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Original Investigation

Association of rs2228570 Polymorphism of Vitamin D Receptor Gene with Lumbar Degenerative Disc Disease

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ABSTRACT

AIM: To investigate the association between the vitamin D receptor (VDR) gene rs2228570 FokI polymorphism and the development of lumbar degenerative disc disease (LDDD) in the Turkish population.

MATERIAL and METHODS: This was a prospective case-control study that included 45 patients with LDDD and 49 healthy individuals (control group). The clinical investigations of the LDDD patients consisted of neurological examinations, lumbar magnetic resonance imaging studies, visual analog scale (VAS) scores, and Oswestry Disability Index scores. The VDR gene rs2228570 FokI polymorphism was analyzed via a real-time polymerase chain reaction.

RESULTS: Individuals with the VDR GG genotype had a significantly increased risk of LDDD, while those with the AG genotype had a significantly decreased risk. In addition, the A allele may have a protective effect against LDDD in the Turkish population. Moreover, the VAS pain results showed that the GG genotype had a significantly higher score than the others.

CONCLUSION: VDR rs2228570 AG genotype is at a decreased risk and the GG genotype is at an increased risk of LDDD in the Turkish population. Since genetic polymorphisms often show ethnic differences, further functional studies are needed to evaluate the genotype and phenotype correlations in large cohorts of various ethnicities.

KEYWORDS: FokI polymorphism, Lumbar degenerative disc disease, Vitamin D receptor

INTRODUCTION

Lumbar degenerative disc disease (LDDD) is the most common cause of low back pain (LBP) and sciatica. Moreover, the degenerative process has been identified as multifactorial, irreversible, and associated with mechanical dysfunction (13). LBP is mainly caused by lumbar disc disease and affects more than 50% of the population during their lifetimes, while the lifetime incidence of sciatica varies from 13% to 40% (3,7,16). Some studies have shown that 20% of LDDD patients require surgical treatment due to persistent or aggrevated leg pain (6,9). LBP generally leads to reduced physical activity and a decreased quality of life; therefore, it brings with it serious economic pressure on society with the overall loss of work (1).

The vitamin D receptor (VDR) gene, which is located on chromosome 12 (12q12–q14), was the first gene reported to be potentially related to the intervertebral degenerative disc disease risk. It is believed to play critical roles in the mechanisms of chondrocyte proliferation and bone mineralization and remodeling (22,23). The VDR is an endocrine member of the nuclear receptor superfamily of steroid hormones and acts as a ligand-activated transcription factor (5). VDR polymorphisms are thought to affect degenerative disc disease, osteoarthritis, and osteoporosis (4).

Based on the abovementioned information, we conducted a case-control study in a Turkish population and investigated the association between the VDR gene rs2228570 FokI polymorphism and the development of LDDD.

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MATERIAL and METHODS

Study Population

This was a prospective case-control study that included 94 individuals. All of the participants were selected from the Neurosurgery Department of Yeditepe University in Istanbul, Turkey. There were 45 patients with a diagnosis of LDDD. The clinical investigations of the LDDD patients consisted of neurological examinations, lumbar magnetic resonance imaging (MRI) studies, visual analog scale (VAS) scores to define the pain level, and Oswestry Disability Index (ODI) scores. The inclusion criteria were leg pain and LBP as a result of lumbar radiculopathy, and lumbar intervertebral disc protrusion as diagnosed via MRI. The exclusion criteria were trauma, infections, congenital anomalies, oncological pathologies, osteoporosis, spinal stenosis, spondylolisthesis, vertebral fractures, and spinal deformities. Each patient was questioned about their level of pain, which was scored using the VAS, and questioned about their quality of life, which was scored using the ODI. A total of 49 healthy individuals were selected for the control group.

The study was approved by Yeditepe University Ethical Committee with the decision number 729 on 24th May, 2017. The clinical data of the patients were recorded and followed-up prospectively, while the demographic characteristics of the patients and controls were obtained from the medical records of the subjects. Informed consent was obtained from all of the patients.

Genetic Analysis

The peripheral blood samples were collected into ethylene-diaminetetraacetic acid (EDTA) tubes. The deoxyribonucleic acid (DNA) extraction was performed using an iPrep Purification Instrument (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA) with 350 µl of peripheral blood and an iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA). The isolated DNA samples were then measured with a NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) using a 1.7–1.9 optical density range for the genotyping and final sample concentrations diluted to approximately 100 ng/µl. The VDR gene rs2228570 FokI polymorphism genotyping was performed using the 7500 Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) with the TaqMan Genotyping Assay, TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA), and 100 ng of sample DNA. The reaction mixture and conditions were used as recommended by the manufacturer: 10 minutes at 95°C for the holding stage, 40 cycles of 15 seconds each at 92°C for the denaturation, and 60 seconds at 60°C for the annealing/extension. The allelic discrimination of the samples was done by collecting and interpreting the fluorescent signals of the hybridization probes using the software from the 7500 Fast Real-Time *polymerase chain reaction* (PCR) instrument.

Statistical Analysis

The statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). The significant differences between the groups according to genotypes were examined with the chi-squared and Fisher's exact tests, and the comparisons of the demographic information were evaluated with the Student's t-test. The statistical significance level was $p<0.05$.

RESULTS

Our analysis included 45 LDDD patients and 49 healthy individuals as a control group. The demographic characteristics of the two groups and values of the VAS and ODI scores are shown in Tables I, II. The mean ages of the patients with LDDD and the healthy controls were 37.33 ± 8.26 years and 34.81 ± 4.53 years, respectively. No significant differences were found between the LDDD and control groups in terms of the mean ages ($p=0.068$). The gender frequency was 46.7% male and 53.3% female for the patients and 38.8% male and 61.2% female for the controls. There were no significant differences with regard to gender ($p=0.44$).

The allele and genotype frequencies for the VDR gene rs2228570 FokI polymorphism in the patients with LDDD and the control group are shown in Table III. The VDR gene rs2228570 FokI genotype frequency comparison between the patients and controls was statistically significant ($p=0.011$). The GG genotype frequency was significantly higher in the patients than in the control group ($p=0.011$), while the AG genotype frequency was significantly higher in the control group than in the patients ($p=0.038$).

Our results indicated that those individuals with the GG genotype had a significantly increased risk of LDDD, and those with the AG genotype had a significantly decreased risk. Therefore, carrying an A allele may have a protective effect against LDDD in the Turkish population.

We also evaluated the relationships of the VAS and ODI scores between the patient genotypes and found that the VAS score was significantly higher in the GG genotype (Table IV). Although there were no significant differences between the ODI scores and patient genotypes, it is worth noting that the lowest value was found with the GG genotype (Table V).

DISCUSSION

LBP is a common disability-causing musculoskeletal problem, and it is the primary cause of activity limitation and work absence throughout the world (25). Intervertebral disc degeneration (IDD) is considered to be the primary cause of LBP, which is an irreversible process within the second decade via molecular changes in the nucleus pulposus (12). The results of this process are the weakened loading ability of the vertebral column or compression and decreased disc height (15). In addition, the upregulation of the degradation systems, like apoptosis, inflammation and matrix metalloproteinase (MMP), cause further damage (20). Genomic research has investigated many genetic polymorphisms, such as MMP, VDR, collagen, apoptosis factors, aggrecans, and interleukins, which have been attributed to an increased risk of developing IDD (10,14,19,29).

The VDR gene, which is located on chromosome 12 (12q12–q14) is the first gene reported to be potentially related to an IDD risk (22,23). The VDR is a nuclear receptor for the vitamin D metabolite 1,25-dihydroxy-vitamin D3. Moreover, the function of the VDR has been hypothesized to be an indirect mechanism for the proliferation of chondrocytes and their effects on proteoglycans (27). TaqI (rs731236), FokI (rs2228570), and ApaI (rs7975232) are examples of single nucleotide polymorphisms (SNP) that affect the expression and function of the VDR in degenerative disc disease (2,6,10,14,21,23,26,27).

The FokI (rs228570) polymorphism is responsible for a substitution at exon 2 of the VDR gene. As a result of this substitution, the methionine amino acid is translated to threonine, which could finally affect the efficacy of the VDR (8). One study reported that a shorter VDR polypeptide was related to the wild-type A variant, which leads to normal VDR function, while the G substitution was attributed to reduced functioning (28).

Videman et al., who studied Finnish twins, reported that the mutant genotype was associated with a 9.3% reduced signal intensity when compared with the wild-type on MRI scans of the T6–S1 region in 1998 (23). Additionally, they found that the heterozygote genotype was associated with a 4.3% reduced signal intensity within the same region (23).

Kanna et al. studied the prevalence of intervertebral modic changes and genetic association of 41 candidate genes in a large Indian cohort and they found that the rs2228570 SNP of VDR (p=0.02) and rs17099008 SNP of matrix metalloproteinase 20 (p=0.03) were significantly associated with intervertebral modic changes in Indian population (11).

Nong et al. reported a meta-analysis about the association between vitamin D receptor, matrix metalloproteinase 3

Table I: Demographic Characteristics of Patient and Control Groups

Parameter	Control group (n=49)	LDDD (n=45)	p
Age (years) (Mean ± SD)	34.81 ± 4.53	37.33 ± 8.26	0.068
Gender (Male/Female)	19/30	21/24	0.44

Table II: Oswestry and VAS Scores of Patients

Score	Minimum	Median	Maximum
Oswestry	6	46	86
VAS	20	70	100

Table III: Genotype and Allele Frequencies Between Patient and Control Groups

Genotype rs2228570	Control group (n=49) n (%)	LDDD (n=45) n (%)	p	OR	95% CI
AA	6 (12.2)	3 (6.7)	0.35	0.521	0.120-2.182
AG	22 (44.9)	11 (24.4)	**0.038**	0.397	0.164-0.960
GG	21 (42.9)	31 (68.9)	**0.011**	2.952	1.265-6.891
Allele		**Allelic Count n (%)**			
A	34 (34.69)	17 (19.31)	**0.011**	0.339	0.145-0.791
G	64 (65.31)	71 (80.39)	0.49	1.953	0.458-8.325

Table IV: Evaluation of VAS Scores Between Genotypes within Patient Group

Genotype rs2228570	VAS (Mean ± SD)	p
AA	43.33 ± 15.27	0.13
AG	54.54 ± 24.64	0.17
GG	67.41 ± 21.59	**0.037**

Table V: Evaluation of ODI Score Between Genotypes within DLDD Group

Genotype rs2228570	ODI score (Mean ± SD)	p
AA	42.66 ± 18.90	0.68
AG	42.09 ± 21.43	0.32
GG	49.48 ± 19.30	0.25

(MMP-3) polymorphisms and the risk of intervertebral disc degeneration susceptibility and they found that FokI, ApaI polymorphisms in the vitamin D receptor gene and MMP-3 polymorphism are not obvious risk factors for intervertebral disc degeneration (17). But they also concluded the study with the discussion that IDD is a multifactor disease determined by the synthetic effect and interactions between race, age, environment, gene and polymorphic loci of the same gene; these interactions may mask or magnify the function of the involved genes (17).

The FokI SNP was not only associated with an increased severity of IDD, but also an increased risk of developing IDD, which is supported by other studies in the literature (2,6,24,28). Several FokI SNP studies have reported that a genetic risk factor could also affect the severity of IDD (18,21,28). All of these data suggest that the FokI polymorphism manifests differently in patients based on ethnicity or race. In our study, we found that the individuals with the VDR GG genotype had a significantly increased risk of LDDD, while those with the AG genotype had a significantly decreased risk, and that carrying an A allele might have a protective effect against LDDD in the Turkish population. We also analyzed the VAS pain scores between the patient genotypes, discovering that the GG genotype had a significantly higher score than the others.

CONCLUSION

This study evaluated the relevance of the VDR gene rs2228570 FokI polymorphism with regard to the risk of LDDD. Our results suggested that carrying the VDR rs2228570 A allele created a decreased risk of LDDD and might have a protective effect against the disease, while carrying the G allele increased the risk of LDDD. Since genetic polymorphisms often show ethnic differences, further functional studies are needed to evaluate the genotype and phenotype correlations in large cohorts of various ethnicities.

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