

Case report**Severe osteoporosis with multiple spontaneous vertebral fractures in a young male carrying triple polymorphisms in the vitamin D receptor, collagen type 1, and low-density lipoprotein receptor-related peptide 5 genes**

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ABSTRACT

Osteoporosis is a common disease with a strong genetic component. Several studies have reported the vitamin D receptor (VDR), collagen type I (COL1A1), and LDL receptor-related protein 5 (LRP5) genes as the most likely candidates. However, most of the studies have been carried out in postmenopausal women and older men and show inconsistent results. CASE PRESENTATION: We report a case of a 26-year old male who presented with severe back pain of acute onset, unrelated to any kind of trauma, and diffuse myalgia. Imaging of the lumbar and the thoracic spine revealed two Grade 3, according to Genant's semiquantitative method, vertebral fractures in T10 and T11 and multiple Grade 1 and 2 fractures from T8 to L2. Measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy) showed severe osteoporosis of the lumbar spine (Z -score=-3.0, BMD = 0.866 gr/cm²). A complete laboratory and biochemical work-up was performed to exclude secondary causes of osteoporosis. Total genomic DNA was extracted from peripheral blood and was used as a template for genotype analysis. The patient was heterozygous for the p.V667M mutation of the LRP5 gene and for the BsmI [g.63980 G→A, rs1544410] and Sp1 polymorphisms [g.6252 G→T, rs1800012] of the VDR and COL1A1 genes, respectively. Further genotype analysis excluded types of osteogenesis imperfecta associated with mutations in the COL1A1 and COL1A2 genes. **CONCLUSION:** We herein show that the co-existence of three polymorphic sites in the VDR,

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COL1A1, and LPR-5 genes in a young male adult caused severe osteoporosis with multiple fractures, suggesting a combined effect and/or interaction between these genes.

Key words: COL1A1 polymorphism, Idiopathic osteoporosis, LRP-5 mutation, Multiple fractures, VDR polymorphism, Young adult

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and abnormal bone microarchitecture leading to increased fracture risk.¹ In postmenopausal women and men aged 50 and above, estrogen deficiency, vitamin D deficiency, secondary hyperparathyroidism, and age-related changes in bone tissue contribute to accelerated bone loss and increased bone fragility.²⁻⁴

Low bone mass in children and young adults does not necessarily imply skeletal fragility unless the patient sustains low trauma or atraumatic fractures.⁵ Low bone mass in young adults could represent either attainment of low peak bone mass in relation to their body size, pubertal timing, and environment during growth,^{6,7} a pathological condition with bone fragility due to chronic diseases and secondary causes of osteoporosis, or a genetically predisposed/idiopathic condition.⁸ Osteoporosis is considered to be a disease with a strong genetic component of about 40-60%. Polymorphisms and/or mutations in vitamin D receptor (*VDR*), the collagen type I alpha1 (*COL1A1*), and the low-density lipoprotein receptor related-protein 5 (*LRP5*) genes have independently shown significant associations with bone mineral density and increased fracture risk. In addition, in genome-wide association studies, several single nucleotide polymorphisms (SNPs) have been identified as being associated with bone density or fracture risk at the genome-wide significance level.⁹⁻¹¹ However, most of these studies concern postmenopausal women and men aged above 50 years, while data on younger individuals are scarce. We present a case of a young male adult with severe osteoporosis and multiple atraumatic vertebral fractures in whom, after exclusion of a chronic disease and other secondary causes of osteoporosis, a genetic background of polymorphisms and mutation in all three *VDR*, *COL1A1*, and *LRP5* genes was revealed.

CASE PRESENTATION

A 26-year old male was referred to our center due to severe back pain of acute onset, unrelated to any kind of trauma, and diffuse myalgia. During the past 6 months, the patient had visited the outpatient clinics of the Rheumatology and the Orthopedic departments several times complaining of diffuse pain of moderate intensity along the spine, which was aggravated when lying in bed or sitting. At that time he was diagnosed with seronegative spondyloarthritis and was prescribed non-steroidal anti-inflammatory drugs and methotrexate. His clinical condition, however, was not improved. His medical record was free of any kind of systemic disease, as was also his family medical record. More specifically, there was no family history of frequent fractures, childhood or adolescent osteoporosis, osteogenesis imperfecta, gross skeletal anomalies, rickets, discoloured sclera, or early onset of hearing loss. Clinical examination revealed no other abnormalities.

X-rays and magnetic resonance imaging (MRI) of the lumbar and the thoracic spine revealed two Grade 3, according to Genant's semiquantitative method, vertebral fractures in Th10 and Th11 and multiple Grade 1 (T12, L1) and Grade 2 (T8, T9, L2) fractures (Figure 1). Measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy) showed severe osteoporosis of the lumbar spine (Z -score=-3.0, $BMD=0.866$ gr/cm²) (Figure 2). A complete laboratory and biochemical work-up was performed to exclude secondary causes of osteoporosis (Table 1). The patient was found to have low-normal levels of 25-OH-vitamin D and was prescribed cholecalciferol 2200units/d per os.

GENETIC ANALYSIS

Total genomic DNA was extracted from peripheral blood and was used as a template for genotype analysis.



Figure 1. MRI of the thoracic and lumbar spine.

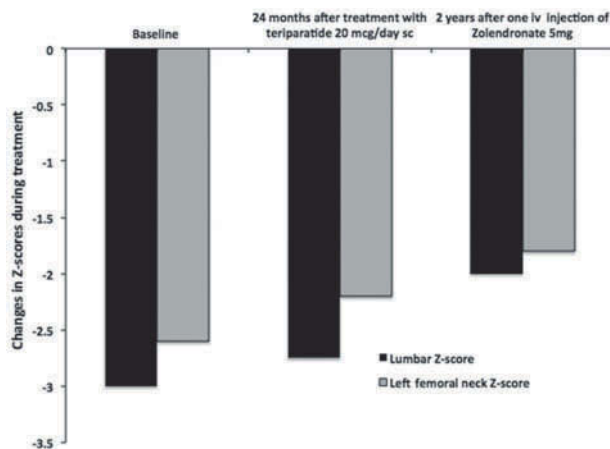


Figure 2. Changes in Z-scores of the lumbar spine and left femoral neck during treatment with teriparatide (20mcg/day sc) and zoledronate (5mg single iv injection).

Table 1. Laboratory work up and baseline hormone profile at the day of admission

Assay	Normal Range	Value
Hematocrit (Hct)	38.8-50.0%	42%
Fasting blood glucose	3.9-5.5 mmol/L	4.2
Alanine aminotransferase (ALT)	7-56 U/L	34
Aspartate aminotransferase (AST)	10-40 U/L	22
Alkaline phosphatase (ALP)	25-100 U/L	44
Serum creatinine levels	80-110 µmol/L	92
Ionized calcium	1.1-1.35 mmol/L	1.2
Phosphate	0.8-1.5 mmol/L	0.92
Parathyroid hormone (PTH) (pg/ml)	10-65	35
25-OH-vitamin D levels	75-250 nmol/L	77
24-Hour urine calcium	15-20 mmol/24h	18.7
Magnesium	1.5-2 mEq/L	1.75
Thyroid-stimulating hormone (TSH) level	0.5-5 mIU/L	3.2
Ferritin	33-450 pmol/L	104
Serum iron	10.7-26.9 pmol/L	15
Testosterone levels	10-25 nmol/L	20
24-Hour urine free cortisol	9.66-124.2 nmol/24h	32
Morning serum Cortisol	7-28 µg/dL	16
Serum protein electrophoresis		
albumin	38-50 g/L	40
alpha-1 globulin	1-3 g/L	2.2
alpha-2 globulin	6-10 g/L	7.5
beta globulin	7-14 g/L	8.1
gamma globulin	7-16 g/L	8.9
Antigliadin antibodies (IGA/IGG)		Not detected
Antiendomysial antibodies		Not detected
Serum tryptase	<11.4 ng/mL	3.2
Rheumatoid factor	<25 IU/ml	17

Types of osteogenesis imperfecta associated with mutations in the COL1A1 and COL1A2 genes were excluded.

We subsequently performed genotype analysis for genes known to correlate with the genetic background of osteoporosis, such as *LRP5*, *VDR* and *COL1A1*, using a novel Real-Time PCR assay based on SimpleProbe® melting curve analysis. The patient was found to be heterozygous for the p.V667M mutation of the *LRP5* gene and for the BsmI [g.63980 G→A, rs1544410] and Sp1 polymorphisms [g.6252 G→T, rs1800012] of the *VDR* and *COL1A1* genes, respectively.

TREATMENT AND FOLLOW-UP

The patient was treated with daily s.c injections of recombinant teriparatide for 24 months and supplementation with calcium (1000 mg/daily) and vitamin D (800 IU/daily). During the first 6 months of treatment, his back pain and diffuse myalgia improved significantly and the patient did not sustain a new vertebral or non-vertebral fracture. After the completion of the 24-month teriparatide-treatment the patient showed significant gains in the bone mass of the lumbar spine (Z -score=-2.2, BMD=0.970 gr/cm²) and received an i.v injection of zoledronate 5 mg. Two years later the patient was free of symptoms: he had a BMD measurement in the osteopenic range in the lumbar spine and left femoral neck (Figure 2), there was no history of new fractures, and there were no new morphometric fractures in the thoracic and lumbar spine based on new X-rays. Serum levels of bone formation and bone resorption markers remained in the lower quartiles (Figure 3). In addition to anti-osteoporotic treatment, the patient was subjected to a rehabilitation program because of his long-lasting immobilization due to pain and fear of falling. The program included strengthening in joint mobility, clearance in the extremities and back muscles, posture exercises, and walking training. At the end of treatment, significant improvements were observed in walking, lower and upper extremity muscle strength levels, and posture.

DISCUSSION

The *BsmI* polymorphic site of the *VDR* gene, as well as the polymorphic Sp1 binding site of the *COL1A1* gene, and the *V667M* mutation of the *LRP5* gene have been independently associated with osteoporosis and fracture risk. We herein show that the co-existence

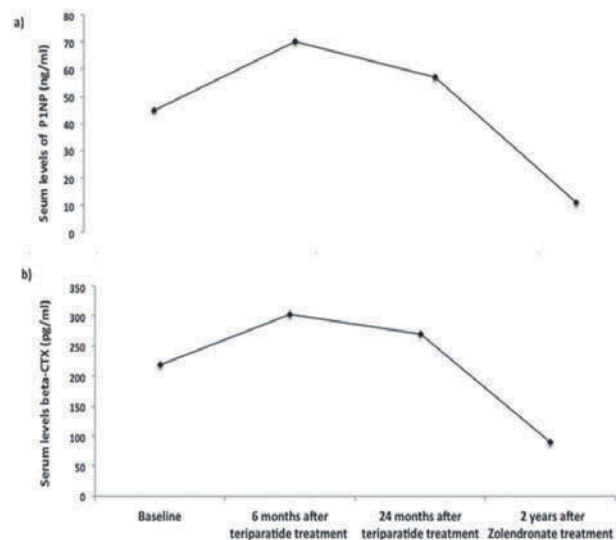


Figure 3. Changes in serum levels of the bone formation markers P1NP (a) and the bone resorption marker beta-CTX (b) during treatment with teriparatide (20mcg/day sc) and zoledronate (5mg single iv injection).

of these three genetic changes in a young male adult caused severe osteoporosis with multiple fractures, suggesting a combined effect and/or interaction between these genes.

The *VDR* gene was among the first candidate genes studied for association with osteoporosis.¹² The active metabolite of vitamin D (1,25 OH D₃) acts through its specific receptor, VDR, which is a nuclear transcription factor regulating the expression of the target genes through binding to vitamin D responsive elements.¹³ Mutations at the key sites of the *VDR* gene have been reported to cause vitamin D deficiency even when vitamin D itself is supplemented adequately.¹⁴ This was a critical finding since insufficient serum levels of 25-OH-vitamin D are a well-established risk factor for osteoporosis and increased fracture risk.¹⁵ In our case, the initial symptoms of diffuse myalgia, which could be attributed to low vitamin D levels, were successfully treated with vitamin D supplementation. The most frequently studied polymorphisms of the *VDR* gene in association with osteoporosis include BsmI, ApaI, TaqI, FokI,¹⁶ and Cdx2.^{17,18} The effect of the VDR genotype on BMD has been found to be stronger in premenopausal women and to decrease with age,¹⁹ but several studies have produced inconsistent results with some showing positive²⁰⁻²³ or no association between VDR gene polymorphisms

and BMD values.²⁴⁻²⁶ Association of fracture risk with *VDR* polymorphisms has also been a matter of debate, with one showing significant association of BsmI and fracture risk and another no association between any genotype of *VDR* polymorphism and fractures.^{27,28} Most of the studies, however, concerned postmenopausal women and older men and thus data regarding the association of the BsmI polymorphism with osteoporosis and fracture risk among the young are scarce.

The *COL1A1* and *COL1A2* genes encode the two alpha chains (alpha 1 and alpha 2, respectively) of collagen type 1 that trimerize to form the procollagen 1 molecule,²⁹ the main component of bone matrix. Mutations in these genes account for approximately 90% of osteogenesis imperfecta (OI), which is a form of inherited osteoporosis in children characterized by low bone mass, fragile bone with increased fracture risk, blue sclerae, and in some cases impaired odontogenesis. Mild cases of OI can result in the diagnosis of osteoporosis in adulthood or at an advanced age³⁰ and, at present, almost 17 genetic causes of OI have been identified.³¹ Several large-scale studies have demonstrated an association between the polymorphism affecting the Sp1 binding site of the *COL1A1* gene and low bone mass and increased fracture risk in the general population.³²⁻³⁴

The *LRP5* gene encodes for the *LRP5* co-receptor of the Wnt signaling pathway, which is of critical importance in bone metabolism regulating osteoblastogenesis and bone formation.³⁵ Wnt ligands bind to the Frizzled-LRP 5/6 receptor complex in the cell membrane and activate intracellular Wnt signaling. Activation is mediated either through translocation of the cytoplasmic protein beta-catenin to the nucleus, where it acts as a transcription factor activating the transcription of the target genes (canonical pathway), or via calcium and cAMP signals (non-canonical pathway). Loss-of-function mutations in the *LRP5* gene cause the osteoporosis-pseudoglioma syndrome,³⁶ an autosomal recessive disorder characterized by severe juvenile-onset osteoporosis and congenital or juvenile-onset blindness.³⁷ Activating mutations, on the other hand, are responsible for the autosomal dominant high bone mass trait.³⁸ In a recent multicenter study of 37,534 participants, it was demonstrated that the V667M (in exon 9) and A1330V (in exon 18) poly-

morphisms of the *LRP5* gene are associated with low BMD values and increased risk of fractures.³⁹

Our patient suffered from unspecific musculoskeletal symptoms, such as arthralgia and diffuse myalgia, which were initially attributed to seronegative spondyloarthritis, thus delaying the final diagnosis. The lack of evidence of a systemic chronic disease and secondary osteoporosis and the severity of his bone disease with multiple spontaneous vertebral fractures led us to look for a genetic background whereby we were able to further exclude inherited forms of juvenile osteoporosis, such as OI. The co-existence of mutations in more than one of the genes related to osteoporosis in young adults has not been evaluated so far and larger studies are needed to elucidate a possible interaction and synergistic effect between the implicated genes. Administration of teriparatide, as the only bone anabolic agent currently available, followed by an antiresorptive agent and calcium plus vitamin D supplementation proved very effective in our patient, who continues to remain free of symptoms and with no new fractures over a long period of time.

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