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Association of *Vitamin D Receptor* Gene Polymorphisms and the Risk of Multiple Sclerosis-A: Meta Analysis

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Background. Previous studies have reported vitamin D receptor (*VDR*) polymorphisms in multiple sclerosis (MS); however, the results remain contradictory. This study aimed to investigate the association between *VDR* polymorphisms and the risk of MS.

Methods. PubMed and Embase databases were searched to obtain eligible studies. Data were calculated by odds ratios (OR) with 95% confidence intervals (CI).

Results. Twenty seven case–control studies with 4879 MS patients and 5402 controls were included. There was no significant association between *ApaI* polymorphisms and MS in the overall population. In Asians, no association was found between *ApaI* polymorphism and MS in the recessive, dominant, Codominant (OR1), Codominant (OR2), Codominant (OR3) models and allele contrast. Similar results were obtained between *BsmI* polymorphisms and MS. The association between *TaqI* polymorphism and MS showed significance in the recessive, homozygous, codominant (OR3) models in the overall population and Caucasians. The dominant model showed no association of *Taq I* polymorphism with MS risk in *HLA-DRB1**15-positive and *HLA-DRB1**15-negative groups. *FokI* polymorphism with MS was found in Codominant (OR3) model in the overall population. In Asians, *FokI* polymorphism showed association with MS in recessive, dominant, Codominant (OR1), Codominant (OR3) models and allele contrast. Subgroup analysis of sex showed no associations between *TaqI* or *FokI* polymorphism and MS risk in males or females in all models or allele contrast.

Conclusions. The *VDR TaqI* polymorphisms showed association with MS risk, especially in Caucasians. In Asians, *ApaI* and *FokI* polymorphisms correlated with MS risk, while *BsmI* polymorphisms showed no association with MS. © 2019 Published by Elsevier Inc. on behalf of IMSS.

Key Words: Multiple sclerosis, Vitamin, D receptor, Gene polymorphisms, Meta-analysis.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder, in which the axonal degeneration affects the central nervous system (CNS). It typically affects young adults, causing intermittent neurological disturbances followed by progressive accumulation of disability (1).

The etiology of MS is still unknown. Multiple factors such as genetic, environmental (infectious or chemical) and geographical elements have been suggested to influence disease susceptibility (2,3). Till date, *HLA-DRB1**15 haplotype (DQB1*0602, DQA1*0102, DRB1*1501, DRB5*0101) is considered as the strongest genetic factor associated with MS, but it cannot completely explain the genetic impact on MS risk (4). Genes lying in the non-HLA regions may also confer susceptibility to MS.

Some studies showed that MS risk is significantly associated with increasing latitude or past low sun exposure, particularly in childhood and early adolescence (5,6). It is now becoming clear that this MS risk factor may arise, at least in part, due to the influence of less ultraviolet ray exposure, which insufficiently synthesizes vitamin D (7,8).

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Vitamin D is a fat soluble pro-hormone supplied in diet and/or formed on skin upon exposure to ultraviolet radiation. 25-hydroxyvitamin D (25[OH]D) is the major circulating vitamin D metabolite and a reliable indicator of vitamin D status. Following hepatic hydroxylation, 25 (OH)D, is converted to its bioactive form 1,25-dihydroxy vitamin D (1,25[OH]2D) in kidney (9) through the ubiquitously expressed vitamin D receptor (*VDR*). Many biological responses, including bone and calcium homeostasis, adequate neuromuscular functions, detoxification pathways, immune system regulation and maintenance were controlled by VDR. Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease that is most commonly used as a model for MS. According to previous reports, absolute inhibition of disease course occurs following the injection of 1,25- (OH)2D (10). While *VDR* knockout mice are crucial for EAE activity (11).

The *VDR* gene is located on chromosome 12q12-14, and consists of 5 promoter regions, 8 protein-coding exons, and 6 untranslated exons. The most studied polymorphisms in the *VDR* gene are *Apa I*, *Bsm I*, *Taq I* and *Fok I* (12−14). *Apa-I* and *Bsm-I* are polymorphic sites that are located in the intron separating exons 8 and 9, while *Taq-I* is located in exon 9 and are without consequences for VDR protein structure. *Fok-I* that is located in exon 2 introduces an initiation code, leading to the addition of 3 aminoacids to the VDR protein. Some case-control studies have shown a significant association between individual single nucleotide polymorphisms (SNPs) (*ApaI*, *TaqI*, *FokI*, and *BsmI*) and risk of MS (15−17), while few other studies were contradictory (12,18).

Vitamin D plays a key role in calcium homeostasis and is also correlated with the regulation of immune system (19). Vitamin D deficiency has a role in the pathogenesis and progression of MS (20−22), making it reasonable for analyzing the possible relationship of *VDR* gene polymorphisms and gene allelic variants and the risk of MS. Despite lack of genome-wide association studies (GWAS), many case−control studies investigated the association between *VDR* polymorphisms (23) and the risk of MS, *BsmI* (rs1544410), *ApaI* (rs7975232), *FokI* (rs10735810), and *TaqI* (rs731236) have been conducted, but the results are contradictory and the role of *VDR* polymorphisms remains unclear.

The reasons for this disparity are due to small sample sizes, low statistical power, differences in ethnicities, extensive geographic variations, interactions with other genetic or environmental factors and/or clinical heterogeneity. Meta-analysis is a statistical analysis technique that obtains data from a number of independent studies and combines them to calculate the estimation of overall pooled effect, which in turn overcomes the limitations of individual studies.

In this meta-analysis, we combined data from published studies to evaluate genetic associations between the common polymorphisms of the *VDR* gene (*ApaI* polymorphism rs7975232A > C, *Bsm-I* polymorphism rs1544410 G > A, *Taq I* polymorphism rs731236 T > C, and *Fok I* polymorphism rs2228570 C > T) and the risk of MS. This was not the first meta-analysis study addressing this issue (24,25), but was an updated study with much more cases and controls. In the previous meta analysis (24), the association between TaqI polymorphism and MS risk was significant in the homozygous model. While in another meta analysis (25), it did not indicate an association between any of the VDR polymorphisms and the risk of MS among overall populations. Hence, to investigate the association between *VDR* gene polymorphisms and MS risk, an updated meta-analysis obtaining data from all eligible studies and combining them to permit the estimation of an overall effect was performed.

Materials and Methods

Search Strategy

A systematic literature search was carried out using online electronic databases such as PubMed and Embase with language restricted to English only before April, 2019. Articles that examined the associations between *VDR* gene polymorphisms of *ApaI*, *BsmI*, *TaqI*, and *FokI* and risk of MS were included. The following keywords were used: ''VDR OR vitamin D receptor'', ''polymorphism OR variant OR variation OR single nucleotide polymorphism OR SNP OR genotype OR rs'', ''multiple sclerosis OR MS''. The references of eligible articles were also checked to identify for any potentially relevant studies. The literature searches were performed by two independent reviewers, and any discrepancies were discussed by reaching a consensus.

Study Selection

All studies were independently evaluated by two reviewers by using a standardized extraction form to avoid selection bias. The eligible studies should meet the following selection criteria: (a) Studies that assessed the association between *TaqI*, *BsmI*, *ApaI* and *FokI* polymorphisms in *VDR* gene and risk of MS; (b) a case-control study design; (c) studies had disease outcome definitions that met the accepted diagnostic guidelines; and (d) case-control studies regarding the number of individual genotypes and/or alleles that are available to calculate the odds ratio (OR) and 95% confidence intervals. If the authors used overlapping sample data regarding the same polymorphism in multiple papers from the same research institution, only the study with the most complete information was selected. Studies were excluded if: (a) they had no control group; (b) there is insufficient published data for extraction; (c) the study had repeated reports on the same population or subpopulation; (d) not relevant to *VDR* polymorphisms with MS; and (e)

the they were reviews, conference abstracts or editorial articles.The quality of these case control studies was evaluated by the Newcastle-Ottawa Scale (NOS), which allowed a total score of 9 points or fewer (9 indicates the highest quality) summarizing 8 aspects of each study. We assigned scores 7–9 as high quality of studies.

Data Extraction

For each eligible study, two investigators independently extracted the data. Any disagreements were further discussed and resolved by reaching a consensus. The following data were extracted from the eligible studies: name of the first author, year of publication, study population (country, ethnicity, sex, and age), sample size (cases and controls), and the number of genotypes in cases and controls. We also tested the distribution of the genotypes in controls by departure from the Hardy-Weinberg equilibrium (HWE) by χ^2 test. If $p > 0.05$, the genotype distribution of control population was conformed to HWE. The study would be excluded from this meta-analysis if the distribution of the genotypes in controls were not in accordance with the laws of HWE.

Statistical Analyses

The raw data for genotypic distribution were used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). Review Manager (version 5.2) and Stata 12.0 (STATA Corp., College Station, TX, USA) were used for conducting meta-analysis. The analyses were performed when sufficient information was reported in at least 3 studies. To measure the strength of associations between *VDR* gene polymorphisms and MS risk, the overall MS risk in the following models was evaluated: the recessive model (AA/Aa + aa) (A represents major allele and a represents minor allele), the homozygotes model (AA + aa/Aa), the dominant model (AA + Aa/aa), the codominant model OR1 (AA/aa), OR2 (Aa/aa) and OR3 (AA/Aa), and the allele contrasts (A/a). Heterogeneity was calculated using the Cochran's Q statistic and the I^2 statistic, $p < 0.10$ and $I^2 > 50\%$ showed evidence of heterogeneity, and so a random effects model was used. Otherwise, the fixed effects model was applied. The pooled OR was determined by a Z test. The level of statistical significance was set at $p < 0.05$. Furthermore, the potential publication bias was evaluated using Egger's test, and $p < 0.05$ was considered to indicate a statistically significant publication bias. In addition, subgroup analyses were performed based on ethnicity (Caucasians and Asians), sex (male and female), and *HLA-DRB1**15 (positive and negative). These subgroup analyses should be reported in at least 3 studies in each subgroup. The stability of the pooled OR was evaluated by using a sensitivity analysis, in which each study was individually removed, recalculating the OR value.

Results

Characteristics of the Included Studies

A flow chart of literature selection process was presented in Figure 1. After applying the inclusion criteria, a total of 27 case–control studies on *VDR* polymorphisms and the risk of MS were finally included in this meta-analysis (9,12,13,15–18,23,26–44). In total, 4879 patients with MS and 5402 controls were included. Assessed by the NOS for case control studies, all the quality scores of the studies were 7–9, indicating high quality of included studies.

We evaluated 4 well-characterized polymorphisms (*Apa-I*, *Bsm-I*, *Taq-I* and *Fok-I*) in this meta-analysis. The genotypic distributions of all SNPs in controls exhibited HWE. The main characteristics of the eligible studies are listed in Supplementary Table 1.

Quantitative Synthesis of Data

ApaI polymorphism rs7975232A > C. Sixteen studies with 2155 cases and 2651 controls were included. Among them, 9 studies were conducted in Caucasians and 7 studies were conducted in Asians. No significant association between *ApaI* polymorphism and MS in the recessive model (AA/Aa + aa), (OR = 0.91, 95% CI [0.75, 1.11], $p = 0.36$), homozygote model (AA + aa/Aa) (OR = 0.96, 95% CI [0.85, 1.08], $p = 0.5$), dominant mode (AA + Aa/aa), (OR = 0.90, 95% CI [0.67, 1.20], $p = 0.46$), codominant model OR1 (AA/aa) (OR = 0.88, 95% CI [0.63, 1.22], $p = 0.43$), OR2 (Aa/aa) (OR = 0.98,95% CI [0.91, 1.05], $p = 0.54$), OR3 (AA/Aa) (OR = 0.91, 95% CI [0.80, 1.04], $p = 0.18$), allele contrast (A/a) (OR = 0.92, 95% CI [0.78, 1.07], $p = 0.28$) was observed in the overall population (Table 1, Figure 2). In subgroup analysis of Caucasians, no significant association of *ApaI* polymorphism and MS was observed in all the models (Table 1, Figure 2). While in Asians, a significant association was found between *ApaI* polymorphism and MS risk in the recessive (OR = 0.66, 95% CI [0.53, 0.82], $p = 0.0002$), dominant (OR = 0.58, 95% CI [0.34, 0.99], $p = 0.05$), Codominant (OR1) (OR = 0.49, 95% CI [0.34, 0.71], $p = 0.0001$), Codominant (OR2) (OR = 0.91, 95% CI [0.85, 0.98], $p = 0.01$), Codominant (OR3) (OR = 0.68, 95% CI [0.56, 0.84], $p = 0.0003$) models and allele contrast (OR = 0.72,95% CI [0.56, 0.91], $p = 0.006$), (Table 1, Figure 2).

Bsm-I Polymorphismrs1544410 G > A. Twelve studies with 1641 cases and 1842 controls were included. Among them, 6 studies were performed in Caucasians and 6 studies in Asians. In the overall population, no association was found between *Bsm-I* polymorphism and the risk of MS in all models: recessive model (BB/Bb + bb) (OR = 0.99, 95% CI [0.76, 1.29], $p = 0.93$), homozygote

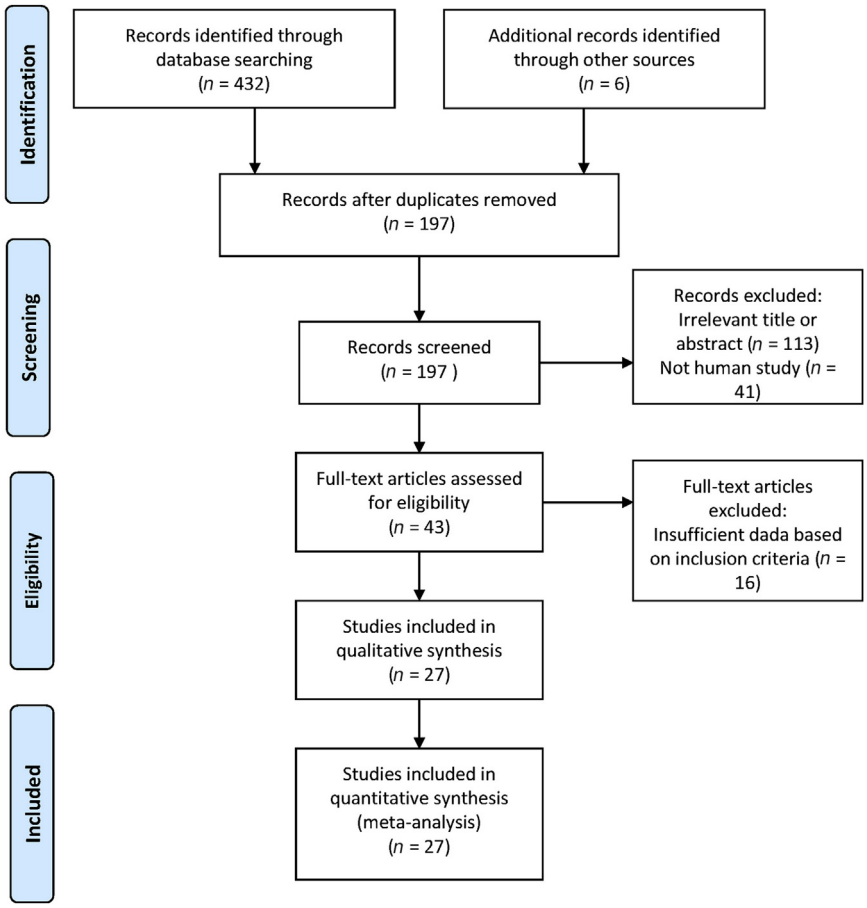

Figure 1. Flow chart of study selection process.

Table 1. *VDR* polymorphisms and multiple sclerosis risk: *ApaI* (rs7975232A > C)

	Genetic model		Stratification	*n*	Case/Control	OR (95% CI)	I^2 %	*Pz*
ApaI	Recessive	AA/Aa + aa	All	16	2155/2651	0.91 (0.75, 1.11)	56	0.36
			Caucasians	9	1527/1897	1.08 (0.93, 1.26)	28	0.33
			Asian	7	628/754	0.66 (0.53, 0.82)	39	0.0002
	Homozygous	AA + aa/Aa	All	16	2155/2651	0.96 (0.85, 1.08)	42	0.5
		Caucasians	9	1527/1897	1.02 (0.89, 1.17)	26	0.8	
		Asian	7	628/754	0.85 (0.61, 1.18)	54	0.34	
	Dominant	AA + Aa/aa	All	16	2155/2651	0.90 (0.67, 1.20)	66	0.46
		Caucasians	9	1527/1897	1.12 (0.82, 1.52)	64	0.47	
		Asian	7	628/754	0.58 (0.34, 0.99)	54	0.05	
	Codominant (OR1)	AA/aa	All	16	1098/1393	0.88 (0.63, 1.22)	67	0.43
		Caucasians	9	755/958	1.19 (0.84, 1.67)	62	0.33	
		Asian	7	343/435	0.49 (0.34, 0.71)	49	0.0001	
	Codominant (OR2)	Aa/aa	All	16	1443/1752	0.98 (0.91, 1.05)	58	0.54
		Caucasians	9	1069/1362	1.02 (0.94, 1.11)	57	0.65	
		Asian	7	375/390	0.91 (0.85, 0.98)	48	0.01	
	Codominant (OR3)	AA/Aa	All	16	1599/2136	0.91 (0.80, 1.04)	41	0.18
			Caucasians	9	892/1315	1.14 (0.95, 1.36)	0	0.16
			Asian	7	707/821	0.68 (0.56, 0.84)	23	0.0003
	Allele	A/a	All	16	4310/5302	0.92 (0.78, 1.07)	68	0.28
			Caucasians	9	3054/3795	1.07 (0.92, 1.24)	50	0.38
			Asian	7	1256/1508	0.72 (0.56, 0.91)	52	0.006

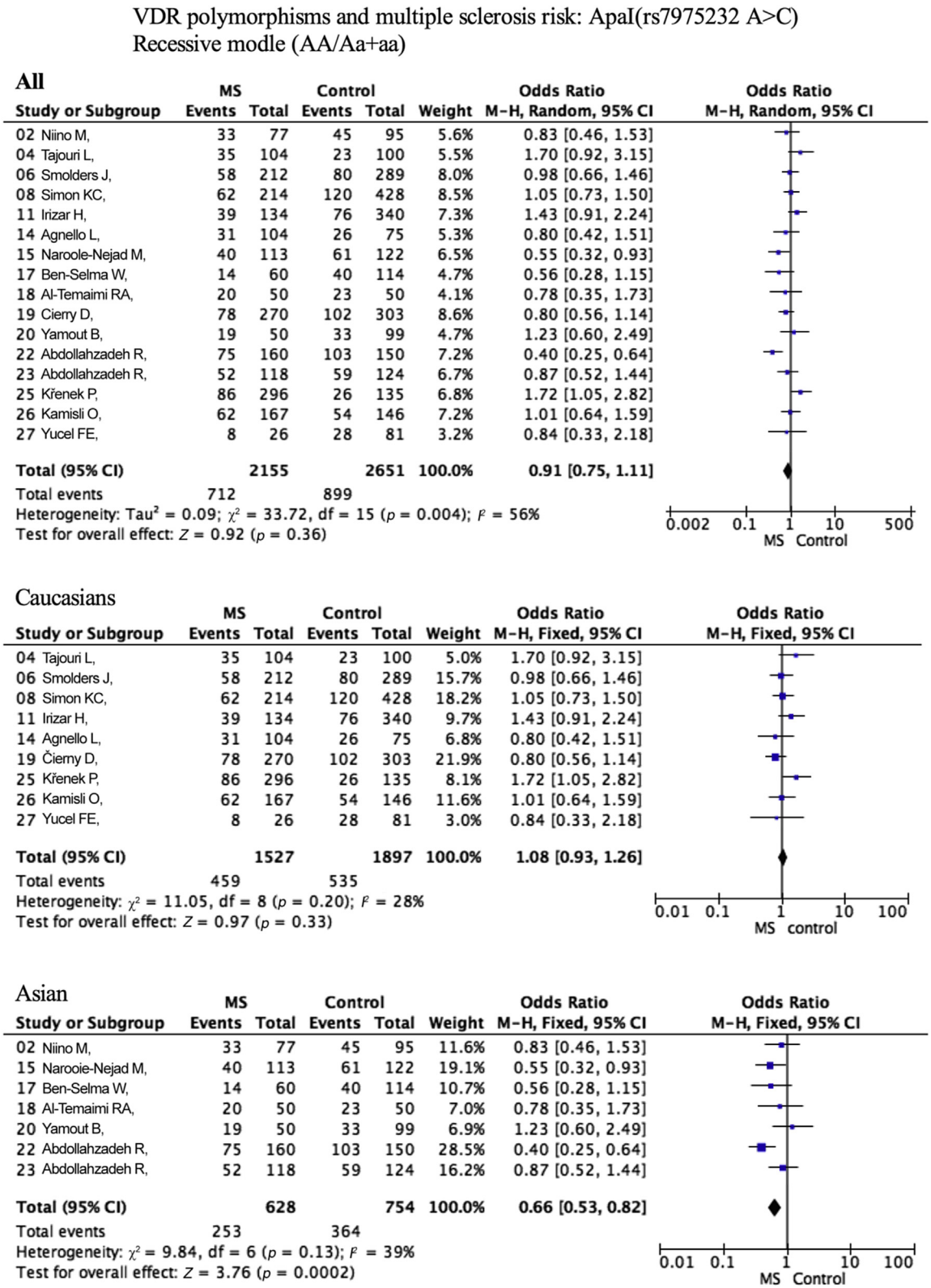

Figure 2. Forest plot for the association of *ApaI* polymorphism and MS risk in recessive model. MS: multiple sclerosis.

model (BB + bb/Bb) (OR = 0.97, 95% CI [0.79, 1.20], p = 0.8), dominant mode (BB + Bb/bb) (OR = 0.97, 95% CI [0.61, 1.55], p = 0.9), codominant model OR1 (BB/bb) (OR = 0.95, 95% CI [0.56, 1.62], p = 0.86), OR2 (Bb/bb) (OR = 1.02, 95% CI [0.65, 1.60], p = 0.95), OR3 (BB/Bb) (OR = 0.97, 95% CI [0.76, 1.23], p = 0.8), allele contrast (B/b) (OR = 1.01,95% CI [0.80, 1.27], p = 0.95). Subgroup analysis of Caucasians and Asians showed no association between *BsmI* polymorphisms and MS risk in all models and allele contrast (Table 2, Figure 3).

Taq I polymorphism rs731236 T > C. Twenty one studies with 3753 cases and 4132 controls discussed *Taq I* polymorphism rs731236 T > C. Among them, 15 studies were performed in Caucasians, while 6 in Asians. In the overall population, significant association was observed between *Taq I* polymorphism and the risk of MS in recessive model (TT/Tt + tt) (OR = 0.76, 95% CI [0.59, 0.97], p = 0.03), homozygote model (TT + tt/Tt) (OR = 0.93, 95% CI [0.89, 0.97], p = 0.0004), and codominant model OR3 (TT/Tt) (OR = 0.77, 95% CI [0.62, 0.94], p = 0.01). According to subgroup analysis of ethnicity, a significant correlation was found between *TaqI* polymorphisms and MS risk in recessive (OR = 0.90, 95% CI [0.81, 0.99], p = 0.04), homozygous (OR = 0.94, 95% CI [0.90, 0.98], p = 0.0006), codominant (OR3) models (OR = 0.87, 95% CI [0.78, 0.97], p = 0.01) in Caucasians. Four studies analyzed Taq I polymorphism in females and 3 studies in males, and the results showed no association (Table 3, Supplementary Figure 2). Three studies analyzed *TaqI* polymorphism in *HLA-DRB1**15-positive and *HLA-DRB1**15- negative MS patients and controls in the dominant model (AA + Aa/aa). The data was then pooled and showed no association of *Taq I* polymorphism with MS risk among *HLA-DRB1**15-positive group and *HLA -DRB1**15-negative group (other models were unavailable because of insufficient information), (Table 3, Supplementary Figure 2).

Fok I polymorphism rs2228570 C > T. Sixteen studies with 3209 cases and 3254 controls discussed *Fok I* polymorphism rs2228570 C > T. Among them, 12 studies were performed in Caucasians, and 4 studies in Asians. In the overall population, a significant association was found between *FokI* polymorphism and the risk of MS in Codominant (OR3) model (FF/Ft) (OR = 0.90, 95% CI [0.80, 1.00], p = 0.04). Based on subgroup analysis of ethnicity, a significant relationship was observed between *FokI* polymorphisms and MS risk in recessive (OR = 0.67, 95% CI [0.51, 0.87], p = 0.003), dominant (OR = 0.48, 95% CI [0.28, 0.85], p = 0.01), Codominant (OR1) (OR = 0.42, 95% CI [0.23,0.74], p = 0.003), Codominant (OR3) model (OR = 0.73, 95% CI [0.55, 0.97], p = 0.03) and allele contrast (OR = 0.68, 95% CI [0.54, 0.85], p = 0.0006) in Asians. Five studies evaluated the association of *FokI* polymorphism with the risk of MS in females and 4 studies in males, and showed negative results (Table 4, Supplementary Figure 3).

Publication Bias

No evidence of publication bias was found by using Egger's test (all p values >0.05) (Supplementary Figure 1).

Table 2. *VDR* polymorphisms and multiple sclerosis risk: *BsmI* (rs1544410 G > A)

	Genetic model		Ethnicity	*n*	Case/Control	OR (95% CI)	I^2 %	*Pz*
BsmI	Recessive	BB/Bb + bb	All	12	1641/1842	0.99 (0.76, 1.29)	67	0.93
			Caucasians	6	1073/1202	0.89 (0.75, 1.06)	44	0.2
			Asian	6	568/640	1.15 (0.65, 2.02)	79	0.64
	Homozygous	BB + bb/Bb	All	12	1641/1842	0.97 (0.79, 1.20)	52	0.8
			Caucasians	6	1073/1202	0.89 (0.68, 1.17)	56	0.4
			Asian	6	568/640	1.06 (0.84, 1.34)	45	0.61
	Dominant	BB + Bb/bb	All	12	1641/1842	0.97 (0.61, 1.55)	76	0.9
			Caucasians	6	1073/1202	0.93 (0.51, 1.68)	81	0.8
			Asian	6	568/640	1.12 (0.50, 2.51)	72	0.78
	Codominant (OR1)	BB/bb	All	12	871/1030	0.95 (0.56, 1.62)	77	0.86
			Caucasians	6	537/645	1.21 (0.73, 2.02)	65	0.46
			Asian	6	334/385	0.77 (0.31, 1.93)	75	0.58
	Codominant (OR2)	Bb/bb	All	12	1003/1095	1.02 (0.65, 1.60)	71	0.95
			Caucasians	6	558/596	0.89 (0.39, 2.03)	80	0.79
			Asian	6	445/499	1.17 (0.68, 2.00)	60	0.57
	Codominant (OR3)	BB/Bb	All	12	1408/1559	0.97 (0.76, 1.23)	56	0.80
			Caucasians	6	926/957	0.82 (0.61, 1.11)	56	0.21
			Asian	6	482/602	1.15 (0.89, 1.49)	42	0.28
	Allele	B/b	All	12	3282/3684	1.01 (0.80, 1.27)	78	0.95
			Caucasians	6	2146/2404	0.94 (0.74, 1.19)	69	0.61
			Asian	6	1136/1280	1.14 (0.71, 1.83)	85	0.59

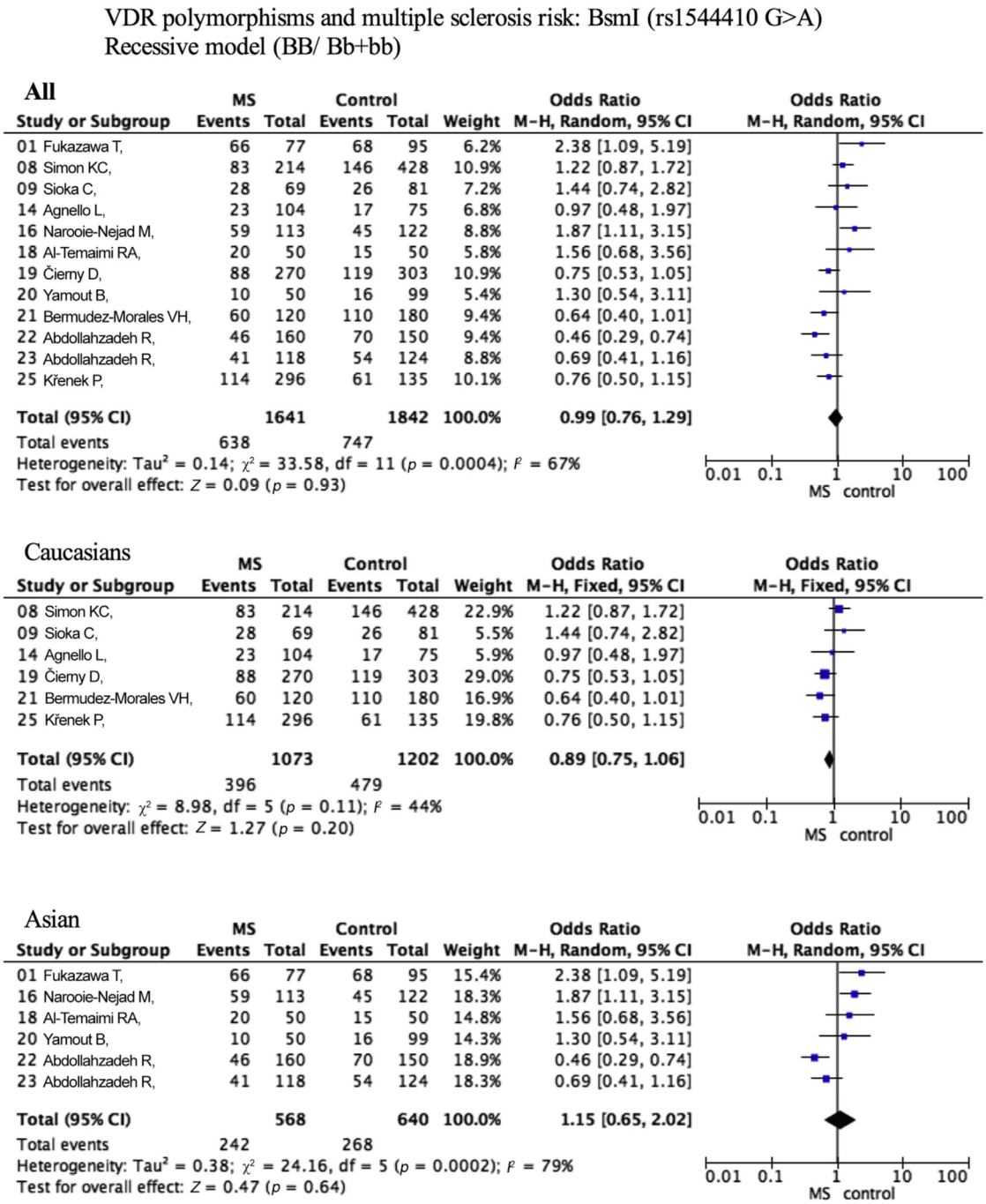

Figure 3. Forest plot for the association of *Bsm-I* polymorphism and MS risk in recessive model. MS: multiple sclerosis.

Heterogeneity Analysis

As shown in Figures 2,3, and Supplementary Figure 2,3, significant heterogeneity was observed in this meta-analysis (I^2 >50%). If all I^2 values were less than 50% between the subgroups of ethnicity, then ethnicity might be considered as a potential source of heterogeneity. Subgroup analysis of ethnicity showed no obvious heterogeneity in the subgroups of Asians and Caucasians (I^2 <50%, Figure 2), suggesting that ethnicity was a possible source of heterogeneity between *ApaI* polymorphism and the risk of MS.

All I^2 values of heterogeneity were not less than 50% between different subgroups. Subgroup analysis of ethnicity did not find any potential sources of heterogeneity between *Bsm-I*, *Taq I*, and *Fok I* polymorphisms and the risk of MS (Figure 3, and Supplementary Figure 2,3). Therefore,

Table 3. *VDR* polymorphisms and multiple sclerosis risk *TaqI* (rs731236 T > C)

	Genetic model		Stratification	*n*	Case/Control	OR (95% CI)	I^2 %	*Pz*
TaqI	Recessive	TT/tt + Tt	all	21	3753/4132	0.76 (0.59, 0.97)	84	0.03
			women	4	763/601	1.09 (0.87, 1.36)	0	0.44
			men	3	220/205	0.81 (0.37, 1.78)	74	0.6
			Caucasians	15	3202/3473	0.90 (0.81, 0.99)	22	0.04
			Asian	6	551/659	0.46 (0.16, 1.34)	94	0.16
	Homozygous	TT + tt/Tt	all	21	3753/4132	0.93 (0.89, 0.97)	33	0.0004
			women	4	763/601	1.00 (0.81, 1.25)	0	0.97
			men	3	220/205	0.97 (0.48, 1.95)	68	0.93
			Caucasians	15	3202/3473	0.94 (0.90, 0.98)	18	0.006
			Asian	6	551/659	0.89 (0.77, 1.04)	53	0.14
	Dominant	Tt + tt/tt	all	21	3753/4132	0.89 (0.67, 1.19)	72	0.44
			women	4	763/601	1.21 (0.87, 1.69)	19	0.26
			men	3	220/205	0.69 (0.39, 1.24)	0	0.21
			Caucasians	15	3202/3473	1.08 (0.94, 1.24)	28	0.3
			Asian	6	551/659	0.38 (0.12, 1.25)	86	0.11
			HLA^+	3	535/609	1.16 (0.82, 1.65)	16	0.39
			HLA^-	3	541/560	0.90 (0.65, 1.26)	0	0.54
	Codominant (OR1)	TT/tt	all	21	1954/2331	0.77 (0.54, 1.11)	80	0.17
			women	4	401/316	1.24 (0.86, 1.77)	12	0.25
			men	3	117/111	0.66 (0.36, 1.22)	16	0.19
			Caucasians	15	1659/1929	1.00 (0.86, 1.17)	30	0.96
			Asian	6	295/402	0.28 (0.06, 1.32)	90	0.11
	Codominant (OR2)	Tt/tt	all	21	2378/2364	1.02 (0.80, 1.30)	56	0.86
			women	4	442/364	1.19 (0.84, 1.69)	11	0.34
			men	3	136/117	0.69 (0.37, 1.28)	0	0.24
			Caucasians	15	1985/2046	1.15 (0.99, 1.34)	31	0.06
			Asian	6	393/318	0.53 (0.23, 1.25)	68	0.15
	Codominant (OR3)	TT/Tt	all	21	3174/3569	0.77 (0.62, 0.94)	75	0.01
			women	4	683/522	1.06 (0.84, 1.33)	0	0.64
			men	3	187/182	0.87 (0.37, 2.03)	75	0.75
			Caucasians	15	2760/2971	0.87 (0.78, 0.97)	24	0.01
			Asian	6	414/598	0.53 (0.22, 1.26)	90	0.15
	Allele	T/t	all	21	7506/8264	0.83 (0.67, 1.03)	90	0.10
			women	4	1526/1202	1.09 (0.93, 1.28)	0	0.27
			men	3	440/410	0.84 (0.54, 1.29)	55	0.42
			Caucasians	15	6096/6718	0.91 (0.80, 1.04)	64	0.17
			Asian	6	1102/1318	0.54 (0.22, 1.36)	96	0.19

sensitivity analyses were further conducted by removing each study to detect the robustness of the re-calculated results. When the studies of Narooie-Nejad M, et al. 2015 in Iran (Asian) (36), Abdollahzadeh R, et al. 2016 in Iran (Asian) (31), Fukazawa T, et al. 1999 in Japan (Asian) (16) were removed, heterogeneity was significantly reduced (I^2 = 35% and p = 0.14), but the re-calculated OR between *Bsm-I* polymorphism and the risk of MS showed no significant change in the recessive model (OR = 0.91, 95% CI = 0.74−1.13, p = 0.4). Similarly, when these 3 studies were deleted, the re-calculated OR was not significantly changed between *Taq I* polymorphism and the risk of MS in the dominant model (OR = 1.09, 95% CI = 0.90−1.32, p = 0.391), with no heterogeneity (I^2 = 33.8%). When the studies of Kamisli O, et al. 2018 in Turkey (Caucasian) (27), Abdollahzadeh R, et al. 2018 in Iran (Asian) (28), and Narooie-Nejad M, et al. 2015 in Iran (Asian) (36) were removed,the re-calculated result showed no significant change between *Fok I* polymorphism and MS in the allele model (OR = 1.00, 95% CI = 0.90−1.10, p = 0.925), with no obvious evidence of heterogeneity (p = 0.123 and I^2 = 32.5%).

Discussion

Genetic and environmental factors, especially T cell-mediated autoimmunity, are believed to play a role in the pathogenesis of MS (45). Vitamin D is regarded as a potent modulator in immune system. *VDR* is responsible for most of the vitamin D functions, and so the status of vitamin D has been explored in MS patients (46). Studies have suggested that vitamin D/*VDR* might be involved in immunopathological processes occurring in MS (46,47). *VDR* polymorphisms may modify the function and metabolism of vitamin D (46). Many studies have investigated the roles of *VDR* polymorphisms in MS risk. *Taq I*, *Apa I*, *Fok I*, and

Table 4. *VDR* polymorphisms and multiple sclerosis risk: *FokI* (rs2228570 C > T)

	Genetic model		Stratification	*n*	Case/Control	OR (95% CI)	I^2 %	*Pz*
ForkI	Recessive	FF/Ff + ff	all	16	3209/3254	0.89 (0.76, 1.04)	54	0.15
			women	5	964/1043	0.86 (0.65, 1.14)	52	0.28
			men	4	286/279	0.79 (0.56, 1.11)	33	0.17
			Caucasians	12	2768/2808	0.95 (0.80, 1.13)	53	0.56
			Asian	4	441/446	0.67 (0.51, 0.87)	30	0.003
	Homozygous	FF + ff/Ff	all	16	3209/3254	0.92 (0.83, 1.01)	25	0.09
			women	5	964/1043	0.87 (0.66, 1.16)	55	0.35
			men	4	286/279	0.77 (0.55, 1.08)	34	0.12
			Caucasians	12	2768/2808	0.94 (0.84, 1.04)	31	0.24
			Asian	4	441/446	0.79 (0.60, 1.04)	0	0.09
	Dominant	Ff + ff/ff	all	16	3209/3254	0.94 (0.81, 1.09)	44	0.40
			women	5	964/1043	0.97 (0.75, 1.26)	34	0.83
			men	4	286/279	1.08 (0.64, 1.82)	44	0.77
			Caucasians	12	2768/2808	0.99 (0.85, 1.16)	43	0.9
			Asian	4	441/446	0.48 (0.28, 0.85)	27	0.01
	Codominant (OR1)	FF/ff	all	16	1755/1871	0.89 (0.68, 1.16)	52	0.38
			women	5	502/585	0.91 (0.69, 1.22)	34	0.54
			men	4	146/162	0.95 (0.54, 1.65)	39	0.84
			Caucasians	12	1481/1569	0.98 (0.76, 1.27)	50	0.88
			Asian	4	274/302	0.42 (0.23, 0.74)	33	0.003
	Codominant (OR2)	Ff/ff	all	16	1875/1781	0.98 (0.83, 1.15)	25	0.77
			women	5	597/604	1.04 (0.79, 1.37)	36	0.8
			men	4	173/149	1.21 (0.70, 2.10)	39	0.49
			Caucasians	12	1670/1618	1.02 (0.86, 1.20)	26	0.83
			Asian	4	205/163	0.59 (0.33, 1.06)	17	0.08
	Codominant (OR3)	FF/Ff	all	16	2788/2856	0.90 (0.80, 1.00)	40	0.04
			women	5	829/897	0.85 (0.63, 1.15)	53	0.3
			men	4	253/247	0.76 (0.53, 1.08)	25	0.13
			Caucasians	12	2385/2429	0.93 (0.82, 1.04)	42	0.2
			Asian	4	403/427	0.73 (0.55, 0.97)	18	0.03
	Allele	F/f	all	16	6418/6508	0.91 (0.80, 1.04)	64	0.16
			women	5	1928/2086	0.92 (0.80, 1.05)	48	0.19
			man	4	572/558	0.90 (0.70, 1.15)	48	0.39
			Caucasians	12	5536/5616	0.97 (0.85, 1.11)	61	0.7
			Asian	4	882/892	0.68 (0.54, 0.85)	42	0.0006

Bsm I polymorphisms are the most commonly reported polymorphisms in *VDR* gene (27). However, the results of *VDR* polymorphisms remained inconsistent in patients with MS. Our study based on more studies with larger sample sizes found that *ApaI* and *BsmI* polymorphisms showed no association with MS risk in the overall population. *TaqI* polymorphism was significantly associated with MS risk in the recessive, homozygous, and Codominant (OR3) models in the overall population. *FokI* polymorphism was related to MS risk in Codominant (OR3) model in the overall population, suggesting that *VDR* polymorphisms, especially for *TaqI* and *FokI* polymorphisms might be related to the pathogenesis of MS. These results are consistent with the previous findings regarding the effects of *ApaI*, *TaqI*, *FokI*, and *BsmI* on the risk of MS (15–17).

VDR polymorphisms during the development of MS might depend on ethnicity. In this study, we performed an ethnicity-specific meta-analysis based on Caucasians and Asians. These results suggested that *VDR TaqI* polymorphism was associated with MS risk, especially in Caucasians. While *ApaI* and *FokI* polymorphisms were associated with MS risk in Asians. So, *VDR* polymorphisms may confer different susceptibilities to MS among different populations. Vitamin D is considered to be a potential treatment for patients with MS (46). We investigated the effect of VDR polymorphisms in different populations to confirm this susceptibility to MS and to obtain more adequate strategic planning for the treatment of MS. This suggested that *ApaI* and *FokI* might be potential treatment strategies in Asians with MS, and *TaqI* might become a potential treatment strategy for Caucasians with MS. But there are many other variants to be also considered as potential medical targets, besides VDR *ApaI*, *FokI* and *TaqI* SNP. In future, clinical trials with large cohorts are needed to conduct the association of *VDR* polymorphism and risk of MS, and ethnic factor should always be considered.

Cierny D, et al. found that *Fok I* polymorphism is more common in females with MS (38). We supposed that sex might be an influential factor in the association of VDR polymorphisms and MS risk. In this meta-analysis, pooled

OR of all eligible studies was performed to analyze the correlation of *TaqI* and *FokI* polymorphisms with risk of MS in males and females. However, no significant sex differences were observed, which was consistent with that of previous findings (13,27). Further studies are warranted to address this issue in future.

The *MHC* gene region is the major area of the genome involved in MS and *HLA-DRB1**15 allele has repeatedly been associated with MS in Caucasians. Three studies analyzed co-segregation of 3 SNPs *ApaI* (rs7975232) (40), *FokI* (rs10735810) (39), and *TaqI* (rs731236) (39–41) of the *VDR* gene with *HLA-DRB1* locus in MS patients and healthy individuals. Irizar H, et al. (41) analyzed the *ApaI* polymorphism in *HLA-DRB1**15-positive and *HLA-DRB1**15- negative MS patients and controls, and the results showed no association. Garcia-Martin E, et al. (39) analyzed the interaction of *HLADRB1**15-positive between *FokI* polymorphism and MS risk, and the results showed a negative association. Agliardi C, et al. (41) reported a decreased risk of MS in TT genotypes and T allele. The pooled ORs of the recessive model (TT/Tt + tt) in these 3 studies found no association of *Taq I* polymorphism with MS risk either in *HLA-DRB1**15-positive group or *HLA -DRB1**15-negative group. These results further supported the role of *MHC* gene in determining *VDR* polymorphisms and MS susceptibility. While the previous meta-analysis (24,25) did not perform subgroup analyses based on *HLA-DRB1**15 (positive and negative).

VDR polymorphisms showed association with prostate cancer risk in only patients with vitamin D deficiency (48). *VDR* polymorphisms may not certainly influence the disease risk directly, but may reflect the role of environmental factors, leading to the uncertainty of genetic association studies and failed to identify the specific ''causal'' genes (49). Serum vitamin D levels might be the potential source of environmental exposure for MS (20). Studies (12,43) have focused on serum vitamin D levels and its association with *VDR* polymorphisms and MS risk. *Fokl* polymorphism is associated with the levels of vitamin D (12), but showed no correlation of *Apal* and *Taql* polymorphisms with vitamin D metabolism in MS (43). Al-Temaimi RA, et al. reported no association between *TaqI* and *ApaI* and vitamin D levels, but *FokI* showed association with vitamin D levels in MS (34). However, Bettencourt A, et al. reported that *FokI* polymorphism was not associated with vitamin D levels in MS (29). Based on these findings, whether VDR polymorphisms, especially for *Fokl* polymorphism, could confer a genetic predisposition to vitamin D levels, and this subsequently is warranted to investigate MS further. Therefore, it is important to include measurement of serum vitamin D levels in future studies in order to better characterize the genetic effects of *VDR* polymorphisms and risk of MS.

Between-study heterogeneity was observed in some comparisons in our meta-analysis. For example, we found that ethnicity might be a potential source of heterogeneity between *ApaI* polymorphism and MS in the recessive model. Three studies (16,31,36)were removed between *Bsm-I* polymorphism and MS in the recessive model in Asians. Similarly, three studies (28,31,37) were deleted between *Taq I* polymorphism and MS in the dominant model in Asians, because Asian population was a possible source of heterogeneity in these models. We did not find potential sources of heterogeneity between *Fok I* polymorphism and MS in the allele models. The reasons for this heterogeneity were not very clear, for example, the use of inappropriate and different methods in the detection of *VDR Fok I* polymorphism might be a possible reason. Additionally, geographical and environmental factors may contribute to heterogeneity. These factors might act as potential sources of heterogeneity.

However, this meta-analysis had several limitations. Firstly, all eligible studies were limited to English language, and other publications in other languages were ignored due to insufficient information, resulting in a selection bias. Secondly, due to insufficiency of available data between *VDR* polymorphisms and clinical profiles, the correlation of *VDR* polymorphisms with these clinical profiles such as disease duration, expanded disability status scale (EDSS) score, treatments received, and progression index (PI) was not evaluated. Additional studies are necessary to further estimate the relationship between *VDR* polymorphisms and clinical profiles of MS. Thirdly, we did not find the required available data in sufficient studies to evaluate the correlation of VDR polymorphisms with MS subtypes. More studies are warranted in future. Finally, as eligible studies were not very sufficient on the fifth polymorphism (*Cdx-2*), the association between *Cdx-2* polymorphism and MS risk was not evaluated. More studies are imperative to analyze the role of *Cdx-2* polymorphism in MS in future.

In conclusion, this meta-analysis showed that *VDR TaqI* polymorphism was related to the risk of MS, especially in the Caucasians. *ApaI* and *FokI* polymorphisms showed association with MS risk in Asian population. *BsmI* polymorphism was not associated with MS. Functional assays are required to understand the role of *VDR* polymorphisms in *VDR* expression or active individuals. Also it is important to investigate the interaction of *VDR* polymorphisms with ethnicity, sex, environmental factors and *MHC* gene in the pathogenesis of MS.

Conflicts of Interest

The authors declare no conflicts of interest related to this manuscript.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.arcmed.2019.10.007.

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