Vitamin D Receptor Polymorphisms in the Turkish Population are not associated with Multiple Sclerosis

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Abstract

Background Multiple sclerosis (MS) is an inflammatory disease characterized by demyelination and axonal degeneration affecting the central nervous system (CNS). Unfortunately, very little is known about the etiology of this disease. Among the genetic factors suggested to be associated with this disease are polymorphisms to the vitamin D receptor (VDR). However, there is disagreement in the literature on this topic. Therefore, we tested the hypothesis that polymorphisms in the vitamin D receptor (VDR) are also associated with MS.

Aim The aim of the study was to investigate the relationship of MS disease with VDR Fok-I, Bsm-I and Taq-I polymorphisms in Turkish population.

Method This study contains 175 MS patients and 183 healthy controls. Blood samples were taken from each subject to isolate genomic DNA by salting out method. VDR gene Fok-I, Bsm-I and Taq-I polymorphism regions for each patient and control were amplified by polymerase chain reaction (PCR). The PCR products were digested, and the genotypes were determined based on size of digested PCR products.

Results No correlation was found between MS disease and VDR Fok-I, Bsm-I and Taq-I polymorphisms for genotype distribution (Pearson test, p>0,05) or allele frequency (Pearson test, p>0,05). No meaningful association was found between subtypes of MS for genotypes distribution and allele frequencies of Fok-I, Bsm-I and Taq-I polymorphisms (Pearson test, p>0,05).

Conclusion There is no association between VDR gene polymorphisms (Fok-I, Bsm-I and Taq-I) and MS disease in Turkish population.

1. Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease, which leads to demyelination and neurodegeneration on the central nervous system [1, 2]. The disease generally affects young adults and causes serious neurological disabilities [3, 4]. Focal demyelination, inflammation, scar formation and various axonal degeneration are involved in the pathology of MS lesions [5–7] and the axonal degeneration is the main reason for non-reversible disability in these patients [8]. While the etiology of MS is not fully understood, environmental, genetic, and geographical factors may play a part in the etiology [8–10]. Specific environmental/metabolic factors including, Epstein Barr virus, seasonality in MS patients' birth, sun exposure, vitamin D levels and cigarettes have been shown to influence epidemiologic patterns in MS [11]. The differences in susceptibility to MS despite of the same environmental exposures indicates the importance of genetical factors in the development of pathogenesis [8]. In recent years, genetic studies suggest that a single susceptible locus is not sufficient to lead to MS, and that MS is a heterogeneous disease [12–13]. Therefore, it is likely that multiple genes mutations are needed to affect the course of this disease [14]. Major gene regions that are associated with the MS susceptibility are located at the major histocompatibility complex (MHC) which is also called the HLA-DRB1*15 haplotype. The promoter region of HLA-DRB1 gene contains a vitamin D response element (VDRE) which is important for gene expression of HLA-DRB1. Variants in the vitamin D receptor (VDR) gene affect the MS susceptibility by the way of changing the interaction of VDRE on the MHC regulatory region [15]. Thus, vitamin D may play an important role in MS.

Furthermore, vitamin D has been shown to impact immunomodulation in the MS pathogenesis [16–17]. The usage of the active form of vitamin D in experimental MS and experimental autoimmune encephalomyelitis (EAE) animal studies was shown to be beneficial [18–19]. Additionally, it has been shown that the VDR gene has an critical role for activity of EAE in rat studies [20]. These findings suggest that VDR and its ligand have immunosuppressive and anti-inflammatory effects which affect MS susceptibility [1, 21]. Finally, there is an inverse correlation between vitamin D blood levels and MS prevalence [22]. Taken together, these studies indicate that vitamin D (or lack thereof) may play an important role in MS.

In addition to vitamin D, the vitamin D receptor (VDR) is hypothesized to play a role in MS; however, this is a controversial topic. Various Single nucleotide polymorphisms (SNP) including Apa-I (rs7975232), Bsm-I (rs1544410), Fok-I (rs2228570), and Taq-I (rs731236) in VDR gene have been investigated for MS susceptibility and are thought to be associated with MS disease [8, 21, 37]. However, these results are inconclusive and there is disagreement among these finding [8, 23]. Several studies indicate that VDR gene polymorphisms are associated with susceptibility to MS [25–27]. Furthermore, these polymorphisms in the VDR gene may change the vitamin D serum levels, vitamin D structure and function such as immune modulatory effect, the mechanisms of the vitamin D and VDR complex [23]. In contrast, several studies suggest that these polymorphisms are not associated with MS as indicated by VDR-mRNA expression or active vitamin D induced target gene expression [8, 24].

Since there is disagreement in the literature, the aim of the current study was to investigate the relationship between the VDR Fok-I (rs2228570) T/C, Bsm-I (rs1544410) G/A, Taq-I (rs731236) T/C polymorphisms and MS disease in Turkish population.

2. Material And Methods

2.1. Patients and controls

A total of 358 participants from the Turkish population were enrolled in the study. Of the 358 participants, 175 were diagnosed with MS (2 PPMS, 120 RRMS and 53 SPMS) and 183 individuals served as healthy controls. All patients referred to Goztepe Training and Research Hospital and were clinically diagnosed with MS according to the McDonald's criteria [28]. A blood sample was collected from each person in order to obtain genomic DNA. The study protocol and consent was approved by Marmara University Medical School Clinical Researches Ethic Committee. Written informed consent was obtained from all of the participants and there was no patient or control younger than at age of 16 in the study.

2.2. Genotyping of polymorphisms

Genomic DNA was extracted by using salting out method as previously described [29]. Polymorphism regions Fok-I (rs2225870), Bsm-I (rs1544410) and Taq-I (rs731236) were amplificated by polymerase chain reaction (PCR) (Techne Tc312) using with specific primers in the Table 1. PCR was carried by total volume of 25 µl reaction containing 0,5 µg of genomic DNA, 2,5 µl 10x buffer, 1,5 mM $MgCl_2$, 0,5 µM forward primer, 0,5 µM reverse primer, 0,2 mM dNTP and 0,5 U Taq polymerase. The PCR sample were denatured at 94 °C for 3 min (1x) for initial denaturation and main pcr cycle is for denaturation at 94 °C for 30 secs, annealing at 69 °C for 30 secs, extension at 72 °C for 45 secs (all cycle 40x) and final extension was done at 72 °C for 10 min (1x) (annealing 69 °C for Fok-I, 66 °C for Bsm-I and 68 °C for Taq-I). The PCR products were digested by Fok-I, Bsm-I and Taq-I restriction enzymes (CutSmart, New England Biolabs inc.). 10 µl PCR product mixed with 5 U restriction enzyme, 3 µl 10X reaction buffer and incubated at 37 °C overnight for Fok-I, at 65 °C for 3 hours for Bsm-I, at 65 °C for 3 hours for Taq-I. The digested PCR products were run on 1,5% agarose gel electrophoreses and genotyping was determined based on fragments size of digested pcr products. Digestion of Fok-I gives C/C (343 bp for homozygote mutant), T/C (343 bp, 267 bp, 76 bp for heterozygote) and T/T (267 bp, 76 bp for homozygote wild type). The digestion of Bsm-I gives A/A (531 bp for homozygote mutant), G/A (531 bp, 329 bp, 202 bp for heterozygote) and G/G (329 bp, 202 bp for homozygote wild type). The digestion of Taq-I gives T/T (479 bp for homozygote wild type), C/T (479 bp, 294 bp, 185 bp for heterozygote) and C/C (294 bp, 185 bp for homozygote mutant).

Table 1
Primers used for amplification of polymorphism sites on the VDR gene.

Polymorphism	Forward Primer	Reverse Primer
Fok-I (rs2228570)	AGGATGCCAGCTGGCCCTGGCAC	TGGCTGTGAGCGCCGCATGTTCCATG
Bsm-I (rs1544410)	TCCTTGAGCCTCCAGTCCAGG	GCAACCTGAAGGGAGACGTAGC
Taq-I (rs731236)	AGAGCATGGACAGGGAGCAAGGC	TAGCTTCATGCTGCACTCAGGCTGG

2.3. Statistical analysis

Comparison of genotype or allele between MS and control or MS subtypes were determined by using Pearson's chi-square test. Values of $p < 0,05$ was considered as significant. Data was analyzed with SPSS 21.0 program.

3. Results

VDR gene polymorphisms (Fok-I, Taq-I and Bsm-I) were determined in both MS and healthy people in Turkish population. The distribution of the genotypes of Bsm-I, Fok-I, and Taq-I polymorphisms between MS/MS subtypes and control groups were shown in Table 2a, Table 3a and Table 4a respectively. Chi-square tests were performed for distribution of VDR gene polymorphisms across MS/MS subtypes and control groups (Table 2a, Table 3a, Table 4a).

Table 2 a-b: Genotype distribution and allele frequency of VDR Bsm-I polymorphism in MS patients and healthy controls.

a)

Bsm-I Genotype					
	G/G	G/A	A/A	Total	*p*
MS-Control					
Control	37,2% (n=68)	47,0% (n=86)	15,8% (n=29)	100% (n=183)	*0,665*
MS	34,9% (n=61)	45,7% (n=80)	19,4% (n=34)	100% (n=175)	
Total	36,0% (n=129)	46,4% (n=166)	17,6% (n=63)	100% (n=358)	
MS subtypes					
Control	37,2% (n=68)	47,0% (n=86)	15,8% (n=29)	100% (n=183)	*0,460*
RRMS	35,0% (n=42)	49,2% (n=59)	15,8% (n=19)	100% (n=120)	
SPMS	34,0% (n=18)	37,7% (n=20)	28,3% (n=15)	100% (n=53)	
PPMS	50,0% (n=1)	50,0% (n=1)	0,0% (n=0)	100% (n=2)	
Total	36,0% (n=129)	46,4% (n=166)	17,6% (n=63)	100% (358)	

b)

Bsm-I Allele				
	G	A	Total	*p*
MS-Control				
Control	60,7% (n=222)	39,3% (n=144)	100% (n=366)	*0,423*
MS	57,7% (n=202)	42,3% (n=148)	100% (n=350)	
Total	59,2%(n=424)	40,8% (n=292)	100% (n=716)	
MS subtypes				
Control	60,7% (n=222)	39,3% (n=144)	100% (n=366)	*0,470*
RRMS	59,6% (n=143)	40,4% (n=97)	100% (n=240)	
SPMS	52,8% (n=56)	47,2% (n=50)	100% (n=106)	
PPMS	75,0% (n=3)	25,0% (n=1)	100% (n=4)	
Total	59,2% (n=424)	40,8% (n=292)	100% (n=716)	

Table 3 a-b: Genotype distribution and allele frequency of VDR Fok-I polymorphism in MS patients and healthy controls.

a)

Fok-I Genotype					
	T/T	T/C	C/C	Total	*p*
MS-Control					
Control	9,8% (n=18)	37,2% (n=68)	53,0% (n=97)	100% (n=183)	*0,956*
MS	10,3% (n=18)	38,3% (n=67)	51,4% (n=90)	100% (n=175)	
Total	10,1% (n=36)	37,7% (n=135)	52,2% (n=187)	100% (n=358)	
MS subtypes					
Control	9,8% (n=18)	37,2% (n=68)	53,0% (n=97)	100% (n=183)	*0,892*
RRMS	10,8% (n=13)	37,5% (n=45)	51,75 (n=62)	100% (n=120)	
SPMS	9,4% (n=5)	41,5% (n=22)	49,1% (n=26)	100% (n=53)	
PPMS	0,0% (n=0)	0,0% (n=0)	100%,0 (n=2)	100% (n=2)	
Total	10,1% (n=36)	37,7% (n=135)	52,2% (n=187)	100% (n=358)	

b)

Fok-I Allele				
	T	C	Total	*p*
MS-Control				
Control	28,4% (n=104)	71,6% (n=262)	100% (n=366)	*0,765*
MS	29,4% (n=103)	70,6% (n=247)	100% (n=350)	
Total	28,9% (n=207)	71,1% (n=509)	100% (n=716)	
MS subtypes				
Control	28,4% (n=104)	71,6% (n=262)	100% (n=366)	*0,613*
RRMS	29,6% (n=71)	70,4% (n=169)	100% (n=240)	
SPMS	30,2% (n=32)	69,8% (n=74)	100% (n=106)	
PPMS	0,0% (n=0)	100,0% (n=4)	100% (n=4)	
Total	29,0% (n=207)	71,0% (n=509)	100% (n=716)	

Table 4 a-b: Genotype distribution and allele frequency of VDR Taq-I polymorphism in MS patients and healthy controls.

a)

Taq-I Genotype					
	C/C	C/T	T/T	Total	*p*
MS-Control					
Control	14,2% (n=26)	45,9% (n=84)	39,9% (n=73)	100% (n=183)	*0,481*
MS	18,9% (n=33)	42,3% (n=74)	38,9% (n=68)	100% (n=175)	
Total	16,5% (n=59)	44,1% (n=158)	39,4% (n=141)	100% (n=358)	
MS subtypes					
Control	14,2% (n=26)	45,9% (n=84)	39,9% (n=73)	100% (n=183)	*0,855*
RRMS	18,3% (n=22)	44,2% (n=53)	37,5% (n=45)	100% (n=120)	
SPMS	20,8% (n=11)	37,7% (n=20)	41,5% (n=22)	100% (n=53)	
PPMS	0,0% (n=0)	50,0% (n=1)	50,0% (n=1)	100% (n=2)	
Total	16,5% (n=59)	44,1% (n=158)	39,4% (n=141)	100% (n=358)	

b)

There were no significant differences of Bsm-I (Table 2a), Fok-I (Table 3a), Taq-I (Table 4a) polymorphisms genotype distribution across MS/MS subtypes and control groups. Distribution of Bsm-I, Fok-I, Taq-I polymorphisms genotypes within any binary comparison of MS/MS subtypes and control groups were similar (Pearson test; $p > 0,05$).

Among 175 MS patients and 183 healthy controls VDR gene allele frequencies (allele Fok-I, allele Taq-I and allele Bsm-I) were established. The proportions of alleles of Bsm-I, Fok-I and Taq-I polymorphisms were shown in Table 2b, Table 3b and Table 4b respectively. Chi-square tests were performed for frequency of VDR gene alleles within MS/MS subtypes and control groups (Table 2b, Table 3b, Table 4b).

There were no significant differences of Bsm-I (Table 2b), Fok-I (Table 3b), Taq-I (Table 4b) polymorphisms allele frequencies across MS/MS subtypes and control groups. Frequency of Bsm-I, Fok-I, Taq-I allele within any binary comparison of MS/MS subtypes and control groups were similar (Pearson test; $p > 0,05$).

4. Discussion

Taq-I Allele				
	T	C	Total	*p*
MS-Control				
Control	62,8% (n=230)	37,2% (n=136)	100% (n=366)	*0,435*
MS	60,0% (n=210)	40,0% (n=140)	100% (n=350)	
Total	61,5% (n=440)	38,5% (n=276)	100% (n=716)	
MS subtypes				
Control	62,8% (n=230)	37,2% (n=136)	100% (n=366)	*0,768*
RRMS	59,2% (n=142)	40,8% (n=98)	100% (n=240)	
SPMS	61,3% (n=65)	38,7% (n=41)	100% (n=106)	
PPMS	75,0% (n=3)	25,0% (n=1)	100% (n=4)	
Total	61,5% (n=440)	38,5% (n=276)	100% (n=716)	

MS is an immune mediated chronic inflammatory demyelinating disease of CNS. While very little is known about the etiology of this disease, vitamin D as well as its receptor VDR, are thought to be associated with MS. However, this is a controversial topic since there is disagreement in the literature. Because of this we evaluated polymorphisms in the vitamin D receptor (VDR) in 175 MS patients and 183 healthy controls to determine if we observed an associated with MS in the Turkish population. Our results showed no significant relationship in the Turkish population between VDR gene polymorphisms with MS or MS subtypes. This was true for three distinct (Bsm-I, Fok-I, and Taq-I) VDR gene polymorphisms.

Previous research evaluating the effect of exogenous vitamin D in prevention of MS development based on genetic tendency has helped to establish the importance of polymorphisms [23]. The Fok-I polymorphism is a T/C allele variation located in exon 2 and translation initiation site of VDR. An interaction was observed between dietary intake of vitamin D and the VDR Fok-I polymorphism on MS risk and it was argued that vitamin D has higher effect on MS prevention in woman carrying T allele [23]. Therefore, determination of immune status by genetic predisposition according to vitamin D intake allowed better assessment of MS [23]. However, there was no association between Fok-I polymorphisms on VDR gene and MS in Australian population [26]. In separate study evaluating the MS patients in the British population, there is a tendency for low VDR expression in people with the Fok-I polymorphism (T/T) genotype on VDR gene. However, the relationship between MS and VDR single nucleotide polymorphisms has not been established as results among the studies differ from each other [30]. Smolders and colleagues, observed a relation between the Fok-I polymorphism in the VDR gene and level of vitamin D. The C allele of the Fok-I polymorphism is associated with decreased 25(OH)D and increased 1,25-dihydroxyvitamin D (1,25(OH)D) levels [31]. Polymorphisms in the VDR gene were found to be associated with the severity and course of the MS as Mamutse and colleagues demonstrate that the Fok-I allele was associated with a decreased 10 year disability level following initial disease development [32]. In contrast a meta-analysis [23, 26–27, 31, 33–34] conducted on Caucasian populations indicates that the risk of MS is independent from Fok-I polymorphisms in dominant, heterozygote and recessive gene models [8].

The Bsm-I polymorphism is located in intron 8 of VDR and has a G/A variation. The first studies to report a relationship between MS and Bsm-I polymorphisms on VDR gene were found in the Japanese population [14, 25]. However, it was later found that there was no association between Bsm-I polymorphisms on VDR gene and MS in Canadian population [22]. In our study it was found that there was no association between Bsm-I polymorphisms on VDR gene and MS in the Turkish population.

The Taq-I polymorphism is found at exon 9 of VDR with a C/T variation. Association was found between Taq-I polymorphisms on VDR gene and MS in Australian population [26]. In contrast to this study there was no association between Taq-I polymorphisms and MS in Canadian population [22]. In our study it was found that there was no association between Taq-I polymorphisms on VDR gene and MS risk in the Turkish population. In summary, we found no significant relationship in the Turkish population between VDR gene polymorphisms (Bsm-I, Fok-I, and Taq-I) with MS or MS subtypes. These data are important since previous reports on this topic are in contrast to one another and more studies are needed. Some of limitations of our study which should be considered include small sample size and different ethnicity compared to other studies. Accordingly, our study adds evidence to the argument that VDR is not associated with MS, at least in certain populations.

Declarations

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Competing Interest Statement

The authors do not have any competing interest or conflict of interest for any aspect of this study.

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