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ORIGINAL ARTICLE

Novel compound heterozygous mutations in *SERPINH1* cause rare autosomal recessive osteogenesis imperfecta type X

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Abstract

Summary We identified novel compound heterozygous mutations in *SERPINH1* in a Chinese boy suffering from recurrent fractures, femoral deformities, and growth retardation, which resulted in extremely rare autosomal recessive OI type X. Long-term treatment of BPs was effective in increasing BMD Z-score, reducing fracture incidence and reshaping vertebrae compression.

Introduction Osteogenesis imperfecta (OI) is a heritable bone disorder characterized by low bone mineral density, recurrent fractures, and progressive bone deformities. Mutation in serpin peptidase inhibitor clade H, member 1 (*SERPINH1*), which encodes heat shock protein 47 (HSP47), leads to rare autosomal recessive OI type X. We aimed to detect the phenotype and the pathogenic mutation of OI type X in a boy from a non-consanguineous Chinese family.

Methods We investigated the pathogenic mutations and analyzed their relationship with the phenotype in the patient using next-generation sequencing (NGS) and Sanger sequencing. Moreover, the efficacy of long-term bisphosphonate treatment in this patient was evaluated.

Results The patient suffered from multiple fractures, low bone mass, and bone deformities in the femur, without dentinogenesis imperfecta or hearing loss. Compound heterozygous variants were found in *SERPINH1* as follows: c.149 T>G in exon 2 and c.1214G>A in exon 5. His parents were heterozygous carriers of each of these mutations, respectively. Bisphosphonates could be helpful in increasing BMD Z-score, reducing bone fracture risk and reshaping the compressed vertebral bodies of this patient.

Conclusion We reported novel compound heterozygous mutations in *SERPINH1* in a Chinese OI patient for the first time, which expanded the spectrum of phenotype and genotype of extremely rare OI type X.

Keywords Bisphosphonates · HSP47 · Osteogenesis imperfecta · *SERPINH1*

Introduction

Osteogenesis imperfecta (OI) is a heritable bone disorder characterized by low bone mineral density (BMD), recurrent fractures, and progressive bone deformity [1]. Extra-skeletal features include blue sclera, hearing loss, dentinogenesis imperfecta, and joint hyperlaxity. As a collagen-related disorder, about 85–90% patients with OI are caused by mutations in genes encoding type I collagen, such as *COL1A1* or *COL1A2*, with an autosomal dominant pattern of inheritance [2]. In addition, OI can be induced by mutations in genes, which regulate the post-translational modification (*CRTAP*, *P3H1*, *PPIB, TMEM38B*), secretion (*SERPINH1*, *FKBP10*), processing (*PLOD2*, *BMP1*) of type I collagen, as well as regulate osteoblast differentiation (*WNT1*, *SP7*) or bone mineralization (*IFITM5*, *SERPINF1*) [3–8].

In 2010, mutation in serpin peptidase inhibitor clade H, member 1 (*SERPINH1*, MIM 600943) was firstly reported to cause OI type X in human. *SERPINH1* is located at 11q13.5 and encodes heat shock protein 47 (HSP47), which is a 418-amino-acid protein and belongs to the serpin

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superfamily [9–11]. HSP47 can prevent local unfolding and aggregate formation of procollagen and regulate the activity of lysyl hydroxylase 2 (LH2) by forming an endoplasmic ergatoplasm (ER) complex with FK506 binding protein (FKBP65) and immunoglobulin heavy-chain-binding protein (BiP) [12]. Inactivation mutations in *SERPINH1* will cause incorrect aggregation and delayed secretion of type I procollagen molecules, which finally result in increased bone fragility and recurrent fractures [13].

To our knowledge, OI type X is extremely rare, and only five patients all over the world have been reported to carry four kinds of mutations in *SERPINH1*, which lead to autosomal recessive OI type X. Till now, no information about mutations in *SERPINH1* is available in Chinese OI patients [13–16]. Moreover, bisphosphonates (BPs) are widely used to treat bone fragility of OI patients, which are demonstrated to be effective in increasing BMD and decreasing bone fracture incidence. Only one patient with *SERPINH1* mutation was reported to receive pamidronate treatment; however, BMD was not increased after 2 years of BP treatment [14].

In the present study, we aim to investigate the phenotype, the pathogenic mutation, and the effects of BP treatment on bones of a Chinese patient with extremely rare OI type X.

Materials and methods

Subjects

A 5-year-old boy was recruited from a non-consanguineous Chinese family of Han origin by department of endocrinology of Peking Union Medical College Hospital (PUMCH) in 2008. He was clinically diagnosed with OI because of recurrent bone fractures, and he had no family history of bone fractures or bone deformities.

The study protocol was approved by the Scientific Research Ethics Committee of PUMCH, and the parents of the patient signed informed consent before they participated in this study.

Phenotype evaluation

The proband received detailed evaluation, including medical history, physical examination, biochemical index, and X-ray films. Bone fracture history and bone X-ray films were assessed to confirm the fracture number and sites. According to the standardized growth charts of Chinese children, age- and sex-adjusted Z score of height and weight were calculated. Fasting blood samples were collected for the biochemical analysis. The serum levels of calcium (Ca), phosphate (P), alkaline phosphatase (ALP), and creatinine (Cr) were measured by an automatic biochemical analyzer. Serum level of β-cross-linked C-telopeptide of type I collagen (β-CTX) was measured utilizing the automated Roche electrochemiluminescence system (Roche Diagnostics, Switzerland) at the central laboratory of PUMCH.

Radiographic assessments

BMD at lumbar spine 2–4 (LS) and femoral neck (FN) was measured using dual-energy X-ray absorptiometry (DXA, GE Lunar, USA) with appropriate pediatric software. Then, BMD Z-score was calculated according to reference data of BMD of Asian children [17]. X-ray films of the skull, thoracolumbar vertebrae, pelvis, and femurs were also examined.

Bisphosphonate treatment

The patient received oral alendronate (Fosamax, Merck Sharp & Dohme) 70 mg per week for 3 years and then received intravenous infusion of zoledronic acid (Aclasta, Novartis Pharma Stein AG) 5 mg annually for 4 years, with an intermittent discontinuation of 2 years because of fractures. During treatment, changes of BMD, serum levels of bone turnover markers (ALP and β-CTX), and fracture incidences were evaluated annually. Treatment safety was also evaluated including adverse effects of BPs, as well as liver and renal functions.

Molecular genetic analysis

Peripheral blood samples were obtained from the proband, his parents, and 100 unaffected healthy individuals. Genomic DNA was extracted from peripheral leukocytes under the standard protocols using the DNA Extraction Kit (QIAamp DNA, Qiagen, Frandfurt, Germany).

The DNA sample of the proband was sequenced by a targeted next-generation sequencing (NGS) panel (Illumina HiSeq2000 platform, Illumina, Inc., San Diego, CA, USA). The panel covered more than 700 genes that were implicated with bone disorders, which included candidate genes of OI (*COL1A1*, *COL1A2*, *BMP1*, *CREB3L1*, *CRTAP*, *FKBP10*, *IFITM5*, *LEPRE1*, *P4HB*, *PLOD2*, *PPIB*, *PLS3*, *SEC24D*, *SERPINF1*, *SERPINH1*, *SP7*, *SPARK*, *TMEM38B*, and *WNT1*). Sequencing was performed to 98.95% coverage for a mean depth of 200× on target regions in each of the chromosomes. NGS data was analyzed according to the protocol that was described previously [18]. Variants including missense mutations, nonsense mutations, frame shift mutations, and splice site mutations were expected to be pathogenic. Missense mutations causing substitution of amino acids were predicted with Polyphen2 software (http://genetics.bwh.harvard.edu/pph2/). Splice site mutations were predicted utilizing NNSPLICE0.9 (http://www.fruitfly.org/) and Human Splicing Finder (http://www.umd.be/HSF3/HSF.html).

To confirm the corresponding mutation in *SERPINH1* gene identified by NGS, we performed Sanger sequencing. Genomic DNA from the proband and his parents were used to amplify the region surrounding exon 2 and exon 5 of *SERPINH1* by polymerase chain reaction (PCR). Primers for PCR were as follows: F 5′-AGAGCTGAGGGTGG TTGTTG-3′, R 5′-GCACGGAATGTCGTCCTAAC-3′ for exon 2; F 5′-TCAGGGTAGTATGGGGTGGA-3′, R 5′-GGGAGAGGTTGGGATAGAGC-3′ for exon 5, which were designed by web-based Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/). Following an initial denaturation at 95 °C for 3 min, reactions were taken for 35 cycles of 95 °C for 30 s, 59–60 °C for 30 s, and 72 °C for 30–60 s. PCR products were then sequenced by ABI 377 DNA automated sequencer (Applied Biosystems) using standard protocols. Sanger sequencing was also performed in the 100 unrelated, healthy individuals to confirm that the mutations were not polymorphisms. The involving fragments were compared with the NCBI reference sequence of *SERPINH1* (NM_001235.3).

Three-dimensional modeling of HSP47 protein

We also investigated the protein change caused by mutations in the *SERPINH1* gene. The three-dimensional (3D) structure of the HSP47-collagen complex (PDB ID: 4AU2) was downloaded from Protein Data Bank. Then, the 3D structure of HSP47 was obtained by extracting the chain A of the PDB file 4AU2. Subsequently, the Lue50Arg and Arg405His mutants of HSP47 were built by mutagenesis module of PyMoL 1.7.6 software (http://www.pymol.org/). Finally, the models were verified by the Ramachandran plot.

Results

Phenotype of the patient

The clinical characteristics of the patient are shown in Table 1. The proband was a 14-year-old boy, the only child of the family. He was born full-term with a birth weight of 3100 g and a length of 50 cm. At 3 years old, he experienced bilateral femoral fracture for the first time. Subsequently, eight times of fractures occurred at his bilateral femurs under minor trauma from 3 to 5 years old. Gradually, his femurs became bowed and the movement of lower limbs was restricted. He came to our clinic at 5 years old. Physical examination revealed short stature, low weight (95 cm and 15 kg, all lower than the 3rd percentile of normal children), and apparent bending femurs. He had no scoliosis, blue sclera, hearing loss, and dentinogenesis imperfecta. X-ray films indicated severe osteoporosis, occipital wormian bone, deformities of proximal femurs, and slender long bone with thin cortical bone (Fig. 1a).

Mutations in *SERPINH1*

For the proband, novel compound heterozygous mutations were identified in *SERPINH1*: c.149 T>G in exon 2 (p.Leu50Arg) and c.1214 G>A in exon 5 (p.Arg405His), both of which led to amino acid substitutions (Fig. 2). These two missense mutations were predicted to be damaging with scores of 0.999 and 1.000 (Polyphen2), respectively. His father was a heterozygous carrier of mutation c.1214 G>A and his mother carried mutation of c.149 T>G. The affected HSP47 sequence was highly conserved across different species (Fig. 3b). The two missense mutations in *SERPINH1* were absent from the 100 healthy controls. Other gene mutations related to OI were not found in this patient.

The changes of amino acid of HSP47 in 3D structure induced by the mutations in *SERPINH1* of our patient are shown in Fig. 4.

Efficacy of BP treatment

At baseline, the patient's serum concentrations of Ca, P, ALP were within normal ranges. Since there was no normal reference of β-CTX in Chinese children, it was difficult to judge the result of β-CTX. His BMD and Z-scores at LS and FN were extremely low (Table 1). He received 3-year regular treatment of oral alendronate from 5 to 8 years old. Then, the treatment became intermittent because of fractures. When he was 10 years old, he received annually intravenous infusion of zoledronic acid for 4 years. After a total of 7 years of BP treatment, his BMD Z-score significantly increased from − 5.34 to 0.93 at LS, from − 5.06 to − 0.19 at FN, respectively (Fig. 1b). Average fracture rates decreased from 1.60 to 0.22 per year and compressed vertebrae were reshaped (Fig. 1a). During the treatment of alendronate, he experienced two fractures at left femur under minor trauma at his 6 and 8 years old, and no fracture happened when he received zoledronic acid treatment. His body length increased from 95 cm (5 years old) to 143 cm (14 years old), but Z-score of height was still low, from − 3.05 to − 3.18. The tolerance of the patients to alendronate and zoledronic acid was quite well and no obvious adverse events were found during the treatment.

Discussion

In the present study, we reported novel compound heterozygous mutations in *SERPINH1* for the first time in Chinese OI patients, which led to the extremely rare OI type X. The boy suffered from early-onset recurrent fractures, bilateral femur deformities, and short stature, without typical extra-skeletal manifestations. We identified novel compound heterozygous mutations in *SERPINH1* (c.149 T>G in exon 2 and c.1214G>A in exon 5) in this proband, and his parents were

Table 1 Phenotypes of the OI patient with *SERPINH1* mutation

	Baseline	After treatment[a]	Reference
Age	5 years	14 years	
Height (cm)	95	143	
Z-score of height	**−3.05**	**−3.18**	
Weight (kg)	15	51	
Z-score of weight	−1.60	−0.21	
Fracture number	8	2	
Scoliosis	No	No	
Clubfeet	No	No	
Camptodactyly	No	No	
Pterygia	No	No	
Color of sclera	White	White	
Dentinogenesis imperfecta	No	No	
Wormian bones	Yes	Yes	
Serum calcium (mmol/L)	2.69	2.56	2.13–2.70
Serum phosphate (mmol/L)	1.67	1.52	1.29–1.94
ALT (U/L)	9	31	9–50
Creatinine (μmol/L)	44	54	18–69
ALP (U/L)	248	273	58–390
β-CTX (ng/mL)	0.948	0.908	0.21–0.44 in adults
LS BMD (g/cm^2)	0.227	0.921	
Z-score of LS BMD	**−5.34**	0.93	
FN BMD (g/cm^2)	0.117	0.861	
Z-score of FN BMD	**−5.06**	−0.19	

Abnormal results were indicated in bold

[a] Seven years of BP treatment and 2 years of treatment intermittence

ALT alanine aminotransferase, *ALP* alkaline phosphatase, *β-CTX* cross-linked C-telopeptide of type I collagen, *LS* lumbar spine 2–4, *FN* femoral neck, *BMD* bone mineral density

proved to be the heterozygous carriers for these mutations. BPs were effective in reducing fracture rate, increasing BMD and reshaping the compressed vertebrae in the proband with *SERPINH1* mutations.

The exact mechanism of mutations in *SERPINH1* causing OI has not been completely elucidated. HSP47, encoded by *SERPINH1*, is an ER (endoplasmic reticulum)-resident molecular chaperone which contributes to proper assembly of the collagen triple helix [19, 20]. HSP47 would effectively prevent local unfolding and aggregation of the procollagen by specifically recognizing its Gly-Xaa-Arg repeats [21, 22]. Moreover, HSP47 can bind the procollagen in the ER, and then dissociate with it in the Golgi compartment in a PH-dependent manner, making sure the correct transportation of type I procollagen [23]. In *SERPINH1*$^{-/-}$ mice, collagen synthesis was defective and the mice died at around 11 days after birth [24]. In a dachshunds model, loss-of-function mutations in *SERPINH1* of OI could cause over modification and increased cross-linking of type I collagen [25, 26]. Therefore, *SERPINH1* mutations could alter post-translational modification of collagen due to aggregation and delayed secretion of procollagen molecules. Moreover, HSP47 could regulate the activity of lysyl hydroxylase 2 (LH2) by forming an endoplasmic ergatoplasm (ER) complex with FK506 binding protein (FKBP65) and immunoglobulin heavy-chain-binding protein (BiP) [12], through which *SERPINH1* mutations would lead to abnormal synthesis of type I collagen.

As far as we know, only six mutations in *SERPINH1* have been reported in OI patients including our study (http://lovd.nl/serpinh1). The mutations including 4 missense mutations (c.233T>C in exon 2, c.710T>C in exon 3, c.149 T>G in exon 2, and c.1214G>A in exon 5) and 2 deletion mutation (c.338_357del22 in exon 2 and c.314_325del12 in exon 2), which are shown in Fig. 3a. In this study, we identified two heterozygous mutations in *SERPINH1* gene in our patient, both of which were missense mutations (c.149 T>G and c.1214G>A). The c.149 T>G mutation caused Leu50Arg and the c.1214G>A led to Arg405His, respectively. Since Leu50 and Arg405 were highly conserved across different species, we speculated that these changes of the amino acids could impair the function of HSP47.

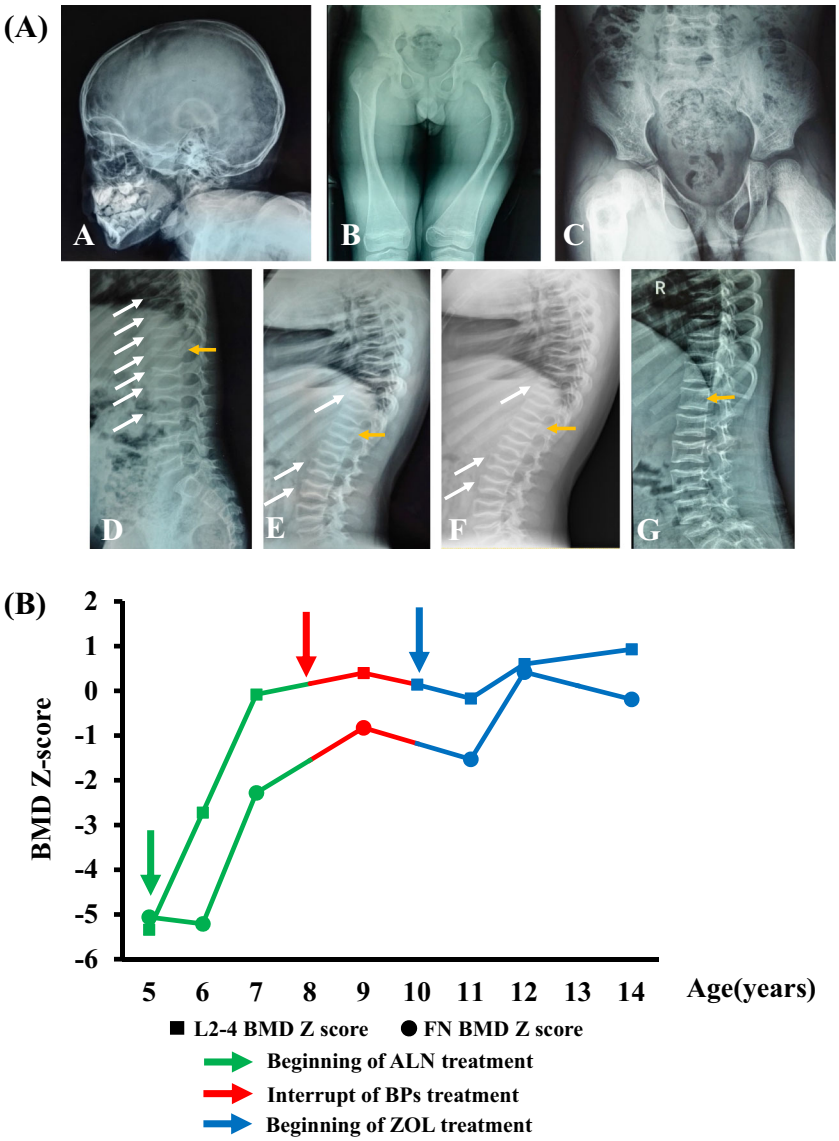

Fig. 1 Phenotype of the patient with *SERPINH1* mutation and effects of bisphosphonate treatment. **a** X ray films of the proband *A. wormian* bones at the occipital region. **b** Deformities in bilateral femur. **c** Low BMD of pelvis. **d** Lateral X-ray of the spine at 5 years old showed diffused vertebral compressions, indicated by white arrows. **e**–**g** Lateral X-ray showed reshape of several compressed vertebral bodies at 11, 13, 14 years old. Yellow arrows indicated the obvious reshape of the12th thoracic vertebra. **b** Changes in BMD Z-score at lumbar spine and femoral neck after treatment with bisphosphonates. ALN: alendronate; BPs: bisphosphonates; ZOL: zoledronic acid

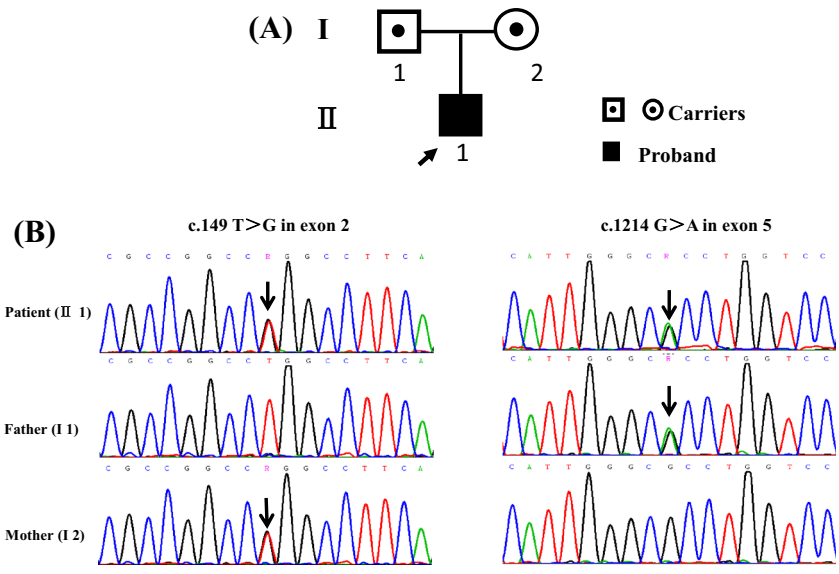

Fig. 2 Pedigree of the family with osteogenesis imperfecta and genetic analysis of *SERPINH1*. **a** The proband was designated with an arrow. **b** Sanger sequencing results of the patient and his parents. In the patient, novel compound heterozygous mutations in *SERPINH1* were identified as c.149T>G in exon 2 and c.1214 G>A in exon 5. The parents were heterozygous carriers for each of these mutations, respectively. Mutations were designated with arrows

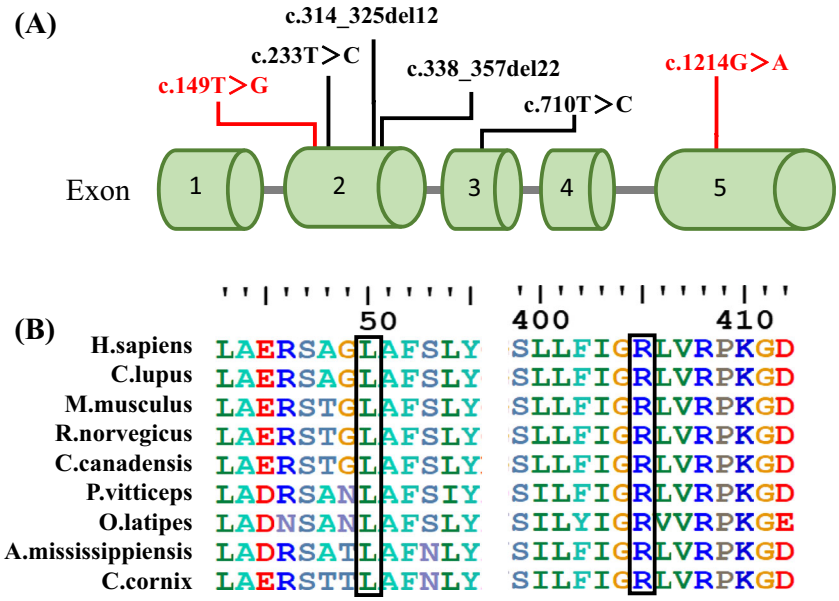

Fig. 3 Schematic view of *SERPINH1* exons structure (NM_001235.3) and sequence conservation in species. **a** The reported positions of *SERPINH1* mutations were indicated by black words. The novel mutations in our patient were indicated by red words. **b** Leu 50 and Arg 405 were highly conserved among 9 different species

HSP47 consisted of nine α-helixes and three β-sheets (A, B, C), with a signal sequence at the amino-terminus, two amino-glycosylation sites, and an ER-retention signal (Arg-Asp-Glu-Leu, RDEL) at the carboxy-terminus. Arg222, Leu381, Tyr383, Asp385 on β-sheet C were suspected to be the key residues of HSP47 binding to collagen peptide [21]. Serine domain was the major functional domain of HSP47, which was responsible for its chaperone function in the folding of fibrillar procollagen molecules. The Leu50Arg affected one of α helix, and Arg405His influenced β sheet, both of which could interfere with physiological function and activity of serine domain. Therefore, these mutations would significantly affect the functions of HSP47, which would lead to OI through impairing the folding of procollagen molecules.

Up to now, only six patients with *SERPINH1* mutations were reported, including our patient [13–16]. The phenotype of patients was obviously heterogeneous, which could vary from moderate to lethal (Table 2). Patient 2 suffered from multiple fractures in the ribs and severe deformities of bone, and finally died from respiratory distress at 3.5 years old. Patient 5 presented with perinatally lethal OI, who died at 8 days after birth. Similar with patients 3, 4, and 6, our patient (patient 1) presented with relatively moderate phenotype, with multiple fractures, obvious femoral deformity, and short stature. Extra-skeletal features, like blue sclera, hearing loss, and dentinogenesis imperfecta, were not found in patients 1, 3, and 4. However, it was difficult to establish genotype-phenotype correlation in OI patients with *SERPINH1* mutations, because the sample size was fairly small.

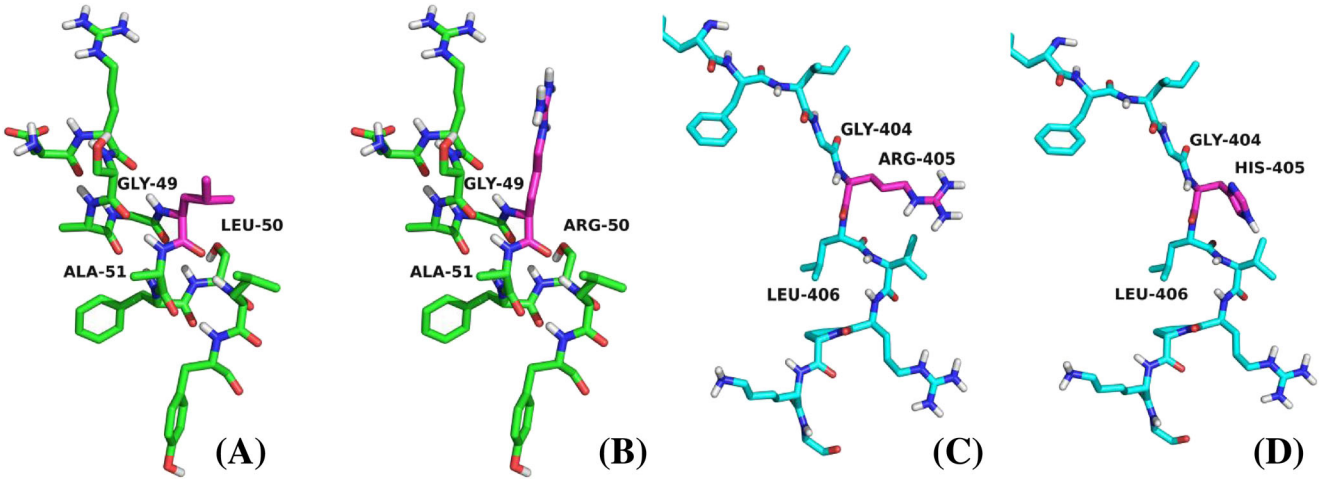

Fig. 4 Close-up of the three-dimensional structural model of HSP47 using the PyMol software (**a**–**d**). **a** Position 50 was a leucine in normal population. **b** Leu50Arg led to a change with an aliphatic straight chain. **c** Position 405 was an arginine in normal population. **d** Arg 405His led to a change with a hydrophobic core. Amino acids in position 50 and 405 were indicated in purple

Table 2 Molecular and clinical findings of this proband and previously reported patients with *SERPINH1* mutations

	Patient 1 (this study)	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Inheritance	Compound heterozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous
Gene mutation	c.149 T>G, c.1214 G>A	c.233T>C	c.710T>C	c.710T>C	c.338_357del22	c.314_325del12
Amino acid change	p. Leu50Arg, p. Arg405His	p. Leu78Pro	p. Met237Thr	p. Met237Thr	premature termination	p.(Glu105_His108del)
Sillence classification	IV	III	IV	IV	II	IV
Sex	Male	Male	Female	Male	Male	Male
Race	Chinese	Saudi Arabian	NA	NA	Mexican	Palestinian
Consanguineous pedigree	–	–	–	–	–	–
Fractures of extremities	+	+	+	NA	+	+
Age at first fracture	3 years	Before birth	4 years	6 months	NA	18 months
Number of fracture	10	Multiple	NA	NA	Multiple	4
Bowing of extremities	+	+	Mild	Mild	+	NA
Wormian bones	+	+	+	+	NA	NA
Color of sclera	White	Blue	White	White	Blue	Blue
Dentinogenesis imperfecta	–	+	–	–	NA	+
Hypermobility of joints	–	+	+	+	NA	NA
Cardiac impairment	–	–	NA	NA	NA	NA
Hearing impairment	–	–	–	–	NA	–
Growth retardation	Severe	Severe	NA	NA	NA	+
Intellectual development	Normal	NA	NA	NA	NA	NA
Others		Triangular face, relative macrocephaly, bitemporal narrowing			Passed away at 8 days old	
Literature	This study	14	13	13	15	16

NA not available

Bisphosphonates (BPs) were demonstrated effective to increase BMD, decrease fracture incidence in children with OI [27, 28]. Long-term treatment of BPs was indicated to be safe and effective in small size of OI patients [29, 30]. In a prospective study, 91 children with OI received 3years of treatment with 70 mg alendronate weekly. During the treatment, the mean annual fracture incidence decreased from 1.2 to 0.2, with the BMD Z-score at LS increasing from − 3.0 to 0.1, and there were no severe adverse events that happened during the treatment [30]. In OI patients with *SERPINH1* mutations, only two patients received BP therapy. Early at the age of 1 month, a patient was administrated with intravenous pamidronate acid every 2–4 months. However, his BMD at lumbar spine decreased from 0.246 to 0.210 g/cm^2 from 1 to 2 years old [14]. In our patient, 7 years of BP treatment was demonstrated to be effective in reducing fracture rates, increasing BMD at lumbar spine and proximal hip, with good tolerance. Additionally, reshape of compressed vertebrae was observed in our patient after the treatment of intravenous infusion of zoledronic acid (Fig. 1a), which was in accord with Palomo's research [31]. In Palomo's research, 37 children with OI were included, who received BP treatment before 5 years old. After more than 10-year follow-up, the rate of vertebrae compression decreased from 35% at baseline to 6% at the last evaluation [31]. However, the efficacy of BP treatment in patients with *SERPINH1* mutations still needed to be investigated in a large sample of patients.

In summary, we identified two missense mutations (c.149 T>G, p. Leu50Arg and c.1214 G>A, p. Arg405His) in *SERPINH1*, which resulted in extremely rare autosomal recessive OI type X. The patient presented with moderate phenotype of OI, including extremely low BMD, recurrent fracture, femoral deformities, and growth retardation. Long-term treatment of BPs was effective in increasing BMD, reducing fracture incidence, and reshaping vertebrae compression. Our findings of the novel mutations in *SERPINH1* would enrich the genetic spectrum of OI type X and emphasize the role of HSP47 in the pathogenesis of OI.

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Compliance with ethical standards

The study protocol was approved by the Scientific Research Ethics Committee of PUMCH, and the parents of the patient signed informed consent before they participated in this study.

Conflict of interests None.

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