CASE REPORT

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Novel Homozygous Nonsense Mutation in the *LRP5* Gene in Two Siblings with Osteoporosis-pseudoglioma Syndrome

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What is already known on this topic?

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by severe juvenile osteoporosis, increased bone fragility and congenital blindness, due to mutations in the *LRP5* genes.

What this study adds?

A novel homozygous nonsense mutation present in two siblings with OPPG but with differing phenotype is described which will add to the spectrum of *LRP5* mutations leading to OPPG.

Abstract

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder characterized by severe osteoporosis and eye abnormalities that lead to vision loss. In this study, clinical findings and genetic study of two siblings with OPPG are presented. Whole exome sequencing of DNA enriched for exonic regions was performed with SureSelect 38Mbp all exon kit v. 7.0. The two siblings presented with different clinical manifestations of OPPG. The younger female sibling had blindness and severe osteoporosis with multiple fractures, while her older brother was also blind but with less severe osteoporosis and no fractures. On analysis, a novel homozygous nonsense mutation (c.351G>A) in exon 2 of *LRP5* (NM_002335) was found, predicted to change a tryptophan at 117 to a stop codon (p.Trp117Ter). Thus, a variable phenotype was associated with an identical variant in these two siblings. The novel mutation reported herein expands the spectrum of the underlying genetic pathology of OPPG.

Keywords: Osteoporosis-pseudoglioma syndrome, *LRP5* gene, nonsense mutation

Introduction

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disease characterized by severe osteoporosis, increased bone fragility, defects in the fetal ocular fibrovascular system, convulsions and intellectual disability. Osteoporosis causes vertebral compression, kyphosis, short stature, bowing of long bones, and vertebral and recurrent long bone fractures that may lead to skeletal abnormalities and physical disabilities (1). Ocular complications, usually presenting at birth or in early infancy, are due to vitreoretinal degeneration and manifest as phthisis bulbi, microphthalmia, retinal detachment, and/or exudative retinopathy, leading to congenital or juvenile blindness (2).

The WNT signaling pathway plays an important role in the regulation of skeletal homeostasis, osteoblast differentiation, and bone formation. A characteristic feature of WNT signaling is dose-dependency, which results in different phenotypic disorders. In the case of WNT activation, binding of WNT ligands to the seven-pass transmembrane Frizzled (Fzd) receptor and its co-receptor, low-density lipoprotein receptor-related protein-5 or -6 (LRP5/6), it triggers a series

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of events. This includes the dimerization of the two receptors on the cell surface and subsequent structural changes in these receptors.

In vertebrates, there are indeed 19 different WNT ligands and 10 Fzd receptors. However, out of these receptors, only two - LRP5 and LRP6 - are directly involved in WNT signaling. LRP5/6 play an important role in the initiation of WNT signal transduction and inhibit their function in WNT/Fzd signaling. The N-terminus of the Fzd receptor is indeed the primary region responsible for binding to WNT ligands. LRP5 and LRP6, as co-receptors of WNT ligands, play a crucial role in canonical WNT signaling. They are key components of the Wnt receptor complex and are essential for transmitting the WNT signal into the cell (3).

Homozygous or compound heterozygous inactivating mutations in the gene encoding low-density lipoprotein receptor-related protein 5 (*LRP5*) causes OPPG, while gain of function mutations in *LRP5* resuls in a high bone mass phenotype (hyperostosis and osterosclerosis) secondary to increased WNT signaling (4,5). Here, we report a novel mutation in the human *LRP5* gene in two Iranian siblings with OPPG.

Case Reports

Case 1

The proposita was a 12-year-old girl as the second child of a consanguineous marriage of Iranian descent. She was born at term with weight, head circumference and length of 3100 g (25% percentile), 35 cm (50% percentile) and 52 cm (75-90% percentile), respectively. At presentation the patient had multiple fractures in the wrist, femur, and tibia without any obvious trauma (Figure 1). She had bilateral microphthalmia, corneal opacities and pseudoglioma and was congenitally blind, which was diagnosed at two months old. Her first recognized long bone fracture was in the femur at the age of two years during occupational therapy. At the age of three years, her wrist broke, but did not have any further fractures until she was seven. After the age of seven years, she had a fracture in her femurs, tibia/fibula or wrists every year. There was no chest deformity. The patient had an autism spectrum disorder and she could neither talk nor communicate with others and was not teachable. Serum calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone, thyroid function tests, lipid profile, liver transaminases and uric acid were in all within the normal range.

Bone turnover markers were not investigated as these were unfortuanetly not available. The bone mineral density (BMD) was investigated with an absorptiometry (DEXA) method using a Hologic Discovery W S/N 83407 (Hologic Inc., Marlboro, MA, USA). BMD measurement showed severe osteoporosis with the absolute value of 0.369 g/cm^2 in the lumbar region (L1-L4) (Z score -3.1) and 0.309 g/cm^2 in the femur (Z score -4.4).

After the diagnosis of OPPG, pamidronate was started with infusions of 1 mg/kg daily for three consecutive days every three months from the age of three years and continued up to 11 years old. In addition, she received 1000 units of vitamin D and 500 mg of oral calcium daily during this period.

During treatment with pamidronate, she had multiple tibial fractures without any obvious trauma, while at the age of 11 years hip fractures occurred. In general, there was no improvement in her physical activity during treatment with pamidronate and she suffered from bone pain and recurrent bone fractures despite pamidronate. BMD at 10 years old showed the absolute value of 0.532 g/cm^2 in the lumbar region and 0.372 g/cm^2 in the femur, while BMD Z score according to height was -1.1. Therefore, no significant increase in BMD was observed despite continuous pamidronate therapy. We declare that the patient's mother has given her informed

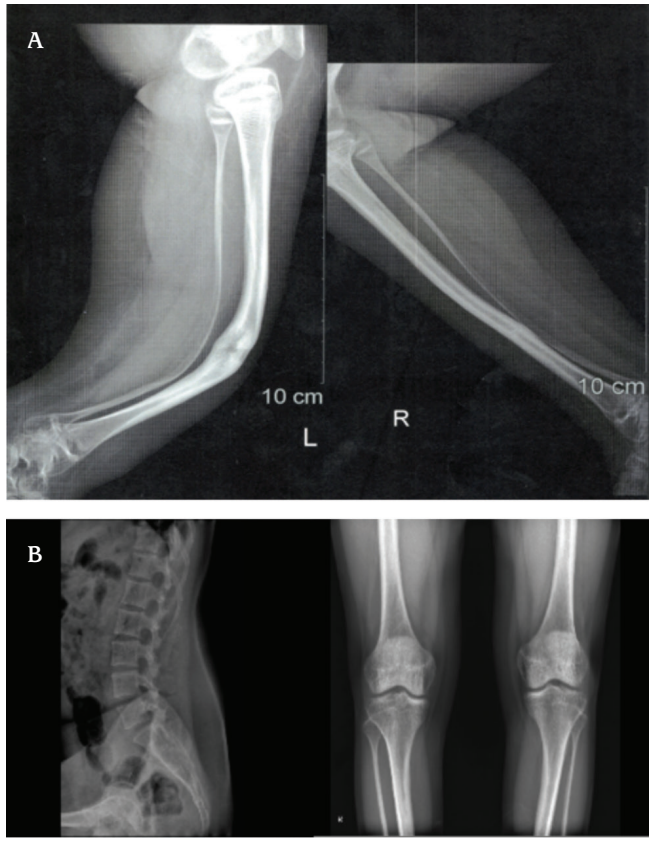

Figure 1. A) X-ray radiography of the patient's tibia and fibula, B) X-ray radiography of the patient's lumbar spine and knees

written consent to the publication of her children's file in accordance with the Helsinki Declaration.

Case 2

The patient, brother of the proposita, was 18 years old at the time of first evaluation and a student at law school. He was born preterm at 33 weeks by normal vaginal delivery, with birth weight, height, and head circumference of 2000 g (<3%), 46 cm (10%), and 33 cm (10%), respectively. His weight and height at presentation were 52 kg [-2 standard deviation (SD)] and 152 cm (-4 SD). In early infancy, ophthalmological evaluation revealed bilateral microphthalmia with corneal opacities, persistent hyperplasia of the primary vitreous and lesions in the anterior and posterior chambers. His cardiovascular and neurological examinations were normal. Of note, he had no history of long bone fractures. Furthermore, the patient did not have any long bone or chest deformities and there was no vertebral compression on his lumbar X-ray (Figure 1B). He did not complain of back or limb pain.

All laboratory data, including biochemical and hormonal tests, including serum calcium, phosphorus, magnesium, alkaline phosphatase, parathyroid hormone, thyroid function tests, lipid profile, liver transaminases and uric acid were normal. At first examination when he was 18 years old, BMD by DEXA showed severe osteoporosis with absolute values of 0.635 g/cm^2 in the lumbar (Z score -4.1) and 0.439 gr/cm^2 in the femur (Z score of -3.6). BMD at 22 years old after three years of treatment with alendronate (70 mg per week, starting at age 19 years) showed absolute value of 0.516 g/cm^2 (Z score -3.0) in the lumbar and 0.615 g/cm^2 (Z score -2.8) in the femur. Thus there there was a relative increase BMD in the lumbar area. The patient received 1000 mg calcium and vitamin D3 1000 IU (25 mcg) daily, in addition to the oral alendronate. The measured height of the mother was 167 cm and the father's height was 174 cm.

Genetic Studies

DNA was extracted from peripheral blood leukocytes using a commercial kit (High Pure PCR Template Preparation, Roche). Whole exome sequencing on DNA enriched for exonic regions was performed with SureSelect 38Mbp All exon kit v. 7.0 (Agilent Technologies, Santa Clara, CA, USA), and the samples were prepared according to manufacturer protocols. Prepared DNA samples were sequenced on HiSeq2000 (Illumina Inc., San Diego, CA, USA) using 75 x 2 bp paired-end sequencing with a mean coverage of 80-120x. The preliminary whole exome data analysis was performed through BWA and GATK software (6,7) to generate a BAM and a VCF file, respectively. Annotations of the VCF files were carried out using the wANNOVAR software, and the data was manually analyzed for the presence of candidate pathogenic variants.

In these patients, a novel, homozygous, nonsense mutation (c.351G>A) in exon 2 of *LRP5* (NM_002335) was found (Figure 2, 3), This was predicted to change tryptophan 117

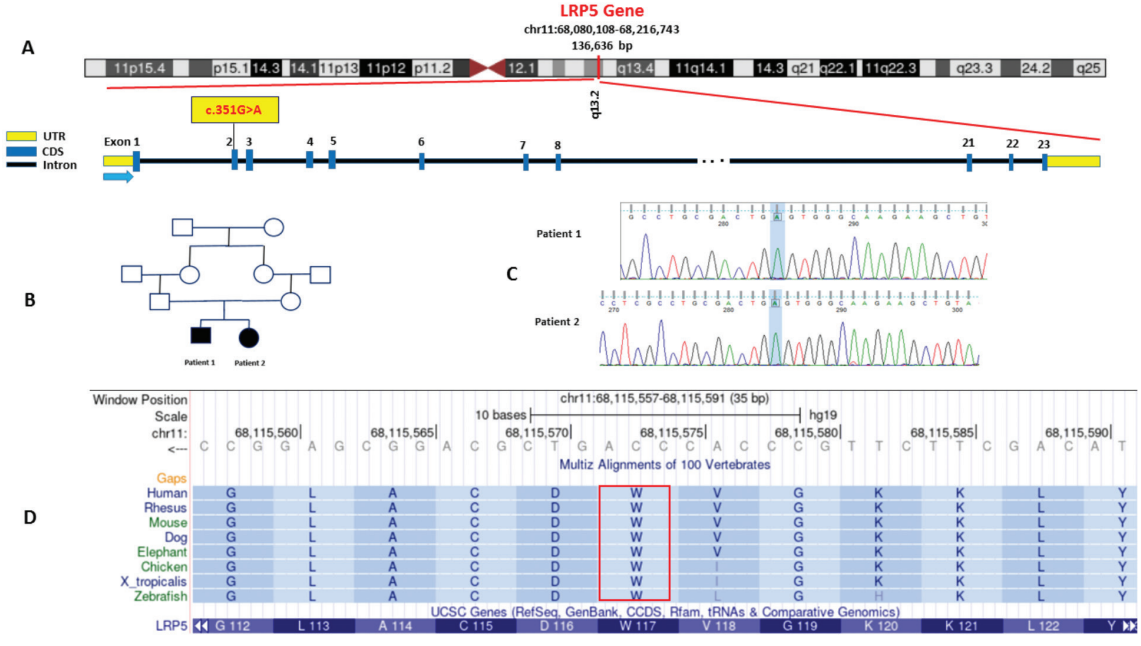

Figure 2. A) *LRP5* gene structure and the mutation. B) Family pedigree. C) Electropherograms from Sanger confirmation in family members showing *LRP5* (c.351G>A, p.Trp117*), homozygous mutant. D) The highly conserved state of the variant amino acid across evolution of species

to a stop codon (p. Trp117Ter). The identified mutation in *LRP5* was validated using Sanger sequencing. Segregation analysis revealed that the mother was heterozygous at the mutation position (Figure 2) but the father was not available for testing (Figure 2). Detailed computational predictive analysis of p. Trp117Ter mutation indicated a disease-causing alteration using PolyPhen-2, SIFT, and Mutation Taster. The local NGS database (8), currently consisting of 1406 whole exome sequencing data from healthy controls as well as public databases including the 1000 Genomes Project, the Genome Aggregation Database (gnome AD), and the Genome Aggregation Consortium (ExAC), were also interrogated and no samples exhibited the identified mutation in *LRP5* found in these siblings, indicating a novel, previously unreported variant.

Discussion

In the present study, the OPPG phenotype was different in two patients from one family with an identical mutation. The sister was blind with multiple bone fractures and was unable to move, while her brother was also blind and had osteoporosis but had never had a bone fracture. Both patients had pseudoglioma and had been blind from the beginning of their lives.

The role of *LRP5* in regulating bone density was first identified as pathogenic mutations in patients with OPPG. Subsequent studies showed that *LRP5*, as a common receptor of the WNT signaling pathway, regulated osteocyte apoptosis in addition to modulating osteoblast differentiation and proliferation (9). *LRP5* mutations are associated with pseudogliomas, hypovascularization of the retina and exudative vitreoretinopathy. WNT signaling regulates the development of retinal vasculature and regression of primary vitreous vessels in the growing eye. The range of ocular involvement of patients ranges from persistent fetal arteries to phthisis bulbi (10,11,12,13). Most patients with OPPG are congenitally blind or become blind in early childhood, and all patients will become blind by the age of 25 years (14). Although a widespread allelic heterogeneity has been reported in OPPG patients, phenotypes do not appear to have significant variation in terms of final ophthalmological outcome (15).

Mutations in *LRP5*-encoding genes can cause a variety of phenotypes, from subtle changes in bone traits to severe changes that cause multiple bone fractures. Moreover, similar gene mutation in single families can result in different phenotypes (2,16). Variable expression and diversity within the family, although usually occurring with autosomal dominant disorders, can also occur in autosomal recessive conditions. Intrafamilial variability of the OPPG disease phenotype has been reported in some families (17).

These patients may also have some degree of muscular hypotonia and ligament laxity (18). Cognitive impairment has also been reported in approximately 25% of patients with OPPG and usually the first bone fracture occurs at about two years of age. Genetic factors in combination with environmental influences may play a role in increasing cognitive dysfunction (19,20). It was notable that the younger female proband had autism and was unable to communicate verbally, while her brother with the same variant was able to continue his university education.

In 2010, Saarinen et al. (21) reported abnormal glucose tolerance test findings and hyperglycemia in patients with the *LRP5* mutation, secondary to beta-cell dysfunction. Additional findings reported by these authors included a high prevalence of hypercholesterolemia in these patients. Neither of the siblings presented herein had hypercholesterolemia or hyperglycemia.

To date, more than 70 cases of OPPG have been reported, most of them are associated with consanguineous marriages, with a prevalence of approximately 1: 2,000,000 (12,13).

In the patients presented in this case report, a novel, homozygous nonsense mutation in exon 2 of *LRP5* gene was found, and based on American College of Medical Genomics standards, there is strong evidence that this mutation is pathogenic (22). The mutation is a G to A substitution in exon 2 of *LRP5*, predicted to change tryptophan 117 to a stop codon and thus causing a modification of the *LRP5* protein sequence.

LRP5 contains 23 exons and encodes a transmembrane, low-density lipoprotein receptor that binds and internalizes ligands in the process of receptor-mediated endocytosis and plays a key role in skeletal homeostasis. The majority of mutations linked to OPPG are found in the second and third of the four YWTD b-propeller domains (23,24), coded for by exons 6 to 12. LRP5 protein consists of 1615 amino acids, of which the first 1384 amino acids form the extracellular part of this receptor. This extracellular part also includes 29 amino acid signal peptide, 20 YWTD spacer repeat domains, interspersed by four EGF-like domains and three LDL receptor-like ligand binding domains.

This variant is located in exon 2 and the extracellular region of this protein, which is indicated by an arrow. Change of tryptophan in position 117 to stop codon by this mutation leads to truncated protein and loss of function of it. On the other hand loss of function is a known mechanism of disease in *LRP5* gene (Figure 3) (25).

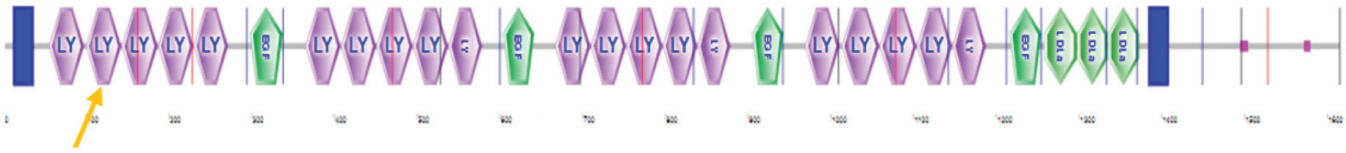

Figure 3. cDNA reference sequence for *LRP5*. The homozygous, nonsense mutation (c.351G > A) in exon 2 of *LRP5* (NM_002335)

Most of the pathogenic *LRP5* mutations are missense (67 out of 93), and there are only two nonsense and six frameshift mutations (8). *LRP5* is one of the most important factors having a remarkable effect in increasing bone mass. Other functions of LRP5 include interaction with sex hormones and growth hormone and IGF-1 (26).

Conclusion

A novel, homozygous nonsense mutation was identified in two siblings with OPPG. These siblings had remarkably different phenotype with one unable to communicate while the other was in higher education. This is the first report of a novel *LRP5* mutation in OPPG in the Iranian population. This report expands the spectrum of *LRP5* mutations that are the cause of OPPG.

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Ethics

Informed Consent: Consent form was filled out by all participants (or families).

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Fatemeh Saffari, Concept: Abolfazl Heidari, Fatemeh Saffari, Design: Fatemeh Saffari, Data Collection or Processing: Ali Homaei, Analysis or Interpretation: Abolfazl Heidari, Fatemeh Saffari, Literature Search: Ali Homaei, Fatemeh Saffari, Writing: Abolfazl Heidari, Ali Homaei Fatemeh Saffari.

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