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*Original Article*

# Association of *COL2A* and *Aggrecan* polymorphisms with the susceptibility of intervertebral disc degeneration

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**Abstract:** Background: To explore the association of *COL2A* and *Aggrecan* polymorphisms and the susceptibility of intervertebral disc degeneration (IVDD). Methods: IVDD patients (n = 354) in Spinal Surgery department of our hospital between June 2010 and June 2013 were allocated to the case group. Healthy people (n = 310) were allocated to the control group. The distribution frequency of *COL2A* and *Aggrecan* were detected by using PCR-RFLP. Haplotype analysis and spearman rank correlation analysis were conducted. Results: Statistical significance was found in *COL2A* (rs2276454) between case group and control group (both $P < 0.05$), while no statistical significance was found in rs2070739 (both $P > 0.05$); subgroup analysis on rs2070739 demonstrated the statistical significance between the male patients and controls (both $P < 0.05$). Statistical significance was found in VNTR between the two groups (all $P < 0.05$). The frequency of TTL was significantly lower in case group than in control group; the frequency of CTS, CCS was significantly higher in case group than in control group (all $P < 0.05$). *COL2A* (rs2276454) was correlated with Schneiderman level, the number of degenerative segments, herniation type and the number of herniated segments (all $P < 0.05$); rs2070739 was only correlated with Schneiderman level ($P < 0.05$); *Aggrecan* polymorphism (VNTR) was associated with Schneiderman level, the number of degenerative segments and the number of herniated segments (all $P < 0.05$). Conclusion: The *COL2A* and *Aggrecan* polymorphisms were associated with the susceptibility of IVDD; TTL, CTS and CCS might be the risk factors of IVDD.

**Keywords:** Intervertebral disc degeneration, *COL2A1*, *aggrecan*, polymorphism, haplotype analysis, correlation analysis

## Introduction

Clinical low back pain which was reported associated with intervertebral disc degeneration (IVDD) which was considered as a chronic common symptom suffered by 70%-90% of the population [1, 2]. IVDD involves a series of morphological and biochemical changes, leading to spinal instability, the compression on spinal cord and nerves [3]. IVDD remains the pathological foundation of most musculoskeletal diseases of the spine, including disc herniation, spinal stenosis, radiculopathy and myelopathy [4]. It was believed the IVDD was related with environmental factors such as age, occupation, and trauma. As previous studies showed, apart from environmental factors, the genetic factors could also influence the development of IVDD [5, 6]. By now, the genes reported relevant to IVDD included the genes related to collagen metabolism, inflammatory factor, bone metabolism and extracellular matrix [7-9].

Type II collagen (Col2), encoded by the COL2A1 gene on human chromosome 12, it is the most abundant extracellular protein produced cartilage-specific collagens [10]. COL2A1 was a specific proliferation gene of collagen type II which plays a critical part in the constitution of articular cartilages and was the major matrix maintaining the function of spine and joints. In recent years, the effect of *COL2A1* expression in the development of IVDD was concerned of increasing value [11, 12]. Aggrecan, a protein encoded by gene Aggrecan, is of critical importance in skeletal development, as a key molecular component of the cartilage templates in the process of endochondral ossification [13].

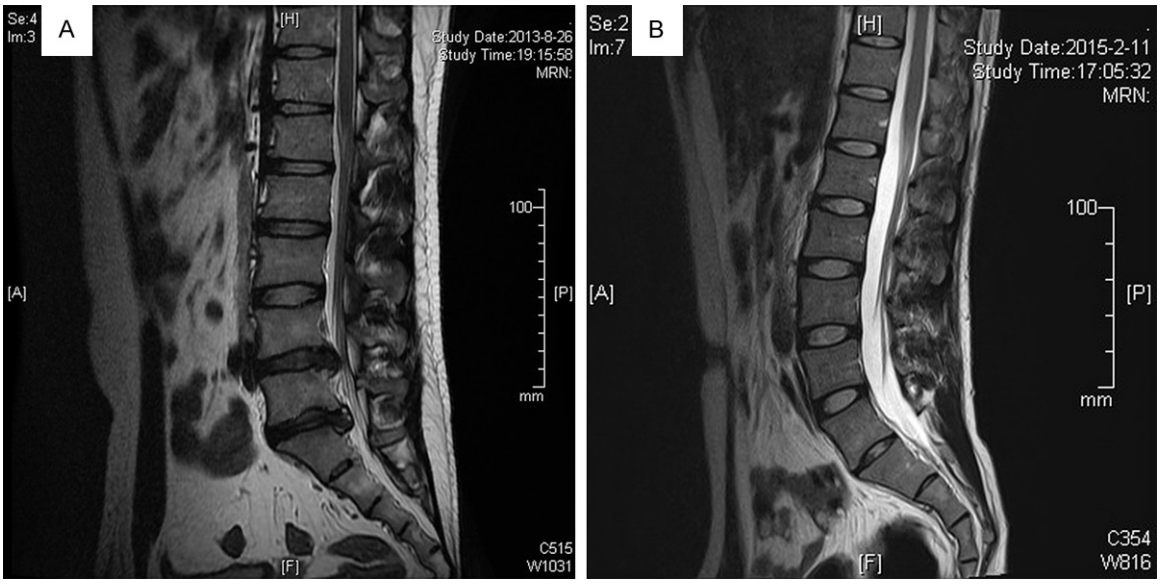


**Figure 1.** The comparison of MRI imaging between case group and control group. (A. Case group; B. Control group; MRI, magnetic resonance imaging).

Preceding gene sequence analysis on Aggrecan demonstrated repetitive sequences in a mode of serine amino-glycine bonding existed in repeating region CSI, which make it easier to produce Aggrecan polymeride, influencing the construction and function of cartilage tissue [14]. So far, some studies have reported that variable number of tandem repeats (VNTR) allele polymorphism may be associated with IVDD [15, 16]. Currently, few study investigated the associations of COL2A and *Aggrecan* polymorphisms and IVDD. In our study, we used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) to detect the polymorphic locus to explore the association between the polymorphisms of *COL2A1* and *Aggrecan* and the susceptibility of IVDD.

## Materials and methods

### *Ethical statement*

This study was approved by the Ethics committee of Rizhao People's Hospital. Study protocols were based on the ethical principles for medical research involving human subjects of the Helsinki Declaration. All participants provided written informed consent before participating in the research.

### *Participants*

IVDD patients (n = 354) in Department of Orthopedics, Rizhao People's Hospital between June 2010 and June 2013 were allocated to the case group, including 281 males and 73 females (age range, 23-72; average age 51.11 ± 13.04; average body mass index (BMI) 23.08 ± 2.8 kg.m$^2$). Healthy people (n = 310) who received physical examination in our hospital during the same period were allocated to the control group, including 248 males and 62 females, aging from 25-70; the average age was 48.56 ± 12.99 and the average BMI was 22.9 ± 3.1 kg.m$^2$. The age, gender, height and weight of both the groups were recorded. Inclusion criteria of case group: (1) Chinese Han people whose magnetic resonance imaging (MRI) presented lumbar IVDD or lumbar disc herniation (LDH) and were diagnosed with IVDD by clinical findings and examinations. (2) No neck shoulder pain or lumbocrural pain were reported before being diagnosed. (3) No diabetes, hypertension or other hereditary disease. Exclusion criteria: patients with obesity, smoking, heavy work intensity and have the medical history of spine trauma, rheumatism, rheumatoid arthritis, ankylosing spondylitis, metabolic bone disease, unequal limb length, tumor, spine infection or the spine deformity diagnosed in youth time. Inclusion criteria of control group: (1) Chinese Han people whose age and gender were matched with the patients in case group. (2) Mild or moderate work intensity. (3) No medical history of discogenic pain. Exclusion criteria: patients who were diagnosed or suspected with severe organic disease, endocrine disease, liver and renal disease, immune dis-

**Table 1.** Primer sequences of *COL2A1* and *Aggrecan*

| Gene | Sequence | |
|---|---|---|
| *COL2A1* rs2070739 | Forward | 5'-CCAGTGTACGTGAACCTGCTA-3' |
| | Reverse | 5'-ACCTACCACTGCAAGAACAG-3' |
| *COL2A1* rs2276454 | Forward | 5'-CTCCAGGTCTTCAGGGAAT-3' |
| | Reverse | 5'-TGAGAGGCTGTAACCTCAGT-3' |
| *Aggrecan* VNTR | Forward | 5'-TAGAGGGCTCTGCCTCTGGAGTTG-3' |
| | Reverse | 5'-AGGTCCCCTACCGCAGAGGTAGAA-3' |

Note: VNTR, variable number of tandem repeats.

ease and spinal or extraspinal deformity diagnosed in youth time.

*Radiographic assessment*

All of the patients in case group were received sagittal and axial lumbar MRI (1.5 T magnetic resonance imaging system, Siemens, German). The slice thickness was 5 mm. TR and TE value were set at 2500 ms and 9 ms respectively and T2 weighted image was selected. The signal of the nuclear of each lumbar IVD was assessed by spine surgeons to make the diagnosis of LDH and the axial image was referenced (**Figure 1**). By the assessment of MRI, no one in control group was found with IVDD or LDH and according to the Schneiderman standard [17], there were 205 patients in the case group diagnosed with IVDD level 2, 121 patients diagnosed with IVDD level 3 and 28 patients diagnosed with level 4 or beyond; There were 134 patients having 1 segment degenerated, 149 patients having 2 segments degenerated and 71 patients having 3 segments degenerated or more. According to the MacNab criteria [18], there were 13 protrusion type, 28 extrusion type and 9 sequestration type LDH patients and there were 152 patients having 1 segment herniated, 146 patients having 2 segments herniated and 57 patients having 3 segments herniated or more.

*Sample collection*

Peripheral venous blood (2 ml) was collected in the morning after the participants fasting 10-12 h, and then added into the anticoagulated vacuum tube with content of sodium citrate, preserved in -80°C. When then experiment started, sample was taken out and unfroze, then 300 μL of the sample was taken and incubated in 37°C water bath after adding in 800 μL cell lysis buffer. Next, TRIS saturated phenol was added and the sample mixture was centrifuged (10000 r/min), the supernate was discarded and isometric phenol-chloroform (1:1) was added, chloroform extraction was conducted. Then 2 ml isopropanol was added, precipitated and centrifuged (12000 r/min), the supernate was discarded. 75% ethanol was used for washing. The sample was dried and dissolved by 40 μL dnase-free waster. The concentration and purity of DNA was detected by ultraviolet spectrophotometer. The remaining sample was preserved in -30°C.

*Primer design for COL2A and Aggrecan and PCR-RFLP detection*

*COL2A* and *Aggrecan* single nucleotide polymorphisms (SNP) was detected by PCR-RFLP. Primer Premier 5.0 was used for the designing and verifying the PCR primer according to each locus of *COL2A* and *Aggrecan.* The primer was synthesized by Shanghai Sangon biotech co., Ltd. The primer sequence and PCR reaction conditions were presented in **Table 1**. The *COL2A1* PCR reaction mix was composed by: 4.0 μL template DNA, 14 μL 2 × Mix PCR buffer, 1.5 μL forward and reverse primers for each. PCR conditions: Initial denaturation at 95°C/5 min and 35 cycles under the following conditions: Denaturation at 94°C/45 s, annealing at 60°C/45 s and extension at 72°C/1 min. *Aggrecan* reaction mix was composed by: 9 μL ultrapure water, 10 × M PCR buffer 2 μL, 1 μL forward and reverse primers for each. 12 μL Premix Taq enzyme (Takara biotech Co., Ltd, Japan; The solution was composed by 0.2 μL Taq DNA polymerase, 3 mmol/L $Mg^{2+}$ and dNTP substrate 2 μL) PCR conditions: Initial denaturation at 95°C/5 min and 35 cycles under the following conditions: Denaturation at 94°C/30 s, annealing at 72°C/30 s and extension at 72°C/7 min. The digested products were run on 15 g/L agarose gel electrophoresis, visualized by staining with ethidium bromide and observed and imaged under ultraviolet gel imaging system. If PvuII restriction site was not found in *COL2A1*, the stripe could be observed near 331 bp. If PvuII exist, the two stripes could be observed near 211 bp and 120 bp. The allele of *Aggrecan* could be determined according to the table presenting the corresponding

**Table 2.** The frequency of *COL2A1* rs2276454 in case group and control group

| Genotype | Case group (n = 354) | Control group (n = 310) | $\chi^2$ | *P* | OR (95% CI) |
|---|---|---|---|---|---|
| rs2276454 | | | | | |
| TT | 50 (14.12%) | 115 (37.10%) | 46.462 | <0.001 | |
| TC | 226 (63.84%) | 142 (45.80%) | | | |
| CC | 78 (22.03%) | 53 (17.10%) | | | |
| TC+CC | 304 (85.88%) | 195 (62.90%) | 46.351 | <0.001 | 3.567 (2.446-5.204) |
| TT | 50 (14.12%) | 115 (37.10%) | | | |
| C | 382 (53.95%) | 248 (40.00%) | 25.741 | <0.001 | 1.755 (1.411-2.183) |
| T | 326 (46.05%) | 370 (60.00%) | | | |
| rs2070739 | | | | | |
| TT | 108 (30.51%) | 109 (35.16%) | 1.886 | 0.393 | |
| TC | 176 (49.72%) | 148 (47.74%) | | | |
| CC | 70 (19.77%) | 53 (17.10%) | | | |
| TT+TC | 284 (80.23%) | 257 (82.90%) | 0.785 | 0.376 | 0.837 (0.564-1.242) |
| CC | 70 (19.77%) | 53 (17.10%) | | | |
| T | 392 (55.37%) | 366 (59.03%) | 1.812 | 0.178 | 0.861 (0.692-1.071) |
| C | 316 (44.63%) | 254 (40.97%) | | | |
| C | 316 (44.63%) | 254 (40.97%) | | | |

Note: OR, odd ratio; CI, confidence interval.

relations between molecular weight and repeating times of VNTR.

*Statistical analysis*

Statistical analysis was processed on SPSS 19.0 (SPSS Inc, Chicago, IL, USA). Enumeration data were presented in the form of percentage or ratio. Chi-square test was used to detect the difference between gene distributions of each group and find if the gene distribution accorded with the Hardy-Weinberg equilibrium. Measurement data were presented as mean ± standard deviation (SD). T-test was used to detect the difference between the two groups. The risk of each genotype for getting IVDD was presented with odds ratio (OR) or 95% confidence interval (95% CI). The haplotype analysis of *COL2A1* and *Aggrecan* was processed on the software Shesis. Spearman rank correlation was used for the correlation analysis. *P* value less than 0.5 was considered statistically significant.

## Results

*The frequency distribution of COL2A1 polymorphism*

Statistical significance was found between case group and control group in the frequency of TT, TC, CC which were the three polymorphism of *COL2A1*, locus rs2276454 ($P < 0001$). The frequency of genotype TC+CC was significantly higher in case group than control group ($P < 0001$). The frequency of allele C was significantly higher in case group than control group ($P < 0.001$) and the risk for IVDD of allele C carriers were 1.755 times than the risk of allele T carriers (OR = 1.755; 95% CI = 1.411-2.183). No statistical significance was found between case group and control group in the rs2070739 polymorphism including the frequency of genotype TT, TC, or CC, genotype TT+TC or the allele T ($P > 0.393$) (**Table 2**). The subgroup analysis on rs2070739 polymorphism according to gender difference found statistical significance between case group and control group in the frequency of genotype TT, TC, CC ($P = 0.029$) and the frequency of genotype TT+TC was significantly higher in case group than control group ($P = 0.008$). The frequency of allele T was significantly higher in case group than control group ($P = 0.041$) and the risk for IVDD of allele C carriers were 0.758 times than the risk of allele T carriers (OR = 0.758; 95% CI = 0.581-0.988) (**Table 3**).

*The frequency distribution of Aggrecan polymorphism*

In present study, 12 different alleles of *Aggrecan* VNTR were detected and the segments repeating times ranged from A21 to A32. The frequency of A27 (25.42%) was found the

**Table 3.** The frequency of *COL2A1* rs2070739 in subgroup of gender

| rs2070739 | Women | | | | Men | | | | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| | Case group | Control group | $\chi^2$ | *P* | Case group | Control group | $\chi^2$ | *P* | |
| TT | 23 (31.50%) | 44 (36.36%) | 2.384 | 0.304 | 85 (29.93%) | 65 (34.39%) | 7.052 | 0.029 | |
| TC | 34 (46.58%) | 43 (35.54%) | | | 145 (51.06%) | 105 (55.56%) | | | |
| CC | 16 (21.92%) | 34 (28.10%) | | | 54 (19.01%) | 19 (10.05%) | | | |
| TT+TC | 57 (78.08%) | 87 (71.90%) | 0.904 | 0.34 | 230 (80.99%) | 170 (89.95%) | 6.982 | 0.008 | 0.476 (0.272-0.833) |
| CC | 16 (21.92%) | 34 (28.10%) | | | 54 (19.01%) | 19 (10.05%) | | | |
| T | 80 (54.79%) | 131 (54.13%) | 0.016 | 0.899 | 315 (55.46%) | 235 (62.17%) | 4.201 | 0.041 | 0.758 (0.581-0.988) |
| C | 66 (45.21%) | 111 (45.87%) | | | 253 (44.54%) | 143 (37.83%) | | | |

Note: OR, odd ratio; CI, confidence interval.

**Table 4.** The frequency of *Aggrecan* VNTR in case group and control group

| Type | The length of PCR products (bp) | Case group | Control group |
|---|---|---|---|
| 21 | 1231 | 19 (5.37%) | 20 (6.45%) |
| 22 | 1288 | 21 (5.93%) | 7 (2.26%) |
| 23 | 1345 | 5 (1.41%) | 6 (1.94%) |
| 24 | 1402 | 19 (5.37%) | 7 (2.26%) |
| 25 | 1459 | 64 (18.08%) | 48 (15.48%) |
| 26 | 1516 | 74 (20.90%) | 47 (15.16%) |
| 27 | 1573 | 90 (25.42%) | 82 (26.45%) |
| 28 | 1630 | 45 (12.71%) | 65 (20.97%) |
| 29 | 1687 | 17 (4.80%) | 17 (5.48%) |
| 30 | 1744 | 0 (0.00%) | 5 (1.61%) |
| 31 | 1801 | 0 (0.00%) | 3 (0.97%) |
| 32 | 1858 | 0 (0.00%) | 3 (0.97%) |

Note: VNTR, variable number of tandem repeats; PCR, polymerase chain reaction.

**Table 5.** The frequency of *Aggrecan* in case group and control group

| Type | Case group (n = 354) | Control group (n = 310) | $\chi^2$ | *P* |
|---|---|---|---|---|
| S | 202 (57.06%) | 135 (43.55%) | 12.29 | 0.001 |
| L | 152 (42.94%) | 175 (56.45%) | | |

Note: S, short sequence (A21-A26); L, long sequence (A26-A30).

highest and the next were A26 (20.90%) and A28 (12.71%) (**Table 4**). No statistical significance was found between case group and control group in the allele of *Aggrecan* VNTR according to the results of chi-square test ($P > 0.05$). After regrouping the alleles according to the VNTR repeating times, we found statistical significance in short sequenced VNTR alleles (A21-A26) and long sequenced VNTR alleles (A27-A32) between case group and control group ($\chi^2 = 12.29$, $P = 0.001$) (**Table 5**).

*Haplotype analysis*

The haplotype frequency distribution of the *COL2A1* locus rs2276454, rs2070739 and *Aggrecan* VNTR was presented in **Table 6**. The software Shesis was used for the haplotype analysis of different loci on *COL2A1* and *Aggrecan.* The haplotypes whose frequency were both less than 3% were neglected. The frequency of haplotype CTS and CCS was significantly higher in case group than control group ($\chi^2 = 9.01$ and 10.78; $P = 0.003$ and $P = 0.001$, respectively); No statistical significance was found between case group and control group in the frequency of haplotype TTL, TTS. TCL, TCS, CTS, CCL and CCS (all $P > 0.05$).

*Correlation analysis*

The correlation analysis on the risk factors of the loci of *COL2A* and *Aggrecan* demonstrated that locus rs2276454 of *COL2A* polymorphism was positively correlated with Schneiderman level, the number of degenerative segments, herniation type and the number of herniated segments (r = 0.193, 0.213, 0.236, 0.174, respectively; all $P < 0.05$), but not correlated with age, gender or BMI (all $P > 0.05$). Locus rs2070739 of *COL2A* polymorphism was positively correlated with Schneiderman level (r = 0.160, $P < 0.05$) but not correlated with every other factors (all $P > 0.05$). VNTR of *Aggrecan* polymorphism was positively correlated with Schneiderman level, the number of degenerative segments and the number of herniated segments (r = 0.258, 0.179,

**Table 6.** The haplotype analysis on *COL2A1* and *Aggrecan* in case group and control group

| Haplotype | | | Case group (n = 354) | Control group (n = 310) | $\chi^2$ | *P* | OR (95% CI) |
|---|---|---|---|---|---|---|---|
| COL2A1 rs2276454 | COL2A1 rs2070739 | Aggrecan VNTR | | | | | |
| T | T | L | 39 (11.02%) | 62 (20.00%) | 5.209 | 0.023 | 0.568 (0.347-0.927) |
| T | T | S | 51 (14.41%) | 48 (15.48%) | 0.151 | 0.698 | 0.919 (0.803-2.705) |
| T | C | L | 31 (8.76%) | 43 (13.87%) | 4.365 | 0.037 | 0.596 (0.365-0.972) |
| T | C | S | 42 (11.86%) | 33 (10.65%) | 0.245 | 0.621 | 1.130 (0.697-1.833) |
| C | T | L | 45 (12.71%) | 41 (13.23%) | 0.039 | 0.844 | 0.956 (0.607-1.504) |
| C | T | S | 60 (16.95%) | 32 (10.32%) | 6.08 | 0.014 | 1.773 (1.120-2.807) |
| C | C | L | 37 (10.45%) | 29 (9.35%) | 0.222 | 0.637 | 1.131 (0.678-1.887) |
| C | C | S | 49 (13.84%) | 22 (7.10%) | 7.874 | 0.005 | 2.103 (1.240-3.567) |

Note: VNTR, variable number of tandem repeats; short sequence (A21-A26); L, long sequence (A26-A30); OR, odd ratio; CI, confidence interval.

**Table 7.** The correlation analysis on *COL2A1* and *Aggrecan*

| | COL2A1 rs2276454 | COL2A1 rs2070739 | *Aggrecan VNTR* |
|---|---|---|---|
| Age | 0.127* | 0.121* | 0.054 |
| Gender | -0.005 | 0.062 | 0.046 |
| BMI | 0.105* | 0.104 | 0.014 |
| Schneiderman level | 0.154* | 0.122* | 0.153* |
| Number of degenerative segments | 0.108* | 0.081 | 0.137* |
| Herniation type | 0.126* | 0.150* | 0.139* |
| Number of herniated segments | 0.165* | 0.150* | 0.109* |

Note: VNTR, variable number of tandem repeats; BMI, body mass index; *means $P < 0.05$.

0.109, respectively; all $P < 0.05$), but not correlated with age, gender, BMI or herniation type (all $P > 0.05$) (**Table 7**).

*Logistic regression analysis of the risk factors of IVDD*

The onset of IVDD was taken as dependent variable and age, BMI, locus rs2276454 and rs2070739 of *COL2A1*, *Aggrecan* VNTR as independent variables. As the logistic regression analysis presented, correlation could be found between IVDD and BMI, locus rs2276454, locus rs2070739 and *Aggrecan* VNTR ($P < 0.001$, OR (95% CI) = 0.578 (0.525-0.637); $P < 0.001$, OR (95% CI) = 8.827 (5.533-14.082); $P < 0.001$, OR (95% CI) = 0.358 (0.191-0.669); $P = 0.004$, OR (95% CI) = 1.916 (1.234-2.977), respectively), which were considered as the independent risk factors of IVDD (**Table 8**).

## Discussion

As a process demonstrating the aging of human tissues, IVDD is regarded as the foundation and basis for the research of LDH [19]. Current evidence indicates that heredity was taken as the main cause of IVDD with the combination of environmental conditions [20]. By researching the mechanism of genetic polymorphism, genetic biotherapy methods could be developed basing on the intervention of gene expression regulation. Consequently, the pathologic progress of IVD could be reversed or corrected and the IVDD could be cured [21]. The aim of our study is to discuss the correlation between the polymorphism of *COL2A1* and *Aggrecan* and the susceptibility of IVDD by our analysis on the experimental data. Our study results suggested that *COL2A* and *Aggrecan* polymorphisms were associated with the susceptibility of IVDD; TTL, CTS and CCS might be the risk factors of IVDD.

*COL2A1* is a gene contributes to the regulation of the synthesis of type II collagen [22]. The declination of *COL2A1* expression would imbalance the stability between type II collagen and proteoglycan, loosening the inner collagen fibers of vertebral pulp which results in destroyed biodynamic of vertebral pulp and finally causing IVDD [23]. The length of COL2A1 is 31538 bp, including 54 exons, encoding the N terminal region of type II collagen, the core region of the DNA triplex structure and the C terminal region. The intron of COL2A1 is 4105 bp in length, including two polymorphic loci, regulating the transcription of COL2A1 [24]. In present study, we respectively allocated 354 IVDD patients and 310 healthy people into

**Table 8.** The logistic regression analysis on the risk factors of IVDD

| Regression coefficient | *P* | OR | 95% CI |
|---|---|---|---|
| Age | 0.172 | 0.012 | 0.995-0.030 |
| BMI | 0.001 | 0.578 | 0.525-0.637 |
| COL2A1 rs2276454 | 0.001 | 8.827 | 5.533-14.082 |
| COL2A1 rs2070739 | 0.001 | 0.358 | 0.191-0.669 |
| Aggrecan VNTR (L/S) | 0.004 | 1.916 | 1.234-2.977 |

Note: IVDD, intervertebral disc degeneration; BMI, body mass index; OR, odd ratio; CI, confidence interval; VNTR, variable number of tandem repeats.

case group and control group and two different polymorphic loci were located and studied. The result demonstrated genotype TC+CC of *COL2A1* rs2276454 was higher in case group than control group, inferring the polymorphism might be associated with IVDD. Besides, the analysis on the polymorphism of rs2070739 indicted the frequency of genotype TT+TC in male participants was higher in case group than control group, inferring rs2070739 might be associated with IVDD in male populations. Furthermore, we proved our deduction by using Spearman rank correlation and regression analysis. As the results presented, rs2276454 and rs2070739 of *COL2A1* were both related predisposing factor of IVDD. Rs2276454 was positively correlated with Schneider man level, the number of degenerative segments, herniation typeand the number of herniated segments and rs2070739 was positively correlated with Schneider man level.

Previous study has reported that Aggrecan, which is considered as the main proteoglycan type lost in IVDD, plays an instrumental role in the weighing capacity of IVD [25]. Aggrecan is a protein expressed by Aggrecan [26]. The polymorphism of *Aggrecan* control region could influence the amount of aggrecan expressed and the declination or ceasing of *Aggrecan* expression might reduce the bonding between proteoglycan and collagen, causing the weakened viscoelasticity of cartilage tissue and resulting in a broken biomechanical integrity of cartilage endplate in IVD which is an initial manifestation of IVDD [27]. Because of the *Aggrecan* VNTR structure, the study on *Aggrecan* was promoted with a molecular biological significance on the research of IVDD [16]. In present study, statistical significance was found in the frequency of *Aggrecan* VNTR between case group and control group and the frequency of short sequence VNTR in case group was higher than long sequence VNTR, indicating the short sequence VNTR of *Aggrecan* might be associated with IVDD. As the haplotype analysis of our study presented, The frequency of TTL was significantly lower in case group than control group and the frequency of CTS, CCS was significantly higher in case group than control group, manifesting the association between TTL, CTS, CCS and IVDD. The further analysis on genetic locus and regression analysis demonstrated the positive correlation between the VNTR polymorphism of *Aggrecan* and some evaluation index of IVDD, including the Schneider man level, number of degenerative segments, herniation type and the number of herniated segments.

The result of our study indicated the association between the polymorphisms of *COL2A1* and *Aggrecan* and the susceptibility of IVDD. Haplotype TTL, CTS and CCS might be the risk factors of IVDD. However, the mechanism of the process in which *COL2A1* and *Aggrecan* contribute to IVDD still remains to discover.

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## Disclosure of conflict of interest

None.

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## References

[1] Kadow T, Sowa G, Vo N, Kang JD. Molecular basis of intervertebral disc degeneration and herniations: what are the important translational questions? Clin Orthop Relat Res 2015; 473: 1903-1912.

[2] Luan S, Wan Q, Luo H, Li X, Ke S, Lin C, Wu Y, Wu S, Ma C. Running exercise alleviates pain and promotes cell proliferation in a rat model of intervertebral disc degeneration. Int J Mol Sci 2015; 16: 2130-2144.

[3] Omlor GW, Bertram H, Kleinschmidt K, Fischer J, Brohm K, Guehring T, Anton M, Richter W. Methods to monitor distribution and metabolic

activity of mesenchymal stem cells following in vivo injection into nucleotomized porcine intervertebral discs. Eur Spine J 2010; 19: 601-612.
[4] Wang WJ, Yu XH, Wang C, Yang W, He WS, Zhang SJ, Yan YG, Zhang J. MMPs and ADAMTSs in intervertebral disc degeneration. Clin Chim Acta 2015; 448: 238-246.
[5] Battie MC, Videman T, Levalahti E, Gill K, Kaprio J. Heritability of low back pain and the role of disc degeneration. Pain 2007; 131: 272-280.
[6] Tang Y, Wang S, Liu Y, Wang X. Microarray analysis of genes and gene functions in disc degeneration. Exp Ther Med 2014; 7: 343-348.
[7] Phillips KL, Jordan-Mahy N, Nicklin MJ, Le Maitre CL. Interleukin-1 receptor antagonist deficient mice provide insights into pathogenesis of human intervertebral disc degeneration. Ann Rheum Dis 2013; 72: 1860-1867.
[8] Wang Z, Weitzmann MN, Sangadala S, Hutton WC, Yoon ST. Link protein N-terminal peptide binds to bone morphogenetic protein (BMP) type II receptor and drives matrix protein expression in rabbit intervertebral disc cells. J Biol Chem 2013; 288: 28243-28253.
[9] Li YY, Diao HJ, Chik TK, Chow CT, An XM, Leung V, Cheung KM, Chan BP. Delivering mesenchymal stem cells in collagen microsphere carriers to rabbit degenerative disc: reduced risk of osteophyte formation. Tissue Eng Part A 2014; 20: 1379-1391.
[10] Niebler S, Bosserhoff AK. The transcription factor activating enhancer-binding protein epsilon (AP-2epsilon) regulates the core promoter of type II collagen (COL2A1). FEBS J 2013; 280: 1397-1408.
[11] Chen K, Wu D, Zhu X, Ni H, Wei X, Mao N, Xie Y, Niu Y, Li M. Gene expression profile analysis of human intervertebral disc degeneration. Genet Mol Biol 2013; 36: 448-454.
[12] Yang L, Sun X, Geng X. Effects of psoralen on chondrocyte degeneration in lumbar intervertebral disc of rats. Pak J Pharm Sci 2015; 28: 667-670.
[13] Aspberg A. The different roles of aggrecan interaction domains. J Histochem Cytochem 2012; 60: 987-996.
[14] Kim NK, Shin DA, Han IB, Yoo EH, Kim SH, Chung SS. The association of aggrecan gene polymorphism with the risk of intervertebral disc degeneration. Acta Neurochir (Wien) 2011; 153: 129-133.
[15] Eser B, Cora T, Eser O, Kalkan E, Haktanir A, Erdogan MO, Solak M. Association of the polymorphisms of vitamin D receptor and aggrecan genes with degenerative disc disease. Genet Test Mol Biomarkers 2010; 14: 313-317.
[16] Cong L, Zhu Y, Pang H, Guanjun TU. The interaction between aggrecan gene VNTR polymorphism and obesity in predicting incident symptomatic lumbar disc herniation. Connect Tissue Res 2014; 55: 384-390.
[17] Macnab I. Negative disc exploration. An analysis of the causes of nerve-root involvement in sixty-eight patients. J Bone Joint Surg Am 1971; 53: 891-903.
[18] Hou T, Zhou Q, Dai F, Luo F, He Q, Zhang J, Xu J. Repeated microendoscopic discectomy for recurrent lumbar disk herniation. Clinics (Sao Paulo) 2015; 70: 120-125.
[19] He X, Liang A, Gao W, Peng Y, Zhang L, Liang G, Huang D. The relationship between concave angle of vertebral endplate and lumbar intervertebral disc degeneration. Spine (Phila Pa 1976) 2012; 37: E1068-1073.
[20] Hanaei S, Abdollahzade S, Khoshnevisan A, Kepler CK, Rezaei N. Genetic aspects of intervertebral disc degeneration. Rev Neurosci 2015; 26: 581-606.
[21] Kalb S, Martirosyan NL, Kalani MY, Broc GG, Theodore N. Genetics of the degenerated intervertebral disc. World Neurosurg 2012; 77: 491-501.
[22] Tarpey PS, Behjati S, Cooke SL, Van Loo P, Wedge DC, Pillay N, Marshall J, O'Meara S, Davies H, Nik-Zainal S, Beare D, Butler A, Gamble J, Hardy C, Hinton J, Jia MM, Jayakumar A, Jones D, Latimer C, Maddison M, Martin S, McLaren S, Menzies A, Mudie L, Raine K, Teague JW, Tubio JM, Halai D, Tirabosco R, Amary F, Campbell PJ, Stratton MR, Flanagan AM, Futreal PA. Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. Nat Genet 2013; 45: 923-926.
[23] Hwang DW, Kim KT, Lee SH, Kim JY, Kim DH. Association of COL2A1 gene polymorphism with degenerative lumbar scoliosis. Clin Orthop Surg 2014; 6: 379-384.
[24] Li N, Yu J, Cao X, Wu QY, Li WW, Li TF, Zhang C, Cui YX, Li XJ, Yin ZM, Xia XY. A novel p. Gly630Ser mutation of COL2A1 in a Chinese family with presentations of Legg-Calve-Perthes disease or avascular necrosis of the femoral head. PLoS One 2014; 9: e100505.
[25] Eser O, Eser B, Cosar M, Erdogan MO, Aslan A, Yildiz H, Solak M, Haktanir A. Short aggrecan gene repetitive alleles associated with lumbar degenerative disc disease in Turkish patients. Genet Mol Res 2011; 10: 1923-1930.
[26] Nia HT, Ortiz C, Grodzinsky A. Aggrecan: approaches to study biophysical and biomechanical properties. Methods Mol Biol 2015; 1229: 221-237.
[27] Sivan SS, Wachtel E, Roughley P. Structure, function, aging and turnover of aggrecan in the intervertebral disc. Biochim Biophys Acta 2014; 1840: 3181-3189.