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Original Article

No association between VDR gene polymorphisms and lumbar disc herniation in a Chinese population

Lijun Li[1], Dongkui Ni[1], Fuliang Zhu[1], Zhuyan Jiang[1], Yubo Shi[1], Yunguo Wang[1], Hamid Abdugheni[1], Donghao Xu[2], Wei Tian[3], Qiang Zong[4]

[1]The Second Hospital of Tianjin Medical University, Tianjin 300211, China; [2]Tianjin First Central Hospital, Tianjin 300192, China; [3]Tianjin Hospital, Tianjin 300211, China; [4]Yantai Affiliated Hospital of Binzhou Medical University, Yantai 264100, China

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Abstract: Objective: To explore the association between vitamin D receptor (VDR) gene [Fok I (rs2228570), Apa I (rs7975232), Taq I (rs731236)] polymorphisms and lumbar disc herniation (LDH). Method: 120 patients with LDH and 120 healthy controls were recruited from different hospitals in Tianjin. Magnetic resonance imaging scans of the lumbar spine were performed for all subjects. Genomic DNA was extracted from peripheral blood samples. The polymorphisms of Fok I, Apa I and Taq I in the VDR gene were detected using SNapShot. The genotype and the gene frequency distribution were detected in both LDH patients and normal controls. Results: Compared with controls, no significant differences were found in the genotype and gene frequency of Fok I, Apa I, Taq I in patients with LDH ($p>0.05$), but BMI was significantly higher in LDH patients ($p=0.014$). After adjusting age, gender and BMI, no significant difference was found in allele Fok I, Apa I and Taq I between LDH patients and controls. After adjusting age and gender, however, it was found that BMI>25 Kg/m^2 was associated with LDH (OR=0.292, 95% CI=0.171-0.500, $p<0.05$). Conclusions: Polymorphisms of Fok I, Apa I and Taq I are not risk factors for LDH in our study. BMI>25 Kg/m^2 may be a risk factor for LDH.

Keywords: Vitamin D receptor, gene polymorphism, lumbar disc herniation

Introduction

Lumbar degenerative disc disease (LDDD), which is a main reason for musculoskeletal problems, is characterized by a series of lumbar degenerative changes, including LDH, lumbar disc bulging, intervertebralun steadiness, and discogenic pain [1, 2]. Studies show that low-back pain (LBP) primarily caused by lumbar disc disease affects more than 50% of population [3], and 20% of the patients with LDDD require operative treatment due to persistent or aggravated leg pain [4, 5]. In America, lumbar disc disease and back pain are the main reasons for activity limitation in people younger than 45 years old [6]. LBP can cause psychological distress, reduce physical activity, decrease quality of life and consume enormous medical resources [6, 7].

However, the pathogenesis of LDH, one major type of LDDD, is still unclear. It is generally believed that both age and environment contribute to its onset and progress. Physical activity, injury and smoking might be risk factors for LDH. Nevertheless, studies reported that those causes cannot explain the mechanism of the disease. In the past decades, researchers dedicated to genome research and found large number of relevant genes, like Collagen I [8], Collagen IX [9], Aggrecan [10], COL9A2 [11], Interleukins [12]. VDR gene, closely related to metabolism of bone and cartilage, has attracted widespread attention.

Since 1998, the VDR gene has been studied as a predictive factor for spine pathologies. The receptor is one of the endocrine members in the nuclear receptor super-family for steroid hormones [13]. It is closely correlated to some physical functions, like maintaining metabolism balance of calcium and phosphorus, regulating cell proliferation and differentiation, etc. [13]. VDR also plays a critical role in bone min-

Table 1. Primers sequences for genotyping of multiple single nucleotide polymorphisms using SNapShot

Number	Locus	Forward primer	Reverse primer	Fragment length and GC content
S01	rs2228570	ACTGACTCTGGCTCTGACCGT	CAGCCTTCACAGGTCATAGCAT	176 bp, GC%: 59.32%
S02	rs7975232	AGCGGATGTACGTCTGCAGTG	ACGGAGAAGTCACTGGAGGG	261 bp, GC%: 62.60%
S03	rs731236	AGCGGATGTACGTCTGCAGTG	ACGGAGAAGTCACTGGAGGG	261 bp, GC%: 62.60%

eralization and remodeling [14, 15]. The VDR gene, located on chromosome 12, is composed of 11 exons, involving in the formation of VDR which mediates $1,25(OH)_2D3$ to produce biological effects. Studies have shown that VDR gene polymorphisms are associated with osteoarthritis, osteoporosis and degenerative disc disease [16]. Currently, several allele polymorphisms have been studied to assess the association between VDR and degenerative disc disease in different populations. Fok I (rs2228570), Apa I (rs7975232), Taq I (rs731236) restriction sites are best-known polymorphisms of the VDR gene. These three loci were believed to affect the expression and stability of mRNA and change the affinity of enhancers to target sites, and eventually change the metabolism of bone and cartilage [17].

However, based on previous studies, the influences of these single nucleotide polymorphisms on symptomatic lumbar disc disease are inconsistent and ambiguous. The association of VDR gene polymorphisms and LDH in a population in North China has not been studied. We, therefore, collected data of 120 LDH patients and 120 controls from four hospitals in Tianjin to explore the correlation between VDR gene polymorphisms and LDH.

Materials and methods

Subjects

A total of 120 LDH patients and 120 controls were recruited from the Second Hospital of Tianjin Medical University, Tianjin People's Hospital, The General Hospital of Tianjin Medical University and Tianjin Hospital of Tianjin City from May 2015 to December 2015. All participants underwent a magnetic resonance imaging (MRI) scan and were diagnosed by two experienced radiologists and two orthopedists. The present study was approved by the medical ethics committee and informed consent was given before collecting blood samples and individual information.

Inclusion and exclusion criteria

The following inclusion criteria were applied: 1) Han population in Tianjin, age >18 years old; 2) patients were diagnosed with LDH based on clinical presentation and spine MRI result; 3) LDH or other spine diseases were ruled out in controls; 4) all participants can cooperate with us. Exclusion criteria were as follows: 1) patients with previous spinal surgery; 2) patients with congenital spinal deformities, previous spine injury or spine tumor.

Main reagents and equipment

DNA extraction kit (Shanghai Generay Biotech CO., Ltd), 3730XL gene sequencing machine, SNapShot (Applied Biosystems (ABI), America), Taq polymerase enzyme, Dntp, ExoI enzyme and FastAP enzyme (MBI Fermentas, Canada) were used.

DNA extraction and genotyping

The genomic DNA was isolated from peripheral white blood cells by standard methods listed in the genomic DNA extraction kit.

According to the published papers [18-20] and the National Center of Biotechnology Information (NCBI) [Fok I: rs2228570 (GGCCTGCTTGCTGTTCTTACAGGGA [C/T] GGAGGCAATGGCGGCCAGCACTTCC); ApaI: rs7975232 (AAGGCACAGGAGCTCTCAGCTGGGC [A/C] CCTCACTGCTCAATCCCACCACCCC); Taq I: rs731236 (CTGGGGTGCAGGACGCCGCGCTGAT [C/T] GAGGCCATCCAGGACCGCCTGTCCA)). Fok I, Apa I, and Taq I polymorphisms were detected using SNapShot. The sequences of specific primers for SNapShot are listed as follows: rs2228570 forward: 5-ACTGACTCTGGCTCTGACCGT-3, reverse: 5-CAGCCTTCACAGGTCATAGCAT-3; rs7975232 forward: 5-AGCGGATGTACGTCTGCAGTG-3, reverse: 5-ACGGAGAAGTCACTGGAGGG-3; rs731236 forward: 5-AGCGGATGTACGTCTGCAGTG-3, reverse: 5-ACGGAGAAGTCACTGGAGGG-3 (**Table 1**).

Table 2. Characteristics of participants ($\bar{X} \pm S$)

	Cases (n=120)	Controls (n=120)	P
Age (year, $\bar{X} \pm S$)	54.29±15.46	55.21±15.83	0.650
Gender (male/female)	72/48	77/43	0.506
Height (cm, $\bar{X} \pm S$)	167.17±7.45	168.58±8.49	0.171
Weight (kg, $\bar{X} \pm S$)	70.94±14.17	68.82±11.96	0.211
BMI (kg/m^2)	25.20±3.59	24.11±3.17	0.014
Smoking (%)	41.67	37.50	0.509
Alcohol (%)	20.83	28.33	0.177
Hypertension (%)	31.67	37.50	0.342
Diabetes (%)	16.67	21.67	0.325

After amplification, polymerase chain reaction (PCR) was performed, containing 1 μL DNA, 1.5 ml 10* buffer, $MgCL_2$ 1.5 μL, DNTP 0.3 μL, 0.3 μL Primers, Taq polymerase 0.3 μL and 9.5 μL H_2O. Thermal cycling used the following conditions: 95°C for 3 min, 11 cycles at 94°C for 15 s, 60°C for 15 s, 72°C for 30 s, every cycle decreased by 0.5°C, then 24 cycles at 94°C for 15 s, 54°C for 15 s, 72°C for 30 s, followed by a final extension at 72°C for 3 min. Then, after PCR amplification, PCR purification was performed in the following conditions: PCR products 3 μL, ExoI 0.2 μL, FastAP 0.8 μL, ExoI buffer 0.7 μL, H_2O 2.37 μL. The purification was operated in 37°C for 15 min, followed by 80°C for 15 min. After purification, extension reaction (ER) was carried out in a volume of 6 μL which contained PCR products 2 μL, SNapShot Mix 1 μL, extended primer 0.3 μL and H_2O 2.7 μL. The ER was performed under 96°C followed by 30 cycles of 96°C for 10 s, 52°C for 5 s and 60°C for 30 s. Then the extended products, after sample loading and degeneration, were analyzed by ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). GeneMapper software (Applied Biosystems) was used to analyze the results. Finally, the assays of genotyping were independently conducted by two authors.

Statistical analysis

SPSS 20.0 (IBM, Armonk, NY, USA) was used. Differences between cases and controls in characteristics, risk factors and frequencies of allele and genotype of each SNP were evaluated by two-sided test (for categorical variables) or Student's t-test (for continuous variables). If a parameter showed significant difference between two groups ($p<0.05$), logistic regression was conducted to evaluate its association with LDH after adjusting for age and sex. Chi-square was used to test the frequencies of allele and genotype of the two groups and the Hardy-Weinberg equilibrium. After adjusting age, sex and BMI, multivariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) to estimate the association between VDR gene polymorphisms and lumbar disc herniation. $p<0.05$ was considered statistically significant.

Results

Characteristics of subjects

Totally, 120 patients and 120 controls from the Tianjin area were recruited in our study. There were 72 men and 48 women (mean age, 54.29±15.46 y) in the patient group and 77 men and 43 women (mean age, 55.21±15.83 y) in control group. Characteristics are shown in **Table 2**. There were no significant differences between the two groups in age, gender, height, weight, and the ratio of smoking, drinking, hypertension and diabetes. LDH patients had a higher BMI than controls (25.20±3.59 *vs* 24.11±3.17 kg/m^2, $p<0.05$).

The genotype and allele distributions of VDR gene polymorphisms

The genotype and allele frequencies of the VDR gene in patients and controls are shown in **Table 3**. All blood samples were successfully genotyped. The frequency distribution of Fok I, Apa I and Taq I polymorphisms in two groups was in accordance with the Hardy-Weinberg Equilibrium (Fok I: LDH: 0.331, Control: 0.250; Apa I: LDH: 0.413, Control: 0.273; Taq I: LDH: 0.779, Control: 0.599).

The genotype frequencies in LDH patients were 36.7% (FF), 44.2% (Ff), 19.2% (ff), 10.8% (AA), 39.3% (Aa), 50.0% (aa), 95.0% (TT), 5.0% (Tt), and in controls, the frequencies were 25.8% (FF), 55% (Ff), 19.2% (ff), 13.3% (AA), 40.0% (Aa), 46.7% (aa), 90.8% (TT), 9.2% (Tt). No significant differences were found between patients and controls ($p>0.05$) in genotype

Table 3. Genotype and allele distributions of Fok I, Apa I, Taq I polymorphisms

Genotype	Cases	Controls	X^2	P*	OR (95% CI)*	HWE
rs2228570						0.250
FF	44	31	3.28	-	1.00	
Ff	53	66	2.82	0.060	1.814 (0.975-3.372)	
ff	23	23	0.00	0.409	1.391 (0.635-3.045)	
F allele	141	128	1.43	-	1.00	
f allele	99	112	-	0.269	1.238 (0.848-1.808)	
rs7975232						0.273
AA	13	16	0.35	-	1.00	
Aa	47	48	0.02	0.395	0.678 (0.277-1.660)	
aa	60	56	0.27	0.527	0.750 (0.307-1.830)	
A allele	73	80	0.47	-	1.00	
a allele	167	160	-	0.417	0.845 (0.562-1.270)	
rs731236						0.599
TT	114	109	1.58	-	1.00	
Tt	6	11	-	0.399	1.591 (0.541-4.681)	
tt	0	0	-	-	-	
T allele	234	229	1.52	-	1.00	
t allele	6	11	-	0.410	1.559 (0.542-4.484)	

*Age, gender and BMI were adjusted with logistic regression.

and alleles. In addition, we did not find the tt genotype when detecting Taq I polymorphism.

Fok I, Apa I, Taq I polymorphisms, BMI and LDH Susceptibility in two groups

The allele and genotype frequencies of Fok I, Apa I and Taq I for the cases and controls are presented in **Table 3**. After adjusting age, gender and BMI, both Ff (adjusted OR=1.814; 95% CI=0.975-3.372.; p=0.060) and ff genotype (adjusted OR=1.391; 95% CI=0.635-3.045; p=0.409) were not associated with increased LDH risk. Additionally, there was no significant association between the Aa/aa genotypes (Aa: adjusted OR=0.678; 95% CI=0.277-1.660; p=0.395; aa: adjusted OR=0.750; 95% CI= 0.307-1.830; p=0.527) and Tt genotype (adjusted OR=1.591; 95% CI=0.541-4.681; p= 0.399), respectively. Allele f, a and t were not associated with LDH using logistic regression. After adjusting age and gender, we found that BMI>25 kg/m^2 was associated with LDH (OR =0.292, 95% CI=0.171-0.500, p<0.05).

Discussion

To our knowledge, this is the first case-control genetic study to explore the association between the Fok I (rs2228570), Apa I (rs7975232), Taq I (rs731236) polymorphisms and LDH in a population in North China.

LDH is one of the main causes for physical disorder, activity limitation and decreasing quality of life and work efficiency [21]. Environment, aging and social stress are possible risk factors for LDH [22, 23]. Genetic polymorphisms are suspected to be related with LDH. VDR gene is the first gene reported to be associated with LDH in 1998 [15, 24]. In a study of monozygotic twins in Finns, Videman first proposed that FokI and TaqI polymorphisms may be risk factors for LDH. Subsequently, studies were performed to explore the correlation between Fok I, Taq I and other loci like Apa I with LDH. The results of these studies, however, were inconsistent and controversial, even in the same race. Previous studies in Japanese and southern Chinese population showed that VDR gene polymorphism is associated with LDDD. Few studies have been performed about the correlation between VDR gene polymorphism and LDH in a population in North China. Therefore, our study is to identify the association of VDR polymorphisms with LDH in a population in North China.

In our study, the results showed that there were no significant differences in basic parameters except BMI in the two groups. No significant difference was found in frequency distribution of Fok I, Apa I and Taq I polymorphisms in two groups, and all statistical data were in accordance with the Hardy-Weinberg equilibrium.

After genotyping, it was found that f and F alleles in our research were 41.25% and 58.75%, respectively, similar to the results in Caucasians reported by Noponen-Hietala [25]. Furthermore, no association was found between Fok I polymorphisms and LDH, similar to Chen's study and some studies on Cau-

casians [14, 18, 26]. This may suggest that there is still some genetic diversity in different races.

In Apa I polymorphism, no alleles were found to be risk factors for LDH in our study, different from Yuan's study in a southern Chinese Han population [27]. This finding may due to the fact that the Apa I polymorphism in case group in Yuan's study was not consistent with the Hardy-Weinberg equilibrium. Sample size and regional difference may also be a possible cause. In our study, results show that Apa I polymorphism is not correlated with LDH, similar to the finding in Chen's research.

In human, tt genotype of Taq I was the first genetic risk factor identified to be associated with LDDD [15]. However, tt genotype was not detected in our study, similar to Yuan's [27] and Xu's [28] findings, in which they did not detect tt genotype in Han population from South China. In Cheung's study [16], 804 participants were recruited in South China, and only one individual was found to have the tt genotype. This may indicate that the tt genotype on Taq I locus rarely exists in Chinese population. In addition, studies show that the frequency of tt genotype in Han population and Caucasians is 0.6% versus 10%-17%, which might show a different distribution in various races [17]. Further studies will be needed to verify these conclusions. In 2002, Kawaguchi reported that compared with TT genotype, Tt genotype of the VDR was more frequently associated with multilevel and severe disc degeneration and disc herniation [29]. In our study, we did not find an association between Tt genotype and LDH (adjusted OR=1.591; 95% CI=0.541-4.681; p=0.399). It may be due to different races recruited in two studies.

Logistic regression was used to adjust age, gender and BMI, and no association was found between Fok I, Apa I, and Taq I polymorphisms and LDH ($p>0.05$). After adjusting age and gender, BMI>25 Kg/m^2 was found to be a risk factor for LDH (OR 0.292, 95% CI 0.171-0.500, $p<0.05$), possibly caused by more pressure on lumbar spine.

There are some limitations in our study. First, because of finite time and participants, there might be a bias. For instance, we did not find tt genotype in Taq I, which may due to the limited sample size. Furthermore, in spite of no remarkable association was found between VDR polymorphisms and LDH in our study, it is undeniable that these polymorphisms may play a role in LDH in different populations.

In summary, no association was found between Fok I, Apa I and Taq I polymorphisms and LDH in our study. BMI>25 Kg/m^2 is correlated with LDH. Furthermore, studies with a larger sample and multi-regions can better explore the association of VDR gene polymorphism and degenerative disease in lumbar spine.

Disclosure of conflict of interest

None.

Address correspondence to: Qiang Zong, Yantai Affiliated Hospital of Binzhou Medical University, Yantai 264100, China. Tel: +8615266570697; E-mail: zq890509@sina.com

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