Original Article / *Özgün Araştırma*

DOI: 10.4274/haseki.galenos.2020.6006
Med Bull Haseki 2020;58:309-314

ACAN Gene VNTR Polymorphism and Intervertebral Disc Degeneration in a Turkish Population

ACAN gen VNTR Polimorfizmi ve Türk Popülasyonundaki İntervertebral Disk Dejenerasyonu

Tuba Öz*, İsmail Kaya**, Ayşe Feyda Nursal***, Hasan Emre Aydın**, Osman Demir****, Serbülent Yiğit*,*****

**Gaziosmanpaşa University Faculty of Medicine, Department of Medical Biology, Tokat, Turkey*

***Kütahya Health Sciences University Faculty of Medicine, Department of Neurosurgery, Kütahya, Turkey*

****Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey*

*****Gaziosmanpaşa University Faculty of Medicine, Department of Biostatictics, Tokat, Turkey*

******Ondokuz Mayıs University Faculty of Veterinary, Department of Veterinary Genetics, Samsun, Turkey*

Abstract

Aim: Intervertebral disc degeneration (IVDD) is caused by several genetic and environmental factors. Aggrecan is the major component of intervertebral disk matrix proteoglycan with multiple functional domains. The aim of this study was to investigate the possible association between *ACAN (coding aggrecan)* gene variable number tandem repeat (VNTR) polymorphism and susceptibility to IVDD.

Methods: Two hundred and sixty subjects participated in this study. The disease group comprised 150 patients diagnosed with symptomatic IVDD. The control group consisted of 110 healthy subjects. The *ACAN* gene VNTR region was analyzed using the polymerase chain reaction (PCR) method.

Results: The most common allele in the patient and the control group was 27 repeat allele (49% and 34.55%, respectively). Allele 26 was more frequent in males compared to females (p=0.030). Allele 21 and 23 were more common in ones living in rural areas (p=0.030) while allele 27 was the most frequent in ones living in urban areas (p<0.001). Allele 26, allele 29 and allele 30 were less frequent in the patient group than in the control group (p=0.013, p=0.001 and p=0.001, respectively) while allele 27 was more common in the patient group compared to the control group (p=0.001).

Conclusion: Our results showed that *ACAN VNTR* allele 27 had a positive relationship with IVDD susceptibility in a Turkish population.

Keywords: Intervertebral disc degeneration, aggrecan, VNTR, PCR

Öz

Amaç: İntervertebral disk dejenerasyonu (İVDD) çeşitli genetik ve çevresel faktörlerden kaynaklanır. Agrekan intervertebral disk matrisi proteoglikanın çoklu fonksiyonel etki alanlarına sahip ana bileşenidir. Bu çalışmanın amacı *ACAN (agrekan kodlayan)* gen değişken sayılı ardışık tekrar (VNTR) polimorfizmi ve İVDD yatkınlığı arasındaki olası ilişkiyi araştırmaktır.

Yöntemler: ACAN VNTR polimorfizmi ve İVDD arasındaki ilişkiyi incelemek için, 260 kişi bu çalışmaya katıldı. Hasta grubu semptomatik İVDD tanısı alan 150 hastadan oluşmuştu. Kontrol grubu 110 sağlıklı kişiden oluşmuştu. *ACAN* geni VNTR polimorfizmi polimeraz zincir reaksiyonu (PZR) yöntemi ile analiz edildi.

Bulgular: Hasta ve kontrol grubunda en sık görülen alel 27 tekrarlı aleldi (sırasıyla, %49 ve %34,55). Alel 26 erkeklerde kadınlara göre daha sıktı (p=0,030). Alel 27 en fazla şehirde yaşayanlarda bulunurken (p<0,001), alel 21 ve 23 köyde yaşayanlarda fazlaydı (p=0,030). Alel 27 hasta grubunda kontrol grubuna göre artmışken (p=0,001), alel 26, alel 29 ve alel 30 hasta grubunda kontrol grubuna göre azdı (sırasıyla, p=0,013, p=0,001, p=0,001).

Sonuç: Sonuçlarımız, Türk popülasyonunda *ACAN VNTR* alel 27'yi taşıyan kişilerin İVDD yatkınlığı ile pozitif ilişkisi olduğunu göstermiştir.

Anahtar Sözcükler: İntervertebral disk dejenerasyonu, agrekan, VNTR, PZR

Address for Correspondence/Yazışma Adresi: Ayşe Feyda Nursal, Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
Phone: +90 364 222 11 00 E-mail: feyda.nursal@gmail.com ORCID: orcid.org/0000-0001-7639-1122
Received/Geliş Tarihi: 19 February 2020 **Accepted/Kabul Tarihi:** 06 April 2020

This study was presented as an oral presentation in the 3rd International GAP Conference on Mathematics – Engineering – Science and Medical Studies in Şanlıurfa, Turkey.

Introduction

Intervertebral disc degeneration (IVDD) is a common chronic disorder affecting 70-90% of the population (1). It is considered a global health problem because it leads to debilitation, negatively affects physical and work activities, decrease quality of life, and causes psychological distress in affected individuals (2). The etiology of degenerative disc disease is complex. Environmental, ergonomic, and anthropometric factors have been determined as causes; several studies have suggested that genetic factors or familial predisposition contribute to IVDD (3). According to Videman et al. (4), the process of disc degeneration involves desiccation, collagen fragmentation, and annulus fibrosus failure, resulting in disc height narrowing.

Aggrecan, a large aggregating proteoglycan, is one of the major structural components of intervertebral disc and cartilage (5). The localized high concentration of aggrecan provides the osmotic property that is necessary for tissue function (6). This function is related to the structure of aggrecan, especially to the large number of chondroitin sulfate (CS) chains that present in their core protein. The CS chains are present in two adjacent regions of the aggrecan core protein, termed the CS1 and CS2 domains. The human *ACAN* gene (encoded for aggrecan core protein) is located on the chromosome 15q26. The human *ACAN* gene variable number of tandem repeat (VNTR) (often referred to as micro- or minisatellite DNA) polymorphism has repeats of 57 nucleotides, encoding each 19-amino acid unit (7). The described alleles range from 13 to 34 repeats. The most common alleles have 26, 27, or 28 repeats (8). Some studies proved that the alleles 13-25 were risk factors for lumbar disc degeneration (9,10), however, some found that alleles 26-27 were associated with lumbar disc degeneration (11,12).

Thus, we aimed to investigate the association between the *ACAN* gene VNTR polymorphism and IVDD in Turkish patients.

Methods

Study population

This case-control study included 150 patients (67 males and 83 females, mean age: 51.46±16.35) with IVDD and 110 age and sex-matched (47 males and 63 females, mean age: 48.29±11.89) unrelated healthy controls. All patients were recruited from the Department of Neurosurgery, Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital. The disease was diagnosed in all individuals via magnetic resonance imaging. Subjects without IVDD, with trauma-related disc degeneration, a previous diagnosis of rheumatic and neurological diseases, spinal infections and metabolic diseases were excluded. All controls were confirmed to be free of discogenic low back pain and history of intervertebral disc disease. Demographic and clinical information of all participants were obtained from medical records. These information included age, gender, living place, working status, disease diagnosis, and spinal level. Ethical committee approval (Hitit University 2017/49) was received, and informed consent was obtained from patients and control subjects before beginning of the study.

Genotyping

5 mL of intravenous blood was collected from the subjects into EDTA vacutainers. The genomic DNA was extracted using a commercial kit, according to the manufacturer's instructions. Genotypic analysis of the ACAN VNTR polymorphism was performed using the polymerase chain reaction (PCR) as described by Casa et al. (13). VNTR polymorphism was determined by PCR using the following primers: 5'-TAGAGGGCTCTGCCTCTGGAGTTG-3' and 5'-AGGTCCCCTACCGCAGAGGTAGAA-3', respectively. Amplification was conducted over 38 cycles, with denaturation at 95 °C for 5 min, followed by annealing at 66 °C for 50 s and extension at 72 °C for 50 s. The amplified products were resolved on a 2% agarose gel, and visualized and documented under ultraviolet light after staining with ethidium bromide gel.

Statistical Analysis

All data were analyzed by using the Statistical Package for the Social Sciences (SPSS) for Windows (version 16.0; SPSS Inc, Chicago, IL, USA). Continuous data were given as mean ± standard deviation and minimum/maximum. The number of repeats in the ACAN VNTR region was determined in reference to a table of repeat number versus amplicon length. The χ^2 test was used to measure significance of differences in the allele frequency and genotype distribution between the groups. Odds ratio and 95% confidence intervals were calculated. A p value of less than 0.05 was considered statistically significant.

Results

A total of 150 IVDD patients and 110 controls were included in the study. Age, gender, living place, working status, disease diagnosis, and spinal level were analyzed in all IVDD patients. Demographic and clinical characteristics of the study participants are shown in Table 1.

Allele frequencies of the patient and the control groups are presented in Table 2. The genotyping identified 11 alleles ranging from 21 to 31 repeats. The most common allele in patients and the control group was 27 repeat allele (49% and 34.55%, respectively). Allele 26, allele 29 and allele 30 were less common in the patient group than in the control group (p=0.013, p=0.001 and p=0.001, respectively) while allele 27 was more common in the

Table 1. The demographical and clinical characteristics of the study subjects					
		Patient group (n=150)	**Control group (n=110)**		
Age		51.46±16.35	48.29±11.89		0.086
Gender	Female	83 (55.3)	63 (57.3)	146 (56.2)	0.756
	Male	67 (44.7)	47 (42.7)	114 (43.8)	
Living place	Rural	22 (14.7)	0 (0)	22 (14.7)	-
	Town	64 (42.7)	0 (0)	64 (42.7)	
	Urban	64 (42.7)	0 (0)	64 (42.7)	
Working status	Working	35 (23.3)	0 (0)	35 (23.3)	-
	Retired	105 (70)	0 (0)	105 (70)	
	Unemployed	10 (6.7)	0 (0)	10 (6.7)	
Diagnosis	Lomber hernia	31 (20.7)	0 (0)	31 (20.7)	-
	Cervical hernia	14 (9.3)	0 (0)	14 (9.3)	
	Thoracic vertebra fracture	6 (4)	0 (0)	6 (4)	
	Spinal stenosis (lomber)	44 (29.3)	0 (0)	44 (29.3)	
	Cervical disc degeneration	6 (4)	0 (0)	6 (4)	
	General medical examination	1 (0.7)	0 (0)	1(0.7)	
	Soft tissue disorders	2 (1.3)	0 (0)	2 (1.3)	
	Spondylolisthesis	7 (4.7)	0 (0)	7 (4.7)	
	Lombar intervertebral disc disorder	30 (20)	0 (0)	30 (20)	
	Cerebrovascular disease	1 (0.7)	0 (0)	1 (0.7)	
	Headache	2 (1.3)	0 (0)	2 (1.3)	
	Hydrocephaly	1 (0.7)	0 (0)	1 (0.7)	
	Lomber vertebra fracture	5 (3.3)	0 (0)	5 (3.3)	
Disc localization	I4	6 (4)	0 (0)	6 (4)	-
	I5	4 (2.7)	0 (0)	4(2.7)	
	I3	1 (0.7)	0 (0)	1(0.7)	
	I2	3 (2)	0 (0)	3 (2)	
	I5-C1	24 (16)	0 (0)	24 (16)	
	I4-I5	40 (26.7)	0 (0)	40 (26.7)	
	I3-4 HNP	7 (4.7)	0 (0)	7 (4.7)	
	L1	4 (2.7)	0 (0)	4 (2.7)	
	L3-4/L4-5/L5-S1	45 (30)	0 (0)	45 (30)	
	T1	1 (0.7)	0 (0)	1 (0.7)	
	T11	4 (2.7)	0 (0)	4 (2.7)	
	T12	2 (1.3)	0 (0)	2 (1.3)	
	L3-4	2 (1.3)	0 (0)	2 (1.3)	
	C4-5-6-7	7 (4.7)	0 (0)	7 (4.7)	
	I	89 (59.3)	0 (0)	89 (59.3)	-
	L	47 (31.3)	0 (0)	47 (31.3)	
	T	7 (4.7)	0 (0)	7 (4.7)	
	C	7 (4.7)	0 (0)	7 (4.7)	

I: Intervertebral region, HNP: Herniated nucleus pulposus, L: Lomber, T: Thoracic, C: Cervical, n: Number

patient group compared to the control group (p=0.001). Allele frequencies of groups according to gender and area of residence are shown in Table 3 and Table 4. Allele 26 was more frequent in males compared to females (p=0.030). Allele 21 and 23 were more frequent in ones living in urban (p=0.030), while allele 27 was the most frequent in those living in rural areas (p<0.001).

Table 2. Allele frequencies of ACAN VNTR polymorphism in patient and control groups

Alleles (repeat number)	Patient group (n=150) (%)	Control group (n=110) (%)	p*
21	6 (2)	1 (0.45)	0.130
22	4 (1.33)	4 (1.82)	0.657
23	9 (3)	5 (2.27)	0.613
24	16 (5.33)	9 (4.09)	0.513
25	56 (18.67)	41 (18.64)	0.993
26	34 (11.33)	42 (19.09)	**0.013**
27	147 (49)	76 (34.55)	**0.001**
28	22 (7.33)	18 (8.18)	0.719
29	5 (1.67)	16 (7.27)	**0.001**
30	1 (0.33)	7 (3.18)	**0.001**
31	0 (0)	1 (0.45)	0.242

VNTR: Variable number tandem repeat, n: Number
*chi-square test
Significant p values are shown in bold

Table 3. Allele frequencies of ACAN VNTR polymorphism according to gender

Alleles (repeat number)	Females (n=146) (%)	Males (n=114) (%)	p*
21	3 (1.03)	4 (1.75)	0.475
22	5 (1.71)	3 (1.32)	0.715
23	7 (2.4)	7 (3.07)	0.638
24	12 (4.11)	13 (5.7)	0.399
25	61 (20.89)	36 (15.79)	0.138
26	34 (11.64)	42 (18.42)	**0.030**
27	127 (43.49)	96 (42.11)	0.751
28	23 (7.88)	17 (7.46)	0.858
29	14 (4.79)	7 (3.07)	0.321
30	5 (1.71)	3 (1.32)	0.715
31	1 (0.34)	0 (0)	0.377

VNTR: Variable number tandem repeat, n: Number
*Pearson chi-square test
Significant p values are shown in bold

Table 4. Allele frequencies of ACAN VNTR polymorphism according to living place

Alleles (repeat number)	Village (n=70) (%)	County (n=210) (%)	Urban (n=20) (%)	p*
21	1 (1.43)	3 (1.43)	2 (10)	**0.030**
22	2 (2.86)	2 (0.95)	0 (0)	0.419
23	2 (2.86)	4 (1.9)	3 (15)	**0.005**
24	6 (8.57)	9 (4.29)	1 (5)	0.384
25	15 (21.43)	34 (16.19)	7 (35)	0.095
26	11 (15.71)	21 (10)	2 (10)	0.418
27	27 (38.57)	117 (55.71)	3 (15)	**<0.001**
28	6 (8.57)	14 (6.67)	2 (10)	0.777
29	0 (0)	5 (2.38)	0 (0)	0.336
30	0 (0)	1 (0.48)	0 (0)	0.807
31	0 (0)	0 (0)	0 (0)	-

VNTR: Variable number tandem repeat, n: Number
*Pearson chi-square test
Significant p values are shown in bold

Discussion

IVDD, a common musculoskeletal disease, is considered a multifactorial disease imposing economic and medical burdens to society. Although the exact mechanism of IVDD is still unknown, recent studies have focused on the role of genetic factors in the etiology of IVDD, and epidemiologic studies suggest that genetic susceptibility is the largest single determinant of disc degeneration (14). In order to understand the role that genetics plays in the development of disc degeneration, it is essential to be familiar with the cellular structure and genetic composition of an intervertebral disc, as well as the biochemical changes that occur through the process of disc degeneration. Intervertebral discs are avascular pads of fibrocartilage lying between adjacent vertebral bodies and allowing movement of the spine (15). Each disc has a specialized structure that provides stability, promotes flexibility, transmits loads through the spinal column, and absorbs external forces applied to the spine (15). The normal functions of the disc are altered due to pathologic changes in the cell morphology of the extracellular matrix (ECM), the biochemical structure, and the cell mechanics (16).

Aggrecan is the main proteoglycan in the intervertebral disc and provides the osmotic properties which assist the disc in resisting mechanical compressive loads transmitted along the spine (17). Its key function is to maintain hydration of the disc structure, attracting water molecules through the highly negatively charged glycosamiglican moieties which are mainly CS chains. Thus, from the structural integrity point of view and the associated loss of water content in a degenerating disc, this ECM molecule is considered a good candidate for genetic association studies. In the human *ACAN* gene, the region coding the CS1 domain exhibits size polymorphism, commonly known as VNTR in exon 12, ranging from 13 to 33 repeats (8). The functional property of aggrecan thus may vary between individuals with different lengths of the VNTR coding for the attachment sites of CS chains with a difference of as many as 40 CS chains per aggrecan core protein between the shortest and the longest *ACAN* alleles.

Casa et al. (13) reported that the most prevalent allele among individuals with lumbar disc herniation and controls, was allele A28, followed by alleles A27

and A29 (28, 27, and 29 repeats). In their study, Kim et al. (18) included 104 Korean subjects (66 men and 38 women) with disc degeneration, of whom 89 had disc herniation. They found that allele 27 was the most common followed by alleles 26 and 28. For the case that the analysis was limited to the subjects aged 40 years or younger, the allele 21 was significantly overrepresented among persons with multilevel (>3) disc degeneration. Mashayekhi et al. (19) showed that the most frequent AGC1 allele was 27, followed by 28, in patients and controls in the Iranian population. Additionally, they found that the shorter alleles (<24 repeats) were more common in patients with IVDD than in controls. It was declared that allele 26 was statistically significantly overrepresented among IVDD patients (12). In their study, Kawaguchi et al. (7) reported that there was a significant difference between the distribution of the allele sizes and severity of the degeneration, but no significant association was found between any of the alleles and number or type of disc herniation. In a study investigating the interaction between aggrecan VNTR and obesity in the susceptibility of symptomatic lumbar disc herniation, it has been reported that individuals carrying one or two alleles ≤25 repeats who were non-obese showed a 1.057-fold increase in risk for symptomatic lumbar disc herniation and participants carrying two alleles >25 repeats who were obese showed an 1.061-fold higher risk while participants carrying one or two alleles ≤25 repeats who were obese showed a 4.667-fold increase in risk for symptomatic lumbar disc herniation (20). Eser et al. (10) found a significant association between short repeated alleles (13-25) and multilevel disc herniation in a Turkish population.

This study was designed to evaluate the association of ACAN VNTR polymorphism with the risk and clinicopathological features of IVDD in a Turkish population. The patient group comprised 150 individuals, of whom 55.3% were women and 44.7% were men. We found 11 alleles ranging from 21 to 31 repeats and allele 27 was the most frequent allele in both groups. Allele 26, 29 and 30 were decreased in patients compared to controls ($p<0.05$). It was thought that these alleles had protective roles against IVDD in our samples. However, allele 27, the most common allele, was more frequent in the patient group than in the control group ($p<0.05$) (Table 2). Our data is not compatible with other results suggesting shorter alleles of ACAN VNTR polymorphism was associated with IVDD. This may be due to regional and ethnic diversity. According to gender, it was found that allele 26 was more common in males than in females ($p<0.05$). Also, allele 27 was more frequent in ones living in urban areas ($p<0.05$).

Conclusion

Our data suggested that carrying the ACAN VNTR variant allele 26, 29, 30 was associated with a decreased risk of IVDD and might have a protective role against the disease, while carrying the allele 27 increased the risk of IVDD. Further association studies investigating the role of ACAN VNTR polymorphism in IVDD are needed to better understand the pathobiology of disc degeneration.

Authorship Contributions

Concept: S.Y., T.Ö. Design: S.Y., T.Ö. Data Collection or Processing: H.E.A., İ.K. Analysis or Interpretation: S.Y., T.Ö., O.D. Literature Search: A.F.N., H.E.A., İ.K. Writing: A.F.N.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article

Financial Disclosure: The authors received no financial support for the research and/or authorship of this article.

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