

## Prevalence of osteoporosis and vitamin D receptor gene polymorphisms (FokI) in an Iranian general population based study (Kurdistan) (IMOS)

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### Abstract

**Background:** Osteoporosis, or porous bone, is a disease characterized by low bone mass density (BMD) and structural deterioration of bone tissue, leading to bone fragility and increased risk of hip, spine, and wrist fractures. There are numerous risk factors for osteoporosis. While many of these factors are non-genetic in nature, there is a definite genetic component responsible for this condition. The main aim of this study was to evaluate the association between VDR (Vitamin D receptor gene) polymorphisms (FokI) A>G (rs2228570) and bone mineral density in an Iranian defined population.

**Methods:** The study participants comprised of 1032 Iranians recruited from the city of Sanandaj during IMOS (Iranian Multi Center Osteoporosis Study). Bone mineral density measurement was performed in all the participants with and without osteoporosis. All samples were genotyped for VDR genes (FokI) polymorphism with polymerase chain reaction, using a predesigned TaqMan allele discrimination assay.

**Results:** There was a significant association between FokI polymorphism and osteoporosis in post-menopausal women, 0.138 (0.025-0.768).

**Conclusion:** It seems that cohort studies, which are more powerful than case-control studies, can be useful in evaluating the roles of genetic variants as risk or protective factors for osteoporosis.

**Keywords:** Osteoporosis; Vitamin D Receptor Gene; FokI; Bone Mineral Density.

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### Introduction

Osteoporosis is a metabolic bone disease, characterized by low bone mass and bone tissue deterioration, which leads to osteoporotic fracture risk (1,2). It is a disease

caused by the interaction of genetic and environmental factors. According to many studies, the contribution of genetic and environmental factors is about 70% and 30%, respectively. The environmental factors;

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however, can control gene expression and the process of the disease (3).

In this regard, several studies have focused on the influence of ethnicity on bone mineral density (BMD) and osteoporosis. About 60% of the bone characteristics are genetic-dependent; the Caucasians and Asians usually have lower bone density than the Negros, Hispanics and Latino Americans (4-6).

Different genes are reported to be linked with osteoporosis, the most important of which is vitamin D receptor gene (VDR) (7). The most common genetic markers that have been investigated in genetic association studies of BMD or osteoporosis are the single nucleotide polymorphisms (SNPs) in the VDR gene (8).

VDR, expressed in certain organs, has a vital role in calcium homeostasis. VDR repress proximal activator of 1, 25 (OH) 2 D3, inducing the expression of 1, 25(OH) 2 D3 through deactivating the enzyme CYP24 (1,25-dihydroxyvitamin D3 24-hydroxylase, mitochondrial) (9).

The human VDR gene is localized on chromosome 12q12-q14 (10). There are more than 100 restriction endonuclease recognition sites in VDR gene such as Fok1, Bsm1, Apa1 and etc. Fok1 is located on exon 2 (11-13). VDR genotypes are associated with the development of several bone diseases as well as osteoporosis and other complex maladies such as osteoarthritis and diabetes (14).

Several studies have evaluated the relationship between VDR polymorphism and BMD. There are controversial reports regarding the relationship between Fok1 and BMD values (15-19). Previous studies have investigated the frequency of Fok1 genotypes and its relationship with BMD or osteoporosis in the Iranian population. However, the results were controversial indicating the necessity of conducting a more comprehensive population based study with a larger sample size (20-23).

The aim of this study was to analyze the association between the Fok1 polymorphism and BMD values in a large group of

Iranians from a defined population of Sanandaj, the twenty-third largest city in Iran and also the capital of Kurdistan Province (24).

### Methods

The Iranian Multi Center Osteoporosis Study (IMOS) was conducted on an Iranian population in three phases. The third phase was performed in two big cities in 2012-2013. All individuals older than 20 years of age were selected using random cluster sampling. We excluded cases with metabolic bone disease, or those taking any medication known to affect bone metabolism such as any kinds of vitamin D. Cases suffering from cancer, acute or chronic renal failure, or advanced liver failure were also excluded.

### Participants

In this study, 1032 Iranians recruited from Sanandaj city during IMOS were included in this study. They were classified into 3 groups according to the World Health Organization (WHO) criteria, and based on BMD measurements at the lumbar (L2-L4) spine, Hip and femoral neck. BMD measurements were performed by dual-energy X-ray absorptiometry (Norland XR46) following manufacturer protocols. A structured questionnaire assessing biological and lifestyle risk factors was completed by each participant; informed consent was obtained from all the participants, and all were subject to full history taking, clinical examination and laboratory investigations. The protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

### Genotyping

Genomic DNA was isolated from peripheral blood leukocytes, and DNA extraction was performed using the phenol chloroform method (25). The VDR SNPs, Fok1 A>G (rs2228570) were genotyped using a Taq-Man 5'-exonuclease SNP allelic discrimination assay with the ABI 7300 Real-Time PCR System (Applied Biosystems, Foster

City, CA, USA). Negative and positive controls were included to ensure the accuracy of genotyping. The reactions were carried out in a total volume of 15  $\mu$ L containing 50 ng DNA. The thermal cycler conditions consisted of an initial hold for 10 min at 95°C, followed by 40 cycles of 15 s at 92°C, and 1 min at 60°C each.

### Statistical Analysis

STATA program version 11.0 was used for data analysis. Data were summarized as mean, SD. One way ANOVA was done to analyze more than two variables, followed by post hoc test to detect the significance. Logistic regression analysis was used to assess the association of Fok1 polymorphism and BMD. The crude association was assessed in model 1, and the association was adjusted for BMI and age in model II. P-value was considered significant at <0.05.

In this study, because of the importance of age and sex in osteoporosis diagnosis, the participants were divided in four categories based on their age and gender: men younger than 50 years of age, men equal and older than 50 years, pre and postmenopausal women.

### Results

Baseline characteristics of the participants are illustrated in Table 1. Overall, 61%

(n=629) of the participants were female. The mean $\pm$ SD age of post-menopausal women was 49.4 $\pm$ 4.05 years.

In total, 11.8% (n= 122) of the participants suffered from osteoporosis. The prevalence of osteoporosis in men over 50 years of age and postmenopausal women was 44.1% and 37%, respectively; and the difference was significant (p< 0.001).

The prevalence of Vitamin D deficiency was 85.1%, and the condition was reported in 92.7%, 81.1%, 85.9% and 73.5% of men younger than 50 years, men over 50 years, pre-menopausal and postmenopausal women, respectively. A significant difference was found between the four groups (p<0.001).

There was a significant relationship between age-sex groups and BMD values at all bone sites, osteoporosis, BMI, height, weight, Vitamin D and calcium (p<0.0001).

Age, Vitamin D serum level, and calcium were significantly different between osteoporotic and non-osteoporotic groups (p<0.05).

The frequency of Fok1 genotypes according to osteoporotic and non-osteoporotic subjects are presented in Table 2. The frequencies of FF, Ff and ff genotypes in participants were 55.5%, 38.3% and 6.2%, respectively.

The frequency of genotypes in osteoporotic and non-osteoporotic group was sig-

Table 1. Baseline Characteristics of the Participants Based On Age-Sex Groups: The IMOS Study in Sanandaj

Characteristics	Men<50 Yr	Men $\geq$ 50 Yr	Premenopausal women	Postmenopausal women	Total
Number	268	134	458	172	1032
Age(Yr)	32.9 $\pm$ 8.60	61.2 $\pm$ 8.90	35.4 $\pm$ 9.	58.1 $\pm$ 7.90	41.9 $\pm$ 14.62
Height(cm)	172.4 $\pm$ 6.85	166.9 $\pm$ 7.19	156.9 $\pm$ 5.92	154.02 $\pm$ 5.80	161.7 $\pm$ 9.67
Weight(kg)	75.9 $\pm$ 12.75	73.5 $\pm$ 12.60	68.0 $\pm$ 12.09	69.3 $\pm$ 11.10	70.9 $\pm$ 12.75
BMI(kg/m <sup>2</sup> )	25.5 $\pm$ 4.01	26.3 $\pm$ 3.90	27.7 $\pm$ 4.92	29.2 $\pm$ 4.6	27.2 $\pm$ 4.70
BMD L2-L4(g/cm <sup>2</sup> )	0.98 $\pm$ 0.150	0.91 $\pm$ 0.150	0.99 $\pm$ 0.1	0.83 $\pm$ 0.150	0.95 $\pm$ 0.16
BMD Femoral Neck (g/cm <sup>2</sup> )	0.93 $\pm$ 0.150	0.81 $\pm$ 0.1	0.88 $\pm$ 0.130	0.77 $\pm$ 0.130	0.87 $\pm$ 0.15
BMD Hip(g/cm <sup>2</sup> )	0.97 $\pm$ 0.150	0.90 $\pm$ 0.1	0.92 $\pm$ 0.130	0.82 $\pm$ 0.150	0.92 $\pm$ 0.14
Osteoporosis	75(17.4)	112(26)	101(23.5)	142(33.0)	430(100)
Non Osteoporosis	195(31.3)	24(3.8)	374(59.9)	31(5.0)	624(100)
Vitamin D (nmol/L)	29.5 $\pm$ 16.40	43.8 $\pm$ 69.	28.2 $\pm$ 32.40	42.9 $\pm$ 48.20	33.0 $\pm$ 39.90
Calcium (mg/dL)	9.7 $\pm$ 0.60	9.4 $\pm$ 0.	9.5 $\pm$ 0.60	9.6 $\pm$ 0.60	9.5 $\pm$ 0.60
Phosphor(mg/dL)	4.0 $\pm$ 0.70	3.7 $\pm$ 0.	3.9 $\pm$ 0.60	4.1 $\pm$ 0.60	3.9 $\pm$ 0.70
Alkaline phosphates(U/L)	190.5 $\pm$ 56.20	187.0 $\pm$ 52.	165.6 $\pm$ 54.90	205.8 $\pm$ 64.10	181.5 $\pm$ 58.50
Bone specific Alkaline phosphates(U/L)	17.8 $\pm$ 9.70	14.1 $\pm$ 5.	13.6 $\pm$ 10.25	17.7 $\pm$ 8.10	15.4 $\pm$ 9.50
Parathyroid hormone(pg/mL)	2.4 $\pm$ 1.20	3.0 $\pm$ 2.0	3.0 $\pm$ 1.70	3.2 $\pm$ 1.90	2.9 $\pm$ 1.70

Table 2. Frequency of Genotypes Based On Osteoporotic and Non-Osteoporotic Groups

Groups	Types	FF	Ff	Ff	p
		No (%)	No (%)	No (%)	
Postmenopausal women	Osteoporotic	80(57.6)	3(2.2)	56(40.2)	0.02*
	Non Osteoporotic	11(35.5)	3(9.7)	17(54.8)	
Premenopausal women	Osteoporotic	52(54.2)	8(8.3)	36(37.5)	0.90
	Non Osteoporotic	198(55.6)	30(8.4)	128(36.0)	
Men<50 Yr	Osteoporotic	40(58.0)	3(4.3)	26(37.7)	0.90
	Non Osteoporotic	111(57.5)	9(4.7)	73(37.8)	
Men≥50 Yr	Osteoporotic	64(58.7)	4(3.7)	41(37.6)	0.90
	Non Osteoporotic	12(56.5)	1(4.5)	9(40.9)	

\*significant

Table 3. Association of Fok1 Genotypes with Osteoporosis according to Different Age-Sex Groups

Groups	Model	FF	ff	Ff
		OR (CI95%)	OR (CI95%)	OR (CI95%)
Postmenopausal women	Model 1	1	0.138(0.025-0.768)*	0.453(0.197-1.041)
	Model 2	1	0.136(0.023-0.810)*	0.513(0.211-1.244)
Premenopausal women	Model 1	1	1.015(0.439-2.346)	1.071(0.663-1.730)
	Model 2	1	1.115(0.466-2.672)	1.078(0.652-1.783)
Men<50 Yr	Model 1	1	0.925(0.238-3.589)	0.988(0.556-1.757)
	Model 2	1	1.320(0.328-5.302)	0.920(0.509-1.662)
Men≥50 Yr	Model 1	1	0.750(0.077-7.306)	0.854(0.331-2.206)
	Model 2	1	0.860(0.087-8.536)	0.745(0.274-2.026)

Model 1: Crude model

Model 2: Adjusted for BMI and Age

\*significant relationship

nificantly different in post-menopausal women ( $p=0.02$ ).

Results of the logistic regression analysis on the association of Fok1 polymorphisms and osteoporosis are presented in Table 3. There was a significant association between osteoporosis and types of Fok1 polymorphisms in postmenopausal women in the crude and adjusted model. The regression analyses have shown that ff has a protective effect on osteoporosis. The possibility of osteoporosis in participants with ff genotype was 87% lower than others.

No significant relationship was found between BMD and types of Fok1 genotypes in men and premenopausal women.

## Discussion

Bone is a metabolically active tissue that experiences continuous remodeling via two reciprocal processes, bone formation and resorption. Osteoclasts, osteoblasts and osteocytes are respectively responsible for bone resorption, formation and maintenance. In osteoporosis, bone density decreases due to the high activity of osteoclasts (26). The prevalence of osteoporosis is different in various ethnicities and thus it is different in multiethnic countries (27,28).

The etiology of osteoporosis is still unknown; however, the importance of age, gene-environment interactions, gene-gene interactions and life-style in the development of osteoporosis has been shown in many studies. Several lines of evidence support the involvement of genetic factors in the development of osteoporosis. These studies show that daughters of women with osteoporosis have lower bone mass than other women of the same age. The sons of men with idiopathic osteoporosis also have lower bone density in comparison with normal men (5,6). Twin research and human genetics studies have shown that osteoporosis and its outcomes are highly heritable (31-34). Animal and human studies have shown that genes can affect bone density in various pathways. The most prominent pathway is related to the exertion of calcium (35,36). The majority of these researches focus on VDR gene and the fact that single point polymorphism in this gene is known to alter metabolic activity of the bone (29).

Several studies have evaluated the association between polymorphisms of vitamin D receptor gene (Fok1, Bsm1, Taq1, Apa1) and low bone mass density or osteoporosis

(37,38).

A systematic review published in 2014 revealed a significant association between the two polymorphisms (Fok1 and Bsm1) and osteoporosis in more than 50% of the available studies. Based on the articles, 60.0% of these studies reported a significant correlation between Fok1 and higher osteoporosis risk (39). They concluded that ethnicity and race, like gender, can influence the risk of osteoporosis and BMD.

In our study, the proportions of Fok1 genotypes were not significantly different in the two genders ( $p= 0.1$ ). This study found that the frequency of FF, Ff and ff genotypes is 55.5%, 38.3% and 6.2% in the studied population, respectively. Similar to the study performed by Hossein-Nezhad in 2009, FF (56.5%) is the most common genotype in the Iranian population (21). As for the Indian population, FF was seen in 59%, Ff in 36%, and ff in 5% of the studied population (40). In the Lampedusa Italian population, the observed proportions of Fok1 genotypes were 33.2% for FF, 32.8% for Ff, and 34% for ff (41). In the Spanish population, the prevalence of these genotypes was 40.4, 48.0 and 11.6% for FF, Ff and ff, correspondingly (42).

In the present study, BMD values in all the participants at either site were higher in ff genotype although the difference did not reach statistical significance. However, in this study, post-menopausal women with ff genotype exhibited a significantly lower risk for osteoporosis, compared with those with Ff and FF genotypes. Other investigators have reported that women with ff genotype experience greater bone loss at hip when compared with those with either Ff or FF genotypes (43-44). The previous studies conducted on Iranian population have shown that participants with ff genotype exhibited a significantly lower hip bone mass (21). Based on Hossein-Nezhad's study, individuals with ff genotype have a significantly lower bone mass at lumbar spine, relative to those with Ff and FF genotypes in pre- and post-menopausal women (45).

As articles have indicated, the results are vice versa. Existing literatures in this regard have shown controversial results and thus have failed to confirm the effect of Fok1 genotypes on bone mass density. However, most studies have shown the effect of confounding factors in accuracy of these results. Conducting a cohort study together with performing a cross-sectional analysis may be a more suitable observational research design for analyzing the genetic markers as risk factors for osteoporosis.

Cohort is perhaps the best-known type of observational controlled research to overcome these deficiencies. Considering the cross-sectional nature of the study and the influential role of environmental factors in the development of osteoporosis, it was not possible to confirm the role of genetic variants as risk or protective factors for osteoporosis. The present study is the first such research to be conducted on a large sample size of both genders.

### Conclusion

In addition to the increasing prevalence of osteoporosis, a surge is also noted in the mortality rate of osteoporotic patients (30). The finding of this report indicated that Fok1 in VDR gene may affect bone mass and predict osteoporosis. In this study, ff genotype was a protective factor just in post-menopausal women. These findings may be useful for better management of osteoporotic patients.

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