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The genetic association of Vitamin D receptor polymorphisms and cervical spondylotic myelopathy in Chinese subjects

Zhan Chao Wang [a], Xiong Sheng Chen [a], Da Wei Wang [b], Jian Gang Shi [a], Lian Shun Jia [a,*], Guang Hui Xu [a], Jian Hou Huang [a], Lei Fan [a]

[a] Department of Orthopedics, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Road, Shanghai, 200003, China
[b] Department of Orthopedics, Liaocheng People's Hospital, No 67, Dongchang West Road, Liaocheng City, Shandong Province, 252000, China

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ABSTRACT

Background: The association of vitamin D receptor (VDR) gene polymorphisms to the lumbar degenerative disc disease has been previously studied; however, the role of VDR gene polymorphisms in cervical spondylosis remains unknown.
Methods: One hundred fifty four patients with cervical spondylotic myelopathy (CSM) and 156 controls were enrolled. The clinical characteristics were collected and the severity of cervical spondylotic myelopathy was evaluated by magnetic resonance imaging (MRI). The VDR polymorphism genotyping was performed.
Results: No significant difference in clinical characteristics was noted between the case and controls. For *Apa*I polymorphism, the cases had a marked higher prevalence of AA genotype (19.5% vs. 8.3%, $P=0.003$) and A allele frequencies (34.4% vs. 22.4%, $P<0.001$) than controls. For *Taq*I polymorphism, the cases had a significant higher prevalence of TT genotype (67.5vs. 44.2%, $P<0.001$) and T allele frequencies (76.9% vs. 54.2%, $P<0.001$) than controls. The odds ratio for CSM was 2.88 for the *Apa*I A allele carriers and 4.67 for the *Taq*I T allele carriers. The *Taq*I genotypes, both TT and TC showed a markedly higher MRI severity grading level than CC genotype (both $P<0.01$, compared with CC genotype).
Conclusion: Certain VDR polymorphism is related in the presence and severity of CSM in Chinese subjects.

1. Introduction

Cervical spondylosis is a highly prevalent musculoskeletal disorder in clinical practice. It encompasses a sequence of degenerative changes in the intervertebral disc space and the surrounding bony anatomy and soft tissues. Cervical spondylosis may affect the entire cervical spine and lead to the development of clinical symptoms if the neural structures, e.g. nerve root or spinal cord were impinged.

A series of environmental factors, such as age, gender, smoking, height, weight were considered to be the risk factors for musculoskeletal degenerative disorders [1–3]. Additionally, the genetic factors were noted as well for their important role in the degeneration process of musculoskeletal disorders, including intervertebral degenerative disc disease [4–7].

Vitamin D is an important factor in bone metabolism and bone development. Through activation of the vitamin D receptor [VDR), vitamin D facilitates the intestinal absorption of calcium, stimulates renal production of 1,25-[OH)2-vitamin D3 and also influences osteoblasts, osteoclasts, and PTH secretion [8].

VDR is encoded by a gene located in chromosome 12q-12. Considering its importance in bone metabolism and development, the VDR gene is one of the most frequently studied genes in the context of the musculoskeletal disorders such as bone density [9–11], osteoarthritis [12,13] and intervertebral degenerative disc disease [14–16].

Several VDR polymorphisms, including *Fok*I, *Bsm*I, *Apa*I, and *Taq*I have been determined. The genetic association of VDR gene polymorphisms to the lumbar degenerative disc disease has been previously studied, which revealed that the certain VDR polymorphisms are associated with the presence or the severity of lumbar degenerative disc disease [14–16]. However, there is to date no study regarding the role of VDR gene polymorphisms in cervical spondylosis was reported.

In the present study, we enrolled Chinese cervical spondylotic myelopathy (CSM) subjects and the age, sex matched controls for a case–control study. Our aim was to explore the genetic association between VDR gene polymorphisms and the occurrence and the severity of CSM.

2. Methods

2.1. Subject enrollment

154 consecutive patients being diagnosed as CSM were enrolled in the study. The diagnoses were established on the basis of findings

* Corresponding author. Tel.: +86 021 81886999; fax: +86 021 63520020.
E-mail address: JiaLianshun@yahoo.com.cn (L.S. Jia).

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from the history, physical examination and confirmed by magnetic resonance imaging (MRI) as previously described [17]. Exclusion criteria included congenital cervical anomalies, trauma, prior cervical surgery, rheumatoid arthritis, infections,tumors, ankylosing spondylitis, ossification of the posterior longitudinal ligament, diffuse idiopathic skeletal hyperostosis, and any other inflammatory disease involving the cervical spine. The control samples consisted of 156 sex and age matched Chinese individuals with negative MRI findings. The clinical characteristics including sex, age, weight, height, body mass index (BMI), profession (long time desk worker or not), smoking status and family history of intervertebral degenerative disc disease were collected. The study protocol was approved by the Ethics Committees Ethical Committee in hospital. Written informed consent was obtained from all patients before participation in the study.

2.2. Evaluation of severity of cervical spondylotic myelopathy

The severity levels were determined according to the MRI finding on T2-weighted images as described previously [14]. The numbers of segmental lesions were calculated as well from MRI image. Based on the clinical symptoms and signs, the severity of neurological deficits of all patients was scored according to the modified Japanese Orthopedic Association (modified JOA) score for CSM [18].

2.3. VDR polymorphisms genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a Genomic DNA Extraction Kit (Qiagen, Hilden, Germany). Primer sequences used for amplification of *Fok*I, *Bsm*I, *Apa*I and *Taq*I polymorphisms were as previously described and listed in Table 1 [19]. The polymerase chain reaction (PCR) reactions were performed at 93 °C for 10 min followed by 35 cycles at 93 °C for 45 s, 64 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were digested overnight with the restriction enzymes *Fok*I at 55 °C, *Bsm*I at 37 °C, *Apa*I at 37 °C, or *Taq*I at 65 °C. The VDR genotypes of each subject were identified according to the digestion pattern and alleles according to the presence of the *Fok*I, *Bsm*I, *Apa*I, and *Taq*I sites, respectively [19].

2.4. Statistical analysis

Single nucleotide polymorphisms (SNP) were assessed for both genotypic and allelic association analysis among cases and controls. The genotype data of all tested SNPs were used to estimate Hardy–Weinberg equilibrium by comparison of genotype frequencies within the 2 groups by a χ^2-test. *P*-values were calculated with SPSS statistical software (SPSS Statistics 17.0; 2008 SPSS Inc, Chicago, IL).

3. Results

Table 2 showed the clinical characteristics of cases and controls. There was no significant difference in age, BMI, sex, smoking and family history status. The percentage of desk workers and the mean deskwork time between the case and controls were similar as well. Table 3 showed the genotype and the allele frequencies of VDR gene polymorphisms in cases and control subjects. All the genotype frequencies were in Hardy–Weinberg equilibrium. Significant differences were noted in *Apa*I and *Taq*I genotype distribution between the cases and controls. For *Apa*I polymorphism, we observed the cases had a marked higher prevalence of TT genotype than controls (19.5% vs. 8.3%, $P=0.003$).The A allele frequencies in cases and controls were 34.4% vs. 22.4%, respectively, $P<0.001$. For *Taq*I polymorphism, the cases had a significant higher prevalence of TT genotype than controls (67.5 vs. 44.2%, $P<0.001$) the allele frequencies were significantly different, indicated by a 76.9% prevalence of T allele in case and 54.2% in controls, $P<0.001$. The odds ratio for developing CSM was 2.88 for the *Apa*I A allele carriers and 4.67 for the *Taq*I T allele carriers. For the *Fok*I and *Bsm*I polymorphism, no difference was observed in genotype distribution and the allele frequencies between cases and controls.

Fig. 1 shows the comparison of the MRI severity of CSM according to the genotype profiles. In order to facilitate the quantification, the A, B, C, and D levels were transformed into 1, 2, 3, and 4. There was no significant difference of the severity grading among the *Fok*I and *Bsm*I as well as *Apa*I genotypes. However, in *Taq*I genotypes, both TT and TC showed a markedly higher grading level than CC genotype (mean grading levels 3.2 and 3.6 respectively, both $P<0.01$ compared with CC genotype). Figs. 2 and 3 showed the mean modified JOA score and the mean numbers of segmental lesions of CSM subjects according to the genotype profiles, respectively. There were no significant differences in the mean modified JOA scores and the mean numbers of segmental lesions among the *Fok*I, *Bsm*I, *Apa*I as well as *Taq*I genotypes.

4. Discussion

In the present study, the VDR gene *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms were selected to evaluate the genetic association between the VDR polymorphisms and cervical spondylosis subjects. Our data showed a significant difference in the *Apa*I and *Taq*I genotype distributions as well as the allele frequencies between the cases and controls. The risk ratio of developing CSM was 2.88 fold higher for the *Apa*I A allele carriers than C allele carriers, and was 4.67 fold higher for the T allele carriers than C allele carriers in *Taq*I. Although the modified JOA score and the mean numbers of segmental lesions were similar among different genotypes, the genotypes of *Taq*I polymorphisms genetically determined the MRI severity of CSM. To our knowledge, this is the first study to examine the association between cervical spondylosis and VDR polymorphisms.

The VDR gene is one of the most frequently studied genes in the context of degenerative intervertebral disc disease. The polymorphisms recognized by *Bsm*I and *Apa*I are located in intron 8 of the VDR gene and the *Taq*I is located in exon 9 that leads to a silent codon change, with ATT and ATC, both coding for isoleucine and has been associated with increased VDR mRNA stability [20]. The *Fok*I polymorphism locates at the translation initiation site in exon 2 of the VDR gene [20].

The association between the VDR polymorphysms and the intervertebral degenerative disc disease has been investigated in

Table 1
Different loci selected within the VDR sequence and their corresponding primers.

	PCR primer	PCR product (bp)	Restriction enzyme	RFLP products (bp)
C/T	F: 5′-CCTGGCACTGACTCTGGCTCTG-3′ R: 5′-GGCTCCCTTCATGGAAACACCT-3′	270	*Fok*I	207/63
G/A	F: 5′-GGTGGGACTGAAGAAGCTGAAC-3′ R: 5′-CTTTGGACCTCATCACCGACAT-3′	613	*Bsm*I	357/256
A/C	F: 5′-GTAGAATAGAAGGAGGGAAGC-3′ R:5′-AGAGGCAGCGGTACTGCTTGGAGTG-3′	662	*Apa*I	424/238
TC	F: 5′-GTAGAATAGAAGGAGGGAAGC-3′ R: 5′-AGCTTCATGCTGCACTCAGGCT-3′	690	*Taq*I	504/186

Table 2
Clinical characteristics of cases and controls.

Characteristics	Cases	Controls	*P*
Age (years)	45.4 ± 3.5	46.1 ± 2.8	NS
Gender (female/male)	55/89	61/92	NS
BMI	25.5 ± 1.4	25.4 ± 1.7	NS
Smoking (%)	67.6	66.4	NS
Desk workers (%)	67.5	66.9	NS
Mean desk work time (h/day)	4.6	4.2	NS