Case Report

Novel Homozygous Nonsense Mutation in *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 low-density lipoprotein receptor-related protein 5 (*LRP5*) genes.

What this study adds?

Hereby we reported a novel homozygous nonsense mutation in two siblings with OPPG which could extend the spectrum of *LRP5* mutations leading to osteoporosis-pseudoglioma syndrome.

Abstract

Osteoporosis-Pseudoglioma Syndrome is a rare autosomal recessive disorder characterized by severe osteoporosis and eye abnormalities that lead to vision loss. In this study, we report clinical findings and genetic study of two siblings with OPPG. Whole exome sequencing on DNA enriched for exonic regions was performed with SureSelect 38Mbp all exon kit v. 7.0. The two siblings showed different clinical manifestations with OPPG; as the female patient had blindness and severe osteoporosis with multiple fractures, while her older brother was blind with less severe osteoporosis and no bone fractures. In these patients, a novel homozygous nonsense mutation (c.351G>A) in exon 2 of *LRP5* gene (NM_002335) was found, changing Tryptophan 117 to stop codon (p. Trp117Ter). A variety of clinical manifestations of OPPG can be observed in a family despite the same gene mutation. The novel mutation reported in this study expands the spectrum of the underlying OPPG genetic pathology.

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

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11.07.2021

25.11.2021

Published: 30.12.2021

Introduction

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

The WNT signaling pathway plays an important role in skeletal homeostasis regulation, osteoblast differentiation, and bone formation. A special feature of WNT signaling is its dose-dependency, which results in different phenotypic disorders.

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* results in high bone mass phenotype (hyperostosis and osteosclerosis) secondary to increased WNT signaling (3,4). Here, we report a novel mutation in the human *LRP5* gene in two Iranian siblings with OPPG.

Case Reports

Case 1

The proband is a 12-year-old girl as the second child of a consanguineous marriage of Iranian descent. She was the result of term birth with weight, head circumference and length of 3.100 g (25%), 35 cm (50%) and 52 cm (75-90%), respectively. 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 2 months old; first noticed long bone fracture was at the age of 2 years in the femur during occupational therapy. At the age of three, her wrist broke, but did not have any bone fractures until she was seven, and after the age of 7, she had a fracture in her femurs, legs or wrists every year. There was no chest deformity. The patient had an autism spectrum disorder; she couldn't talk or communicate with others and was not teachable. Serum calcium, phosphorus, magnesium, alkaline phosphatase, parathormone, thyroid function tests, lipid profile, SGOT, SGPT and uric acid were in the normal range. Bone turnover markers were not able to be studied. The BMD was performed by absorptiometry method (DEXA) with HOLOGIC machine (model: discovery W S/N 83407). BMD measurement showed severe osteoporosis with the absolute value of 0.369 gr/cm² in the lumbar (Z score of - 3.1) and 0.309 gr/cm² in the femur (Z score of - 4.4).

After the diagnosis of OPPG, pamidronate treatment was started with 1mg/kg daily infusions for 3 days continuously every 3 months from 3 years old and continued up to 11 years old.

During treatment with pamidronate, she had multiple tibial fractures without any obvious trauma and also hip fractures happened in 11 years old. In overall, there was no improvement in physical activity during treatment with pamidronate and the patient suffered from bone pain and recurrent bone fractures inspite of receiving pamidronate.

BMD in 10 years old showed the absolute value of 0.532 gr/cm² in the lumbar and 0.372 gr/cm² in the femur, in addition, BMD Z score according to height was -1.1. Therefore, no significant increase in bone mineral density was observed.

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 and his current weight and height are 52 kg (-2SD) and 152 cm (-4SD). At early infancy, ophthalmological evaluation revealed bilateral microphthalmia with corneal opacities, persistent hyperplasia of the primary vitreous and lesions in the anterior and posterior chambers. He has no history of long bone fracture and his cardiovascular and neurological examinations were normal. The patient has not had any long bone or chest deformities and there was no vertebral compression in his lumbar X-ray (figure 1B). In addition, he did not complain of back and limb pain.

All laboratory data including chemical and hormonal tests (Serum calcium, phosphorus, magnesium, alkaline phosphatase, parathormone, thyroid function tests, lipid profile, SGOT, SGPT and uric acid) were normal. The BMD was performed by absorptiometry method (DEXA). BMD measurement showed severe osteoporosis with the absolute value of 0.635 gr/cm² in the lumbar (Z score of -4.1) and 0.439 gr/cm² in the femur (Z score of -3.6). BMD in 22 years old showed the absolute value of 0.516 gr/cm² (Z score of -3.0) in the lumbar and 0.615 gr/cm² (Z score of -2.8) in the femur and after 3 years treatment with alendronate, there was a relative increase in bone mineral density in the lumbar area.

The patient was treated with oral alendronate in a dose of 70 mg per week from 19 years old to present, 1000 mg calcium and Vitamin D3 1000 IU (25 mcg) Tablet daily. The height of the mother is measured as 167 cm and the father's height was 174 cm.

Genetic study

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.) using 75 ×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 to generate a BAM and a VCF file, respectively. Annotations of the VCF files were carried out using the WANNNOVAR software, and the data was manually analyzed for the presence of candidate pathogenic variant. In these patients, a novel homozygous nonsense mutation (c.351G>A) in exon 2 of *LRP5* gene (NM_002335) was found (Figure 2), changing Tryptophan 117 to stop codon (p. Trp117Ter). The identified mutation in the *LRP5* gene was validated using Sanger sequencing. Segregation analysis revealed that the mother showed heterozygosity at the mutation position (Figure 2) but the father was not available for testing (Figure 2). Detailed computational analysis of p. Trp117Ter mutation revealed it as a disease-causing alteration using prediction methods (PolyPhen-2, SIFT, Mutation Taster). The local NGS database (5), currently consisting of 1406 whole exome sequencing data from healthy controls as well as public databases [the 1000 Genomes Project, the Genome Aggregation Database (gnome AD), the Genome Aggregation Consortium (ExAC)], were also investigated and no samples exhibited the identified novel mutation in the *LRP5* gene.

Results

In these patients, a novel homozygous nonsense mutation (c.351G>A) in exon 2 of *LRP5* gene (NM_002335) was found, changing Tryptophan 117 to stop codon (p. Trp117Ter).

Discussion

In the present study, the manifestations of the disease were different in two patients of the same family; the girl patient had blindness with multiple bone fractures and was unable to move, while her brother had only blindness and osteoporosis and never had a bone fracture. Both patients reported in our study had pseudoglioma and were blind from the beginning of their lives. The researchers identified the role of *LRP5* in regulating bone density by identifying pathogenic mutations in patients with OPPG. Subsequent studies showed that *LRP5*, as a common receptor of the WNT signaling pathway, in addition to modulating osteoblast differentiation and proliferation, also regulates osteocyte apoptosis (6). *LRP5* mutations are associated with pseudogliomas, hypovascularization of the retina and exudative vitreoretinopathy. WNT signaling regulates the retinal vasculature development and the regression of primary vitreous vessels in the growing eye. The range of ocular involvement of patients ranges from persistent fetal arteries to phthisis bulbi (7, 8-10). Most patients with OPPG are congenitally blind or become blind in early childhood, and all patients will become blind by the age of 25 (11). Although a widespread allelic

heterogeneity has been reported in OPPG patients, Patients' phenotypes do not appear to have significant variation in relation to final eye involvement (12).

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; also a similar gene mutation can cause different phenotypes of the disease in a family (2, 13). 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 (14).

These patients may also have some degree of muscular hypotonia and ligament laxity (15). Cognitive impairment has also been reported in approximately 25% of patients with OPPG and usually the first bone fracture occurs in about 2 years old. Genetic factors in combination with environmental influences may play a role in increasing cognitive dysfunction (16, 17). **One** of our patients had autism and was unable to communicate verbally, while his brother was able to continue his university education. In 2010, Saarinen et al. reported abnormal glucose tolerance test and hyperglycemia in patients with the *LRP5* mutation secondary to beta-cell dysfunction; in addition, they reported a high prevalence of hypercholesterolemia in these patients (18). Whereas none of our patients had hypercholesterolemia or hyperglycemia.

To date, more than 70 cases with OPPG have been reported, most of them are due to consanguineous marriages, with a prevalence of approximately 1: 2,000,000 (9, 10).

In our patients, a novel homozygous nonsense mutation in exon 2 of *LRP5* gene was found, based on American College of Medical Genomics (ACMG) standards, there is strong evidence that this mutation is pathogenic (19).

The mutation is a G to A substitution in exon 2, changing tryptophan 117 to stop codon and causing a modification in the *LRP5* protein sequence by creating a premature stop codon. *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 YTDW b-propeller domains (20, 21), coded for by exons 6 to 12. Most of the pathogenic *LRP5* mutations are missense (67 out of 93), and there are only two nonsense and six frameshift mutations (5). The *LRP5* is one of the most important factors having a remarkable effect in increasing bone mass, together with sex and growth hormones, and IGF-1.

Conclusion

Novel homozygous nonsense mutation was demonstrated in two siblings with OPPG. Our report expands the spectrum of *LRP5* mutations that cause osteoporosis-pseudoglioma syndrome. Our patients had different disease severities with a similar gene mutation. This is the first report of novel mutation of OPPG in Iranian population.

Acknowledgments

We thank the patient's family for their cooperation in this study. This study was funded by Qazvin Rehabilitation Organization, Qazvin-Iran. The authors are thankful to the staff of the Clinical Research Center at Qazvin Children Hospital, affiliated to Qazvin University of Medical Sciences for their help in preparing this paper.

Ethics

Approval ID: IR.QUMS.REC.1399.345

Ethics Informed consent: We declare that the patient's mother has given her informed written consent to the publication of her children's file in accordance with the Helsinki Declaration.

Disclosure of interest

The authors declare no competing financial interests

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Figure 1A. X-ray radiography of the patient's tibia and fibula
B

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.