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Analysis of genetic polymorphisms associated with intervertebral disc degeneration

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Abstract: Intervertebral disc degeneration (IVDD) is a common degenerative spinal condition. Recent studies have shown that the incidence of disc herniation and disc degeneration may be explained by genetic factors. In this study, we investigated the link between various polymorphic variants of the vitamin D receptor (VDR), matrix metalloproteinase 2 (MMP2), and insulin like growth factor 1 receptor (IGF1R) genes and IVDD in patients with IVDD, in Turkey. We examined and genotyped 199 patients with IVDD and 197 healthy individuals. Genomic DNA was isolated from the peripheral blood leukocytes of all participants, and analyzed using real-time PCR. Via melting curve analysis, VDR, MMP2, and IGF1R polymorphism variant distributions were determined. The patients with IVDD showed higher frequencies of the VDR ApaI A allele genotype as compared to the control group; however, there were no significant differences in the frequencies or allelic distributions of the IGF1R and MMP2 genotypes between the IVDD patients and the control group. The incidence of IVDD in these Turkish patients is correlated with the VDR ApaI gene polymorphism, but not with the IGF1R and MMP2 polymorphisms.

Key words: Insulin like growth factor 1 receptor; Intervertebral disc degeneration; Matrix metalloproteinase-2; Polymorphism; Vitamin D receptor.

Introduction

Lower back pain (LBP) negatively affects quality of life, increases the rate of sick leave, and is a leading cause of disability worldwide. It is estimated that 50 % to 80 % of adult experience LBP at some point during their lifetimes. One of the most common causes of LBP is intervertebral disc degeneration (IVDD) (1-4).

Intervertebral discs (IVDs), the soft tissues found between the vertebrae of the spine, are complex structures made up of the nucleus pulposus, annulus fibrosus, and cartilage endplates (5). These IVD tissues interact with each other and provide mechanical support to the spine (2,6). IVDD is characterized by the dysfunction and deterioration of the nucleus pulposus and annulus fibrosus and is diagnosed using magnetic resonance imaging (MRI). Disc degeneration is graded using T2-weighted MRI images. This five-level grading system of disc degeneration is measured by disc structure, distinction of the nucleus and the annulus, signal intensity, and the height of the intervertebral discs (7,8).

IVDD is associated with various factors; these include age, body weight, mechanical loading of the spine, physical activity, and smoking. Recently, it has been shown that genetic factors also play a role in the development of IVDD (9-11). Increasingly, the evidence suggests that collagen I (COL1A1), collagen IX (COL9A2 and COL9A3), collagen XI (COL11A2), interleukin 1 (IL-1A and IL-1B), interleukin 6 (IL-6), vitamin D receptor (VDR), matrix metalloproteinase (MMP2 and MMP3), aggrecan, extracellular matrix-degrading enzyme, and insulin like growth factor 1 receptor (IGF1R) genes are linked to degenerative disc disease (10-12). However, these associations have only been shown in a few ethnic groups, and therefore, need to be confirmed in additional ethnic population studies.

Therefore, the objective of this study was to evaluate the association between VDR, MMP2, and IGF1R gene polymorphisms and the risk of developing IVDD in individuals from Turkey. Here, we analyze these genes to determine the relationship between specific polymorphisms, the development of IVDD, and IVDD grade in unrelated patients diagnosed with IVDD as compared to those without IVDD.

Materials and Methods

Study protocols were approved by the Clinical Research Ethics Committee of Necmettin Erbakan University, Meram Medical Faculty (Approval Number: 2013/22). All participants signed a written informed consent form. The study group consisted of 199 patients (105 females and 94 males) diagnosed with IVDD, as defined by the International Society for the Study of the Lumbar Spine, and 197 healthy control patients (93 females and 104 males), at the Neurosurgery Clinic of Beysehir State Hospital. The patient and control groups were matched by age and gender. The control group had no history back problems.

The demographic data of the study participants are presented in Table 1. The grade of disc degeneration was determined according to the methods used by Pfirrmann et al. (7) using MRI. MRI scores are shown in Table 2. Patients with synovial cysts, spondylolisthesis,

Table 1. Demographic characteristics of IVDD patients and the control group.

	IVDD (mean ± SD)	Control (mean ± SD)	p
Total number	199	197	
Age (years)	43.9 ± 10.2	42.6 ± 11.8	NS
Gender (female/male)	105 / 94	93 / 104	NS
BMI (kg/m2)	26.9 ± 4.3	26.1 ± 4.4	< 0.05
Smoking (Yes/No)	74 / 125	78 / 119	NS
Exercise (Yes/No)	28 / 171	39 / 58	NS
Back injury (Yes/No)	32 / 167	6 / 191	< 0.001

BMI: body mass index, IVDD: intervertebral disc degeneration, NS: non-significant.

spinal tumors, spondylosis, trauma, inflammatory diseases, morbid obesity, and people aged 65 years and older were excluded from the study.

Blood samples were collected from each participant and placed in tubes containing ethylenediaminetetraacetic acid (EDTA). All samples were stored at 4 °C. Genomic DNA was isolated from the blood samples using the High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions. The purity and concentration of the DNA samples was quantified using a nano-drop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Genotyping of the VDR ApaI (rs7975232), IGF1R (rs11247361), and MMP2 (rs243865) polymorphisms was performed using a real-time PCR (LightCycler 480, Roche Diagnostics, GmbH, Mannheim, Germany) (13-15). Specific primers and hybridization probes for each polymorphism were used in combination with the LightCycler DNA Master Hybridization Probes Kit (Roche Applied Science, Germany). The amplification conditions were as follows: polymerase activation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60 °C for 10 s, and elongation at 72 °C for 15 s. Polymorphic genotypes were identified by the specific melting temperatures (Tm) of the resulting amplicons.

All statistical analysis was performed using SPSS statistical software, version 21.0 (SPSS Inc. Chicago, USA). Values of $p < 0.05$ were defined as statistically significant. All data are expressed as the mean ± standard deviation (SD). Comparisons between groups were performed using the Mann Whitney U test, and discrete variables were compared using the Pearson chi-square test.

Results

In this study, we examined 396 unrelated patients with or without IVDD. The median ages of the patients with IVDD and the control group were 43.9 ± 10.2 and 42.6 ± 11.8, respectively. No correlations were found between the control and patient groups in terms of age, gender, smoking, and exercise. However, body mass index (BMI) measurements and the incidence of back injury were higher in the IVDD patient group than in the control group ($p < 0.05$ and $p < 0.001$ respectively). A comparison of these characteristics is presented in Table 1.

The AA, AC, and CC polymorphism distributions of VDR ApaI (rs7975232) were found to be 55.3 %, 33.7 %, and 11.1 %, respectively, in the IVDD patient group, and 39.6 %, 41.1 %, and 19.3 %, respectively, in the control group. IVDD patients had a higher frequency of the VDR ApaI A allele at 72.1 %, as compared to 27.9 % in the control group ($p < 0.001$; Table 3). The IGF1R (rs11247361) distribution of the CC, CG, and GG polymorphisms was found to be 42.2 %, 48.2 %, and 9.5 %, respectively, in the IVDD patients, and 46.2 %, 41.6 %, and 12.2 %, respectively, in the control group. The MMP2 (rs243865) distribution of the CC, CT, and TT polymorphisms was 62.8 %, 31.2 %, and 6 %, respectively, in the IVDD patients, and 67 %, 28.9 %, and 4.1 %, respectively, in the control group. However, there were no significant differences between the IVDD patient and control group distributions of IGF1R (rs11247361) and MMP2 (rs243865) polymorphisms (Table 3). We also compared the relationship of the VDR ApaI, IGF1R, and MMP2 polymorphism distributions to IVDD grade; however, we found no statistically significant correlations (Table 4).

Discussion

Intervertebral disc degeneration (IVDD) is associated with environment and genetic factors. The structure of the disease is complex, and its genetic mechanisms are mostly unknown. Elmasry et al. (16) suggested that smoking is related to numerous pathological conditions, and benefits to disc health associated with cessation of smoking were limited. Mohammed et al. (17) reported

Table 2. Grades of disc degeneration in patients with IVDD.

Grade	Disc Structure	Distinction of Nucleus-Annulus	Signal intensity of IVD	Height of IVD
1	Homogenous, bright white	Clear	Hyperintens/with CSF isointens	Normal
2	Non-homogenous, with or without horizontal gray bands	Clear	Hyperintens/with CSF isointens	Normal
3	Non-homogenous, gray	Unclear	İntermediate	Normal/Slightly decrease
4	Non-homogenous, gray-black	Lost	İntermediate-Hypointens	Normal/Moderately decrease
5	Non-homogenous, black	Lost	Hypointens	Collapse

CSF: Cerebrospinal fluid, IVD: intervertebral disc, IVDD: intervertebral disc degeneration.

Table 3. Distribution and allelic frequency of the VDR ApaI (rs7975232), IGF1R (rs11247361) and MMP2 (rs243865) gene polymorphisms in IVDD patients and the control group.

Polymorphisms	Genotype Haplotype	Patients, n (%)	Control, n (%)	p
VDR ApaI (rs7975232)	AA	110 (55.3)	78 (39.6)	< 0.001
	AC	67 (33.7)	81 (41.1)	
	CC	22 (11.1)	38 (19.3)	
	A	287	237	
	C	111	157	
IGF1R (rs11247361)	CC	84 (42.2)	91 (46.2)	> 0.05
	CG	96 (48.2)	82 (41.6)	
	GG	19 (9.5)	24 (12.2)	
	C	264	264	
	G	134	130	
MMP2 (rs243865)	CC	125 (62.8)	132 (67)	> 0.05
	CT	62 (31.2)	57 (28.9)	
	TT	12 (6)	8 (4.1)	
	C	312	321	
	T	89	73	

IGF1R: insulin like growth factor 1 receptor, MMP2: matrix metalloproteinase, VDR: vitamin D receptor

Table 4. Relationship of VDR ApaI, IGF1R, and MMP2 genotypes to the grade of disc degeneration in IVDD patients.

Genotype		Grade 2	Grade 3	Grade 4	Grade 5	Total	p
VDR ApaI (rs7975232)	AA (%)	1 (0.9)	31 (28.2)	67 (60.9)	11 (10)	110 (100)	> 0.05
	AC (%)	1 (1.5)	15 (22.4)	44 (65.7)	7 (10.4)	67 (100)	
	CC (%)	0 (0)	4 (18.2)	14 (63.6)	4 (18.2)	22 (100)	
IGF1R (rs11247361)	CC (%)	0 (0)	24 (28.6)	54 (64.3)	6 (7.1)	84 (100)	> 0.05
	CG (%)	2 (2.1)	24 (25)	56 (58.3)	14 (14.6)	96 (100)	
	GG (%)	0 (0)	2 (10.5)	15 (78.9)	2 (10.5)	19 (100)	
MMP2 (rs243865)	CC (%)	2 (1.6)	33 (26.4)	74 (59.2)	16 (12.8)	125 (100)	> 0.05
	CT (%)	0 (0)	15 (24.2)	42 (67.7)	5 (8.1)	62 (100)	
	TT (%)	0 (0)	2 (16.7)	9 (75)	1 (8.3)	12 (100)	
		2 (1)	50 (25.1)	125 (62.8)	22 (11.1)	199 (100)	

IGF1R: insulin like growth factor 1 receptor, MMP2: matrix metalloproteinase, VDR: vitamin D receptor.

that the disc degeneration-induced back pain was higher in chronic smokers as compared with non-smoker. In addition, Zawalli et al. (18) reported that smoking and occupational factors such as prolonged sitting, twisting/bending, whole body vibration, and lifting heavy objects were significantly associated with disc degeneration. However, in the present study, no difference with respect to smoking was found in the patient and control groups. On the other hand, we observed that back injury is significantly associated with IVDD, the results being consistent to those of the study of Zawalli et al. (18). In addition to these factors, an increased BMI was determined in the patient group compared to healthy control individuals. Therefore, back injury and BMI can be considered risk factors of IVDD, but not smoking.

Rajasekaran et al. (19) suggested that seventy-one single-nucleotide polymorphisms (SNPs) of 41 candidate genes including VDR, IGF1R, COX2, MMP1 and 7 etc. may be associated with MRI findings of disc degeneration. Furthermore, the study indicated that different phenotypes completely change SNP associations. A steroid hormone receptor, VDR, is an endocrine member of the nuclear receptor superfamily and binds to the most active form of vitamin D, 1α,25-dihydroxyvitamin D3 (20). VDR is a significant factor for normal bone mineralization and remodeling as other members of steroid receptor family, and its polymorphism is associated with diseases, such as osteoporosis, osteoarthritis, and degenerative disc diseases (21).

Recent studies have shown that polymorphisms in the gene encoding VDR are associated with the development of IVDD. It has also been shown that the FokI (rs2228570), TaqI (rs731236), ApaI (rs7975232), and BsmI (rs1544410) VDR polymorphic sites are associated with degenerative disc disease in Japanese, Chinese, Italian, Finnish, and Australian populations (22-24). Furthermore, Cheung et al. (24) showed that the VDR TaqI polymorphism leads to an increased risk of IVDD, especially in individuals under 40 years of age. In this study, we examined the VDR ApaI polymorphism in Turkish patients with IVDD. Similar to other studies, our results showed that the AA genotype is significantly higher in IVDD patients (13,15).

Matrix metalloproteinases (MMPs) degrade the extracellular matrix (ECM). Increased expression of these MMPs, including MMP1, MMP2, MMP3, and MMP9, has been shown in patients with IVDD (25,26). Furthermore, Rutges et al. (27) showed that the activity of MMP2 (gelatinase A) is associated with the development of IVDD, and that MMP2 is activated by increased MMP14 expression.

Price et al. (28) reported that a functional single nucleotide polymorphism (SNP) in the promoter regions of the MMPs changes the Sp1-binding site, and causes a reduction in transcriptional activity. MMP2 levels have been shown to be higher in individuals who carry the

CC genotype as compared to those who carry the CT or TT genotypes. In a study of Chinese young adults investigating the MMP2-1306C/T polymorphism, Dong et al. (15) found an association between this polymorphism and disc degeneration. Similarly, in another study, it is suggested that the extracellular matrix-degrading enzymes, such as MMP1, MMP2, MMP3, MMP9, MMP14, ADAMTS-4, and ADAMTS-5 are also associated with IVDD (29). In contrast to these studies, we did not find any significant correlation between the MMP2 polymorphism and the incidence of IVDD; therefore, it remains unclear if the MMP2 polymorphism is a genetic risk factor in the Turkish population diagnosed with IVDD.

Insulin growth factor 1 (IGF1) is an anabolic growth factor, which regulates chondrocytic metabolic function (30). It has been reported that IGF1 can reduce tissue resorption via proteoglycan synthesis and matrix degradation (31,32). Urano et al. (14) showed there is an association between IGF1R polymorphisms and disc degeneration in healthy postmenopausal Japanese women. In a study using mice, it was reported that IGFR1 activity is associated with IVDD, and reduced expression of IGF-1R may result in the accelerated degeneration of IVDs (33). However, our results show that the distribution of IGF1R genotypes is similar in both patients diagnosed with IVDD and the control group. Therefore, the IGF1R gene mutation may not be related to the incidence of IVDD in the Turkish population. Furthermore, our evaluation of the relationship between these three gene variants of VDR (ApaI, MMP2, and IGF1R) and the grade of IVDD, revealed no significant correlations.

In conclusion, we investigated the link between various environmental risk factors and the VDR ApaI, IGF 1R, and MMP2 gene polymorphisms in IVDD patients and healthy control individuals in Turkey; we found that BMI and back injury were significant risk factors for IVDD in this population. As compared to the healthy individuals, the VDR Apa I gene mutation was observed more frequently in the patients with IVDD. Therefore, the VDR ApaI A allele may be associated with the incidence of IVDD in the Turkish population; however, unlike other studies, we did not find MMP2 or IGF1R gene mutations to be linked to IVD. Therefore, additional studies in different ethnic populations are necessary to clarify the specific genetic factors associated with IVDD.

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Conflict of interest
The all authors declare there is no conflict of interest.

Author's contribution
The experimental design: Aynur Acar and Ayse Gul Zamani.
The collection of sample: Serkan Kitis
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