

Novel mutations of the *SERPINF1* and *FKBP10* genes in Chinese families with autosomal recessive osteogenesis imperfecta

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Abstract. The aim of the present study was to characterize the clinical manifestations and identify the mutations of Serpin family F member 1 (*SERPINF1*) and FK506 binding protein 10 (*FKBP10*) genes in Chinese patients with osteogenesis imperfecta (OI). Using whole-exome sequencing in the first and third probands, a novel mutation was identified in *SERPINF1* and a novel compound heterozygous mutation was revealed in *FKBP10*. Using Sanger sequencing, an additional novel mutation in *SERPINF1* was identified in a proband of family 2. In family 1, the proband presented with a novel homozygous missense mutation of the *SERPINF1* gene, c.1067T>A (V356E). In family 2, the proband had a novel homozygous deletion mutation of the *SERPINF1* gene, c.283+473_643+104del (p.Ala96_Gly215del). Serum pigment-epithelium-derived factor concentration was not detected in probands with OI type VI. For both families, the proband's father was demonstrated to have a heterozygous mutation of *SERPINF1*, whereas no mutations were detected in the probands' mothers. An assessment of allelic copy numbers revealed a deletion of *SERPINF1* in the mother of family 1. The results of the present study demonstrate that patients may have mild symptoms of OI with a large fragment deletion in the *SERPINF1* gene. Thus, the phenotype of Chinese patients with type VI OI is milder than that of Caucasian and Korean patients. In family 3, the

proband displayed a novel compound heterozygous mutation in *FKBP10*, c.813_814delGA (p.Glu271AspfsX101) and c.831delC (p.Gly278AlafsX20), and did not have Bruck syndrome. Codon 831 of the *FKBP10* gene may represent a mutation hotspot for human OI. These results extend both the phenotypic and the genotypic contents of OI patients with *SERPINF1* or *FKBP10* mutations.

Introduction

Osteogenesis imperfecta (OI) is a clinically heterogeneous and heritable connective tissue disorder that is characterized by bone fragility and susceptibility to fractures following minimal trauma (1). Sillence *et al* (2) initially categorized OI individuals into four types based on clinical features and disease severity: Type I [(Mendelian Inheritance in Man (MIM) 166200], type II (MIM 166210), type III (MIM 259420), and type IV (MIM 166220). For the majority of patients, OI is associated with mutations of *COL1A1* (MIM 120150) or *COL1A2* (MIM 120160), which encode type I collagens (3). Glorieux *et al* (4) described a group of patients with OI that presented with a discrete phenotype, including hyperplastic callus formation and calcification of the interosseous membrane; this was designated as OI type V (MIM 610967). In 2002, Glorieux *et al* (5) described another novel form of OI that presented with osteoid accumulation due to a mineralization defect; this was designated OI type VI (MIM 613982). Patients with OI type VI typically sustain their first fracture after the age of 6 months (6). Their sclerae are white or faintly blue and dentinogenesis imperfecta (DI) is uniformly absent (6). These characteristic features of OI type VI are readily apparent in histological bone samples, including bone biopsy specimens that have a 'fish-scale' pattern, hyperostoidosis, prolonged mineralization lag time and decreased mineral apposition rate (5). Becker *et al* (7) first reported that Serpin family F member 1 (*SERPINF1*) mutations cause severe autosomal recessive OI in 2011. FK506 binding protein 10 (*FKBP10*) mutations were first reported by Alanay *et al* (8) in 2010 in autosomal-recessive OI. Kelley *et al* (9) concluded that *FKBP10* mutations also cause Bruck syndrome, which is characterized

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by congenital joint contractures. Autosomal-recessive OI with *FKBP10* mutations was designated as OI type XI (10). As an increasing number of virulence genes have been discovered, the OI classification has expanded to 15 types (10).

Previous studies have reported that the most prevalent pathogenic genes responsible for OI in Chinese patients were *COL1A1* and *COL1A2* (11,12). To the best of our knowledge, only one report of Chinese patients with *SERPINF1* mutations and one with *FKBP10* mutation have previously been reported, respectively (13,14). In the present study, two novel mutations in *SERPINF1* and one novel compound heterozygous mutation in *FKBP10* were identified in three unrelated Chinese families with autosomal recessive OI type VI and XI.

Materials and methods

Subjects. None of the probands belonged to consanguineous families. Three unrelated Chinese families with OI (Fig. 1) and 250 healthy control donors were included in the present study. Patients were recruited between May 2010 and May 2015 from our outpatient clinic, Tianlin Street Community Centre and Fengin Street Community Centre. The control donors included 129 females (66.6±10.0 years) and 121 males (59.3±17.9 years). All patients and control subjects belonged to the Han ethnic group.

Ethics statement. The present study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China). All adult participants provided written informed consent prior to beginning the study. In addition, written informed consent was provided by the parents on behalf of the children enrolled in the present study.

Bone densitometry. The bone mineral density (BMD; g/cm²) of the lumbar spine (L1-L4) was measured using dual-energy X-ray absorptiometry (DXA). The probands of families 1 (proband 1) and 3 (proband 3) were assessed using Lunar Prodigy equipment (GE Lunar Corp., Madison, WI, USA). The Lunar device was calibrated daily and the coefficient of variability (CV) value of the DXA measurements at L1-4 was 1.39% (15). The proband of family 2 (proband 2) was assessed using Hologic Discovery A equipment (Hologic, Bedford, MA, USA). The machine was calibrated daily. The CV value of the DXA measurements at L1-4 was 0.9% (15). Lumbar spine BMD results were converted to age- and sex-specific Z-scores according to the reference data (16). There are 3 DXAs in the department, including 2 GE Lunar equipment and 1 Hologic Discovery A equipment. The patients were examined using the DXAs depending on availability.

Laboratory tests. Fasting blood samples were obtained between 8 and 10 am. Blood was stored for 2-3 h at 25°C and centrifuged at 4°C for 10 min at 4,000 × g to obtain the serum. The following markers of calcium metabolism were measured: Serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (PTH), and 25 hydroxy vitamin D3 [25(OH)D]. Ca, P and ALP were measured using a HITACHI7600-020 automatic biochemistry analyser (Hitachi, Ltd., Tokyo, Japan). All other compounds

were measured using the following kits: Intact PTH kit (Roche Diagnostics GmbH, Mannheim, Germany) and a 25(OH)D kit (Roche Diagnostics GmbH, Mannheim, Germany). The intra- and inter-assay CVs, respectively, were 1.5 and 2.0% for Ca, 2.1 and 2.3% for P, 2.5 and 4.5% for ALP, 5.7 and 7.3% for 25(OH)D and 1.4 and 2.9% for PTH as previously reported (17). Pigment-epithelium-derived factor (PEDF) was measured using an ELISA reader (R&D Systems, Inc., Minneapolis, MN, USA). The intra- and inter-assay coefficients of variation were 8.3 and 9.2%, respectively.

Whole exome capture and massively parallel DNA sequencing. The exome of proband 1 and proband 3 were sequenced to identify the pathogenic gene. Exon-enriched DNA was sequenced using an Illumina Genome Analyser II platform according to the manufacturer's protocol (Illumina, Inc., San Diego, CA, USA). The raw image files were processed using Illumina Base Calling Software v. 1.7 with default parameters and the sequence of each individual DNA fragment was reported as 101-bp paired-end reads. The sequencing reads were aligned to the NCBI human reference genome (NCBI36.3; https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.12/) using SOAPaligner 2.21 (18-20). The SOAPsnps results were filtered using the following standards: The base quality was ≥20, the sequencing depth was between 50-200 and the distance between two single-nucleotide polymorphisms (SNPs) was >5 bp (21-23). Approximately 114 million reads were quantified and mapped to the hs37d5 human reference genome DNA sequence, giving an average read depth of 70.0-96.5 for the exome of each proband. The percentage of the official target covered with at least 20X was 89.06%. All 1000G and dbSNP records, the filter region (including exonic or splicing) and the overlapping non-coding RNA regions were filtered out. Approximately 11.4 Gb of high-quality data were aligned to the target regions of the proband with a per-base mismatch rate of 0.52%. As a result, the mean coverage sequencing depth on the official target was 79. Reads that were among the target regions for SNP identification were collected for subsequent analysis. The consensus sequence and quality of each allele were calculated by SOAPsnps.

Sanger sequencing analysis. As whole-exome sequencing is expensive, Sanger sequencing of *SERPINF1* was used for other OI probands without *COL1A1* and *COL1A2* mutations. Mutations in *SERPINF1* for proband 2 were assessed. The results of exome sequencing for families 1 and 2 were verified using Sanger sequencing analysis of the *SERPINF1* gene. All eight exons and the exon-intron boundaries of *SERPINF1* were amplified from genomic DNA via polymerase chain reaction (PCR). Patient sequences were referenced to the Ensembl gene sequence ENSG00000132386 (*SERPINF1*; https://www.ncbi.nlm.nih.gov/nuccore/NC_000017.11?from=1761965&to=1777565&report=genbank).

The results of next-generation Sanger sequencing analysis of the *FKBP10* gene were used to verify Family 3. All eleven exons and the exon-intron boundaries of *FKBP10* were amplified from genomic DNA via PCR. Patient sequences were referenced to the Ensembl gene sequence ENSG00000141756 (*FKBP10*; https://www.ncbi.nlm.nih.gov/nuccore/NC_000017.11?from=41812262&to=41823217&report=genbank).

Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit, v. 3.1 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the sequencing was analysed with an ABI 3730XL automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). SNPs were identified using Polyphred (<http://droog.gs.washington.edu/polyphred>). Furthermore, Protein Variation Effect Analyzer (PROVEAN; <http://provean.jcvi.org/>) and Polymorphism Phenotyping (POLYPHEN-2; <http://genetics.bwh.harvard.edu/pph2/>) tools were used to predict whether an amino acid substitution had an impact on the biological function of the protein.

Quantitative PCR (qPCR) and detection of the allelic copy numbers of the two families to verify deletion. The following primers were used in the present study and were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi): Primers corresponding to the head site of the targeted gene *SERPINF1*, forward 5'-GCCTGCTGGACGCTGGATTA-3' and reverse 5'-CACCCAGCCTAGTCCCTCTAAGC-3'; primers corresponding to the mutagenic site of proband 2 on the targeted gene *SERPINF1*, forward 5'-CCATCATTCACCGGGCTCTCT-3' and reverse 5'-CGGGAGGCACTCTTGAGGTTC-3'; primers corresponding to the tail site of the targeted gene *SERPINF1* forward, 5'-TGGCTTTGAGTGGAACGAGGA-3' and reverse 5'-TGATAGTCCAGCGGAAGGTG-3'. The mutagenic site of proband 1 was near the tail of the gene region; as such, family 1 only needed two pairs of primers corresponding to the head and tail regions. However, family 2 required two pairs of primers corresponding to the head, tail and mutated regions. All DNA samples from the 2 families were detected using 2X SYBR Quant Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) qPCR assays and the reference gene was RPP14. Thermo cycling conditions were as follows: 95°C for 2 min followed by 40 cycles of 95°C for 15 sec and 60°C for 15 sec followed by the dissociation step. All reactions were performed in triplicate. The allelic copy number of the markers in the muted region was finally determined with the comparison of normalized data of control samples and patients using the $2^{-\Delta\Delta C_q}$ method (24).

Results

Clinical features Family 1. Proband 1 was an 11-year-old girl, the first daughter of non-consanguineous parents. She was the product of a full-term pregnancy with a normal delivery (Fig. 1), and her birth weight and length were within normal limits. Her height and weight at the time of the study were 132.6 cm (Z score, -1.7) and 35.4 kg (Z score, -0.3), respectively. She experienced her first fracture in the femur at the age of 2 years and has since experienced 5 subsequent fractures, including both femurs between 8 and 11 years. Proband 1 has evident deformities in both lower limbs. Blue sclera, brittle teeth and hearing loss were not observed and no family history of bone fragility was identified. Radiographs revealed thin cortices and normal diaphyseal modelling with bending deformities in both femurs (Fig. 2A). Radiographs of the thoracic vertebra and lumbar vertebra of proband 1 revealed multiple vertebral wedging, she did not feel any pain (Fig. 2B). Proband 1 was

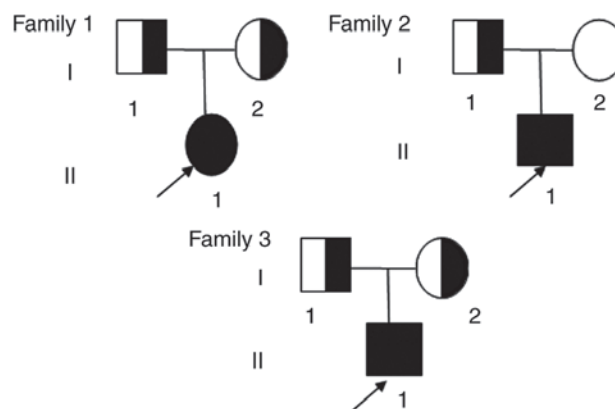


Figure 1. Pedigrees of the families included in the present study. Arrows indicate probands. Shaded symbols represent patients with osteogenesis imperfecta, half shaded symbols represent asymptomatic carriers. Squares represent males and circles represent females.

demonstrated to have a lower height and lumbar spine bone mass compared with healthy donors of the same age (Table I). Biological test results, including serum Ca, P, ALP and PTH were within normal ranges, whereas 25(OH)D levels were below the normal range (Table I). Her parents did not show any symptom of OI, except that her father was below average height (159.9 cm; Z score, -1.9).

Family 2. Proband 2 was a 4-year-old boy, the only son of non-consanguineous parents (Fig. 1). He was the product of a full-term pregnancy with a normal delivery, and his birth weight and length were within normal limits. His height and weight at the time of the study were 102.4 cm (Z score, -0.3) and 16.1 kg (Z score, -0.1), respectively. He experienced his first fracture in the left upper femur at the age of 3 years. Subsequently, he experienced another fracture in the left middle femur at the age of 4 years. Proband 2 presented with blue sclera without brittle teeth or hearing loss. Radiographs revealed thin cortices, normal diaphyseal modelling in the left femur and multiple vertebral wedging (Fig. 2C and D). Proband 2 had a decreased lumbar spine bone mass compared with healthy controls of the same age. All biological test results were within normal ranges (Table I). His parents did not exhibit any symptoms of OI.

Family 3. Proband 3 was a 12-year-old boy, the only son of non-consanguineous parents (Fig. 1). He was the product of a full-term pregnancy with a normal delivery, and his birth weight and length were within normal limits. His height and weight at the time of the study were 140.0 cm (Z score, -1.9) and 34.0 kg (Z score, -0.7), respectively. He experienced his first fracture in the right upper femur at the age of 10 years, with 4 subsequent fractures of the right femur. Proband 3 was unable to walk without the aid of auxiliary equipment following the first fracture. He did not present with blue sclera, brittle teeth, hearing loss or congenital large joint contracture. No family history of bone fragility was identified. Radiographs revealed thin cortices, normal diaphyseal modelling and bending deformities in the right femur, whilst a chest X-ray identified light scoliosis (Fig. 2E and F). Proband 3 had a lower lumbar spine bone mass compared with healthy controls of the

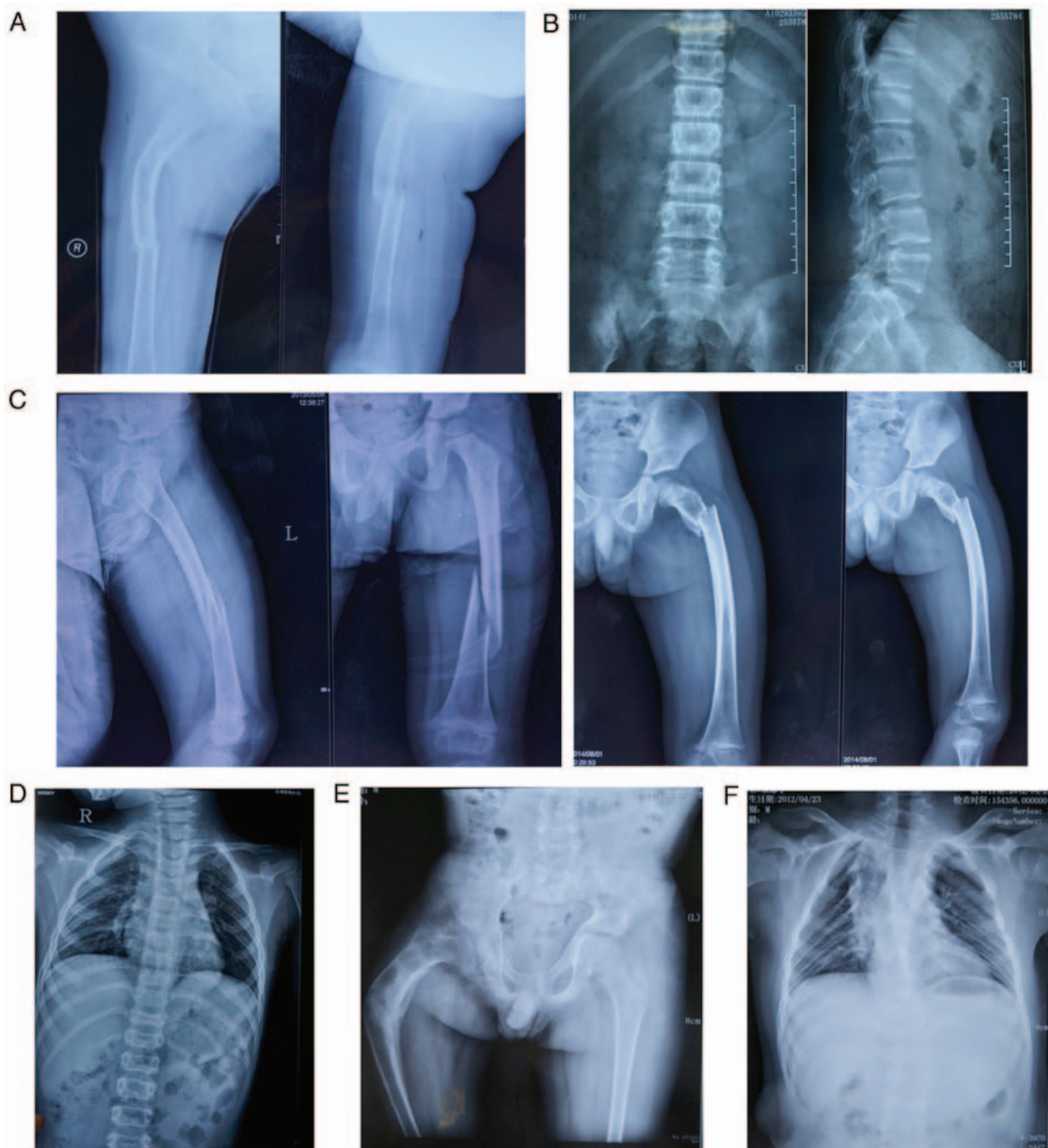


Figure 2. Radiographs of disease manifestations in three probands. (A) Anteroposterior and lateral images of the right leg of proband 1 with severe bowing of the right femur and multiple old and new fractures. (B) Radiographs of the thoracic and lumbar vertebrae of proband 1 with multiple vertebral wedging. (C) Radiographs of the left leg of proband 2, with two fractures and bowing of the left femur. (D) Chest radiograph of proband 2 revealed multiple vertebral wedging of thoracic vertebrae 11 and 12 and lumbar vertebra 1 with no signs of rib or clavicular fractures. (E) Radiographs of the right leg of proband 3 revealed severe bowing and multiple fractures of the right femur. (F) Chest radiograph of proband 3 revealed light scoliosis.

same age. Biological test results, including serum Ca, P, ALP and PTH, were within normal ranges. However, proband 3 was identified to have a 25(OH)D deficiency (Table I). His parents did not show any other symptoms.

Whole exome capture and massively parallel DNA sequencing. A novel missense substitution was identified in the *SERPINF1* gene of proband 1. This mutation was homozygous according to the autosomal recessive mode of inheritance.

The homozygous T to A transition at c.1067 in exon 8 of *SERPINF1* (Fig. 3) resulted in a valine to glutamic acid substitution at p.356 (V356E). This homozygous missense mutation occurred at a highly conserved position (Fig. 4). The results revealed that this missense mutation was deleterious with a PROVEAN score of -4.334. A similar result was obtained when using POLYPHEN-2.

A novel compound heterozygous mutation that consisted of a nonsense mutation (c.813_814delGA, p.Glu271AspfsX101)

Table I. General features and laboratory findings of the probands.

Parameter	Proband 1	Proband 2	Proband 3	Normal values
Sex	F	M	M	
Age (years)	11	4	12	
Blue sclera	-	+	-	
Dentinogenesis imperfecta	-	-	-	
Hearing loss	-	-	-	
Site of fracture	B	L	R	
First fracture age (years)	2	3	10	
Times of fracture	6	2	5	
Height Z-score	-1.7 ^a	-0.3	-1.9 ^a	>-1
Weight Z-score	-0.3	-0.1	-0.7	>-1
Lumbar spine 1-4 Z-score	-2.9 ^a	-2.6 ^a	-5.9 ^a	>-1
Calcium (mmol/l)	2.42	2.49	2.40	2.08-2.60
Phosphate (mmol/l)	1.39	1.27	1.02	0.80-1.60
Alkaline phosphate (U/l)	224	365	350	116-380
Parathyroid hormone (pg/ml)	58.0	29.3	54	15.0-65.0
25(OH)D (ng/ml)	16 ^a	34	18 ^a	>30

^aParameters outside of the normal range with clinical significance. F, female; M, male; B, both femurs; L, left femur; R, right femur.

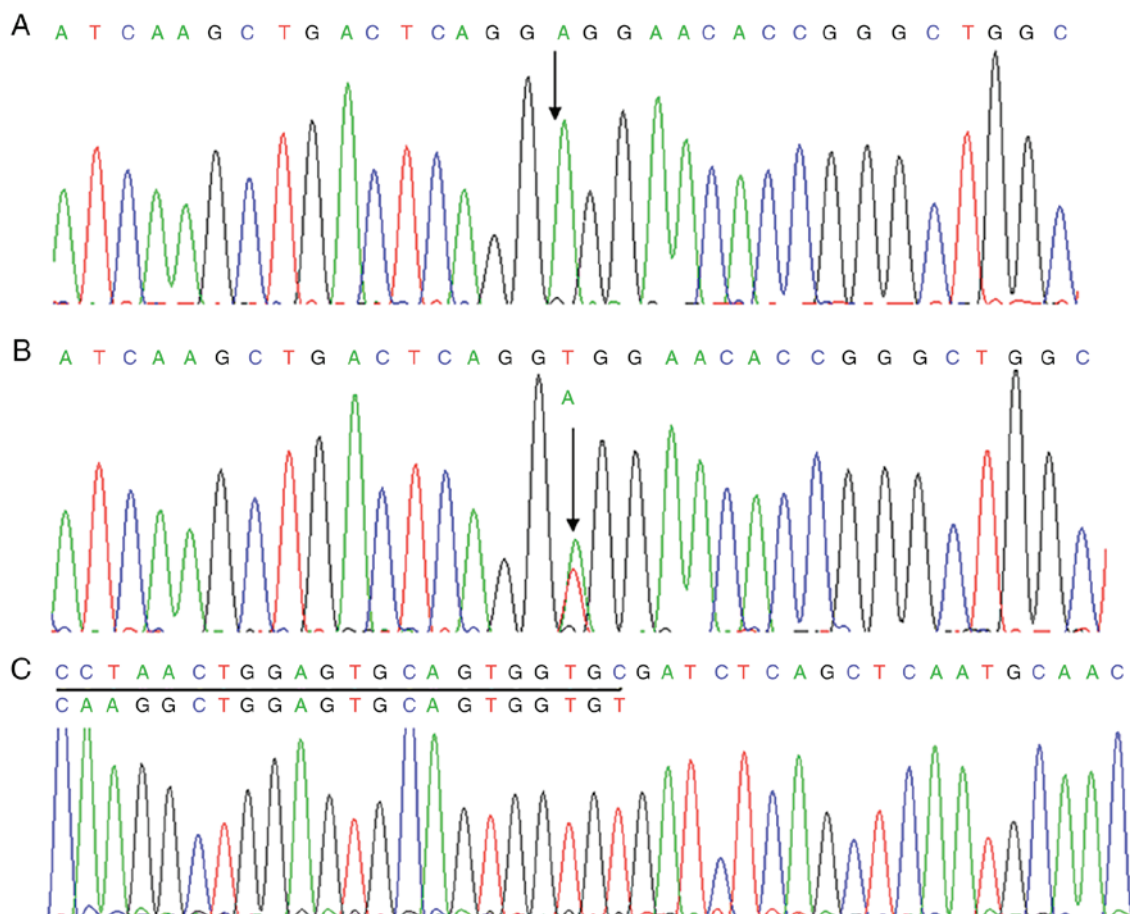


Figure 3. Genetic analysis of the Serpin family F member 1 gene mutation in family 1 and family 2. (A) Direct DNA sequencing of proband 1. Arrow indicates the homozygous missense mutation c.1067T>A (V356E). (B) Mutation screening was performed in the father of proband 1, a heterozygous carrier. Arrow indicates the site of the heterozygous missense mutation c.1067T>A (V356E). (C) Direct DNA sequencing of proband 2 and his father. The proband had homozygous deletion of a 1,657 bp base sequence including exon 4, intron 4, exon 5 and parts of intron 3 and 5, c.283+473_643+104del (p.Ala96_Gly215del). The DNA sequencing chart identified the chain with deletions in the proband's father, who had heterozygous deletion.

	338	p. Val356Glu
	I	
Human	FDSPDFSKITGKPIKLTQVE	
Rat	FESPDFSKITGKPVKLTQVE	
Mouse	FESPDFSKITGKPVKLTQVE	
Cattle	FDAPDFSKITGKPIKLTQVE	
Sheep	FDAPDFSKITGKPIKLTQVE	
Western clawed frog	FATPDFSKISSKPLKLSYV	
Zebrafish	LAETDTKITSQPVKLNAVH	
Dog	FDSPDFSKITGKPIKLTQVE	
Cynomolgus monkey	FDSPDFSKITGKPIKLTQVE	
Sarcophilus harrisii	FDSPDLSKITGKAIKLTQVE	

Figure 4. Homologous sequences of *SERPINF1* across species. The missense mutation p.Val356Glu observed in proband 1 occurs at a highly conserved position in *SERPINF1*. The bases that are identical to those in *Homo sapiens* are displayed in the box. *SERPINF1*, Serpin family F member 1.

and another nonsense mutation (c.831delC, p.Gly278AlafsX20) was identified in exon 5 of the *FKBP10* gene in proband 3 (Fig. 5A and B).

Validation of the *SERPINF1* and *FKBP10* germline mutation. The Sanger sequencing results for the *SERPINF1* gene were consistent with the whole exome sequencing results. A homozygous V356E (c.1067T>A) mutation was identified in proband 1. The heterozygous V356E (c.1067T>A) mutation was found in proband 1's father (Fig. 3B). However, no mutation in *SERPINF1* was observed in the proband's mother, and no V356E mutation was identified in the control subjects. According to OI variant databases (<https://oi.gene.le.ac.uk/>

variants.php?select_db=*SERPINF1*&action=view_all), the identified mutation is novel.

A homozygous p.Ala96_Gly215del (c.283+473_643+104del) mutation of *SERPINF1* was identified in proband 2 (Fig. 3C). The same heterozygous mutation was identified in the proband's father, whereas no mutation was observed in the proband's mother. The same compound heterozygous mutation was identified in proband 3 by further Sanger sequencing. The heterozygous mutation (c.831delC, p.Gly278AlafsX20) of *FKBP10* was present in proband 3's father, whereas the heterozygous mutation (c.813_814delGA, p.Glu271AspfsX101) was identified in the proband's mother.

In the present study, qPCR was repeatedly performed to detect allelic copy numbers of both families and verify whether there were deletions. The results for family 1 revealed a deletion of *SERPINF1* in the proband and her mother. However, the results for family 2 demonstrated that there was no *SERPINF1* deletion in the proband or her mother. Furthermore, the genotypes of all SNPs of proband 2 were homozygous. This suggests that, due to the uniparental disomy, the region of the chromosome of the proband of family 2 was derived from the father although the total copy number was unchanged.

Detection of serum PEDF. PEDF was undetectable in serum samples from proband 1. Serum samples were not obtained from her parents as initially only the whole blood of the parents was reserved and they did not return to have the serum extracted. Serum PEDF was also undetectable in proband 2. However, for proband 2's father and mother, the PEDF serum concentration was 13.8 and 37.8 $\mu\text{g/ml}$, respectively. PEDF serum concentrations were determined for 10 normal subjects. The median value was 22.4 $\mu\text{g/ml}$, the minimum was 12.0 $\mu\text{g/ml}$ and the maximum was 39.5 $\mu\text{g/ml}$.

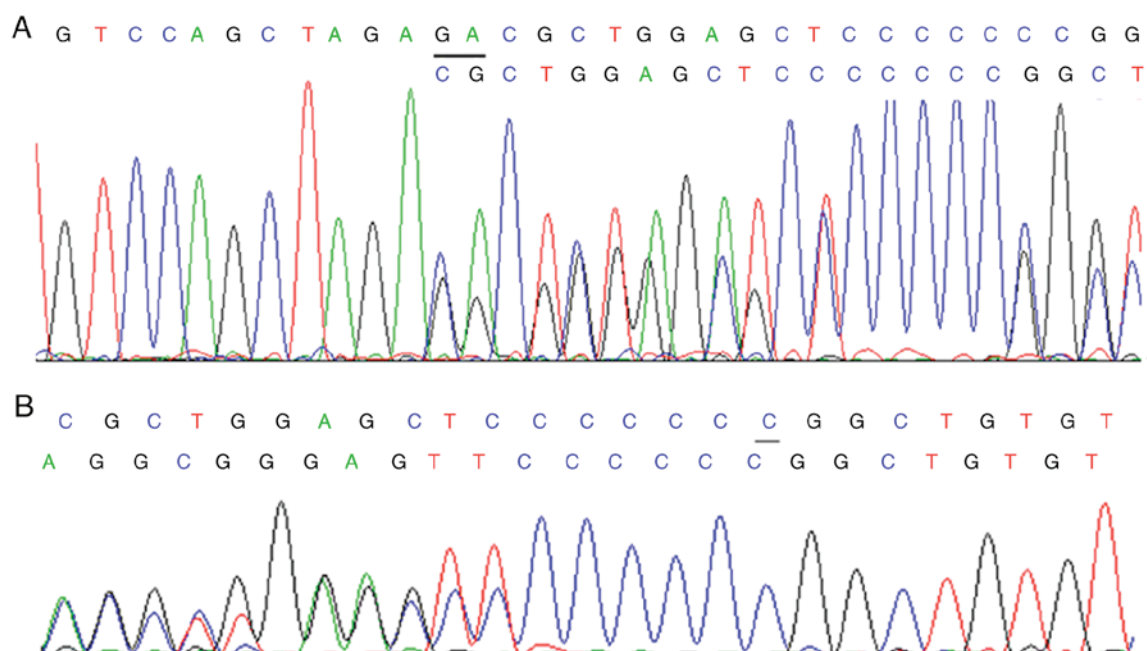


Figure 5. Direct DNA sequencing of proband 3. The proband exhibited a compound heterozygous mutation in FK506 binding protein 10 (A) c.813_814delGA, (p.Glu271AspfsX101) deletion mutation in exon 5 (B) and the other deletion mutation in exon 5 c.831delC(p.Gly278AlafsX20).

Discussion

The first causal gene of recessive OI, *CRTAP* (MIM 605497), was reported in 2006 (25,26). A number of additional genes, including *LEPRE1* (MIM 610339), *PPIB* (MIM 123841), *FKBP10* (MIM 607063), *SERPINF1* (MIM 600943), and *SP7* (MIM 606633) mutations, have since been reported to cause severe or lethal autosomal recessive OI (8,27-30). Becker *et al* (7) identified a lack of homozygosity of the *SERPINF1* gene product, PEDF, which caused severe OI in four patients. Alanay *et al* (8) first discovered homozygosity for mutations in *FKBP10* in a cohort of five consanguineous Turkish families and a Mexican-American family, causing autosomal-recessive OI.

The *SERPINF1* gene is located on chromosome 17p13.3 and encodes PEDF, which is a glycoprotein of the Serpin superfamily secreted by retinal pigment epithelial cells (7). In the musculoskeletal system, collagen-1 is bound by PEDF, which may act as an inhibitor of bone resorption by inhibiting osteoclast maturation via osteoprotegerin and receptor activator of nuclear factor- κ B ligand (7). Li *et al* (31) observed that PEDF suppressed the expression of genes that inhibit mineralization, leading to enhanced osteoblastic differentiation and increased matrix mineralization. In the present study, probands 1 and 2 experienced their first fracture when they were older than 1 year. Both probands suffered recurrent fractures of the femur, with proband 1 more severely affected than proband 2. Proband 1 was 11 years old and proband 2 was 4 years old at the time of the present study; this discrepancy in age may be responsible for the difference in phenotypic severity of OI. The sclera of proband 1 was white, whereas that of proband 2 was faintly blue. Both probands lacked dentinogenesis imperfecta. The phenotype of Chinese patients in the present study was similar to that of other patients reported in previous studies (5,7,13,32-35). Both probands were able to walk independently during the intermittent period between fractures, although they fractured their femurs many times. However, Caucasian patients with OI typically experience severe limb deformity and are mostly unable to walk independently (5,7,32-35). Phenotypes of Korean patients with type VI OI and Inuit patients have also been reported to be more severe compared with those presented in this study (5,35). Probands 1 and 2 presented with multiple vertebral wedging, however they did not report experiencing backache or lumbago. Moderate to severe bone fragility was observed in Chinese patients by Wang *et al* (13), further supporting the hypothesis that the phenotype of Chinese patients with OI is less severe compared with Caucasian and Korean patients.

To date, a total of 82 mutations in the *SERPINF1* gene, including 37 substitutions, 23 deletions, 20 duplications and 2 insertions, have been described (https://oi.gene.le.ac.uk/variants.php?select_db=SERPINF1&action=view_unique). The novel missense mutation identified in proband 1 consisted of a homozygous T to A transition at c.1067 in exon 8 (V356E) of *SERPINF1*. In proband 2, the novel deletion mutation identified comprised a homozygous p.Ala96_Gly215del (c.283+473_643+104del) deletion of *SERPINF1*, including the loss of intron 4, exon 4, exon 5 and part of introns 3 and 5. At present, this mutation is the only large deletion identified that

has been identified among mutations of the *SERPINF1* gene. The father of proband 2 was demonstrated to have a heterozygous deletion of *SERPINF1* with no symptoms of OI and normal stature. This indicates that carriers are asymptomatic despite the large fragment deletion. Proband 2 had comparatively mild symptoms; he was able to walk, jump and play with other children during the intermittent period between fractures. Furthermore, his height is within the normal range. The association between the severe mutation type and relatively mild phenotype of this proband should be further studied. Rauch *et al* (36) reported that the absence of circulating PEDF was specific to OI type VI. In the present study, probands 1 and 2 had undetectable serum concentrations of PEDF, which indicates that their mutations were pathogenic. In addition, the mutations occurred at a highly conserved position and were predicted as deleterious by PROVEAN and polyphen software.

Interestingly, the families of probands 1 and 2 in the present study exhibited nonpaternity. Both probands had homozygous mutations in the *SERPINF1* gene, whilst the fathers from both families showed heterozygous mutations. However, both mothers had no mutation on this allele. The mothers may therefore have a deleted *SERPINF1* allele. The results of allelic copy number detection revealed that proband 1 and her mother exhibited a *SERPINF1* deletion, meaning that family 1 did not deviate from Mendelian inheritance. However, no deletion of *SERPINF1* was observed in family 2. Furthermore, the genotypes of all SNPs of proband 2 were homozygous. It was therefore assumed that the uniparental disomy of proband 2 was in part because his chromosome originated from his father, although the total copy number was unchanged. Wang *et al* (13) also reported this phenomenon in a family that included a proband with a homozygous in-frame duplication, a heterozygous carrier mother and a father without mutations. It was suggested that Sanger sequencing of this phenomenon may reveal nonpaternity or partial *de novo* mutation.

The first *FKBP10* mutations were identified in families with a moderate to severe form of OI without contractures or webbing (8). Steinlein *et al* (37) also described patients with *FKBP10* mutations affected by severe autosomal recessive OI without contractures. However, Shaheen *et al* (38) discovered two brothers with Bruck syndrome, in which multiple joint contracture was caused by a mutation in the *FKBP10* gene. Kelley *et al* (9) concluded that *FKBP10* mutations are the cause of recessive OI and Bruck syndrome. Zhou *et al* (14) also reported a Chinese patient with OI and Bruck syndrome resulting from mutations in the *FKBP10* gene. In the present study, the patients presented with moderate OI without contractures or webbing. The majority of patients with OI experience their first fractures soon after birth or in infancy (8,14,37). However, in the present study, proband 3 suffered his first fracture when he was 10 years old, consistent with the phenotype described by Schwarze *et al* (39). Proband 3 did not have blue sclerae or dentinogenesis imperfecta and presented with scoliosis that developed during adolescence. His fracture was restricted on the right femur and resulted in deformity in this area. The results of the present study verified that *FKBP10* may cause Bruck syndrome or isolated OI. In addition, Schwarze *et al* (39) reported that isolated OI and Bruck syndrome may occur in siblings of the same family.

A total of 119 variants in the *FKBP10* gene have been identified, including 32 substitutions, 17 deletions, 62 duplications and 8 insertion/deletions and mutations primarily located in exon 5 (32.04%) and exon 6 (22.33%) (https://oi.gene.le.ac.uk/variants_statistics.php). The novel compound heterozygous mutation in proband 3 described herein was located in exon 5. Of these mutations, c.831dupC and c.948dupT occur most frequently, having been reported 21 and 19 times, respectively (https://oi.gene.le.ac.uk/variants.php?select_db=FKBP10&action=view_unique). In particular, the mutation c.831dupC has been reported in different origins, including the families of Turkish, Mexican, South African and Caucasian origin (8,9,39). In the present study, proband 3 also had a nonsense mutation located at c.831, with a deleted C instead of a duplicate C. These results suggest that codon 831 of the *FKBP10* gene may represent a mutation hotspot for human OI.

In conclusion, the results of the present study indicate that a novel missense mutation, c.1067T>A, in exon 8 and a large fragment deletion mutation, c.283+473_643+104del, in the *SERPINF1* gene cause type VI autosomal recessive OI in Chinese patients. The present study also demonstrated that a patient may experience mild symptoms of OI despite having a large fragment deletion in the *SERPINF1* gene. Furthermore, the phenotype of Chinese patients with type VI OI appears to be less severe compared with Caucasian and Korean patients. Deletion of the *SERPINF1* gene, as observed in one of the proband's parents, or the uniparental disomy of the proband may result from one heterozygous parent carrier and one parent without identified mutation producing a child with a homozygous mutation. The novel compound heterozygous mutations c.831delC and c.813_814delGA in *FKBP10* are responsible for moderate OI without Bruck syndrome in Chinese patients. Codon 831 of the *FKBP10* gene may represent a mutation hotspot for OI in humans. The results of the present study may improve the clinical and pathogenic gene spectrum of recessively inherited forms of OI in China.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZZ recruited patients, designed the study, revised and approved the final version of the manuscript. HZ recruited patients, performed experiments, and wrote the manuscript. YX, HY and CW performed experiments and revised the manuscript. JG, JH, WF and WH performed experiments and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China). All adult participants provided written informed consent prior to beginning the study. In addition, written informed consent was provided by the parents on behalf of the children enrolled in the present study.

Consent for publication

All the probands and their parents consented to publication of this study.

Competing interests

The authors declare that they have no competing interests.

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