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Research article

DOI:10.2478/rrlm-2018-0028

Vitamin D receptor gene polymorphisms in multiple sclerosis disease: A case-control study

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Abstract

Multiple sclerosis (MS) is a common neurologic disorder that is a chronic inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS). Its etiology remains unknown. Several recent studies have found that decreased susceptibility to vitamin D deficiency is also associated with a decreased risk of MS. The role of vitamin D receptor (VDR) gene and its polymorphisms are highlighted as susceptible components. In this study, we aimed to identify the relationship between ApaI (rs7975232), BsmI (rs 1544410), and TaqI (rs731236) gene polymorphisms with MS. ApaI, BsmI, and TaqI genotypes were determined in 70 patients with MS and in 70 control subjects. DNA was isolated from blood samples, and then ApaI, BsmI and TaqI gene polymorphisms were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The distribution of BsmI and TaqI polymorphisms did not show any significant differences in MS patients and controls; however, increased A allele of ApaI polymorphism was found in MS patients. Our findings suggest that the ApaI gene polymorphism might be associated with MS. Investigation of a larger population and functional work on these gene structures and function in MS patients are recommended.

Keywords*: multiple sclerosis, vitamin D, vitamin D receptor (VDR) gene polymorphism*

Received: 5th March 2018; Accepted: 10th July 2018; Published: 22nd July 2018

Introduction

Multiple sclerosis (MS) is a central nervous system disease characterized by demyelination. Although the precise etiology of MS is not known, it has been reported that genetic, environmental, and geographical factors may be causative (1-3). Recently, studies showed an association between vitamin D levels and high risk for autoimmune diseases such as systemic lupus erythematosus and MS. Some studies have determined a correlation between MS risk and serum vitamin D levels. In addition, there is a positive correlation between serum vitamin D level and Vitamin D receptor (VDR) (3-7).

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The primary form of vitamin D, colecalciferol (vitamin D_3), is synthesized in the skin upon exposure to ultraviolet radiation (UVR). This form of vitamin D is enzymatically converted in the liver to 25-hydroxyvitamin D (25(OH) D), which is regulated by the vitamin D receptor (VDR). The mechanism underlying the correlation between vitamin D and autoimmunity is the immunomodulatory function of vitamin D as well as the presence of the VDR on the most immune cells. Impaired vitamin D signaling, inadequate vitamin D intake, and/or environmental factors (e.g. insufficient sunlight exposure) may contribute to the onset and progression of autoimmunity in MS patients (1, 2, 4).

The vitamin D receptor gene (VDRG) is mapped to chromosome 12q (12–12q14). Although more than 30 polymorphisms in the VDR gene have been identified, ApaI (rs7975232), BsmI (rs 1544410), and TaqI (rs731236) are the most widely studied. These single nucleotide polymorphisms (SNPs) are located close to the 3' terminus of the gene and do not result in structural changes in the VDR protein (5-7).

Previous studies have shown that the VDR gene may be a significant predictor of MS; however, the results have been inadequate. The relationship between polymorphisms in the VDR gene and the risk of MS were determined in different studies (4, 11, 12).

The aim of this study was to evaluate VDR ApaI (rs7975232), BsmI (rs1544410), and TaqI (rs731236) alleles and genotypes in MS patients in comparison with controls.

Materials and Methods

Study population

The study was planned as a case-control study. The MS population was obtained from patients in university hospitals in Canakkale Onsekiz Mart, Turkey, and the diagnosis was made by a neurologist according to the Revised McDonald Criteria (8). Approval for the study was obtained from the Ethics Committee of Canakkale University Faculty of Medicine. Peripheral venous blood samples were collected from 70 patients with MS and from 70 age- and gender-matched healthy controls.

Genetic analaysis

DNA was isolated from blood samples using AccuPrep Genomic DNA extraction kits (Bioneer, Daejeon, South Korea). DNA purity and quantity were assessed by absorbance values using a spectrophotometer (DeNovix DS-11+, Thermo Scientific, USA).

To amplify a 740 bp fragment of the ApaI (rs7975232) polymorphism, the following primers were used: 5′CTGCCGTTGAGTGTCTGTGT3′ (forward) and 5′TCGGCTAGCTTCTGGATCAT3′ (reverse). Amplification was performed in a final volume of 50 μL reaction mixture containing 200 μM of dNTPs, 10 pmol each of forward (F) and reverse (R) primers, 1 U HotStarTaq DNA polymerase (Bioneer), 1× PCR buffer (Bioneer), and 100 ng genomic DNA. The thermal profile was as follows: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 30 s, and final extension step at 72°C for 2 min. Then, the PCR products (740 bp) were digested at 25°C for 2 h with 5.0 U of ApaI (Thermo Fisher Scientific), and electrophoresed on a 2% agarose gel stained with ethidium bromide. Digestion of the PCR product by ApaI yields 740 bp for AA, 520/220 bp for aa, and 740/520/220 bp bands for Aa genotypes.

The BsmI (rs1544410) polymorphism was genotyped by PCR-RFLP. The primers used were 5′GGGAGACGTAGCAAAAGGAG3′ (forward) and 5′CCATCTCTCAGGCTCCAAAG3′ (reverse). Amplification was accomplished with a 50 μL reaction mixture containing 100 ng template DNA, 10 pmol each of forward (F) and reverse (R) primers, 0.2 mM each dNTP,

1 U HotStarTaq DNA polymerase (Bioneer), and 1× PCR buffer (Bioneer). The PCR profile consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (30 seconds), annealing temperature (94°C, 56°C and 72°C, respectively, 50 seconds) and extension (72°C, 2 minutes). Then, the amplified PCR products (191 bp) were cleaved with 5.0 U of BsmI (Thermo Fisher Scientific) and electrophoresed on a 3% agarose gel stained with ethidium bromide. Digestion of the PCR product by BsmI yields 191 bp for BB, 115/76 bp for bb and 191/115/76 bp for Bb genotypes.

The primers designed for both ends of the TaqI (rs731236) polymorphism allele were 5′CCCATGAAGCTTAGGAGGAA3′ (forward) and 5′TCATCTTGGCATAGAGCAGGT3′ (reverse). PCR was achieved in a 50 µL volume containing 100 ng of template DNA, 1 U HotStarTaq DNA polymerase (Bioneer), 1× PCR buffer (Bioneer), and 10 pmol of each primer. The reaction conditions consisted of an initial melting step at 95°C for 5 min, followed by 35 cycles of 94°C for 45 s, 72°C for 45 s, 72°C for 30 s; and final extension step at 72°C for 2 min. Then, the PCR products (740 bp) were digested at 25°C for 2 h with 5.0 U of TaqI (Thermo Fisher Scientific) and electrophoresed on a 2% agarose gel stained with ethidium bromide. Digestion of the PCR product by TaqI yields 495/45 bp for TT, 290/245/205 bp for tt, and 495/290/245/205bp for Tt genotypes.

Statistical analysis

Statistical analysis was performed using SPSS version 19.0. (SPSS, IBM Company). Kolmogorov-Smirnov test was used to assess the normality of data distribution. Normally distributed data are reported as mean±standard deviation. Genotype frequencies were tested on Hardy-Weinberg equilibrium with the χ^2 test. A statistical difference was considered significant when the p-value was less than 0.05. The associations between these genotypes and the risk of MS were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from binary logistic regression analysis.

Results

The study population comprised 70 unrelated patients (19 males and 51 females; mean age=44.4±11.2 years) with a clinical diagnosis of MS recruited consecutively and prospectively from those who were treated and followed-up in the Neurology Department of Canakkale Onsekiz Mart University Research Hospital, Çanakkale, Turkey. Of the MS group, 47 patients had relapsing-remitting MS (RRMS), 14 patients had secondary progressive MS (SPMS), and 9 patients had primary progressive MS (PPMS). A total of 70 unrelated healthy subjects (22 males and 48 females; mean age=38.2±9.5 years) were recruited consecutively. The healthy controls were matched for age and gender with MS patients **(Table 1).**

For both cases and controls, the distribution frequency of each genotype and allele is summarized in **Table 2.** The frequencies of aa, Aa and AA genotypes were 21.4, 38.6 and 40.0% in the patient group and 41.4, 30.0 and 28.6% in the control group, respectively. There was a statistically significant difference in the frequencies of genotypes between patient and control groups (p=0.04, ref (aa); OR: 2.49 , CI: 1.07-5.79, (Aa); CI: 2.71, OR: 1.16-6.32 (AA)). The A allele of ApaI polymorphism was more frequent in cases than in controls (59.3%, and 43.6%, respectively) (p=0.01). The BsmI and TaqI polymorphisms were not significantly different in the frequencies of genotypes between patient and control groups. There was not a statistically significant difference in the distribution of the VDR genotypes in the disease course of MS patients **(Table 3).**

Table 1. Demographic and clinical details of MS patients and healthy controls

	Case (n=70)	Control (n=70)
Age (mean years ± SD)	44.4 ± 11.2	38.2 ± 9.5
Sex (n (%))		
Male	19 (27.1)	22 (31.4)
Female	51 (72.9)	48 (68.6)
MS-Type (n (%))		
RRMS	47 (67.1)	
PPMS	9 (12.9)	
SSMS	14 (20.0)	

SD: Standard Deviation; RRMS: Relapsing-remitting MS; PPMS: Primary progressive MS; SPMS: Secondary progressive MS

Table 2. Distribution of VDR of genotypes and alleles in cases and controls

Genotypes		Case		Control		p	OR (95%CI)
		n=70	%	n=70	%		
ApaI	aa	15	21.4	29	41.4	**0.04**	**Ref =1.00**
	Aa	27	38.6	21	30.0		2.49 (1.07-5.79)
	AA	28	40.0	20	28.6		2.71 (1.16-6.32)
	a	57	40.7	79	56.4	**0.01**	**Ref=1.00**
	A	83	59.3	61	43.6		1.89 (1.17-3.03)
BsmI	bb	20	28.6	22	31.4	0.79	Ref=1.00
	Bb	36	51.4	37	52.9		1.07 (0.50-2.29)
	BB	14	20.0	11	15.7		1.40 (0.52-3.79)
	b	76	54.3	81	57.9	0.55	Ref=1.00
	B	64	45.7	59	42.1		1.16 (0.72-1.85)
TaqI	tt	9	12.9	5	7.1	0.52	Ref=1.00
	Tt	41	58.6	45	64.3		0.51 (0.16-1.64)
	TT	20	28.6	20	28.6		0.56 (0.16-1.95)
	t	81	57.9	85	60.7	0.63	Ref=1.00
	T	59	42.1	55	39.3		1.13 (0.70-1.81)

Table 3. Distribution of VDR genotypes in the disease course of MS patients

Genotypes		PPMS (9)		SPMS (14)		RRMS (47)		p
		n	%	n	%	n	%	
ApaI	aa (wild type)	2	2.9	5	7.1	8	11.4	0.36
	Aa	5	7.1	3	4.3	19	27.1	
	AA	2	2.9	6	8.6	20	28.6	
BsmI	bb (wild type)	2	2.9	4	5.7	14	20.0	0.22
	Bb	3	4.3	6	8.6	27	38.6	
	BB	4	5.7	4	5.7	6	8.6	
TaqI	tt (wild type)	2	2.9	2	2.9	5	7.1	0.47
	Tt	6	8.6	6	8.6	29	41.4	
	TT	1	1.4	6	8.6	13	18.6	

PPMS: Primary progressive MS, SPMS: Secondary progressive MS, RRMS: Relapsing-remitting MS

Discussion

MS is an immune-mediated disease of the central nervous system caused by genetic and environmental factors. A possible effect on MS susceptibility of gene polymorphisms of several vitamin D-related proteins has been investigated. Polymorphisms of the VDR gene have been associated with neurological diseases such as MS, Alzheimer's, and Parkinson's. Recently, studies have reported the role of vitamin D with the course and progression of MS. They have showed that vitamin D is regulated by the VDR gene (1, 2, 4, 6, 9-12).

Our study shows no association of the specific VDR gene polymorphisms BsmI and TaqI in MS.

In previous studies, the association between VDR gene polymorphisms and MS was investigated in several populations (11, 13, 14). Our results are consistent with some studies reported in the literature for various populations. A study in Iran reported that there was a significant difference in genotype distribution between case and control groups for polymorphism and allelic frequency for ApaI, BsmI and TaqI (15). A Japanese group found a higher frequency of the ApaI AA genotype and A allele in MS (16). Sioka et al. showed no association of the ApaI, BsmI and TaqI polymorphisms of the VDR gene (17). The work of Abdollahzadeh et al. was similar to the study by the Japanese group (15). The results of our study are consistent with those found by Sioka and colleagues; in addition, our ApaI A allele frequency findings are compatible with the Japanese group and Abdollahzadeh (15-17).

Bermúdez-Morales et al. found a positive association of the genetic polymorphisms of VDR, specifically TaqI (rs731236) and BsmI (rs1544410). They suggested that more epidemiological and genetic studies are necessary (10).

One limitation of our study is that vitamin D status was not determined. However, another case-control study of MS in a similar population showed no differences in serum (25(OH)D) concentrations between cases and controls (22.3+6.4 ng/mL and 23.3+6.3 ng/mL, respectively), indicating no association between vitamin D status and MS risk in Mexican adults (5, 10).

Thus far, VDR has been investigated in many studies to determine the relationship with MS, but there are contradictory conclusions (12, 18-20). Small sample sizes, low statistical power, differences in ethnicities, extensive geographic variation, interactions with other genetic or environmental factors, and/or clinical heterogeneity may be the reasons for this disparity.

In summary, this study suggests that the ApaI polymorphisms of the VDR gene may be associated with MS risk**.**

Abbreviations

bp	= base pair
CI	= Confidence Intervals
CNS	= Central Nervous System
MS	= Multiple Sclerosis
PCR	= Polymerase Chain Reaction
UVR	= Ultraviolet Radiation
VDR	= Vitamin D Receptor
VDRG	= Vitamin D Receptor Gene
OR	= Odds Ratio

Acknowledgement

We thank the staff of the Neurology Unit of Çanakkale Onsekiz Mart University Hospital for collecting blood samples from patients with MS.

Conflict of Interest

There are no conflicts of interest.

Authors' contributions

Conceptualization, S.C. and H.I.O.K.; Methodology, S.C.; Formal analysis, S.C.; Investi-

gation, S.C., O.O., A.O., and S.Y.; Resources, S.C., H.I.O.K., O.O., A.O., and S.Y.; Writing – original draft preparation, S.C., H.I.O.K., O.O., A.O., and S.Y.; Writing – review and editing, S.C., H.I.O.K., O.O., A.O., and S.Y.; Visualization, S.C. and H.I.O.K.; Supervision, S.C.; Project administration, S.C.; Funding acquisition, S.C. and H.I.O.K.

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