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The association between vitamin D receptor polymorphisms and multiple sclerosis in a Turkish population

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system (CNS). Genetic and environmental factors are important in disease development. Many studies have investigated the relationship between MS and *VDR* polymorphisms. VDR gene polymorphism has not been previously studied in Turkish MS patients. We aimed to investigate the relationship between MS and VDR genotypes Taq I, Apa I and Fok I polymorphisms in a Turkish population.

Methods: 167 MS patients and 146 healthy control subjects were included in the present study. MS and the *VDR* *Taq*I *(rs731236)*, *Apa*I *(rs7975232)*, and *Fok*I (rs2228570) polymorphisms were investigated.

Results: The study enrolled 167 patients (121 females, 46 males) with MS and 146 healthy individuals (88 females, 58 males). The frequency of only the *Fok I* polymorphism differed significantly between the two groups ($p = 0.002$). The *TaqI* (rs731236) and *ApaI* (rs7975232) genotype distributions were not significantly different between MS patients and healthy controls ($p = 0.626$ and $p = 0.990$, respectively). Also there were no significant gender difference between patients and controls for *Taq I* and *Apa I*.

Conclusion: In conclusion, we found a significant association between MS and the *Fok*I polymorphism in our region of Turkey. However, the results may be different in other populations. More epidemiological and genetic studies are needed to explain the association between genetic factors and MS.

1. Introduction

Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system (CNS) that develops in young adults (Sioka et al., 2009). Although the etiology of the disease remains poorly understood, it is thought that both autoimmune and infectious mechanisms play a role. Genetic and environmental factors are important in disease development (McFarland and Martin, 2007). Viral infections, smoking, and Vitamin D deficiency have been described as environmental risk factors for MS (Bettencourt et al., 2017). Auto-reactive T cells and antibodies against the CNS are believed to play a major pathogenic role in the development of inflammation and tissue damage (McFarland and Martin, 2007).

Vitamin D is a steroid molecule that has regulatory and functional effects in humans. Vitamin D is important in the development of the nervous system, and the active form of vitamin D has extensive immunomodulatory and anti-inflammatory functions (Bettencourt et al., 2017; Adorini and Penna, 2008; Zella and DeLuca, 2003). It plays a regulatory role within the immune system by reducing the presentation of major histocompatibility complex II by monocytes and T cells. Vitamin D also reduces T cell proliferation and pro-inflammatory cytokine release (Gorman et al., 2007). MS patients were reported to have lower serum vitamin D levels than healthy controls. Vitamin D also has positive effects in modulating the risk of MS development (Zhang et al., 2017).

Vitamin D exerts effects on the immune system by binding to the nuclear vitamin D receptor (VDR). Specific variants of the *VDR* gene are associated with alterations in vitamin D function and metabolism (Ates et al., 2011). Many studies have investigated the relationship between MS and *VDR* polymorphisms (Smolders et al., 2009; Harada and Nakanuma, 2007; Fukazawa et al., 1999; Lotti et al., 2005; Dickinson et al., 2009; Simon et al., 2010; Cox et al., 2012; Sioka et al., 2011). Typically, studies have investigated associations between MS and the *Taq*I, *Apa*I, *Fok*I, and *Bsm*I polymorphisms, which are the most common

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single nucleotide polymorphisms (SNPs) of the *VDR* gene.

An Australian case–control study found significant differences in the frequencies of the *Taq*I and *Apa*I polymorphisms in MS patients (Tajouri et al., 2005). Cox et al. found that the *Taq*I polymorphism increased the risk of MS development (Cox et al., 2012). In a Tunisian study of 60 patients with relapsing–remitting MS and 114 controls, the T allele of the *Taq*I polymorphism was found to protect against the development of MS in an age- and gender-specific manner (Ben-Selma et al., 2015). The association of VDR polymorphisms with MS has been investigated in many countries, but the results are still equivocal.

The frequencies of these polymorphisms have not been studied in a Turkish population. Therefore, we investigated the association between MS and the *VDR Taq*I *(rs731236)*, *Apa*I *(rs7975232)*, and *Fok*I (rs2228570) polymorphisms in people living in Malatya, Turkey.

2. Materials and methods

2.1. Subjects

This case–control study enrolled 167 Turkish MS patients and 146 age- and sex-matched healthy control individuals. Patients were recruited from the Neurology Department of the Medical School of Inonu University, and molecular analyses were carried out in the Department of the Molecular Biology and Genetics. All MS patients had diagnoses confirmed using the McDonald criteria. The study was performed in accordance with the guidelines of the Declaration of Helsinki and was approved by our local ethics committee. All participants were fully informed about the study procedures and gave written consent before the study started.

2.2. DNA isolation and genotyping

Genomic DNA was extracted from venous blood stored with an anticoagulant using a commercial kit (PureLink® Genomic DNA Mini Kit; Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. We coded all blood and DNA samples to ensure anonymity. All samples were genotyped using the TaqMan® SNP Assay (Applied Biosystems, Foster City, CA, USA): C_12060045_20 for *rs2228570*, C_2404008_10 for *rs731236*, and C_28977635_10 for *rs7975232*. We used the StepOnePlus™ Real-Time PCR System (Applied Biosystems,).

2.3. Statistical analysis

SPSS (ver. 17.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analyses. Data were summarized using numbers and percentages. Hardy–Weinberg equilibrium was approximated by the chi-square distribution with one degree of freedom. Differences between groups by allelic and genotypic distribution were analyzed using Pearson's exact test or Pearson's chi-square test. In all comparisons, the significance level was considered to be 0.05.

3. Results

The study enrolled 167 patients (121 females, 46 males) with MS and 146 healthy individuals (88 females, 58 males). The demographic and clinical data are summarized in Table 1. Tables 2–5 show the VDR SNP genotypes and allelic distributions.

The frequency of only the *Fok*I polymorphism differed significantly between the two groups ($p = 0.002$). *Fok*I differed significantly between both female and male patients and controls ($p = 0.048$).

Each SNP was analyzed separately for its association with MS risk and the study variables. The *TaqI* (rs731236) and *ApaI* (rs7975232) genotype distributions were not significantly different between MS patients and healthy controls ($p = 0.626$ and $p = 0.990$, respectively). There were no significant gender differences between patients and controls for *Taq*I and *Apa*I.

Table 1
Demographic characteristics of patients and controls.

	Patients (n = 167)	Controls (n = 146)
Age (years)	39.96 ± 9.46	33.81 ± 7.12
Gender (male/female, n)	46/121	58/88

4. Discussion

Vitamin D is a potential modulator of the immune system and many studies have explored the vitamin D status of patients with MS (Zhang et al., 2017; Fernandes de Abreu et al., 2009). Vitamin D binds to VDR and modulates the transcription of target genes in response to 1,25 (OH) 2D3 (Amital, 2013). The *VDR* gene is a pleiotropic gene that is associated with multiple autoimmune and allergic diseases. Its polymorphisms affect the structure and function of vitamin D. Many studies have investigated the relationship between VDR polymorphisms and MS (Smolders et al., 2009; Harada and Nakanuma, 2007; Fukazawa et al., 1999; Lotti et al., 2005; Dickinson et al., 2009; Simon et al., 2010; Cox et al., 2012; Sioka et al., 2011). The *Taq*I, *Apa*I, *Fok*I, and *Bsm*I polymorphisms are the most studied SNPs in MS patients (Zhang et al., 2017; Sioka et al., 2011; Tajouri et al., 2005; Ben-Selma et al., 2015).

The relationship between MS and the *VDR Bsm*I polymorphism was first studied in 1999 in Japan (Fukazawa et al., 1999), and then in several other studies (Sioka et al., 2011; Tajouri et al., 2005; Ben-Selma et al., 2015; Ban and Taniyama, 2000; Al-Temaimi et al., 2015; Uitterlinden et al., 2004; Steckley et al., 2000). Simon et al. (2010) studied 214 patients and 428 controls in the United Kingdom and found that patients with the *Fok*I polymorphism and low vitamin D intake were at high risk of developing MS. The frequencies of the *Taq*I and *Apa*I polymorphisms did not differ in a Canadian study (Steckley et al., 2000). Similarly, the frequencies of the *Bsm*I and *Taq*I polymorphisms did not differ in a Greek study (Sioka et al., 2011). (Dickinson et al. 2009) studied 136 MS patients and 235 controls and found no relationship between any of the *Fok*I or *Taq*I polymorphisms and MS. The same study found a relationship between the G allele and a reduced MS risk in patients exposed to the sun for fewer than 2 h/day, suggesting that the association between *VDR* gene polymorphisms and MS may be attributable to historical sunlight exposure. In a recent study in Portugal, Bettencourt et al. (2017) found an association between the *Fok*I ff genotype and MS susceptibility .

To our knowledge, ours is the first study to explore the association of MS with *Taq*I, *Apa*I, and *Fok*I polymorphisms in a Turkish population. In this study, the frequency of the *Fok*I polymorphism differed significantly between controls and patients ($p = 0.002$). It seems likely that this difference is caused by the decrease in the frequency of the GG genotype in patients, suggesting that it protects against individuals' developing MS. This difference was also supported by the differences in allele frequencies ($p = 0.001$). When the analysis was done for the two genders, the allele frequencies differed significantly in both females and males ($p = 0.016$ for both) compared with controls. However, the *Taq*I and *Apa*I polymorphism analyses did not show a significant difference between MS patients and controls. There was also no significant difference between genders for the *Taq*I and *Apa*I polymorphisms. *Bsm*I, *Apa*I and *Taq*I polymorphisms are in the intron location between exon 8 and 9 of the VDR gene, they do not cause the structural change of VDR protein. However, the *Fok*I polymorphism is located in exon 2 and it might alterate the VDR protein structure and transcriptional activity (Chen et al., 2017).

The association of VDR polymorphisms with MS has been investigated in many countries, but the results are still equivocal. Two previous meta-analyses performed by Huang et al. (2012) and Garcia-Martin et al. (2013) showed that *VDR* gene polymorphisms were not associated with susceptibility to MS. However, a recent meta-analysis

Table 2
VDR SNP genotypes and allelic distributions.

Taq I	AA	AG	GG	p	Hardy-Weinberg	A	G	p
	n (%)	n (%)	n (%)		p	n (%)	n (%)	
Controls	59(40,4)	65 (44,5)	22 (15,1)	0,626	0,558	183(62,7)	109(37,3)	0,451
Patients	71(42,5)	77 (46,1)	19 (11,4)		0,784	219(65,6)	115(34,4)	
Fok I	AA	AG	GG	p	Hardy-Weinberg	A	G	
	n (%)	n (%)	n (%)		p	n (%)	n (%)	
Controls	6 (4,1)	46 (31,5)	94 (64,4)	**0,002**	0,901	58 (19,9)	234(80,1)	**0,001**
Patients	15 (9,0)	77 (46,1)	75 (44,9)		0,447	107(32,0)	227(68,0)	
Apa I	AA	AC	CC	p	Hardy-Weinberg	A	C	
	n (%)	n (%)	n (%)		p	n (%)	n (%)	
Controls	54(37,0)	67 (45,9)	25 (17,1)	0,997	0,591	175(59,9)	117(40,1)	0,990
Patients	62(37,1)	76 (45,5)	29 (17,4)		0,495	200(59,9)	134(40,1)	

Table 3
*Fok*I genotype and allelic distributions for gender.

Fok I Female	AA	AG	GG	p	A	G	p
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	4 (4,5)	30(34,1)	54(61,4)	**0,048**	38(21,6)	138(78,4)	**0,016**
Patients	11(9,1)	56(46,3)	54(44,6)		78(32,2)	164(67,8)	
Fok I Male	AA	AG	GG	p	A	G	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	2 (3,4)	16(27,6)	40(69,0)	**0,049**	20(17,2)	96 (82,8)	**0,016**
Patients	4 (8,6)	21(45,7)	21(45,7)		29(31,5)	63 (68,5)	

Table 4
*Apa*I genotype and allelic distributions for gender.

Apa I Female	AA	AC	CC	p	A	C	p
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	34(38,7)	39(44,3)	15(17,0)	0,867	107(60,8)	69 (39,2)	0,603
Patients	44(36,4)	53(43,8)	24(19,8)		141(58,3)	101(41,7)	
Apa I Male	AA	AC	CC	p	A	C	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	20(34,5)	28(48,3)	10(17,2)	0,641	68 (58,6)	48 (41,4)	0,418
Patients	18(39,1)	23(50,0)	5 (10,9)		59 (64,1)	33 (35,9)	

Table 5
*Taq*I genotype and allelic distributions for gender.

Taq I Female	AA	AG	GG	p	A	G	p
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	37(42,0)	37(42,0)	14(16,0)	0,511	111(63,1)	65(36,9)	0,578
Patients	51(42,2)	57(47,1)	13(10,7)		159(65,7)	83(34,3)	
Taq I Male	AA	AG	GG	p	A	G	
	n (%)	n (%)	n (%)		n (%)	n (%)	
Controls	22(37,9)	28(48,3)	8 (13,8)	0,846	72 (62,1)	44(37,9)	0,640
Patients	20(43,5)	20(43,5)	6 (13,0)		60 (65,2)	32(34,8)	

by Tizaoui et al. (2015) showed that the *Apa*I and *Fok*I VDR polymorphisms were significantly associated with the pathogenesis of MS. Two new meta-analyses were recently reported by Zhang et al. (2017) and Chen et al. (2017). Zhang et al. (2017) showed that the *Bsm*I, *Fok*I, *Apa*I, and *Taq*I VDR polymorphisms did not play a role in the pathogenesis of MS, whereas Chen et al. (2017) found that the *Taq*I polymorphism was directly associated with MS risk. The results are still contradictory. Ethnic differences, geographical features, other genetic or environmental interactions, small sample sizes, and clinical heterogeneity may be the reasons (Zhang et al., 2017; Tizaoui et al., 2015).

In conclusion, our preliminary results suggest that the VDR gene *Fok*I polymorphism is associated with an elevated MS risk in our region in Turkey. Our study limitations are that we did not examine the relationship between these results and Vitamin D levels, we did not evaluate the BSMI polymorphism, and our sample size was relatively small. MS is a complex disease, and multiple genetic factors interact with each other and with diverse environments. Future epidemiological and genetic studies that examine gene–environment interactions are needed to determine the risk of MS.

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Conflicts of interest

none.

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