Case Report / Olgu Sunumu

Turkish Journal of Pediatric Disease
Türkiye Çocuk Hastalıkları Dergisi

Heterozygous Vitamin D Receptor Gene Polymorphism in an Osteogenesis Imperfecta Type IV Case

Osteogenezis İmperfekta Tip 4'lü Bir Olguda Heterozigot Vitamin D Reseptör Geni Polimorfizmi

Ayça TÖREL ERGÜR[1], Suzan AKYILDIZ[2], Semra BAYKAL GÖKÇE[3]

[1]Kırıkkale University, Faculty of Medicine, Department of Pediatric Endocrinology, Kırıkkale, Turkey
[2]Atatürk Research Hospital, Department of Molecular Genetics, Ankara, Turkey
[3]Atatürk Research Hospital, Department of Biochemistry, Ankara, Turkey

ABSTRACT

Osteogenesis imperfecta (OI) type IV is a clinical entity with autosomal dominant inheritance in type 1 collagen genes; collagen, type I, alpha 2 (COL1A2) and more rarely collagen, type I, alpha 1 (COL1A1) point mutation or small deletion, and short stature, osteoporosis and/or diffuse osteopenia, repeating fractures and observed as normal sclera and more rarely seen compared to other type of osteogenesis imperfecta. On the physical examination of a 15-year old male child admitted to our clinic three times last year with minor traumas and complaints of fractures, the weight was 59 kg, height 150 cm, height standard deviation score (height SDS) -2.31, and upper/lower segment ratio 1.03. The sclera color was normal and other physical examination findings also normal. The serum alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), parathormone (PTH) levels were within the normal range. The patient's serum 25(OH)D level was 16.7 mcg/L but his 1.25-(OH)2D3 level (30 pg/ml) was normal. The lumbar bone mineral density (BMD) of our patient who had a repeating fracture history conformed with osteoporosis (BMI z score:-3.1). After amplifying the DNA samples obtained from the patient's serum, the molecular analysis made by using the reverse hybridization method helped determine a heterozygous COL1A1 spl (s/s) and homozygous Vitamin D receptor (VDR) Bsml (B/B) gene polymorphism.

This result brings to mind the contribution of the VDR anomalies in the development of the disorders accompanying hereditary osteoporosis. In other words, the determination of the risk alleles of VDR and COL1A1 together is important in identifying a hereditary component of osteoporosis.

Key Words: COL1A1, Osteogenesis imperfecta, Vitamin D reseptör gen polimorfizmi

ÖZET

Çalışmada dört yıl içerisinde hastanemizde doğan bebekler içinde immün olmayan hidrops fetalis tanısı alan olguların Osteogenezis imperfekta (OI) tip 4 otozomal dominant geçişli, tip1 kollajen genlerinde; kollajen tip I alfa 2 (COL1A2) ve çok daha nadir olarak kollajen tip I alfa 1 (COL1A1); nokta mutasyon veya küçük delesyonun saptandığı, kısayapı, osteoporoz ve /veya diffüz osteopeni ,tekrarlayan kırıklar ve normal skleranın gözlendiği ve diğer osteogenez imperfekta tipleri ile kıyaslığında çok daha nadir gözlenen klinik bir antitedir. Yazıda son 1 yıl içinde minör travmalarla 3 kez kırık oluşumu nedeniyle kliniğimize başvuran 15 yaşında erkek olgu sunulmuştur. Olgunun fizik muayenesinde vücut ağırlığı: 59 kg, boy: 150 cm, boy standart deviasyon skoru (boy SDS):-2.31, üst/ alt segment oranı:1.03 ve sklera rengi normaldi. Diğer sistem bulguları normaldi. Serum biyokimyası ve kalsiyum (Ca), fosfor (P), alkalen fosfataz (ALP), parathormon (PTH) normal aralıktaydı. 25(OH)D düzeyi 16.7 mcg /L ve 1,25-(OH)2D3 düzeyi (30 pg/ml) normaldi. Tekrarlayan kırık öyküsüne sahip olan olgumuzun lumbal kemik mineral dansitesi (BMD) osteoporoz ile uyumluydu (BMI z skoru: -3.1). Hastanın serumundan alınan DNA örnekleri amplifiye edildikten sonra revers hibridizasyon metodu kullanılarak yapılan moleküler analizinde COL1A1 heterozigotluğu ve Vitamin D reseptörü (VDR) gen polimorfizmi saptandı.

Bu sonuçlar bu hastalıkta VDR anomalisinin de osteoporoza katkıda bulunduğunu düşündürmüştür. Diğer bir deyişle, VDR ve COL1A1 risk allallerinin saptanması osteoporozun herediter oluşunu belirlemede önem taşımaktadır.

Anahtar Sözcükler: COL1A1, Osteogenezis imperfekta, Vitamin D receptor gene polymorphism

Correspondence Address / Yazışma Adresi:
Ayça TÖREL ERGÜR
Kırıkkale Üniversitesi, Tıp Fakültesi, Pediatrik Endokrinoloji Anabilim Dalı, Kırıkkale, Turkey
E-mail: aycaergur@superonline.com

Received / Geliş tarihi : 03.04.2014
Accepted / Kabul tarihi : 06.05.2014
Elektronik yayın tarihi : 26.01.2015
Online published
DOI: 10.12956/tjpd.2015.115

INTRODUCTION

Osteogenesis imperfecta is the clinical manifestation of genetic errors in the synthesis of collagen type 1 present not only in the bone but also in the skin, ligaments, sclera, and teeth (1). Biochemical and molecular genetic studies have shown that the vast majority of affected individuals have mutations (loss of function, insertions, duplications, frameshifts, or point mutations) in either COL1A1 or COL1A2, genes that encode the chains of type1 procollagen, leading to a decrease in bone formation, osteopenia and increased fracture rate (1). Recent studies in which bone mineral density has been associated with genetic variation at a number of candidate genes are promising but these studies are too premature yet to be used clinically (2) In this report we defined OI type IV case by showing heterozygous COL1A1 Sp1(S/s) and homozygous VDR BsmI (B/B) gene polymorphism.

CASE REPORT

A 15-year-old boy (Figure 1) presented with history of repeated fractures following minor trauma during last year. On physical examination; weight was 56 kg (<3rd percentile), height 150 cm (<3rd percentile), BMI: 24.89 (90-95th percentile), height SDS -2.31, maternal height 150 cm, paternal height 175 cm, upper/lower segment ratio 1.03, armspan-height difference: 149 cm and puberty stage Tanner 5. Other physical examination findings were normal and color of the sclerae was also normal. The bone age of the patient who had a disproportional shortness of height was in conformity with the chronological age (bone age: 15 years). The mother and aunt also had a history disproportionate shortness of height. On the laboratory examination of the patient, the serum Ca (9.2 mg/dl), P (5.1 mg/dl) and the ALP (212 IU) and PTH (53.3 pg/ml) levelswere normal. The patient's serum 25(OH)D level was low (16.7 mcg/L) but his 1.25-$(OH)_2D_3$ (30 pg/ml) level was within normal limits.

Lumbar dual energy X-Ray Absorptiometer (DEXA) analysis was found to be in conformity with osteoporosis (BMI z score: -3.1SD). The Wormian bones could not be determined in the cranial graphs.

Determination of COL1A1 and VDR genotypes

Using DNA isolated from whole blood, two PCRs were first performed. The characterization of the amplified gene fragments was carried out in a hybridization reaction with sequence-specific oligonucleotide probes that are immobilized on nitrocellulose strips (reverse hybridization). The nitrocellulose strips had gene probes for the wild type and mutated alleles of both gene loci as well as various control zones. During hybridization, the denatured amplified DNA, mixed from both PCRs with PN-VDR nd PN-COLIA, bound to the gene probes attached to the strips. A highly specific washing procedure ensured that the hybrids only survived if the probes sequence was 100% complementary to that of the amplified DNA (3). This kit from the company GenID GmbH detects the Sp1(S/s) polymorphism in the COL1A1 gene and the BsmI (B/B) in the VDR gene by two parallel polymerase chain reactions and subsequent hybridization. Accordingly, heterozygous COL1A1 Sp1(S/s) and homozygous VDR BsmI (B/B) gene polymorphisms were determined in the patient (Figure 2).

The patient was diagnosed as OI type IV and oral vitamin D and calcium was initiated, followed a month later with oral alendronate treatment (10 mg/day).

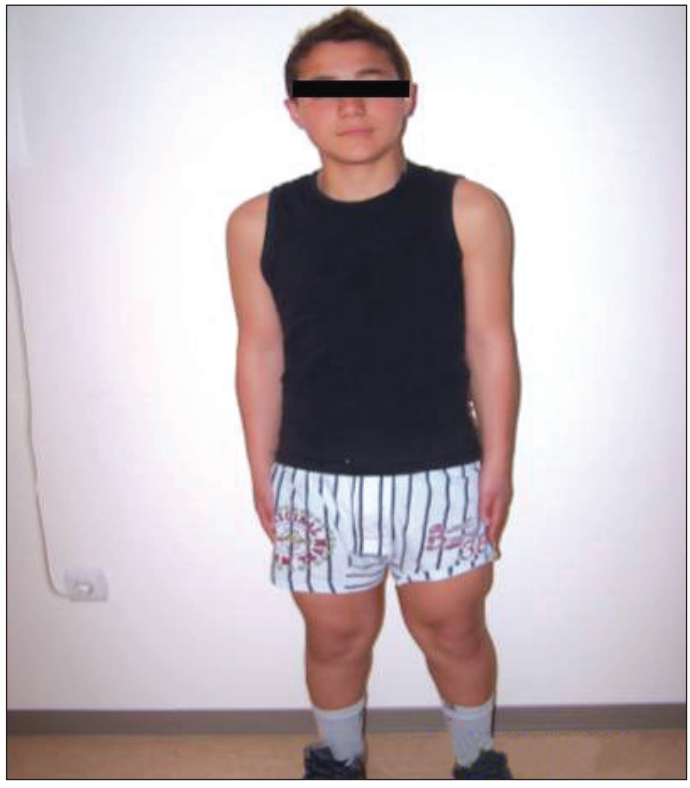

Figure 1: Characteristics of the case.

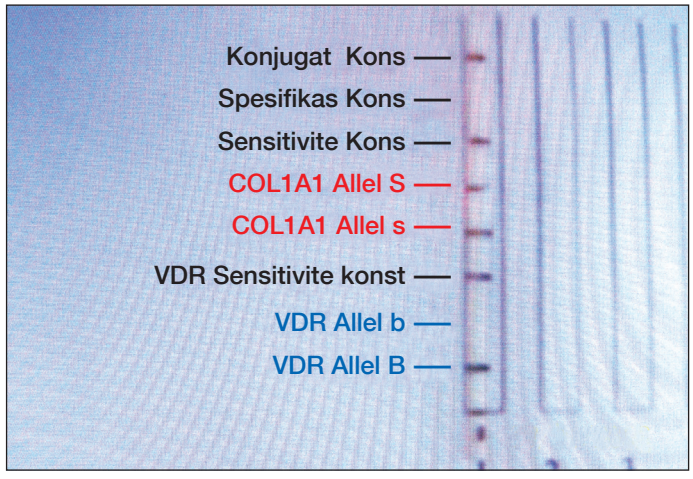

Figure 2: Molecular analysis of case, COL1A1 Allele Ss (heterozygous), VDR Allele BB (homozygous).

Table I: Clinical and laboratory characteristics in our patient.

Findings of OI type IV	Case
Mutations in COL1A1 or COL1A2 (more)	Mutation in COL1A1
Short stature	+
Normal sclerae	+
Variable ages of onset of fractures (from birth to adult life)	Adolescent age
Dentinogenesis imperfecta positive or negative	Negative
Diffuse osteopenia or osteoporosis	Osteoporosis

DISCUSSION

OI type IV should be differentiated from the other OI types, juvenile idiopathic osteoporosis (JIO), osteoporosis with pseudogliomatous blindness and inborn errors of metabolism characterised by osteopenia (Cockayne syndrome, Thompson syndrome, Fanconi pancytopenia, homosistinuria, Lowe-oculo-cerebro-renal syndrome…) (17). It may be difficult to differentiate juvenile idiopathic osteoporosi from mildly affected cases of OI type IV (with normal teeth) as in our case. Studies of collagen synthesis have been normal in children with JIO. On the other hand there is no positive family history in JIO. In our case, there was a positive family history. Osteogenesis imperfecta type II and III are severe forms of OI. These subgroups are characterised by extreme bone fragility leading to intrauterine or early infant death (as in OI type II) or recurrent fractures leading to progressive bone deformities often apparent at birth (as in OI type III) (17). We did not observe these findings in our case.

Twin and family studies have suggested that bone mineral density (BMD) has a strong genetic component, besides being influenced by candidate genes (4). Among the candidate genes in relation to BMD are the genes for collagen type I (COLIA1), VDR, and the estrogen receptor (ER) (5-9). The VDR gene is important to human stature, as it mediates metabolic pathways, calcium homeostasis, and phosphate homeostasis, which influence growth (10). Although the mechanism(s) for the gene-gene interaction unknown, it is conceivable from a physiological point of view. 1.25-$(OH)_2D_3$ is an important factor in estrogen biosynthesis and might thus influence local equilibrium between estrogens and androgens (11). Furthermore, 1,25-$(OH)_2D_3$ regulates ER expression in osteoblast-like cells (11). In this way 1,25-$(OH)_2D_3$ might regulate the effect of estradiol (E_2) on bone metabolism. In vitro and in vivo studies have shown that several biological responses to treatment with vitamin D, such as intestinal calcium absorption and osteocalcin production, are VDR genotype dependent (11). If 1,25-$(OH)_2D_3$ influences the effect of E_2 on bone metabolism, this effect might also be VDR genotype dependent. Although contrary reports have been published, two metaanalyses have shown a weak relation between the VDR gene and BMD (12,13). Others have found a significant association between VDR polymorphisms and fracture risk, although other studies could not confirm such an association (14-16). We report case of OI type IV in a fifteen-year-old boy either VDR-B and COLIA1-ss risk alleles determined. The most rapid period of skeletal development occurs over several years in childhood and adolescence, accounting for 40 to 50% of the total accrual of skeletal mass Osteoporosis is a common disease with a strong genetic component, characterized by reduced bone mass and increased fracture risk. Current evidence suggest that the inheritance of bone mass is under polygenic control but the genes responsible are poorly defined. Colin et al. (12) reported the combined influence of polymorphisms in the estrogen receptor (ER) gene and the vitamin D receptor (VDR) gene on the susceptibility to osteoporotic vertebral fractures in 634 women aged an interlocus interaction in relation to BMD and fractures between two important candidate genes in osteoporosis. Another study by Uitterlinden et al. (9) demonstrated an interaction between VDR and another candidate gene, the COLIA1 gene, with respect to fracture risk in 1004 postmenopausal women. They found that both the VDR and the COLIA1 polymorphisms are genetic markers for osteoporotic fracture. Recently, a G to T polymorphism in an Sp1 site in the COLIA1 gene was found to be associated with reduced BMD and with increased fracture risk (16). These findings underscore the polygenic character of osteoporosis and the importance of the contribution of gene interactions in determining fracture risk. The lumbar BMD of our patient who had a repeating fracture history was in conformity with osteoporosis. As his family history was positive, the patient was diagnosed OI type IV with the physical features and genetic investigation results. The findings supporting the OI type IV diagnosis are shown in Table I.

Although Turkey is in a geographic location with abundant sunlight and daily vitamin D supplementation (400 IU) is being recommended for infants between 15 days to 1 year old, vitamin D deficiency and nutritional rickets is still major problems in our country. In our case, the vitamin D level was quite low. There are various studies in the literature that discuss the effect of vitamin D on bone structure in patients with OI. Edourt et al. (18) and Wilsford et al. (19) investigated the relationship between bone mineralization and structure and vitamin D levels in OI cases. In both of these studies, there was no association between the fractures and low levels of vitamin D in this patient group.

The most important therapeutic advance is the introduction of biphosphonate treatment for moderate to severe forms of OI.

However, at present the best treatment regimen and long –term outcomes of biphosphonate therapy are unknown. We started oral alendronate treatment in our case. The number of reports regarding the use of oral biphosphonates in children with OI has increased (20). The usage of oral biphosphonates is inexpensive and easy to administer in contrast to the intravenous form. Maasalu et al. (21) reported that the BMD of l5 cases with OI administered oral biphosphanate increased while a significant decrease in fracture frequency was observed. In conclusion, we suggest that the examination of the risk alleles VDR-B and COLIA1-s helps to determine diagnosis and plan treatment for a hereditary disposition for osteoporosis during childhood at an early stage.

REFERENCES

1. Gajko-Galicka A. Mutation in type 1 collagen genes resulting in osteogenesis imperfecta in humans. Acta Biochim Pol 2002;49:433-41.
2. Tinkle BT, Wenstrup RJ. A genetic approach to fracture epidemiology in childhood. Am J Med Genet C Semin Med Genet 2005;15:38-54.
3. Ferrari S, Rizzoli R, Bonjour JP. Gentic aspects of osteoporosis. Curr Opin Rheumatol 1999;11:294-300.
4. Emery AEH, Rimoin DL. Principles and Practice of Medical Genetics. 2nd ed. Edinburgh: Churchill Livingstone, 1990:933-8.
5. 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. Nature Genetics 1996;14:203-5.
6. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 1996;11:306-11.
7. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367:284-7.
8. Uitterlinden AG, Pols HA, Burger H, Huang Q, Van Daele PL, Van Duijn CM, et al. A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. J Bone Miner Res 1996;11:1242-8.
9. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA, et al. Relation of alleles at the collagen type I gene to bone density and risk of osteoporotic fracture in postmenopausal women. N Engl J Med 1998; 338:1016-21
10. Uitterlinden AG, Weel AEAM, Burger H, Fang Y, Van Duijn CM, Hofman A, et al. Interaction between the vitamin D receptor gene and collagen type I gene in susceptibility for fracture. J Bone Miner Res 2001;16:379–85.
11. Xiong DH, Xu FH, Liu PY, Shen H, Long JR, Eize L, et al. Vitamin D receptor gene polymorphisms are linked to and associated with adult height. Journal of Medical Genetics 2005;42:228-34.
12. Colin EM, Uitterlinden AG, Meurs JB, Bergink AP, van de Klift M, Fang Y, et al. Interaction between vitamin D receptor genotype and estrogen receptor genotype influences vertebral fracture risk. J Clin Endocrinol Metab 2003; 88: 3777-84.
13. Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone density? J Bone Miner Res 1996;11:1841-9.
14. Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW, et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. Osteoporos Int 1999;9:55-64.
15. Feskanich D, Hunter DJ, Willet WC, Hankinson SE, Hollis BW, Hough HL, et al. Vitamin D receptor genotype and the risk for fractures in women. Epidemiology1998;9:535-9.
16. Houston LA, Grant SFA, Reid DM, Ralston SH, Vitamin D. receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: Studies in a UK population. Bone 1996;18:249-52.
17. White CP, Nguyen TV, Jones G, Morrison NA, Gardiner EM, Kelly PJ, et al. Vitamin D receptor alleles predict osteoporotic fracture risk. J Bone Miner Res 1994;9:263.
18. Edouard T, Glorieux FH, Rauch F. Relationship between vitamin D status and bone mineralization, mass, and metabolism in children with osteogenesis imperfecta: Histomorphometric study. J Bone Miner Res 2011;26:2245-51.
19. Wilsford LD, Sullivan E, Mazur LJ. Risk factors for vitamin D deficiency in children with osteogenesis imperfecta. J Pediatr Orthop 2013;33:575-9.
20. Unal E, Abacı A, Böber E, Büyükgebiz A. Oral alendronate in Osteogenesis Imperfecta. Indian Pediatrics 2005;42:17:1156-60.
21. Maasalu K, Haviko T, Martson A. Treatment of children with osteogenesis imperfecta in Estonia. Acta Pediatr 2003;92:452-5.