (WES) and Sanger sequencing were used to identify the pathogenic gene variants and for cosegregating analysis.

Results: We described in detail the clinical features of the collected family with autosomal dominant OI-I, and firstly identified a pathogenic splicing variant (c.2344-1G>T) in intron 33 of *COL1A1* in a Chinese family. The molecular analysis suggested that the mutation might cause splice site changes that result in a loss of gene function. The proband, who suffered from otosclerosis and presented two-side middle-severe conductive hearing loss, benefitted significantly from successive bilateral middle ear surgery.

Conclusions: The diagnosis of OI-I in a Chinese family was established by clinical and genetic investigation. A heterozygous pathogenic splicing variant in *COL1A1* was directly responsible for the bone fragility and hearing loss of this family. Otosclerosis surgery should be suggested to rehabilitate conductive hearing impairment in OI patients.

KEYWORDS
COL1A1, osteogenesis imperfecta, otosclerosis, splicing pathogenic variant, whole-exome sequencing

1 | INTRODUCTION

Osteogenesis imperfecta (OI) is a rare collagen-related bone disorder with clinical and genetic heterogeneity. It has an incidence of 1:10,000–1:25,000 worldwide and is characterized by bone fragility, blue sclerae, dentinogenesis imperfecta (DI), valvular, or cardiac failure, bone pain, and hearing loss (Marini et al., 2017). Although many classifications of OI focusing on gene mutations and the severity of clinical features have been proposed, the classification published by Sillence (types I–IV) in 1987 remains the most widely accepted (Sillence et al., 1979; Van Dijk et al., 2010). Based on the radiographic findings, clinical presentation, and patterns of inheritance, OI types I–IV comprise approximately 80~85% of cases and have a strong genetic component (Schleit et al., 2015). According to the classification system, OI types I–IV are caused by mutations in *COL1A1* (OMIM #120150) and *COL1A2* (OMIM #120160), the genes that encode the α-1 chain and α-2 chain, respectively, in the type I collagen triple helix (Kuivaniemi et al., 1997; Van Dijk & Sillence, 2014). To date, over sixteen hundred OI pathogenic variants in *COL1A1* and *COL1A2* have been included in the Osteogenesis Imperfecta Variant Database (LOVD, https://oi.gene.le.ac.uk).

OI Type I (OI-I, OMIM #16620) is the mildest and most common form of OI, characterized by susceptibility to bone fracture, near-normal stature, blue sclera, dental problems, triangular face shape, tendency toward spinal curvature, and early hearing loss (Willing et al., 1994). OI requires both clinical management and genetic analysis. OI-I is inherited in an autosomal dominant manner and is always challenging to diagnose because of the variable phenotypes among OI-I patients. Almost all OI-I cases are caused by mutations in *COL1A1* (NM_000088.4, NP_000079.2) (Augusciak-Duma et al., 2018), which encodes the pro-α1 chain of type I collagen; *COL1A1* is located on chromosome 17q21.33 and composed of 51 exons (Augusciak-Duma et al., 2018). To date, more than 300 hundred mutations associated with OI-I have been reported in different groups and in different regions, and few of these mutations are recurrent (HGMD, http://www.hgmd.cf.ac.uk). The spatial relationships between the ligand-binding sites and mutation positions still had not been examined. To date, no treatment or medicine can completely cure OI-I (Vidal-Ruiz et al., 2018). Treatment of OI-I is supportive and based on the features and symptoms present in each person. The main goal of treatment is to correct symptoms, diminish the fracture rate, and allow patients to achieve functional independence and associated high quality of life.

In this study, we revealed the comprehensive clinical features of a four-generation Chinese family with OI-I and identified a rare c.2344–1G>A splicing mutation in *COL1A1* by molecular investigation.

2 | MATERIALS AND METHODS

2.1 | Family data

The proband (III-2, Figure 1A) of this family (GX-052) was identified in the Otology Clinic at the Affiliated Hospital of Guangxi Medical University. All of the study participants provided written informed consent and underwent detailed clinical and physical examinations performed by experienced orthopedists, ophthalmologists, pediatricians, endocrinologists, and otologists. Syndromic manifestations were given special attention. All genomic DNA (gDNA) samples were extracted from whole-blood samples using a DNA kit (TIANGEN BIOTECH, Beijing, China) and stored at −80 °C for genetic testing. A custom-designed form was used to collect the comprehensive clinical history, including aging, trauma, infections, surgery, ototoxic drugs, hearing, consanguineous marriage, pregnancy, birth complications, and clinical symptoms. An additional 250 race-matched normal subjects were enrolled in this study.

2.2 | Clinical evaluation

A series of audiological examinations, including pure-tone audiometry (PTA), acoustic immittance, otoacoustic emission, and the Gelle test (GT), were performed as the standard protocol in the current study. Using the common criteria established by the WHO (1997), the grades of deafness were evaluated on the basis of the audiological results. Objective medical examinations were performed on two affected family members, the proband (III-2, Figure 1A) and her son (IV-1, Figure 1A). High-resolution computed tomography (HRCT) of the head, temporal bone, and lung was performed, and X-ray imaging of the whole spine, upper and lower limbs, chest, abdomen, and joints was carried out to evaluate bone development and growth. Dual-energy X-ray absorptiometry (DEXA) was used to evaluate bone strength and fracture risk. Blood biochemical analysis, thyroid ultrasound, and cardiac ultrasound were performed to determine physiological and biochemical states and investigate potential issues. The common *Sillence classification* of OI was used to classify the degree of disease severity in this family.

2.3 | WES

gDNA from three individuals (III-1, III-2, and IV-1, Figure 1A) from family GX-052 was subjected to whole-exome sequencing (WES) performed at Novogene Inc., Beijing, China. The Agilent SureSelect Human All Exon V6
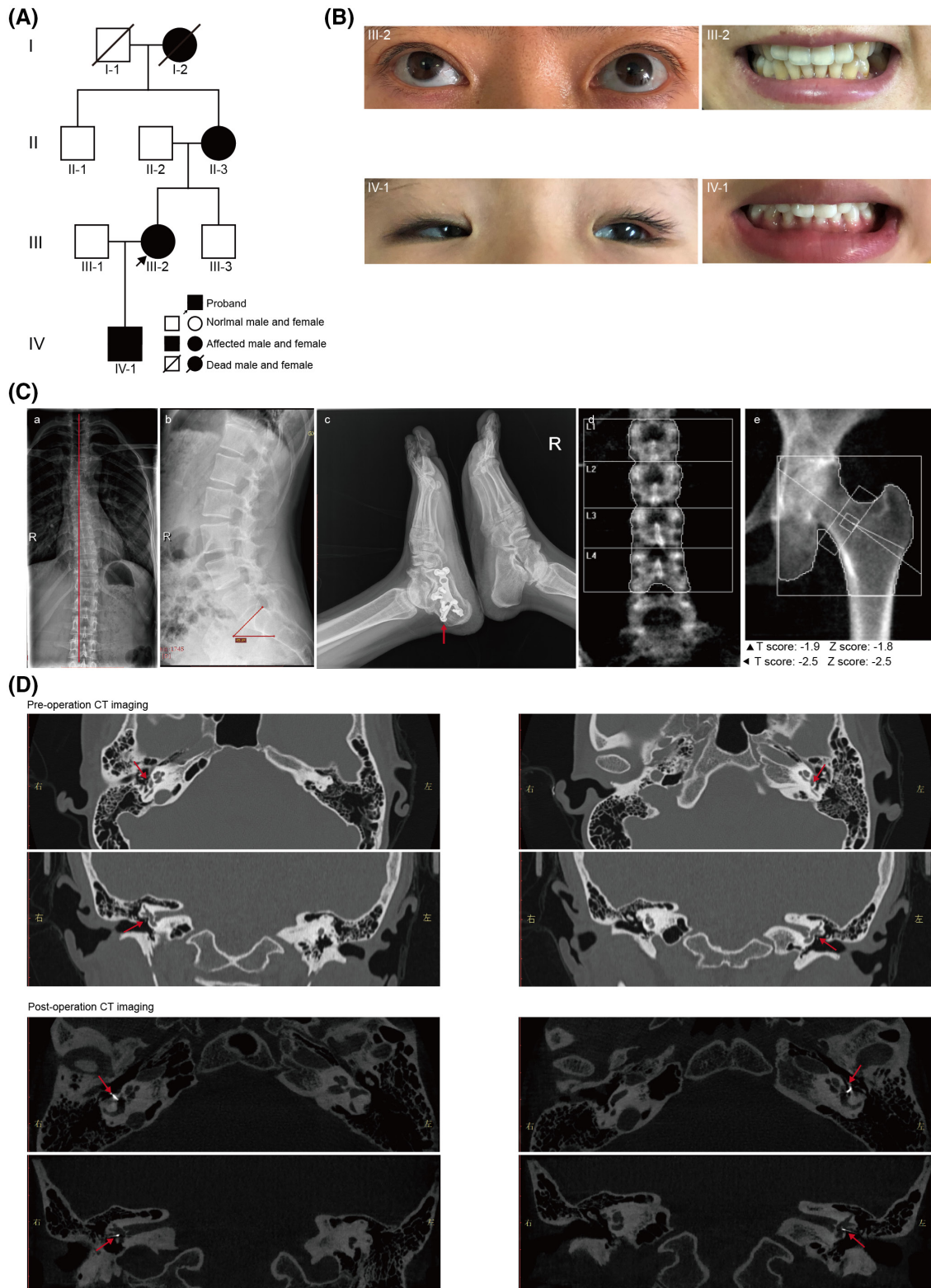
FIGURE 1 Chinese pedigree with OI-I and clinical features. (A) The pedigree is shown. The squares indicate men, and the circles indicate women. The filled black quadrants indicate affected individuals, and the arrow symbol denotes the proband. The diagonal lines indicate deceased individuals. (B) Photographs of the eyes and teeth of two family members (III-2 and IV-1). Both presented bilateral blue sclera and normal teeth. (C) Imaging examinations of the proband (III-2). The proband had mild thoracic scoliosis (a), increased lumbosacral angle (b), left calcaneal fracture (c), and decreased bone mineral density (d). (D) HRCT imaging of head and temporal bone (III-2). Red arrowheads in a and b show thickening of the footplate in the left and right ear. Red arrowheads in (c) and (d) show the location of the artificial ossicles in the left and right ear.

kit and Illumina HiSeq PE150 (Illumina) were used to capture the targeted regions and sequence the DNA library at a mean coverage of 100X depth. The raw image files obtained from the HiSeq PE150 were processed with the Illumina pipeline for base calling, and then the clean reads were aligned to the human reference genome sequence (UCSC hg19 and NCBI database) by Burrows-Wheeler Aligner (BWA) software, Samtools and GATK. The identified variants were analyzed according to the dominant inheritance model and filtered against public databases (the 1000 Genomes, Human Gene Mutation Database [HGMD], Exome Variant Server, and dbSNP databases). The Filter-based annotation was performed according to a standard protocol.

2.4 | *COL1A1* mutation analysis

For validation and cosegregation analyses by Sanger sequencing, primers flanking the candidate loci were designed using Primer 5 and synthesized by Sangon Biotech, Shanghai, China. The forward primer sequence was 5′-CCTCTTGTCCCTTGCTC-3′, and the reverse primer sequence was 5′-CCCAGTCGGTGATGAA-3′. PCR amplification was performed to analyze the coding region and exon-intron splice junctions. PCR products were sent to Sangon Biotech for purification (Axygen) and direct sequencing (ABI 3730XL). DNASTAR (version 7.1) and Chromas (version 2.6.5) software were used to analyze the obtained sequence data. Human Genome Variation Society (HGVS) nomenclature was used to name the identified causative nucleotide changes, which were numbered corresponding to their positions in *COL1A1* messenger RNA (NM_000088.4).

2.5 | Biological molecular analysis

Splice Predictor (DK, http://www.cbs.dtu.dk/services/NetGene2/) and MutationTaster (http://www.mutationtaster.org/) were applied to the functional prediction of the *COL1A1* pathogenic variant.

3 | RESULTS

3.1 | Clinical characteristics

This four-generation family GX-052 of Han origin with OI-I was found in Guangxi (China), and the pedigree was constructed (Figure 1A). The pattern of inheritance suggested autosomal dominant inheritance.

The proband is a 33-year-old Chinese female, 158 cm tall in height and 50 kg in weight. She presents with bilateral blue sclera, left calcaneal fracture, flat feet, hip pain, increased lumbosacral angle (45.3° > 30°), mild thoracic scoliosis, and stapedial otosclerosis (Figure 1B–E). Teeth and vision are normal. A DEXA scan for bone density showed a T-score of −2.5 and a Z-score of −2.5 in the spine and a T-score of −1.9 and a Z-score of −1.8 in the hips, indicating a low bone density.

The proband's son (IV-1, Figure 1A) is a 5-year-old boy, 105 cm tall in height and 16 kg in weight. He exhibited bilateral blue sclera (Figure 1B), three fractures (the tibia, ulna, and radius), flat feet, and mild lumbar scoliosis. Hearing, teeth, and vision are normal. He also underwent a DEXA scan and had an abnormal result. Blood and ultrasound analysis of subject IV-1 excluded additional diseases. Subject II-3 showed bilateral blue sclera, flat feet, and three spine fractures. Subject I-2 died, and no more information could be obtained. According to the standard definitions of bone density levels (WHO), the affected family members all had a high risk of fracture on orthopedic evaluation and were diagnosed with OI-I according to her clinical presentation and genetic diagnostics. Pedigree analysis revealed that the inheritance of OI-I was consistent with an autosomal dominant pattern.

3.2 | Middle ear surgery

The proband suffered from progressive bilateral conductive hearing loss (Figure 2) since her first pregnancy in 2015 and came to our department for help in 2016. Based on 2 years of follow-up audiological data, she was found to present with middle-severe conductive hearing loss (SHL) at all frequencies with a large air-bone gap. The results of acoustic immittance measures, GT, otoscopy, and CT excluded secretory otitis media, tympanic membrane perforation, tympanosclerosis, and ossicular chain disruption. HRCT examination of the temporal bone showed thickening of the footplate of the stapes (Figure 1D). Based on detailed family clinical examinations and medical histories, the proband was diagnosed with bilateral otosclerosis and underwent successive surgery in the left and right ears in 2017 and 2021, respectively. The proband was satisfied with the results of stapes surgery. The four-year follow-up audiograms (Figure 2) indicated that middle ear surgery significantly improved the proband's conductive hearing loss.

3.3 | Mutational analysis

Three peripheral blood samples (III-1, III-2, and IV-1) were collected from this family. WES analysis revealed a hemizygous mutation (c.2344-1G>T) in intron 33 of the *COL1A1* gene. This variant was confirmed by Sanger
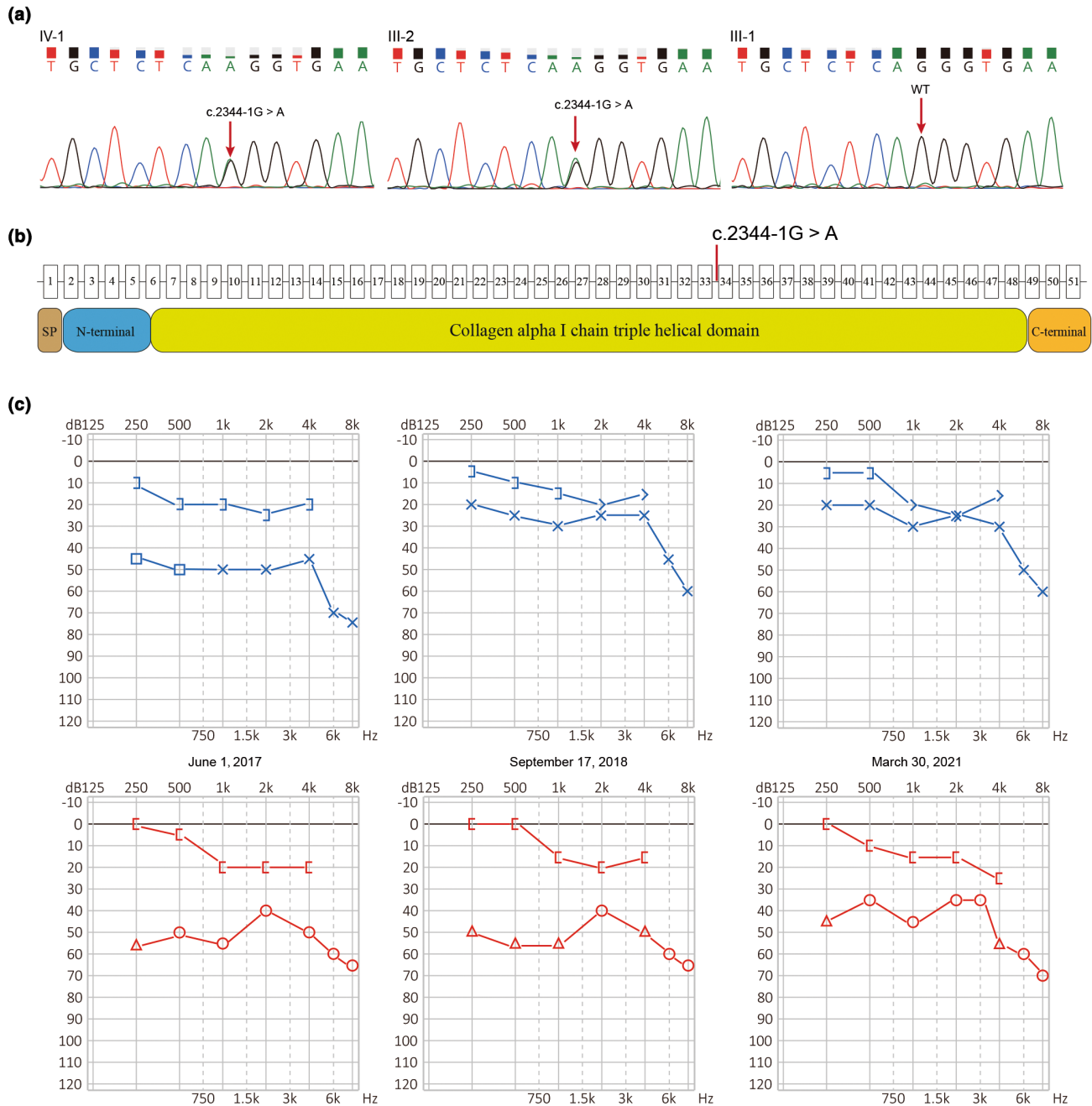
FIGURE 2 Genetic information of the OI-I family and follow-up audiograms of the proband. (a) Sequence chromatography. The heterozygous change, c.2344-1G>A, was identified in two family members (IV-1 and III-2). However, unaffected member (III-1) and healthy controls are wild-type at this position. (b) Schematic representation of COL1A1 protein with the signal peptide (SP), N-terminal propeptide, collagen alpha I chain triple-helical domain and C-terminal propeptide. The arrow highlights the c.2344-1G>A mutation site. (c) Pre- and postoperative audiograms of the proband (III-2). The bone conduction thresholds of the two ears were improved by middle ear surgery, and the air-bone gap decreased in the low and middle frequencies (below 4000 Hz)

sequencing, cosegregated with the family phenotype, and was absent from 200 normal controls. By searching public databases, we found that this variant has been reported in two sporadic cases of OI-I (Schleit et al., 2015) (Table 1). The two boys in these sporadic cases showed blue sclera, multiple/one fractures, and hypermobility, the same phenotypes as the proband's son (IV-1) in the current family.

3.4 | *COL1A1* protein bioinformatic analysis

The *COL1A1* c.2344-1G>A variant was predicted by MutationTaster to cause splice site changes and affect protein features. The NetGene2 server determined that the mutation could cause the loss of an acceptor splice

TABLE 1 Phenotypes and genotypes of probands with OI type I due to variants which alter splicing of *COL1A1*

Intron	Mutation	Age	Scleral Hue	Height/Weight	Fractures	Bone Deformity	Hearing Loss	Dentinogenesis Imperfecta	Family History	Misc	Reference
33	c.2344-1G>A	1y	Blue	–	1	–	–	–	No	–	Schleit et al. (2015)
		2y	Blue	–	Multiple	–	–	–	No	Hypermobility	Schleit et al. (2015)
		5y	Blue	105 cm/16 kg	3	Lumbar spine	No	No	Yes	No	This study
		33y	Blue	158 cm/50 kg	2	Thoracic scoliosis	Yes	No	Yes	No	This study

Note: – indicates a lack of data for that variable.

site, which would cause abnormal pre-mRNA splicing resulting in the insertion of intron 33 between exons 33 and 34 in transcripts of the *COL1A1* gene. According to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG), the *COL1A1* c.2344-1G>A variant was suggested to be pathogenic (PVS1 + PS2_M + PM2 + PP4).

4 | DISCUSSION

As a genetic disorder, OI-I exhibits two groups of clinical symptoms, primary and associated characteristics. Fractures and osteoporosis are the main primary presentations. Wide variability exists in associated characteristics, which include the blueness of the sclerae, hearing impairment, progressive skeletal deformity, scoliosis, and basilar impression, short stature, and cardiopulmonary dysfunction, which is the main direct cause of death in these patients. According to the update of the *Sillence classification* in 2015, blue sclerae is a determinant feature of OI-I, whereas the other characteristics are not common (Van Dijk et al., 2010).

In the current study, spine deformity was shown in three affected subjects of family GX-052, and pregnancy coincided with accelerated the hearing loss in the proband. No limb calcification of the interosseous membrane, dentinogenesis imperfecta, or long bone deformity was seen in these patients. The proband's mother (II-3, Figure 1A) displayed multiple spinal fractures after menopause. The proband presented left calcaneal fracture and hip pain after delivery of her first baby. The proband's son is susceptible to fracture upon minimal trauma and suffered from multiple fractures and mild lumbar scoliosis during childhood. A change in the lumbosacral angle could increase the shearing stress at the lumbosacral joint. Progressive deformity of the skeleton needs to be considered and prevented. Muscular strengthening exercise might benefit these patients and should be suggested to prevent progressive spine deformity. Although the different phenotypes in affected individuals could be due to clinical heterogeneity, the possibility of genetic anticipation might not be excluded in this family.

Following a thorough clinical investigation and relevant examinations, family GX-052 was diagnosed with OI-I. Because multiple body systems are affected in OI-I, different medical doctors need to work in conjunction to achieve the final treatment goals. Fracture management should be given the most attention to achieve good stabilization and prevent further deformities. Contact sports and high-intensity exercise should be avoided even in mildly affected individuals. When hearing loss

impacts patient quality of life, good surgery therapy is necessary to restore hearing. In this study, artificial ossicle implantation was confirmed to be a useful treatment for the proband's conductive hearing loss due to stapedial otosclerosis. Then, surgery could provide functional benefit for patients with hearing loss and should be considered to address hearing problems, especially stapedial otosclerosis.

Splicing pathogenic variants are less commonly described than missense, nonsense, and frameshift variant types (Marini et al., 2017; Stenson et al., 2017). Many splicing mutations lead to frameshifts and premature termination in eliminating downstream exons (Han et al., 2020), which is often uncovered as a pathological mechanism. RNA splicing and exon bonding are known to be significantly important in gene translation and expression. The correct splicing sites, including a donor site (5′-end of the intron), a branch site (near the 3′-end of the intron) and an acceptor site (3′-end of the intron), play key roles in these reactions (Madhani & Guthrie, 1994; Nilsen, 1994). The splice donor site usually includes an almost invariant sequence GU at the 5′-end of the intron. The splice acceptor site at the 3′-end of the intron normally terminates the intron with an almost invariant AG sequence.

Sanger sequencing showed that the *COL1A1* mutation c.2344-1G>A cosegregated with the bone disease in this family. This mutation is located at the boundary of exon 34 and intron 33. Because the correct recognition of flanking intronic regions determines alternative splicing (Patel & Steitz, 2003), nucleotide changes in splice sites could cause splicing defects and/or amino acid changes (Scotti & Swanson, 2016). Many causative variants of splicing donor/acceptor sites have been identified in OI cases (Han et al., 2020; Schleit et al., 2015). Splice site mutations accounted for 20% of helix mutations, were rarely lethal, and often led to a mild phenotype (Marini et al., 2007). The identified splicing variant c.2344-1G>A was predicted to cause the loss of the 3′ acceptor site of intron 33, which might lead to intron 33 retention. The resulting in-frame insertion could create an atypical protein with abnormal function, resulting in haploinsufficiency or a dominant-negative effect, both of which are well-known hypotheses of pathogenic mechanisms in OI-I. The former may be the main contributor to OI-I cases mild phenotype of (Rauch et al., 2010), as shown in the current family.

Genetic counseling and surgical management are essential components of complete care for OI patients. Any child of the proband of family GX-052 will have a 50% chance of inheriting the causative mutation and developing some manifestations of OI-I. Prenatal molecular genetic testing and ultrasound examination in potentially affected pregnancies in this family are suggested to reduce the risk of having another baby with the same birth defects.

5 | CONCLUSION

A rare splicing mutation c.2344-1G>A in the *COL1A1* gene was firstly identified in a Chinese family with OI-I. The identification of new OI-I families and novel mutations could contribute to comprehensively establishing the relationship between the genotype and phenotype of OI-I. Surgical treatment should be performed as necessary for OI-I patients with otosclerosis. Our findings could help family members understand the risk of having another child with OI and of their child's risk of having their own child with OI and also provide support to parents as they learn to care for their child's needs.

AUTHOR CONTRIBUTIONS
Study design: Anzhou Tang and Zhijie Niu. *Data collection*: Yongjing Lai, Lingyuan Liu, and Wenwen Zhou. *Data analysis*: Fen Tang, Yupei Su, and Yanglong Xu. *Contribution of new reagents or analytical tools*: Songhua Tan, Guangyao He, Jingyu Li, Lei Liu, Lihong Xie, and Qin Fang. *Manuscript preparation*: Zhijie Niu. All authors reviewed the manuscript.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

ETHICS STATEMENT
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (protocol code 2019-KY-067, date 6 March 2019). All subjects participating in the image acquisition signed the consent form.

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