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<sup>1</sup>Inonu University, School of Medicine,  
Department of Neurology Malatya, Turkey

<sup>2</sup>Inonu University, Department of Molecular  
Biology and Genetics Malatya, Turkey

**Corresponding Author:** Ozden Kamisli, MD.  
Department of Neurology, Inonu University,  
Malatya, Turkey. ORCID ID: <http://www.orcid.org/0000-0003-1114-7860>. Phone:  
00904223410660-4905. E-mail: [okamisli@yahoo.com](mailto:okamisli@yahoo.com) 0000-0003-1114-7860

# Analysis of Vitamin D Receptor Polymorphisms in Patients with Familial Multiple Sclerosis

Fatma Ebru Yucel<sup>1</sup>, Ozden Kamisli<sup>1</sup>, Ceren Acar<sup>2</sup>, Mert Sozen<sup>2</sup>, Mehmet Tecellioğlu<sup>1</sup>, Cemal Ozcan<sup>1</sup>

## ABSTRACT

**Objective:** Genetic and environmental factors are important in the development of the multiple sclerosis (MS). Vitamin D shows its effects on the immune system with the vitamin D receptor (VDR) in the nucleus. Single nucleotide polymorphisms (SNPs) in the VDR gene can lead to alterations in vitamin D functions and metabolism. Taq I, Apa I, Fok I and Bsm I polymorphisms and MS associations have been investigated in many studies. VDR gene polymorphism has not been previously studied in patients with familial MS. **Aim:** We aimed to investigate the relationship between familial MS patients present in Turkish population and VDR genotypes Taq I, Apa I and Fok I polymorphisms. **Methods:** 29 patients with a family history of MS and 120 healthy control subjects were included in the present study. We studied present VDR genotypes Taq I, Apa I and Fok I polymorphisms. **Results:** We observed a significant difference between controls and patient group only in Taq I polymorphism (p: 0.025). Homozygosity of G allele was not seen in the patients whereas in controls frequency of that genotype was p:0.208. When gender was considered males show significant difference for GG genotype. There were no significant association for the Apa I and Fok I polymorphisms. **Conclusion:** Although our findings suggest association between VDR Taq I polymorphism and the familial MS, additional studies are needed to establish detailed relationships. **Keywords:** Multiple Sclerosis, Familial Multiple Sclerosis, Vitamin D, Vitamin D receptor polymorphisms.

## 1. INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) in young adults (1). Although the etiology of the disease remains poorly understood, it is thought that both autoimmune and infectious mechanisms may play a role. This idea is supported by data from studies indicating that both genetic and environmental factors are involved in disease development (2). Although the factors triggering MS remain unknown, auto-reactive T cells and antibodies against CNS are believed to play major pathogenic roles in the development of inflammation and tissue damage. Similar mechanisms are implicated in the progression of neurological disability and functional deficits (1, 2).

Vitamin D is a steroid hormone that exerts regulatory and functional effects in humans (3). Vitamin D plays a regulatory role within the immune system by reducing the presentation of major histocompatibility complex (MHC)II by monocytes and T cells, and stimulating the proliferation of B

cells, differentiation of plasma cells, release of immunoglobulin E and M, production of memory B cells, and apoptosis of active B cells. Vitamin D also reduces T cell proliferation and pro-inflammatory cytokine release. The low levels of vitamin D in patients with MS suggest that vitamin D modulates the Th1-mediated immune response (3, 4).

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 (5). The relationship between MS and VDR polymorphisms has been investigated in many studies (6-13). In 2005, an Australian study explored the relationships between MS and the *Taq I*, *Apa I*, and *Fok I* polymorphisms (14). A similar study was conducted in Greece in 2011 (13). In addition, studies have been performed in Tunisia (15), Kuwait (16), South-east Iran (17), and Slovakia (18). Usually, associations between MS and the *Taq I*, *Apa I*, *Fok I*, and *Bsm*

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I polymorphisms are investigated; these polymorphisms are the most common single nucleotide polymorphisms (SNPs) of the VDR gene. The frequencies of these polymorphisms have not been previously studied in patients with familial MS in our population. We therefore explored the relationship between familial MS in our country and the VDR *Taq I* (*rs731236*), *Apa I* (*rs7975232*), and *Fok I* (*rs2228570*) polymorphisms.

In this study we aimed to study the presence of association between familial MS and three polymorphisms of VDR gene in a Turkish population living in Malatya Turkey.

## 2. MATERIALS AND METHODS

### Subjects

This study was performed in the Department of Neurology of Medical School and the Molecular Biology and Genetics Department of Inonu University. Twenty-nine patients with a family history of MS and 120 healthy controls were enrolled. MS patients aged 18-60 years, with no other known disease, were included. The controls were healthy Turkish volunteers in the same age group. There was no relationship between the patients and the control subjects. 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 and gave written consent prior to study commencement.

### 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) 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): C\_12060045\_20 for *rs2228570*, C\_2404008\_10 for *rs731236*, and C\_28977635\_10 for *rs7975232*. We employed a StepOnePlus™ Real-Time PCR System [Applied Biosystems, USA].

### Statistical analysis

SPSS software version 17.0 (Chicago, IL) was used for all statistical analyses. Hardy–Weinberg Equilibrium (HWE) was explored using Pearson’s goodness-of-fit chi-square test. The significance level for deviation from HWE was set at p-value less than 0.01 (19). Allele and

genotype frequencies are shown as counts with percentages. The level of significance was set at  $p < 0.05$ . Pearson’s, exact, and continuity-corrected chi-square tests were used to make comparisons. As the numerical data were not normally distributed, medians were defined by reference to the minimum and maximum values, and the Kruskal - Wallis test was used to compare the medians.

## 3. RESULTS

Twenty-nine patients with MS with family histories of the disease and 120 healthy individuals were enrolled in this study. Of the 29 patients, 21 were females and 8 were males. The control group included 61 females and 59 males. The demographic and clinical data are summarized in Table 1.

	Patients (n = 29)	Controls (n = 120)
Age (years)	33.7 ± 10.7	33.1 ± 8.5
Gender (male/female, n)	8/21	59/61
Duration of disease ( years)	7.6 ± 5.6	
MS subtype (n)	*RRMS, 23 *PPMS, 1 *SPMS, 5	

**Table 1. Demographic and clinical characteristics of patients and controls. \*RRMS: Relapsing Remitting Multiple Sclerosis. \*PPMS: Primary Progressive Multiple Sclerosis. \*SPMS: Secondary Progressive Multiple Sclerosis**

Tables 2 shows the allele and genotype frequencies of the *Taq I* (*rs731236*), *Fok I* (*rs2228570*), and *Apa I* (*rs7975232*) polymorphisms in cases and controls. The frequency of only the *Taq I* polymorphism differed significantly between the patient and control groups ( $p = 0.025$ ). Homozygosity of GG was absent in patients but occurred at a frequency of 0.208 in controls. The other two polymorphisms were not significantly associated with in our group of familial MS patients from Malatya.

## 4. DISCUSSION

Multiple Sclerosis is a chronic demyelinating disease of the CNS and is one of the most common causes of disability in young and middle-aged adults (20). Genetic and environmental factors, especially T cell-mediated autoimmunity, are thought to play roles in MS pathogenesis (2). Vitamin D is a potential modulator of immune

Taq I	AA	AG	GG	p	Hardy–Weinberg p	A	G	p
	n (%)	n (%)	n (%)			n (%)	n (%)	
Controls	31 (43.1)	26 (36.1)	15 (20.8)	0.025	0.041	88 (61.1)	56 (38.9)	0.149
Patients	13 (46.4)	15 (53.6)	0 (0)			0.053	41 (73.2)	
Fok I	AA	AG	GG	p	Hardy–Weinberg p	A	G	p
	n (%)	n (%)	n (%)			n (%)	n (%)	
Controls	8 (7)	34 (29.8)	72 (63.2)	0.422	0.168	50 (21.9)	178 (78.1)	0.233
Patients	1 (3.4)	6 (20.7)	22 (75.9)			0.483	8 (13.8)	
Apa I	AA	AC	CC	p	Hardy–Weinberg p	A	C	p
	n (%)	n (%)	n (%)			n (%)	n (%)	
Controls	28 (34.6)	37 (45.7)	16 (19.8)	0.956	0.553	93 (57.4)	69 (42.6)	0.963
Patients	8 (30.8)	13 (50)	5 (19.2)			0.945	29 (55.8)	

**Table 2. VDR Polymorphisms genotypes and alleles frequencies**

system; therefore, many studies have explored vitamin D status in patients with MS (21). Vitamin D plays regulatory and functional roles in humans. Vitamin D binds to VDR and the membrane-associated fast-response steroid-binding receptor (MARRS). Certain polymorphisms of the *VDR* gene modify the function and metabolism of vitamin D (22). Many studies have explored the effects of *VDR* polymorphisms in various ethnic groups. Associations between *Taq I*, *Apa I*, *Fok I*, and *Bsm I* polymorphisms and MS, are usually examined; these polymorphisms are the most commonly studied SNPs in the *VDR* gene. These polymorphisms have not previously been studied in patients with familial MS. We evaluated the frequencies of *Taq I*, *Apa I*, and *Fok I* polymorphisms in patients with familial MS in our region.

Several studies focused on these three SNPs in their cohorts. An Australian case-control study (104 patients and 104 controls) found significant difference between groups in the frequencies of the *Taq I* and *Apa I* polymorphisms; the latter polymorphism predisposed patients to primary and secondary progressive MS (14). Cox et al., found that *Taq I* polymorphism increased the risk of MS in the UK (12). In another study involving 60 patients with RRMS and 114 controls in Tunisia, the T allele of the *Taq I* polymorphism was found to protect against the risk of MS in an age- and gender-specific manner. Thus, VDR status may affect the risk of MS. However, no significant association was evident between the *Apa I* polymorphism and MS (15).

In a Canadian case-control study allele or genotype frequencies of the *Taq I* and *Apa I* polymorphisms did not differ (23). Similarly, the frequencies of the genotypes or alleles of *Taq I* polymorphism showed no difference in a Greek study (13). The latter study showed that the bone mineral densities of the hip and lumbar vertebrae were lower in patients with MS than in controls. No significant association was apparent between low bone mineral density and the *Taq I* polymorphism (13).

Dickinson et al. studied 136 patients and 235 controls. No relationship was evident between any of the *Cdx-2*, *Fok I*, or *Taq I* polymorphisms and MS. However, low-level winter sun exposure during childhood was associated with the *Cdx-2* polymorphism and MS development. The same study found a 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 (10).

Simon et al. studied 214 patients and 428 controls in the United Kingdom. Patients with the *Fok I* polymorphism and low vitamin D intake were at high risk of MS development. No association was evident between *VDR* SNPs (*Apa I*, *Taq I*, *Bsm I*, and *Fok I*) and MS (11).

Ciorny et al., working in Slovakia, found that the *Fok I* polymorphism was more common in female patients with MS (18). In the present study, no significant gender difference was observed.

In the present study we analysed if there is an association between three SNPs located in *VDR* gene and familial MS. We set our hypothesis as “there is no significant

difference in genotypes and allele frequencies between familial MS patients and healthy controls for three VDR SNPs we have screened in this study.”

Our results showed that, the frequency of the *Taq I* polymorphism differed significantly between controls and patients ( $p = 0.025$ ). In addition, the *Taq I* polymorphism analysis suggested that the GG genotype may protect against the familial MS and this may be a positive marker for familial MS disease. The frequency of the *Apa I* and *Fok I* polymorphisms did not differ significantly between the two groups. Since there were a few patients in the subgroups we couldn't analyze our data by MS subtype. Neither Australian (14) nor our study found significant difference between case and control groups for *Fok I* polymorphism.

The incidence of familial MS in the community is low (4% in first-degree relatives of patients with MS) (24); we enrolled only 29 patients with familial MS. The lack of associations between the *Fok I* and *Apa I* polymorphisms and MS may indicate that these polymorphisms in this patient group are in fact not associated with the disease, or that our patient number was too small. Our work is preliminary in nature; additional studies with more patients with a family history of MS are needed to obtain statistically significant results.

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- **Author contribution:** FEY and OK conceived and wrote the initial study proposal and contributed substantially to conception and design of the research study. CA and MS made substantial contributions to acquisition of data. FEY, OK and CA performed data analysis and drafted the manuscript. FEY, OK, CA, and MS participated in results interpretation and editing of the manuscript. All authors approved the final version of the manuscript.
- **Conflict of interest:** The authors declare no conflicts of interest related to this manuscript.

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