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Vitamin D receptor gene variants contribute to hip and knee osteoarthritis susceptibility

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Abstract: Vitamin D receptor (*VDR*) gene polymorphisms could play a significant role in the susceptibility and pathogenesis of osteoarthritis (OA), the most common degenerative joint disorder in humans. The current study involved 94 OA patients and 100 healthy, asymptomatic controls. *VDR* variants FokI (rs2228570), TaqI (rs731236), ApaI (rs7975232) and EcoRV (rs4516035) were genotyped using TaqMan-based real-time PCR. Adjusted odds ratio (OR) analysis showed that *VDR* TaqI and FokI polymorphisms are significantly associated with susceptibility to OA (OR=1.986, P=0.001 and OR=1.561, P=0.017, respectively). Joint-specific analysis showed that the *VDR* TaqI polymorphism was associated with risk of hip OA (OR=1.930, P=0.005) and knee OA (OR=1.916, P=0.028), while the *VDR* FokI polymorphism was associated with higher risk of knee OA (OR=2.117, P=0.012). *VDR* TaqI and FokI polymorphisms are associated with the occurrence of persistent pain (P=0.005 and P=0.027, respectively), while ApaI was associated with a family history of OA (p=0.004). The *VDR* FokI and TaqI genetic variants significantly contribute to osteoarthritis susceptibility, the occurrence of persistent pain, and potentially to joint-specific OA risk.

Keywords: vitamin D; vitamin D receptor; *VDR* gene; genetic polymorphisms; osteoarthritis

INTRODUCTION

Osteoarthritis (OA) is the most common degenerative, progressive and irreversible joint disorder, affecting about 3% of the human population [1]. It is the single most common cause of disability in older adults, characterized by inflammation of the synovium and progressive cartilage degradation [2,3]. The most affected joints are the knee and hip, and the risk factors for the primary OA are age above 60 years, female gender (especially after menopause), obesity, diet, genetic predisposition and joint-level factors, such as occupational exposure/high physical activity and joint injuries [4].

Studies found associations of different genetic factors with OA pathogenesis and predisposition. More than half of the OA cases can be attributed to genetic factors, and the prevalence of this disease in siblings of patients showed a 2- to 3-fold higher risk of OA in comparison with the general population [5].

One of the factors that could play a key role in OA is vitamin D [6]. Calcitriol (1,25-dihydroxyvitamin D3, $1,25(OH)_2D3$), a bioactive form of vitamin D, is a steroid hormone known for its central role in bone mineralization and basal serum calcium and phosphorus level maintenance. Also, vitamin D3 exerts diverse immunomodulatory and antiinflammatory effects [7-10]. The biological effects of vitamin D3 are mediated through binding to the vitamin D receptor (*VDR*), which acts as a ligand-activated transcription

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factor. Several *VDR* gene polymorphisms have a functional immunomodulatory impact [7] and influence rheumatoid arthritis risk [8] and intervertebral disc degeneration [9] in different populations and could therefore play a significant role in the pathogenesis of OA. About 500 single nucleotide polymorphisms (SNPs) in the human *VDR* gene have been described so far, and the most extensively studied variants are FokI, TaqI, ApaI, and EcoRV [11]. FokI polymorphism (C>T, rs2228570) is located in exon 2 of the *VDR* gene and codes for a receptor that is three amino-acids shorter, but with a 2-fold higher transactivation capacity [7]. *VDR* TaqI polymorphism (T>C, rs731236) is the result of a synonymous substitution at codon 352 (isoleucine), in exon 9. This polymorphism is related to the regulation of *VDR* mRNA stability and serum 25-hydroxyvitamin D levels [11]. The *VDR* ApaI polymorphism (C>A, rs7975232) is formed by substitution in intron 8 and could potentially affect gene expression through an alteration of mRNA stability, a change of splice sites or intronic regulatory elements [7,11]. EcoRV (A>G, rs4516035) polymorphism is located in the *VDR* promoter region, and the A allele of the substitution contains a binding site for the transcription factor GATA-3 [11].

Several studies have investigated the association of vitamin D levels and polymorphisms in the vitamin D receptor with osteoarthritis susceptibility, but have yielded inconsistent findings [12-20]. To the best of our knowledge, EcoRV polymorphism has not been previously investigated in OA, while *VDR* polymorphisms have not been investigated in OA patients of the Serbian population. The current study aimed to analyze the association of vitamin D receptor gene polymorphisms with susceptibility to and clinicopathological features of osteoarthritis patients in the Serbian population.

MATERIALS AND METHODS

Ethics statement

Informed consent was obtained from all subjects, individual data was protected during research, and the study was approved by the Institutional Review Board (IRB), the Ethics Committee of the Military Medical Academy, Belgrade, Serbia, approvals No. 09/07/2014. and No. 56/2019. All procedures were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Table 1. Baseline characteristics of the studied cohort of osteoarthritis patients.

Variable		Total OA (N=94)	Hip OA (N=60)	Knee OA (N=34)
Gender	male	35	26	9
	female	59	34	25
Age* (years)	<70	45	26	19
	>70	49	34	15
Smoking	Yes	17	49	28
	No	77	11	6
High physical activity	Yes	20	47	27
	No	74	13	7
Injuries	Yes	33	38	23
	No	61	22	11
Family history	Yes	38	34	21
	No	55	25	13
Early onset OA	<55	52	33	19
	>55	42	27	15
BMI (kg/m²)	≤25	32	24	8
	25-30	40	23	17
	>30	22	13	9
Menopause	Yes	55	32	23
	No	4	2	2
Early menopause# (<45 years)	Yes	15	9	6
	No	40	23	17

OA – osteoarthritis; BMI – body mass index; * – age median; # – number of menopausal women with early onset of menopause

Study participants and biological samples

Our case-control, hospital-based study involved 94 OA patients that had undergone total hip or knee joint replacement, as well as the control group of 100 healthy individuals. Baseline characteristics are presented in Table 1. From the total of 94 patients with radiographically confirmed primary OA, 60 patients (64%) underwent total hip replacement while 34 patients (36%) had total knee replacement at the Clinic for Orthopedic Surgery and Traumatology of our institution in the period from 2015-2018. Excluding criteria in the group of OA patients were secondary osteoarthritis from trauma or surgery to the joint structures, congenital syndromes, congenital

Table 2. Genotype frequencies of the studied *VDR* gene variants in controls and osteoarthritis (OA) groups.

VDR gene variants	Genotype	Controls (N=100)	OA Patients					
			Total OA (N=94)	*P**	Hip OA (N=60)	*P**	Knee OA (N=34)	*P**
TaqI (rs731236)	TT	46	22	***0.003***	15	***0.009***	7	***0.030***
	TC	40	48		27		21	
	CC	14	24		18		6	
FokI (rs2228570)	TT	28	13	*0.053*	9	*0.091*	4	*0.067*
	TC	41	47		34		13	
	CC	31	34		17		17	
ApaI (rs7975232)	CC	25	35	*0.120*	24	*0.110*	11	*0.526*
	CA	56	48		29		19	
	AA	19	11		7		4	
EcoRV (rs4516035)	AA	30	27	*0.702*	18	*0.435*	9	*0.908*
	AG	38	41		28		13	
	GG	32	26		14		12	

OA – osteoarthritis; * bold values show statistically significant P-values (P<0.05)

hip dysplasia and developmental disorders, infection, gout, rheumatoid arthritis and hormone or metabolic disorders. In the control group of 100 healthy individuals, the exclusion criteria were the occurrence of clinical manifestations of OA or other systemic inflammatory or autoimmune diseases and a history of malignancy. All participants were Caucasians of the same ethnicity.

DNA isolation and *VDR* variants analysis

Blood samples were collected from both OA patients and the control group and were stored at -20°C until further use. DNA was isolated by the PureLink Genomic DNA Kit (US). Gene variants were determined by the Applied Biosystems Real-Time PCR 7300 in a reaction volume of 20μL, comprised of 2×Universal Master Mix (Applied Biosystems, US), 0.5 μL 40× TaqMan SNP Genotyping Assays (*VDR* TaqI (rs2228570, TaqMan Assay ID C_2404008_10), FokI (rs731236, TaqMan Assay ID C_12060045_20), ApaI (rs7975232, TaqMan Assay ID C_28977635_10) and EcoRV (rs4516035, TaqMan Assay ID C_2880805_10), and 2.0 μL of genomic DNA. The amplification protocol was utilized under the following conditions: polymerase activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s. Genotypes were analyzed by SDS software (v.2.3).

Statistical analysis

Statistical analysis was performed using SPSS version 20.00. The difference or association between the categorical variables, the allele and genotype frequencies between the patient group and controls, as well as the association of FokI, TaqI and ApaI *VDR* gene polymorphisms with demographic and etiological data were calculated by nonparametric Chi-square (χ^2) or the Fisher's exact test. Association between FokI, TaqI, ApaI, and EcoRV *VDR* polymorphisms and increasing genetic susceptibility to OA were assessed with adjusted odds ratios (OR). OR and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression analysis, adjusted for age and gender considered as the potential confounding factors. The recessive genetic model (wild-type genotype vs. combined heterozygous and mutated genotype) as well as dominant (combined wild-type and heterozygous vs. mutated genotype) and additive genetic models of analyzed *VDR* variants were included in the calculation of OA risk. P values of less than 0.05 were regarded as statistically significant.

RESULTS

To increase statistical power, in the selection of *VDR* polymorphisms, we incorporated prior information from *VDR* association studies and included

Table 3. Vitamin D receptor gene variant association with osteoarthritis (OA) risk.

VDR gene variants	Model	Genotype	OA Cases		Hip OA Cases		Knee OA Cases	
			Adjusted OR [95% CI]‡	*P**	Adjusted OR [95% CI]‡	*P**	Adjusted OR [95% CI]‡	*P**
TaqI (rs731236)	**Genotype**	**TT**	1.000	*Ref.*	1.000	*Ref.*	1.000	*Ref.*
		TC	2.539 [1.299-4.962]	***0.006***	1.935 [0.885-4.229]	*0.098*	3.921 [1.474-10.431]	***0.006***
		CC	3.662 [1.561-8.592]	***0.003***	3.724 [1.472-9.423]	***0.006***	3.056 [0.848-11.022]	*0.088*
	Recessive	**TT vs. TC+CC**	1.678 [1.222-2.303]	***0.001***	2.396 [1.159-4.955]	***0.018***	1.923 [1.199-3.084]	***0.007***
	Additive	-	1.986 [1.302-3.030]	***0.001***	1.930 [1.216-3.063]	***0.005***	1.916 [1.071-3.427]	***0.028***
FokI (rs2228570)	**Genotype**	**TT**	1.000	*Ref.*	1.000	*Ref.*	1.000	*Ref.*
		TC	2.507 [1.144-5.494]	***0.022***	2.792 [1.140-6.839]	***0.025***	2.215 [0.648-7.568]	*0.205*
		CC	2.347 [1.031-5.341]	***0.042***	1.592 [0.605-0.190]	*0.346*	4.556 [1.325-15.672]	***0.016***
	Recessive	**TT vs. TC+CC**	1.561 [1.082-2.252]	***0.017***	2.231 [0.964-5.162]	*0.061*	3.097 [0.995-9.686]	*0.052*
	Additive	-	1.443 [0.972-2.145]	*0.069*	1.181 [0.754-1.848]	*0.468*	2.117 [1.182-3.793]	***0.012***
ApaI (rs7975232)	**Genotype**	**CC**	1.000	*Ref.*	1.000	*Ref.*	1.000	*Ref.*
		CA	0.618 [0.324-1.176]	*0.142*	0.542 [0.263-1.117]	*0.097*	0.774 [0.316-1.899]	*0.576*
		AA	0.424 [0.171-1.049]	*0.063*	0.408 [0.144-1.153]	*0.091*	0.470 [0.128-1.728	*0.256*
	Recessive	**CC vs. CA+AA**	0.754 [0.554-1.028]	*0.074*	0.509 [0.255-1.017]	*0.056*	0.833 [0.541-1.284]	*0.409*
	Additive	-	0.644 [0.416-0.997]	***0.049***	0.613 [0.372-1.013]	*0.056*	0.704 [0.382-1.297]	*0.260*
EcoRV (rs4516035)	**Genotype**	**AA**	1.000	*Ref.*	1.000	*Ref.*	1.000	*Ref.*
		AG	1.236 [0.622-2.456]	*0.546*	1.284 [0.594-2.774]	*0.525*	1.164 [0.435-3.113]	*0.763*
		GG	0.881 [0.422-1.843]	*0.737*	0.725 [0.305-1.727]	*0.468*	1.238 [0.452-3.389]	*0.677*
	Recessive	**AA vs. AG+GG**	1.035 [0.759-1.411]	*0.830*	1.012 [0.712-1.439]	*0.947*	1.095 [0.704-1.701]	*0.688*
	Additive	-	0.939 [0.650-1.356]	*0.738*	0.863 [0.566-1.317]	*0.494*	1.111 [0.674-1.831]	*0.681*

‡ – Adjusted by age and gender; bold values show statistically significant P-values (p<0.05); Ref. – referent genotype

high- and medium-powered SNPs, which are likely to be involved in OA. For the available sample size of 94 cases and 100 controls, according to the minor allele frequency of individual *VDR* SNPs in cases and controls in our cohort, the average power of the study was 0.83, with a significance level of 0.05.

Genotype frequencies of investigated *VDR* polymorphisms in OA patients and the control group are shown in Table 2. The results pointed to a significant difference in *VDR* TaqI genotype distributions between the total group of OA patients and healthy controls (P=0.003), with a tendency toward different genotype prevalence in *VDR* FokI polymorphism (P=0.053) (Table 2). Joint-specific analysis revealed significant differences in *VDR* TaqI genotype distributions in patients with hip OA (P=0.009) and knee OA (P=0.030), compared to the controls (Table 2).

No differences in genotype distribution for *VDR* ApaI and EcoRV polymorphisms were observed (Table 2).

Logistic regression was assessed to evaluate OA risk. Age and gender were included as confounding factors since they are well-known risk factors for primary OA. The results of the adjusted OR analysis showed that *VDR* TaqI polymorphism is significantly associated with an increased OA risk in the recessive model (TC+CC genotypes vs. TT (OR=1.678, P=0.001)), as well as in the additive model (OR=1.986, P=0.001), while no association was found in the dominant genetic model (Table 3). Comparison of OA patients with control subjects indicated that individuals with TC and CC genotypes of *VDR* TaqI polymorphism had a higher OA risk (OR=2.539, P=0.006, and OR=3.662, P=0.003, respectively).

VDR FokI polymorphism significantly increased OA risk in the recessive model (OR=1.561, P=0.017), but not in the dominant or additive genetic model (Table 3). Compared to the wild-type TT genotype, TC and CC genotypes of *VDR* FokI polymorphism had an elevated OA risk (OR=2.507, P=0.022, and OR=2.347, P=0.042, respectively) (Table 3).

In the joint-specific analysis, in the additive model, *VDR* TaqI polymorphism was associated with significantly increased risk of both hip (OR=1.930, P=0.005) and knee OA (OR=1.916, P=0.028), while *VDR* FokI polymorphism was associated with a significantly increased risk of knee OA (OR=2.117, P=0.012), but not hip OA (OR=1.181, P=0.468). The joint-stratified analysis showed that the CC genotype of *VDR* TaqI was associated with an increased prevalence of hip OA (OR=3.724, P=0.006), while the TC genotype was associated with an increased risk of knee OA (OR=3.921, P=0.006) (Table 3). The TC genotype of *VDR* FokI was associated with an increased hip OA risk (OR=2.792, p=0.025), while the homozygous CC-mutated genotype was associated with increased knee OA risk (OR=4.556, P=0.016) (Table 3). Our study showed no association of EcoRV polymorphism with OA susceptibility or clinical features of OA patients.

In the additive model, *VDR* ApaI polymorphism was associated with decreased OA risk (OR=0.644, P=0.049). A tendency towards lower OA prevalence in the AA genotype compared to the CC genotype was observed (OR=0.424, P=0.063).

Analysis of associations between *VDR* variants and demographic and risk factors for OA indicated that *VDR* TaqI and FokI polymorphism were significantly associated with the occurrence of persistent, chronic pain (P=0.005 and P=0.027, respectively) (Table 4). The ApaI *VDR* polymorphism was associated with a family history of OA (P=0.004) (Table 4).

DISCUSSION

Osteoarthritis (OA) is the most common degenerative and multifactorial joint disorder in humans. Although the molecular mechanisms of OA are not fully elucidated, more than half of disease predisposition in the population can be explained by genetic factors [5]. Due to its role in bone metabolism and inflammation, vitamin D and its receptor (*VDR*) could play a crucial role in osteoarthritis susceptibility. In the current study, we investigated the four common polymorphisms of the *VDR* gene, FokI, TaqI, ApaI and EcoRV, previously reported to alter *VDR* function and modulate the immune response [7,11].

Our findings indicate that both *VDR* TaqI (rs731236) and FokI (rs2228570) *VDR* gene polymorphisms significantly increase OA risk. Individuals with TC/CC genotypes of *VDR* TaqI polymorphism have a 2.5-/3.6-fold significantly increased OA risk in comparison with the wild-type genotype. *VDR* FokI TC and CC genotypes were associated with >2-fold increased risk. Joint-specific analysis pointed to differences in hip and knee susceptibility for *VDR* TaqI and FokI polymorphisms, indicating potential joint-specific effects of gene variants. Moreover, joint-specific stratification revealed differences in OA prevalence between heterozygous and mutated homozygous genotypes for the *VDR* TaqI and FokI polymorphisms. In the additive model, *VDR* TaqI polymorphism was associated with an almost 2-fold increased risk of both hip and knee OA, while *VDR* FokI polymorphism was associated with a 2-fold increased risk for knee OA but not hip OA. Also, our findings show that *VDR* TaqI and FokI polymorphisms are significantly associated with the occurrence of persistent pain. Interestingly, our results reveal that the *VDR* ApaI polymorphism is associated with a family history of OA and decreased OA risk, and that it could exert a protective effect in this degenerative joint disorder.

Table 4. *VDR* gene variant association with demographic features and OA risk factors.

Demographic and risk factors		Total N	TaqI (rs731236)			FokI (rs2228570)			ApaI (rs7975232)			EcoRV (rs4516035)		
			TT	TC	CC	TT	TC	CC	CC	CA	AA	AA	AG	GG
Gender	Male	35	7	15	13	6	18	11	14	17	4	7	18	10
	Female	59	15	33	11	7	29	23	21	31	7	20	23	16
	P		*0.138*			*0.666*			*0.911*			*0.321*		
Age median	<70	45	13	23	9	5	24	16	17	21	7	12	25	8
	>70	49	9	25	15	8	23	18	18	27	4	15	16	18
	P		*0.342*			*0.718*			*0.489*			***0.050***		
Smoking	Yes	17	5	8	4	1	10	6	7	9	1	20	36	21
	No	77	17	40	20	12	37	28	28	39	10	7	5	5
	P		*0.811*			*0.529*			*0.704*			*0.349*		
High physical activity	Yes	20	3	9	8	4	7	9	8	10	2	20	33	21
	No	74	19	39	16	9	40	25	27	38	9	7	8	5
	P		*0.220*			*0.303*			*0.941*			*0.783*		
History of injury	Yes	33	7	13	13	3	19	11	11	16	6	18	24	19
	No	61	15	35	11	10	28	23	24	32	5	9	17	7
	P		*0.071*			*0.467*			*0.350*			*0.466*		
Family history	Yes	38	10	19	9	3	22	13	22	13	3	14	28	13
	No	55	12	28	15	9	25	21	13	35	7	13	12	13
	P		*0.857*			*0.362*			***0.004***			*0.179*		
Early onset	<55	52	9	27	16	9	26	17	23	25	4	17	22	13
	>55	42	13	21	8	4	21	17	12	23	7	10	19	13
	P		*0.211*			*0.495*			*0.189*			*0.612*		
BMI (kg/m²)	≤25	32	11	17	4	2	16	14	12	17	3	7	16	9
	25-30	40	8	20	12	8	20	12	14	22	4	13	15	12
	>30	22	3	11	8	3	11	8	9	9	4	7	10	5
	P		*0.182*			*0.499*			*0.779*			*0.785*		
Menopause	Yes	55	14	30	11	7	26	22	20	29	6	19	21	15
	No	4	1	3	0	0	3	1	1	2	1	1	2	1
	P		*0.583*			*0.520*			*0.682*			*0.887*		
Early menopause# (<45 years)	Yes	15	4	8	3	4	8	3	6	6	3	7	6	2
	No	40	10	20	8	3	18	19	14	23	3	12	15	13
	P		*0.991*			*0.068*			*0.321*			*0.307*		
Persistent pain	Yes	56	17	31	8	3	30	23	23	26	7	9	19	9
	No	37	5	16	16	9	17	11	12	21	4	17	22	17
	P		***0.005***			***0.027***			*0.617*			*0.518*		

BMI – body mass index; bold values show statistically significant P-values (P<0.05); # – number of menopausal women with early onset of menopause

VDR gene polymorphisms affect the activity of the VDR protein by altering the calcitriol binding sites and could influence bone mineralization and the antiinflammatory functions of vitamin D [7,11]; therefore, they could influence genetic susceptibility to OA. Even though *VDR* is one of the best candidate genes for OA susceptibility, the results of association studies are still inconsistent. Our findings are in agreement with earlier studies indicating the potential role of *VDR* variants in the predisposition to OA susceptibility [12,13]. Another study showed that *VDR* TaqI polymorphism is associated with increased risk of primary knee OA in the Mexican Mestizo population [15], and with symmetrical hand OA susceptibility in Finnish women, which could be modified by calcium intake [21]. Moreover, *VDR* BsmI and TaqI polymorphisms were associated with susceptibility of the spine to OA [22]. However, other studies and

meta-analyses did not show the association of *VDR* variants with OA susceptibility [19,20,22-24]. A meta-analysis of European and Asian populations showed a significant association of ApaI and OA susceptibility in Asians [18], but a more recent study reported a lack of association of this polymorphism with OA risk [·22]. The lack of association with *VDR* ApaI and TaqI polymorphisms was also observed in Turkish patients with OA of the temporomandibular joint [14].

VDR polymorphisms have yielded inconsistent results in different populations. To the best of our knowledge, *VDR* EcoRV polymorphism has not been previously investigated in OA, while *VDR* polymorphism studies have not been performed on OA patients of the Serbian population. The discrepancies between different case-control association studies of VDR and OA, as well as other degenerative and inflammatory joint diseases, may be explained by genetic heterogeneity among different populations, since risk alleles often show ethnic stratification, as demonstrated in Asian OA patients but not in Europeans [18]. The *VDR* FokI polymorphism was associated with intervertebral disc degeneration among Caucasians but not Asians [9]. Moreover, different *VDR* polymorphisms exerted opposite effects on susceptibility to rheumatoid arthritis in different populations [8]. Inconsistent findings in studies could also be a result of the criteria applied for OA patients' inclusion, clinical heterogeneity, age or gender discrepancies among cases and controls, and the differences in affected joint-types, weight-bearing joints, such as knees and hips, as opposed to other joints [4,25]. Moreover, variable effects of the *VDR* risk alleles might be modulated by nutrition and depend on country-specific deficiencies of vitamin D. Thus, the association of *VDR* polymorphisms with OA risk should be population-based, with age-matched healthy controls, and stratified by the skeletal site of the affected joint.

Our results of joint-specific differences in risk prevalence may be the result of differences in gene expression between the cartilage from different joints, as demonstrated in the comparison between normal and OA cartilage of knees and hips [26,27]. A comprehensive gene expression analysis has revealed differential gene expression at the individual gene status between hip and knee cartilage in OA patients [26]. These findings indicate that risk alleles for OA could more frequently show joint-specific effects and rarely have systemic effects.

Our findings of *VDR* TaqI and FokI polymorphism correlation with the prevalence of persistent pain are consistent with previous findings that pointed to the FokI variant and low vitamin D3 level association with pain in knee OA [24]. Vitamin D deficiency is associated with pain in OA patients [28,29]. In the early stages of OA, the pain is activity-related and linked to nociceptive activity, while chronic pain in advanced OA extends beyond neurobiological mechanisms that could include the role of vitamin D in optimizing neurological functioning and reducing inflammation [30].

Since OA is the most common progressive degenerative joint disease worldwide, therapeutic options need to be expanded. Vitamin D3 supplementation could be a promising dietary factor in the prevention or treatment of OA due to its role in bone mineralization and antiinflammatory effects. Previous findings showed that OA progression could be associated with low vitamin D3 levels and that vitamin D3 supplementation exerts a positive effect on joint structure in OA patients [31]. Vitamin D3 treatment causes long-lasting inhibition of cell proliferation and cytokine production in synovial stromal cells cultured from OA patients [32]. Vitamin D3 supplementation in elderly OA patients reduces pain and improves the quality of life [33, 34]. However, some studies have showed inconsistent results, indicating that vitamin D3 supplementation might more likely show its effects in slowing down OA progression rather than stopping it or reversing the symptoms. The individual responses to vitamin D supplementation may be dictated by genetic polymorphisms and might be the source of an inconsistency between studies.

To summarize, the results obtained in this study could contribute to further elucidation of the role of vitamin D and *VDR* variants in OA. Our findings show that *VDR* TaqI (rs731236) and FokI (rs2228570) polymorphisms are significantly associated with OA susceptibility. *VDR* polymorphisms could predispose joint-specific OA, and chronic, persistent pain in OA. Given the key role of vitamin D in bone metabolism and inflammation, future integrative analysis

combining a wider panel of genetic changes, epigenetic and transcriptomics data will be required to elucidate the roles of vitamin D and VDR in OA. Future directions for OA management could include vitamin D supplementation according to *VDR* polymorphisms and serum vitamin D status, to potentially delay the onset of OA, to slow down its progression and to reduce pain in OA patients.

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