RESEARCH ARTICLE

Association of *VDR-FokI* and *VDBP-Thr420Lys* polymorphisms with cervical spondylotic myelopathy: A case-control study in the population of China

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Background: Cervical spondylotic myelopathy (CSM), a common degenerative disorder, is characterized by chronic progressive compression of the cervical spinal cord. The present case-control study aimed to explore the potential role of *VDR-FokI* and *VDBP-Thr420Lys* polymorphisms in the susceptibility to CSM in the Chinese population.

Methods: The study enrolled 318 CSM patients and 282 healthy individuals whose clinical data were retrospectively analyzed. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to genotype *VDR-FokI* and *VDBP-Thr420Lys* polymorphisms. The severity of CSM was assessed using the Japanese Orthopaedic Association (JOA) score with magnetic resonance imaging (MRI) of cervical vertebra. A nonconditional binary logistic regression model was conducted for assessing the risk factors of CSM.

Results: Patients in the CSM group had longer time duration to bend over desk working than the control group. The ff genotype and f allele frequency of *VDR-FokI* were elevated in CSM patients. Elevated Ff + ff genotype and f allele frequency of *VDR-FokI* might increase the risk of CSM. The *VDR-FokI* polymorphism was associated with nucleus pulposus capillary invasion, necrosis, hyaline degeneration and fibrosis, genesis and hyperplasia of cartilage-like cells, and fibrocyst in the fibrous ring. The *VDR-FokI* and *VDBP-Thr420Lys* genotypes conformed to Hardy-Weinberg equilibrium which showed that *VDR-FokI* and *VDBP-Thr420Lys* had group representation characteristics.

Conclusion: Binary logistic regression analysis confirmed that *VDR-FokI* polymorphism and the time to bend over desk working were risk factors of CSM. Our results indicate that *VDR-FokI* polymorphism may be closely associated with the risk of CSM.

KEYWORDS

cervical spondylotic myelopathy, *FokI*, polymorphism, *Thr420Lys*, *VDBP*, *VDR*

1 | INTRODUCTION

Cervical spondylotic myelopathy (CSM), defined as a clinical symptomatic entity, is a degenerative condition which causes nontraumatic, progressive, and chronic compression injury of cervical spinal cord, which is a leading cause of spinal cord impairment in the world.¹ CSM is the most prevalent cause of reversible spinal cord dysfunction predominantly affecting males, and the Asian population (individuals over 55 years of age).² A recent study showed that the incidence figure of patients with CSM treated surgically is at least 1.6 per 100 000 per year.³ CSM has been extensively accepted as a group of closely related disorders caused by multiple factors, including disk herniation, degenerative changes in the spine, infolding of the ligamentum flavum and facet joint capsule, and congenital spinal canal stenosis.⁴ Furthermore, as a spinal cord dysfunction, CSM leads to clinical symptoms such as loss of dexterity, poor coordination, motor weakness, and/or sensory loss.⁵ A close association has been observed between vitamin D receptor (VDR) gene and skeletal and cartilage metabolism; meanwhile, vitamin D binding protein (VDBP) gene serves as a potential novel marker of renal interstitial inflammation and fibrosis.^{6,7} Hence, the association of VDR and VDBP gene polymorphisms with CSM requires high clinical attention.

Vitamin D receptor, a member of the nuclear receptor superfamily, plays an important role in cellular differentiation and proliferation in a variety of cell types.⁸ The gene encoding human VDR is located on chromosome 12 and covers approximately about 100 kb of genomic DNA.⁹ Recent studies showed a correlation between VDR gene polymorphism and individual growth and development, which includes VDR TaqI (rs731236), VDR-FokI (rs2228570), VDR BsmI (rs1544410), and VDR Apal (rs7975232).^{10,11} Previous studies support evidence stating the close relation between VDR-FokI and bone density.^{12,13} Besides, VDR-FokI gene polymorphism affects vitamin D status and may affect the development risk of spinal tuberculosis.¹⁴ It has been previously reported that bone density is correlated with CSM,¹⁵ whereas no correlation was observed between the VDR-FokI and CSM; therefore, this study aimed to explore whether the VDR-FokI is associated with CSM. On the other hand, there are BsmI, TaqI, and Apal sites on the VDR gene except for the FokI site, and studies have shown a positive correlation between VDR TaqI and VDR Apal with CSM.¹⁶ VDBP, a glycosylated alpha-globulin, functions via transporting vitamin D metabolites through the circulation.⁷ VDBP gene is located on the long arm of chromosome 4 (4q12-q13), which contains 13 exons and 12 introns.¹⁷ No correlation was observed between VDR-FokI and VDBP-Thr420Lys polymorphisms in American and European populations which lead to the susceptibility to CSM,¹⁸ but some studies in the Asian population showed a potential correlation between them.^{16,19} Several genetic polymorphisms have been ascertained regarding the susceptibility to CSM, such as *BMP-4*, *HIF-1 α* , and *OPN* genetic polymorphism.²⁰⁻²² However, few studies were conducted concerning the relationship of CSM with VDR and VDBP genetic polymorphisms. Therefore, the aim of this study was to explore the correlations of VDR-FokI and VDBP-Thr420Lys polymorphisms with the susceptibility to CSM.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The current study was performed with approval of the Ethics Committee of the People's Hospital of Mengyin County. Informed consents from parents or legal guardians of study subjects were obtained. The study protocols were strictly based on the ethical principles for medical research involving human subjects of the Helsinki Declaration.

2.2 | Study subjects

From June 2013 to June 2015, a total of 318 patients diagnosed with CSM in the People's Hospital of Mengyin County were enrolled as the CSM group, comprising 192 males and 126 females (aging from 25 to 72 years, with a mean age of 48.28 ± 8.08 years). Inclusion criteria for the selected CSM group were as follows: (a) patients with progressive and chronic numbness of limb, packing feeling, bilateral clumsy hands, and spastic gait; (b) patients with no clinical manifestations observed in X-ray, but with visible diseased intervertebral space stenosis and hyperostosis of vertebral posterior; (c) patients with curved spinal cord in front and the compression of spinal cord caused by segment multiple sclerosis lesions observed under magnetic resonance imaging (MRI) examination; and (d) patients undergoing surgical treatment. Exclusion criteria for the CSM group were described as follows: (a) patients with lumbar disk protrusion; (b) patients without complete clinical data; and (c) patients with HIV infection, diabetes, cancer, chronic pneumonia, or other diseases. Diagnostic criteria for CSM included the following: (a) clinical compression of the spinal cord; (b) canal stenosis in cervical sagittal diameter, hyperostosis, and instability of cervical spine segment in X-ray; and (c) results of extensive examinations such as CT, MRI, and digital subtraction according to the condition of illness. Meanwhile, 282 healthy individuals including 178 males and 104 females (aged from 25 to 68 years, mean age of 47.35 ± 8.57 years) confirmed by physical examination in the corresponding period were enrolled to serve as the control group. All healthy controls exhibited no neck and shoulder discomfort, autoimmune disease, allergic disease, liver and kidney diseases, or other autoimmune diseases. The characteristics were comparable between the CSM group and the control group, including factors such as the basal status of disease and age.

2.3 | Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Venous blood samples (5 mL) were collected from all subjects after overnight fasting and placed in tubes with ethylenediaminetetraacetic acid dipotassium (EDTA-K) for anticoagulation. Genomic DNA of each subject was extracted from the white blood cells using the sodium iodide (NaI) method. DNA content was determined using a spectrophotometer and preserved at -20°C . Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

analysis was conducted to analyze VDR-FokI and VDBP-Thr420Lys polymorphisms. The PCR primer sequences are shown in Table 1. PCR amplification was conducted in the reaction system (50 μ L) containing 5 μ L of 10 $\times$ PCR buffer, 3 μ L of 25 mmol/L $MgCl_2$, 0.5 μ L of 10 mmol/L diethyl-nitrophenyl thiophosphate (dNTP), 1 μ L of forward primer and 1 μ L of reverse primer, 0.2 μ L of 5 U/ μ L Taq DNA polymerase, and 39.3 μ L of ultrapure water. PCR conditions were as follows: predenaturation at 95°C for 5 minutes, 35 cycles of amplification (95°C for 45 seconds, 59°C for 45 seconds and 72°C for 45 seconds, and 72°C for 45 seconds), and a final extension at 72°C for 5 minutes. After verification by performing agarose gel electrophoresis, the VDR was enzyme-digested with FokI. The restriction sites of VDR-FokI are 5'-GGATG(N)₉-3' and 3'-CCTAC(N)₁₃-5'. VDBP-Thr420Lys was digested with restriction enzyme Styl with the recognition sequence of 5'-CCWWGG-3'.

2.4 | Magnetic resonance imaging (MRI) and the Japanese Orthopaedic Association (JOA) score

According to assessment criteria as previously reported and the MRI imaging results of the cervical spine, the number of affected cervical spine segments was recorded. The Japanese Orthopaedic Association (JOA) score was adopted to assess the severity of neurological dysfunction in patients with CSM.²³ The JOA scoring system primarily includes motor function of upper and lower extremities (ranging from 0 points [completely lost] to 4 points [completely normal]); sensory function of upper extremities, trunk, and lower extremities (ranging from 0 points [disordered] to 2 points [normal]); and bladder function (ranging from 0 points [dysuria] to 3 points [normal]).

2.5 | Pathological observation

After surgery, the intervertebral disk tissues of all subjects were collected and separated into nuclear pulposus and fibrous ring, which were numbered and pathologically detected. Hematoxylin and eosin (HE) staining was employed to stain all pathological samples. The pathological changes of nuclear pulposus and fibrous ring were observed under an electron microscope (SEM; ZEISS EVO18; Carl Zeiss, Oberkochen, Germany). The pathological indexes included the following: (a) decreased chondrocytes in nuclear pulposus, and

hyperplasia of fibroblasts in different degrees; (b) necrosis, hyaline degeneration, and fibrosis in nuclear pulposus tissues; (c) capillary invasion in nuclear pulposus; (d) infiltration of inflammatory cells in nuclear pulposus; (e) genesis and hyperplasia of cartilage-like cells in fibrous ring; (f) tumor-like lesion in fibrous ring mucus; and (g) genesis of fibrocyst in fibrous ring.

2.6 | Statistical analysis

The statistical data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software (IBM Corp. Armonk, NY, USA). Count data were expressed as percentage or ratio, and measurement data were presented as mean $\pm$ standard deviation (SD). The genotype and allele frequencies were examined using the chi-square test. Moreover, Hardy-Weinberg equilibrium was employed to verify whether the samples had group representation. Nonconditional binary logistic regression analysis was used to analyze the risk factors for CSM, with odds ratio and 95% confidence interval (CI) as the risk of CSM. All *P* values were bilateral, and *P* < 0.05 was considered to be statistically significant.

3 | RESULTS

3.1 | Baseline characteristics of subjects

A total of 318 patients with CSM and 282 healthy individuals with no history of CSM were enrolled in this study. No statistical differences were observed in age (*P* = 0.172), gender (*P* = 0.490), body mass index (BMI) (*P* = 0.876), smoking history (*P* = 0.605), and average working time (*P* = 0.549) between the CSM and control groups (all *P* > 0.05), while the time to bend over desk working was significantly higher in the CSM group compared to the control group (*P* = 0.003 < 0.05) (Table 2).

3.2 | Genotyping of VDR-FokI and VDBP-Thr420Lys polymorphisms

Genomic DNA was amplified by PCR. The PCR products, namely VDR gene segments, were digested by restriction enzyme FokI in a water bath, followed by electrophoresis in 3% agarose gel and analysis using the BIO-RAD gel imaging system. The allele of FokI changed from T to A/C/G (also from f to F); that is, if genotype was TT (hereinafter as ff), it was considered as the recognition site of FokI; the genotype CC/GG/AA (FF) would maintain its original size. The PCR products included three segments, 54 bp, 286 bp, and 340 bp (Figure 1A). The ff homozygote included a segment of 286 bp, FF homozygote included a segment of 340 bp, and Ff heterozygote included two segments of 286 bp and 340 bp (Figure 1B). The allele of Thr420Lys changed from C to A, and the genotype was AA, which was the recognition site of Thr420Lys. The AA genotype was digested into two segments with one segment of 355 bp and the other of 199 bp. The CC genotype could not be digested; hence, it maintained its original size (554 bp). Half of the heterozygotes CA

TABLE 1 Primer sequences of VDR-FokI and VDBP-Thr420Lys polymorphisms

Polymorphism	Primer sequence
VDR-FokI	F: 5'-TGACTCTGGCTCTGACCG-3' R: 5'-AAGACCCTCTGCTCCTG-3'
VDBP-Thr420Lys	F: 5'-GAAGAGAAGGTGAAAGGTTAGG-3' R: 5'-CCTGTACATAATGGCATCTCAAT-3'

F, forward; R, reverse; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

Characteristic	CSM group (n = 318)	Control group (n = 282)	t/χ^2	P
Age (y)	48.28 ± 8.08	47.35 ± 8.57	0.172	0.172
Gender (male/female)	192/126	178/104	0.476	0.490
BMI (kg/m ²)	23.05 ± 3.32	23.09 ± 2.93	0.156	0.876
Smoking history (%)	55.35	57.45	0.268	0.605
Working time at table (hours/d)	5.43 ± 1.03	5.19 ± 0.96	2.941	0.003
Average working time (hours/d)	6.90 ± 0.63	6.87 ± 0.59	0.560	0.549

BMI, body mass index; CSM, Cervical spondylotic myelopathy.

TABLE 2 Comparison of baseline characteristics of subjects in the case and control groups

FIGURE 1 Electrophoretogram of polymerase chain reaction (PCR) products of VDR-FokI (A) and VDBP-Thr420Lys (B) polymorphisms. Note: VDR, vitamin D receptor; VDBP, vitamin D binding protein

could be recognized; therefore, all three segments of 355 bp, 199 bp, and 554 bp existed (Figure 1).

3.3 | Distribution of genotype and allele frequencies of VDR-FokI and VDBP-Thr420Lys polymorphisms in the case and control groups

The Hardy-Weinberg equilibrium testing was performed to test the distribution of the genotypes of VDR-FokI and VDBP-Thr420Lys in the case and control groups, and the results showed that the CSM group (VDR-FokI: $\chi^2 = 2.476$, $P = 0.116$; VDBP-Thr420Lys: $\chi^2 = 1.209$, $P = 0.272$) and the control group (VDR-FokI: $\chi^2 = 2.086$, $P = 0.149$; VDBP-Thr420Lys: $\chi^2 = 0.211$, $P = 0.646$) were in conformity with the Hardy-Weinberg equilibrium, which indicated that VDR-FokI and VDBP-Thr420Lys were representative (Table 3).

As shown in Table 4, in comparison with the control group, the ff genotype (FF vs ff: $\chi^2 = 8.696$, $P = 0.003$) and f allele frequencies (F vs f: $\chi^2 = 8.696$, $P = 0.003$) of VDR-FokI were higher in the CSM group (both $P < 0.05$), and the Ff + ff genotype (FF vs Ff + ff: $\chi^2 = 6.985$, OR = 1.569; 95% CI = 1.122 ~ 2.192; $P = 0.008$) and f allele (F vs f: $\chi^2 = 10.030$, OR = 1.453; 95% CI = 1.153 ~ 1.831; $P = 0.002$) would increase the risk of CSM (both $P < 0.05$). However, no significant difference was observed in the distribution of genotype (CC vs CA: $\chi^2 = 0.187$, $P = 0.665$; CC vs CA + AA: $\chi^2 = 0.126$, $P = 0.723$) and allele frequencies (C vs A: $\chi^2 = 0.044$, $P = 0.834$) of VDBP-Thr420Lys between the case and control groups (all $P > 0.05$).

3.4 | JOA scores and the number of cervical spine segments in CSM patients with different genotypes of VDR-FokI and VDBP-Thr420Lys polymorphisms

Further analysis was conducted to investigate the association of patients with CSM carrying different genotypes of VDR-FokI and VDBP-Thr420Lys to evaluate the severity of CSM. As shown in Table 5, the JOA scores in CSM patients with different genotypes of VDR-FokI ($F = 1.032$, $P = 0.358$), the number of cervical spine segments in CSM patients with different genotypes of VDR-FokI ($F = 0.554$, $P = 0.575$), the JOA scores in CSM patients with different genotypes of VDBP-Thr420Lys ($F = 0.607$, $P = 0.546$), and the number of cervical spine segments in CSM patients with different genotypes of VDBP-Thr420Lys ($F = 2.162$, $P = 0.117$) indicated that VDR-FokI and VDBP-Thr420Lys polymorphisms were irrelevant to the severity of CSM.

3.5 | Correlations of VDR-FokI and VDBP-Thr420Lys polymorphisms with the pathological features of CSM patients

As shown in Table 6, VDR-FokI polymorphism was associated with nucleus pulposus capillary invasion, necrosis, hyaline degeneration and fibrosis, genesis and hyperplasia of cartilage-like cells, and fibrocyt in the fibrous ring (all $P < 0.05$), and it was not correlated with other pathological features (all $P > 0.05$). Evidently there was

Polymorphism	Genotype	CSM group (n = 318)		P	Control group (n = 282)		χ ²	P
		Observed value	Predicted value		Observed value	Predicted value		
VDR-FokI	FF	100 (31.45)	93 (29.26)		118 (41.84)	112 (35.33)		
	Ff	144 (45.28)	158 (49.67)	0.116	120 (42.55)	131 (41.29)	2.086	0.149
	ff	74 (23.27)	67 (21.08)		44 (15.60)	38 (12.06)		
VDBP-Thr420Lys	CC	176 (55.35)	172 (54.15)		152 (53.90)	150 (47.32)		
	CA	116 (36.48)	124 (38.88)	0.272	108 (38.30)	111 (34.92)	0.211	0.646
	AA	26 (8.18)	22 (6.98)		22 (7.80)	20 (6.44)		

no association of *VDBP*-Thr420Lys with the pathological features of CSM (all $P > 0.05$).

With CSM as a dependent variable, the baseline characteristics of individuals in the case and control groups (age, gender, BMI, smoking history, time to bend over desk working, and average working time), and VDR-FokI and VDBP-Thr420Lys polymorphisms as independent variables, these factors were enrolled into the multivariate logistic regression model, and binary logistic regression analysis was performed to determine the risk factors for CSM. As shown in Table 7, time to bend over desk working (OR = 1.279, 95% CI = 1.083 ~ 1.511; $P = 0.004$) and VDR-FokI polymorphism (OR = 1.461, 95% CI = 1.162 ~ 1.837; $P = 0.001$) were the risk factors for CSM, while parameters such as age, gender, BMI, smoking history, average working time, and VDBP-Thr420Lys polymorphism were irrelevant to CSM (all $P > 0.05$).

Cervical spondylotic myelopathy is the most prevalent spinal disorder, affecting patients over the age of 50 years. The complex pathophysiology of CSM includes mechanical spinal cord compression due to anterior compressive osteophytes, spinal canal stenosis, and spinal hypermobility.²⁴ Recently, several studies have focused on exploring the correlation between CSM and gene polymorphism.^{21,22,25} In this study, we explored the association of the distribution of genotypes and allele frequency of *VDR*-FokI and *VDBP*-Thr420Lys with CSM through the case-control method. As a result, the study was indicative of a close association between *VDR*-FokI polymorphism and susceptibility to CSM.

The results demonstrated that the ff genotype and f allele frequencies of VDR-FokI were higher in the CSM group than those in the control group, which were suggestive of an increased risk of CSM due to the Ff + ff genotype and f allele. A combination of vitamin D with VDR in the cytoplasm forms a hormone receptor compound, which later combines with vitamin D response elements in the nucleus to activate or inhibit genes containing vitamin D response elements.²⁶ A recent study noted that the polymorphisms of VDR have a close association with the susceptibility to, risk of, and severity of CSM.²⁷ Moreover, polymorphisms in different regions exercised different effects. VDR rs2228570 polymorphism, a T-C transition at the translation initiation codon of VDR, caused a shorter protein with increased biological activity, while VDR rs11568820, in the 5' promoter region of VDR, led to reduced transcriptional activity of the promoter and affected calcium absorption in the intestine.²⁸ FokI (rs2228570/rs10735810) in exon 2 at 5' end of VDR is considered as the most potential functional polymorphic site among all the VDR polymorphic sites.²⁹ The F allele (with no FokI restriction site) initiates translation at the second ATG site and encodes

TABLE 4 Distribution of genotype and allele frequencies of VDR-FokI and VDBP-Thr420Lys polymorphisms in the case and control groups

Polymorphism	Genotype/allele	CSM group (n = 318, %)	Control group (n = 282, %)	χ^2	OR (95% CI)	P
VDR-FokI	FF	100 (31.45)	118 (41.84)	Ref		
	Ff	144 (45.28)	120 (42.55)	1.127	0.763 (0.462~1.258)	0.289
	ff	74 (23.27)	44 (15.60)	8.696	1.985 (1.255~3.139)	0.003
	Ff + ff	218 (68.55)	164 (58.16)	6.985	1.569 (1.122~2.192)	0.008
	F	344 (54.09)	356 (63.12)	Ref		
	f	292 (45.91)	208 (36.88)	10.030	1.453 (1.153~1.831)	0.002
VDBP-Thr420Lys	CC	176 (55.35)	152 (53.90)	Ref		
	CA	116 (36.48)	108 (38.30)	0.187	0.928 (0.660~1.304)	0.665
	AA	26 (8.18)	22 (7.80)	0.004	1.021 (0.556~1.875)	0.947
	CA + AA	142 (44.65)	130 (46.10)	0.126	0.943 (0.684~1.302)	0.723
	C	468 (73.58)	412 (73.05)	Ref		
	A	168 (26.42)	152 (26.95)	0.044	0.973 (0.753~1.257)	0.834

CI, confidence interval; CSM, cervical spondylotic myelopathy; OR, odd ratio; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

TABLE 5 Comparison of the JOA scores and the number of cervical spine segments in CSM patients with different genotypes of VDR-FokI and VDBP-Thr420Lys polymorphisms

Polymorphism	JOA score	F	P	Number of cervical spine segments	F	P
VDR-FokI		1.032	0.358		0.554	0.575
FF	9.56 ± 1.96			2.45 ± 0.52		
Ff	9.51 ± 2.04			2.43 ± 0.52		
ff	9.24 ± 1.78			2.51 ± 0.58		
VDBP-Thr420Lys		0.607	0.546		2.162	0.117
CC	9.36 ± 1.86			2.46 ± 0.54		
CA	9.59 ± 2.11			2.41 ± 0.53		
AA	9.65 ± 1.87			2.65 ± 0.49		

CSM, cervical spondylotic myelopathy; JOA, Japanese Orthopaedic Association; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

for short-length protein lacking three -NH₂-terminal amino acids.⁹ The variant of VDR protein (lacking 3 amino acids) is a more potent transcriptional activator, which could perform its functions via regulating cell proliferation and division compared to the ff genotype.²⁹ The f allele (with restriction site) initiates translation at first ATG site and encodes full-length VDR protein, while the F allele encodes for the more functional shorter VDR protein. In addition, recent studies observed a close association between VDR gene polymorphism and intervertebral disk degeneration (IDD), which highlighted the functioning of genetic factors in the occurrence and development of IDD.^{30,31} Furthermore, polymorphisms in VDR gene were previously associated with IDD process, and the combination of wild-type alleles of TaqI, Apal, and FokI polymorphisms decreased the risk of IDD.³² Moreover, the results were indicative of no evident association between VDBP-Thr420Lys polymorphism and CSM. Contrarily, a recent individual study reported the ability of VDBP to be transformed into a potent macrophage-activating factor and linked with

an actin-scavenging protein in the vascular and extracellular system.³³ Additionally, VDBP-Thr420Lys has been observed to be more frequent in the healthy population than in inflammatory bowel disease cases.³⁴ Therefore, it is essential to further explore whether VDBP-Thr420Lys polymorphism is associated with CSM.

In addition, our data indicated the association between VDR-FokI polymorphism and nucleus pulposus capillary invasion, necrosis, hyaline degeneration and fibrosis, genesis and hyperplasia of cartilage-like cells, and fibrocyst in the annulus fibrosis. Annulus fibrosis cells exhibit a fibroblast-like morphology in the outer region but resemble chondrocyte within, whereas the nucleus pulposus cells resemble chondrocyte more, with single cells in matrix-surrounded lacunae.³⁵ Gruber HE et al demonstrated that human annulus fibrosis cells display VDR expression both in vivo and in vitro and the effects of administration of vitamin D metabolites in vitro are investigated on annulus cells.³⁶ Colombini et al further demonstrated that annulus fibrosis and nucleus

TABLE 6 Associations of VDR-FokI and VDBP-Thr420Lys polymorphisms with the pathological features of CSM patients

Feature	VDR-FokI		χ^2	P	VDBP-Thr420Lys		χ^2	P
	FF	Ff + ff			CC	CA		
Decreased chondrocytes and fibroblast hyperplasia (160)	46 (46.00)	114 (52.29)	1.086	0.297	95 (53.98)	65 (45.77)	2.115	0.146
Capillary invasion in nuclear pulposus (185)	48 (48.00)	137 (62.84)	6.208	0.013	103 (58.52)	82 (57.75)	0.019	0.889
Inflammatory cell invasion in nuclear pulposus (121)	34 (34.00)	87 (39.91)	1.015	0.314	60 (34.09)	61 (42.96)	2.621	0.105
Necrosis, glass-like degeneration, and fibrosis in nuclear pulposus tissues (162)	41 (41.00)	121 (55.50)	5.771	0.016	92 (52.27)	70 (49.30)	2.749	0.598
Genesis and hyperplasia of cartilage-like cells in fibrous ring (235)	65 (65.00)	170 (77.98)	5.990	0.014	136 (77.27)	99 (69.72)	2.325	0.127
Tumor-like lesion in fibrous ring mucus (228)	67 (67.00)	161 (73.85)	1.587	0.208	129 (73.30)	99 (69.72)	0.496	0.481
Genesis of fibrocyst in fibrous ring (99)	22 (22.00)	77 (35.32)	5.674	0.017	50 (28.41)	49 (34.51)	1.363	0.243

CSM, cervical spondylotic myelopathy; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

pulposus both express VDR. The intragenic polymorphisms in the VDR gene are correlated with the features of disk degeneration, which suggests that alterations in the vitamin D-mediated signaling can affect the pathology and physiology of the disk and signify the importance of disk health during the interaction between disk cells and vitamin D metabolites.³⁷ Moreover, the results demonstrated that time to bend over desk working was a risk factor for CSM, which was confirmed by the binary logistic regression analysis, while gender, BMI, and smoking were not independent risk factors of CSM. Several research studies have shown the contribution of many environmental factors and susceptibility genes to the morbidity of CSM.³⁸ Ossification of the posterior longitudinal ligament, a hyperostotic condition of the spine related to severe neurologic dysfunction, glutamatergic toxicity, ischemic injury, and apoptotic neurons and oligodendrocytes are key risk factors for CSM.^{39,40} Due to the limitation of a relatively small sample size

and selected susceptibility genes, further studies are needed to validate the effects of various factors such as age, environment, and time to bend over desk working at the table on CSM.

5 | CONCLUSION

In conclusion, our study provided evidence highlighting the close association of VDR-FokI polymorphism with the susceptibility to CSM. Novel evidence for the role of time to bend over desk working in the development and progression of CSM was discovered and provided. However, the sample size in our study was very small and limited to discover the effects of the environmental factors and the interaction of genes on CSM. Nevertheless, our future studies will emphasize on large sample sizes to explore profoundly the mechanisms of other susceptibility genes in the occurrence and development of CSM.

TABLE 7 Logistic regression analysis of risk factors for CSM

Risk factor	B	SE	Wald	Sig.	OR	95% CI
Gender (male/female)	0.094	0.198	0.227	0.634	1.099	0.746 ~ 1.619
Age (y)	0.247	0.174	2.010	0.156	1.281	0.910 ~ 1.802
BMI (kg/m ²)	0.204	0.169	1.456	0.228	1.226	0.881 ~ 1.706
Smoking history (%)	0.156	0.199	0.616	0.433	1.169	0.792 ~ 1.726
Working time at table (hours/d)	0.246	0.085	8.382	0.004	1.279	1.083 ~ 1.511
Average working time (hours/d)	0.100	0.139	0.525	0.469	1.106	0.843 ~ 1.451
VDR-FokI	0.379	0.117	10.534	0.001	1.461	1.162 ~ 1.837
VDBP-Thr420Lys	0.071	0.152	0.220	0.639	0.931	0.692 ~ 1.254

BMI, body mass index; CI, confidence interval; CSM, cervical spondylotic myelopathy; OR, odd ratio; S.E., standard error; Sig., P value; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

ACKNOWLEDGMENTS

We would like to acknowledge the helpful comments on this article received from our reviewers.

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REFERENCES

- Karadimas SK, Gatzounis G, Fehlings MG. Pathobiology of cervical spondylotic myelopathy. *Eur Spine J*. 2015;24:132-138.
- Karadimas SK, Erwin WM, Ely CG, Dettori JR, Fehlings MG. Pathophysiology and natural history of cervical spondylotic myelopathy. *Spine*. 2013;38:21-36.
- Boogaarts HD, Bartels RHMA. Prevalence of cervical spondylotic myelopathy. *Eur Spine J*. 2015;24:139-141.
- Baptiste DC, Fehlings MG. Pathophysiology of cervical myelopathy. *Spine J*. 2006;6:S190-S197.
- Harrop JS, Naroji S, Maltenfort M, et al. Cervical myelopathy: a clinical and radiographic evaluation and correlation to cervical spondylotic myelopathy. *Spine*. 2010;35:620.
- Ling Y, Lin H, Aleteng Q, et al. Cdx-2 polymorphism in Vitamin D Receptor gene was associated with serum 25-hydroxyvitamin D levels, bone mineral density and fracture in middle-aged and elderly Chinese women. *Mol Cell Endocrinol*. 2016;427:155-161.
- Mirković K, Doorenbos CR, Dam WA, et al. Urinary vitamin D binding protein: a potential novel marker of renal interstitial inflammation and fibrosis. *PLoS One*. 2013;8:e55887.
- Zhao B, Zhang W, Du S, Zhou Z. Vitamin D receptor BsmI polymorphism and osteoporosis risk in post-menopausal women. *Arch Med Sci*. 2016;12:25-30.
- Marchwicka A, Cebrat M, Łaskiewicz A, et al. Regulation of vitamin D receptor expression by retinoic acid receptor alpha in acute myeloid leukemia cells. *J Steroid Biochem Mol Biol*. 2016;159:121-130.
- Anic GM, Thompson RC, Nabors LB, et al. An exploratory analysis of common genetic variants in the vitamin D pathway including genome-wide associated variants in relation to glioma risk and outcome. *Cancer Causes Control*. 2012;23:1443-1449.
- Cakir OO, Yilmaz A, Demir E, et al. Association of the BsmI, Apal, TaqI, Tru9I and FokI polymorphisms of the vitamin D receptor gene with Nephrolithiasis in the Turkish population. *Urol J*. 2016;13:2509.
- Wang D, Liu R, Zhu H, et al. Vitamin D receptor Fok I polymorphism is associated with low bone mineral density in postmenopausal women: a meta-analysis focused on populations in Asian countries. *Eur J Obstet Gynecol Reprod Biol*. 2013;169:380-386.
- Zhang C, Wang C, Liang J, et al. The vitamin D receptor Fok1 polymorphism and bone mineral density in Chinese children. *Clin Chim Acta*. 2008;395:111-114.
- Selvaraj P, Kurian SM, Chandra G, et al. Vitamin D receptor gene variants of BsmI, Apal, TaqI, and FokI polymorphisms in spinal tuberculosis. *Clin Genet*. 2004;65:73-76.
- Yan D, Wang Z, Deng S, Li J, Soo C. Anterior corpectomy and reconstruction with titanium mesh cage and dynamic cervical plate for cervical spondylotic myelopathy in elderly osteoporosis patients. *Arch Orthop Trauma Surg*. 2011;131:1369-1374.
- Wang ZC, Chen XS, da Wang W, et al. The genetic association of vitamin D receptor polymorphisms and cervical spondylotic myelopathy in Chinese subjects. *Clin Chim Acta*. 2010;411:794-797.
- Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin Chim Acta*. 2006;372:33-42.
- Colombini A, Cauci S, Lombardi G, et al. Relationship between vitamin D receptor gene (VDR) polymorphisms, vitamin D status, osteoarthritis and intervertebral disc degeneration. *J Steroid Biochem Mol Biol*. 2013;138:24-40.
- Ma R, Gu Y, Zhao S, et al. Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies. *Am J Physiol Endocrinol Metab*. 2012;303:E928-E935.
- Wang D, Liu W, Cao Y, et al. BMP-4 polymorphisms in the susceptibility of cervical spondylotic myelopathy and its outcome after anterior cervical corpectomy and fusion. *Cell Physiol Biochem*. 2013;32:210-217.
- Wang ZC, Hou XW, Shao J, et al. HIF-1 α polymorphism in the susceptibility of cervical spondylotic myelopathy and its outcome after anterior cervical corpectomy and fusion treatment. *PLoS One*. 2014;9:e110862.
- Wu J, Wu D, Guo K, Yuan F, Ran B. OPN polymorphism is associated with the susceptibility to cervical spondylotic myelopathy and its outcome after anterior cervical corpectomy and fusion. *Cell Physiol Biochem*. 2014;34:565-574.
- Rhodes CT, Hone RE. Automated assay of drugs in body fluids. *Drug Metab Rev*. 1973;2:221-238.
- Amanda LS, Giachelli CM, Marta S. The role of osteopontin in inflammatory processes. *J Cell Commun Signal*. 2009;3:311-322.
- Abodeiyamah KO, Stoner KE, Grossbach AJ, et al. Effects of brain derived neurotrophic factor Val66Met polymorphism in patients with cervical spondylotic myelopathy. *J Clin Neurosci*. 2016;24:117-121.
- Mizwicki MT, Menegaz D, Yaghmaei S, Henry HL, Norman AW. A molecular description of ligand binding to the two overlapping binding pockets of the nuclear vitamin D receptor (VDR): structure-function implications. *J Steroid Biochem Mol Biol*. 2010;121:98-105.
- Wang G, Cao Y, Wu T, et al. Genetic factors of cervical spondylotic myelopathy-a systemic review. *J Clin Neurosci*. 2017;44:89-94.
- Frederiksen B, Liu E, Romanos J, et al. Investigation of the vitamin D receptor gene (VDR) and its interaction with protein tyrosine phosphatase, non-receptor type 2 gene (PTPN2) on risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). *J Steroid Biochem Mol Biol*. 2013;133:51-57.
- Tang C, Chen N, Wu M, Yuan H, Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. *Breast Cancer Res Treat*. 2009;117:391.
- Zhu GQ, Shi KQ, Huang S, et al. Systematic review with network meta-analysis: the comparative effectiveness and safety of interventions in patients with overt hepatic encephalopathy. *Aliment Pharmacol Ther*. 2015;41:624-635.
- Toktaş ZO, Ekşi MŞ, Yılmaz B, et al. Association of collagen I, IX and vitamin D receptor gene polymorphisms with radiological severity of intervertebral disc degeneration in Southern European Ancestor. *Eur Spine J*. 2015;24:2432-2441.
- Vieira LA, Dos Santos AA, Peluso C, et al. Influence of lifestyle characteristics and VDR polymorphisms as risk factors for intervertebral disc degeneration: a case-control study. *Eur J Med Res*. 2018;23:11.
- Chupeerach C, Tungtrongchitr A, Phonrat B, et al. Association of Thr420Lys polymorphism in DBP gene with fat-soluble vitamins and low radial bone mineral density in postmenopausal Thai women. *Biomark Med*. 2012;6:103-108.
- Eloranta JJ, Wenger C, Mwinyi J, et al. Association of a common vitamin D-binding protein polymorphism with inflammatory bowel disease. *Pharmacogenet Genomics*. 2011;21:559-564.
- Lazebnik M, Singh M, Glatt P, et al. Biomimetic method for combining the nucleus pulposus and annulus fibrosus for intervertebral disc tissue engineering. *J Tissue Eng Regen Med*. 2011;5:e179.

36. Gruber HE, Hoelscher G, Ingram JA, et al. 1,25(OH)₂-vitamin D₃ inhibits proliferation and decreases production of monocyte chemoattractant protein-1, thrombopoietin, VEGF, and angiogenin by human annulus cells in vitro. *Spine*. 2008;33:755.
37. Colombini A, Lanteri P, Lombardi G, et al. Metabolic effects of vitamin D active metabolites in monolayer and micromass cultures of nucleus pulposus and annulus fibrosus cells isolated from human intervertebral disc. *Int J Biochem Cell Biol*. 2012;44:1019-1030.
38. Yamazaki T, Yanaka K, Sato H, et al. Cervical spondylotic myelopathy: surgical results and factors affecting outcome with special reference to age differences. *Neurosurgery*. 2003;53:122-126.
39. Ahn JS, Lee JK, Kim BK. Prognostic factors that affect the surgical outcome of the laminoplasty in cervical spondylotic myelopathy. *Clin Orthop Surg*. 2010;2:98-104.
40. Matsunaga S, Komiya S, Toyama Y. Risk factors for development of myelopathy in patients with cervical spondylotic cord compression. *Eur Spine J*. 2015;24(Suppl 2):142-149.

How to cite this article: Song D-W, Wu Y-D, Tian D-D.

Association of VDR-FokI and VDBP-Thr420Lys polymorphisms with cervical spondylotic myelopathy: A case-control study in the population of China. *J Clin Lab Anal*. 2019;33:e22669.

<https://doi.org/10.1002/jcla.22669>