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Predisposing role of vitamin D receptor (VDR) polymorphisms in the development of multiple sclerosis: A case-control study

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS) with a complex etiology. Given the Vitamin D receptor (VDR) gene, it is considered an outstanding risk component associated with MS. The aim of the present study has been to explore and emphasize the role of *Apa*I, *Bsm*I, *Taq*I and *Fok*I polymorphisms of VDR gene in susceptibility to MS in an Iranian case-control population including 160 patients and 150 healthy controls. All cases were clinically diagnosed with relapsing-remitting (RR) form, and the controls were age, gender, and race matched which were completely in agreement with the case group. PCR-R FLP was conducted for all the SNPs genotyping. The findings of the study showed a significant difference in allele frequency between the cases and controls for *Apa*I ($p < 0.0002$), *Bsm*I ($p < 0.0002$) and *Taq*I ($p < 0.0001$), while no significant difference was observed for *Fok*I ($P > 0.0125$). The results also showed that AA genotype polymorphism of *Apa*I and *Bsm*I (OR = 4.6 and OR = 2.52, respectively), CC genotype of TaqI (OR = 2.41) and AC genotype of *Apa*I (OR = 1.79) are associated with the disease status. Nevertheless, the results revealed the protective role of TT genotype of TaqI (ORs < 1), CC genotype of *Apa*I, and GG genotype of *Bsm*I (ORs < 1). VDR polymorphisms seem to have a notable connection with MS pathogenesis, however, study of more big population and functional work on the gene structure and its function are recommended.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory, an autoimmune and demyelinating disease of the central nervous system (CNS). It is estimated that over 2.1 million people throughout the world suffer from MS episodes [1]. The overall prevalence of MS in Asia is approximately 51.5 per 100,000 people and the average age of onset is 28.54 years [2]. According to the latest data, MS prevalence in Iran is 45 per 100,000 people and age of onset has decreased. Another fact is that female patients are in majority in Iran [3].

Although the exact etiology of MS is not clear, it seems to be developed as the result of complex interaction between genetic and environmental factors, whether synergistically or independently [4]. So far, association and linkage studies have shown that the human leukocyte antigen (HLA) locus is the most powerful predisposing genetic factor to MS, but it cannot fully explain the genetic etiology of MS [5]. None-HLA genes including vitamin D receptor play an important role in susceptibility to the disease and a large number of variants has been identified through genome-wide association studies (GWAS) [6–8].

Vitamin D, as the environmental factors, is considered a causative factor in the pathogenesis of neurodegenerative diseases such as MS [9]. Several studies regarding to relation of serum vitamin D levels with MS have shown that 25 (OH) D and 1, 25 $(OH)_2$ D are considerably lower in MS subjects than in healthy people [10,11]. In an alternative study, treatment of a mouse model of MS with the vitamin D metabolites significantly improved the course and progression of the disease [12]. On the other hand, Vitamin D serves as both a regulator of the immune system and an anti-inflammatory factor in the body [13]. It has an inverse relationship with proinflammatory interferon gamma (IFN-ϒ) production, while it shows a direct connection with anti-inflammatory factors including IL-4, IL-5 and IL-10 [14]. Also, vitamin D changes the *T*-lymphocyte proliferation, especially by regulating *T*-regulatory cells

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[15]. Hence, the incorrect action and deficient levels of vitamin D is likely to influence neuroprotective and immunomodulatory events, and lack of adequate amount of vitamin D plays a role in the development of MS [16].

Vitamin D is principally produced from 7-dehydrocholesterol after exposure to sunlight and through the direct dietary intake. The active form of Vitamin D (1, 25-dihydroxyvitamin D_3) exerts its functions by binding to the nuclear vitamin D receptor (VDR). VDR gene is located on 12q13.1 and consists of 9 exons and 8 introns encoding a polypeptide belonging to the steroid and thyroid hormone receptors family. VDR works as homodimers (ADR/ADR) or heterodimers (VDR/RXR) and regulates the downstream targets involved in variety of biological activities like immune response through specific binding to the VDR response element in the promoter region of under control genes [17]. Thus, the defect in this gene can disrupt the function of vitamin D and consequently increase the risk of autoimmune diseases including MS. Experimental studies have also confirmed that the knocking out of VDR is critical for autoimmune encephalomyelitis [15].

A lot of studies have been carried out on the association of VDR gene variants with autoimmune disorders such as MS, which showed contradictory results [17]. Single nucleotide polymorphisms (SNPs) may alter VDR function and change the susceptibility of individuals to MS. Therefore, more studies need to be conducted in different populations to gather more information on VDR role and these variants which are different among individuals. Accordingly, the purpose of the current study is to explore the association of the four SNPs located in VDR gene including *Bms*I (rs1544410), *Apa*I (rs7975232), TaqI (rs731236) and *Fok*I (rs10735810) with MS in an Iranian population.

2. Materials and methods

2.1. Patients

The current study has been approved by the Research Ethic Committee of Tehran University of Medical Science (Tehran, Iran) and a signed informed consent form (according to the Declaration of Helsinki) was taken from all the patients. The patients of the study consisted of 160 people confirmed with McDonald criteria referred to Imam Khomeini Hospital (Tehran, Iran). One hundred and fifty (150) matched healthy controls without any family history of neurologic and systemic disorder were selected for the study.

2.2. DNA extraction and SNPs genotyping

Peripheral blood was taken from each of the participants and DNA extraction was performed with High Pure PCR Template Preparation Kit (Roche Applied Science, USA). All the SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers sequences designed for amplifying the target DNA containing *Apa*I (rs7975232), *Bsm*I (rs1544410), *Taq*I (rs731236) and *Fok*I (rs222870) polymorphisms are shown in Table 1.

PCR reactions of final volume of 25 μl were run in Veriti® Thermal Cycler (Applied Biosystems, USA) as follows: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 58 (*Apa*I), 57 (*Bsm*I), 62 (*Fok*I) and 60 °C (*Taq*I), extension at 72 °C for 30 s and a final extension at 72 °C for 7 min. After amplification, each PCR product was digested with the specific restriction enzymes and digested products were then electrophoresed on 2% agarose gel. The genotypes of all the SNPs were determined based on digestion pattern.

3. Statistical analysis

Data were analyzed using SPSS Software version 16.0 (Chicago, IL) and allelic and genotype distribution between the cases and the control subjects were calculated for deviation from Hardy-Weinberg Equilibrium. Chi-square ($\chi 2$) and independent sample T-tests were used to evaluate differences in allelic and genotype distribution between the patients and healthy controls. Descriptive statistics were reported as the mean ± the standard deviation and as the median (minimum–maximum) for normally and non-normally distributed variables. Since four SNPs were tested, *P*-values less than 0.0125 were considered statistically significant. Odds ratio (OR) and 95% confidential interference (95% CI) were determined for genetic risk of each allele and genotype.

4. Results

In the present study, 160 MS patients (120 females and 40 males) with a mean age of 35.9 ± 2.3 years and 150 healthy subjects (112 females and 38 males) with a mean age of 36.8 ± 1.8 years were genotyped for mentioned VDR gene polymorphisms. Patients and controls were not significantly different in age and sex ($P > 0.05$). The frequency of each genotype and allele are shown in Tables 2 and 3. The healthy controls and patients were also in Hardy-Weinberg equilibrium for all the polymorphisms ($p > 0.0125$).

As it has been shown in Tables 2 and 3, *Apa*I (rs7975232) allele and genotype frequencies showed highly significant difference between the healthy subjects and the patients. Positive association was observed in AC ($P = 0.016$; OR = 1.79, 95% CI in 1.12–2.88) and AA ($P = 0.006$; OR = 4.6, 95% CI in 1.52–14.00) genotypes with MS, while there was no significant relationship between the CC genotype and the disease. Allele frequency of A allele of *Apa*I was also significantly higher in the cases than in the controls (*P*-value < 0.0001; OR = 2.31, 95% CI in 1.58–3.39); it can be concluded that allele A is a predisposing allele for MS. For SNP of *Bsm*I (rs1544410), there were also significant differences in the allelic and genotype frequencies (excluding heterozygous AG genotype, $P = 0.2$). For this SNP, combination of AG and AA (AG + AA) against GG yielded an OR = 1.92 for MS (95% CI = 1.21–3.06, $P = 0.006$). AA genotype ($P = 0.005$; OR = 2.52, 95% CI in 1.31–4.83) and allele A frequency ($P = 0.0002$; OR = 1.88, 95% CI in 1.35–2.60) of *Bsm*I polymorphism were higher in MS patient in comparison to the controls and showed association with the disease. GG genotype (OR = 0.45, 95% CI in 0.28–0.72) and allele G (OR = 0.53, 95% CI in 0.38–0.73) showed negative association (protective) with MS. Like *Bsm*I polymorphisms, genotype and allele frequencies of *Taq*I showed significant differences between the healthy subjects and patients (excluding heterozygous TC, $P = 0.4$). The homozygote CC genotype

Table 1
Primer sequences used to amplify the target fragments containing *Apa*I, *Bsm*I, *Taq*I and *Fok*I polymorphisms.

*Apa*I	**Forward: 5′CTGCCGTTGAGTGTCTGTGT3′**
	Reverse: 5′TCGGCTAGCTTCTGGATCAT3′
*Bsm*I	**Forward**: **5′GGGAGACGTAGCAAAAGGAG3′**
	Reverse: **5′CCATCTCTCAGGCTCCAAAG3′**
*Taq*I	**Forward**: **5′CCCATGAAGCTTAGGAGGAA3′**
	Reverse: **5′TCATCTTGGCATAGAGCAGGT3′**
*Fok*I	**Forward**: **5′CTGGCACTGACTCTGGCTCT3′**
	Reverse: **5′TGCTTCTTCTCCCTCCCTTT3′**

Table 2
Distribution of *Apa*I, *Bsm*I, *Taq*I and *Fok*I alleles between the patients and healthy controls.

Polymorphism	Allele	MS patients no.	Controls	*P*-value	Odds ratio (95% CI)
*Apa*I	C (%)	217 (67)	249 (83)	<0.0001	0.43 (0.30–0.63)
	A (%)	103 [33]	51 (17)	<**0.0001**	2.31 (1.58–3.39)
*Bsm*I	G (%)	171 (53)	205 (68)	0.0002	0.53 (0.38–0.73)
	A (%)	149 (47)	95 (32)	<**0.0002**	1.88 (1.35–2.60)
*Fok*I	T (%)	96 (30)	81 (27)	0.41	1.15 (0.82–1.64)
	C (%)	224 (70)	219 (73)	0.41	0.86 (0.61–1.22)
*Taq*I	T (%)	156 (53)	195 (65)	0.0001	0.51 (0.37–0.70)
	C (%)	164 (47)	105 (35)	<**0.0001**	1.95 (1.41–2.69)