ASSOCIATION OF COL1A1 SP1 AND FOK-I VDR GENETIC POLYMORPHISMS IN YOUNG MALE IDIOPATHIC OSTEOPOROSIS

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Abstract

Introduction. Primary osteoporosis during childhood and adolescence represents an uncommon condition, and secondary forms are more likely to manifest at this age due to chronic disease and adverse effects of medical treatment.

Case report. The authors report the case of a young male patient with a history of multiple idiopathic non-vertebral fragility fractures in addition to a family history of maternal osteoporosis and fracture, in whom osteoporosis was confirmed according to 2013 International Society for Clinical Densitometry (ISCD) criteria. Bone markers indicated low bone formation marker osteocalcin. Genetic testing revealed homozygosity for Sp1 COL1A1 gene polymorphism in combination to Fok-I vitamin D receptor (VDR) heterozygous polymorphism, to contribute to low bone mass and increased fracture risk. Severe premenopausal osteoporosis was present in the patient's mother, who was also tested positive for both gene polymorphisms.

Conclusion. This case report highlights the association between COL1A1 and VDR candidate gene polymorphisms and fragility fractures in a family. Individual genetic testing might be of clinical value in idiopathic osteoporosis in young patients, identifying subjects at increased fracture risk.

Key words: gene polymorphisms, Sp1 binding site, vitamin D receptor, type I collagen, idiopathic osteoporosis, juvenile osteoporosis.

INTRODUCTION

During childhood, the peak bone mass rests on several aspects, mainly calcium intake, body mass index (BMI) and genetic factors. Genetic and environmental determinants have a contribution of 70% and 30%, respectively. Twin, familial and genome-wide association studies (GWAS) have shown that bone

mass is predicted by genetics in approximately 60-80% of cases (1). Bone mass can be affected by genes in two ways; they control body intakes and uptakes (e.g. calcium modulates particularly bone mass accrual), and due to an intrinsic genetic defect (2, 3).

Osteoporosis in children seems to be caused by an intrinsic genetic anomaly in bones, i.e. primary osteoporosis, or as a result of a medical treatment or chronic disease, known as secondary osteoporosis. Typically, osteogenesis imperfecta, in 95% of the cases explained by dominant mutations in COL1A1 and COL1A2 genes, may present in mild forms of osteoporosis and variable degrees of fractures (4). Primary osteoporosis in children is a rare event. However, adequately powered studies on the epidemiologic aspect are lacking, with most cases of idiopathic juvenile osteoporosis published in the form of case reports. In contrast, fragility fractures develop among 16% of children with acute lymphoblastic leukemia (5) and 7% of children initiating glucocorticoid treatment for rheumatologic diseases (6).

CASE REPORT

We report the case of an 18 years-old male patient that had been admitted to our clinic for joint pain in the dorsal spine and muscle contractures of the shin. From the medical history, we recorded that he was diagnosed at the age of 10 with primary hypothyroidism and was treated since with thyroid hormones replacement therapy. Of note, during childhood and adolescence, he experienced multiple fragility fractures, including the left forearm at the age of 1 and both bones of the right forearm and the right clavicle at the age of 13.

Furthermore, the family history revealed

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osteoporosis and a hip fracture sustained by the patient's grandmother in addition to a maternal history of osteoporosis and primary hypothyroidism. The patient's mother was diagnosed with postpartum osteoporosis, with vertebral collapse at the age of 23. During childhood, she also experienced forearm and clavicle fractures. Recent magnetic resonance imaging (MRI) evaluation, at age 43, confirmed vertebral fractures at D10-L1 in the mother, in addition to low BMD and she was under chronic bisphosphonates therapy.

At clinical examination, the patient had normal weight with a BMI of 24.8 kg/m² and sitting blood pressure of 110/60 mmHg and with a resting heart rate of 76 bpm. Apart from a mild dorsal kyphosis, no other changes were recorded.

Laboratory tests evidenced low ionized serum and urinary calcium levels, while parameters of liver function, serum creatinine, glucose, lipid profile, serum electrolytes and complete blood count were all in the appropriate reference ranges (Table 1). Endocrine investigations revealed normal serum TSH and FT4 concentrations thus indicating well-controlled hypothyroidism under L-thyroxin 75 µg/day in addition to normal PTH and lowered 25-hydroxyvitamin D₃ levels. Bone turnover markers were assessed, and a slightly decreased level of osteocalcin (OC) was recorded, with no changes in serum β-Cross Laps (Table 1).

Table 1. Laboratory investigations of the calcium-phosphorus metabolism, bone turnover markers, and thyroid hormones tests

Investigation	Result	Normal reference
Total calcium (mg/dL)	9.6	8.4-10.2
Ionized calcium (mg/dL)	3.92	4.2-5.4
Urinary calcium (mg/24h)	79.8	100-300
Urinary phosphorus (g/24h)	0.81	0.4-1.3
25-hydroxyvitaminD ₃ (ng/mL)	17.4	30-80
PTH (pg/mL)	25.6	11-67
Osteocalcin (ng/mL)	22.7	24-70
Beta Cross Laps (β-CTX) (ng/mL)	0.38	≤0.584
TSH (µUI/mL)	2.86	0.5-4
FT4 (ng/dL)	1.41	0.89-1.76

RBC-Red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), platelet count (PLT), erythrocyte sedimentation rate (ESR), triglycerides (TG), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ glutamyl transferase (GGT), alkaline phosphatase (ALP) were all within normal values.

Dual-energy X-ray absorptiometry (DXA) examination revealed a lumbar spine L1-L4 Z-score, calculated and adjusted for age, of -2.96 SD with a BMD of 0.839 g/cm², indicating low bone mineral density (BMD). According to 2013 International

Society for Clinical Densitometry (ISCD) criteria, the patient was diagnosed with primary osteoporosis and therapy was initiated to prevent further fractures.

Considering the personal and family history, the patient was evaluated for common polymorphisms associated with primary osteoporosis. A helix α-1 Sp1 binding site single-nucleotide-polymorphism (SNP) of collagen type I (COL1 α1), with homozygote "ss" genotype was confirmed (Fig. 1a). Moreover, a Fok-I polymorphism of the vitamin D receptor (VDR Fok-I) with "Ff" heterozygote genotype was identified (Fig. 1b). Given these results, the patient's mother was also genetically tested and was found heterozygote for both SNPs, i.e. "Ss" and "Ff" genotypes (Figs 1 a,b).

Figure 1a. RFLP (Restriction-Fragment Length Polymorphism) analysis of genomic DNA for COL1A1 Sp1 (G>T) intron 1 gene polymorphism revealed homozygous ss genotype in the index case (ID3478) and heterozygous Ss genotype in the patient's mother (ID3623).

Figure 1b. Fok-I VDR C>T exon 2 gene polymorphism genotyping (RFLP) revealed Ff heterozygosity in both boy and mother (ID3478 and ID3623, respectively). DNA extraction was performed using a commercial kit (Quiagen DNeasy Blood & Tissue, CA, USA). PCR conditions for COL1A1 were as follows: initial denaturation at 95°C for 10 min; 30 cycles of denaturing at 95°C for 1 min, annealing at 60°C for 30s and extension at 72°C for 1 min; and a final extension at 72°C for 7 min. PCR conditions for Fok-I VDR were as follows: initial denaturation at 94°C for 10 min; 35 cycles of denaturing at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 2 min; and a final extension at 72°C for 7 min. PCR products were digested with the restriction endonucleases Apal and FokI, respectively, and then the products and their restriction fragments were separated on 3% agarose gels.

Anti-fracture therapy was commenced in our patient with zoledronic acid (Aclasta®) 5 mg as an annual intravenous infusion, in addition to calcium and vitamin D supplementation. The clinical outcome at 3 years was favorable, without joint pain or fracture. Moreover, DXA follow-up at 3 years revealed a gain

in bone mass with Δ BMD of 3.6% (BMD 0.869 g/cm² and Z-score -2.6 SD), above the least significant change (LSC) limit of the equipment, to attest for improved evolution.

DISCUSSION

The greatest deficit of bone and mineral mass during childhood and adolescence occurs mainly due to genetic defects such as osteogenesis imperfecta and X-linked hypophosphatemic rickets, followed by enzymatic defects (e.g. hypophosphatasia, homocystinuria) and rare disorders of copper transport, including Wilson's disease and Menkes' kinky hair syndrome. Other uncommon causes of pediatric osteoporosis include idiopathic juvenile osteoporosis, featuring several fragility fractures during childhood that usually cease in adults, and early-onset of Paget disease; thus, secondary osteoporosis is more common in children due to chronic illness, medical treatment, neoplasia (7).

Calcium absorption consists of a transcellular and a paracellular pathway, the former depending on the modulation by 1,25(OH)₂vitamin D₃ and the intestinal vitamin D receptor (VDR). VDR is expressed in the thyroid and kidneys, but the highest concentration is found in the duodenum, where the transcellular transport of calcium primarily occurs; this is the reason why regulation of the VDR gene is crucial in calcium absorption efficacy.

The VDR gene has been located on chromosome 12q13.11 (1,8) and VDR genotypes (Fok-I, BSMI, Apal, Taq1 polymorphisms and others, as they are more than 100 endonuclease restriction sites in the VDR gene) have been associated with multiple sclerosis, osteoporosis, and vitamin D-dependent rickets type II and other disorders (1).

Fok-I polymorphism represents a C to T transition within the VDR translation initiation site and generates upstream initiation codon follow-on in VDR molecules extended by three amino acids (f allele) compared with downstream site (F allele). Ames *et al.* reported in 1999 a study of VDR gene Fok-I SNP and bone metabolism and bone mass in children, showing that Fok-I polymorphism was associated with BMD and calcium absorption. FF homozygotes children had mean calcium absorption 41.5% greater than ff homozygotes children. Children of FF genotype exhibited 17% greater absorption than Ff heterozygotes. Furthermore, FF genotypes had an 8.2% greater BMD in comparison to ff genotypes and 4.8% compared to Ff genotypes,

respectively (9). In a large systematic review, 60% of the studies reported a significant link between Fok-I polymorphism and osteoporosis risk (1).

A polymorphism in the COL1A1 gene, localized in the long (q) arm of chromosome 17 at position 21.33 (17q21.33) increases the risk of developing osteoporosis (10). It was reported that the association between COL1A1 alleles and fractures further persists after improvement of BMD (11). This polymorphism is a G/T SNP that occurs in a regulatory region of the COL1A1 gene and affects the production of type I collagen. Several studies have shown that subjects with Ss genotypes are more likely to have low BMD and a higher prevalence of bone fractures and this was confirmed by a large meta-analysis (12) showing an increased risk of any fracture in Ss subjects (OR = 1.26) and to a greater extent in ss subjects (OR = 1.78). Indeed, a higher ratio of $\alpha(1)/\alpha(2)$ protein chains was confirmed in Ss vs. SS individuals, which results in reduced strength of bone in former (13), overall supporting the clinical value of genotyping at the Sp1 site in detecting individuals at risk of osteoporotic fractures in human (14).

The ss genotype of the COL1A1 Sp1 binding site polymorphism is linked to lower BMD of the total body and lumbar spine in early puberty girls (15) and significantly bone formation markers bone alkaline phosphatase (B-ALP), amino-terminal propeptide of type I collagen (PINP) and OC to suggest it may contribute to inadequate acquisition of peak bone mass and explain low BMD in children and teens. In contrast, in smaller, pre-pubertal children aged 4.5-6.5 years from the Iowa Bone Development Study COL1A1 Sp1 was not associated with any of the BMD/BMC measure (16). In our case, the patient was positive for α -1 Sp1 polymorphism of COL1A1, with a homozygote genotype, to suggest a key role of genetic determinants in the pathogenesis of his osteoporosis. A bone formation defect is apparently involved in the pathogenesis of osteoporosis in this young male, as supported by low serum OC.

Furthermore, Fok-I polymorphism of the VDR gene with heterozygote genotype was concomitantly detected in our clinical case, and the association of these two polymorphisms had not been reported previously in a young male patient with primary osteoporosis. In adolescents, VDR Fok-I is related to calcium absorption and BMD (17,18) to suggest a link to bone mineral accretion during puberty via an influence on calcium absorption.

It remains unclear if this association had a

cumulative deleterious influence on bone mass and studies do not have a sufficient number of subjects to conclude on the interaction of the different genotypes. A significant contribution of another genetic mechanism to the low BMD and increased fracture risk cannot be excluded, yet.

Bisphosphonates may suppress bone turnover, preserve the bone architecture, prevent bone loss and inhibit bone resorption (19). Zoledronic acid is the only once-a-year osteoporosis treatment with approval in postmenopausal and male osteoporosis as well as several forms of secondary osteoporosis, and is under trial in children (1-17 years) with osteogenesis imperfecta. His main advantages include an increase in BMD and the reduction of both vertebral and non-vertebral fractures (20). Treatment with zoledronic acid was well-tolerated by our patient in whom no fragility fractures developed under therapy.

In addition, nutrition status influences osteoporosis; overweight or grade 1 obesity seems to protect via a mechanism related to leptin (21).

In conclusion, idiopathic osteoporosis in young male patients is an uncommon condition, which might be related to genetic predisposition, as suggested in this clinical case by the positive familial history. It remains unclear if the association of $\alpha 1$ Sp1 polymorphism of the COL1A1 gene with VDR Fok-I gene polymorphism had a cumulative negative influence on bone mass. Further studies are needed to elucidate this association in large populations.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

References

1. Mohammadi Z, Fayyazbakhsh F, Ebrahimi M, Amoli MM, Khashayar P, Dini M, Zadeh RN, Keshtkar A, Barikani HR. Association between vitamin D receptor gene polymorphisms (Fok1 and Bsm1) and osteoporosis: a systematic review. *J Diabetes Metab Disord* 2014; 13(1):98.
2. Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM, Zhou Y, Deng H, Heaney R, Recker RR. Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. *J Bone Miner Res* 2000; 15(7):1243-1252.
3. Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I in iliac crest bone matrix in premenopausal women with idiopathic osteoporosis. *Exp Clin Endocrinol Diab* 2004; 112(01):38-43.
4. Ma NS, Gordon CM. Pediatric osteoporosis: where are we now? *J Pediatr* 2012; 161(6):983-990.
5. Halton J, Gaboury I, Grant R, Alos N, Cummings EA, Matzinger M, Shenouda N, Lentle B, Abish S, Atkinson S, Cairney E, Dix

- D, Israels S, Stephure D, Wilson B, Hay J, Moher D, Rauch F, Siminoski K, Ward LM, Canadian SC. Advanced vertebral fracture among newly diagnosed children with acute lymphoblastic leukemia: results of the Canadian Steroid-Associated Osteoporosis in the Pediatric Population (STOPP) research program. *J Bone Miner Res* 2009; 24(7):1326-1334.
6. Huber AM, Giannini EH, Bowyer SL, Kim S, Lang B, Lindsley CB, Pachman LM, Pilkington C, Reed AM, Rennebohm RM, Rider LG, Wallace CA, Feldman BM. Protocols for the initial treatment of moderately severe juvenile dermatomyositis: results of a Children's Arthritis and Rheumatology Research Alliance Consensus Conference. *Arthritis Care Res (Hoboken)* 2010; 62(2):219-225.
7. Cimaz R, Stagi S. Managing pediatric osteoporosis. *Int J Clin Rheumatol* 2013; 8(5):509-512.
8. VDR gene. Vitamin D (1,25- dihydroxyvitamin D3) receptor. U.S. Department of Health & Human Services, 2017. (Accessed April 12, 2017, at <https://ghr.nlm.nih.gov/gene/VDR#>.)
9. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *J Bone Miner Res* 1999; 14(5):740-746.
10. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet* 1996; 14(2):203-205.
11. Stewart TL, Ralston SH. Role of genetic factors in the pathogenesis of osteoporosis. *J Endocrinol* 2000; 166(2):235-245.
12. Mann V, Ralston SH. Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. *Bone* 2003; 32(6):711-717.
13. Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, Aspden RM, Ralston SH. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest* 2001; 107(7):899-907.
14. Langdahl BL, Ralston SH, Grant SF, Eriksen EF. An Sp1 binding site polymorphism in the COL1A1 gene predicts osteoporotic fractures in both men and women. *J Bone Miner Res* 1998; 13(9):1384-1389.
15. Suuriniemi M, Kovanen V, Mahonen A, Alen M, Wang Q, Lyytikäinen A, Cheng S. COL1A1 Sp1 polymorphism associates with bone density in early puberty. *Bone* 2006; 39(3):591-597.
16. Willing MC, Torner JC, Burns TL, Janz KF, Marshall T, Gilmore J, Deschenes SP, Warren JJ, Levy SM. Gene polymorphisms, bone mineral density and bone mineral content in young children: the Iowa Bone Development Study. *Osteoporos Int* 2003; 14(8):650-658.
17. Abrams SA, Griffin IJ, Hawthorne KM, Chen Z, Gunn SK, Wilde M, Darlington G, Shypailo RJ, Ellis KJ. Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. *J Bone Miner Res* 2005; 20(6):945-953.
18. Zhang C, Wang C, Liang J, Zhou X, Zheng F, Fan Y, Shi Q. The vitamin D receptor Fok1 polymorphism and bone mineral density in Chinese children. *Clin Chim Acta* 2008; 395(1-2):111-114.
19. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster JY, Scientific Advisory Board of the European Society for C, Economic Aspects of O, Osteoarthritis, the Committee of Scientific Advisors of the International Osteoporosis F. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int* 2013; 24(1):23-57.
20. Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol* 2014; 142:155-170.
21. Oros S, Ianas O, Vladoiu S, Giurcaneanu M, Ionescu L, Neacsu E, Voicu G, Stoiceanu M, Rosca R, Neamtu C, Badiu C, Dumitrache C. Does Obesity Protect Postmenopausal Women Against Osteoporosis? *Acta Endocrinologica-Bucharest* 2012; 8(1):67-76.