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High bone mass due to novel *LRP5* and *AMER1* mutations

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ABSTRACT

WNT signaling is a key regulator of bone metabolism and its increased or decreased activity leads to skeletal disorders. Here we describe two patients with high bone mass (HBM) caused by novel mutations in two different WNT pathway components.

The first patient is a 53-year-old male with HBM. He was diagnosed at adult age based on significantly increased bone mineral density (BMD). He has undergone several surgeries due to excessive bone in ear canals, bilateral jaw exostoses and mandibular tori. Radiographs show severe cortical thickening of cranial and long bones. Sanger sequencing identified a novel heterozygous mutation c.592A>T (p.N198Y) in *LRP5* (Low-density lipoprotein receptor-related protein 5). The second patient, an adolescent female, was diagnosed with skeletal dysplasia in early childhood. She had macrocephaly (head circumference +6.0 SD), facial dysmorphism, delayed motor development, laryngomalasia and epilepsy. Radiographic findings were consistent with osteopathia striata with cranial sclerosis. A novel heterozygous frameshift mutation c.655del (p.E219Rfs*63) in *AMER1* (APC Membrane Recruiting Protein 1) was identified. Although both mutations are predicted to lead to increased WNT signaling with a consequent increase in bone formation, the resulting phenotypes are different; cranial sclerosis versus macrocephaly, long bone cortical thickening versus vertical striations and discordant neurological development. This report underscores the diversity of genotypes and phenotypes of HBM and facilitates their differential diagnosis.

1. Background

Canonical WNT/β-catenin pathway plays an essential role in bone health. It affects both osteoblast and osteoclast differentiation and function and partakes in bone homeostasis from prenatal skeletal development to adult age. Dysregulated WNT signaling is a known cause of various forms of inherited bone mass disorders. For example, early-onset osteoporosis can be caused by loss-of-function mutations in the ligand *WNT1* (Wnt Family Member 1) (Laine et al., 2013; Makitie et al., 2016) or in the co-receptor protein LDL Receptor Related Protein 5 (*LRP5*) (Gong et al., 2001; Laine et al., 2011; Saarinen et al., 2010). On the other hand, increased WNT signaling due to, for instance, gain-of-function mutations in *LRP5*, leads to increased bone formation with excessive cortical thickening, dense long bones and craniofacial abnormalities (Balemans et al., 2007). Two high bone mass (HBM) disorders, van Buchem disease (Loots et al., 2005) and sclerosteosis (Balemans et al., 2001), are caused by loss-of-function mutations in *SOST*, encoding the WNT antagonist sclerostin. Furthermore, mutations in the gene encoding the WNT inhibitor APC membrane recruitment 1 (*AMER1*) cause a specific form of HBM called osteopathia striata with cranial sclerosis (OSCS) (Holman et al., 2011; Jenkins et al., 2009).

Here we describe two patients affected by HBM due to novel mutations in two different WNT pathway–related genes. These heterozygous mutations in *LRP5* and *AMER1* are predicted to lead to increased WNT signaling. The resulting conditions both associate with HBM but differ in several clinical and radiographic features, as illustrated by the two cases.

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2. Patients data/material

Patients were identified during the course of an ongoing research program on skeletal dysplasias in Finland. The study was approved by the Institutional Ethics Board at Children's Hospital, Helsinki University Hospital, Finland. Hospital data were reviewed retrospectively and genetic studies performed for diagnostic purposes. Peripheral blood genomic DNA was extracted using standard methods.

3. Methods

For Patient 1 PCR was performed using Taq DNA polymerase (ThermoFisher Scientific) and primers were designed to amplify exons 2–4 of the *LRP5* gene (reference sequence: NM_002335.3). Primer sequences are available from authors upon request. Sanger sequencing was performed according to standard procedures. For Patient 2, genomic DNA was evaluated for a panel of genes associated with osteopetrosis at Bristol RGC, UK Genetic Testing Network. The panel included 21 genes (*ANKH*, *AMER1*, *CA2*, *CLCN7*, *CTSK*, *FAM20C*, *FERMT3*, *IKBKG*, *LEMD3*, *LRP5*, *OSTM1*, *PLEKHM1*, *PTH1R*, *RASGRP2*, *SNX10*, *SOST*, *TCIRG1*, *TGFB1*, *TNFRSF11A*, *TNFSF11* and *TYROBP*).

4. Results

4.1. Patient I

This 53-year-old male had a history of ulcerative colitis, for which he was treated with oral mesalazine and methylprednisolone periodically. To exclude secondary osteoporosis he underwent dual-energy X-ray absorptiometry (DXA). Unexpectedly, he had markedly increased bone mineral density (BMD) with T-scores for femoral neck +7.2 and for lumbar spine +10.1. Based on subsequent clinical and radiographic evaluations he was diagnosed with HBM. Prior to diagnosis he had undergone several operations: two ear canal operations due to excessive bone formation, and surgical removal of bilateral jaw exostoses and mandibular tori. He had no hearing deficits but had recurrent headache. He had chronic back pain and stiffness and no previous fractures.

Skeletal plain radiographs showed generalized thickening of the skull (Fig. 1A) and diaphysis of long bones (Fig. 2A and C). Head magnetic resonance imaging (MRI) showed cranial hyperostosis with significant thickening of all skull bones but no cranial nerve compression and no macrocephaly. Spinal MRI showed dense vertebral bodies, cervical and lumbal foraminal stenosis at several levels and lumbar disc prolaps with spinal canal stenosis at L3-L4. The bone formation marker amino-terminal propeptide (PINP) of type I collagen, was increased 93 μg/l (normal 20–76 μg/l). All other laboratory findings including hematology and markers of calcium homeostasis (calcium, phosphate, alkaline phosphatase, parathyroid hormone) were normal.

We performed Sanger sequencing on genomic DNA to look for potential changes in *LRP5* (Supplemental Fig. S1). A novel heterozygous *LRP5* missense mutation, c.592A>T (p.N198Y), was identified in exon 3 (reference sequence: NM_002335.3) (Supplemental Fig. S1-S2). Based on the mutation's location it was predicted to be an activating mutation leading to increased WNT signaling. The index patient's mother and two sisters, all clinically asymptomatic, were negative for the mutation; the father had deceased and no DNA was available for investigation (Supplemental Fig. S1).

4.2. Patient II

This presently 17-year-old female was born to healthy parents preterm at 35 weeks of gestation. Her birth measurements were 46 cm (−2.4 SD) and 2510 g (−2.4 SD) and the head circumference was 35.5 cm (+0.2 SD). She had triangular face, hypertelorism, micrognatia, short neck, flat nasal bridge, large open fontanelles, and two-parted xyphoid process (bifidi xiphoid). Brain ultrasound and MRI were performed due to rapidly increasing head circumference. White matter was decreased and lateral ventricles widened but there were no signs of hydrocephaly and the cause of macrocephaly remained unknown. Laryngobronchoscopy at age 1 year revealed choanal stenosis with laryngomalasia and subglottic stenosis. Her ear and nose canals were narrow leading to recurrent ear infections. Abdominal ultrasound showed no apparent abnormalities. She had normal hearing but no stereovision. The motor development was delayed. Treatment-responsive epilepsy was diagnosed at 13 years. Later in life, she had otherwise normal growth (height +1.0 SD, BMI 23.6 kg/m^2) and pubertal development. Macrocephaly has persisted at +6.0 SD. Dental problems have necessitated orthodontic treatment for over 10 years. A clinical diagnosis of pycnodysostosis was made in early childhood but later diagnosis of OSCS was considered based on radiographic findings and craniofacial characteristics.

Radiography displayed severe hyperostosis with longitudinal striations and uneven bone mineralization (Fig. 2B and D). Cranial plain radiographs depicted severe macrocephaly with underdeveloped sinuses, small jaw and open fontanels (Fig. 1B). Long bones and phalanges were abnormal in shape. Spinal images showed normal vertebrae, mild scoliosis, and no bone-in-bone features. DXA measurement at 17 years showed increased BMD with Z-scores +3.0 for lumbar spine and +2.4 for femoral neck, confirming generalized high bone mass.

Extensive studies were performed in childhood to determine the cause of her skeletal phenotype. Biochemical parameters of calcium homeostasis were normal. Chromosomes, fibroblast lyzosomal enzyme activity, and organic amino acids were normal. A 21 osteopetrosis gene panel at Bristol RGC, UK Genetic Testing Network, found a novel X-chromosomal heterozygous deletion c.655del (p.E219Rfs*63) in *AMER1* (APC Membrane Recruiting Protein 1) (reference sequence: NM_152424.3) (Supplemental Fig. S3). The one-base deletion likely leads to premature termination of the gene product. This putative loss of function (LOF) mutation has not been described before but the gene's other previously described mutations are associated with OSCS (OMIM #: 300373). No parental samples were available for testing but the mutation was determined *de novo* as the parents and younger brother were healthy.

5. Discussion

High bone mass (HBM) is a rare skeletal condition characterized by increased BMD. Excessive bone deposition is usually most prominent in cranial and tubular bones but the clinical characteristics are heterogeneous. The underlying molecular and genetic mechanisms are also diverse and both, malfunctioning osteoclasts and subsequent decrease in bone resorption as well as increased osteoblast function, with consequent excessive bone deposition can lead to similar phenotypes. This study describes two novel mutations in two patients with HBM and portrays how mutated *LRP5* and *AMER1* and consequent increase in WNT signaling cause excessive bone deposition with differences in phenotypes.

We adopted the candidate gene approach to identify the genetic defect causing HBM in our first index patient. The most commonly mutated gene in HBM is *LRP5* and 14 activating mutations have been identified in this gene thus far (Supplemental Fig. S2). The previously identified mutations are all missense, except one small deletion (Supplemental Fig. S2). Furthermore, HBM *LRP5* mutations

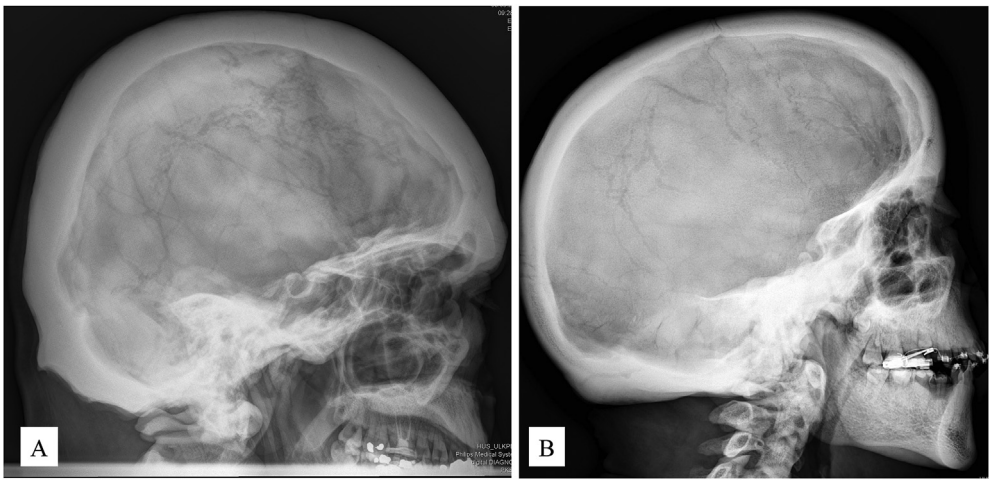

Fig. 1. Cranial radiographs from two patients with different genetic defects in WNT pathway components. **A)** Cranial X-ray of a 53-year-old male with high bone mass due to a heterozygous missense mutation p.N198Y in *LRP5* showing cranial hyperostosis with thickened skull bones, underpneumatized paranasal sinuses and prominent jaw. **B)** Cranial X-ray of a 17-year-old female with OSCS due to novel X-chromosomal heterozygous frameshift deletion p.E219Rfs*63 in *AMER1*. Figure shows enlarged head circumference (+6 SD), dense cranial bones and prominent jaw.

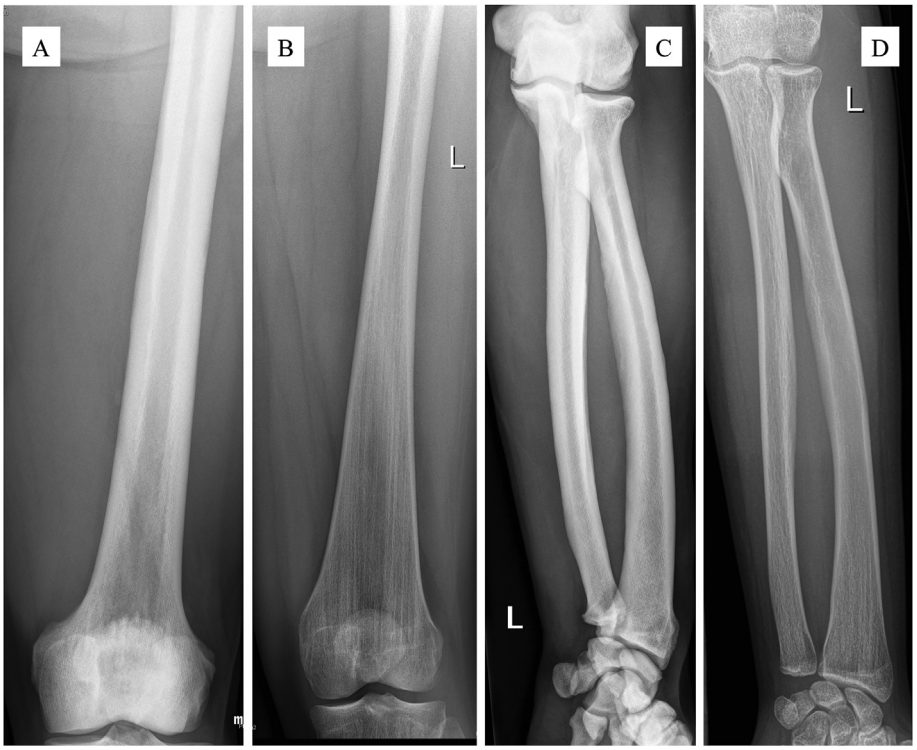

Fig. 2. Radiographs of long bones from two patients with different genetic defects in WNT pathway components. **A)** and **C)** are lower and upper extremity radiographs, respectively, from a 53-year-old male with high bone mass due to a heterozygous missense mutation p.N198Y in *LRP5* showing severe hyperostosis, cortical thickening in diaphyses and slightly abnormal shape. **B)** and **D)** are lower and upper extremity radiographs, respectively, from a 17-year-old female with OSCS due to a novel X-chromosomal heterozygous deletion p.E219Rfs*63 in *AMER1* showing striated patterning and abnormal shape.

have only been identified in exons 2–4 corresponding to the β-propeller 1 in the protein (Gregson et al., 2016; Supplemental Fig. 2). For this reason we sequenced only these exons and identified a novel missense mutation, c.592A>T (p.N198Y), in exon 3. Another mutation in the same codon (p.N198S) has already been reported as pathogenic (Gregson et al., 2016); this has been defined as one of the most important among the HBM mutations since Asparagine 198 strictly interacts with the Asparagine of the "NXI" motif of the SOST/DKK1 inhibitors (Bourhis et al., 2011). The previously identified mutation converts Asparagine into Serine, two amino acids that belong to the same chemical group and causes a loss of affinity for the inhibitors due to the different amino acid structure (Gregson et al., 2016). Our mutation instead converts Asparagine into Tyrosine. These two amino acids have different chemical-physical characteristics and in this way this mutation may have an even more deleterious impact on the protein. The mutation locates in the β-propeller 1, where the Dickkopf WNT Signaling Pathway Inhibitor 1 (*DKK1*) binds (Ai et al., 2005). *DKK1*

encodes an antagonist of the WNT co-receptors *LRP5* and *LRP6*. Sclerostin (encoded by *SOST*) is also an antagonist for these co-receptors. LOF mutations in *SOST* lead to upregulation of the WNT/β-catenin pathway and bone formation is promoted. The WNT pathway can be also up-regulated by gain of function mutations of *LRP5* because they impair the binding of the ligand *DKK1* to its co-receptor. In this way the canonical WNT pathway escapes inhibition and BMD increases (Balemans et al., 2008). This mechanism is likely to cause the disease in our index patient. Furthermore, his skeletal phenotype, characterized by hyperostosis not restricted to skull but affecting the whole skeleton, overlaps with the phenotype of the other patients with HBM and supports the presence of a germline gain of function mutation (Gregson et al., 2016). Usually, patients with HBM have torus palatinum but instead our patient had torus mandibularis.

Our second patient is affected by OSCS, which is a rare form of skeletal dysplasia. Mutations in *AMER1* were first discovered as the underlying cause in 2009 and since then many other patients and families have been described with novel mutations and varying clinical characteristics (Supplemental Fig. S3) (Jenkins et al., 2009). Our patient's phenotype is in congruence with the common traits in female patients and diagnosis is confirmed by radiographic signs of sclerosis of skull and long bones with longitudinal striations. These striations may be the result of a random X inactivation in the osteoblast lineage cells since they are usually absent in male patients with *AMER1* mutations (Ciceri et al., 2013; Jenkins et al., 2009; Perdu et al., 2010). Due to X-linked dominant inheritance, the condition is significantly more severe in males and usually associated with fetal or neonatal lethality. Surviving males exhibit severe multiorgan malformations and hyperostosis (Holman et al., 2011; Jenkins et al., 2009). AMER1 functions intracellularly to suppress WNT signaling by promoting the ubiquitination and degradation of β-catenin (Tanneberger et al., 2011). Inactivating mutations in *AMER1* lead to accumulation of active cytoplasmic β-catenin and consequently translocation of β-catenin to the nucleus, increased target gene transcription and excessive bone formation (Major et al., 2007). Although *AMER1,* also referred to as WTX (Wilms tumor X-linked), is a tumor suppressor gene and somatic mutations lead to Wilms tumor (OMIM #: 194070), OSCS patients with germline mutations do not seem to be predisposed to cancer (Jenkins et al., 2009). However, there are exceptions: a female patient with OSCS also developed hepatoblastoma (Fujita et al., 2014). For this reason careful follow up to exclude malignancies, such as Wilms tumor, is indicated in patients with OSCS. Concerning our patient, no malignancies or suspicious symptoms have thus far been observed and follow-up will continue.

Differentiating between various HBM disorders can appear complex. Both of our patients went through several examinations or surgeries and biochemical tests before arriving at their final diagnosis. The recent advances in understanding the genetic and molecular mechanisms governing bone health, expansion of available genetic tests and the data on rare variants have increased our understanding and enable early diagnosis, genetic counseling and proper management. Although our study lacks *in vitro* validations there is good evidence from the literature that the observed novel mutations in *LRP5* and *AMER1* cause HBM through increased WNT signaling.

In conclusion, we identified a novel missense *LRP5* mutation and a novel X-chromosomal heterozygous deletion in *AMER1* both causing HBM. Our findings expand the spectrum of WNT pathway-related mutations causing HBM. Furthermore, our work elucidates how defects in the same pathway but in two different proteins give rise to parallel phenotypes with different modes of inheritance and careful clinical and radiological evaluation is needed for accurate diagnosis.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2017.09.001.

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