ATYPICAL FEMORAL FRACTURE IN OSTEOPOROSIS PSEUDOGLIOMA SYNDROME ASSOCIATED WITH TWO NOVEL COMPOUND HETEROZYGOUS MUTATIONS IN \textit{LRP5}†

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ABSTRACT

Osteoporosis pseudoglioma syndrome (OPPG) is a rare autosomal recessive condition of congenital blindness and severe childhood osteoporosis with skeletal fragility, caused by loss-of-function mutations in the *LRP5* (Low-density lipoprotein receptor-related protein 5) gene. We report the first case of atypical (subtrochanteric) femoral fracture (AFF) in OPPG, occurring in a 38 year old man within the context of relatively low bone turnover and trabecular osteoporosis on bone histology. We identify two novel *LRP5* mutations: *R752W* is associated with low bone mass density (BMD), as demonstrated by the heterozygous carriage identified in his 57 year old mother; however, the combination of this *R752W* mutation with another novel *W79R* mutation, causes a severe case of compound heterozygous OPPG. We undertake three-dimensional homology modelling of the four extracellular YWTD β-propeller/EGF-like domains (E1-E4) of LRP5, and show that both novel mutations destabilise the β-propeller domains that are critical for protein and ligand binding to regulate Wnt signalling and osteoblast function. Whilst AFFs have been reported in other rare bone diseases, this is the first in a genetic condition of primary osteoblast dysfunction. The relatively low bone turnover observed, and knowledge of *LRP5* function, implicates impaired bone remodelling in the pathogenesis of AFF.

KEY WORDS

Atypical femoral fracture, compound heterozygote, LRP5, OPPG, protein model
INTRODUCTION

Atypical femoral fractures (AFF) have been reported in patients taking anti-resorptive therapy, particularly for prolonged durations, although they can occur in bisphosphonate-naive individuals. The pathogenesis of AFF remains unclear and much anxiety exists regarding the association between commonly used osteoporosis treatments and AFF\(^{(1)}\). Studying rare genetic diseases, such as *LRP5* disorders, can offer insights into the understanding of more common diseases.

Loss-of-function *LRP5* mutations cause osteoporosis pseudoglioma syndrome\(^{(2)}\) (OPPG, OMIM:259770), a rare autosomal recessive disorder, characterised by congenital or juvenile-onset blindness, severe juvenile-onset osteoporosis\(^{(3,4)}\), skeletal fragility\(^{(5)}\) and occasionally learning difficulties\(^{(6)}\). Approximately 70 cases have been reported worldwide\(^{(6-10)}\). Ocular abnormalities are detected at birth or in the first few years of life. Short stature and bone deformities can develop. Biochemical markers of bone resorption and formation are usually normal\(^{(5;11)}\). Heterozygous carriers often have decreased bone mass but normal eye sight\(^{(2;12;13)}\). Activating *LRP5* mutations confer the opposing phenotype of high bone mass and fracture resistance (HBM, OMIM:601884)\(^{(14-17)}\).

*LRP5* is a cell-surface receptor which activates the canonical Wnt/\(\beta\)-catenin pathway, regulating osteoblastic bone formation\(^{(10)}\). The *LRP5* protein carries an extracellular domain composed of four \(\beta\)-propellers and EGF motifs and three LDL-receptor domains, a 23-amino acid membrane-spanning segment and an intracellular domain\(^{(18)}\). Most loss-of-function mutations contributing to OPPG, including homozygous mutations and compound heterozygotes, have been described in the highly conserved second \(\beta\)-propeller binding domain of the *LRP5* protein\(^{(19)}\).

Here we describe the first report of an atypical femoral fracture in a case of OPPG, explained by two novel missense *LRP5* mutations, and discuss the insights this provides. We further developed and validated LRP5 protein models showing both heterozygous mutations destabilise their respective LRP5 \(\beta\)-propellers; domains critical for protein and ligand binding.
CASE REPORTS

We report the case of a 40-year old white European man with OPPG. Congenital blindness was diagnosed at 6 months of age and osteoporosis aged 11 following his first 3 fractures (wrist aged 7, wrist aged 8, humerus aged 10). He suffered a low trauma fracture of the femur aged 16 leading to an abnormal gait due to unequal leg length. A bone biopsy at this time showed normal cortical thickness, normal mineralization, but severe trabecular osteoporosis. He sustained further fractures of the humerus aged 23 and then a unilateral atypical subtrochanteric femoral fracture aged 38 years when he slipped off a low curb whilst walking (Figure 1); he had received no anti-resorptive therapy before this time and had no prodromal pain. The fracture was managed by open reduction and internal fixation with a plate and screws (Supplemental figure S1).

Although a non-smoker, his alcohol consumption is high averaging 64 units per week; diet otherwise normal with unlimited exercise tolerance. He has no children. Extensive corneal scarring and iris hypoplasia in his right eye now limits his visual perception to light and dark. His left eye vision is severely limited by a persistent embryonic hyaloid amblyopia with convergent squint, nystagmus, raised intraocular pressure and cataract. On examination, he was obese (height 163cm, weight 105kg, BMI 39.5kg/cm², arm span 184cm), with both mild kyphosis and pectus carinatum, a mild leg length deficit, but no signs of joint disease. He had normal nails, sclera, hearing, dentition and cardiovascular and neurological examinations. Serum calcium, phosphate, alkaline phosphatase and fasting glucose were all normal, biochemistry showed no evidence of renal, inflammatory or endocrine disease. 25(OH) vitamin D level was 43 nmol/L; bone turnover markers were low [serum C-terminal collagen crosslinks (CTX) 0.02 μg/L and N-terminal procollagen (P1NP) 24 μg/L] before anti-resorptive treatment. Dual Energy X-ray Absorptiometry (DXA) scanning measured L1-L4 T-score -3.1, with femoral neck T-score -1.3 (Supplemental Figure S2). He received 9 months of oral alendronate with supplementary calcium and vitamin D after his atypical femoral fracture (aged 38), but this was discontinued after specialist review.

Our index case reported a 36-year old sister with very similar phenotype (Supplemental Figure S3): congenital blindness, early onset kyphosis, short stature, and four fractures to date, but no DNA sample was available. In contrast, their 57-year old mother, without ophthalmological disease, had only experienced a greenstick forearm fracture aged 8. After menarche at 13 and menopause at 53 years, she developed diet-controlled type II diabetes.
mellitus. She consumes 30 units alcohol/week and smokes 30 cigarettes/day. Her DXA L1-L4 T-score was -2.7 and femoral neck T-score -1.7 (Supplemental Figure S4); FRAX assessment recommend lifestyle advice. There are no other cases of OPPG in her family. Our index case reported his 60-year old father (Supplemental Figure S3) to have no eye disease but, although fracture-free, he has developed a kyphosis with height loss. He has two further offspring by a second relationship; none show an OPPG phenotype. There was no history of consanguinity.

The family has never previously sought genetic testing as the mother had ascribed her offspring’s phenotypes to her use of the antiemetic Debendox (dicuclomine-doxylamine-pyridoxine) during pregnancy, despite a series of epidemiological studies in the late 1970s finding no evidence of teratogenicity\(^{20-22}\).

**METHODS**

**LRP5 Sequencing**

Genomic DNA from blood was obtained using QIAamp DNA blood extraction kit (Qiagen, GmbH, Hilden, Germany) following manufacturer’s indications. Written informed consent was obtained from the index case and his mother. Research complied with the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. We amplified by PCR and directly sequenced, using Sanger methodology, all 23 exons of human \(LRP5\) (RefSeq NM_002335) as well as the relevant exon-intron boundaries. Primers for PCR and sequencing were designed using Primer3 v4.0.0 software\(^{23,24}\) (Supplemental Table S1). Mutations found were confirmed on two independent PCR products.

**In silico analysis of novel LRP5 mutations**

Novel missense \(LRP5\) mutations were analysed using PolyPhen-2\(^{25}\), SIFT\(^{26}\), Pmut\(^{27}\), PANTHER\(^{28}\), Align-GVGD\(^{29}\), and MutationTaster\(^{30}\). Modelling of the four extracellular YWTD \(\beta\)-propeller/EGF-like domains of human \(LRP5\) (E1-E4) was undertaken using Modeller 9v12\(^{31,32}\) based upon highly similar crystal structure template domain-fragments of LRP6 – E1-E2 (PDB ID: 4DG6) and E3-E4 (PDB ID: 4A0P). The \(LRP5\) E1-E2 and E3-E4 domains share \(~76\%\) and \(~72\%\) sequence identity (\(~86\%\) sequence similarity in both cases) with the template \(LRP6\) sequences, respectively. The target-template alignment was based upon multiple sequence alignment among related \(LRP5/6\) protein sequences using ClustalX\(^{33}\). Fifty models were generated using
Modeller 9v12, and the one with the lowest objective function score selected as the representative model in each case. The models were assessed using PROCHECK\textsuperscript{(34)}, WHAT IF\textsuperscript{(35;36)} and MetaMQAPII\textsuperscript{(37)}. Details about the protein model quality assessment are provided in the Supplementary Table S2. FoldX\textsuperscript{(38;39)} was used to estimate the free energy difference (stability change, expressed as $\Delta\Delta G$) upon mutagenesis from wild-type for the mutations.

**RESULTS**

PCR amplification and sequencing of *LRP5* revealed two novel missense mutations in our OPPG index case. We found in both the index case and his mother, a novel heterozygous c.2254C>T (p.R752W) mutation (Supplemental Figure S5A, S5B). Our OPPG index case also carried a second novel heterozygous missense mutation, c.235T>C (p.W79R) (Supplemental Figure S5C). *In silico* bioinformatic analysis suggested both mutations are deleterious (Supplemental Table S3). Both substituted amino acids are evolutionarily strictly conserved among LRP5 orthologues and also among related LRP4 and LRP6 sequences\textsuperscript{(40)} (Supplemental Figures S6 and S7).

In order to further assess potential impact of the mutations on the structure and function of LRP5, we created and validated three-dimensional (3-D) homology models for the four extracellular YWTD $\beta$-propeller/EGF-like domains (E1-E4) of LRP5. Mutation W79R is located in the second $\beta$-strand of blade 2 in the E1 domain, within the highly conserved “YWTD” signature motif, and is completely buried in the core of the $\beta$-propeller (Figure 2A). Protein stability calculations using FoldX on the 3-D model of LRP5-E1-E2 predict this mutation to severely destabilise structure (mean $\Delta\Delta G = \sim5$ kcal/mol), based upon previously published studies\textsuperscript{(39;41;42)} ($\Delta\Delta G >1.6$ kcal/mol infers severely reduced structural stability). The R752W mutation, located in blade 3 of E3 domain, near the interface with E4 (Figure 2B), is also predicted to destabilise structure, albeit to a lesser extent (mean $\Delta\Delta G = \sim2$ kcal/mol).

**DISCUSSION**

To our knowledge, this is the first report of an atypical subtrochanteric femoral fracture occurring in a case of OPPG. Furthermore, we have identified two novel missense *LRP5* mutations explaining this compound
heterozygous case of OPPG, supported by 3-D protein models showing significant deleterious structural consequences to the LRP5 protein.

This AFF occurred within the context of relatively low bone turnover, impaired Wnt signalling due to predicted destabilisation of LRP5, and trabecular osteoporosis with normal mineralization on histology, which implicates impaired bone remodelling in the pathogenesis of AFF. Given our knowledge of LRP5 function, we hypothesize that this relatively low bone turnover state in adult OPPG may be chronic. Prolonged suppression of bone turnover with bisphosphonates, used to treat osteoporosis, has also been associated with AFF\textsuperscript{(1)}.

The underlying pathogenesis of AFF remains unclear. AFFs may represent a stress or insufficiency fracture, developing from accumulated cortical microcracks in response to repetitive loading, repair of which is hampered if bone remodelling is impaired\textsuperscript{(4)}\textsuperscript{(1)}. However, in contrast to stress fractures, AFFs usually start from the lateral (rather than medial) cortex, and fracture with a smooth transverse (rather than oblique) edge, suggestive of ‘brittle bone’, as seen in Figure 1. Femoral alignment, varus neck-shaft angle and shorter hip-axis length have been associated with AFF\textsuperscript{(43;44)}, with the initial cortical lesions occurring at the lateral femoral site under greatest tensile load\textsuperscript{(45)}. Whether our index cases’ mild leg-length deficit altered his lower limb biomechanics to increase tensile stress over the left lateral femur, predisposing to AFF, is unclear.

AFFs are thought to develop from a cortical defect. Earlier our index cases’ bone biopsy had shown normal cortical thickness, but with trabecular osteoporosis. Clinical vertebral fractures have been reported more commonly in a handful of AFF cases\textsuperscript{(46)}. Whether a weakened trabecular scaffold within the femoral subtrochanteric region predisposes to cortical microcrack propagation is not known. Further lowering bone turnover by bisphosphonate therapy for OPPG, proposed by some in earlier case reports\textsuperscript{(11;47)}, is inadvisable in our case, where arguably anabolic therapy would be the most appropriate therapeutic intervention.

AFFs have been reported in other rare bone diseases causing (i) impaired bone resorption due to osteoclast dysfunction with increased bone density e.g. pycnodysostosis\textsuperscript{(48)}, autosomal dominant type II osteopetrosis\textsuperscript{(49-51)} (2 of 3 osteopetrosis cases occurred after high trauma accidents), and (ii) impaired mineralization due to hypophosphatasia\textsuperscript{(52)}. Until now AFF has never been reported in a genetic condition of primary osteoblast dysfunction.
We have identified two novel missense mutations in *LRP5*. Mutation *R752W*, within the E3 domain, is predicted to destabilise protein structure; however, the pathological effect is comparatively subtle, conferring only low BMD (no eye disease) in our postmenopausal mother who has other risk factors for low BMD, consistent with the mean ΔΔG just above the predicted destabilisation threshold (only ~0.4kcal/mol above the 1.6kcal/mol threshold). Interestingly, a *R752G* mutation has been described in familial exudative vitreoretinopathy[^40]; a rare retinal disorder with some cases showing reduced BMD[^53]. In addition to *R752W*, our index case has a second novel missense mutation; *W79R*, located within the core of the E1 β-propeller domain, is predicted to severely destabilise structure because of inappropriate burial of the polar/charged Arginine residue within a key conserved structural (YWTD) motif. Since we could not sequence either the father (normal vision) or sibling (with the same OPPG phenotype) we could not determine whether this second mutation is *de novo* or paternally inherited. Paternal inheritance is likely given the sibling’s affection status and the father’s height loss with kyphosis suggestive of osteoporosis.

*LRP5* regulates bone formation through activation of osteoblastic Wnt/β-catenin canonical signalling[^54]. Development of OPPG has been associated with homozygous and compound heterozygous mutations in *LRP5*[^2;6]. No phenotypic differences have been reported between homozygotes and compound heterozygotes[^6]. Interestingly, common polymorphisms in *LRP5* influence BMD in the general population[^55-60]; the genetic architecture of individuals within the general population sustaining AFF is unknown.

It has been suggested that *LRP5* regulates bone mass through duodenal serotonin synthesis[^61], although subsequent analyses have not all supported this finding[^62;63]. Furthermore, serotonin levels in high bone mass, due to activating *LRP5* mutations, have been reported to be normal[^64]; whether the same holds for OPPG remains to be determined.

In conclusion, we report the first case of atypical femoral fracture in a case of OPPG within the context of relatively low bone turnover, trabecular osteoporosis and impaired osteoblast function. The novel mutation *R752W* is associated with low BMD, but the combination of this mutation with another novel *W79R* mutation, causes a severe case of compound heterozygous OPPG, due to destabilisation of the β-propellers of the *LRP5* protein that are required for protein and ligand binding to regulate Wnt signalling and osteoblast function[^65].
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REFERENCES


**FIGURE LEGENDS**

**Figure 1.** X-ray showing a left atypical femoral fracture sustained by the index case aged 38. A subtrochanteric, transverse, non-comminuted fracture with medial spike is seen. Generalised increases in cortical thickness are seen in the diaphyses.

**Figure 2.** A) The 3-D model of LRP5 E1-E2 showing location of missense mutation W79R (sphere representation) in the second $\beta$-strand of blade 2 in the E1 domain. W79 is completely buried within the core of the $\beta$-propeller and the mutation is predicted to severely destabilise structure. A transparency setting was used for the surface representation to show the location of the buried residue on the secondary structure cartoon representation. Each of the blades that make up the six-bladed $\beta$-propeller structure in E1, and the four $\beta$-strands that make up blade 2 are labelled. B) Side-view of the 3-D model of LRP5 E3-E4 showing missense mutation R752W (sphere representation). R752 is located in blade 3 of the E3 domain, near the interface with E4.
Figure 1
Figure 2b