**Citation:** IBMS BoneKEy **13**, Article number: 824 (2016) | doi:10.1038/bonekey.2015.137

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## CLINICAL CASES

# Duplication of 17p13.3 associated with an unclassified type of metaphyseal dysplasia

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**We report a 17p13.3 duplication detected on whole-exome sequencing (WES) analysis in a patient with an unclassified type of metaphyseal dysplasia. A 12-year-old male presented with short stature and associated genu valgum and coxa valga. Radiographic findings showed multiple lytic and sclerotic metaphyses. He had bilateral distal femoral and proximal tibial medial hemiepiphysiodesis and two bilateral triple pelvic osteotomies, after which the orthopedic surgeon noted 'somewhat soft bones'. He also had progressive linear growth deceleration (height −2.3 s.d., weight +0.8 s.d.), upper-to-lower segment ratio of +3.5 s.d., no blue sclerae, normal cognitive function and no history of fractures. His endocrine evaluation and *RMRP* and *COL10A1* gene sequencing were normal, but WES analysis and oligonucleotide-single-nucleotide polymorphism chromosomal microarray revealed a *de novo* 608-kb duplication of 17p13.3. This duplication included multiple genes including *SERPINF1*. *SERPINF1* encodes pigment epithelium-derived factor, which has a critical role in collagen type-I binding, with haploinsufficiency resulting in severe autosomal recessive osteogenesis imperfecta. There are no reports of this gene duplication resulting in an unclassified type of metaphyseal dysplasia. However, this finding could suggest the possibility that this gene might be dosage sensitive.**

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### Introduction

We describe a patient with an unclassified type of metaphyseal dysplasia associated with short stature, progressive linear growth deceleration, normal cognition, genu valgum, coxa valga, a 2-cm leg length discrepancy and lower extremity radiographs significant for lytic and sclerotic metaphyses. Whole-exome sequencing of this patient revealed a *de novo* duplication of 17p13.3, which included *SERPINF1*, and an oligonucleotide-single-nucleotide polymorphism (SNP) array confirmed that the duplication involved a total of 19 genes. Although previous reports have demonstrated a link between severe autosomal recessive osteogenesis imperfecta (OI) and loss-of-function *SERPINF1* mutations, to our knowledge this is the first report of a *SERPINF1* gene duplication potentially associated with an unclassified type of metaphyseal dysplasia, suggesting that this gene involved in bone morphogenesis might be dosage sensitive.

### Patient Characteristics

This patient is a 12-year-old Korean male born at 37 weeks gestation via uncomplicated spontaneous vaginal delivery to a 30-year-old, gravida 2, para 2 female and her unrelated 34-year-old husband. His birth length was noted to be 49.5 cm, with a birth weight of 3105 g and a head circumference of 35 cm. He initially presented at age 2 years with hip pain and in-toeing. He was subsequently noted to have short stature with progressive linear growth deceleration (height −2.3 s.d., weight +0.8 s.d.) starting at 4 years of age, with an average calculated mid-parental target height of 174 ± 5 cm (**Figure 1**). Additional clinical findings included an upper-to-lower segment ratio of +3.5 s.d., normal cognitive function, non-blue sclerae and lack of fracture history despite an active lifestyle. Endocrine evaluation was normal, including the following: calcium 8.6 mg dl$^{-1}$ (normal: 8.8–10.8 mg dl$^{-1}$), intact parathyroid hormone (iPTH) 52.3 pg ml$^{-1}$ (normal: 10–71 pg ml$^{-1}$), alkaline phosphatase 327 U l$^{-1}$ (normal: 185–562 U l$^{-1}$), 25-hydroxy vitamin D 22 ng ml$^{-1}$ (normal: 30–100 ng ml$^{-1}$), 1,25-hydroxy vitamin D 70 pg ml$^{-1}$ (normal: 30–83 pg ml$^{-1}$), thyroid-stimulating hormone 1.12 mIU ml$^{-1}$ (normal: 0.5–5 mIU ml$^{-1}$), fT4 SO 1.5 ng dl$^{-1}$ (normal: 1–2.4 ng dl$^{-1}$), insulin-like growth factor-1 (IGF-1) 168 ng ml$^{-1}$ (−1.2 s.d.; normal: 123–497 ng ml$^{-1}$), Insulin-like growth factor binding protein-3 (IGFBP-3) 5.2 mg l$^{-1}$ (normal: 2.4–8.4 mg l$^{-1}$) and bone age equivalent to chronological age. As part of the genetic work-up, he was noted to have lytic and sclerotic distal

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Received 18 August 2015; revised 7 October 2015; accepted 30 October 2015; published online 20 July 2016

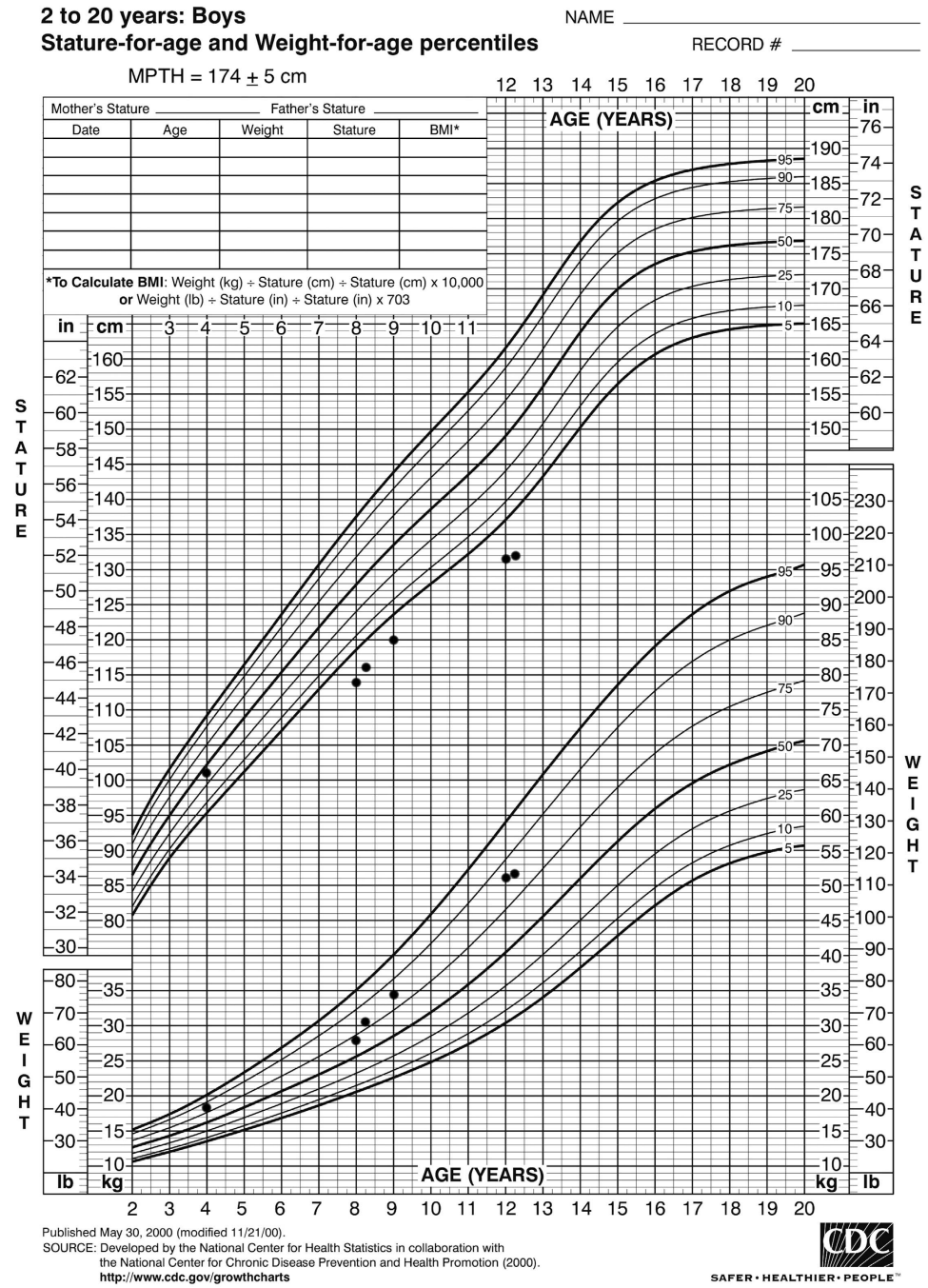


**Figure 1** This figure demonstrates the patient's progressive linear growth deceleration starting at 4 years of age with preservation of weight. Note that his height percentile is significantly lower than his calculated mid-parental target height of 174 ± 5 cm, with bone age equivalent to chronological age.

femoral, proximal tibial and distal tibial metaphyses on lower extremity radiographs (**Figure 2**), and he was subsequently diagnosed by a skeletal dysplasia radiologist with an unclassified type of metaphyseal dysplasia associated with genu valgum, coxa valga and a 2-cm leg length discrepancy with rhizomelic limb shortening. To correct his bone abnormalities, he underwent bilateral distal femoral and proximal tibial medial hemiepiphysiodesis with stapling, as well as two bilateral triple pelvic osteotomy procedures, with 'somewhat soft bones' noted by the orthopedist after surgery.

Computed tomography (CT) bone density was performed later and revealed the cortical bone density (measured at the midshaft of femur) of 1953 mg cm$^{-3}$ (normal 2000 ± 60 mg cm$^{-3}$) and the mean cancellous vertebral bone density of 243 mg cm$^{-3}$ (normal for pubertal children: 285 ± 45 mg cm$^{-3}$). Gene testing for known types of metaphyseal dysplasia such as the McKusick and Schmid types of metaphyseal chondrodysplasia (*RMRP* and *COL10A1*) was normal. Whole-exome sequencing was then performed and revealed a small *de novo* interstitial duplication that included

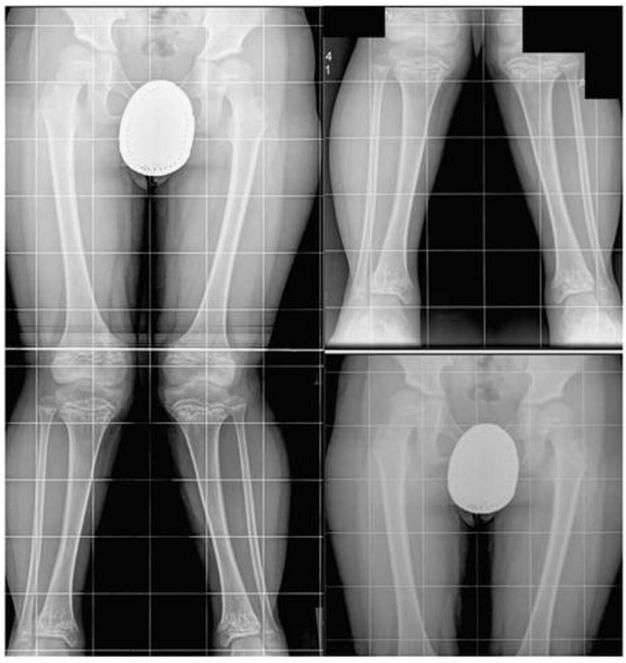

**Figure 2** Lower extremity radiographs at 9 years of age. Lower extremity radiographs demonstrating lytic and sclerotic distal femoral, proximal tibial and distal tibial metaphyses consistent with metaphyseal dysplasia. Additional associated findings notable on the radiograph include genu valgum and coxa valga.

*SERPINF1* on chromosome 17p13.3, and oligonucleotide-SNP chromosomal microarray at 1.15-kb resolution utilizing 2.67 million probes confirmed a *de novo* 608-kb duplication of 17p13.3 that included 19 genes: *SLC43A2, SCARF1, R1LP, PRPF8, TLCD2, MIR22HG, MIR22, WDR81, SERPINF2, SERPINF1, SMYD4, RPA1, RTN4RL1, DPH1, OVA2, MIR132, MIR212, HIC1* and *SMG6*. As a secondary finding, a p.G98S variant in the *LDLR* gene (*LDLR*; OMIM 606945) was detected. The patient had elevated total cholesterol (215; normal 125–170 mg dl$^{-1}$), normal high-density lipoprotein (HDL) cholesterol (47; normal 38–76 mg dl$^{-1}$), elevated triglycerides (189; normal 33–129 mg dl$^{-1}$) and elevated low-density lipoprotein cholesterol (130; normal $<$110 mg dl$^{-1}$) with a normal cholesterol/HDL cholesterol ratio of 4.6 (normal$<$5.0) and elevated non-HDL cholesterol (168; normal $<$12 mg dl$^{-1}$). However, we believe that there is no correlation of LDLR mutation and patient's hyperlipidemia.

## Discussion

We report the first occurrence of a potential association between a chromosome 17p13.3 duplication and an unclassified type of metaphyseal dysplasia. Whole-exome sequencing and oligonucleotide-SNP array in this patient revealed a *de novo* interstitial duplication within cytogenetic band 17p13.3, with the duplicated interval containing 19 genes including *SERPINF1*. Although we recognize that many genes were identified in this duplication, only four of them are associated with a known clinical disorder, including *SERPINF2* (autosomal recessive (AR) hemorrhagic diathesis), *PRPF8* (autosomal dominant (AD) retinitis pigmentosa), *WDR81* (AR cerebellar ataxia with intellectual disability) and *SERPINF1* (AR OI-VI), and, considering that this patient only exhibited an abnormality with his bones, *SERPINF1* is the most likely associated genetic duplication with his condition. *SERPINF1* is one of the many genes located on chromosome 17p13.3, with autosomal recessive loss-of-function mutations in *SERPINF1* known to cause a severe type of OI (OI-type VI).[1,2] *SERPINF1* encodes pigment epithelium-derived factor (PEDF), which has a multitude of roles in bone development and growth. Specifically, it appears that PEDF has a critical role in collagen type-I binding and angiogenesis inhibition.[3,4] Furthermore, studies have shown that PEDF promotes osteoblastic cell differentiation and inhibits bone resorption.[5] Mice lacking PEDF have decreased trabecular bone volume, increased unmineralized bone matrix and increased bone fragility.[6] Similarly, humans with OI-type VI who are missing PEDF secondary to *SERPINF1* loss-of-function mutations have excess unmineralized bone matrix on bone histology and increased bone fragility.[7,8]

Larger 17p13.3 duplications have been described by Curry *et al.*[9] involving both *YWHAE* and *LIS1* genes, with patients exhibiting a distinct facial phenotype, structural brain abnormalities and autism spectrum disorder. *SERPINF1* was also involved in the duplication described in the Curry *et al.* article, but there was no mention of a bone phenotype, and thus this is the first description of a 17p13.3 microduplication involving *SERPINF1* associated with an unclassified type of metaphyseal dysplasia. Interestingly, *SERPINF1* and the gene leading to Schmid metaphyseal dysplasia, *COL10A1*, both have been implicated in chondrocyte differentiation, which may shed additional light as to how *SERPINF1* gene duplications could lead to metaphyseal dysplasia. Dehne *et al.*[10] found an association with *SERPINF1* and chondrocyte proliferation, and *COL10A1*, which encodes collagen type X,[11] is considered a biomarker for hypertrophic chondrocytes.[12] Hence, both *SERPINF1* and *COL10A1* may cause metaphyseal dysplasia by impacting chondrocyte differentiation.

Interestingly, another duplicated gene found on 17p13.3 was *SMG6*, which has been associated with bone mineral density in both the femoral neck and lumbar spine, according to genome-wide meta-analysis study by Estrada *et al.* However, it was not found to be associated with any specific skeletal phenotype or the molecular bone signaling pathway.[13] As a result, the duplication of *SMG6* was unlikely to be a candidate gene responsible for the metaphyseal dysplasia in this patient, but further study of this particular gene in association with other bone disease is needed in the future.

Therefore, based on the associated loss-of-function *SERPINF1* gene mutation with severe OI[1,2] and our currently reported findings of a *SERPINF1* duplication associated with metaphyseal dysplasia but no bone fragility (as evidenced by normal bone density from a CT bone density scan), this may suggest that *SERPINF1* gene is dosage sensitive. However, further functional validation of this gene either *in vitro* or animal experiment should be warranted to confirm the candidacy of this gene.

## Materials and Methods

Exome sequencing was performed commercially at GeneDx, Gaithersburg, Maryland. Using the proband's and parental genomic DNA, the Agilent SureSelect XT2 All Exon V4 kit

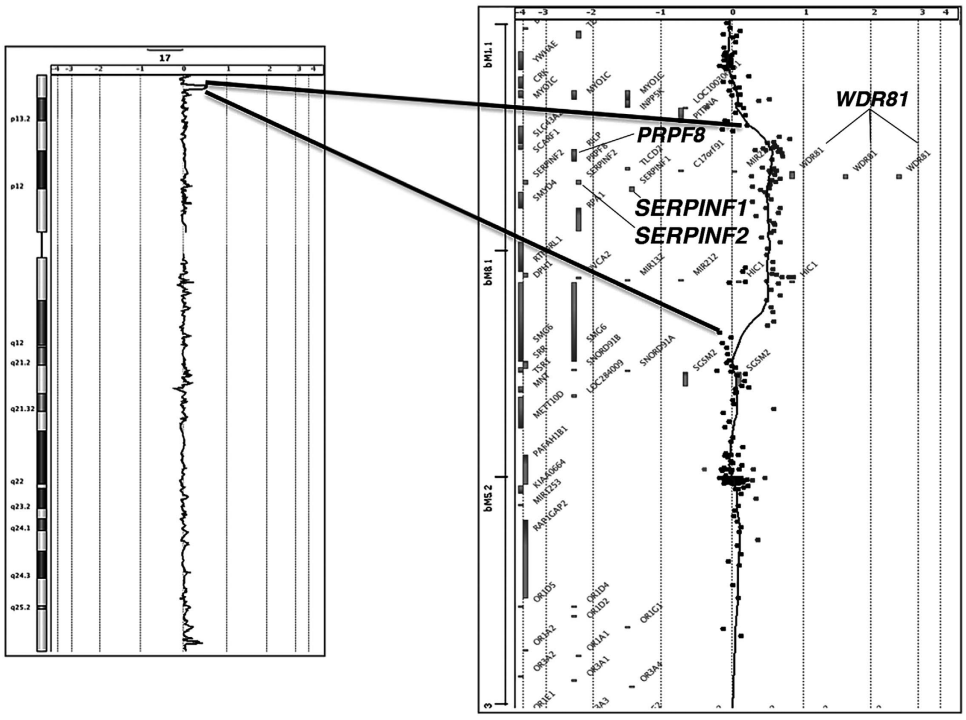


**Figure 3** *De novo* 17p13.3 duplication of *SERPINF1*. Whole-exome sequencing demonstrating a *de novo* 17p13.3 duplication, which includes *SERPINF1* (autosomal recessive OI-VI), *SERPINF2* (autosomal recessive hemorrhagic diathesis), *PRPF8* (autosomal dominant retinitis pigmentosa) and *WDR81* (autosomal dominant cerebellar ataxia with intellectual disability).

(Agilent Technologies Inc, Santa Clara, CA, USA) was used to target the exon regions of their genomes (exome). The targeted region was sequenced using the Illumina HiSeq 2000 sequencing system (Illumina, San Diego, CA, USA) with 100-bp paired-end reads. The DNA sequence was mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. The XomeAnalyzer (GeneDx, Gaithersburg, MD, USA) was used to evaluate sequence changes in this individual compared with other sequenced family members. Copy-number changes were detected directly from exome capture data by comparing the relative coverage on a per-exon basis for the proband sample with the expected relative coverage calculated from a series of $>1000$ exomes generated using the same capture-based protocol and sequencing platform. The reported copy-number variation in the proband was confirmed using a 180-K whole-genome oligonucleotide array CGH + SNP (GenomeDx v5, GeneDx). To confirm gene duplication within this region, quantitative PCR was performed on one of the genes within this duplicated region (*PRPF8*). Using a primer targeted to exon 5 of the *PRPF8* gene in cytogenetic band 17p13.3, the number of *PRPF8* copies present in the parents was compared with the patient to confirm gene duplication within this region. In addition, a 1.15-kb resolution oligo-SNP array (Affymetrix CytoScan HD, Santa Clara, CA, USA) independently confirmed this duplication as arr[hg 19] 17p13.3(1,501,331–2,109,569)x3.

Whole-exome sequencing analysis identified a *de novo* interstitial duplication of at least 588 kb within the cytogenetic band 17p13.3. The duplicated interval contained at least 15 genes, 4 of which (*PRPF8, WDR81, SERPINF2* and *SERPINF1*) are associated with a clinical disorder (**Figure 3**). Specifically, *SERPINF2* mutations lead to an autosomal recessive hemorrhagic diathesis, *WDR81* mutations cause an autosomal recessive form of cerebellar ataxia and intellectual disability, *PRPF8* missense variants cause autosomal dominant retinitis pigmentosa and *SERPINF1* loss-of-function mutations lead to an autosomal recessive form of OI.

## Conflict of Interest

The authors declare no conflict of interest.

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