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Vitamin D receptor gene polymorphism in Egyptian multiple sclerosis patients

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Abstract

One of the most common neurological illnesses in the world is multiple sclerosis (MS), a chronic autoimmune demyelinating disease of the central nervous system (CNS). MS has both a genetic and an environmental origin. In terms of environmental factors, vitamin D deficiency is one of the most important risk factors and closely connected with gene polymorphisms involved in vitamin D metabolism, transport, or activity. Since vitamin D activity requires a receptor-mediated response, any changes to the vitamin D receptor (VDR) may have an effect on the pathophysiology of the disease. In this study, we aimed to identify the relationship between VDR gene polymorphisms, FokI A>G (rs2228570), ApaI A>C (rs7975232) and BsmI C>T (rs1544410) and MS. FokI, ApaI and BsmI genotypes were determined in 50 patients with relapsing remitting MS (RRMS) and in 50 control subjects. DNA was isolated from blood samples, and then FokI, ApaI and BsmI gene polymorphisms were identified using allelic discrimination real time polymerase chain reaction (PCR) assay. The distribution of FokI, ApaI and BsmI polymorphisms did not show any significant differences between MS patients and controls. Thus, we concluded that there is no association between the studied VDR gene polymorphisms and MS.

Keywords: MS, SNP, Vitamin D receptor (VDR), FokI, ApaI and BsmI

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Introduction

One of the most frequent neurological conditions worldwide is multiple sclerosis (MS). It is a chronic, autoimmune demyelinating condition of the central nervous system (CNS), characterized by a progressive loss of motor, sensory, and mental abilities.[1] In Egypt, a research study by Hussein et al., 2019,[2] estimated that there are 59 MS cases for every 100,000 people. In the same study, women made up 75% of MS cases, while men made up 25%. According to the study, primary progressive MS accounts for 10% of instances, while secondary progressive and relapsed MS account for 25% and 65% of cases, respectively.

The etiology of MS is recognized as a complex illness in which both genetic and environmental variables may have an impact on how an individual's immune system develops and responds, predisposing them to MS onset.[3]

Regarding environmental factors, vitamin D insufficiency is one of the most significant risk factors and is closely correlated with gene polymorphisms that are involved in vitamin D metabolism, transport, or activity.[4]

The pathogenesis of the disease may be impacted by changes in the vitamin D receptor (VDR) since vitamin D activity necessitates a receptor-mediated response.[5] Variants in the VDR gene were investigated in relation to immune modulation, autoimmune disorders, and MS, especially the single nucleotide polymorphisms (SNPs) ApaI (rs7975232), BsmI (rs1544410), FokI (rs2228570), and TaqI (rs731236).[6] The polymorphisms ApaI (rs7975232), BsmI (rs1544410), and TaqI (rs731236) are situated close to the VDR gene's 3' end, whereas FokI (rs10735810) is situated close to the VDR gene's 5' end. The FokI is located in exon 2. The A to G substitution eliminates the translation initiation site, producing a final protein of 424 amino acids instead of the 427 amino acids of the wild-type product. This shorter isoform has a higher transcriptional activity; because it interacts with the transcription factor IIB (TFIIB) more effectively.[7,8] The ApaI, BsmI, and TaqI SNPs are in substantial linkage disequilibrium (LD) but do not alter the structure of the VDR protein.[9,10] While neither ApaI nor BsmI SNPs alter the VDR protein's amino acid composition, they may have an impact on the messenger ribonucleic acid (mRNA) stability and, consequently, on the gene expression of VDR.[11] Therefore, in the present study, we aimed to study FokI, ApaI and BsmI VDR SNPs in a cohort of Egyptian MS patients and compare them with normal controls.

Subjects and Methods

Subjects

This case-control study included 50 relapsing remitting MS (RRMS) patients diagnosed according to McDonald's criteria for the diagnosis and classification of MS (2017).[12] They were recruited from the MS unit, Department of Neurology, Ain Shams University Hospitals. They were 9 male patients and 41 female patients, and their ages ranged from 18 to 48 years. The control group consisted of 50 age, sex and ethnicity matched apparently healthy subjects. They were 15 males and 35 females, and their ages ranged from 18 to 40 years.

Full medical history was taken and assessment of functional disability using the "Extended Disability Status Scale" (EDSS) score was performed for all studied MS patients.[13] Detection of FokI (rs2228570), ApaI (rs7975232) and BsmI (rs1544410) VDR gene polymorphisms by allelic discrimination real time PCR was performed for all subjects enrolled in this study at the Department of Clinical Pathology, Immunology unit, Ain Shams University Hospitals from September 2019 to July 2020.

Sample collection and VDR SNPs analysis

Whole venous blood (2 mL) samples were collected from study subjects under complete aseptic conditions, then added to sterile EDTA vacutainers. Samples were transported to the laboratory then stored at -20°C for DNA extraction and subsequent genotyping.

DNA extraction was done using commercial kits (cat. No. 51104, QIAamp DNA blood mini kits, QIAGEN, Strasse 1, 40724 Hilden, Germany), according to the manufacturer' instructions. The extracted DNA was amplified using TaqMan Genotyping master mix (cat. No. 4371353, Applied Biosystems Inc, 850 Lincoln Centre Drive Foster City, CA 94404, USA) and ready-made TaqMan SNP genotyping assay for rs2228570, rs7975232 and rs1544410 (cat. No. 4351379, Applied Biosystems Inc, 850 Lincoln Centre Drive Foster City, CA 94404, USA), according to the manufacturer instructions. DNA amplification and genotyping were performed using a thermal cycler (Rotor-Gene Q MDx, QIAGEN, Strasse 1, 40724 Hilden, Germany).

Statistical Analysis

The collected data were revised, coded, tabulated, and introduced to analysis using the Statistical Package for Social Science (SPSS) for windows (IBM Corp. Released 2019. Version 26.0. Armonk, NY). Data were expressed as median and percentiles for numerical non-parametric data and as frequency and percentages for non-numerical data. Chi-square test was used to

determine differences in the VDR allele frequencies and genotype distribution between patients and controls For non-parametric data, Wilcoxon Rank Sum test was used to assess the statistical significance of the difference between two groups and Kruskal-Wallis test was used to assess the statistical significance of the difference between more than two groups. The Hardy-Weinberg Equilibrium (HWE) of the genotype distribution in patients and controls was assessed using the Chi square test. The strength of association between the VDR gene FokI, ApaI and BsmI polymorphisms and susceptibility to MS was evaluated by odds ratio (OR) and 95% confidence intervals (95% CI). Values of $p< 0.05$ were considered statistically significant.

Results

Descriptive characteristics of the study participants

The main demographic and clinical characteristics of MS patients are shown in Table 1.

Table 1. Demographic and clinical data of the 50 MS patients.

	MS patients' group	
Gender	Male n (%)	Female n (%)
	9 (18%)	41 (82%)
	Median (25^{th} – 75^{th} percentile)	
Age (in years)	30 (23.75 - 36.00)	
Age of disease onset (in years)	24 (20 - 30)	
Duration of the disease (in months)	48 (24 - 84)	
No. of relapses	3.5 (2.00 - 6.25)	
EDSS	2.0 (1.5 - 3.5)	

EDSS: Extended Disability Status Scale

Distribution of FokI, ApaI and BsmI genotypes showed no significant difference when MS patients were compared to control group (X^2= 1.462, p= 0.481, X^2= 1.539, p= 0.463, X^2= 1.073, p= 0.300, respectively). No deviation was observed from Hardy-Weinberg equilibrium (HWE) in the genotypic distribution of the VDR-FokI and VDR-ApaI polymorphisms in all the study subjects, however, VDR-BsmI polymorphism deviated from HWE. Risk assessment of the FokI dominant genetic model (AA vs AG+GG) (OR= 0.375, p=0.255) and the recessive genetic model (AA+AG vs GG) (OR= 0.786, p= 0.548) showed no increased MS risk. Similarly, the risk assessment of ApaI dominant genetic model (AA vs AC+CC) (OR= 1.494, p= 0.318) as well as the recessive genetic model (AA+AC vs CC) (OR= 1.833, p= 0.279) did not show increased MS risk. The risk assessment of BsmI CC vs CT genotypes (OR= 0.649, p= 0.301) showed similar results. (Table 2).

Allele frequencies of FokI, ApaI and BsmI showed no difference when MS patients were compared to the control group. Also, MS patients showed no increased occurrence of G allele of FokI (OR= 0.741, p= 0.345), C allele of ApaI (OR= 1.500, p= 0.178) or T allele of BsmI (OR= 0.792, p= 0.447) (Table 3).

Table 2. Risk assessment and comparison of the distribution of FokI, ApaI and BsmI genotypes between MS patients and the control group.

SNP Genotype		MS patients' group (n=50)		Control group (n=50)		*p*-value (Pearson Chi square test)	Risk assessment	
		N	%	N	%		OR (95% CI)	*p*-value
*FokI	AA	5	10%	2	4%	NS	Reference	
	AG	21	42%	21	42%		0.400 (0.069-2.296)	NS
	GG	24	48%	27	54%		0.355 (0.063-2.004)	NS
Dominant model	AA	5	10%	2	4%	-	0.375 (0.069-2.031)	NS
	AG+GG	45	90%	48	96%			
Recessive model	AA+AG	26	52%	23	46%		0.786 (0.358-1.724)	NS
	GG	24	48%	27	45%			
HWE		*p*-value Chi square test		*p*-value Chi square test				
		NS		NS				
SNP Genotype		MS patients' group (n=50)		Control group (n=50)		*p*-value Pearson Chi square test	Risk assessment	
		N	%	N	%		OR (95% CI)	*p*-value
**ApaI	AA	22	44%	27	54%	NS	Reference	
	AC	18	36%	17	34%		1.299 (0.544-3.100)	NS
	CC	10	20%	6	12%		2.045 (0.642-6.512)	NS
Dominant model	AA	22	44%	27	54%	-	1.494 (0.679-3.285)	NS
	AC+CC	28	56%	23	46%			
Recessive model	AA+AC	40	80%	44	88%		1.833 (0.610-3.127)	NS
	CC	10	20%	6	12%			
HWE		*p*-value Chi square test		*p*-value Chi square test				
		NS		NS				
SNP Genotype		MS patients' group (n=50)		Control group (n=50)		*p*-value Pearson Chi square test	Risk assessment	
		N	%	N	%		OR (95% CI)	*p*-value
***BsmI	CC	21	42%	16	32%	NS	Reference	
	CT	29	58%	34	68%		0.649 (0.286-1.472)	NS
HWE		Chi square test *p*-value		Chi square test *p*-value				
		0.015		0.001				

OR: odds ratio CI: Confidence interval. *FokI: AA: homozygous wild type, AG: heterozygous, GG: homozygous variant. **ApaI: AA: homozygous wild type, AC: heterozygous, CC: homozygous variant. ***BsmI: CC: homozygous wild type, CT: heterozygous. $p > 0.05$ is not significant (NS).

Table 3. Risk assessment and comparison of the frequencies of FokI, ApaI and BsmI alleles between MS patients and the control group.

SNP	Allele	MS patients' group (n=50)		Control group (n=50)		*p*-value Pearson Chi square test	Risk assessment	
		N	%	N	%		OR (95% CI)	*p*-value
FokI*	A	31	31%	25	25%	NS	Reference	
	G	69	69%	75	75%		0.741 (0.399-1.379)	NS
ApaI**	A	62	62%	71	71%	NS	Reference	
	C	38	38%	29	29%		1.500 (0.830-2.710)	NS
BsmI***	C	71	71%	66	66%	NS	Reference	
	T	29	29%	34	34%		0.792 (0.435-1.442)	NS

*FokI: A: wild allele, G: variant allele. **ApaI: A: wild allele, C: variant allele. ***BsmI: C: wild allele, T: variant allele.
p > 0.05 is not significant (NS).

Patients with the different genotypes of FokI, ApaI and BsmI, showed no difference regarding the age of disease onset, disease duration, number of relapses and the EDSS scores (Table 4).

Table 4. Comparison between genotypes of FokI, ApaI and BsmI according to the clinical data of MS patients.

FokI genotypes	AA	AG	GG	*p*-value Kruskal Wallis test
	Median (25^{th} – 75^{th} percentile)	Median (25^{th} – 75^{th} percentile)	Median (25^{th} – 75^{th} percentile)	
Age (years) of disease onset	24 (18-31)	28 (21-30)	22 (20-28.5)	NS
Duration (months)	36 (25-96)	60 (30-90)	48 (24-69)	NS
No. of relapses	3 (1.5-8)	4 (2-6.5)	3 (2.25-5)	NS
EDSS	1.5 (1.5-3.75)	2.5 (1.75-3.5)	1.5 (1.5-3.375)	NS
ApaI genotypes	**AA**	**AC**	**CC**	***p*-value Kruskal-Wallis test**
	Median (25^{th} – 75^{th} percentile)	Median (25^{th} – 75^{th} percentile)	Median (25^{th} – 75^{th} percentile)	
Age of onset (years)	22.5 (18.75-29.5)	26.5 (20.75-30)	24 (19.75-30.5)	NS
Duration (months)	60 (21-84)	60 (24-78)	48 (33-87)	NS
No. of relapses	3 (1.75-5)	4 (2.75-7.25)	3.5 (2-7.5)	NS
EDSS	1.75 (1.5-3)	2.25 (1.5-3.5)	2.5 (1.5-3.625)	NS
BsmI genotypes	**CC**		**CT**	***p*-value Wilcoxon Rank Sum test**
	Median (25^{th} – 75^{th} percentile)		Median (25^{th} – 75^{th} percentile)	
Age of onset (years)	24 (21.5-30)		24 (18-30)	NS
Duration (months)	48 (30-96)		48 (24-78)	NS
No. of relapses	4 (2.5-7.5)		3 (2-5)	NS
EDSS	2 (1.5-3.5)		2 (1.5-3)	NS

p > 0.05 is not significant (NS).

Discussion

MS is an autoimmune disease with a multifactorial etiology. Vitamin D is suggested to have a possible role in the pathogenesis of MS. Single nucleotide polymorphisms in the VDR gene may alter the vitamin D immune-regulatory pathway, which would then have an impact on MS risk.[14] The VDR gene polymorphism and the risk of MS have been studied in a number of studies, but the results were inconsistent and inconclusive. In the present study, we aimed to investigate the role of FokI, ApaI and BsmI VDR polymorphisms, specifically allelic and genotypic frequencies, in the development and clinical course of RRMS in Egyptian patients.

The concept of HWE was employed to assess genetic variation equilibrium in a population. According to the equation, in the absence of disturbing circumstances, the level of genetic variation in a population will remain constant from one generation to the next.[15] In our investigation, the genotype distribution of FokI and ApaI SNPs exhibited HWE in both controls and patients, while the distribution of VDR-BsmI was not in HWE. This deviation could be attributed to factors such as the limited sample size of the study, potential genetic drift, gene flow between generations, and the presence of mutations or SNPs in the VDR gene within the population. Our observations align with a study by Narooie-Nejad et al., 2015,[16] where ApaI polymorphisms were in HWE in MS patients and controls. Conversely, a meta-analysis by Zheng et al., 2018[17] revealed that two studies involved BsmI, two studies involved ApaI and one study for FokI SNPs were not in HWE.

The FokI VDR SNP (rs2228570) resides in exon-2 and is associated with a shorter VDR protein isoform, potentially impacting its transactivation capacity as a transcription factor.[18] The study by Van Etten et al., 2007[19] showed that VDR FokI polymorphism altered immune cell behavior with a more responsive immune system for the short VDR, emphasizing its function in immunological-mediated illnesses. In our study, we found no difference in the distribution of FokI genotypes as well as allele frequency between MS patients and normal controls. Also, we could not find a significant risk association when studying the variant allele (G allele) of FokI SNP in both the dominant genetic model (GG+AG vs AA, OR= 0.375, *p*= 0.255) and the recessive genetic model (AA+AG vs GG, OR= 0.786, *p*= 0.548) and MS development. Our findings are in line with studies by Agnello et al., 2016,[20] Al-Temaimi et al., 2015,[21] Křenek et al., 2018[22] and Tajouri et al., 2005[23] they did not observe a link between FokI and MS risk. Additionally, in 2019, a meta-analysis research reported no association was proven between FokI polymorphisms and MS.[24] Another meta-analysis, conducted by Garcia-Martin et al., 2013[25] included ten studies involving 2944 MS patients and 3166 control subjects, found no association between the FokI SNP and the incidence of MS. Similarly, a meta-analysis by Mohammadi et al., 2020[6] evaluated FokI in 576 MS Iranian cases and 738 controls, but no association with MS risk was detected under all the genetic models.

However, our findings are inconsistent with the results of other studies that showed an association between VDR FokI polymorphisms and MS pathogenesis. An Egyptian study by Hassab et al., 2019[26] found a statistically significant higher percentage of FokI genotypes (AG+GG) in the healthy controls compared to MS cases with a statistically significant association of the "G" allele in the control group, indicating the possibility that the "G" allele is associated with lower MS risk (OR= 0.42, *p*= 0.015). The meta-analysis of 13 case-control studies by Tizaoui et al., 2015[27] reported an association of the wild AA genotype of FokI with an increased susceptibility of MS in a total of 3300 MS patients and 3194 healthy control subjects. In addition, the study by Cierny et al., 2015[8] reported an association of AG genotype with an increased incidence of MS in Slovak women (OR= 1.48, *p*= 0.042). Kamisli et al., 2018[28] suggested a protective role of GG genotype against MS. Another study by Bulan et al., 2023[29] reported an association of AA genotype and A allele with increased MS risk (A allele OR= 1.391, $p < 0.05$). Again, a study by Moosavi et al., 2021[30] revealed an association between FokI A allele of VDR and MS risk (OR=

1.6, *p*= 0.0236). It is notable that a population's exposure to vitamin D may influence whether a VDR polymorphism is associated with MS in that population. A study by Simon et al., 2010[31] in the USA found no relationship between FokI and MS, but it showed a protective benefit of increased vitamin D supplementation, which was noticeable in women with the "AA" genotype who had a significant 80% lower risk of MS. Hypothetically, a VDR gene variant that confers protection against MS development could not influence the disease status in a population that is often deficient in vitamin D. Therefore, it is noticeable that groups with known links between MS and VDR gene SNPs are exposed to relatively high levels of vitamin D in their environment.[5]

The ApaI polymorphism, located at the 3' untranslated region of the VDR gene, can influence gene expression through mRNA stability and translation efficiency. Genotypic distribution and allele frequency of VDR-ApaI polymorphism in the present study did not reveal any statistically significant difference between MS cases and controls. The dominant genetic model for the variant allele (C) of ApaI SNP (AC+CC vs AA) as well as the recessive genetic model (AA+AC vs CC) showed no significant risk association for MS development (OR= 1.494, *p*= 0.318, OR= 1.833, *p*= 0.279, respectively). Similar results were reported by Agnello et al., 2016,[20] Cancela Díez et al., 2021,[32] Kamisli et al., 2018[28] and Smolders et al., 2009[9] who found no influence of the *ApaI* gene polymorphisms on the risk of developing MS in their patients. In addition, the meta-analysis of Zheng et al., 2018,[17] after performing the sensitivity analysis, rejected any association between the ApaI SNP and risk of MS. In addition, they found no association between ApaI SNP genotypes and MS susceptibility in Asians, or Caucasians population. On the contrary, the study of Tajouri et al., 2005[23] detected a predominance for the A allele in MS, although the genotype distribution was not significantly different between patients and controls. Similarly, Hassab et al., 2019[26] found a significant association of A allele with MS risk (OR= 2.47, *p*= 0.008). The meta-analysis by Tizaoui et al., 2015[27] suggested that the homozygote wild AA genotype was a significant MS risk factor. In contrast, Cakina et al., 2018[33] showed a statistically significant higher frequency of the variant C allele in MS cases compared to healthy controls (OR= 1.89, *p*= 0.01). In another meta-analysis by Imani et al., 2019,[24] found no evidence that ApaI polymorphism increased MS risk in the pooled results. However, subgroup analysis detected significant association between the presence of ApaI SNP and MS risk in Asian population in comparison with European population. Inversely, in the meta-analysis by Mohammadi et al., 2020,[6] reported that allelic genetic model revealed that the wild A allele was significantly associated with a lower risk of MS (OR= 0.54, *p*= 0.00), and the homozygote genetic model (AA vs CC) showed the same association (OR= 0.28, *p*= 0.00). BsmI (rs1544410) VDR SNP, situated in intron 8, is characterized by the C > T change, with the ancestral allele being C.[34] Regarding its functional impact, it might cause a change in the VDR intron regulatory elements or the splice sites for mRNA transcription.

In the current study, VDR-BsmI polymorphism did not reveal any statistically significant difference between MS cases and controls as regards the distribution of its genotypes and allele frequency. Also, MS risk association of the variant allele (T) of VDR-BsmI SNP was insignificant (CT vs CC genotypes OR= 0.649, *p*= 0.301 and T allele OR= 0.792, *p*= 0.447). The first study to report a relationship between MS and BsmI polymorphism was based on the Japanese population, it showed that the TT genotype as well as the T allele were significantly higher in MS group compared to the control group (OR= 2.38, *p*= 0.0263 and OR= 2.45, *p*= 0.0138, respectively).[35] However, a study on Canadian population found no association between BsmI polymorphisms on the VDR gene and MS.[36]

The findings of the present study are consistent with those reported by other studies done by Abdollahzadeh et al., 2017,[37] Agnello et al., 2016,[20] Bulan et al., 2023,[29] Cakina et al., 2018,[33] Cancela Díez et al., 2021[32] and Hassab et al., 2019.[26] Similarly, studying the Greek population, which is epidemiologically comparable to the Middle East and North

Africa, where Egypt is located, revealed no association between the BsmI mutation and MS.[38]. In addition, the study of Zheng et al., 2018[17] did not find any association between BsmI polymorphisms and MS susceptibility in the overall populations, Asians, and Caucasians.

On the other hand, in 2019, the meta-analysis by Imani et al., 2019[24] showed no association between BsmI polymorphisms and MS in the pooled results, but subgroup analysis revealed that BsmI polymorphism may increase the risk of MS in Asian population compared to European population. The authors attributed the contradictory findings among Asian, European, and the overall population to the individuals' exposure to regional environmental factors and varying genetic backgrounds, which may have exaggerated impacts on MS risk.

On the other hand, Mohammadi et al., 2020[6] stated that BsmI polymorphism has a negative association with MS risk in allelic C vs T (OR= 0.69, *p*= 0.01), genotypes TT vs CC (OR= 0.46, *p*= 0.01), and CC vs CT+TT (OR= 0.56, *p*= 0.00) models after performing sensitivity analysis. A recent Egyptian study done by Sultan et al., 2023[39] showed that the genotype distribution of the BsmI VDR polymorphism did not differ significantly between healthy controls and MS patients. However, they could reveal a statistically significant higher percentage of the C allele in MS patients (OR= 1.8, *p*= 0.045) and an increase in the risk of MS with CC genotype (OR= 3.00, *p*= 0.124). Their study findings suggested that the VDR gene variant BsmI C allele may increase the risk of the development of MS.

In terms of disease characteristics, our investigation did not identify statistically significant associations between any of the studied VDR gene polymorphisms and age of disease onset, disease duration, relapse frequency, or Expanded Disability Status Scale (EDSS) scores. Similarly, Garcia-Martin et al., 2013[25] did not find any significant difference between FokI genotypes regarding the age of disease onset and/or EDSS. Also, in the study of Cierny et al., 2015[8] there was no significant association between FokI SNP and the rate of disease disability progression. The study by Bettencourt et al., 2017[40] detected the GG genotype of FokI to be more in MS patients with no difference in disease course or disability progression, even though they were unable to rule out FokI role in MS. On the other hand, Mamutse et al., 2008[41] showed that the FokI G allele was associated with a decreased 10-year disability level, following the disease onset.

The following could be the main reasons why VDR gene polymorphism may have varying effects in different studies or among different ethnic groups. 1- The MS diagnostic criteria vary from study to study, 2- the different characteristics and classification of included MS patients between studies, 3- many reviews and meta-analyses studies included case-control studies which showed deviation from HWE, 4- the patients' varied ethnic contextual features may play a significant role in these variations, 5- gender may serve as a potential source of variation, although both male and female respondents were included in the majority of studies, 6- it was proposed that VDR SNPs were associated with basal levels of 1,25 $(OH)_2$ D (the active form of vitamin D) and vitamin D structure and function, which could affect the susceptibility to MS, 7- the included studies used a variety of genotyping techniques; some used PCR-RFLP and others TaqMan assay. Finally, MS is thought to be a polygenic condition, hence it is expected that several gene loci would interact in the pathogenesis of MS.

Numerous epidemiological studies have proposed that vitamin D deficiency and sunlight contributes to the incidence of MS in temperate regions. In addition to numerous other factors, vitamin D deficiency or sufficiency may operate as a risk or protective factor, respectively, and may be constantly modulating the global MS susceptibility from the mother's pregnancy to adulthood. Although it appears that vitamin D's primary function in MS is immunomodulatory, affecting various T and B cell subsets in the immune system as a whole, the CNS may also be affected by neurotrophic and neuroprotective effects.[42,43] Unfortunately, we could not investigate this point due to the lack of data in the patients' files about their vitamin D levels.

In conclusion, our results showed no association between the FokI, ApaI, and BsmI

polymorphisms of the VDR gene and MS risk or disease severity in the studied Egyptian population. The intricate nature of MS etiopathology makes it challenging to draw definitive conclusions.

Author Contributions

RRM was involved in the study design and approval of the research protocol. NMZ and AAM were involved in protocol design, laboratory oversight, and approval of the manuscript. AA, supervision of sample collection, clinical data review, diagnosis, and patient classification. NSW was involved in monitoring of data collection, laboratory work interpretation, and reviewed the manuscript. NRA, sample collection, clinical data compilation, laboratory work, statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 214/2019).

Informed consent

All study participants provided written informed consent before being included in the study.

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