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Novel mutations in *BMP1* induce a rare type of osteogenesis imperfecta

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ABSTRACT

Background: Osteogenesis imperfecta (OI) is a group of hereditary disorders characterized by low bone mass and recurrent fractures. OI patients of autosomal recessive inheritance are extremely rare, of which OI type XIII is attributable to mutation in *BMP1* gene.

Case report: Here, we detect the pathogenic mutations and analyze their relation to the phenotypes in a Chinese family with OI using next-generation sequencing (NGS) and Sanger sequencing. We also evaluate the efficacy of alendronate treatment in the patient with OI type XIII. The clinical phenotypes of the patient included recurrent fractures, muscle weakness, bone deformity, macrocephaly and elbow contractures, but no blue sclera or dentinogenesis imperfecta. High-resolution peripheral quantitative computed tomography revealed high bone mineral density and bone volume, but reduced trabecular numbers, increased porosity and comprised strength in this patient. Novel heterozygous mutations of c.1324G > T (p.Asp442Tyr) and c.148 + 1G > A in *BMP1* gene were found in the proband, which would affect the CUB2 domain and the prodomain of mutant proteins. The parents were heterozygous carriers for the two mutations respectively, but with normal phenotype.

Conclusions: We report for the first time that the novel pathogenic mutations in *BMP1* can lead to the extremely rare OI type XIII, which exhibit unique characters of high bone mass, but with impaired bone microstructure and comprised bone strength. Alendronate is beneficial in increasing bone mineral density and decreasing bone resorption biomarkers, but concerns still remain whether it can reduce fracture incidence in this rare type of OI.

1. Introduction

Osteogenesis imperfecta (OI) is the most common heritable skeletal dysplasia which is characterized by increased bone fragility, low bone mass and recurrent fractures [1]. An array of extra-skeletal features can also be present, including blue sclera, hearing loss, dentinogenesis imperfecta, joint hyperlaxity, pulmonary function impairment and cardiac valve abnormalities [2,3]. A major of OI patients follow an autosomal dominant pattern of inheritance, and > 80% of patients are caused by mutations in *COL1A1* and *COL1A2* gene, which encode the chains of type I collagen [4]. Recent studies have also identified a number of genes related to other autosomal dominant, recessive and X-linked forms of OI. These genes encode proteins which are either involved in the post-translational modification (*CRTAP*, *P3H1*, *PPIB* and *TMEM38B*) or folding (*SERPINH1*, *FKBP10*, *PLOD2* and *BMP1*) of type I collagen, or affecting formation and homeostasis of bone (*SP7*, *SERPINF1*, *IFITM5, WNT1*, *CREB3L1, SPARC, P4HB, SEC24D, MBTPS2* and *FAM46A*) [5–20].

As we know, type I collagen is the most important structural protein in bone matrix and act as the template for mineral deposition [21]. Once the protein is assembled and secreted into extracellular matrix (ECM), the collagen molecules will be arranged into fibrils after proteolytic removal of their carboxyl- and amino-terminal propeptides [21]. Cleavage of the proα(I) C-terminal propeptide (PICP) is conducted by the BMP1/TLD-like (bone morphogenetic protein-1/Tolloid-like) family of metalloproteases, which comprise two alternative splicing products of the *BMP1* gene and two tolloid-like proteins coded by the *TLL1* and *TLL2* gene [22]. Of these four enzymes, it is reported BMP1

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and mammalian TLD (mTLD) present the highest PICP-proteinase efficiency [22].

Located on chromosome 8p21.3, *BMP1* gene encodes the two proteins of BMP1 (730 amino acids) and mTLD (986 amino acids). The common amino-terminal portion of BMP1/mTLD (1-702aa) is coded by exons 1–15, while the different carboxyl-terminal ends are coded by exon 16a for BMP1 and exons16b-20 for mTLD [23]. Based on its key roles in collagen and bone formation, *BMP1* was firstly identified as the pathogenic gene of autosomal recessive OI type XIII (OMIM 614856) in families with progressive deforming bones in 2012 [24,25]. Phenotypic characteristics of OI type XIII included numerous fractures with varying bone mineral density and short stature with rhizomelia. Recent bone tissue assessment also revealed twisted, intermingled and disorganized architecture of type I collagen fibrils [26,27]. Up to now, only 14 families with mutations in *BMP1* gene have been reported, which come from various ethnic groups including Caucasians, Turks, Egyptian, French-Canadian and Asians [24–31]. However, few information about *BMP1* mutation and its phenotype has been reported in Chinese patients with OI.

Herein, we report a Chinese Han family suffering from recurrent fractures, high bone mass, muscle weakness and joint contractures. We detect the pathogenic mutations, and assess the clinical phenotypes and the effects of bisphosphonates (BPs) treatment on bone of this patient.

2. Materials and methods

2.1. Subjects

A fifteen-year-old boy suffering from fragility fractures came to the clinic of endocrinology department of Peking Union Medical College Hospital (PUMCH) in 2015. The present study included the patient and his parents. An additional unrelated 100 healthy individuals were recruited as controls for this genetic study. The study was approved by the Scientific Ethics Committee of PUMCH (2015 S-778). Signed informed consents were obtained from the members of this family and controls before their participations in this study.

2.2. Phenotypic evaluation

A detailed medical history was collected and physical examination was completed. Fracture was suspected by medical history and confirmed by X-ray films of bone. Age- and sex-adjusted height and weight were calculated based on the standardized growth charts for Chinese children [32]. The levels of serum calcium (Ca), phosphorus (P), alanine aminotransferase (ALT) and creatinine (Cr) were measured by an automatic analyzer (Siemens, Munich, Germany). Serum concentrations of β-cross linked C-telopeptide of type I collagen (β-CTX), total alkaline phosphatase (TALP), 25-hydroxyvitamin D (25OHD) and intact parathyroid hormone (PTH) were measured using an automated electrochemiluminescence system (Roche Diagnostics, Basel, Switzerland). Serum levels of pigment epithelium-derived factor (PEDF) were quantified using a DuoSet enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) [12].

Bone mineral density (BMD) at lumbar spine 2–4 (LS), femoral neck (FN) and total hip (TH) was measured by dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Healthcare, Madison,WI, USA) with appropriate pediatric software. BMD *Z*-scores were calculated based on data from age- and gender-matched normal Chinese children [33]. High-resolution peripheral quantitative computed tomography (HR-pQCT) was performed using an Xtreme CT II (SCANCO Medical AG, Bruttisellen, Switzerland) as previously described [34]. Trabecular volumetric BMD, trabecular bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and separation (Tb.Sp) were measured at the 4% site of the radial metaphysis. Results of HR-pQCT were compared to the average values of normative young reference databases (Asian and Caucasian) [34,35].

2.3. Genetic analysis by next-generation sequencing (NGS)

Genomic DNA of the proband and their parents was extracted from peripheral leukocytes with a QIAamp DNA Mini Kit (50) (Qiagen, Duesseldorf, Germany). A targeted NGS panel was designed to capture all sequences of 700 genes involved in skeletal disorders, including 19 OI-related genes (*COL1A1, COL1A2, IFITM5, SERPINF1, CRTAP, P3H1, PPIB, SERPINH1, FKBP10, SP7, BMP1, TMEM38B, WNT1, CREB3L1, SPARC, PLOD2, PLS3, P4HB* and *SEC24D)* and 9 genes related to osteopetrosis (*TCIGR1, CLCN7, CTSK, OSTM1, RANKL, CAII, PLEHKM1, NEMO* and *LRP5*). NGS was performed using the Illumina HiSeq2000 (Illumina Inc., San Diego, CA, USA). To detect the variants at high sensitivity and accuracy, the coverage target for sample was set at a minimum average deep of 150 × .

Variants were filtered using SAMtools (version 1.4) and SOAPsnp software (version 2.0) to exclude variants with a minor allele frequency (MAF) ≥ 0.005 in the Single Nucleotide Polymorphism Database, Exome Variant Server, 1000 Genomes Project, National Heart, Lung, and Blood Institute, or UCSC common SNP database and inner control database. We only analyzed variants considered to produce damaged proteins, such as frameshift, nonsense and missense variants, or variants affecting acceptor and donor splice sites [9,36]. Pathogenic prediction of missense variants were performed using Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/), and potential splice site variants were predicted with NNSPLICE0.9 (http://www.fruitfly.org/) and NetGene2 Server (http://www.cbs.dtu.dk/services/NetGene2/).

2.4. Sanger sequencing

Polymerize chain reaction (PCR) and Sanger sequencing were used to confirm the variants and to provide quality assurance. According to the results of NGS, corresponding fragments of *BMP1* gene were amplified by PCR. Primers were designed using Primer3 software (Table S1). PCR was performed under the following protocol: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30s, 59–61 °C for 30s, and 72 °C for 60s. Direct sequencing of PCR products were performed by an ABI 3130 automatic sequencer (Applied Biosystems, Foster City, CA, USA) under the standard operating procedures. Sanger sequencing was also performed for his family members, and 100 unrelated, healthy subjects to confirm that the mutations were not merely polymorphisms. Chromatograms were aligned and compared with the reference sequence of *BMP1* (NM_001199.3 and NM_006129.4). Novel mutations were identified by their absence from the Osteogenesis Imperfecta Variant Database [37].

2.5. Reverse transcription-PCR

To evaluate the changes of mRNA induced by the splicing mutation, reverse transcriptase-PCR amplification was performed. Total RNA was isolated from whole blood using the RNA Extraction Kit (Qiagen, Duesseldorf, Germany). The cDNA was then generated from total RNA by reverse transcriptase-PCR reaction using the Goscript™ Reverse Transcription system kit (Promega, Madison, WI, USA). Specific primers for PCR amplification of *BMP1* cDNA was designed (Table S1). PCR was performed using the similar methods as above.

3. Results

3.1. Clinical phenotype

The proband (II-2), a fifteen-year-old boy, was the second child of a non-consanguineous couple, who was born full-term by caesarean delivery with a birth weight of 3300 g (50th centile) and a length of 48 cm (10th centile). Neither his parents nor his brother had history of bone fracture. The patient was able to sit up at seven months old, speak at

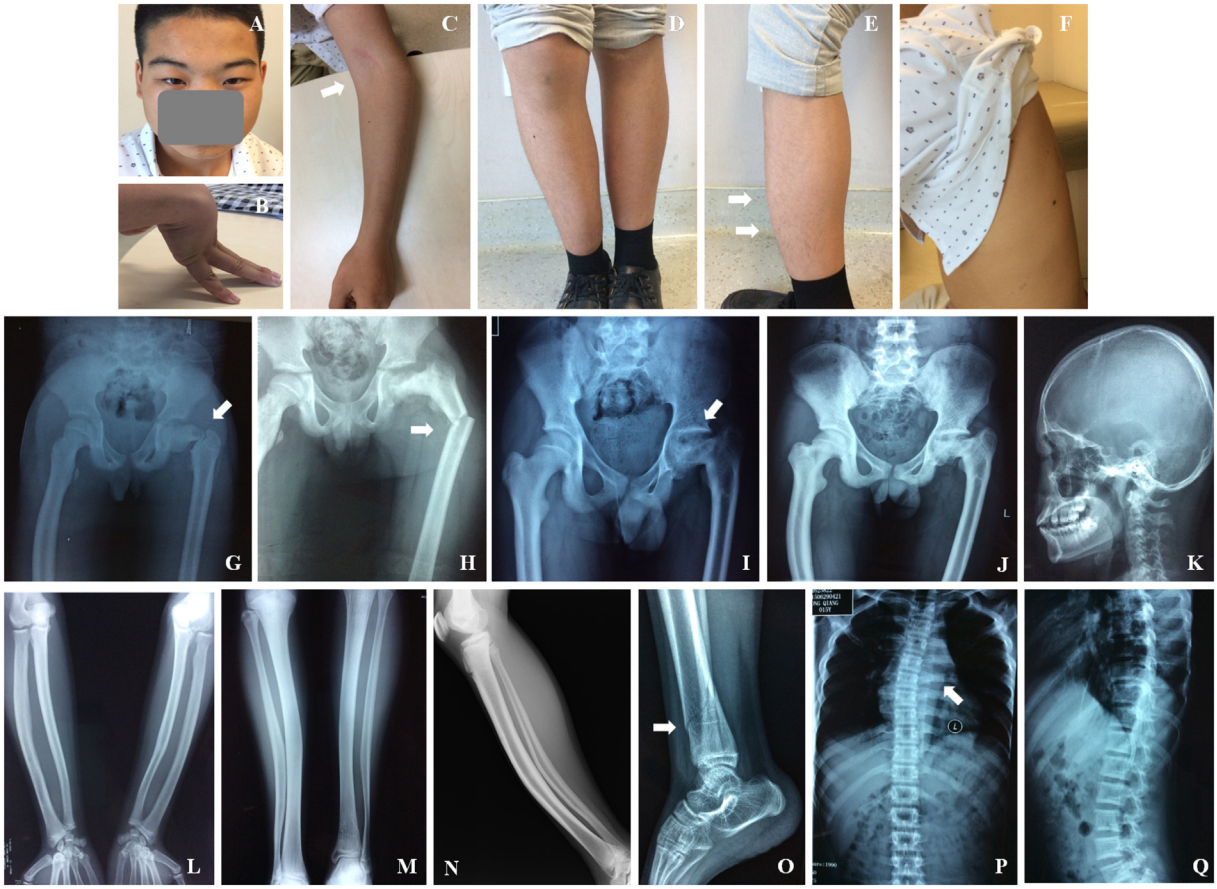

Fig. 1. Clinical and radiological phenotypes of the patient with OI.

twelve months old, and walk at fifteen months old. Intelligence and language skills were satisfying, and motor development was normal. At 22 months old, his first fracture occurred in the left femur under mild trauma, and then he experienced additional nine fractures in long bone (clavicle, forearm, femurs, tibia and fibula) as well as two hand fractures in the following 13 years. Healing process of fracture appeared normal. In addition to fractures, he presented muscle weakness in recent 3 years, but could perform the most activities of daily living. Physical examination indicated a relatively short stature (162 cm at −1.5SD), rather macrocephaly, broad forehead, square face and mild hypertelorism. He had joint hyperlaxity, but no blue sclera, dentinogenesis imperfecta, deafness or bruising skin. Notably, there were bilateral elbow contractures, swaying gait and mildly bowed tibias, with a leg length discrepancy of 5 cm (Fig. 1A–F). Tanner stage of this boy was assessed as stage IV.

X-ray films revealed a generalized increase in bone mineral density, slender and bent long bones with dense cortices, mild scoliosis, but no vertebral compressed fractures and no wormian bones (Fig. 1G–Q). The apparent elevated bone mass was also confirmed by the DXA and HR-pQCT examinations. The *Z*-scores of areal BMD at proximal hip were as high as +2.7, compared with −3.3 ± 2.2, −3.6 ± 2.3 in subjects with *COL1A1/COL1A2* mutations and other autosomal recessive gene mutations in our Chinese cohort. The trabecular volumetric BMD at distal radius was 273.45 mg/cm^3, which was 31% higher than the mean value in age and gender-matched healthy Caucasian population (Table 1) [34]. Furthermore, HR-pQCT showed high BV/TV and Tb.Th, but low Tb.N and increased Tb.Sp values, indicating an impaired bone microstructure (Table 1). Serum levels of calcium, phosphorus, TALP and PEDF were within normal ranges, whereas serum level of β-CTX was elevated. Vitamin D deficiency and mild secondary hyperparathyroidism were also found (Table 1).

3.2. Treatment and follow-up

Treatment with weekly oral alendronate 70 mg (Fosamax; Merck, Hunterdon, NJ, USA), plus daily vitamin D3 200IU and elemental calcium 500 mg (Calcichew D; GE healthcare, Chicago, IL, USA) were administered, as the patient had recurrent fractures, increased bone resorption biomarker, and impaired bone microstructure [38]. During 12-month treatment, we observed improved 25OHD and PTH levels; serum β-CTX decreased from 1.0 to 0.7 ng/ml; areal BMD significantly increased by 27%, 28%, 21% at LS, FN and TH respectively (Table 1); and no new fractures occurred. But he did not experience any catch-up growth, corresponding to −1.7SD of height at the particular age. BPs therapy was then discontinued because his bone appeared abnormally dense on various bone images (Fig. 1J, Table 1).

3.3. Mutations in BMP1 gene

Sequence analysis revealed the patient harbored novel compound heterozygous mutations in *BMP1*: a c.1324G > T in exon 11, leading to the substitution of aspartate at position 442 by tyrosine (p.Asp442Tyr), and c.148 + 1G > A mutation among the first two strictly conserved nucleotides in the donor splice site of intron 1 (Fig. 2A). The missense mutation was predicted as “probably damaging” with a score of 0.984 (PolyPhen2), and the affected sequence was highly conserved across species and astacin-like metalloproteases (Fig. 2B). The mutation c.148 + 1G > A was predicted to cause aberrant splicing with donor splice scores decreasing to 0, compared with the 0.89 score in reference sequence (NNSPLICE0.9). We also performed reverse transcription-PCR, but failed to amplify *BMP1* mRNA from peripheral blood sample of both the patient and healthy controls. Segregation analysis showed the heterozygous presence of c.1324G > T in the mother, whereas

Table 1
Clinical characteristics and bone parameters of the patient with OI.

	Baseline	12 m of visit	28 m of visit	Reference values
Age (year)	15.7	16.7	18.0	
Height (cm) (Z-score)	160 (−1.5)	162 (−1.7)	164 (−1.5)	
Weight (kg) (Z-score)	52 (−0.7)	48 (−1.2)	45 (−2.0)	
Serum calcium (mmol/L)	2.47	2.45	2.47	2.13–2.70
Seum phosphate (mmol/L)	1.67	1.62	1.02	1.29–2.26
Serum TALP (U/L)	341	209	133	42–390
Serum β-CTX (ng/ml)	0.969	0.739	0.471	0.21–0.44
PTH (pg/mL)	104	45.7	32.8	12.0–65.0
25OHD (ng/mL)	15.3	21.2	28.1	20.0–50.0
PEDF (ug/ml)	10.45	N/A	N/A	see note below[c]
Serum creatinine (μmol/L)	48	50	55	18–62
Serum ALT (U/L)	21	19	20	9–50
LS BMD (g/cm^2) (Z-score)	1.067 (+2.1)	1.355 (+4.6)	1.371 (+1.4)	
FN BMD (g/cm^2) (Z-score)	1.069 (+1.9)	1.373 (+4.1)	1.428 (+3.9)	
TH BMD (g/cm^2) (Z-score)	1.239 (+2.7)	1.500 (+4.6)	1.595 (+5.0)	
Tb.BMD(mg HA/cm^3)	273.45	N/A	N/A	208.98[a]
BV/TV	0.270	N/A	N/A	0.162 (0.154–0.169)[b]
Tb.N (1/mm)	1.38	N/A	N/A	1.92 (1.85–1.98)[b]
Tb.Th (mm)	0.203	N/A	N/A	0.085 (0.081–0.088)[b]
Tb.Sp (mm)	0.681	N/A	N/A	0.448 (0.430–0.466)[b]

Biochemical and bone mineral density results outside the reference ranges were shown in bold.
TALP, total alkaline phosphatase; β-CTX, β-cross linked C-telopeptide of type I collagen; PTH, parathyroid hormone; 25OHD, 25 hydroxy-vitamin D; PEDF: pigment epithelium-derived factor; ALT, alanine aminotransferase; BMD, bone mineral density; LS, lumbar spine 2–4; FN, femoral neck; TH, total hip; Tb.BMD, trabecular bone mineral density; HA, hydroxyapatite; BV/TV, trabecular bone volume fraction; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; N/A, not available.
Reference range for the results given as Z-scores: −2 to +2 SD.
[a] Mean value for age-specific Caucasian males at distal radius (age: 15.7 year) [34].
[b] Mean outcomes (95% CI) for Asian males at distal radius (age: 17.2 ± 1.5 year) [35].
[c] Serum PEDF level in healthy controls (n = 24) established in our laboratory: 2.13–12.59μg/ml [12].

c.148 + 1G > A was inherited from the father (Fig. 2A).

These two mutations in *BMP1* were absent from 100 healthy controls, and did not match polymorphisms in any of the public databases. No pathogenic variants were identified in other genes associated with OI or osteopetrosis.

4. Discussion

OI type XIII is an extremely rare autosomal recessive, moderate-to-severe form of OI that is attributed to mutations in *BMP1* gene. We report for the first time that mutations in *BMP1* lead to early-onset fractures, joint contractures, muscle weakness, high bone mass and impaired bone microstructure in a Chinese boy, which expand the phenotypic spectrum of mutations in *BMP1*. The causative mutations are novel compound heterozygous mutations in *BMP1* (c.1324G > T and c.148 + 1G > A), and his parents are heterozygous carriers of these mutations. Treatment with alendronate and calcium is effective to increase BMD, but efforts are still needed to confirm its anti-fracture efficacy in this rare form of OI.

Until now, sixteen unique sequence variants have been identified in *BMP1* gene (Fig. 3). In the present study, we identified biallelic mutations of c.1324G > T in exon 11 and c.148 + 1G > A in intron 1 of *BMP1*, which were both novel pathogenic mutations of OI. The encoding BMP1/mTLD, mainly expressed in mineralized and soft connective tissues, acts as an astacin metalloprotease and takes responsibility for the proteolytic removal of C-terminal propeptide from procollagen type I, II and III and the N-terminal propeptide from type V and XI procollagen [23]. It is reported the postnatal and adult BMP1 expression is essential and important in osteogenesis, ECM and cartilage formation [23,39]. This suggests *BMP1* mutations may promote bony fragility or contracture, roles consistent with the phenotypes in our patient. Loss of functional mutations in *BMP1* is demonstrated to impair PICP cleavage, hinder small leucine-rich proteoglycan (SLRP) prodecorin processing, and lead to abnormal type I collagen fibrils assembly and disorganized heterotypic extracellular matrix [24,27]. In the present study, the p.Asp442Tyr variation in *BMP1* affects the highly conserved CUB2 domain (Fig. 2B and 3), then BMP1 protein interactions will be disturbed and proteinase activity will be impaired [23]. Another variation in a splice site (c.148 + 1G > A) might introduce alternatively spliced transcripts and prodomain disruption; As prodomain is essential for BMP1 latency, processing and activity [23,40], this mutant will be severely functionally compromised. Future reverse transcriptase-PCR performed with fibroblasts and function verification is still worthwhile [29].

The phenotypes of our patient with *BMP1* mutations are somewhat similar to those of patients with OI type XIII (Table 2). Moreover, the boy presents muscle weakness, swaying gait, joint contractures, as well as some dysmorphic features, which further expands the phenotypic spectrum of this rare type of OI. Recently, it has been shown that BMP signaling took part in muscle maintenance, growth and atrophy, and it might be a positive regulator of skeletal muscle mass [41,42]. We then infer that mutant BMP1 protein may potentially be involved in the phenotype of muscle weakness and swaying gait in our patient. Altogether, 19 patients with *BMP1* mutations are now identified, 12 of whom are diagnosed with severe form, whereas 7 patients with mild to moderate bone fragility. Our patient has moderate phenotypes resembling Sillence type IV of OI [43]. Among all *BMP1*-related OI, we note the patients with homozygous polyadenylation site mutation (c.*241 T > C in FM4–6, Table 2 and Fig. 3) show the mildest phenotype, including late onsets of fractures (10 m to 4 yr of age), low fracture rates (1–2 fractures per year) and mild or absent bone deformities. These may be attributed to the distinct variant location leading to isolated BMP1 dysfunction but remaining compensatory mTLD [27]. Similarly, patients with the homozygous SP mutation (p.G12R in FM2–3, Table 2 and Fig. 3) also present a less severe phenotype, because this variant is not so important to protein activity. By contraries, patients with mutant alleles affecting both isoforms are relatively more severe, no matter in homozygote or compound heterozygote, tending to have multiple fractures, short stature, limbs deformity and severe defects of collagen matrix assembly in fibroblast assay [26]. Notably, we

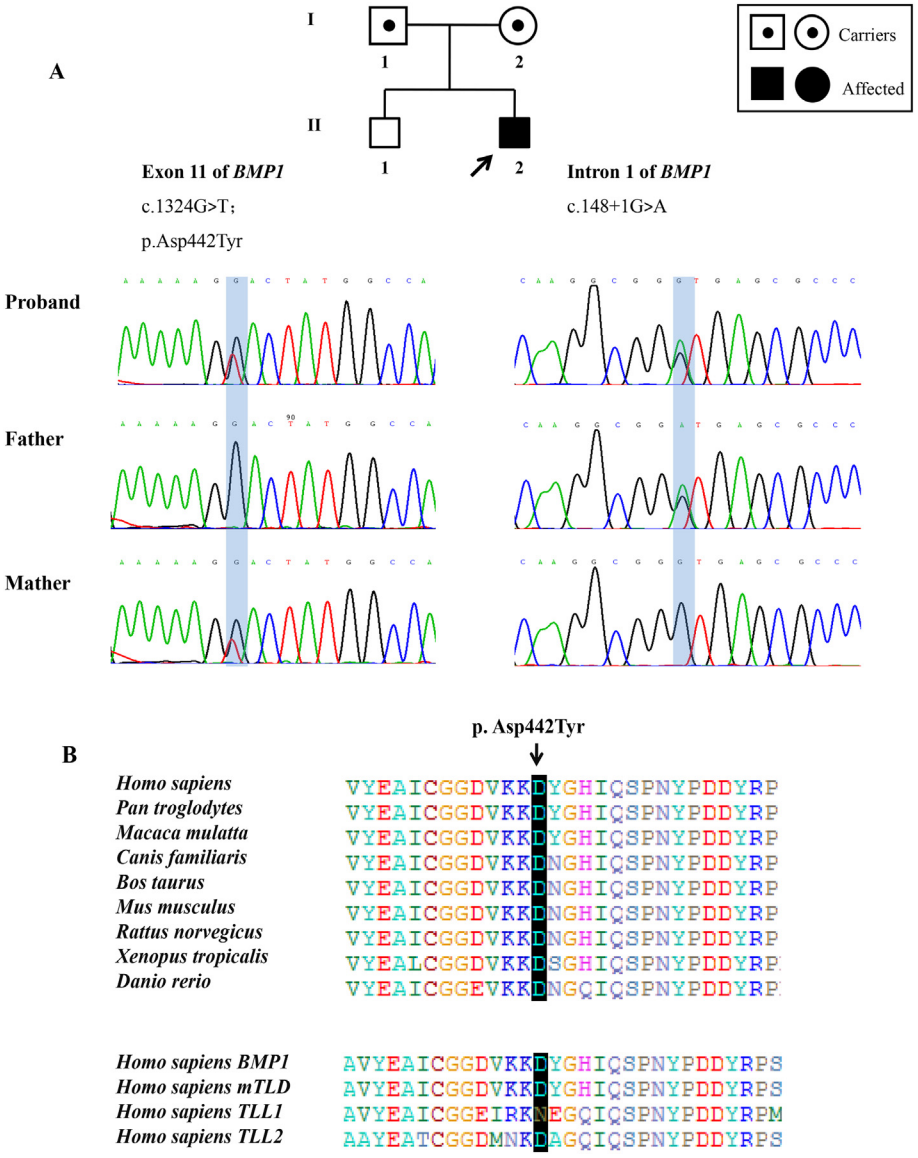

Fig. 2. Molecular findings in the OI family with *BMP1* mutations.

find patients with *BMP1* mutations could also present delayed fracture healing (FM12, Table 2), which are in line with a critical role of BMP1 in bone fracture repair and vascular remodeling provided by recent studies [39,44]. Also, next to skeletal features, knockdown of *BMP1* in mice shows diverse extra-skeletal manifestations, including periodontal defects, fragile skin and delayed wound healing [45,46], all of which are not identified in human patients. These evidences highlight the importance of future assessing extra-skeletal tissues in *BMP1*-related OI.

Interestingly, our patient presented unusually dense bones revealed by X-ray, DXA and HR-pQCT in context of recurrent fractures. HR-pQCT further indicated impaired bone microstructure, including elevated trabecular volume and thickness, but reduced trabecular numbers and increased porosity. The substantial deficits could explain the patient's comprised bone strength and increased bone fragility in vivo for the first time. Previous studies identified four patients with increased BMD and five patients with normal BMD (*Z*-scores from −0.9 to +4.2) [25,26,28,30]. However, homozygous substitution (p.P249L) in the BMP1 protease domain and compound heterozygous mutations (p.E703Q and c.*241 T > C) in CUB3 and polyadenylation site showed somehow osteoporotic outcomes [24,28]. These results indicated a complex heterogeneity with respect to bone mineral density, but we failed to find clear genotype-phenotypic correlations in our study or literature review. The exact mechanism for these varying BMD in *BMP1*-related OI remains unclear. Recent bone biopsy analysis from an individual with p.G12R mutation in *BMP1* suggested two potential mineralization defects [47]. On one hand, the delayed ECM maturation resulted in delayed mineralization at new bone formation; whereas the immature collagen with PICP, on the other hand, led to increased mineral incorporation and hypermineralizaiton at older bone sites [47]. The areas of low and high bone mineral density were also identified in *BMP1a* mutant zebrafish and *BMP1/Tll1* knockout mice [24,48]. Potentially, we think the varying effects of *BMP1* on bone mineralization may partially contribute to BMD heterogeneity.

BPs is demonstrated to increase BMD and reduce bone fracture incidence of patients with OI [3]. In our patient, one-year treatment with alendronate and calcium was effective in inhibiting bone resorption and increasing BMD at lumbar spine and proximal hip. Kinds of BPs therapy were also started in previous patients with *BMP1* mutations, and continued for one year to five years [24–31]. In three patients from FM2 and 7 (Table 2), higher BMD and fewer fractures were observed; whereas in four patients from FM6, 12, 13 and 14 (Table 2), recurrent fractures occurred, even with “chalk-stick” patterns during follow-up.

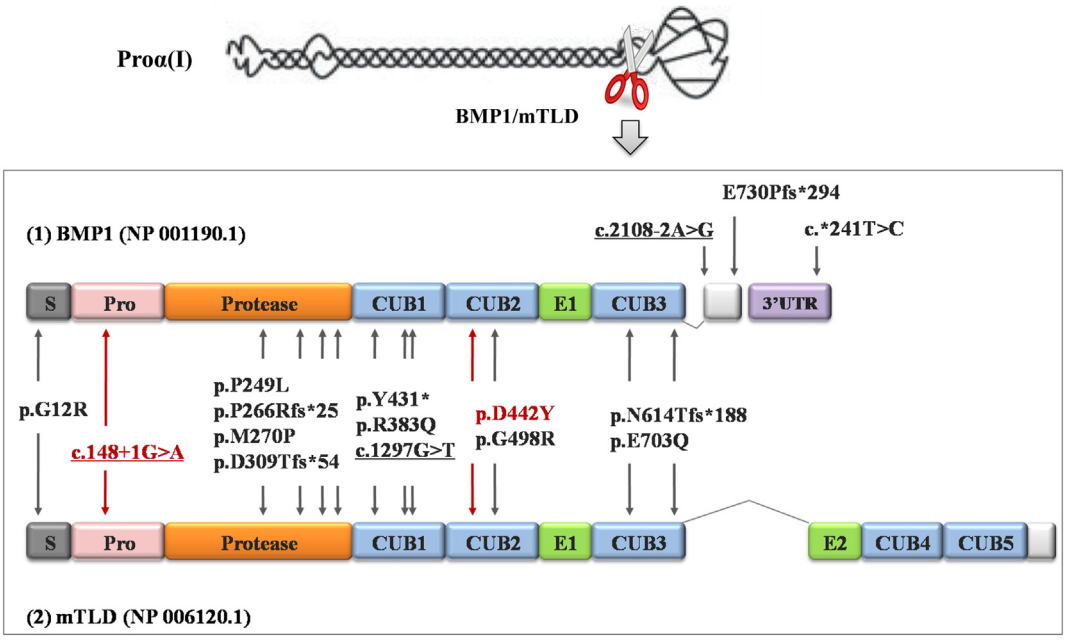

Fig. 3. Location of novel and known BMP1/mTLD variants in protein level.

As we know, some studies indicate that BPs is insufficient to improve bone stiffness and intrinsic bone material quality in OI [49], and they may simultaneously suppress bone modeling/remodeling, increase bone mineralization and allow microdamage accumulation [50]. Several recent studies identified atypical femoral fractures (AFF) in OI patients receiving treatment with BPs [51,52]. Given the high bone mass and hypermineralization in OI type XIII, along with the potential risk of AFF and delayed fracture healing under BPs treatment, whether or not to administrate BPs treatment to patient with OI type XIII raises a dilemma. Due to these concerns, we discontinued alendronate therapy. So the efficacy and safety of BPs in growing patients with rare *BMP1*-related OI requires further investigation over longer periods.

The present study is limited by no in-depth investigation of the splicing defect of c.148 + 1G > A in *BMP1*. In future, we should complete the functional study about the mutant BMP1/mTLD in cultured fibroblasts, to reveal the underlying mechanism of c.148 + 1G > A leading to OI. Besides, the patient refused re-examination of pQCT after treatment; however, it will be worthwhile to follow the bone microstructure changes during BPs treatment in our patient.

5. Conclusions

We identify two novel heterozygous variants in *BMP1* in a Chinese un-consanguineous family with autosomal-recessive OI. In contrast to previously reported patients with *BMP1* mutations, our patient presents with moderate OI, macrocephaly, elbow contractures, increased BMD and comprised bone microstructure, implying a wide phenotypic spectrum in OI type XIII. One-year treatment with alendronate and calcium is effective in reducing bone resorption and increasing BMD, but concerns still remain whether this therapy can reduce bone fracture risk in this rare type of OI.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.11.004.

Declaration of interest

The authors declare that no conflict of interests exist regarding the publication of this paper.

Authors' contributions

Xiaojie Xu, Fang Lv, Yuwen Song, Lujiao Li, Asan, Xiuxiu Wei, Xiuli Zhao, Yan Jiang, Ou Wang, Xiaoping Xing, Weibo Xia, Mei Li have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; participating in drafting the manuscript or revising it critically for content; and have approved the final version of the submitted manuscript.

Physical findings: (A) A relative macrocephaly, broad head, square face, mild hypertelorism and white sclera; (B) joint hyperlaxity of fingers; (C) mild deformity of the left upper limb with contracture (with arrow); (D-E) bowing of bilateral lower limbs (with arrows) with length inequality; (F) No ithyokyphosis.

Radiological findings: (G-H) Transverse fractures through left proximal and mid-femur (white arrows) at the age of 11 and 12 years, respectively; at 15 years, (I) generalized dense and thickened cortices, and abnormally shaped femoral head (with arrow), (K) no obvious wormian bones, (L-N) slender and bent long limbs, with fuzzy bone trabecula, (O) partially healed fracture in the distal tibia (with arrow), (P-Q) mild curved thoracic scoliosis (with arrow), without compression fractures; after 12-month alendronate treatment, (J) bone become abnormally denser than before.

(A) Sequence analysis of the novel variants (c.1324G > T, c.148 + 1G > A) in *BMP1*. The patient was a compound heterozygote, and his parents were heterozygous carriers of these mutations. The proband was indicated by arrow. (B) Multiple sequence alignments of the associated domains from different species and different astacin-like metalloproteases, showing conservation of the Asp442 residue. The mutated residue was shaded in black.

Transcript-specific domains of BMP1/mTLD were in gray; chevrons denote two alternative splicing products from the same gene. Novel and known variants (according to translation BMP1) were indicated in red and black, respectively; underlined mutations were considered to alter splice sites. S: signal peptide; Pro: prodomain; CUB: of complement/Uef/BMP1 domain; E: epidermal growth factor-like motif.

Acknowledgments

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Table 2
Clinical and molecular finding summary of the reported individuals with *BMP1* mutations.

Family	Patient (n)	Origin	Age at 1st fracture	Fracture rates	Bone density	Deformity	BS	DI	Mobility	BPs treatment	Mutation	OISillencetype	Ref
FM-1	2	Egypt	birth	10–15/yr[a]	decreased [a]	severe	(+)	(−)	wheelchair	yes	p.P249L	III	[24]
FM-2	2	Turkey	14-23 m	>15/yr [a]	increased [a]	mild	(−)	(−)	walk	yes	p.G12R	IV	[25]
FM-3	1	Pakistan	4 m	10/yr[a]	normal[a]	(−)	(−)	(−)	wheelchair	yes	p.G12R	IV	[26]
FM-4	1	French -Canadian	4 yr	1.0/yr	normal	N/A	(−)	(−)	N/A	no	c.*241 T > C	I-IV	[28]
FM-5	1	above	10 m	0.7/yr	increased	mild	(−)	(−)	N/A	no	c.*241 T > C	I-IV	[28]
FM-6	1	above	2.5 yr	1.8/yr [a]	normal [a]	(−)	(−)	(−)	N/A	yes	c.*241 T > C	I-IV	[28]
FM-7	1	above	birth	2.2/yr[a]	decreased[a]	N/A	(−)	(−)	N/A	yes	c.*241 T > C; p.E703Q	III-IV	[28]
FM-8	1	Korea	Birth	>10/yr	N/A	Moderate	(−)	(−)	can't sit	Yes	p.M270P; c.1297G > T[b]	III	[29]
FM-9	1	Belgium	8 m	>3.0/yr	N/A	Severe	(+)	(−)	Wheelchair	No	p.D309Tfs*54; p.G498R	III	[27]
FM-10	1	Portugal	Birth	3.8/yr	N/A	Severe	(−)	(+)	Wheelchair	Yes	p.G12R; p.N614Tfs*188	III	[27]
FM-11	2	Scotland	Upon walking	>2.0/yr	N/A	Moderate	(−)	(−)	Wheelchair/walker	No, N/A	p.G12R; P. E730Pfs*294	III	[27]
FM-12	2	Asian	7-12 m	2–3.0/yr [a]	Normal [a]	N/A	(+/−)	(−)	Wheelchair	Yes	P. E730Pfs*294	III-IV	[30]
FM-13	1	North European	2.9 yr	7.0/yr	Increased [a]	N/A	(−)	(−)	N/A	Yes	p.Y431*; p.R383Q	III	[30]
FM-14	1	Thailand	3 m	N/A	N/A	Severe	(−)	(−)	N/A	Yes	p.R266Rfs*25; c.2108-2A > G[b]	III	[31]

BPs, bisphosphonates; N/A, not available.
[a] Parameters before treatment.
[b] Mutations cause aberrant splicing.

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