

ORIGINAL ARTICLE

# Vitamin D Receptor Gene BsmI, FokI, ApaI, and TaqI Polymorphisms in Multiple Sclerosis Patients in Iran: A Case-Control Study

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## ABSTRACT

**Background:** Multiple sclerosis (MS) is a major inflammatory and demyelinating disease of the central nervous system that is dependent on both environmental and genetic susceptibility factors. The single-nucleotide polymorphisms of the vitamin D receptor (VDR) gene may be related to MS risk.

**Materials and Methods:** This cross-sectional study was conducted on 80 MS patients and 50 age-matched healthy controls in Iran. For the purpose of the study, the VDR gene BsmI, FokI, ApaI and TaqI polymorphisms were studied on the DNA of the participants using the polymerase chain reaction-restriction fragment length polymorphism method.

**Results:** Our results demonstrated that the genotype frequency of the VDR ApaI and BsmI polymorphisms were significantly different between the MS patients and controls (OR=2.5, 95%CI: (1.146-5.40), P=0.042 and OR=2.7, 95%CI: (1.08-6.75), P=0.023, respectively). However, the genotype and allele distribution of the VDR gene FokI and TaqI polymorphisms did not differ between the MS patients and controls.

**Conclusion:** The findings of the present study indicated that the VDR ApaI and BsmI polymorphisms were more prevalent in the Iranian MS patients.

**Key Words:** Multiple sclerosis, Single nucleotide polymorphism (SNP), Vitamin D receptor

➤ How to cite this paper:

Abdolrahim Sadeghi A, Shojaipour M, Ghassami K, Mosayebi Gh. Vitamin D Receptor Gene BsmI, FokI, ApaI, and TaqI Polymorphisms in Multiple Sclerosis Patients in Iran: A Case-Control Study. Journal of Iranian Clinical Research. 2015; 1(1): 28-32.

## INTRODUCTION

Multiple sclerosis (MS) is a neurological inflammatory disorder with demyelination of central nervous system [1]. The full etiology of this disease remains unknown; however, the genetic and environmental factors play important roles in susceptibility to this disease [2]. Some studies reported that the low serum levels of vitamin D<sub>3</sub> (25[OH] D) in the MS patients might be associated with the enhancement of MS risk and higher severity of disease [3-4].

The results of the experimental and clinical studies indicated that vitamin D acts as an anti-inflammatory and immunomodulatory agent [5-6] through the vitamin D receptor (VDR) that is expressed by most of the immune cells [4]. Several studies have investigated the association of VDR gene polymorphisms with MS in various

ethnic populations and reported different results [7-11].

In our previous study, we demonstrated that the serum level of vitamin D (25[OH] D) was very low in the Iranian MS patients. The treatment of these patients with vitamin D (300,000 IU/month) for six months increased the serum levels of vitamin D; nevertheless, it did not affect the expanded disability status scale scores or the number of gadolinium-enhancing lesions. However, certain polymorphisms of the VDR gene, which affect the affinity of VDR in binding to vitamin D may alter the function of the activated form of vitamin D [12].

In spite of the high incidence of MS in the central parts of Iran, there is limited information on VDR gene single nucleotide polymorphisms (SNPs) in the Iranian MS patients. The SNPs is the

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most common type of genetic variation among the humans, which represents a difference in a single nucleotide. For example, a SNP may replace the nucleotide cytosine with the nucleotide thymine in a certain stretch of DNA.

Most commonly, these variations are found in the DNA located between the genes. They can act as biological markers, helping scientists locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they might play a more direct role in certain diseases by affecting the gene's function [13].

Due to the importance of SNPs, the aim of this study was to investigate the FokI, BsmI, ApoI, and TaqI polymorphisms of the VDR gene in the Iranian MS patients living in Markazi province, Iran.

## MATERIALS AND METHODS

### Patients

This bi-group cross-sectional study was conducted on 80 MS patients and 50 healthy individuals as control, who were matched for ethnicity, gender, and age within 2010-2012. The patients were diagnosed with MS using the McDonald criteria [14], and the diagnoses were confirmed by a neurologist. All the patients and controls were Iranian and residents of Arak, located in the central part of Iran.

### Genotype analysis

Blood samples were collected using EDTA tube, and the genomic DNA was extracted from peripheral blood leukocytes using DNG™-plus kit (Cat DN8118c, Sinaclone Company, Iran). Primer sequences were chosen based on the previous studies [11, 15]. The polymerase chain reaction (PCR) tests included cycles of initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55-60°C (depending on different SNPs) for 30 sec, extension at 72°C for 1 min, and final extension at 72°C for 5 min.

The amplification reactions were performed in a final volume of 50 µL using a Primus 96 (PiqLab, Germany) thermal cyclor. The reaction contained 200 µM deoxynucleotides, 10 pM of each primer, 0.8 mM MgCl<sub>2</sub>, 2.5 U Taq polymerase (Sinaclone, Iran), and 50 ng DNA templates. The PCR products were confirmed using 1% agarose gel electrophoresis and visualized by ethidium bromide staining. Subsequently, they were photographed with a UV transillumination camera. The amplification conditions of the gene fragments are illustrated in Table 1.

Then, restriction fragment length polymorphism (RFLP) testing was performed on the remaining product with Bsm I, Fok I, Apa I, and Taq I restriction enzymes (Thermo Scientific Company, USA). This step was performed using one unit of enzyme in the enzyme-specific buffer, sterile distilled water, and the PCR product. The mixtures were incubated at 37 °C for 16 h. The digested reaction products were electrophoresed in 8% polyacrylamide gel. Finally, the genotypes of the VDR gene polymorphisms were determined.

### Statistical analysis

The statistical analysis was performed using the SPSS version 16. The Chi-square test was employed to determine significant differences in polymorphisms frequencies for alleles and genotypes. Furthermore, the associations between the disease and genotypes were assessed by calculating odds ratios and 95% confidence intervals. Additionally, to analyze the association between the disease and VDR gene polymorphisms, the logistic regression was applied. P-value less than 0.05 was considered statistically significant.

### Ethical consideration

The study was performed considering the Helsinki declaration on human subject research. Additionally, the protocol of this study was approved by our Institutional Ethics Committee (AUMSEC-85-13/7).

**Table 1.** Amplification conditions of gene fragments based on the primers used in this study

Name of primer*	Sequences (5' to 3')	Annealing primer temperature (0 °C)	Fragment size for genotypes	References for primers
BsmI F	CAACCAAGACTACAAGTACCGCGTCAGTGA	58	BB 825	15
BsmI R	AACCAGCGGGAAGAGGTCAAGGG		Bb 825,650,175 bb 650,175	
FokI F	GATGCCAGCTGGCCCTGGCACTG	60	FF 272	11
FokI R	ATGGAAACACCTTGCTTCTTCTCCCTC		Ff 272,198,74 ff 198,74	
Apa I F	CAGAGCATGGACAGGGAGCAAG	59	AA 740	11
Taq I F			Aa 740,515,225 aa 515,225	
Apa I R	GCAACTCCTCATGGCTGAGGTCTCA		TT 490,250	11
Taq I R			Tt 490,250,290,205 tt 290,250,205	

\* One primer pair was used for ApaI and TaqI.

**Table 2.** Demographic and clinical characteristics of the multiple sclerosis patients and controls

	Multiple sclerosis patients (n=80)	Controls (n=50)
Male/Female	63/17	39/11
Age range (years)	18-60	18-60
Expanded disability status scale [mean(SD)]	2.1 (1.23)	-
Number of gadolinium enhancing lesions [mean(SD)]	1.5 (1.00)	-
Duration of disease (years) [mean(SD)]	4.15 (3.30)	-

**Table 3.** Distributions of vitamin D receptor variants in multiple sclerosis patients and control group

Enzyme	Groups	Genotype frequency N(%)			Allele frequency N(%)	
		B/B	B/b	b/b	B	b
Bsm I	MS	12 (15)	51(63.75)	17 (21.25)	75 (53.12)	85 (46.87)
	Control	16(32)	29( 58)	5(10)	61 (61)	39(39)
Fok I	MS	47 (58.8)	32 (40)	1(1.3)	126 (78.75)	34 (21.25)
	Control	20 (40)	26 ( 52)	4 (8)	66 (66)	34 (34)
Apa I	MS	22 (27.5)	53 (66.25)	5 (6.25)	97 (60.62)	63( 39.37)
	Control	23 (46)	22 (44)	5 (10)	68 (68)	32 (32)
Taq I	MS	38 (47.5)	41 (51.25)	1 (1.25)	117 (73.12)	43 (26.87)
	Control	14 (28)	34 (68)	2 (4)	62 (62)	38 (38)

MS: multiple sclerosis

## RESULTS

The age range of the patients was 18-60 Years and 63% of them were male. The details of demographic characteristics of the MS patients and control group are shown in Table 2.

According to the results of the study, there was no significant difference between the MS patients and control group in terms of the age and gender. The frequencies of BsmI, FokI, ApaI, and TaqI genotypes in the MS patients and the controls are displayed in Table 3. The genotypes were defined as A, B, T, and F (in the absence of restriction sites) or a, b, t, and f (in the presence of restriction sites).

The statistical analysis of the genotype prevalence demonstrated significant differences between the MS patients and controls regarding the BsmI and ApaI genotypes (OR=2.703, 95%CI: (1.08-6.75), P=0.023 and OR=2.489, 95%CI: (1.146-5.40), P=0.045, respectively). However, the genotype and allele distributions of the FokI and TaqI VDR gene polymorphisms did not differ significantly between the MS patients and controls (P>0.05).

## DISCUSSION

As the results of the present study indicated, the VDR BsmI and ApaI polymorphisms were associated with increased risk of MS in Markazi province of Iran.

The VDR is a transcription factor that responds to 1,25-dihydroxyvitamin D in a wide variety of tissues [16, 17]. The allelic variants and their

haplotypes have been widely studied as markers of predisposition to osteoporosis, breast cancer, prostate cancer [17], diabetes, and cardiovascular diseases [18].

Furthermore, there was no association between the MS patients and control group regarding the FokI and TaqI polymorphisms of VDR gene.

However, there is a controversy over the association between VDR gene polymorphisms and MS in some studies. Several clinical studies have indicated the protective effects of increased levels of 1,25 (OH) 2D3 against multiple sclerosis [19]. In addition, in our previous study and some other studies conducted on animal model of MS (i.e., experimental autoimmune encephalomyelitis [EAE]), vitamin D status was found to be associated with disease severity [3].

The administration of vitamin D to mice could prevent the initiation and progression of EAE, and conversely, vitamin D deficiency increased the susceptibility to EAE [20-22]. Although the recent studies have revealed the association between MS in EAE models and vitamin D, this cannot be generalized to the relationship between vitamin D and MS in the humans.

Considering the fact that in MS, the immune system attacks the body tissues, vitamin D may have immunomodulation effect on the immune responses [3].

There are several studies investigating the relationship between VDR polymorphisms and MS disease. In this regard, Fukazama et al. have reported a significant association between BsmI

polymorphism and the disease in Japanese patients [8]. Furthermore, in another study conducted on the Australian patients, a significant association was indicated between the TaqI polymorphism and MS [11].

However, this relationship was not observed in some other studies carried out in different geographical locations [22, 23]. In a study conducted in England, no relationship was reported between TaqI polymorphism and MS [10]. In addition, in a study conducted by Niksresht et al. in the southern regions of Iran, no difference was observed between the MS patients and healthy individuals regarding the genotype distribution of VDR BsmI polymorphism [24].

These differences between our findings and those of other studies can be due to the differences in the patients' race, sample size, research methods, and type of MS disease targeted in these studies.

Expanded disability status scale and number of gadolinium enhancing lesions was not possible to be investigated in the controls due to ethical issue and can be considered as study limitation.

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## CONCLUSION

The findings of the current study demonstrated that the VDR ApaI and BsmI polymorphisms seems to be more prevalent in the Iranian MS patients.

Given the controversy over the relationship of VDR gene polymorphism with MS in different populations, further controlled studies are recommended to investigate the association of VDR polymorphism and severity of disease in the MS patients.

## ACKNOWLEDGEMENTS

We would like to thank and appreciate all colleagues who helped us in conducting this study. This study was supported by a research grant (No. 389) from the Arak University of Medical Sciences, Arak, Iran.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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