Association between Caspase-9 promoter region polymorphisms and discogenic low back pain

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Abstract

Caspase-9 (CASP-9) is an initiator caspase protease for apoptosis, and plays an important role in the development and progression of lumbar disc disease (LDD). The expression and/or activity of CASP-9 are significantly enhanced in the degenerated disc. The polymorphism in the promoter region of CASP-9 enhances the transcriptional activity of this gene, thereby modulating the susceptibility to LDD. The current study investigated the relationship between the CASP-9 -1263A/G (rs4645978) and -712C/T (rs4645981) polymorphisms and discogenic low back pain (LBP). The CASP-9 -1263A/G and -712C/T genotypes in this study were defined by polymerase chain reaction in 154 patients with discogenic LBP and 216 controls that were frequency-matched by age, gender, and occupation. The results showed that the CASP-9 -1263 GG genotype, compared with the AA and AG genotypes [odds ratio (OR) = 1.997, 95% confidence interval (95% CI) = 1.216–3.279, \( p = 0.006 \)] or the AA genotype (OR = 2.760, 95% CI = 1.464–5.203, \( p = 0.002 \)), is associated with a significant increased risk of discogenic LBP, but the -712 TT or TT and CT genotypes do not contribute to discogenic LBP compared with the CC genotype (OR = 0.547, 95% CI = 0.200–1.494, \( p = 0.234 \) and OR = 0.669, 95% CI = 0.439–1.021, \( p = 0.062 \), respectively). These results indicated that the CASP-9 -1263A/G polymorphism is associated with a high risk of discogenic LBP.

Keywords: Caspase-9, disc degeneration, discogenic low back pain, gene polymorphism, association

INTRODUCTION

Low back pain (LBP) affects 70–80% of all population during their lifetime and is the most common cause for activity limitation in individuals younger than 45 years of age [1]. The annual prevalence ranges from 15% to 45%. The inappropriate degeneration of intervertebral discs in the lumbar spine is a primary cause of LBP. Discogenic LBP is the most common disease of chronic LBP, accounting for 39% of its incidence [2]. Lumbar disc herniation (LDH) represents less than 30% of cases, and other causes, such as zygapophysial joint pain, are responsible for an even lower proportion of LBP cases [3,4].

Lumbar disc disease (LDD) refers to the drying out of the spongy interior matrix of an intervertebral disc in the spine. The pathological degeneration of disc initiates LDD. However, the understanding of the pathogenesis of LDD has been very limited and still controversial. Increasing evidence suggests that genetic factor plays an important role in leading to symptomatic intervertebral disc diseases. LDD shows a strong familial predisposition [5], and the association between predisposing genetic factors and degenerative discs has been identified [6,7]. Sun et al. [8] observed the correlation between matrix metalloproteinase-9 (MMP-9) gene polymorphism and the etiology of LDD, and demonstrated that MMP-9 -1562 TT genotype is significantly related to increased risk of LDD. Solovieva et al. [9,10] have found that interleukin-1 gene locus polymorphisms have an influence on the pathogenesis of LDD. Many other reports also reveal predispositions in association with certain genes, such as Taq I polymorphism of vitamin-D receptor [11], collagen IX (COL9A2 and COL9A3) [12,13], collagen I (COL1A1) [14], aggrecan [15], asporin (ASPN) [16], MMP-1 [17], MMP-2 [18], and MMP-3 [19].

Cells are vital machinery for synthesizing and maintaining the function of matrix in all tissues including the intervertebral discs within the spine. Apoptosis is a genetically controlled mechanism considered to be important for tissue homeostasis. At present, the in vivo apoptosis indices of human intervertebral disc cells as reported by some investigators are too high to be reasonable [20,21]. Inappropriate cellular apoptosis...
MATERIALS AND METHODS

Study Population

The subjects of this case–control study were selected from more than 1787 patients of lumbar spine treated in the Department of Orthopaedics Surgery at the First Affiliated Hospital of Xi’an Jiaotong University and the Department of Spinal Surgery of Xi’an Tangchengu Hospital from August 2007 to November 2009. The patients that suffered from sciatica of disc herniation or protrusion, zygapophysial joint pain, lumbar spinal stenoses or spondylolistheses, previous fractures of lumbar spine, malignancies or tuberculoses involving intervertebral discs contributed to the initiation and development of disc degeneration [22]. Apoptotic cell death is orchestrated by the activation of a cascade of enzymes called caspases (CASP). Caspase-9 (CASP-9) is an important protease in the principal death-signalling pathway of disc cells [23]. Recently, several candidate polymorphisms in CASP-9 gene have been reported in the public databases (http://www.ncbi.nlm.nih.gov/SNP) [24]. As the expression and/or activity of CASP-9 are influenced by its genotypes, it has been potentially associated with the risk of LDD. Therefore, we hypothesized that CASP-9 gene locus polymorphisms may cause predisposition to the development of LDD due to the increased activity of CASP-9. To test this hypothesis, a case–control study was performed. We investigated the relationship between the -1263A/G (rs4645978) and -712C/T (rs4645981) polymorphisms in promoter region of CASP-9 gene and symptomatic subjects with discogenic LBP.

of chronic LBP but did not show the evidence of nerve root compression and disc herniation on computer tomography (CT) or magnetic resonance imaging (MRI). All patients showed annular disruption and pain duplication on injection of contrast medium during discography. The conventional MRI scan was performed after discography in each case to identify the location of annular tears and the grade of disc degeneration.

Discography was performed in cases by standard posterolateral extrapedicular approach using a single 22-gauge or 25-gauge spinal needle. The contrast agent was injected under fluoroscopy when the needle tip reached the center of disc. To lower the false-positive rate, the discography was also performed in accordance with the following standards: (1) the contrast agent injected at the open pressure ≤50 psi and the speed ≤0.08 ml/sec [25,26], (2) the amount of injected contrast agent 1.5–3 ml, (3) the visual analog scale (VAS) of consistent pain ≥27, and (4) the negative control of the near disc.

The MRI examination was applied after discography (Magnetom 1.5 T, Siemens AG, Kemnath, Germany). All scans were performed for sagittal images using T1-weighted spin echo and T2-weighted turbo spin echo sequences with a slice thickness of 5 mm. The T1-weighted images were acquired with an echo time (TE) of 30 msec and a repetition time (TR) of 500 msec. The T2-weighted images were acquired with a TE of 90 msec and a TR of 2500 msec. MRI scans after discography showed pain reproduction and abnormal morphology with annular tears extending either well into or through the outer third of the annulus fibrosus. The main clinical data of the studied subjects are summarized in Tables 1 and 2.

All the recruited subjects had received written information about the study procedures and provided informed consent before participation. The protocol of the present study was approved by the Ethical
Committees of both the Public Health Department of Shaanxi province and Xi’an Jiaotong University.

**SNP Genotyping**

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes by proteinase K digestion and phenol/chloroform extraction. DNA quality and quantity were measured by spectrophotometry (NanoDrop ND-100, Foster, USA). The Mass ARRAY AssayDesign software (Sequenom, Salt Lake City, USA) was used to design the amplification and allele-specific primer extension (ASPE). Oligonucleotide primers, including -1263A/G forward primer 5′-GGGAATACTTTCCTGGCAGG-3′, reverse primer 5′-GTCTTCCATTCCCTCTTCCG(C-G)TC-3′, -712C/T forward primer 5′-AGTCGCGGAGGTGC- CGCCTT-3′, and reverse primer 5′-AGGGCTAGCTCGTGCCAG(C-G)C-3′, were used to amplify the DNA fragments. The polymerase chain reaction (PCR) mixture contains 5 ng genomic DNA (5 ng/μl), 1 μl of each specific primer, 2.5 mM of each dNTP, 3.25 mM MgCl₂, and 0.5 U of Hot-StarTaq DNA polymerase (5 U/μl, Qiagen, Valencia, CA, USA). The PCR was performed at 94°C for 15 min, 45 cycles of 94°C for 20 sec, 56°C for 30 sec, and 72°C for 1 min, followed by 72°C for 3 min. The final products were analyzed using a modified Brucker Autoflex MALDI-TOF mass spectrometer (Brucker, Billerica, MA, USA).

**Statistical Analysis**

All data were analyzed by SPSS 17.0 software (SPSS Company, Chicago, IL, USA). Hardy–Weinberg equilibrium was observed throughout to ensure population representation of the discogenic LBP group and the healthy control group. Gene frequency distribution was tested for Hardy–Weinberg equilibrium. Hardy–Weinberg expectations were determined using the PowerStat version 1.2 spreadsheet, as described by Tereba [28]. Comparison of genotype and allele frequencies between cases and controls was carried out using χ² test. Measurement data were expressed with mean ± SD, and the statistical significance of the differences adopted ρ < 0.05.

**RESULTS**

The distributions of the CASP-9 -1263A/G and -712C/T genotypes among the cases were different from those among the controls. And the distributions of genotype frequencies of the -1263A/G and -712C/T in the case group and the control group were consistent with the Hardy–Weinberg equilibrium law (Table 3). The observed values were compared with the expected values using Hardy–Weinberg equilibrium, and the genetic equilibriums were tested in the discogenic LBP case group and the control group, respectively. The observed values of the -1263A/G and -712C/T genotype frequencies in either group demonstrated no significant differences compared with the corresponding expected values (ρ > 0.05). Linkage analysis suggested that the -1263A/G and -712C/T of CASP-9 gene were in linkage disequilibrium (ρ = 0.482).

The data on alleles and genotypes of CASP-9 (-1263A/G and -712C/T) are shown in Table 4. The allele frequency of the -1263G is higher in discogenic LBP cases than in controls (56.2% versus 44.9%). In contrast, the frequency of the -1263A is 43.8% in each specific. Each specific, 2.5 mM of each dNTP, 3.25 mM MgCl₂, and 0.5 U of Hot-StarTaq DNA polymerase (5 U/μl, Qiagen, Valencia, CA, USA). The PCR was performed at 94°C for 15 min, 45 cycles of 94°C for 20 sec, 56°C for 30 sec, and 72°C for 1 min, followed by 72°C for 3 min. The final products were analyzed using a modified Brucker Autoflex MALDI-TOF mass spectrometer (Brucker, Billerica, MA, USA).

**Table 3. Hardy–Weinberg equilibrium of the CASP-9 -1263A/G and -712C/T genotypes in discogenic LBP cases and controls**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>-1263A/G</th>
<th>-712C/T</th>
<th>p-Value</th>
<th>χ²-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1263 Discogenic LBP (n = 154)</td>
<td>26 (30)α</td>
<td>83 (76)</td>
<td>45 (48)</td>
<td></td>
</tr>
<tr>
<td>Controls (n = 216)</td>
<td>59</td>
<td>120 (107)</td>
<td>37 (43)</td>
<td></td>
</tr>
<tr>
<td>-712 Discogenic LBP (n = 154)</td>
<td>97 (98)</td>
<td>51 (50)</td>
<td>6 (6)</td>
<td></td>
</tr>
<tr>
<td>Controls (n = 216)</td>
<td>115 (117)</td>
<td>88 (84)</td>
<td>13 (15)</td>
<td></td>
</tr>
</tbody>
</table>

αExpected count.
discogenic LBP cases and 55.1% in controls. The G allele is significantly different from the A allele (OR = 1.572, 95% CI = 1.171–2.110, \(p = 0.030\)). The genotype frequency of the -1263 GG is borderline, significantly higher in cases (29.2%) than in controls (17.1%). The genotypes of the -1263 AA and AG decrease in frequency in discogenic LBP group (16.9% and 53.9%, respectively) compared with those in control group (27.3% and 55.6% , respectively). For the -712C/T, T allele frequency is lower in cases than in controls (20.5% versus 26.4%), and C allele frequency is 79.5% in case group and 73.6% in control group, but the T allele is not significantly different from the C allele (OR = 0.717, 95% CI = 0.506–1.018, \(p = 0.062\)). The genotype frequencies of the CC, CT, and TT are 63.0%, 33.1%, and 3.9% in case group and 53.2%, 40.7%, and 6.0% in control group, respectively. From Table 3 it can be seen that the -1263 GG genotype is associated with a significantly increased risk of discogenic LBP compared with the AA and AG genotypes (OR = 1.997, 95% CI = 1.216–3.279, \(p = 0.006\)) or the AA genotype (OR = 2.760, 95% CI = 1.464–5.203, \(p = 0.002\)), but the -712 TT or TT and CT genotypes are not statistically different from the CC genotype (OR = 0.547, 95% CI = 0.200–1.494, \(p = 0.234\) and OR = 0.669, 95% CI = 0.439–1.021, \(p = 0.062\), respectively).

**DISCUSSION**

Disc degeneration of lumbar spine is a process that begins early in human life, and it is a result of the common effect of a variety of genetic and environmental factors as well as normal aging. The pathological intervertebral disc degeneration is considered to be one of the prevalent causes of chronic LBP [4]. Discogenic LBP is the most common symptomatic degenerative intervertebral disc disease of lumbar spine, and part of the patients with chronic LBP can be discogenic LBP. A reported study suggests that heredity is the largest single determinant of disc degenerative etiology [29]. Inspired by this, we investigated the heredity of symptomatic discogenic LBP patients. It is well known that CASP-9 is an initiator CASP in the apoptosome-driven apoptosis pathway and plays an important role in the development and progression of LDD. Polymorphisms in the promoter region of CASP-9 gene may influence its promoter activity, thereby modulating the susceptibility to LDD. Our present study has shown that the promoter polymorphism of the CASP-9 -1623A/G is associated with discogenic LBP, whereas that of the CASP-9 -712C/T does not necessarily contribute to this ailment. In our study, the genotype frequency of the CASP-9 -1263 GG in the discogenic LBP patients is higher than that in the healthy control group, and the genotype frequencies of the -712 TT or the TT and CT are not statistically different between the two groups. The results suggest that the CASP-9 -1263 GG genotype is contributory to discogenic LBP.

CASP-9, which is mapped on the short arm of chromosome 1p36 in humans, is composed of nine exons [30]. It triggers the intrinsic pathway for apoptosis. Evidence from CASP-9 gene knockout mouse models shows that CASP-9 is essential to the regulation of cell homeostasis through cleavage of many key players involved in apoptosis [31]. Many proapoptotic stimuli engage the apoptotic machinery in the cells and generate the apoptosome consequently. Apoptosome then activates the CASP-9 cascade downstream with effector caspases, leading to apoptosis [32]. Recent studies have demonstrated that apoptosome with aberrant function not only contributes to carcinogenesis but also has been implicated in the inappropriate apoptosis characteristic of various degenerative disorders [33,34]. Some polymorphisms within this gene have

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Table 4. CASP-9 genotypes and allele frequencies of cases and controls and their association with the risk of discogenic LBP

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Discogenic LBP n (%)</th>
<th>Controls n (%)</th>
<th>(p) value</th>
<th>(\chi^2) value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>26 (16.9)</td>
<td>59 (27.3)</td>
<td>0.100</td>
<td>2.701</td>
<td>1.0</td>
</tr>
<tr>
<td>AG</td>
<td>83 (53.9)</td>
<td>120 (55.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>45 (29.2)</td>
<td>37 (17.1)</td>
<td>0.002</td>
<td>10.08</td>
<td></td>
</tr>
<tr>
<td>-1263</td>
<td>AA + AG</td>
<td>109 (70.8)</td>
<td>0.006</td>
<td>7.619</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td></td>
<td>45 (29.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>A</td>
<td>135 (43.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>173 (56.2)</td>
<td>238 (55.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>CC</td>
<td>97 (63.0)</td>
<td>0.030</td>
<td>9.122</td>
<td>1.0</td>
</tr>
<tr>
<td>CT</td>
<td>51 (33.1)</td>
<td>115 (53.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>6 (3.9)</td>
<td>88 (40.7)</td>
<td>0.093</td>
<td>2.828</td>
<td>1.0</td>
</tr>
<tr>
<td>-712</td>
<td>CT + TT</td>
<td>57 (37.0)</td>
<td>0.062</td>
<td>3.490</td>
<td>1.0</td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>245 (79.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>63 (20.5)</td>
<td>318 (73.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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also been described. Andreoli et al. [35] reported the association between CASP-9 gene locus polymorphisms and multiple sclerosis, indicating that CASP-9 (Ex5 + 32G/A) GG genotype increases the risk of multiple sclerosis and also is associated with the severity of the disease. Another study [36] suggests that the CASP-9 promoter polymorphism is associated with a decreased risk of non-Hodgkin lymphoma with some evidence that the effects were specific to lymphoma subtypes. It is found that CASP-9 gene encodes for a glutamine to arginine amino acid change at codon 221 and thus may have decreased functional significance. Park et al. [24] also conducted a study on the CASP-9 polymorphisms and the risk of primary lung cancer in a Korean population. The findings have revealed that the CASP-9 -1263 GG genotype is associated with a significantly decreased risk of lung cancer compared with the -1263 AA genotype or the combined -1263 AA and AG genotypes, but individuals with at least one T allele of -712C/T are at a significantly increased risk of lung cancer compared with those harboring the CC genotype. Inactivation of apoptosis is one of the hallmarks of cancer because it allows cells prone to intervertebral disc degeneration. Under accelerated degeneration, the radial tear of disc may finally occur.

Our study by now is the first report on the association of polymorphisms in the promoter region of CASP-9 gene with discogenic LBP. The overall sample size of our study was small and the haplotype analysis of -1263A/G and -712C/T was not performed. These limitations might have some influence on our results, but the association between CASPASE-9 -1263A/G polymorphism and a high risk of discogenic LBP is still evidently revealed. In summary, the polymorphism might not directly contribute to the pathogenesis of LDD, but it could instead be a potential genetic marker that is in linkage disequilibrium with a disease predisposing locus nearby [9]. Our findings suggest that the CASP-9 -1263A/G promoter polymorphism is involved in the pathogenesis of discogenic LBP.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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