Case Report

Whole-exome sequencing reveals a heterozygous LRP5 mutation in a 6-year-old boy with vertebral compression fractures and low trabecular bone density

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Abstract

Juvenile osteoporosis (JO) is characterized by bone fragility during development, low bone mass and absence of extraskeletal features. Heterozygous loss-of-function mutations in LRP5 have been found in a few patients, but bone tissue and bone material abnormalities associated with such mutations have not been determined. Here we report on a 6-year-old boy who presented with a history of seven low-energy long-bone fractures starting at 19 months of age and absence of extraskeletal involvement. Spine radiographs revealed multiple vertebral compression fractures. Despite tall stature (95th percentile), lumbar spine areal bone mineral density was low (z-score = −3.2). Trabecular volumetric bone mineral density, measured by peripheral quantitative computed tomography at the distal radius, was low (z-score = −5.1), but cortical bone thickness at the radial diaphysis was normal. Iliac bone histomorphometry demonstrated low bone formation activity in trabecular but not in cortical bone. Quantitative backscattered electron imaging showed normal material bone density in trabecular bone, but elevated results in the cortex. Whole-exome sequencing revealed a heterozygous insertion of a nucleotide in exon 12 of LRP5. This mutation had previously been reported in another JO patient and had been shown to lead to nonsense-mediated decay. Thus, heterozygous loss-of-function mutations in LRP5 can be associated with a bone formation deficit that affects mostly the trabecular compartment and can result in bone fragility during the first years of life.

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Introduction

The diagnostic category of primary osteoporosis in children comprises a group of mostly heritable disorders that lead to low bone mass and bone fragility in the absence of underlying extraskeletal pathology [1]. In many cases, primary osteoporosis in children is associated with extraskeletal connective tissue symptoms, such as blue sclerae and dentinogenesis imperfecta, leading to a diagnosis of osteogenesis imperfecta (OI) [2]. When no such extraskeletal disease manifestations are found, a clinical diagnosis of juvenile osteoporosis (JO) is made [1]. On the bone tissue level, JO is typically characterized by decreased osteoblast activity that mainly affects trabecular bone [3,4]. To date, mutations in only one gene, LRP5 (low-density lipoprotein receptor-related protein 5) are known to cause JO, leading to an autosomal dominant mode of inheritance [5].

LRP5 is a plasma membrane protein that acts as a co-receptor for Wnt proteins and, when activated, stimulates bone formation [6]. Heterozygous loss-of-function mutations cause osteoporosis-pseudoglioma syndrome (OPPG), a disorder characterized by childhood osteoporosis and blindness [7]. The features of OPPG are also observed in homozygous Lrp5-deficient mice [8]. Heterozygous Lrp5 knockout mice have an isolated bone mass deficit and their bone tissue responds less to mechanical stimuli than that of wild type mice [8,9].

Most carriers of heterozygous loss-of-function mutations in LRP5 have been detected by screening asymptomatic family members of patients with OPPG [7], but such mutations have also been reported in 5 children and adolescents presenting with a JO phenotype [5,10]. Nevertheless, LRP5 mutations seem to be an infrequent cause of pediatric bone fragility, as two studies including a total of 93 fracture-prone children and adolescents did not reveal any LRP5 mutations [11,12].

The skeletal effects of LRP5 defects have been studied intensely in mice, but in humans the available information on the skeletal consequences of heterozygous loss-of-function of LRP5 is largely limited to reports of low bone mineral density (BMD) and fracture history [5,7,10,13]. In particular, there is presently no information about bone properties on the tissue and material level in humans affected by heterozygous loss-of-function mutations in LRP5. Abnormalities in material bone density have been found in several pediatric bone fragility conditions [14,15]. Transgenic mice that overexpress Lrp5 under the control of the Coll1 promoter have an increased relative ash weight, indicating...
that LRP5 could be involved in the regulation of material bone density [16].

Here we describe the skeletal phenotype of a 6-year-old boy in whom whole-exome sequencing revealed a heterozygous loss-of-function mutation in LRP5.

Materials and methods

The patient was assessed at the Shriners Hospital for Children in Montreal. Clinical data were extracted by retrospective chart review. Informed parental consent was provided. The study was approved by the Institutional Review Board of McGill University.

Dual-energy X-ray absorptiometry was performed in the anterior–posterior direction at the lumbar spine (L1–L4) using a Hologic QDR Discovery device (Hologic Inc., Waltham, MA, USA). Lumbar spine areal BMD results were transformed to age- and gender-specific z-scores using reference data provided by the densitometer manufacturer [17,18]. Peripheral quantitative computed tomography (XCT–2000, Stratec Inc., Pforzheim, Germany) was performed at the metaphysis (4% site) and at the diaphysis (65% site) of the radius as described, and z-scores were calculated based on reference data established by one of the authors [19–22]. Volumetric BMD data were compared to age- and gender-matched reference data, as these measures are independent of bone size, whereas cortical thickness, bone and muscle cross-sectional areas and bone mineral content were compared to height- and gender-matched reference data, as these measures are dependent on bone size [19–22].

An iliac bone sample was obtained before the start of bisphosphonate treatment, at a site 2 cm posterior of the superior anterior iliac spine. Tetracycline double labeling was performed prior to biopsy. Sample preparation and histomorphometric analyses were performed using previously described procedures [23]. Results were compared to the average value of the age- and gender-specific reference range using reference data established in our laboratory [23].

The bone mineralization density distribution in trabecular and cortical bone from this sample was analyzed by quantitative backscattered electron imaging, as described [24]. We have previously validated this method and applied it to analyze bone samples representing a wide variety of clinical and experimental situations [25]. Results were compared to a pediatric reference data base that we have established previously [26].

Whole-exome capture was carried out on 3 μg of genomic DNA, using the SureSelect Human Exome Kit version 3 (Agilent Technologies, Inc., Santa Clara, CA, USA), which targets 50 Mb of coding exon sequences annotated by the GENCODE project, CCDS and RefSeq databases as well as 10 base pairs of flanking sequence of each exonic region and non-coding RNA. The captured and amplified exome library was sequenced with 100 bp paired-end reads on an Illumina HiSeq2000 DNA sequencer.

Exome sequencing data were analyzed using an in-house bioinformatics pipeline as previously described [27]. Briefly, ~132 million high quality trimmed paired-end sequences were generated and aligned to the human genome assembly hg19 by the Burrows-Wheeler Alignment tool version 0.5.9 [28]. The mean read depth for coding bases defined by CCDS (consensus coding sequence) was 94X, and 95% of bases were covered by ≥5 reads as determined by the Genome Analysis Toolkit [29].

The variants were called by SAMtools version 0.1.7 [30], mpileup and varfilter with the base alignment quality adjustment disabled, leading to the identification of approximately 500K variants. In order to narrow down the list of candidate rare variants, a minimum of two variant reads and >2% single-nucleotide variants or >15% Indel (small insertions or deletions) variant reads were considered for each called position. Subsequent variants were then filtered against the results of 460 individuals with diseases other than pediatric bone fragility disorders who had previously undergone exome sequencing at the Genome Quebec center, in order to remove common polymorphism and false positive variants.

The remaining variants were then annotated by ANNOVAR [31] for the type of mutations, presence in dbSNP132, minor allele frequency in the 1000 Genomes project, EVS (Exome variant server), SIFT, Polyphen-2 and PHASTCONS scores. Subsequently, the variants with an allele frequency less than 0.05 in the 1000 Genomes database and predicted to be nonsynonymous (i.e., missense, nonsense, frameshift or canonical splice site changes) were kept in the final list (n = 576). All potential candidate genes were manually examined using IGV [32]. To confirm the presence of the LRP5 mutation in the index patient and test other family members for the presence of this mutation, exon 12 of LRP5 was amplified by polymerase chain reaction. The sequencing reaction was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The nucleotide sequence was determined using an Applied Biosystems 3100 DNA sequencer. Sequence traces were aligned with the GenBank reference sequences of the LRP5 genomic DNA (GenBank accession number NG_015835.1). Known non-synonymous polymorphisms in LRP5 (Q89R, V667M, A1330V) [12] were assessed in subjects positive for the exon 12 mutation using the same methodology. In addition, all 23 coding exons of LRP5 including exon/intron boundaries were sequenced in the proband, his mother and his father.

Results

The boy was born after 38 weeks of gestation by elective repeat cesarean section. Birth weight was 3940 grams (75th percentile for gestational age), and length was 54 cm (90th percentile). No abnormalities were noted. He was the second child of nonconsanguineous Caucasian parents. The mother had sustained a forearm fracture at 3 years of age but had no history of fractures since. His father and older sister did not have a history of fractures or other significant health issues.

The boy developed normally, but between the age of 19 months and 6 years, he sustained a total of 7 low-energy long-bone fractures (radius/ulna, humerus, tibia/fibula), which all healed without complications. When first evaluated at our institution at the age of 6 years, he was a healthy-looking boy with a height of 126 cm (95th percentile) and a weight of 26.1 kg (90th percentile). Extremities and back were straight. Sclerae were white. Teeth appeared normal on inspection, and there was no joint or skin hyperlaxity. Radiographs revealed the presence of multiple vertebral compression fractures (Figs. 1A and B). Areal BMD at the lumbar spine corresponded to an age- and gender-matched z-score of −3.2. Peripheral qualitative computed tomography of the left radius (Fig. 1E) showed a trabecular volumetric BMD z-score of −5.1 at the metaphysis (‘4% site’). Cortical volumetric BMD (z-score = −0.6) and cortical thickness (z-score = −0.1) at the radial diaphysis (‘65% site’) were normal, but the total bone cross-sectional area was reduced (z-score = −2.9). Bone mineral content was therefore low (z-score = −3.1). Muscle cross-sectional area was normal (z-score = −0.9).

A transiliac bone biopsy specimen was obtained. The trabecular compartment was not preserved intact due to the paucity of trabecular bone; therefore, trabecular bone volume could not be determined (Fig. 1F). Histomorphometric analyses revealed low mineral apposition rate and low bone formation rate in trabecular bone (Table 1). However, parameters of intracortical bone formation were normal. The bone resorption parameter eroded surface per bone surface was normal in both trabecular and cortical bone. Cortical thickness was above average, but porosity was normal.

Quantitative backscattered electron imaging (Fig. 1F, Table 2) showed that in cancellous bone, CaMean and CaPeak (describing mean and the most frequent calcium concentration of the bone mineral density distribution) were close to the mean result of the reference population. CaWidth (reflecting heterogeneity of mineralization), Calow (the percentage of bone areas with low mineralization) and CalHigh
(the percentage of highly mineralized bone areas), were increased. In cortical bone, CaMean, CaPeak and CaHigh were distinctly elevated, while CaLow was markedly low. Only CaWidth was similar to reference values in cortical bone.

Following bone biopsy, intravenous treatment with zoledronic acid was initiated at a dose of 0.05 mg per kg body weight and repeated every 6 months. Up to the time of the last follow-up visit (30 months after the first zoledronic acid infusion), no more long-bone fractures have occurred and there appeared to be some reshaping of compressed vertebra (Fig. 1C). Height was at 146 cm (3 cm above the 95th percentile) and the lumbar spine areal BMD \(z\)-score was \(-1.2\) (\(+2.0\) compared to baseline). Peripheral quantitative computed tomography of the left distal radius showed a trabecular volumetric BMD \(z\)-score of \(-2.3\) (\(+2.8\)). At the radial diaphysis, \(z\)-scores were \(+0.6\) (\(+1.2\)) for cortical volumetric BMD, \(+0.9\) (\(+1.0\)) for cortical thickness, \(-3.1\) (\(-0.2\)) for total bone cross-sectional area, \(-1.8\) (\(+1.3\)) for bone mineral content and \(-0.6\) (\(+0.3\)) for muscle cross-sectional area. No repeat iliac bone biopsy was performed.

In order to identify the mutation underlying bone fragility in this boy, whole-exome sequencing was performed. No variants were located within 13 genes (\(COL1A1\), \(COL1A2\), \(CRTAP\), \(LEPRE1\), \(PPIB\), \(SERPINH1\), \(FKBP10\), \(SP7\), \(SERPINF1\), \(BMP1\), \(ITIM5\), \(TMEM38B\), \(WNT1\)) known to be associated with autosomal-dominant and recessive OI. However, the analysis discovered a heterozygous \(c.2737_2738\text{insT}\) mutation in exon 12 of \(LRP5\) (Fig. 2). The same mutation had been reported before in a patient with JO and results in a frameshift at amino acid position 913 (p.C913LeufsX73) [5]. As expected, functional studies had shown that this mutation leads to nonsense-mediated decay of mRNA [5].

The mutation was confirmed by Sanger sequencing of exon 12 in this patient and was also found in his mother and his maternal grandfather, but not in his sister or his father (Fig. 2). Following the unexpected observation that the patient’s mother carried the same \(LRP5\) mutation, she...
underwent bone densitometry. Normal results for areal BMD at the lumbar spine (z-score = −1.4), trabecular volumetric BMD at the distal radius (z-score = +0.1), cortical volumetric BMD (z-score = −0.3) and cortical thickness (z-score = +0.4) at the radial diaphysis were found. Nevertheless, she had a grade I anterior wedge fracture of thoracic vertebra 11 (Fig. 1D). The maternal grandfather, who also was positive for the LRP5 frameshift mutation, did not have a history of fractures, but could not be evaluated by bone densitometry or radiographically. However, all three family members carrying the LRP5 mutation underwent ophthalmologic evaluation. Fundoscopy did not reveal abnormalities in the retina or retinal vasculature in any of the three subjects.

To assess why the phenotypic consequences of the LRP5 mutation differed between affected family members, we determined single nucleotide polymorphisms in LRP5 (Q89R, V667M, A1330V) that may influence LRP5 expression or function [33–35]. However, the index patient, his mother and his maternal grandfather all were homozygous for the major allele at each site. We also sequenced all 23 coding exons of LRP5 in the proband, his father and his mother, to determine whether the proband had inherited any LRP5 sequence changes from the father that might have aggravated the bone fragility. However, no sequence differences other than the heterozygous c.2737_2738 insertion in the proband and the mother was found between the three individuals.

Discussion

In this study, we used whole-exome sequencing to assess the genetic basis for severe bone fragility in a 6-year-old boy and discovered a heterozygous frameshift mutation in LRP5. This mutation had previously been detected in a child with JO and had been shown to lead to nonsense-mediated decay of mRNA [5]. In addition, a patient with OPPC has been reported who was compound heterozygous for the same mutation and another frameshift mutation in LRP5 [36]. The present case nevertheless contributes new information regarding the role of LRP5 in human bone development.

Iliac bone histomorphometry revealed a low mineral apposition rate in trabecular bone, indicating low osteoblast function. This is compatible with findings in heterozygous LRP5 knockout mice that also have decreased bone formation activity in trabecular bone [8,37]. It is noteworthy, however, that the bone formation defect seemed to be limited to trabecular bone in our patient, as the amount of cortical bone and the activity of cortical bone formation were normal in the iliac bone specimen. The seven different bone surfaces of a transiliac bone sample can differ markedly with regard to cellular activities [38]. It is unclear why the LRP5 mutation in this boy was associated with differential effects on trabecular and intracortical bone cell function, but we have previously made similar observations in a series of 9 patients with JO [3,4].

In accordance with these histomorphometric findings, pQCT at the radius showed a very low amount of trabecular bone but normal cortical thickness, even though external bone size was below the reference range. At the material level, there was a dissociation in matrix mineralization between the trabecular and cortical bone compartment. Cortical bone was more highly mineralized than trabecular bone, while in the reference population cortical bone is slightly less mineralized than trabecular bone [26]. The higher mineral concentration found in cortical bone can lead to more brittleness of the bone material and thus might contribute to fragility fractures in long bones [39].

The identification of the heterozygous frameshift insertion within LRP5 using whole-exome sequencing on a single affected individual demonstrates the power of the technology to discover causative genes with limited number of patients and families. With the decreasing cost of exome sequencing, it may become the method of choice in molecular and clinical diagnoses of predominantly monogenic defects.

After the heterozygous LRP5 frameshift mutation had been identified in the proband’s mother, a lateral spine radiograph was obtained that showed a vertebral compression fracture. This was a surprising finding, as she had no history of back pain and her bone density results were all within normal limits. It thus seems advisable to screen carriers of loss-of-function mutations in LRP5 for vertebral compression fractures, regardless of bone densitometric results.

Even though the proband’s mother had a mild compression fracture, the consequences of the LRP5 mutation seemed to be far more severe in her son, who had severe bone fragility and very low bone density at an early age. We therefore searched for other sequence changes in LRP5 that might explain why mother and son varied in disease severity. However, our analyses failed to detect any sequence differences between these two individuals. It is possible that a second sequence variant in a noncoding region of LRP5 or in another gene contributes to the bone fragility in our index patient.

When the boy described here received treatment with intravenous zoledronic acid, marked increases occurred in lumbar spine areal BMD

### Table 1

<table>
<thead>
<tr>
<th>Trabecular bone</th>
<th>External cortex</th>
<th>Internal cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Controls</td>
<td>Patient</td>
</tr>
<tr>
<td>Cortical width (μm)</td>
<td>1420</td>
<td>747 (327)</td>
</tr>
<tr>
<td>Cortical porosity (%)</td>
<td>12.2</td>
<td>9.3 (3.3)</td>
</tr>
<tr>
<td>Osteoid thickness (μm)</td>
<td>5.3</td>
<td>5.8 (1.4)</td>
</tr>
<tr>
<td>Osteoid surface per bone surface (%)</td>
<td>24</td>
<td>34 (7)</td>
</tr>
<tr>
<td>Mineralizing surface per bone surface (%)</td>
<td>7.7</td>
<td>12.5 (4.5)</td>
</tr>
<tr>
<td>Mineral apposition rate (μm/d)</td>
<td>0.74</td>
<td>1.04 (0.17)</td>
</tr>
<tr>
<td>Bone formation rate per bone surface (μm²·yr⁻¹)</td>
<td>21</td>
<td>48 (19)</td>
</tr>
<tr>
<td>Eroded surface per bone surface (%)</td>
<td>16</td>
<td>15 (4)</td>
</tr>
</tbody>
</table>

Results in controls are given as means (SD).

### Table 2

<table>
<thead>
<tr>
<th>Trabecular bone</th>
<th>Cortical bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Difference (%)</td>
</tr>
<tr>
<td>CaMean (14)</td>
<td>20.42</td>
</tr>
<tr>
<td>CaPeak [%wt Ca]</td>
<td>21.32</td>
</tr>
<tr>
<td>CaWidth [%Δwt Ca]</td>
<td>4.16</td>
</tr>
<tr>
<td>CaLow [%]</td>
<td>11.00</td>
</tr>
<tr>
<td>CaHigh [%]</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median [25th percentile; 75th percentile]. Diff indicates the difference of the patient’s result to the median of the reference population.
and trabecular volumetric BMD at the radius. The increase in areal BMD was similar to what had been observed in children with homozygous LRP5 mutations or in children with OI. However, one aspect that is likely to lead to bone weakness in this boy, small long-bone diameter, did not seem to be affected by the treatment with zoledronic acid, as total bone cross-sectional area at the radial diaphysis remained low.

In conclusion, this case report shows that a heterozygous loss-of-function mutation in LRP5 can be associated with a bone formation deficit that affects mostly the trabecular compartment and can result in bone fragility during the first years of life.

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Roles of the authors: SF and JM performed the whole-exome sequencing analysis and contributed the genetic aspects of the report; PR was responsible for Sanger sequencing analyses; PR and KK performed and interpreted quantitative backscattered electron imaging analyses; FR conceptualized the project, contributed patient information and wrote the report. All authors have read and approved of the final version of the manuscript.

References


[27] Web Resources


