BJMG 25 (1), 2022 • 41-50
DOI: 10.2478/bjmg-2022-0003
sciendo

ORIGINAL ARTICLE

VITAMIN D RECEPTOR POLYMORPHISMS AMONG THE TURKISH POPULATION ARE ASSOCIATED WITH MULTIPLE SCLEROSIS

Bulan B[1], Hoscan AY[1], Keskin SN[1], Cavus A[1], Culcu EA[1], Isik N[2], List EO[2], Arman A*[4]

*Corresponding Author: Dr. Ahmet Arman, The Department of Medical Genetics, Marmara Teaching and Research Hospital, Marmara University, 34899, Pendik, Istanbul/TURKEY. Tel #: +90-216-657 0606, Fax #: +90-216-414 47 31, e-mail: ahmetarman@marmara.edu.tr, ORCID Id: https://orcid.org/0000-0001-5547-0024

ABSTRACT

Multiple sclerosis (MS) is an inflammatory disease characterized by demyelination and axonal degeneration affecting the central nervous system. Among the genetic factors suggested to be associated with this disease are polymorphisms to the vitamin D receptor (VDR) gene. We tested the hypothesis that polymorphisms in the vitamin D receptor (VDR) gene are associated with MS. The aim of the study was to investigate the relationship of MS with the VDR gene Fok-I, Bsm-I and Taq-I polymorphisms among the Turkish population. This study contains 271 MS patients and 203 healthy controls. Genomic DNA was isolated from the samples and the VDR gene Fok-I, Bsm-I and Taq-I polymorphism regions were amplified by polymerase chain reaction (PCR). The PCR products were digested, and the genotypes were determined based on size of digested PCR products. Our results demonstrate associations between MS and the distribution of the VDR gene Fok-I T/T polymorphism genotype in a dominant model, VDR gene Fok-I T allele frequency, distribution of VDR gene Taq-I C/C polymorphism genotype in a dominant model and VDR gene Taq-I C allele frequency (Pearson test, $p<0.05$). However, there was no association between MS and the VDR gene Bsm-I polymorphisms for the genotype distribution (Pearson test, $p>0.05$) or allele frequency (Pearson test, $p>0.05$). Fok-I and Taq-I VDR gene polymorphisms are significantly associated with MS in dominant, homozygote and heterozygote inheritance models among the Turkish population.

Key Words: VitD Receptor; Polymorphism; Multiple Sclerosis

INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory disease which leads to demyelination and neurodegeneration of the central nervous system(CNS) [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 [4, 5, 6]. Axonal degeneration is the main reason for this non-reversible disability in MS patients [7]. While the etiology of MS is not fully understood, environmental, genetic, and geographical factors may play a part [7, 8, 9]. 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 [10, 11]. The differences in susceptibility to MS, despite the same environmental exposures, indicates the involvement of genetic factors in the development of pathogenesis [7]. 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 gene mutations are needed to contribute additively to the course of this disease [14]. In major gene regions, most of the loci associated with 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

[1] Medical School, Marmara University, Istanbul/Turkey.
[2] Clinic of Neurology, Istanbul Okan University, Istanbul, Turkey.
[3] The Edison Biotechnology Institute, Ohio University, Athens, OH, USA.
[4] Department of Medical Genetics, Medical School, Marmara University, Istanbul/Turkey.

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 developing MS. Furthermore, vitamin D has been shown to impact immunomodulation in the MS pathogenesis [11, 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 [11, 18, 19]. Additionally, studies in mice 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 [11]. Taken together, these studies indicate that vitamin D (or lack thereof) may play an important role in the development of MS.

In addition to vitamin D, the vitamin D receptor (*VDR*) is hypothesized as playing 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 the *VDR* gene have been investigated for MS susceptibility, and are they thought to be associated with the MS disease [7, 21]. However, these results are inconclusive and there is disagreement among these findings [7, 22]. Several studies indicate that the *VDR* gene polymorphisms are associated with susceptibility to MS [23, 24, 25]. Furthermore, these polymorphisms in the *VDR* gene may change the vitamin D serum levels, vitamin D structure, and function as such with an immune modulatory effect; these are the mechanisms of the vitamin D and *VDR* complex [22]. By 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 [7, 26].

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.

MATERIAL AND METHODS

Patients and controls

A total of 474 ethnically matched participants from the Turkish population were enrolled in the study. Of the 474 participants, 271 were diagnosed with MS. Within the MS patients 2 of them had Primary Progressive Multiple Sclerosis (PPMS), 184 of them had Relapsing Remitting Multiple Sclerosis (RRMS) and 85 of them had Secondary Progressive Multiple Sclerosis (SPMS). 203 individuals served as healthy controls. All patients were referred to Goztepe Training and Research Hospital and were clinically diagnosed with MS, according to the McDonald's criteria [27]. A blood sample was collected from each person in order to obtain genomic DNA. The study protocol and consent were approved by Marmara University Medical School Clinical Research Ethic Committee. Written informed consent was obtained from all of the participants and there was no patient or control younger than the age of 16 in the study.

Genotyping of polymorphisms

Genomic DNA was extracted by using the salting out method, as previously described [28]. Polymorphism regions Fok-I (rs2225870), Bsm-I (rs1544410) and Taq-I (rs731236) were amplified by a polymerase chain reaction (PCR) (Techne Tc312) using specific primers, visible in Table 1. PCR was carried by a 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 the main PCR cycle for denaturation is at 94°C for 30 secs, annealing at 69°C for 30 secs, extension at 72°C for 45 secs (all cycles 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 of PCR product was mixed with 5 U restriction enzyme, 3 µl 10X reaction buffer and incubated overnight at 37°C 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 fragment 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)

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

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).

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 a 95% confidence interval were also used. Values of $p<0.05$ were considered significant. Data was analyzed with the SPSS 21.0 program. The statistical power of this study was calculated by using the G*Power program version of 3.1.9.6.

RESULTS

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

There were significant differences in Fok-I (Table 3a), Taq-I (Table 4a) polymorphism genotype distributions across MS/MS subtype group and control group. Distribution of the Fok-I polymorphism T/T genotype was 22.5 % (n=61) in the MS group and 13.3% (n=27) in the control group. Otherwise, the distribution of the Fok-I polymorphism C/C genotype was 41.7% (n=113) in the MS group and 47.8 % (n=97) in the control group (Pearson test; $p<0.05$). The distribution of the Taq-I polymorphism T/T genotype was 26.6 % (n=72) in MS group and 16.7 % (n=34) in the control group. Otherwise, the distribution of the Taq-I polymorphism C/C genotype was 32.8 % (n=89) in the MS group and 37.5 % (n=76) in the control group (Pearson test; $p<0.05$). Distribution of Fok-I (Table 6a-d) and Taq-I polymorphisms (Table 7a-d) in the MS and control groups differ significantly in dominant, heterozygote, and homozygote inheritance models (Pearson test; $p<0.05$). However, Fok-I and Taq-I polymorphism genotypes within any binary comparison of

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*	*Power (%)*
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	*p*	*Power (%)*
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**					
	T/T	T/C	C/C	Total	*p*	*Power (%)*
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	*p*	*Power (%)*
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**					
	C/C	C/T	T/T	Total	*p*	*Power (%)*
MS-Control						
Control	16.7% (n=34)	45.8% (n=93)	37.5% (n=76)	100%(n=203)	*0.040*	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)	*0.101*	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	*p*	*Power (%)*
MS-Control					
Control	60.3% (n=245)	39.7% (n=161)	100% (n=406)	*0.027*	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)	*0.087*	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)		

Table 5a-d: Genotype distribution of *VDR* Gene Bsm-I polymorphisms in different inheritance models in the MS patients and control group

a)	**Bsm-I Recessive Model**			
	G/G or G/A	A/A	Total	*p*
MS-Control				
Control	83.3% (n=169)	16.7% (n=34)	100% (n=203)	*0.379*
MS	80.1% (n=217)	19.9% (n=54)	100% (n=271)	
Total	81.4%(n=386)	18.6% (n=88)	100% (n=474)	

b)	**Bsm-I Dominant Model**			
	G/G	G/A or A/A	Total	*p*
MS-Control				
Control	36.9% (n=75)	63.1% (n=128)	100% (n=203)	*0.796*
MS	35.8% (n=97)	64.2% (n=174)	100% (n=271)	
Total	36.3%(n=172)	63.7% (n=302)	100% (n=474)	

c)	**Bsm-I Homozygote Model**			
	G/G	A/A	Total	*p*
MS-Control				
Control	68.8% (n=75)	31.2% (n=34)	100% (n=88)	*0.442*
MS	64.2% (n=97)	35.8% (n=54)	100% (n=172)	
Total	33.8%(n=88)	66.2% (n=172)	100% (n=260)	

d)	**Bsm-I Heterozygote Model**			
	G/G	G/A	Total	*p*
MS-Control				
Control	44.4% (n=75)	55.6% (n=94)	100% (n=169)	*0.950*
MS	44.7% (n=97)	55.3% (n=120)	100% (n=217)	
Total	55.4%(n=214)	44.6% (n=172)	100% (n=386)	

Table 6a-d: Genotype distribution of *VDR* Gene Fok-I polymorphisms in different inheritance models in the MS patients and control group

a)	**Fok-I Recessive Model**			
	T/C or TT	C/C	Total	*p*
MS-Control				
Control	52.2% (n=106)	47.8% (n=97)	100% (n=203)	*0.187*
MS	58.3% (n=158)	41.7% (n=113)	100% (n=271)	
Total	55.7%(n=264)	44.3% (n=210)	100% (n=474)	

b)	**Fok-I Dominant Model**			
	T/T	T/C or C/C	Total	*p*
MS-Control				
Control	13.3% (n=27)	86.7% (n=176)	100% (n=203)	*0.011*
MS	22.5% (n=61)	77.5% (n=210)	100% (n=271)	
Total	18.6%(n=88)	81.4% (n=386)	100% (n=474)	

c)	**Fok-I Homozygote Model**			
	T/T	C/C	Total	*p*
MS-Control				
Control	21.8% (n=27)	78.2% (n=97)	100% (n=124)	*0.013*
MS	35.1% (n=61)	64.9% (n=113)	100% (n=174)	
Total	29.5%(n=88)	70.5% (n=210)	100% (n=298)	

d)	**Fok-I Heterozygote Model**			
	T/T	T/C	Total	*p*
MS-Control				
Control	25.5% (n=27)	74.5% (n=79)	100% (n=106)	*0.026*
MS	38.6% (n=61)	61.4% (n=97)	100% (n=158)	
Total	33.8%(n=88)	66.7% (n=176)	100% (n=264)	

Table 7a-d: Genotype distribution of *VDR* Gene Taq-I polymorphisms in different inheritance models in the MS patients and control group

a)	**Taq-I Recessive Model**			
	T/C or T/T	C/C	Total	*p*
MS-Control				
Control	62.6% (n=127)	37.4% (n=76)	100% (n=203)	*0.299*
MS	67.2% (n=182)	32.8% (n=89)	100% (n=271)	
Total	65.2%(n=309)	34.8% (n=165)	100% (n=474)	

b)	**Taq-I Dominant Model**			
	T/T	T/C or C/C	Total	*p*
MS-Control				
Control	16.7% (n=34)	83.3% (n=169)	100% (n=203)	*0.011*
MS	26.6% (n=72)	73.4% (n=199)	100% (n=271)	
Total	22.4%(n=106)	77.6% (n=368)	100% (n=474)	

c)	**Taq-I Homozygote Model**			
	T/T	C/C	Total	*p*
MS-Control				
Control	30.9% (n=34)	69.1% (n=76)	100% (n=110)	*0.022*
MS	44.7% (n=72)	55.3% (n=89)	100% (n=161)	
Total	39.1%(n=106)	60.9% (n=165)	100% (n=271)	

d)	**Taq-I Heterozygote Model**			
	T/T	T/C	Total	*p*
MS-Control				
Control	26.8% (n=34)	73.2% (n=93)	100% (n=127)	*0.020*
MS	39.6% (n=72)	60.4% (n=110)	100% (n=182)	
Total	34.3%(n=106)	65.7% (n=203)	100% (n=309)	

Table 8a-c: Distribution of *VDR* gene Polymorphisms within gender in MS patients

a)	**Bsm-I Genotype**				
	G/G	G/A	A/A	Total	*p*
Gender					
Female	37.2% (n=70)	43.6% (n=82)	19.1% (n=36)	100% (n=188)	*0.740*
Male	32.5% (n=27)	45.8% (n=38)	21.7% (n=18)	100% (n=83)	
Total	35.8% (n=97)	44.3% (n=120)	19.9% (n=54)	100% (n=271)	

b)	**Fok-I Genotype**				
	T/T	T/C	C/C	Total	*p*
Gender					
Female	22.3% (n=42)	33.0% (n=62)	44.7% (n=84)	100% (n=188)	*0.262*
Male	22.9% (n=19)	42.2% (n=35)	34.9% (n=29)	100% (n=83)	
Total	22.5% (n=61)	35.8% (n=97)	41.7% (n=113)	100% (n=271)	

c)	**Taq-I Genotype**				
	C/C	C/T	T/T	Total	*p*
Gender					
Female	34.6% (n=65)	41.0% (n=77)	24.5% (n=46)	100% (n=188)	*0.449*
Male	28.9% (n=24)	39.8% (n=33)	31.3% (n=26)	100% (n=83)	
Total	32.8% (n=89)	40.6% (n=110)	26.6% (n=72)	100% (n=271)	

MS subtype group and control group were similar (Pearson test; p>0.05). There was no significant difference in Bsm-I (Table 2a, Table 5a-d) polymorphism genotype distribution across MS/MS subtype group and the control group in any inheritance models (Pearson test; p>0.05). Of the 271 MS patients and 203 healthy controls, the *VDR* gene allele frequencies (allele Fok-I, allele Taq-I and allele Bsm-I) were established.

Table 9a-c: Distribution of *VDR* gene allele within gender in MS patients

a)	**Bsm-I Allele**			
	G	A	Total	*p*
Gender				
Female	61.7% (n=153)	38.3% (n=95)	100% (n=248)	*0.019*
Male	48.0% (n=49)	52.0% (n=53)	100% (n=102)	
Total	57.7%(n=202)	42.3% (n=148)	100% (n=350)	

b)	**Fok-I Allele**			
	T	C	Total	*p*
Gender				
Female	29.0% (n=72)	71.0% (n=176)	100% (n=248)	*0.800*
Male	30.4% (n=31)	71.0% (n=69.6)	100% (n=102)	
Total	55.7%(n=264)	70.6% (n=247)	100% (n=350)	

c)	**Taq-I Allele**			
	T	C	Total	*p*
Gender				
Female	37.5% (n=93)	37.4% (n=76)	100% (n=248)	*0.137*
Male	46.1% (n=47)	53.9% (n=55)	100% (n=102)	
Total	40.0%(n=140)	60.0% (n=210)	100% (n=350)	

The proportions of the alleles of Bsm-I, Fok-I, and Taq-I polymorphisms are shown in Table 2b, Table 3b and Table 4b, respectively. Chi-square tests were performed for frequency of the *VDR* gene alleles within MS/MS subtype group and the control group (Table 2b, Table 3b, Table 4b). There were significant differences of Fok-I (Table 3b) and Taq-I (Table 4b) polymorphism allele frequencies across MS/MS subtype group and the control group. The frequency of the Fok-I T allele was 40.4 % (n=219) in the MS group and 32.8 % (n=133) in the control group (MS/control odds ratio=1.391; CI 95%=1.063-1.821). Otherwise, the frequency of the Fok-I C allele was 59.6% (n=323) in the MS group and 67.2 % (n=273) in the control group (MS/control odds ratio=0.719; CI 95%=0.549-0.940) (Pearson test; $p<0.05$). The frequency of the Fok-I T allele was 42.4 % (n=72) in the SPMS subtype group and 39.9 % (n=147) in the RRMS subtype group. Otherwise, the frequency of the Fok-I C allele was 57.6% (n=98) in the SPMS subtype group; 60.1% (n=221) in the RRMS subtype group and 100% (n=4) in the PPMS subtype group (Pearson test; $p<0.05$). The frequency of Taq-I C allele was 46.9% (n=254) in the MS group and 39.7% (n=161) in the control group (MS/control odds ratio=1.342; CI 95%= 1.034-1.742). Otherwise, the frequency of the Taq-I T allele was 53.1% (n=288) in the MS group and 60.3 % (n=245) in the control group (MS/control odds ratio=0.745; %95 CI= 0.574-0.967) (Pearson test; $p<0.05$). However, the frequency of the Taq-I allele within any binary comparison of the MS subtypes group and the control group were similar (Pearson test; $p>0.05$). There was no significant difference in the Bsm-I (table 2b) polymorphism allele frequencies across the MS/MS subtype group and control group.

Among the MS patients, there were 188 males and 83 females. Distribution of the *VDR* gene genotypes and allele frequencies did not differ across genders in the MS patients (Table 8a-c and Table 9a-c). However, only the frequency of the Bsm-I alleles (G and A) were distributed significantly between males and females (Table 9a). The frequency of the Bsm-I G allele was 61.7% among females and the frequency of Bsm-I A allele was 52% among males (Pearson test; $p<0.05$).

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 gene, *VDR*, are thought to be associated with MS. However, this is a controversial topic since there is disagreement in the literature. Therefore, we have evaluated the polymorphisms in the vitamin D receptor (*VDR*) among 271 MS patients and 203 healthy controls to determine if we observed any association with it and MS in the Turkish population. Our results showed a significant relationship in the Turkish population between *VDR* gene polymorphisms among 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 [22]. The Fok-I polymorphism is a T/C

allele variation located in exon 2 which is in the translation initiation site of *VDR*. An interaction was observed between the dietary intake of vitamin D and the *VDR* Fok-I polymorphism and risk of MS. It was argued that vitamin D has a higher effect on MS prevention in women carrying the T allele [22]. Therefore, the determination of immune status by genetic predisposition, according to vitamin D intake, allowed for better assessment of MS risk [22]. However, there was no association between Fok-I polymorphisms on the *VDR* gene and MS in the Australian population [25]. In a separate study evaluating 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 the *VDR* gene. However, the relationship between MS and *VDR* single nucleotide polymorphisms has not been established, as results in the studies differ from each other [29]. Smolders et al. observed a relation between the Fok-I polymorphism in the *VDR* gene and the 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 [29]. Polymorphisms in the *VDR* gene were found to be associated with the severity and course of MS, as Mamutse et al. demonstrate that the Fok-I allele was associated with a decreased 10 year disability level, following initial disease development [30]. By contrast, a meta-analysis [22, 23, 25, 29, 31, 32] conducted on the Caucasian population indicates that the risk of MS is independent of Fok-I polymorphisms in dominant, heterozygote and recessive gene models [7]. Another study found that distribution of the *VDR* gene Fok-I polymorphisms was associated with MS in the Turkish population [33]. In a meta-analysis covering the research up to 2019, there was no association found between Fok-I polymorphisms and MS [34]. In our study, it was found that the Fok-I T/T polymorphism on the *VDR* gene in a dominant inheritance model 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 the *VDR* gene were found in the Japanese population [14, 24]. However, it was later found that there was no association between Bsm-I polymorphisms on the *VDR* gene and MS in the Canadian population [35]. In the meta-analysis of studies up to 2019, there was no association between Bsm-I polymorphisms and MS, but when compared with the European population and the Asian population, there was an association between Bsm-I polymorphisms and MS [34]. In our study, it was found that there was no association between Bsm-I polymorphisms on the *VDR* gene and MS in the Turkish population.

The Taq-I polymorphism is found at exon 9 of *VDR* with a C/T variation. An association was found between the Taq-I polymorphisms on the *VDR* gene and MS in the Australian population [25]. In contrast to this study, there was no association between the Taq-I polymorphisms and MS in the Canadian population [35]. A similar study found that there was no association between Taq-I polymorphisms on the *VDR* gene and MS in the Turkish population [33]. The aforementioned meta-analysis includes the research on this subject until 2019, and there was an association between Taq-I polymorphisms and MS only in the heterozygote model, but not in other inheritance models [34]. The results of our study showed that Taq-I C/C polymorphism on the *VDR* gene in dominant, homozygote and heterozygote inheritance models, 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. A similar study among the Turkish population found an association between *VDR* gene Fok-I polymorphisms and MS. According to this study, however, there was no association between Taq-I *VDR* gene polymorphisms and MS. These data are important, since previous reports on this topic differ from one another, and more studies are needed. Some of the limitations of our study which should be considered include: small sample size and a different ethnicity, compared to other studies. These limitations might be the reason for the contradictions between our study and the study of Kamisli et al. among the Turkish population. Accordingly, our study adds further evidence to the argument that *VDR* is associated with MS, at least in certain populations.

Acknowledgements

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

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Author Contributions

BB: conducted the experiments and analyzed the data; HAY: conducted the experiments and developed the methodology; KSN: developed the methodology and provisioned the materials;

CA: developed the methodology and provisioned the materials; CEA: provisioned the materials; IN: provisioned the materials; ELO: prepared the published work; AA: planned, executed the research and prepared the published work.

REFERENCES

1. Kalman B, Toldy E. Genomic binding sites and biological effects of the vitamin D--VDR complex in multiple sclerosis. Neuromolecular Med. 2014; 16(2): 265-79.
2. Librau RS, Singer SM, McDevitt HO. Th1 and Th2 CD41 T cells in the pathogenesis of organ-specific autoimmune diseases. Immunol Today. 1995; 16: 34–48.
3. Hasheminia SJ, Tolouei S, Zarkesh-Esfahani SH, Shaygannejad V, Shirzad HA, Torabi R, Hashem Zadeh Chaloshtory M. Cytokines gene expression in newly diagnosed multiple sclerosis patients. Iran J Allergy Asthma Immunol. 2015; 14(2): 208-16.
4. Brück W. The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage. J Neurol. 2005; 252(5):v3–9.
5. García-Martín E, Agúndez JA, Martínez C, Benito-León J, Millán-Pascual J, Calleja P, et al. Vitamin D3 receptor (VDR) gene rs2228570 (Fok1) and rs731236 (Taq1) variants are not associated with the risk for multiple sclerosis: results of a new study and a meta-analysis. PLoS One. 2013; 8(6): e65487.
6. Herrero-Herranz E, Pardo LA, Gold R, Linker RA. Pattern of axonal injury in murine myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Neurobiol Dis. 2008; 30: 162–73.
7. Huang J, Xie Z. Polymorphisms in the vitamin D receptor gene and multiple sclerosis risk: A meta-analysis of case–control studies, Journal of the Neurological Sciences. 2012; 313: 79–85.
8. Goldenberg MM. Multiple sclerosis review. P & T. 2012; 37(3): 175–84.
9. Kurland LT. The frequency and geographic distribution of multiple sclerosis as indicated by mortality statistics and morbidity surveys in the United States and Canada. Amer. J. Hygiene. 1952; 55: 457–76.
10. Pugliatti M, Harbo HF, Holmøy T, Kampman MT, Myhr KM, et al. Environmental risk factors in multiple sclerosis. Acta Neurol Scand Suppl. 2008; 188: 34–40.
11. Martina B. Sintzel . Mark Rametta . Anthony T. Reder. Vitamin D and Multiple Sclerosis: A Comprehensive Review. Neurol Ther. 2018; 7: 59–85.
12. Ebers GC, Kukay K, Bulmann DE, et al. A full genome search in multiple sclerosis. Nat Genet. 1996; 13: 472–6.
13. Sawcer S, Jones HB, Feakes R, et al. A genome screen in multiple sclerosis reveals susceptibility loci on chromosomes 6p21 and 17q22. Nat Genet. 1996; 13: 464–8.
14. Fukazawaa T, Yabeb I, Kikuchib S, Sasakib H, Hamadaa T, Miyasaka K, et al. Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. Journal of the Neurological Sciences. 1999; 166: 47–52.
15. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA–DRB1*1501 is regulated by vitamin D. PloS Genet. 2009; 5: e1000369.
16. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Rheumatic Disease Clinics of North America. 2012; 38(1): 125–39.
17. Jurutka PW, Bartik L, Whitfield GK, et al. Vitamin D receptor: key roles in bone mineral pathophysiology, molecular mechanism of action, and novel nutritional ligands. Journal of Bone and Mineral Research. 2007; 22(2): v2-10.
18. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. J Clin Invest. 1991; 87: 1103–7.
19. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci USA. 1996; 93: 7861–4.
20. Meehan TF, DeLuca HF. The vitamin D receptor is necessary for 1α,25-dihydroxyvitamin D3 to suppress experimental autoimmune encephalomyelitis in mice. Archives of Biochemistry and Biophysics. 2002; 408(2): 200–4.
21. Narooie-Nejad M, Moossavi M, Torkamanzehi A, Moghtaderi A. Positive Association of Vitamin D Receptor Gene Variations with Multiple Sclerosis in South East Iranian Population. Hindawi Publishing Corporation BioMed Research International. 2015; Article ID 427519: 1-4.
22. Simon K, Munger K, Yang X, Ascherio A. Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis. Multiple Sclerosis. 2010; 16(2): 133–8.
23. Cox MB, Ban M, Bowden NA, Baker A, Scott RJ, Lechner-Scott J. Potential association of vitamin D receptor polymorphism Taq1 with multiple sclerosis. Mult Scler. 2012; 18(1): 16-22.

24. Niino M, Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Tashiro K. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. Journal of the Neurological Sciences. 2000; 177(1): 65–71.
25. Tajouri L, Ovcaric M, Curtain R, et al. Variation in the vitamin D receptor gene is associated with multiple sclerosis in an Australian population. J Neurogenet. 2005; 19(1): 25–38.
26. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004; 338: 143–56.
27. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS: Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001, 50: 121-127.
28. Nasiri H, Forouzandeh M, Rasaee MJ and Rahbarizadeh F. Modified salting-out method: High-yield, high-quality genomic DNA extraction from whole blood using laundry detergent. J Clin Lab Anal. 2005; 19(6): 229-32.
29. Smolders J, Peelen E, Thewissen M, Menheere P, Tervaert J, Hupperts R, Damoiseaux J. The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. Autoimmunity Reviews. 2009; 8: 621–6.
30. Mamutse G, Woolmore J, Pye E, Partridge J, Boggild M, Young C, et al. Vitamin D receptor gene polymorphism is associated with reduced disability in multiple sclerosis. Mult Scler. 2008; 14: 1280–3.
31. Dickinson JL, Perera DI, van der Mei AF, Ponsonby AL, Polanowski AM, Thomson RJ, et al. Past environmental sun exposure and risk of multiple sclerosis: a role for the Cdx-2 Vitamin D receptor variant in this interaction. Mult Scler. 2009; 15: 563–70.
32. Partridge JM, Weatherby SJ, Woolmore JA, Highland DJ, Fryer AA, Mann CL, et al. Susceptibility and outcome in MS: associations with polymorphisms in pigmentation-related genes. Neurology. 2004; 62: 2323–5.
33. Ozden Kamisli, Ceren Acar, Mert Sozen, Mehmet Tecellioglu, Fatma Ebru Yücel, Dilara Vaizoglu et al. The association between vitamin D receptor polymorphisms and multiple sclerosis in a Turkish population: Multiple Sclerosis and Related Disorders. 2018; 20: 78-81.
34. Danyal Imani, Bahman Razi, Morteza Motallebnezhad, Ramazan Rezaei. Association between vitamin D receptor(VDR) polymorphisms and the risk of multiple sclerosis (MS): an updated meta-analysis. Imani et al. BMC Neurology. 2019; (19): 339.
35. Orton SM, Ramagopalan SV, Para AE, Lincoln MR, Handunnetthi L, Chao MJ et al. Vitamin D metabolic pathway genes and risk of multiple sclerosis in Canadians. J Neurol Sci. 2011; 305(1-2): 116-20.