The Egyptian Journal of Neurology, Psychiatry and Neurosurgery

RESEARCH

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Investigating the role of VDR gene variants in multiple sclerosis susceptibility: a case–control study in Egypt

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Abstract

Background Multiple sclerosis (MS) is a chronic inflammatory disorder. Vitamin D has a major role in preventing inflammatory disorders as well as its role in the pathophysiology of MS. Vitamin D initiates its biological responses by binding to the nuclear vitamin D receptor (VDR). Several studies have been conducted over the last decade to investigate the relationship between VDR gene variants and the risk of MS, but the results have been inconsistent and inconclusive. The objective of this study is to investigate the association between the VDR gene variants (c.1025-49C>A) and (c.1056A>G) and MS susceptibility in a sample of the Egyptian population, and to shed light on its potential role in preventing inflammatory disorders and its impact on clinical outcomes and treatment using TaqMan Real-Time Polymerase Chain Reaction (PCR). This case-control study was conducted on 100 participants, categorized into two groups. The first group included 50 patients diagnosed with relapsing-remitting multiple sclerosis (RRMS) based on the Revised McDonald MS criteria, and the second group included 50 matched healthy participants. After collecting the blood samples, deoxyribonucleic acid (DNA) was extracted and detection of the VDR: c.1025-49C>A and VDR: c.1056A>G gene variants was done using TaqMan Real-Time PCR on all involved individuals.

Results The distribution of the genotypes and alleles of VDR gene variants (c.1025- 49C>A) and (c.1056A>G) did not differ significantly between MS patients and healthy participants (P>0.05 in both).

Conclusion Here we show in this study that there was no association between the risk of MS and the VDR gene variants (c.1025-49C>A) and (c.1056A>G) in a group of the Egyptian population which may have impact on MS therapy and outcome.

Background

Multiple sclerosis (MS) is an autoimmune disease that often affects young and middle-aged adults. It is characterized by demyelination of the central nervous system (CNS) matter, both grey and white leading to disorganization of the nervous system conduction. MS is one of the most common causes of non-traumatic disability among young and middle-aged adults [1].

The etiology of MS is still unsettled, but it is assumed that environmental, geographical, and genetic factors may have a role in its etiology and progression [2].

MS is still an incurable disease that affects an estimated 2.8 million people globally. It most commonly happens during one of the most productive times of life, namely young adulthood, making the condition responsible for lowering the quality of life not only for those affected but also for society as a whole [3].

The diagnosis of MS is made according to the revised McDonald criteria, which is based on neurological symptoms and signs, as well as evidence of dissemination of

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CNS lesions in space and time. Modern revolutionary techniques such as immunohistochemistry and MRI allow the recognition of multiple sclerosis lesions. These lesions appear throughout the CNS matter as focal areas of demyelination, inflammation, and glial reaction. It is denoted by fully or partially reversible incidents of neurological disability, usually lasting days to weeks [4].

Clinical researchers in the last decade have drawn attention to vitamin D deficiency and its role in MS pathophysiology. Vitamin D has broad regulatory effects on cells of the adaptive and innate immune system, such as reducing T cell proliferation and shifting the balance of T cell differentiation from the Th1 and Th17 pathways to the Th2 and regulatory T cell (Treg) pathways [5].

The effect of vitamin D is dependent on the nuclear vitamin D receptor (VDR), so any changes in vitamin D can be influenced by mutations in the VDR gene, such as single nucleotide variants (SNVs) [6].

The risk of developing MS has associated with certain class I and class II alleles of the major histocompatibility complex (MHC), particularly the HLA-DRB1 locus. The presence of a vitamin D response element (VDRE) located in the promoter region of many but not all HLA-DRB1 alleles suggests that environmental differences in vitamin D might interact with HLA-DRB1 to influence the risk of MS. The VDRE enhances gene expression when stimulated by vitamin D. However, other factors related to HLA variation may have more impact on MS risk than vitamin D regulation of HLA-DR expression [3]. Mounting evidence suggests that the risk of MS is associated with multiple genes related to vitamin D metabolic pathway of modest effect (such as, *DHCR7, NADSYN1, CYP2R1, CYP27A1, CYP3A4, CYP24A1, VDBP*) and genes related to the mechanism of action (*VDR, RXR, and MARRS)* [7].

The vitamin D receptor gene is located on chromosome 12q13.1 and is approximately 100 kb in size and is divided into 8 introns and 9 exons. The first exon contains the gene promoter, exon 2–3 code for the DNA binding domain, and exon 6–9 for the ligand-binding domain [8].

The $1,25(OH)_2$ D_3 binds to *VDR* with high affinity and selectivity as it exerts its effect through a series of cell-signaling reactions. To exert the genomic effect, $1,25(OH)_2$ D_3 dissociates from vitamin D-binding protein and then diffuses across the plasma membrane migrating towards the nucleus. The interaction of $1,25(OH)_2$ D_3 hormone with *VDR* initiates a complex cascade of molecular events that activates a range of biological functions or mediates the suppression of gene transcriptions [9, 10].

Gene variants can be defined as subtle sequence variations transpiring in at least 1% of the population. Variants alter gene expression, thereby affecting protein levels of the *VDR* gene, leading to functional changes. Despite over 30 variants discovered within the *VDR* gene, four SNVs have been studied as the major variants involved in autoimmune diseases such as MS, including rs2228570 (*VDR*:c.2 T > C), rs1544410 (*VDR*:c.1024 + 283G > T), rs7975232 (*VDR:*c.1025-49C > A) and rs731236 (*VDR:*c.1056A > G) [8].

Some well-known VDR gene variants are rs2228570 (VDR: c.2 T > C), rs1544410 (VDR: c.1024 + 283G > T), rs7975232 (VDR: c.1025-49C > A), and rs731236 (VDR: c.1056A > G). In general, the majority of variants in the *VDR* gene are found to be in regulatory areas such as the 5' promoter area and the 3' UTR region rather than in coding exons [6].

The rs2228570 (*VDR*:c.2 T > C) variant is located in the translation initiation site in exon 2 (alteration in the start codon) causing shortened *VDR* protein. The rs1544410 (*VDR*:c.1024 + 283G > T) and rs7975232 (*VDR*:c.1025-49C > A) variants are located in the intron between exon 8–9 at the 3′ end. The rs731236 (*VDR*:c.1056A > G) is located in exon 9 at the 3′ end. These variants do not affect VDR protein but are involved in the regulation of the stability of *VDR* mRNA. Regarding their functional effect, they could generate an alteration in the splice sites for mRNA transcription or a change in the intron regulatory elements of *VDR* [11, 12].

The rs731236 (*VDR:* c.1056A > G) gene variant (which was known as TaqI, referred to the used, Thermus aquaticus restriction enzyme) is a transition substitution. It results from a T (thymine) replacement by a C (cytosine) nucleotide, where the ATT codon transitions to ATC generating a silent mutation, as both encode the amino acid isoleucine. Nonetheless, this SNV may alter some functional characteristics of the protein as it is involved in the regulation of mRNA stability and correlates with transcriptional activity [13, 14].

While rs7975232 (*VDR:*c.1025-49C > A) gene variant, was defined using the *Acetobacter pasteurianus* restriction enzyme, therefore was known as ApaI, is a transversion substitution. It presents a change of A (alanine) instead of C (cytosine) nucleotide but there is no change in the amino acid sequence of the *VDR* protein [15].

There are many discrepancies in the study results regarding these variants; while some studies have established an association between VDR gene variants and vitamin D levels, other studies have not found any such association [1, 16–20].

The aim of this study was to investigate the association between the VDR gene variants c.1025-49C > A and c.1056A > G and MS susceptibility in a sample of the Egyptian population, and to study its effect on clinical outcomes using TaqMan Real-Time PCR. The findings of

the study could have implications for the treatment and prevention of multiple sclerosis in Egypt.

Methods

This case–control study was conducted on two groups: Group I, consisting of relapsing–remitting multiple sclerosis (RRMS) patients recruited from Multiple Sclerosis unit of Kasr el Ainy, Cairo University between March 2021 and March 2022, and Group II, consisting of healthy control individuals of matching age and sex.

Sample size calculation was done using the comparison of the prevalence of VDR: c.1056A > G (rs731236) and VDR: c.1025-49C > A (rs7975232) genotypes between Egyptian patients with multiple sclerosis (MS) and matched healthy controls, as reported in previous publications [21, 22].

Inclusion criteria included patients diagnosed with RRMS based on the Revised McDonald MS criteria of 2017 [23]. Both sexes were included with an age range between 18 and 52 years. Exclusion criteria included patients with neurological, inflammatory, and autoimmune diseases other than MS, as well as smokers.

All patients were subjected to the following history taking, clinical, and neurological examination including the Expanded Disability Status Scale (EDSS) [23].

Blood samples were taken for molecular analysis of VDR gene variants: c.1056A > G (rs731236) and c.1025-49C > A (rs7975232) using TaqMan Real-Time polymerase chain reaction in the Molecular Research and Diagnosis Unit at the Chemical Pathology Department of Kasr el Ainy, Cairo University.

Regarding collection of samples, Peripheral venous blood samples were taken and dispensed into 3 mL tubes containing 5.4 mg of ethylene diamine tetra acetic acid (EDTA), then they were stored at – 20 °C for DNA extraction.

The test was done in two main steps, DNA extraction in which Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leucocytes according to the manufacturer's instructions using QIAamp DNA Extraction Mini Kit provided by Thermo Fisher Scientific. The extracted DNA was stored at – 20 °C for further use. The purity and concentration of the DNA was detected by measuring the absorbance at 260 nm (A260) to the absorbance at 280 nm (A280) By Nanodrop. Then Detection of the VDR c.1056A > G (rs731236) and c.1025-49C > A (rs7975232) gene variants was performed using the Real-Time PCR System (Applied Biosystems, USA). Vitamin D receptor SNVs (rs731236, rs7975232) were genotyped by fluorogenic TaqMan SNV technology from a ready to use assays library (Applied Biosystems, Foster City, CA, USA) with the TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA) in a 20 µl reaction volume. The final concentration of genomic DNA for all samples in the experiment sample was 10 ng/µl [25–29].

Table 1 Participant characteristics

		Mean ± SD	Median (IQR)
Age	Patients ($n=50$)	33.6 ± 8.8	32 (27–41)
	Controls ($n=50$)	34 ± 9.6	36.5 (26–40)
Age at onset	Patients	25.3 ± 7.9	23 (20–28)
Duration of disease (years)	Patients	5.6 ± 4.7	4 (2–8)
Total number of attacks	Patients	3.5 ± 1.6	3 (2–5)
EDSS	Patients	2.7 ± 2.1	2 (1–4.375)

IQR: Interquartile range, EDSS: Expanded Disability Status Scale

IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA) was used for statistical analysis. Numerical data were expressed as mean and standard deviation, or median and range, as appropriate. Qualitative data were expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test was used to examine the relationship between qualitative variables. For normally distributed quantitative data, a comparison between two groups was done using the Student's t-test, while a comparison between more than two groups was done using ANOVA test. For not normally distributed quantitative data, a comparison between three groups was done using the Kruskal–Wallis test (non-parametric ANOVA). All tests were two-tailed, and a P-value < 0.05 was considered statistically significant. Odds ratios were used to present the strength of association between risk factors and outcomes.

The study was approved by the Institutional Review Board of the Faculty of Medicine (IRB No. MS-279-2021) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent were obtained from all participants before they enrolled to participate in the study.

Results

This study included 50 patients with RRMS and 50 healthy, age- and sex-matched controls showing demographic data in Table 1.

Regarding the VDR c.1056A > G (rs731236) genotype analysis and alleles distributions for all studied subjects by real-time PCR (Table 2, Fig. 1).

Regarding the genotype analysis and alleles distribution of VDR c.1025-49C > A (rs7975232) by TaqMan Real-Time PCR (Table 3, Fig. 2).

No statistically significant difference was found when comparing different VDR c.1056A > G

Table 2 The distribution of VDR c.1056A > G (rs731236) variant genotypes and alleles in study groups

Genotypes	Group 1 Percentage	Group 2 Percentage	*P*-value	95% CI	*P*-value
A/A	24	26	0.657*	0.37–2.18	0.817
A/G	46	52		0.35–1.72	0.548
G/G	30	22		0.61–3.74	0.363
Alleles	**Group 1**	**Group 2**	***P*-value**	**95% CI**	***P*-value**
A	47	52	0.479*	0.46–1.42	0.479
G	53	48		0.7–2.1	0.479

A: adenine, G: guanine, P ≤ 0.05 is considered significant. **P* value from Chi-square test, CI: confidence interval

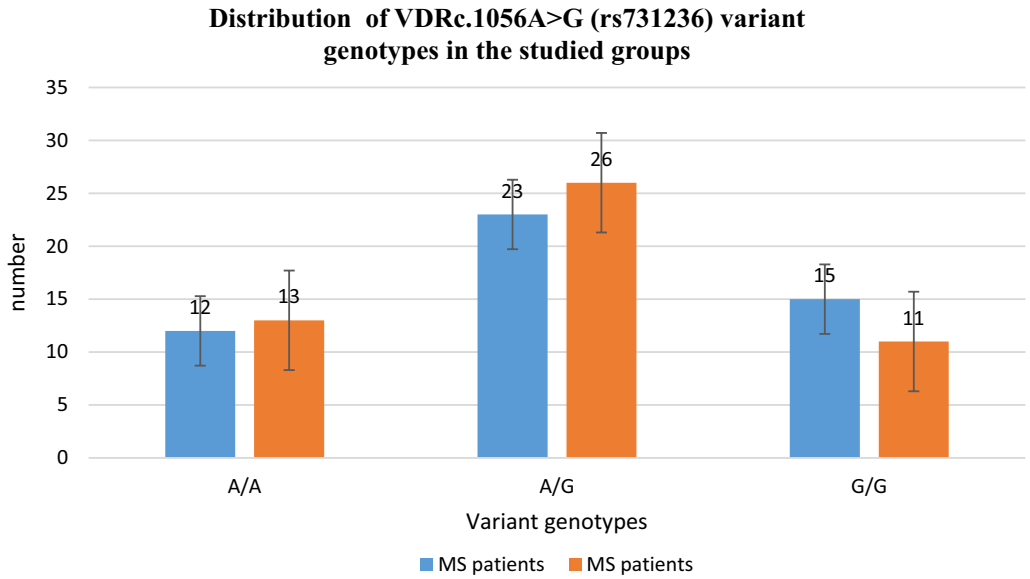

Fig. 1 Genotype distribution of *VDR* c.1056A > G (rs731236) variant among study groups

Table 3 The distribution of *VDR* c.1025-49C > A (rs7975232) variant genotypes and alleles in study groups

Genotypes	Group 1 Percentage	Group 2 Percentage	*P*-value	95% CI	*P*-value
A/A	52	42	0.592*	0.67–3.29	0.317
A/C	36	42		0.34–1.73	0.538
C/C	12	16		0.22–2.2	0.565
Alleles	**Group 1**	**Group 2**	***P*-value**	**95% CI**	***P*-value**
A	70	63	0.294*	0.7–2.4	0.295
C	30	37		0.4–1.3	0.295

A: adenine, C: cytosine, P ≤ 0.05 is considered significant. **P* value from Chi-square test, CI: confidence interval

gene variants regarding the main clinical parameters in MS patients; the same was reported regarding VDR c.1025-49C > A gene variants, as shown in Table 4.

The haplotyping between VDR c.1056A > G and VDR c.1025-49C > A gene variants was done, as shown in Table 5.

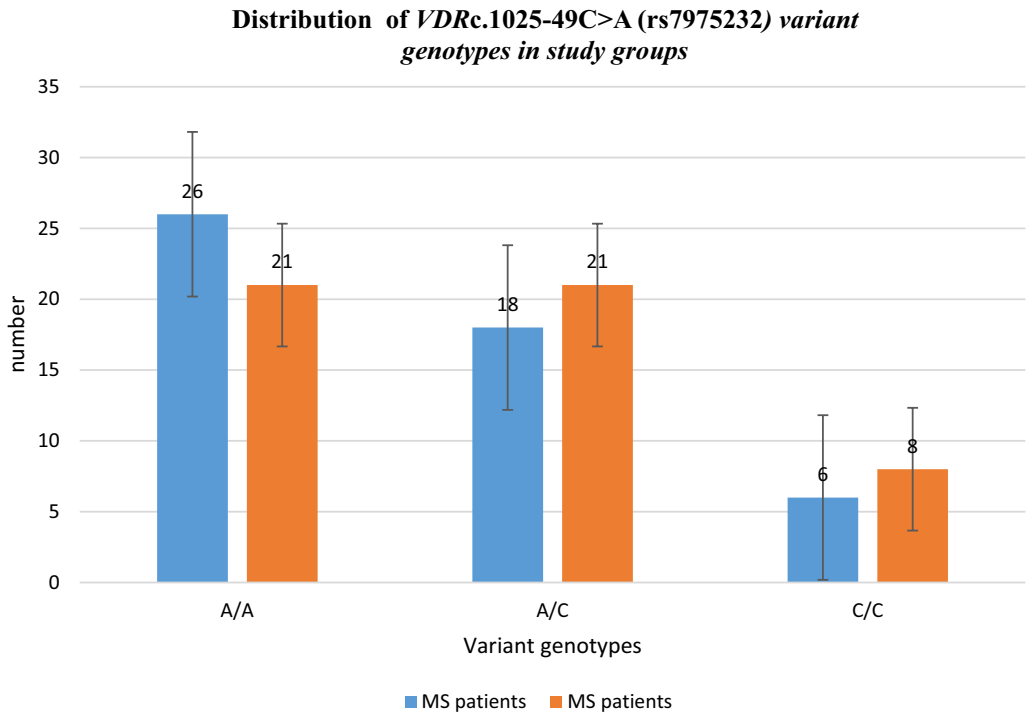

Fig. 2 Genotype distribution of *VDR* c.1025-49C > A (rs7975232) variant among study groups

Table 4 Comparison of different VDR c.1056A > G and c.1025-49C > A gene variants regarding main clinical parameters in MS patients

Gene		*n*	Age	Age at onset	Duration of illness (years)	EDSS score	Total no. of attacks
			Mean ± SD	Mean ± SD	Median (IQR)	Median (IQR)	Median (IQR)
VDR c.1056A > G	A/A	12	30.3 ± 10	26.3 ± 9.6	2 (1.75–4.25)	2.75 (1–5.625)	3 (2–4.25)
	A/G	23	30.7 ± 8.5	24.6 ± 8.1	5 (2–9)	1.5 (1–3.75)	3.5 (2–5)
	G/G	15	32.3 ± 9.3	25.7 ± 6.6	6 (3–8.5)	2.5 (1–4.75)	3 (2.5–4.5)
P-value			0.738*	0.601*	0.240**	0.712**	0.855**
VDR c.1025-49C > A	A/A	26	29.7 ± 7.6	23.6 ± 5.6	6 (1.25–8.75)	2.5 (1.125–5.875)	3 (2–5)
	A//C	18	32.3 ± 9.2	26.7 ± 8.8	4 (2–6.75)	1.25 (1–3.5)	3 (2–4.75)
	C/C	6	33.3 ± 13.5	28.8 ± 12.7	4 (2.5–6.25)	1.5 (1–3.5)	3.5 (3–4.75)
P-value			0.599*	0.471*	0.887**	0.339**	0.856**

A: adenine, C: cytosine, G: guanine, EDSS: Expanded Disability Status Scale, no: number, IQR: interquartile range, **P* value from ANOVA test, ***P* value from Kruskal–Wallis test

Table 5 Haplotype analysis between VDR c.1056A > G and c.1025-49C > A gene variants

Haplotype frequencies estimation (*n* = 100)								
	Haplotype	Total	Cases	Control	Cumulative frequency	OR	95% CI of OR	*P* value
1	GA	0.48	0.48	0.47	0.48	1		
2	AC	0.30	0.25	0.36	0.78	1.4	0.7–2.6	0.310
3	AA	0.19	0.22	0.16	0.97	0.8	0.4–1.7	0.570
4	GC	0.03	0.05	0.01	1	0.4	0.04–2.8	0.330

A: adenine, C: cytosine, G: guanine, *P* value < 0.05 is considered significant, OR: odds ratio, CI: confidence interval

In summary, our findings demonstrated an increase in the G allele in the MS group, which is thought to be the hazardous allele; however, this difference was not statistically significant in VDR:c.1056A > G (rs731236). However, in the VDR:c.1025-49C > A (rs7975232) variant allelic distribution A hazardous allele was found to be more prevalent in the MS group, although it did not achieve statistical significance. In both variations, haplotype analysis and genotype distribution demonstrate a non-significant relationship to MS etiology.

Discussion

Multiple sclerosis is a demyelinating neurodegenerative autoimmune illness. Motor dysfunction, autonomic symptoms, and psychbehavioral aspects of MS include gait difficulties, paresthesia, visual issues, vertigo, incontinence, sexual problems, pain, cognitive dysfunctions, emotional disturbances, and depression. MS biomarkers and gene variants certainly may be able to help identify various stages of MS and build personalized treatment plan [30, 31].

This study aimed to investigate the association between the VDR c.1056A > G and c.1025-49C > A SNVs and susceptibility to MS in a group of Egyptian population which is an important point provides insights into the potential role of vitamin D in preventing inflammatory disorders and its impact on clinical outcomes. The findings of the study could have implications for the treatment and prevention of MS in Egypt. Our results revealed that there was an increase in the G allele in the MS group, which was suggested to be the risky allele; however, this difference did not reach statistical significance in VDR c.1056A > G (rs731236). On the other hand, in VDR c.1025-49C > A (rs7975232), the variant allelic distribution of the A allele, which was supposed to be the risky allele, was increased in the MS group but did not reach statistical significance. The genotype distribution in both variants showed no significant relation to MS pathogenesis. Most of the published work agreed with part of our data and disagreed with others.

Regarding the VDR c.1056A > G genotype distribution analysis, in agreement with our results, Mazroei-Arani et al. (2022) conducted a study on 101 MS patients and 101 healthy subjects in the Iranian population, and Moosavi et al. (2021) conducted a study on 160 MS patients and 162 healthy personnel and found that the genotype frequency of VDRc.1056A > G did not differ between the patients and controls ($P=0.348$ and $P=0.092$, respectively) [1, 18]. Additionally, Hassab et al. (2019) and Zayed et al. (2019) (in which 50 and 63 Egyptian MS patients were recruited, respectively) revealed no statistically significant association between MS and VDRc.1056A > G ($P=0.945$ and $P=0.845$, respectively) [22, 32].

In other studies, conducted on the Turkish population, there was nostatistically significant difference between 167 MS patients and 146 healthy controls, and between 70 MS patients and 70 healthy controls, respectively [33, 34]. Additionally, Zhang et al. (2018) conducted a meta-analysis which included 24 case–control studies with a total of 4013 cases and 4218 controls and found that the association between the VDRc.1056A > G variant and MS was not significant under dominant, recessive genotypes, and allele contrast ($P=0.078$, $P=0.314$, and $P=0.127$, respectively) in overall populations [35]. Similarly, Imani et al. (2019) conducted a meta-analysis with a total of 30 case–control studies and detected no significant association across different genotype models [18].

In contrast to our findings, Al-Temaimi et al. (2015) found that the genotype distribution of Kuwaiti VDR c.1056A > G in 50 MS patients was significantly different from that of 50 healthy controls ($P=0.0008$) [21].

In addition, Mohammadi et al. (2020) discovered a statistically significant negative relationship between the VDR c.1056A > G variant and risk of MS in the homozygote A/A genetic model (OR = 0.28, 95% CI: 0.08–0.9; $P=0.04$) [36]. Additionally, Abdollahzadeh et al. (2018) found that the homozygote G/G genotype carriers for the VDR c.1056A > G variant have a predisposition to MS (OR = 2.18, 95% CI = 1.05–4.52) [37]. Furthermore, Zhang et al. (2019) conducted a meta-analysis, which included 27 case–control studies with 4879 MS patients and 5402 controls and observed that the G/G genotype is associated with the risk of MS (OR = 0.76, 95% CI = 0.62–0.94) [38].

Concerning the allelic distribution, similar to our findings, Mohammadi et al. (2020) conducted a meta-analysis which showed that in allelic comparison, no statistical association between allele G and risk of MS was found in 1206 Iranian patients ($P=0.07$) [36].

However, Moosavi et al. (2021) found that the G allele was more prominent in 160 MS patients than in the 162 control individuals, increasing the risk of disease susceptibility by 1.6 times (OR = 1.6, $P=0.0232$) [1]. According to Abdollahzadeh et al. (2018), the G allele had a positive correlation with MS (OR = 1.98, 95% CI = 1.36–2.87; $P=0.003$), while the A allele had a negative association (OR = 0.51, 95% CI = 0.39–0.73; $P=0.003$) [36]. Additionally, Al-Temaimi et al. (2015) demonstrated that the G allele was associated with MS risk (OR = 1.7, 95% CI = 1.2–2.4; $P=0.003$) [21].

Regarding the VDR c.1025-49C > A genotype distribution analysis, in agreement with our results, many studies and meta-analyses done on Turkish and Iranian

populations have agreed with our results, showing no evidence of an association between VDR c.1025-49C > A and MS risk [17, 35, 37, 38].

In contrast to our findings, a statistical analysis of a study done by Mazrouei-Arani et al. (2022) revealed a significant association between VDR c.1025-49C > A genotypes and MS disease ($P=0.05$), showing that MS patients had an A/A genotype 2.54 times higher than the CC genotype (OR = 2.54, $P=0.029$) [18].

Upon evaluating the genotypes by Cetinel et al. (2021), a statistically significant correlation was found with VDRc.1025-49C > A A/A, C/C, and A/C ($P<0.01$, 0.01, and $P<0.01$, respectively) in the SPMS group and with VDR:c.1025-49C > A A/A and A/C genotypes ($P=0.01$ and 0.04, respectively) in the PPMS group, but no significant difference was found in the genotypes within the RRMS group [16].

In a meta-analysis study conducted on 1206 cases and 1402 controls, a statistically significant association was also found between the VDR c.1025-49C > A homozygote genetic model of A/A and G/G and the risk of MS (OR = 3.48, 95% CI = 1.7–6.9; $P=0.00$) [36]. Additionally, Zhang et al. (2019) performed a meta-analysis revealed a significant association between the VDRc.1025-49C > A variant and MS risk in Asians in the recessive model C/C genotype (OR = 0.66, 95% CI = 0.53–0.82; $P=0.0002$) [37].

Regarding the allelic distribution, like our findings, Imani et al. (2019) meta-analysis showed no statistically significant difference in allelic discrimination [17].

However, Mohammadi et al. (2020) meta-analysis showed a statistically significant relationship between the A allele and decreased risk of MS in Iran (OR = 0.54, 95% CI = 0.37–0.79, $P=0.00$) [36]. Additionally, Hassab et al. (2019) study detected a statistically significant association between the A allele and MS cases (OR = 2.47, 95% CI = 1.25–4.88, $P=0.008$) [22]. Furthermore Zhang et al. (2018) conducted a meta-analysis which observed that the A allele was associated with MS risk in Asian populations (OR = 1.267, 95% CI = 1.074–1.496; $P=0.005$) [35].

The contradictory results of the studies may be because; small sample sizes, differences in ethnicities, extensive geographic variation, interactions with other genetic or environmental factors, and/or clinical heterogeneity.

The significance of this manuscript is that it presents a research study that looks into the relationship between VDR gene variants and MS in the Egyptian population. The study analyzes genetic data using TaqMan Real-Time Polymerase Chain Reaction (PCR) which is accurate method and provides insights into the potential role of vitamin D in preventing inflammatory disorders and its impact on clinical outcomes. The findings of the study could have implications for the treatment and prevention of MS in Egypt. The research adds to the body of knowledge on the subject and may help guide future research and clinical practice. It is recommended that the results obtained by this study be studied on a wider scale with a larger sample size, taking into consideration the presence of different ethnicities. It is also advised that other single nucleotide variants (SNVs) of the vitamin D receptor (VDR) affecting multiple sclerosis (MS) should be studied. This will help in clarifying the genetic role in the development of MS and achieving more accurate results.

Conclusion

Vitamin D receptor gene variants had been studied in many studies to assess its relationship with MS, but the results are contradictory. Data from this study suggested that there was no association between the risk of MS and the *VDR* gene variants regarding *VDR* c.1025-49C > A and *VDR* c.1056A > G in a group of Egyptian population.

Abbreviations

A	Adenine
C	Cytosine
G	Guanine
MS	Multiple sclerosis
VDR	Vitamin D receptor
DNA	Deoxyribonucleic acid
RRMS	Relapsing–remitting multiple sclerosis
PCR	Polymerase chain reaction
CNS	Central nervous system
MRI	Magnetic resonance imaging
Treg	Regulatory T cells
SNVs	Single nucleotide variants
REC	Research Ethics Committee
EDSS	Expanded Disability Status Scale
EDTA	Ethylene diamine tetra acetic acid

Acknowledgements

The authors would like to express their gratitude to the patients for their participation and cooperation in this study.

Author contributions

HAH was the idea founder, shared in the patient collection, and the supervisor in all the steps. AMF shared in the patient collection did the data analysis, wrote and revised the manuscript and is the submitting and corresponding author. NWI shared in the patient collection and did the laboratory work. SAS shared in the patient collection and supervision. All authors read and approved the final manuscript.

Funding

This research received no specific grant from any funding agency from commercial or not-for-profit sectors.

Availability of data and materials

The datasets generated during the current study are not publicly available due to the hospital policy and because the data will be used in future multi-center research to generate a nationwide statistic. However, these datasets are available from the corresponding author (Hala Ashraf) on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Faculty of Medicine, Cairo University (IRB No. MS-279-2021) and was performed in accordance

with the principles of the Declaration of Helsinki. Written informed consents were obtained from all participants before the enrollment to participate in the study.

Consent for publication
Not applicable.

Competing interests
No potential conflict of interest relevant to this article was reported.

Received: 16 August 2023 Accepted: 16 January 2024
Published online: 09 April 2024

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