Vitamin D Receptor Polymorphisms in the Turkish Population are associated with Multiple Sclerosis

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Research article

Keywords: VitD Receptor, Polymorphism, Multiple Sclerosis

Posted Date: May 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-28927/v1>

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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 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 271 MS patients and 203 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: Our results demonstrate associations between MS disease and distribution of VDR Fok-I T/T polymorphism genotype, VDR Fok-I T allele frequency, distribution of VDR Taq-I C/C polymorphism genotype and VDR Taq-I C allele frequency (Pearson test, $p < 0,05$). However, there was no relationship between MS disease and VDR Bsm-I polymorphisms for genotype distribution (Pearson test, $p > 0,05$) or allele frequency (Pearson test, $p > 0,05$). Furthermore, there was also an association between subtypes of MS disease and VDR Fok-I allele frequency (Pearson test, $p < 0,05$) but distribution of VDR Fok-I genotype was not associated with subtypes of MS (Pearson test, $p > 0,05$). No meaningful association was found between subtypes of MS for genotypes distribution and allele frequencies of Bsm-I and Taq-I polymorphisms (Pearson test, $p > 0,05$).

Conclusion: Fok-I and Taq-I VDR gene polymorphisms are significantly associated with MS disease in the Turkish population. However, there is no association between MS and the VDR gene Bsm-I polymorphism.

1. Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease, which leads to demyelination and neurodegeneration of 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 [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, studies in rats indicate that the VDR gene has a critical role in EAE activity [20]. These findings suggest that VDR and its ligand have immunosuppressive and anti-inflammatory properties 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 the Turkish population.

2. Material And Methods

2.1. Patients and controls

A total of 474 participants from the Turkish population were enrolled in the study. Of the 474 participants, 271 were diagnosed with MS (2 PPMS, 184 RRMS and 85 SPMS) and 203 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 (rs2228570), Bsm-I (rs1544410) and Taq-I (rs731236) were amplified 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 MgCl₂, 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).

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. The odds ratio and 95% confidence interval were also used. Values of $p < 0,05$ was considered as significant. Data was analyzed with SPSS 21.0 program. Statistical power of this study was calculated by using G*Power program version of 3.1.9.6.

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).

There were significant differences of Fok-I (Table 3a), Taq-I (Table 4a) polymorphisms genotype distribution across MS/MS subtypes and control groups. Distribution of Fok-I polymorphism T/T genotype was 22,5 % (n=61) within MS group and 13,3% (n=27) within control group. Otherwise, distribution of Fok-I polymorphism C/C genotype was 41,7 % (n=113) within MS group and 47,8 % (n=97) within control group (Pearson test; $p < 0,05$). Distribution of Taq-I polymorphism C/C genotype was 26,6 % (n=72) within MS group and 16,7 % (n=34) within control group. Otherwise, distribution of Taq-I polymorphism T/T genotype was 32,8 % (n=89) within MS group and 37,5 % (n=76) within control group (Pearson test; $p < 0,05$). However, Fok-I and Taq-I polymorphisms genotypes within any binary comparison of MS subtypes and control groups were similar (Pearson test; $p > 0,05$). There was no significant difference of Bsm-I (Table 2a) polymorphism genotype distribution across MS/MS subtypes and control groups (Pearson test; $p > 0,05$). Among 271 MS patients and 203 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 significant differences of Fok-I (Table 3b), Taq-I (Table 4b) polymorphisms allele frequencies across MS/MS subtypes and control groups. Frequency of Fok-I T allele was 40,4 % (n=219) within MS group and 32,8 % (n=133) within control group (MS/control odds ratio=1,391; CI 95%=1,063-1,821). Otherwise, frequency of Fok-I C allele was 59,6% (n=323) within MS group and 67,2 % (n=273) within control group (MS/control odds ratio=0,719; CI 95%=0,549-0,940) (Pearson test; $p < 0,05$). Frequency of Fok-I T allele was 42,4 % (n=72) within SPMS subtype group and 39,9 % (n=147) within RRMS subtype group. Otherwise, frequency of Fok-I C allele was 57,6% (n=98) within SPMS subtype group; 60,1% (n=221) within RRMS subtype group and 100% (n=4) within PPMS subtype group (Pearson test; $p < 0,05$). Frequency of Taq-I C allele was 46,9% (n=254) within MS group and 39,7% (n=161) within control group (MS/control odds ratio=1,342; CI 95%= 1,034-1,742). Otherwise, frequency of Taq-I T allele was 53,1% (n=288) within MS group and 60,3 % (n=245) (MS/control odds ratio=0,745; %95 CI= 0,574-0,967) (Pearson test; $p < 0,05$). However, frequency of Taq-I allele within any binary comparison of MS subtypes and control groups were similar (Pearson test; $p > 0,05$). There was no significant difference of Bsm-I (table 2b) polymorphism allele frequencies across MS/MS subtypes and control groups.

4. Discussion

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 271 MS patients and 203 healthy controls to determine if we observed an association with MS in the Turkish population. Our results showed significant relationship in the Turkish population between VDR gene polymorphisms with MS or MS subtypes. This was true for two distinct (Fok-I and Taq-I) VDR gene polymorphisms. However, there was no significant relationship between VDR gene Bsm-I polymorphism with MS or MS subtypes in our study.

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]. In our study, it was found that Fok-I T/T polymorphism on VDR gene and Fok-I T allele frequency were significantly associated with MS in Turkish population.

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]. The results of our study showed that Taq-I C/C polymorphism on VDR gene and C allele frequency were significantly associated with MS in the Turkish population. In summary, we found that a significant relationship in the Turkish population exists between VDR gene polymorphisms (Fok-I, and Taq-I) and MS. 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 associated with MS, at least in certain populations.

Declarations

Acknowledgments

This work was supported by the grant from The Marmara University Research Foundation.

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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Tables

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

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

a)

	Bsm-I Genotype			Total	<i>p</i>	<i>Power</i> (%)
	G/G	G/A	A/A			
MS-Control						
Control	37,0% (n=75)	46,3% (n=94)	16,7% (n=34)	100% (n=203)	0,677	100
MS	35,8% (n=97)	44,3% (n=120)	19,9% (n=54)	100% (n=271)		
Total	36,3% (n=172)	45,1% (n=214)	18,6% (n=88)	100% (n=474)		
MS subtypes						
Control	37,0% (n=75)	46,3% (n=94)	16,7% (n=34)	100% (n=203)	0,622	100
RRMS	35,9% (n=66)	46,7% (n=86)	17,4% (n=32)	100% (n=184)		
SPMS	35,3% (n=30)	38,8% (n=33)	25,9% (n=22)	100% (n=85)		
PPMS	50,0% (n=1)	50,0% (n=1)	0,0% (n=0)	100% (n=2)		
Total	36,3% (n=172)	45,1% (n=214)	18,6% (n=88)	100% (474)		

b)

Bsm-I Allele					
	G	A	Total	<i>p</i>	<i>Power</i> (%)
MS-Control					
Control	60,1% (n=244)	39,9% (n=162)	100% (n=406)	0,503	100
MS	57,9% (n=314)	42,1% (n=228)	100% (n=542)		
Total	58,9%(n=558)	41,1% (n=390)	100% (n=948)		
MS subtypes					
Control	60,1% (n=244)	39,9% (n=162)	100% (n=406)	0,589	100
RRMS	59,2% (n=218)	40,8% (n=150)	100% (n=368)		
SPMS	54,7% (n=93)	45,3% (n=77)	100% (n=170)		
PPMS	75,0% (n=3)	25,0% (n=1)	100% (n=4)		
Total	58,8% (n=558)	41,2% (n=390)	100% (n=948)		

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

a)

	Fok-I Genotype			Total	<i>p</i>	<i>Power</i> (%)
	T/T	T/C	C/C			
MS-Control						
Control	13,3% (n=27)	38,9% (n=79)	47,8% (n=97)	100%(n=203)	0,037	100
MS	22,5% (n=61)	35,8% (n=97)	41,7% (n=113)	100%(n=271)		
Total	18,6% (n=88)	37,1% (n=176)	44,3% (n=210)	100%(n=474)		
MS subtypes						
Control	13,3% (n=27)	38,9% (n=79)	47,8% (n=97)	100%(n=203)	0,074	100
RRMS	23,3% (n=43)	33,2% (n=61)	43,5% (n=80)	100%(n=184)		
SPMS	21,1% (n=18)	42,4% (n=36)	36,5% (n=31)	100%(n=85)		
PPMS	0,0% (n=0)	0,0% (n=0)	100%,0 (n=2)	100%(n=2)		
Total	18,6% (n=88)	37,1% (n=176)	44,3% (n=210)	100%(n=474)		

b)

Fok-I Allele					
	T	C	Total	<i>p</i>	<i>Power</i> (%)
MS-Control					
Control	32,8% (n=133)	67,2% (n=273)	100% (n=406)	0,016	100
MS	40,4% (n=219)	59,6% (n=323)	100% (n=542)		
Total	37,1% (n=352)	62,9% (n=596)	100% (n=948)		
MS subtypes					
Control	32,8% (n=133)	67,2% (n=273)	100% (n=406)	0,030	100
RRMS	39,9% (n=147)	60,1% (n=221)	100% (n=368)		
SPMS	42,4% (n=72)	57,6% (n=98)	100% (n=170)		
PPMS	0,0% (n=0)	100,0% (n=4)	100% (n=4)		
Total	37,1% (n=352)	62,9% (n=596)	100% (n=948)		

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

a)

	Taq-I Genotype			Total	<i>p</i>	<i>Power(%)</i>
	C/C	C/T	T/T			
MS-Control						
Control	16,7% (n=34)	45,8% (n=93)	37,5% (n=76)	100%(n=203)	<i>0,040</i>	99
MS	26,6% (n=72)	40,6% (n=110)	32,8% (n=89)	100%(n=271)		
Total	22,4% (n=106)	42,8% (n=203)	34,8% (n=165)	100%(n=474)		
MS subtypes						
Control	16,7% (n=34)	45,8% (n=93)	37,5% (n=76)	100%(n=203)	<i>0,101</i>	100
RRMS	23,9% (n=44)	43,5% (n=80)	32,6% (n=60)	100%(n=184)		
SPMS	32,9% (n=28)	34,2% (n=29)	32,9% (n=28)	100%(n=85)		
PPMS	0,0% (n=0)	50,0% (n=1)	50,0% (n=1)	100%(n=2)		
Total	22,4% (n=106)	42,8% (n=203)	34,8% (n=165)	100%(n=474)		

b)

Taq-I Allele					
	T	C	Total	<i>p</i>	<i>Power(%)</i>
MS-Control					
Control	60,3% (n=245)	39,7% (n=161)	100% (n=406)	<i>0,027</i>	99
MS	53,1% (n=288)	46,9% (n=254)	100% (n=542)		
Total	56,2% (n=533)	43,8% (n=415)	100% (n=948)		
MS subtypes					
Control	60,3% (n=245)	39,7% (n=161)	100% (n=406)	<i>0,087</i>	100
RRMS	54,3% (n=200)	45,7% (n=168)	100% (n=368)		
SPMS	50,0% (n=85)	50,0% (n=85)	100% (n=170)		
PPMS	75,0% (n=3)	25,0% (n=1)	100% (n=4)		
Total	56,2% (n=533)	43,8% (n=415)	100% (n=948)		