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Phenotypic Variability in Patients with Osteogenesis Imperfecta Caused by BMP1 Mutations

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Phenotypic Variability in Patients with Osteogenesis Imperfecta Caused by *BMP1* Mutations

Running Title: *BMP1* mutations in Osteogenesis Imperfecta

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ABSTRACT

Osteogenesis Imperfecta (OI) is an inherited bone fragility disorder most commonly associated with autosomal dominant mutations in the type I collagen genes. Autosomal recessive mutations in a number of genes have also been described, including the *BMP1* gene that encodes the mammalian Tolloid (mTLD) and its shorter isoform bone morphogenic protein-1 (BMP1). To date, less than 20 individuals with OI have been identified with *BMP1* mutations, with skeletal phenotypes ranging from mild to severe and progressively deforming. In the majority of reported patients, bone fragility was associated with increased bone mineral density, however the full range of phenotypes associated with *BMP1* mutations remains unclear.

Here we describe three children with mutations in *BMP1* associated with a highly variable phenotype: a sibship homozygous for the c.2188delC mutation that affects only the shorter BMP1 isoform and a further patient who is compound heterozygous for a c.1293C>G nonsense mutation and a c.1148G>A missense mutation in the CUB1 domain. These individuals had recurrent fractures from early childhood, are hypermobile and have no evidence of dentinogenesis imperfecta. The homozygous siblings with OI had normal areal bone mineral density by dual energy X ray absorptiometry whereas the third patient presented with a high bone mass phenotype resembling osteopetrosis. Our findings demonstrate that bone mass in *BMP1*-related OI is highly variable and that OI should be considered as a possibility in individuals presenting with a high bone mass phenotype and a significant fracture history.

KEYWORDS: Osteogenesis Imperfecta, bone fragility, *BMP1*, Bone morphogenic protein-1, high bone mass.

INTRODUCTION

Osteogenesis Imperfecta (OI) is a rare inherited connective tissue disorder characterised by an increased tendency to fracture, often with minimal or no apparent trauma. Extra-skeletal features can include short stature, skin and joint hyper-extensibility, blue sclerae, deafness and dentinogenesis imperfecta. Other features are bone pain, deformities, scoliosis and impaired mobility.

Genetic characterisation of families affected with OI has shown that autosomal dominant mutations in the genes that encode the alpha chains of type I collagen (*COL1A1* and *COL1A2*) can be identified in approximately 90% of affected individuals. Mutations in a variety of other genes encoding proteins involved in type I collagen biosynthesis, bone cell differentiation, bone formation and bone remodelling are known to result in rare forms of autosomal recessive OI [Marini and others 2014; Mendoza-Londono and others 2015]. A hallmark of OI at the tissue level is increased bone mineralisation density [Rauch and Glorieux 2004] .

Mutations in the *BMP1* gene have been described in a small number of individuals with OI. The *BMP1* gene (OMIM 112264) is alternatively transcribed to produce two proteins, Mammalian Tolloid (mTLD) and its shorter isoform bone morphogenic protein-1(BMP1). The BMP1/mTLD protein acts as an astacin metalloprotease whose functions include the proteolytic removal of the carboxyl-terminal propeptide from procollagen type I, II and III and the amino-terminal propeptide from types V and XI procollagen. Studies in BMP1/mTLD deficient patients with OI have demonstrated delayed cleavage of type I collagen C-propeptide [Valencia and others 2014] and disorganization of type I/V collagen fibrils as well as impaired processing of the SLRP prodecorin [Syx and others 2015].

The OI phenotype of individuals with *BMP1* mutations has been described as recurrent fractures, generalized bone deformity, osteopenia and Wormian bones [Martínez-Glez and others 2012], and also as bone fragility associated with an increase in areal bone mineral density (BMD) as measured by dual-energy absorptiometry (DXA) [Asharani and others 2012], similar to mutations that affect the C-propeptide domain of type I collagen [Lindahl and others 2011]. At the tissue level, bone of

one OI patient with *BMP1* mutation was found to be even more hypermineralized than OI caused by collagen mutations. (Hoyer-Kuhn, as above)

However, less than 20 indiviudals have been described with *BMP1*-associated OI and therefore the full range of phenotypes associated with mutations in this gene is not well established. Here we describe three patients that further expand the phenotypic spectrum of *BMP1*-associatied OI; a sibship presenting with OI and normal aBMD and a further patient presenting with a high bone mass phenotype resembling osteopetrosis.

MATERIALS AND METHODS

Clinical Information and DNA extraction

Clinical information was obtained from the patients' medical records. The patients and their parents provided informed consent. Total genomic DNA was isolated from 2 to 5 ml peripheral blood taken from patients and parents using standard extraction methods.

DNA sequencing and mutation analysis

Targeted exome sequencing using SureSelect XT (Agilent Technologies) and Illumina MiSeq platform was used to sequence all coding regions and intron/exon boundaries of genes previously described in OI. The variants identified in the BMP1/mTLD gene were compared to reference sequences NM_001199.3 and NM_006129.4.

RESULTS

Clinical Characteristics

Patient 1, the first child of consanguineous parents of Asian origin was born at 37 weeks gestation weighing 2.5kg. At 6 years of age, he was referred to the metabolic bone clinic for investigation having sustained 8-10 low trauma long bone fractures, the first of which occurred at age 12 months. He had no other significant medical conditions, normal hearing and cognitive development. Due to his recurrent fractures, he mobilised using a wheelchair. On clinical examination there were no dysmorphic features. He had white sclerae, hypermobile fingers and no evidence of dentinogenesis

imperfecta but severe dental decay requiring multiple tooth extractions. Bone biochemistry was normal. A diagnosis of OI was thought to be likely and bisphosphonate treatment was commenced but he continued to have long bone fractures. Due to non-union of a right tibial fracture, he required intramedullary nailing which was followed by delayed osteotomy healing. Further intramedullary nailing of left tibia and femur was required because of mid-shaft fractures. Lateral spine X-ray at age 8.5 years confirmed a compression fracture at L1 (Figure I). He initially received pamidronate (1.5mg/kg/day over 2 days 3 monthly) for the first 2 years followed by zoledronic acid (0.05mg/kg/day single dose 6 monthly). His areal bone mineral density (BMD) by DXA increased in response to bisphosphonate therapy (Table I). He is currently growing along the 10th centile for height and less than 3rd centile for weight.

His sister, **Patient 2,** was born at 36 weeks gestation weighing 2.42kg. She presented at 7 months of age with bilateral ulnar and radial fractures following a fall down the stairs whilst in the arms of her older sister. Over the following 3 years she had three low impact tibia fractures necessitating right tibial rodding. Zoledronic acid infusions (0.035mg/kg/day single dose 4 monthly) were started at 3 years of age. On clinical examination, she had grey sclerae, hypermobile fingers and no evidence of dentinogenesis imperfecta. She had no other medical conditions, normal hearing and cognitive and gross motor development. Her height and weight are on the 3rd centile for age. Her bone profile and vitamin D level at the time of diagnosis were normal. No DXA measurements are available.

Patient 3 is a 7 year old female, the only child of healthy non-consanguineous parents of North European origin. She was born following IVF treatment at 39 weeks gestation with a birth weight of 2.976 kg and her early developmental assessments were normal. Her motor development was delayed; she sat up at 1 year of age and walked around 17 months of age. She was diagnosed with bilateral dislocated hips at 22 months of age for which she had surgery twice, and was immobilised in the hip spica. The patient's first fracture, of the right fibula, occurred at the age of 2 years and 11 months. Further fractures included a spiral fracture of the tibia and three metatarsal fractures in the left foot. Her

lumbar spine (L1-4) BMD was 0.726 g/cm^2 at the age of 3 years 2 months (BMAD Z score +4.2, calculated retrospectively using Kröger method and ALPHABET study dataset [Crabtree NJ 2013; Kroger and others 1993]). Lateral thoracic and lumbar spine radiographs done at the same time did not show any vertebral deformity, osteopenia or clear evidence of increased density. Her lower-limb fragility fractures were attributed to immobilisation in hip spica; she was empirically treated with pamidronate at age 3¾ years (1mg/kg on three consecutive days, three monthly). She received 4 cycles (total dose 12 mg/kg) before treatment was discontinued as her long bones now appeared too dense on radiographs, and she started to suffer apparent 'chalk stick fractures' of her tibiae & fibulae (Figure 2). At the age of 5½ years, bone mineral density measurements were undertaken using various imaging techniques (Table II). The girl's distal radial total and trabecular volumetric BMD Z score, measured by peripheral computed tomography, were markedly elevated. At the lumbar spine (LS), her bone mineral apparent density (BMAD) Z score measured by DXA, but not volumetric trabecular BMD Z score measured by QCT, was elevated. This apparent discrepancy suggests that the trabecular compartment in the LS is less affected than that at the distal radius, however different reference data used to calculate Z scores by these two techniques may be a contributing factor. Radiograph of the spine and vertebral fracture assessment by DXA (Figure 2) did not reveal vertebral fractures. A provisional diagnosis of a mild form of osteopetrosis was suggested, however genetic testing for a panel of 21 genes, including *CLCN7* and *LRP5* was negative.

On clinical examination, she had white sclerae and normal teeth, hearing and spine. She has a bossed forehead and mild left sided ptosis. She has generalised hypermobility with a Beighton score of 8/9 with soft, velvety and very stretchy skin.

Identification of *BMP1* mutations

Mutations in the *COL1A1* and *COL1A2* genes were excluded in all 3 patients. Targeted exome sequencing for a panel of additional genes associated with OI revealed that patient 1 and 2 were homozygous for the c. 2188dupC mutation. The parents were confirmed to be heterozygous carriers.

Patient 3 was compound heterozygous for two novel mutations, a c.1293C>G,p.(Tyr431*) non-sense mutation and a p.(Arg383Gln) missense mutation in the CUB1 domain of *BMP1*. Segregation analysis demonstrated that the c.1293C>G,p.(Tyr431*) was present in the mother and c.1148G>A,p.(Arg383Gln) in the father. The pathogenic effect of the p.Arg383Gln variant was assessed using Alamut Visual version 2.6 (Interactive Biosoftware, Rouen, France).

DISCUSSION

To date the majority, but not all, of individuals described with *BMP1*-associated OI have presented with bone fragility associated with an increased aBMD although no clear genotype-phenotype correlation has yet emerged.

The c.2188dupC identified in patients 1 and 2 is predicted to have different outcomes dependent on the gene transcript. In the shorter BMP1 transcript, this mutation would lead to the creation of an extended protein (p.Gln730Profs*294), whereas in the longer mTLD transcript this mutation is predicted to result in an intronic duplication (c.2108-605dupC). Two individuals who are compound heterozygous for this change and the recurring signal peptide mutation, p.Gly12Arg, have previously been described [Syx and others 2015] . These individuals are reported to have a severe progressive form of OI. Patient 1 and patient 2 presented with a phenotype suggesting that the mutant protein may have residual C-propeptide cleavage activity and the c.2188dupC may therefore represent a relatively 'mild' mutation, with normal aBMD.

The markedly increased bone mass and 'chalk-stick' pattern of long-bone fractures of patient 3 initially suggested a diagnosis of osteopetrosis. To date, similar compound heterozygous changes that result in a 'null' allele and a mutation in a CUB domain have been associated with severe OI phenotypes. No data is available for the associated BMD for these patients [Syx and others 2015]

It remains unclear why some mutations are associated with increased BMD and others with normal or reduced BMD. Areal BMD measurements provide a composite value for bone mass within a given area, and do not reflect tissue mineralisation density – the combination of increased bone

material density with reduced bone mass (as is typical in OI) can give values for aBMD that sit within the normal range for age in children. However, this is clearly not the case for patient 3, where size corrected LS BMD (BMAD) and distal radius volumetric BMD are elevated (table II). Mineral crystals in OI patients are known to be smaller, have high calcium content and are more densely packed than in normal bone. Tissue mineralisation density may be a reflection of the degree of matrix disorganisation; some of the highest values are in type VI OI, where patients have a severely disrupted lamellar structure [Land and others 2007]. The multiple potential effects on matrix organisation resulting from mutations in BMP-1 could be similarly disruptive.

Bone tissue analysis of trabecular and cortical bone from an individual homozygous for the *BMP1* p.(Gly12Arg) signal peptide mutation demonstrated increased regions of unmineralised matrix at sites of new bone formation, possibly caused by a delay in matrix maturation necessary for mineralisation. In contrast, hypermineralisation was observed at older bone sites hypothesised to result from an increase in matrix space caused by retention of the C-propeptide in collagen fibrils which is subsequently filled by mineral crystals [Hoyer-Kuhn and others 2013].

Functional studies have largely focused on the C-propeptide cleavage activity of *BMP1* mutations but BMP1/mTLD is also involved in processing of additional extra cellular matrix components, in particular the processing of the SLRP prodecorin by impaired removal of the prodomain [Syx and others 2015] . Decorin is known to influence both collagen assembly and regulate matrix mineralization [Mochida and others 2009]. The CUB domains of BMP1/mTLD are essential for C-proteinase activity; thus, these regions may be contributing to the increased mineralization in these patients through interaction with SLRPs. Potentially, this explains why our patients with the c.2188dup, where the CUB domains are intact, do not show a high bone mass phenotype.

Conclusion

Our patients demonstrate that bone mass in *BMP1*-related OI is highly variable and that OI should be considered as a possibility in individuals presenting with a high bone mass and a significant

fracture history. In addition, careful monitoring of response to bisphosphonates therapy in these patients is recommended. As further mutations are identified the relationship between *BMP1* mutations and those in the type I collagen C-propeptide, and their functional consequence, may become clearer.

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Conflicts of Interest: None to declare

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Figure legends

Figure 1: Patient 1 X-rays A. Lateral spine showing compression fracture at L1 B. Patient 2 Normal lateral spine and C. left femur

Figure 2: Patient 3 X-rays A. Vertebral fracture assessment by GE iDXA at 5.5 years of age . Note absence of vertebral compression fractures. B. 'Chalk stick' fractures through right mid-tibia & mid-fibula, with soft tissue swelling. Note dense & thickened cortices. Three 'Pamidronate lines' are visible at proximal and distal tibial metaphyses.

Table I Response in lumbar spine (LS) and total body less head (TBLH) BMD (by DXA) Z-scores during intravenous bisphosphonate therapy in patient 1.

Age in years	6 Pre-Treatment	7	8	9	10	12
LS BMD	-0.9	-0.8	-1.1	-0.8	-0.4	0.3
LS BMAD (L1 –L4)	-0.6	-0.6	-0.6	0.4	1.3	2.1
TBLH BMD	NA	-0.5	-1.7	-0.4	-0.8	0.3

LS BMD – lumbar spine bone mineral density, LS BMAD - Lumbar spine bone mineral apparent density, TBLH – total body less head

Table II: Volumetric Bone Mineral Density Z-scores of Patient 3 measured by peripheral quantitative computed tomography (distal radius), DXA (lumbar spine) and quantitative computer tomography (lumbar spine). Pamidronate was started at 3.8 years of age and she remained on treatment for 12 months before raised BMD was identified (see details below).

Age in years	3.2	5.5	6.5	7
Distal radial total volumetric BMD	Not measured	+8.7	+7.1	+7.2
Distal radial trabecular volumetric BMD	Not measured	+9.2	+6.9	+6.6
LS BMAD (L1 –L4)	+4.2	+4.3	+3.1	+3.4
LS volumetric trabecular BMD (L1 –L3)	Not measured	+0.35	Not measured	Not measured

BMD – Bone mineral density, LS – lumbar spine, BMAD - bone mineral apparent density

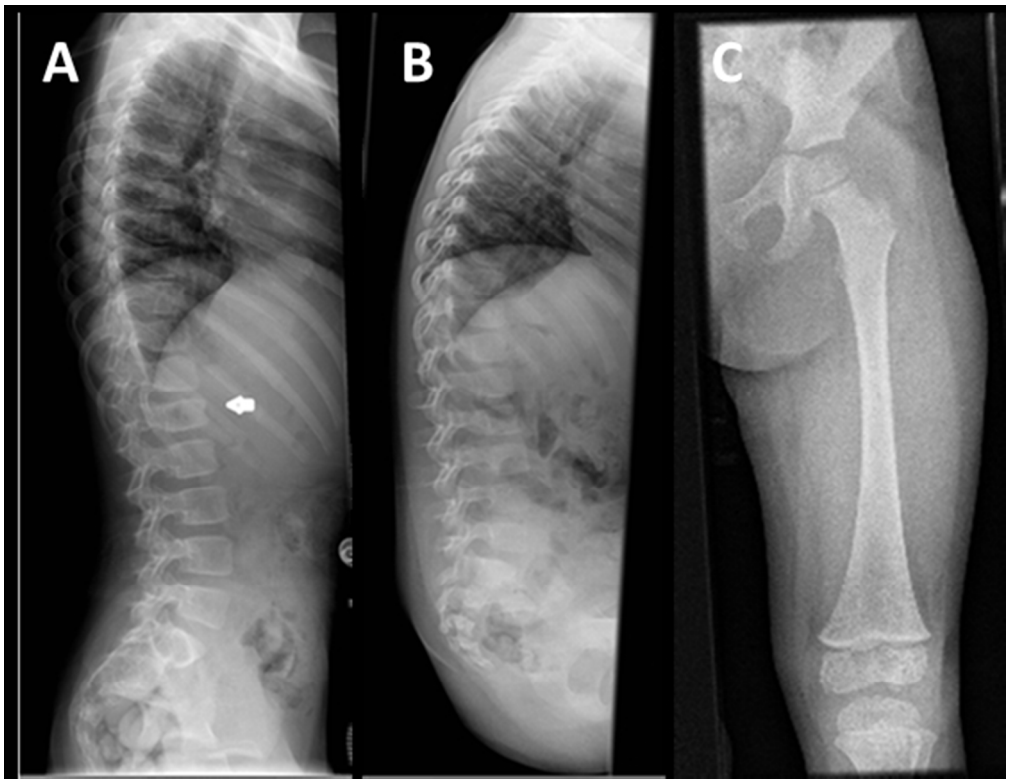

Figure 1: Patient 1 X-rays A. Lateral spine showing compression fracture at L1 B. Patient 2 Normal lateral spine and C. left femur
104x80mm (150 x 150 DPI)

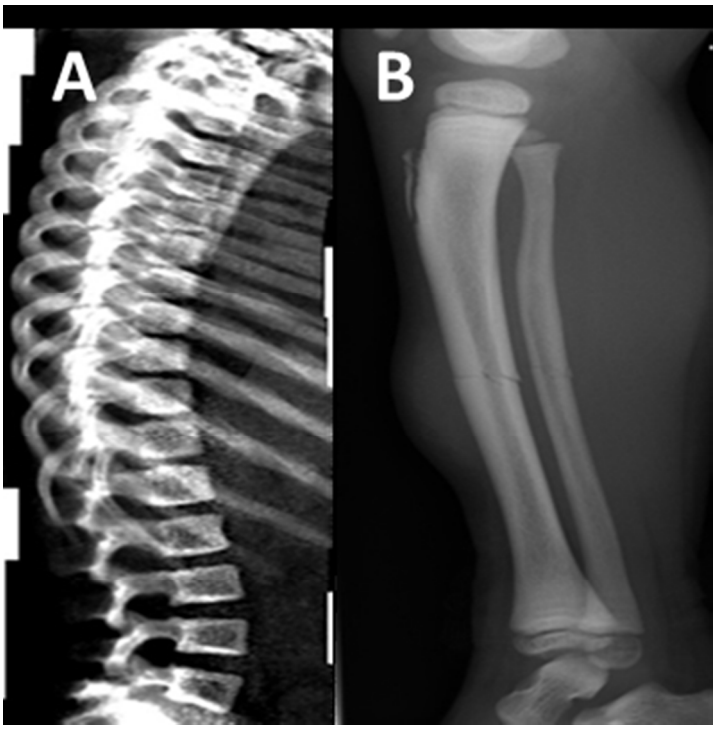

Figure 2: Patient 3 X-rays A. Vertebral fracture assessment by GE iDXA at 5.5 years of age . Note absence of vertebral compression fractures. B. 'Chalk stick' fractures through right mid-tibia & mid-fibula, with soft tissue swelling. Note dense & thickened cortices. Three 'Pamidronate lines' are visible at proximal and distal tibial metaphyses.
59x60mm (150 x 150 DPI)