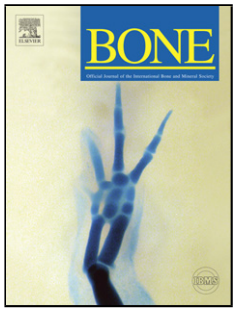

Juvenile Paget's disease with compound heterozygous mutations in *TNFRSF11B* presenting with recurrent clavicular fractures and a mild skeletal phenotype

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Juvenile Paget's disease with compound heterozygous mutations in *TNFRSF11B* presenting with recurrent clavicular fractures and a mild skeletal phenotype

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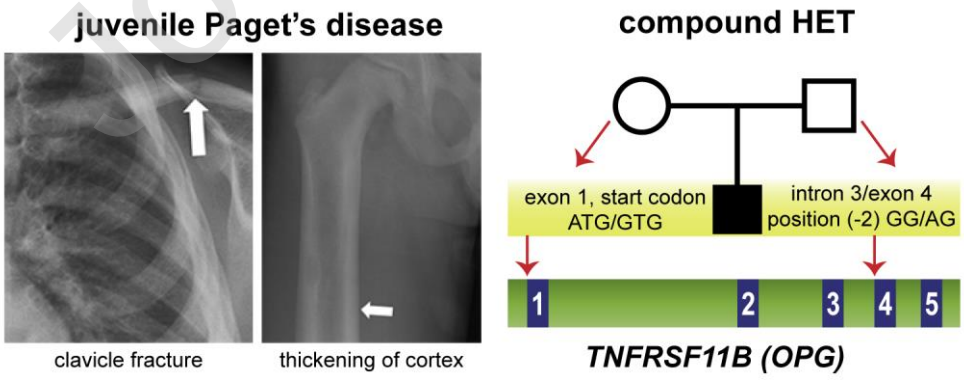
Graphical Abstract



Highlights

- Most cases of Juvenile Paget's disease result from homozygous mutations in the gene *TNFRSF11B.*
- In a boy with recurrent clavicular fractures, but an otherwise mild skeletal phenotype, we identified novel heterozygous mutations in *TNFRSF11B.*
- Sequencing of parental DNA confirmed a compound heterozygous genotype, with one mutation maternally inherited and the other paternally inherited

Abstract

Juvenile Paget's disease (JPD) is a rare recessively-inherited bone dysplasia. The great majority of cases described to date have had homozygous mutations in *TNFRSF11B*, the gene encoding osteoprotegerin. We describe a boy who presented with recurrent clavicular fractures following minor trauma (8 fractures from age 2 to 11). He was of normal height and despite mild lateral bowing of the thighs and anterior bowing of the shins he remained physically active. Abnormal modelling was noted in ribs and humeri on clavicular radiographs, and a skeletal survey at the age of 7 showed generalised diaphyseal expansion of the long bones with thickening of the periosteal and endosteal surfaces of the cortices. On biochemical evaluation, serum alkaline phosphatase was noted to be persistently elevated. The diagnosis of JPD was confirmed by the finding of compound heterozygous mutations in *TNFRSF11B*: a maternally-inherited A>G missense mutation at position 1 of the first amino acid codon (previously reported) and a paternally-inherited splice acceptor site mutation in intron 3 at a highly conserved position (not previously reported). Bioinformatics analysis suggested both mutations were disease-causing. Compound heterozygote mutations in *TNFRSF11B* causing JPD have been previously reported only once – in a boy who also had a relatively mild skeletal

phenotype. The milder features may lead to delay in diagnosis and diagnostic confusion with other entities, but the extraskeletal features of JPD may nonetheless develop.

Keywords: Juvenile Paget's disease**,** *TNFRSF11B***,** osteoprotegerin, mutation

1. Introduction

Juvenile Paget's disease (JPD; MIM #239000) is a rare, autosomal recessively-inherited disorder first described in 1956 [1]. Skeletal manifestations appear first in its natural history, with a characteristic generalised increase in bone turnover that leads to bone expansion, progressive bone deformity, and increased risk of low-energy fracture [2]. The severity of the skeletal phenotype disease varies, and Chong *et al.* have proposed a classification of severity based on the age at which symptoms first develop, the degree of skeletal deformity and the effects on mobility and growth [3]. Extra-skeletal manifestations appear in late childhood and adolescence and include hearing loss (due to skull involvement), a distinctive retinal dystrophy, calcification of the pinna, vascular calcification and internal carotid artery aneurysms [2, 4, 5].

JPD is most commonly caused by bi-allelic inactivating mutations in the *TNFRSF11B* gene [2, 6], although one subject with an activating mutation in *TNFRSF11A* and a JPD-like phenotype has been described [7]. *TNFRSF11B* encodes osteoprotegerin (OPG), a key regulator of osteoclastogenesis. Binding of receptor activator of nuclear factor kappa B (RANK), which is expressed in immature osteoclasts, to RANK-ligand (RANKL), which is expressed by osteoblasts, is central to driving osteoclastogenesis; OPG acts as a soluble decoy receptor that binds to RANKL and prevents its interaction with RANK, thus acting as a brake on osteoclastogenesis [8]. Inactivating mutations in OPG cause upregulation of bone resorption with a secondary increase in bone formation, resulting in the marked elevation of serum alkaline phosphatase, characteristic of JPD.

Approximately 80 cases of JPD have been reported in the literature. A total of 13 different *TNFRSF11B* mutations have been identified to date - with the great majority having homozygous mutations, occurring either where a 'bottleneck' in population growth has occurred or consanguineous marriage is practiced [2]. To our knowledge there has been only one person with JPD identified as having compound heterozygous mutations, and his skeletal phenotype was at the milder end of the disease spectrum [9-12]. We describe here a further patient with a relatively mild skeletal phenotype, who was found to have compound heterozygous mutations in *TNFRSF11B*.

2. Materials and Methods

2.1 Clinical Report

The proband is an 11-year-old boy, one of two children born to healthy unrelated parents of normal stature. His father had a history of surgery to the right humerus at 11 years of age for a bone cyst, and a single sport-related fracture of his right upper fibula, but there was no other family history of note. The proband was born normally at term following an uneventful pregnancy. He weighed 3.47kg and had no neonatal problems. He passed his newborn hearing screen and his developmental milestones were normal. He was first referred at 7 years of age having had five clavicular fractures involving both clavicles on different occasions, at 2, 4, 6 and twice at 7 years of age. Each time the fracture had occurred following a fall onto an outstretched arm.

Otherwise he was in good general health, physically active and achieving well in a mainstream school. He reported no pain other than that directly related to his fractures. At 7.8 years of age he had a height of 124.9cm (25-50th centile) with a span:height ratio of 1.02 and a normal head circumference (54 cm) with a normal facial appearance. He had a relatively short trunk

(upper:lower segment ratio 0.89; about 2 SD below normal for this age), with a barrel-shaped chest, loss of the normal lumbar lordosis, long slim legs with bowing of both femora and, to a lesser extent, the tibiae. His hands, feet and joints appeared normal. Dental examination was normal other than some discrete white patches involving the enamel on a few of the permanent teeth, but the enamel surface was intact. Ophthalmology assessment at the age of 10y11mo found mild myopia, with normal anterior poles, lenses and retinae.

At presentation his calcium, phosphate, renal and liver function, PTH, and 25-hydroxy vitamin D were all normal but his alkaline phosphatase was raised at 1192 U/L (normal range for boys age 8-9; 150-350 U/L). Alkaline phosphatase electrophoresis showed two isoenzyme fractions, initially interpreted as being consistent with transient hyperphosphatasaemia of infancy and childhood. His urinary N-terminal telopeptide excretion (NTx) was high at 4580 nM/mM creatinine (upper limit of normal in children < 16 years is 325 nmol/mmol creatinine). Audiology assessment showed normal ear canals and tympanic membranes and tympanometry, absent oto-acoustic emissions bilaterally and bilateral high frequency hearing loss with notches on the pure tone audiograms at 4000 kHz down to 35dB on the right and 40 dB on the left. His raised alkaline phosphatase and urinary NTx have all persisted at similar levels while his urea, electrolytes, calcium, phosphate, magnesium, liver function tests, thyroid function, PTH, IGF1 and IGFBP-3 have all been normal. Between the ages of 7 and 11 he sustained three further clavicular fractures but was otherwise well. On assessment at age 11.7 he remained fully mobile and enjoying sport including cycling and football. He had minimal deformity with mild femoral and tibial bowing and a barrel chest. His growth remains normal (height 139.1 cm; 25-50th centile).

On initial assessment the possibility of mild Camurati-Engelmann syndrome was considered based on his body habitus and radiology. However, molecular genetic panel testing on a 21 gene osteopetrosis panel including *TGFB1* was normal. Other genes included in the panel

were: *ANKH, CA2, CLCN7, CTSK, FAM123B, FAM20C, FERMT3, IKBKG, LEMD3, LRP5, OSTM1, SNX10, SOST, TCIRG1, PLEKHM1, PTH1R, RASGRP2, TNFRSF11A (*RANK*), TNFSF11 (*RANKL*)* and *TYROBP*. The possibility of atypical osteogenesis imperfecta was also considered but molecular genetic screening of *COL1A1* and *COL1A2* showed no pathogenic changes.

2.2 Mutation analysis

Genomic DNA was extracted from leukocytes using standard laboratory procedures. PCR primers used were designed to amplify the five exons, including the intron/exon boundaries, of *TNFRSF11B* (Gene ID 4982, mRNA accession NM_002546.4) [13]. One additional primer was designed and used as the forward primer for amplification of exon 2, as it permitted clearer reading of the intron1-exon2 boundary. The sequence of this primer was 5'-CACTGCTATCTGCATTCCTGG. PCR amplification was carried out by HOT FIREPol® DNA Polymerase (Solis BioDyne, Tartu, Estonia). Following amplification, DNA fragments were separated by electrophoresis on 1% agarose gels, bands were purified from the gels by High pure PCR product purification kit (Roche) and submitted for direct Sanger sequencing on a 3130XL Genetic Analyzer (Life Technologies).

2.3 Serum RANKL and OPG measurements

Serum concentrations of soluble RANKL (sRANKL) and OPG were measured in the proband and both his parents using a sandwich enzyme-linked immunosorbent assay (ELISA) (Biomedica Medizinprodukte GmbH, Vienna, Austria). Intra- and inter-assay precision were ≤10% for both assays. Limit of detections were 0.14 pmol/L and 0.01 pmol/L for OPG and sRANKL respectively. Normal interquartile ranges for these assays were taken from the paper of Ali et al [14].

The parents were closely involved in the decision to discuss the clinical features in an international expert forum and following that, to send the samples for research-based testing.

3. Results

3.1 Radiological findings

Radiographs obtained of the left shoulder at presentation with clavicular fractures aged 3 and 5 revealed a diffuse sclerotic appearance with thickening of the humeral cortex (Figure 1). There was no hypoplasia of the clavicles. A skeletal survey at age 7 showed widespread diaphyseal expansion of the long bones with cortical thickening and some endosteal thickening (Figure 2). The hands were spared, and the spine and pelvis appeared normal. There was mild thickening of the cranial vault, confirmed on a cranial CT performed one year later (Figure 3). The CT scan also showed areas of bilateral otic capsule demineralisation. Bone density was evaluated by DXA (GE Lunar Prodigy Advance, Software: enCORE v14.1) at aged 9: the lumbar spine z-score was low of -3.4, but whole body (less head) densitometry was normal (z-score -0.2).

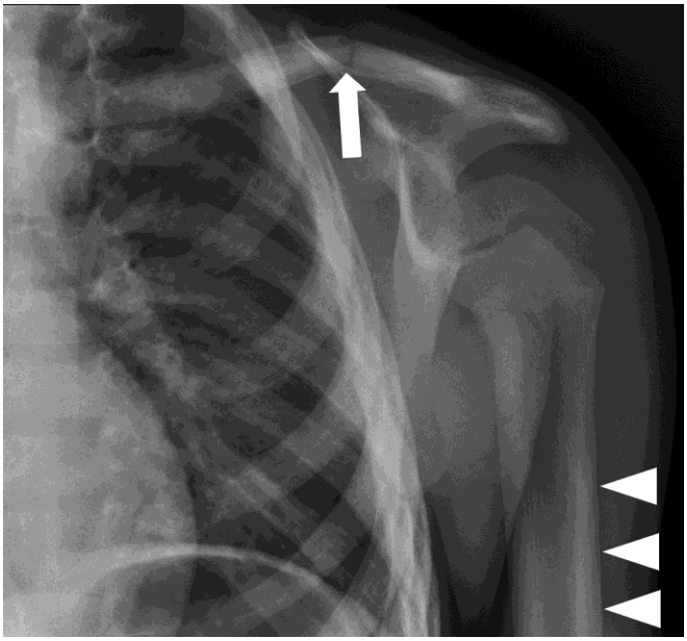


Figure 1. Radiograph of left shoulder at aged 5. There is a left clavicle fracture (arrow). Cortical thickening in the humerus is present (arrowheads)

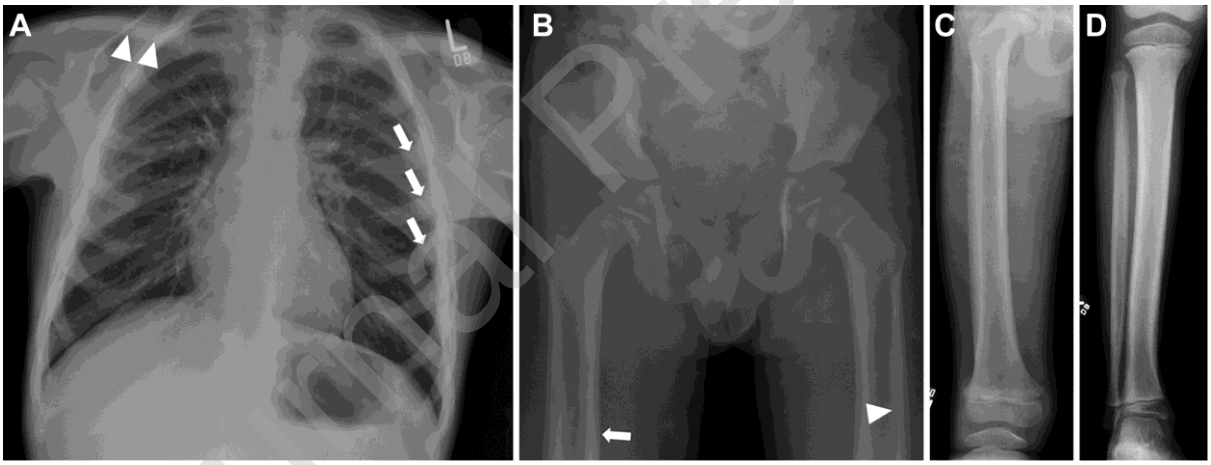


Figure 2. Skeletal survey at aged 7. a) Chest radiograph showing thickening of the central poritions of the ribs (arrows) and clavicles (arrowheads). b) Pelvic radiograph shows normal pelvic bones; there is thickening of the femoral diaphyseal cortex involving both the cortical (arrow) and endosteal (arrowheads) surfaces. Radiographs of right femur (c) and tibia (d) show widespread diaphyseal thickening. The metaphyses and epiphyses are spared.

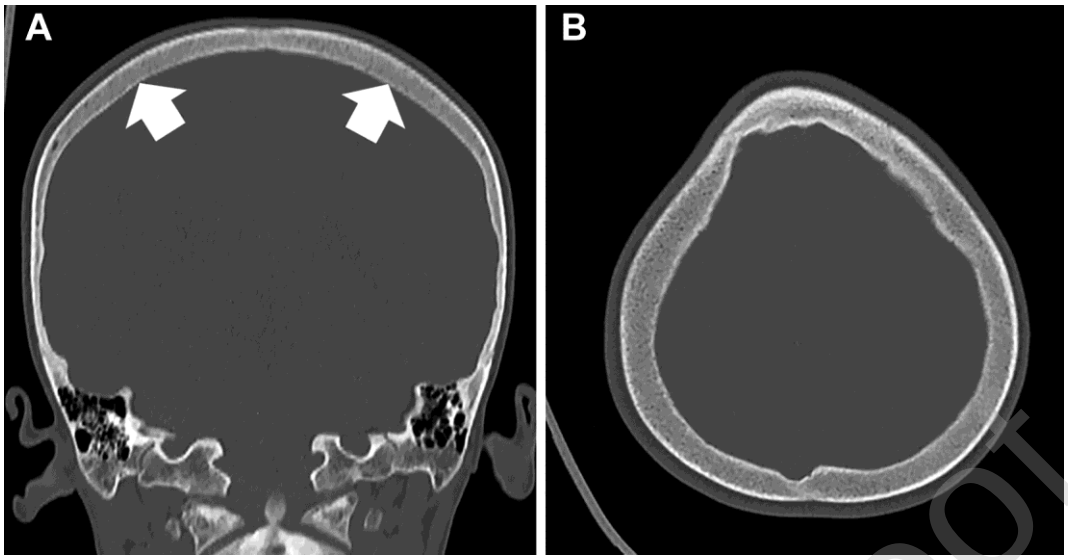


Figure 3. Cranial CT at aged 8. Coronal (a) and axial (b) reformats showing generalised mild thickening of the cranial vault (white arrows). The skull base was not involved.

3.2 Genetic analysis

Sequence analysis of the five exons and flanking regions of *TNFRSF11B* in the patient's DNA identified no homozygous mutations. However, heterozygosity was found in two critical loci: c.1A>G in position 1 of the first amino acid codon of OPG, and c.592-2A>G in the splice acceptor site in intron 3 (Figure 4). The c.1A>G initiation codon variant produces a missense mutation p.M1V, and has been previously documented as SNP rs1461751500 in position chr8:118951821, with an unknown frequency (NCBI dbSNP). The second variation identified in intron 3, position chr8:118926720, has not been reported previously.

Parental DNA was obtained for segregation analysis. As shown in Figure 4, the mother was heterozygous for the mutation A>G in exon 1, and the father was heterozygous for the A>G splice acceptor site mutation in intron 3. In addition, both parents are heterozygous for the variant c.C9G (p.N3K) at the third amino acid of OPG, which is a documented benign SNP (rs2073618). The child inherited the C allele in this position from both parents.

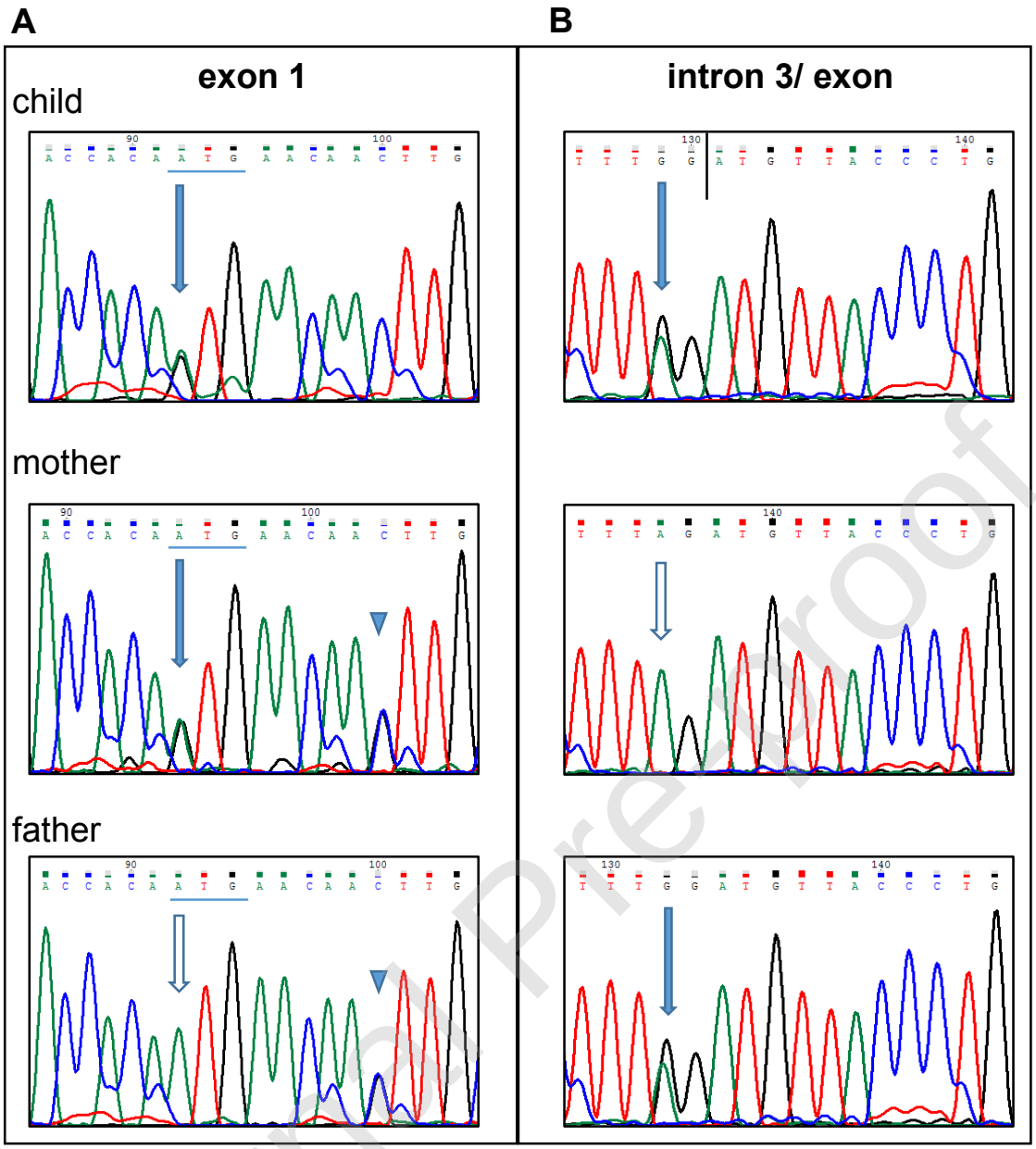


Figure 4. Mutation and segregation analyses of *TNFRSF11B*. (A) DNA sequence of the region in exon 1 that includes the OPG start codon (underlined). The child and mother are heterozygous A/G in position 1 of the first amino acid, while the father is homozygous for the wild type A allele. Both parents are heterozygous C/G in position 3 of the third amino acid (a benign SNP). The child is homozygous C in this position. (B) DNA sequence of intron 3/ exon 4 boundary region. The boundary is indicated by the black vertical line. The second to last position in the intron is heterozygous G/A in the child and the father, while the mother is homozygous for the wild type A allele. All the sequences have been confirmed on the complementary strands.

Blue arrow- heterozygous loci; white arrow- homozygous, wild type loci; arrow heads- heterozygous loci of the known benign SNP.

3.3 Serum concentrations of OPG and sRANKL

Immunoreactive OPG and sRANKL were detectable in the proband and both his parents. OPG concentrations were low in both the proband and his father, and sRANKL concentrations elevated in all three. The ratio of sRANKL to OPG, an index of the driving force to osteoclastogenesis, was high in both the father and the proband, but particularly so in the latter (Table 1).

Table 1

Serum concentrations of OPG and sRANKL, and their ratio in the proband and his parents.

Subject	**OPG pmol/L**	Normal IQR	**sRANKL pmol/L**	Normal IQR	**sRANKL/OPG molar ratio**	Normal IQR
Proband	1.4	2.88-4.04	>2	0.21-0.41	>1.43	0.06-0.14
Mother	4.6	2.61-4.08	0.42	0.14-0.31	0.09	0.04-0.10
Father	0.2		0.69		0.29	

IQR – interquartile range using this assay [14].

4. Discussion

At presentation the diagnosis of this child's bone disease, characterised by recurrent clavicular fractures, mild deformity of the lower limbs with diaphyseal expansion and cranial hyperostosis was not clear. The high alkaline phosphatase levels did raise the possibility of juvenile Paget disease, but this was at first considered unlikely given the absence of pain, normal stature and near normal phenotype. Radiography in typical cases of JPD shows enlarged bones with thick cortices, fractures and bowing of long bones, kyphosis, scoliosis, acetabular protrusion and severe hyperostosis of the skull (commonly leading to deafness), and in our patient these features were either absent or present only in an attenuated form.

Clavicular fractures are common in young children but repeated fractures, such as this boy sustained, are unusual. The radiological appearances raised several other possibilities. These included Camurati-Engelmann disease (CED) which is characterised radiologically by diaphyseal bone expansion with both cortical and endosteal bone proliferation, sparing the epiphyses and metaphyses [15] – all features that were present in this case. The lack of involvement in the hands and spine is also seen in CED, but children this age with CED typically present with bone pain and limb girdle weakness, and recurrent fractures are not a feature. However, it is possible that mild juvenile Paget's may be responsible for some cases previously labelled as CED in whom mutation testing for *TGFB1* is negative.

Given the presentation with fractures, other bone fragility states with bone modelling abnormalities were also entertained. A rare high bone mass form of osteogenesis imperfecta, due to mutations at the C-propeptide cleavage site, has recently been elucidated [16]. This may present with abnormal bone modelling with diaphyseal expansion, particularly of the short tubular bones. Despite recurrent fractures, spinal bone density is usually elevated. However, in this case, spinal bone density was low and there were no mutations in *COL1A1* or *COL1A2*.

We also considered Pyle disease, a rare disorder due to mutations in *SFRP4*, which may manifest osteopenia and bone fragility [17]. Pyle disease exhibits marked metaphyseal undermodelling rather than expansion of the diaphyses, thus distinguishing it from juvenile Paget's disease. Cleinocranial dysplasia due to *RUNX2* mutations is characterised by hypoplasia of the clavicles, but not fractures, and our patient had none of the other clinical features of this syndrome [18]. Gnathodiaphyseal dysplasia is a rare disorder caused by heterozygous mutation in the *ANO5*. Bone fragility with recurrent fractures, high bone turnover and bowing and sclerosis of tubular bones are features of this condition, but our patient did not have the characteristic fibro-osseous lesions of the jawbones [19].

Mutation analysis of DNA from the child determined heterozygous variations in two critical loci of the *TNFRSF11B* gene: in the first amino acid codon and in the acceptor splice site of intron 3. Analysis of DNA from the parents revealed that one mutation was inherited from each, establishing that the affected child had no wild type copy of *TNFRSF11B*. The phenotype was mild as judged by the criteria suggested by Chong et al [3] with deformity recognised after 2 years of age, normal mobility and normal growth.

To our knowledge there has been only one previously reported case of a patient with JPD with compound heterozygous mutations in *TNFRSF11B*. This patient was described in a number of earlier clinical papers: by the age of 5-years he had sustained eight fractures, but remained mobile and was of normal stature [9], thus the skeletal phenotype was mild. However, the extraskeletal features were not benign; by the age of 10 he had developed cutaneous changes of pseudoxanthoma elasticum and retinal angioid streaks. The retinal disease progressed over the next 11 years with sub-retinal haemorrhages causing significant visual impairment [11]. In an abstract published in 2003 he was shown to have a 3-base pair deletion in exon 2 and a nonsense mutation in exon 4 of *TNFRSF11B* [12]. Similarly, individuals with who are homozygous for the so-called Balkan mutation in *TNFRSF11B* (966_969delTGACinsCTT) also have a relatively mild skeletal phenotype, but severe retinal disease may develop with age [20].

To date, thirteen *TNFRSF11B* mutations that had been described in JPD patients. The severity of the phenotype is related to the nature of the mutation. Severe phenotypes (defined as deformity recognised by age 18 months, independent walking not maintained past the age of 5 years, and height <3rd centile) are seen in patients with large deletions in *TNFRSF11B* or mutations in cysteine residues crucial for the tertiary structure of OPG [2, 3].

Using the bioinformatics software MutationTaster [21] to evaluate the pathogenic potential of the DNA sequence alterations identified here, each of these mutations was predicted to cause major disruptions of the expression and function of OPG. The phenotypes of JPD patients carrying *TNFRSF11B* mutations previously described in the literature also support the view that mutations in these two sites are detrimental. A homozygous mutation in the second position of the start codon c.2A>G (p.1M>R) was reported by Grasemann *et al* in a patient with a severe JPD phenotype [22]. That patient was diagnosed in early infancy with demineralised, broadened bones with a coarse trabecular pattern, and severe coxa vara with shepherd's crook deformity of the proximal femur and serum OPG serum levels were below the assay detection limit. The novel mutation in the acceptor site junction of intron 3 is in position (-2), a highly conserved and nearly invariant position according to a comprehensive *in silico* analysis of human splice sites [23]. A homozygous splice site mutation causing a 20bp deletion in the donor site of exon 3 was described by Chong *et al* [3]. That patient also presented in infancy with a severe phenotype, with deformity that limited the development of walking. Similar to the mutation found in the new case we describe, this deletion was predicted to cause a failure in the splicing of exons 3 and 4, and loss of function of OPG.

Given that both mutations we identified are potentially associated with severe phenotypes, it is perhaps surprising that the skeletal phenotype is mild. Our data do not provide a clear answer to this. It is possible that as a consequence of the splice site mutation, alternative spicing may have produced a mutant OPG protein with retained activity, but this remains speculative. This issue could perhaps be addressed by using the proband's mRNA to determine the outcome of the splice site mutation. The proband had low but detectable serum concentrations OPG though it should be borne in mind that the assay detects fragments of OPG, monomeric and dimeric OPG as well as OPG/RANKL complexes. Circulating sRANKL levels were higher than normal in the proband, a finding that has been previously described in patients with JPD [2]. The

proband's sRANKL/OPG ratio was clearly elevated, suggesting there was a powerful drive to osteoclastic activity. His mother's OPG and sRANKL measurements were normal, but his father (who had the splice site mutation) had a relatively low OPG level and high sRANKL/OPG ratio.

Bisphosphonates have been used in several children with JPD and it seems probable that skeletal deformity can be ameliorated by suppressing the greatly accelerated bone turnover during growth [24], but there is no evidence that it prevents the vascular and ocular complications. In theory, denosumab, a RANK-ligand antibody, might be more effective, but to date only short term studies of its use have been reported [22, 25].

Declarations of interest: none

Acknowledgements

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References

1. Bakwin H, Eiger MS (1956) Fragile bones and macrocranium. J Pediatr 49:558-564
2. Polyzos SA, Cundy T, Mantzoros CS (2018) Juvenile Paget disease. Metabolism 80:15-26
3. Chong B, Hegde M, Fawkner M, Simonet S, Cassinelli H, Coker M, Kanis J, Seidel J, Tau C, Tuysuz B, Yuksel B, Love D, Cundy T (2003) Idiopathic hyperphosphatasia and TNFRSF11B mutations: relationships between phenotype and genotype. J Bone Miner Res 18:2095-2104
4. Gottesman GS, Madson KL, McAlister WH, Nenninger A, Wenkert D, Mumm S, Whyte MP (2016) Auricular ossification: A newly recognized feature of osteoprotegerin-deficiency juvenile Paget disease. Am J Med Genet A 170A:978-985
5. Kerr NM, Cassinelli HR, DiMeglio LA, Tau C, Tuysuz B, Cundy T, Vincent AL (2010) Ocular manifestations of juvenile Paget disease. Arch Ophthalmol 128:698-703
6. Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, Mumm S. Osteoprotegerin deficiency and juvenile Paget's disease. N Eng J Med 2002;347:175-84
7. Whyte MP, Tau C, McAlister WH, Zhang X, Novack DV, Preliasco V, Santini-Araujo E, Mumm S (2014) Juvenile Paget's disease with heterozygous duplication within TNFRSF11A encoding RANK. Bone 68:153-161
8. Boyle JW, Simonet S, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423:337-342
9. Woodhouse NJ, Fisher MT, Sigurdsson G, Joplin GF, MacIntyre I (1972) Paget's disease in a 5-year-old: acute response to human calcitonin. Br Med J 4:267-269
10. Doyle FH, Woodhouse NJ, Glen AC, Joplin GF, MacIntyre I (1974) Healing of the bones in juvenile Paget's disease treated by human calcitonin. Br J Radiol 47:9-15

11. Sharif KW, Doig WM, Kinsella FP (1989) Visual impairment in a case of juvenile Paget's disease with pseudoxanthoma elasticum: an eleven year follow up. J Pediatr Ophthalmol Strabismus 26:299-302

12. Mumm S, Banze S, Pettifor J, Tau C, Schmitt K, Ahmed A, Whyte MP (2003) Juvenile Paget's disease: molecular analysis of TNFRSF11B encoding osteoprotegerin indicates homozygous deactivating mutations from consanguinity as the predominant etiology. In: ASBMR 2003 Annual Meeting. J Bone Miner Res, 18; S373, (Minneapolis, Minnesota, USA)

13. Naot D, Choi A, Musson DS, Simsek Kiper PO, Utine GE, Boduroglu K, Peacock M, DiMeglio LA, Cundy T (2014) Novel homozygous mutations in the osteoprotegerin gene TNFRSF11B in two unrelated patients with juvenile Paget's disease. Bone 68:6-10

14. Ali SA, Kang H, Olney R, Ramos-Platt L, Ryabets-Lienhard A, Georgia S, Pitukcheewanont P (2019). Quantifying RANKL and OPG levels in healthy children: a large cross-sectional analysis. Bone 127: 215-219.

15. Janssens K, Vanhoenacker F, Bonduelle M, Verbruggen L, Van Maldergem L, Ralston S, Guanabens N, Migone N, Wientroub S, Divizia MT, Bergmann C, Bennett C, Simsek S, Melancon S, Cundy T, Van Hul W (2006) Camurati-Engelmann disease: review of the clinical, radiological, and molecular data of 24 families and implications for diagnosis and treatment. J Med Genet 43:1-11

16. Cundy T, Dray M, Delahunt J, Hald JD, Langdahl B, Li C, Szybowska M, Mohammed S, Duncan EL, McInerney-Leo AM, Wheeler PG, Roschger P, Klaushofer K, Rai J, Weis M, Eyre D, Schwarze U, Byers PH (2018) Mutations that alter the carboxy-terminal-propeptide cleavage site of the chains of type I procollagen are associated with a unique osteogenesis imperfecta phenotype. J Bone Miner Res 33:1260-1271

17. Kiper POS, Saito H, Gori F, Unger S, Hesse E, Yamana K, Kiviranta R, Solban N, Liu J, Brommage R, Boduroglu K, Bonafe L, Campos-Xavier B, Dikoglu E, Eastell R, Gossiel F, Harshman K, Nishimura G, Girisha KM, Stevenson BJ, Takita H, Rivolta C, Superti-Furga A, Baron R (2016) Cortical-bone fragility--insights from sFRP4 deficiency in Pyle's disease. N Engl J Med 374:2553-2562

18. Mundlos S. Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 1999; 36: 177-182.

19. Rolvien T, Koehne T, Kornak U, Lehmann W, Amling M, Schinke T, Oheim R. A novel *ANO5* mutation causing gnathodiaphyseal dysplasia with high bone turnover osteosclerosis. J Bone Miner Res. 2017;32:277-284.

20. Whyte MP, Singhellakis PN, Petersen MB, Davies M, Totty WG, Mumm S (2007) Juvenile Paget's disease: the second reported, oldest patient is homozygous for the *TNFRSF11B* "Balkan" mutation (966_969delTGACinsCTT), which elevates circulating immunoreactive osteoprotegerin levels. J Bone Miner Res 22:938-946

21. Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014) MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 11:361-362

22. Grasemann C, Schundeln MM, Hovel M, Schweiger B, Bergmann C, Herrmann R, Wieczorek D, Zabel B, Wieland R, Hauffa BP (2013) Effects of RANK-ligand antibody (denosumab) treatment on bone turnover markers in a girl with juvenile Paget's disease. J Clin Endocrinol Metab 98:3121-3126

23. Ma SL, Vega-Warner V, Gillies C, Sampson MG, Kher V, Sethi SK, Otto EA (2015) Whole exome sequencing reveals novel PHEX splice site mutations in patients with hypophosphatemic rickets. PLoS One 10:e0130729

24. Cundy T, Wheadon L, King A (2004) Treatment of idiopathic hyperphosphatasia with intensive bisphosphonate therapy. J Bone Miner Res 19:703-711

25. Polyzos SA, Singhellakis PN, Naot D, Adamidou F, Malandrinou FC, Anastasilakis AD, Polymerou V, Kita M (2014) Denosumab treatment for juvenile Paget's disease: results from two adult patients with osteoprotegerin deficiency ("Balkan" mutation in the *TNFRSF11B* gene). J Clin Endocrinol Metab 99:703-707