Original Article
Relationship between the exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration
in a Chinese population

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Abstract: It is estimated that at least 30% of the population has some degree of vertebral disc degeneration by the time they reach 40 years of age, and a finding of disc degeneration is often considered normal in patients over the age of 60. Despite the frequency at which disc degeneration occurs and its often painful and debilitating consequences, the exact cause of disc degeneration remains unclear. To investigate the relationship between the exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration in a Chinese population, 239 patients were enrolled in this study: 119 patients with lumbar disc degeneration and 120 without intervertebral disc disease. All study participants were patients in our hospital between July 2013 and May 2015. Genomic DNA was isolated from all subjects, and COL9A2 genotypes were determined by polymerase chain reaction (PCR) and direct DNA sequencing in a case-control study. Statistical analysis was performed using a chi-square test and Fisher’s exact test. Gln326 replacement with Trp was observed in 6 patients in the case group and 13 patients in the control group. The difference in the prevalence of Gln326 replacement to Trp between the case group and the control group was not statistically significant (P > 0.05). Gln326 replacement with Arg was observed in 72 patients in the case group and 64 patients in the control group. The difference in the prevalence of Gln326 replacement to Arg between the case group and the control group was not statistically significant (P > 0.05). MRI analysis of patients with lumbar disc degeneration showed no association between Arg326, Gln326, or Trp326 and the location of disc degeneration or the number of degenerated segments (both P > 0.05). In brief, the data do not support an association between exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration in this Han Chinese population.

Keywords: Lumbar disc degeneration, collagen XI, polymorphism

Introduction
Degeneration of the lumbar intervertebral disc is a general term for the accumulated biological changes that occur in the lumbar intervertebral disc tissue over a patient’s lifetime and for a variety of reasons. Degeneration causes changes in the mechanical properties of the disc so that the adjacent bones and joints experience corresponding degenerative changes. The overall results may include spinal instability and even compression of nerve roots, the spinal cord, and vertebral arteries. These changes cause a corresponding syndrome of clinical symptoms and signs, and serve as the pathological basis for most spinal column diseases [1]. The pathogenesis of vertebral disc degeneration is not entirely clear, but with the progress of research in genetics and molecular biology it has been found that specific genes are associated with some intervertebral disc lesions. Solovieva, et al., indicated that single nucleotide polymorphism (SNP) of interleukin-1 had an association with the degeneration of intervertebral discs [2]. Battié, et al., found that genetic factors had a significant effect on intervertebral disc degeneration, suggesting that heredity may explain as much as 74% of the variance in disc degeneration in adult populations [3]. Other genes associated with intervertebral disc degeneration include type XI collagen Al (COL11A1), matrix metalloproteinase-3 (MMP3), MMP9, aggrecan 1 (AGC1), cartilage intermediate layer protein (CILP), and thrombospondin (THBS2) [4-9].
Olmarker, et al., confirmed that the COL9A2 gene was closely associated with degenerative disease of intervertebral discs [10]. The relationship between the Gln326Trp polymorphism of the COL9A2 gene and intervertebral disc degeneration was reported by Hirose, et al., who noted that the prevalence of the polymorphism of specific genes varies in different regions and ethnic groups [11]. The current study investigated the relationship between the Gln326 polymorphism of the COL9A2 gene of Chinese Han people and the degenerative lesions of lumbar intervertebral discs.

Subjects and methods

Study subjects

The case group was made up of 119 patients with a clinical diagnosis of lumbar disc hernia who received inpatient treatment at the Department of Spine Surgery of the Second People’s Hospital of Liaocheng City from July 2013 to May 2015. Among them, 95 were men (79.8%) and 24 were women (20.2%). The mean age was 32.5 ± 8.2 years. The control group was made up of 120 patients with non-lumbar disc hernia. Among them, 92 were men (76.7%) and 28 were women (23.3%). The mean age was 34.1 ± 7.9 years. For all members of the case group, clinical examination and magnetic resonance imaging (MRI) confirmed the diagnosis of lumbar disc hernia, and imaging verified that the Pfirrmann’s grade was over grade III. All patients in the control group were volunteers with other diseases without obvious degeneration of the lumbar intervertebral discs visible on MRI. The patients in both the case group and the control group were free from chronic diseases such as hypertension and diabetes mellitus as well as other genetic diseases. There was no significant difference between the case group and the control group in terms of the patients’ sex and age (P > 0.05), and there was a comparability between the two groups. This study was approved by the Ethics Committee of the hospital and obtained the informed consent of all subjects.

Method

Main instruments and reagents: PCR Master Mix (Fermentas Corporation), Tris saturated phenol (PolePolar Biotechnology Co., LTD.), cell lysis buffer (0.5% SDS, 3 mmol EDTA, 0.1 mmol NaCl, 0.05 mol Tris, pH 8.0), agarose, spectrophotometer (Germany Eppendorf), thermal cycler (Germany Eppendorf), electrophoresis apparatus, low-temperature high-speed centrifuge, etc. Gene sequencing and primer synthesis were completed by the Beijing Microread Gene Technology Co., Ltd.

Magnetic resonance imaging (MRI) evaluation: Patients underwent lumbar vertebral MRI scan (slice thickness of 4 mm; GE 1.5 T, Milwaukee), sagittal T2WI (echo time of 98-110 ms and repetition time of 2640-2800 ms). Results were analyzed by radiologists unrelated to this study. An evaluation was performed according to Pfirrmann’s intervertebral disc degeneration grading system [12]. The degeneration grading of discs L1/2-L5/S1 as well as the position and grade of the most degenerative segments were recorded.

Extraction of genomic DNA: Blood samples (2 ml) were collected from each patient, combined with sodium citrate, and stored at -80°C. DNA was extracted by combining 300 μl of blood with 800 µl of lysis buffer and incubating at 37°C for 4 hours. Next, 500 µl of Tris-saturated phenol was added and the samples were centrifuged at 10,000 rpm for 20 minutes. The supernatant was extracted and combined with an equal volume of 1:1 phenol-chloroform. Following chloroform extraction, 1 ml of dimethyl carbinol was added to precipitate the DNA, and samples were centrifuged for 10 min at 12,000 rpm. The supernatant was discarded and the precipitate was washed with 75% ethanol. The precipitates were dried, and then suspended in 40 µl of DNAse-free water. DNA was quantified with an ultraviolet spectrophotometer and stored at -30°C.

Amplification and sequencing of target segments

Premier 5 software was used for primer design. The sequence of the downstream primer was 5’-CAAGA GGTGG TGATT GAGCA AGAGC-3’. The sequence of the upstream primer was 5’-TGGAT CTCAG TTTCC CTACCTG-3’. For DNA amplification 4 ml template was combined with 25 mL of 2x mix PCR buffer, 1.5 mL of the upstream and downstream primers (10 pmol/L) and sterile deionized water to a total volume of 50 mL. The PCR reaction procedure was as follows: pre-degeneration at 94°C for 5 minutes,
COL9A2 polymorphisms and lumbar disc degeneration

Table 1. Analysis of the collagen IXA2 gene for sequence variations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gln326</th>
<th>Arg326</th>
<th>Trp326</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient group (n=119)</td>
<td>41</td>
<td>72</td>
<td>6</td>
</tr>
<tr>
<td>Control group (n=120)</td>
<td>43</td>
<td>64</td>
<td>13</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>0.355*</td>
<td>1.854#</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>0.551*</td>
<td>0.173#</td>
<td></td>
</tr>
</tbody>
</table>

Note: * means Gln326 vs. Arg326, # means Gln326 vs. Trp326.

Table 2. Analysis of Pfirrmann’s grade in patients with lumbar disc degeneration

<table>
<thead>
<tr>
<th>Grade</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln326 (n=41)</td>
<td>9</td>
<td>32</td>
<td>0.250</td>
</tr>
<tr>
<td>Arg326 (n=72)</td>
<td>26</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Trp326 (n=6)</td>
<td>1</td>
<td>5</td>
<td></td>
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</table>

Note: * Fisher’s exact test.

Table 3. Analysis for location of intervertebral disc degeneration in patients with lumbar disc degeneration

<table>
<thead>
<tr>
<th>Location</th>
<th>L4/5</th>
<th>L5/S1</th>
<th>$P^*$</th>
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<tbody>
<tr>
<td>Gln326 (n=30)</td>
<td>10</td>
<td>20</td>
<td>0.560</td>
</tr>
<tr>
<td>Arg326 (n=62)</td>
<td>27</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Trp326 (n=4)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Note: * Fisher’s exact test.

Table 4. Analysis of the number of degenerated intervertebral discs in patients with lumbar disc degeneration

<table>
<thead>
<tr>
<th>Discs</th>
<th>Single</th>
<th>Multiple</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln326 (n=41)</td>
<td>30</td>
<td>11</td>
<td>0.134</td>
</tr>
<tr>
<td>Arg326 (n=72)</td>
<td>62</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Trp326 (n=6)</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Note: * Fisher’s exact test.

Gene polymorphism analysis on the 19th exon of the COL9A2 chain of all subjects found that the replacement from Gln326 to Trp was present in 6 patients in the case group and 13 patients in the control group. The difference between the case group and the control group in terms of the replacement frequency from Gln326 to Trp was of no statistical significance ($P > 0.05$). The replacement from Gln326 to Arg was present in 72 patients in the case group and 64 patients in the control group. The difference between the case group and the control group in terms of the frequency of replacement from Gln326 to Arg was of no statistical significance ($P > 0.05$). See Table 1.

Analysis of the relationship of Arg326, Gln326, and Trp326 with the degree of lumbar intervertebral disc degeneration

MRI analysis was performed on the lumbar spine of all patients in the case group. There were no significant differences in degree of degeneration, position of degenerative segments, or the number of degenerative segments among patients with Arg, Gln, or Trp at position 326 in the COL9A2 gene ($P > 0.05$). See Tables 2-4; Figure 1.

Discussion

The function of human intervertebral discs includes buffering stresses from all directions during normal activity [13]. Degeneration of intervertebral discs can begin at a relatively early age. Patients can experience intervertebral disc degeneration as early as age 20, thereby inducing pathological changes such as herniated discs and zygapophysial joint degeneration. These changes can lead to clinical degenerative changes such as vertebral canal stenosis and protrusion of the intervertebral disc [14]. When attempting to identify the factors that induce degenerative changes in intervertebral discs, current studies frequently focus on gene polymorphisms. Many of the can-
COL9A2 polymorphisms and lumbar disc degeneration

Candidate genes for degenerative diseases of lumbar intervertebral discs are genes related to the structure, composition, and metabolism of the discs.

One candidate, IX collagen, is encoded by the COL9A1, COL9A2, and COL9A3 genes, and is a heterotrimer consisting of 3 chains. The synthesis and cellular source of type IX collagen in human intervertebral discs are similar to those seen in articular cartilage, suggesting that collagen type IX in human intervertebral discs has similar functions. In the cases of intervertebral disc degeneration, the expression of the type IX collagen gene decreases or stops. This induces decreased stability between proteoglycan and type II collagen. The adhesion capacity between the collagen filaments within the pulpiform nucleus decreases, thereby undermining the biomechanical integrity of the pulpiform nucleus and leading to the degeneration of the disc [15]. Seki, et al, indicated that COL9A2 haplo-
COL9A2 polymorphisms and lumbar disc degeneration

type was associated with serious intervertebral disc diseases. They suggest that the differences in the distribution of genetic susceptibility among different regions and different races were of statistical significance [16]. The great differences among different regions and different races in terms of COL9A2 gene polymorphism increase the difficulty of studying this possibility. This study was a case control study, in which the relationship between the polymorphism of Gin326 in the COL9A2 gene of Chinese Han people and the degenerative lesions of lumbar intervertebral discs were observed.

The study found that the 19th exon of the COL9A2 chain had Gin326Arg and Gin326Trp polymorphisms. The difference between the case group and the control group in terms of the Gin326Arg and Gin326Trp polymorphism was of no statistical significance (P > 0.05). This suggested that, among the Chinese Han people, the polymorphism of the 19th exon of COL9A2 chain might be unrelated to the degenerative lesions of lumbar intervertebral discs. The results of the studies carried out by Knoeringer, et al, were similar: a study carried of 288 German patients who underwent surgery for protrusion of lumbar intervertebral discs indicated that COL9A2 allelic gene was unrelated with lumbar intervertebral disc degeneration among the German population [17]. In the present study, further analysis on the case group indicated that the Gin326Arg and Gin326Trp polymorphism of the 19th exon of COL9A2 were unrelated to the degree of lumbar intervertebral disc degeneration, position of degenerative segments, and quantity of degenerative segments.

Higashino, et al, indicated that adults under the age of 40 with COL9A2 allelic gene Trp2 were more apt to experience degeneration of lumbar intervertebral discs when compared with the normal control group [18]. The results indicated that there was a difference among difference races in terms of the predisposing genes of degeneration of lumbar intervertebral discs, and the genes predisposing the Chinese patients to the degeneration of lumbar intervertebral discs are unique. In the meantime, due to the different genetic backgrounds of the Chinese population in different regions such as the different frequencies of some allelic genes among the difference ethnic groups and Han groups in North China and South China, the characteristics and nature of a certain allelic gene may be affected.

In conclusion, among the Chinese Han people, the polymorphism of the 19th exon of COL9A2 chain might be unrelated with the degenerative lesions of lumbar intervertebral discs.

Disclosure of conflict of interest

None.

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References


