Association between the -1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults

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Abstract Matrix metalloproteinase-2 (MMP-2) has been shown to play a pivotal role in the pathophysiology of lumbar disc disease (LDD). Increased expression and activity of MMP-2 has been documented in degenerative discs. The polymorphism -1306C/T in the promoter region of MMP-2 gene was reported to influence gene transcription and expression. The objective of this study was therefore to investigate the possible association of MMP-2 -1306C/T polymorphism with the occurrence and the clinical characteristics of LDD. MMP-2 genotypes were determined by polymerase chain reaction (PCR) and direct DNA sequencing in a case-control study involving 162 younger patients with LDD and 318 age- and sex-matched healthy adults. The results showed that the frequency of MMP-2 -1306CC genotype was significantly higher in LDD patients when compared with controls. Subjects with the CC genotype had nearly threefold increased risk for LDD (odds ratio 3.08; 95% confidence interval 1.84–5.16) compared with subjects carrying at least one variant T allele. Furthermore, this genotype was found to correlate with more severe grades of disc degeneration observed on magnetic resonance imaging scan. These findings suggest that MMP-2 -1306C/T polymorphism may be a genetic risk

factor related to LDD susceptibility in the young adult population.

Keywords Intervertebral disc degeneration · Lumbar disc disease · Matrix metalloproteinase-2 · Polymorphism

Introduction

Lumbar disc disease (LDD) is a highly prevalent musculoskeletal disorder, caused by degeneration of intervertebral discs of the lumbar spine. Although various environmental and anthropometric risk factors have been implicated in the pathogenesis of disc degeneration, increasing evidence shows that LDD has strong genetic determinants [1]. Several studies have found familial predisposition to LDD, including early onset sciatica and lumbar disc herniation. In addition, mutations or polymorphisms in several genes have been reported to be associated with either the presence or severity of disc degeneration, including gene coding for collagen IX and XI [2, 12, 13], aggrecan [9], cartilage intermediate layer protein [18], vitamin D receptor [22], interleukin-1 [19], matrix metalloproteinase-3 (MMP-3) [20], tissue inhibitor of metalloproteinase-1 (TIMP-1) [21], and cyclooxygenase-2 [21].

The development of disc degeneration is a complex, multistage process in which the degradation of the disc matrix is one of the key steps. The groups of matrix-degrading enzyme, the matrix metalloproteinases (MMPs), are assumed to play a critical role in the excessive breakdown of the extracellular matrix (ECM) during disc degeneration [4]. MMP-2 (gelatinase A), an important member of the MMP family, is of particular importance to

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intervertebral discs homeostasis. Increased expression and activity of MMP-2 has been documented in disc tissue with degenerative lesions [3, 15].

Recently, a functional single nucleotide polymorphism (SNP) in the promoter region of MMP-2 was reported to influence gene transcription and expression. The C→T transition at -1306 disrupts Sp1-binding site and results in decreased transcriptional activity, whereas the presence of the Sp1 promoter site in the -1306C allele may enhance transcription [14]. Therefore, MMP-2 protein expression would be higher in individuals who carry the CC genotype than those who carry the TT or CT genotype.

MMP-2 is known to be potentially related to the pathophysiology of LDD, and its genetic polymorphism is thought to influence the expression and activity of this enzyme. The objective of this study was therefore to investigate the possible association of MMP-2 gene -1306C/T polymorphism with the occurrence and the clinical characteristics of LDD.

Materials and methods

Subjects

One hundred and sixty-two patients with LDD (48.8% females, age 25.4 ± 3.5) and 318 age and sex-matched healthy adults (50.6% female, age 24.1 ± 2.7), were enrolled in this case-control study protocol. LDD cases were symptomatic patients with surgically or radiologically proven degenerative and/or herniated discs of the lumbar spine. All patients had discogenic low-back pain and/or unilateral pain radiating from the back along the femoral or sciatic nerve to the corresponding dermatome of the nerve root with a duration of at least 1 month, recruited from february 2005 to may 2006 in the Department of Orthopedic Surgery at the Second Affiliated Hospital of Harbin Medical University.

Sagittal magnetic resonance imaging (MRI) scan (Magnetom 1.5 T, Siemens AG, Germany) was performed with a slice thickness of 5 mm. A T2-weighted image with a repetition of 2,500 ms and an echo time of 90 ms of the lumbar spine was taken in all the participants. Films were assessed by an experienced physician blinded to the results of the genetic analysis and clinical history. The grade of disc degeneration was determined according to Schneiderman's classification [17]. Grade zero indicated a normal disc with a hyperintense signal within the nucleus; Grade 1, a slight decrease in signal intensity in the nucleus pulposus; Grade 2, a generalized hypointense nucleus; and Grade 3, a hypointense nucleus with disc space narrowing.

Primary exclusion criteria included synovial cysts, spondylolisthesis, spinal tumor, spondylosis, trauma and inflammatory disease. Individuals who had known

environmental risk factors, including heavy physical loading, occupational driving, cigarette smoking or obesity (body mass index $> 25 \text{ kg/m}^2$), were also excluded.

The control sample consisted of 318 unrelated Chinese individuals with no history of back problems and with negative MRI findings, selected among medical students and hospital staff.

The study was approved by Ethics Committee of Harbin Medical University and written informed consent was obtained from all subjects.

Genotype analysis

Genomic DNA was isolated from peripheral blood by the standard phenol–chloroform method. MMP-2 genotypes were determined by PCR and direct DNA sequencing. Oligonucleotide primers (forward, 5'-CTG ACC CCC AGT CCT ATC TGC C-3'; reverse, 5'-TGT TGG GAA CGC CTG ACT TCA G-3') were used to amplify genomic DNA fragments [14]. PCR was accomplished in a total volume of 25 μl containing 100 ng DNA, 1.0 μM each primer, 0.2 mM dNTP, 2.0 mM MgCl_2 , 1.0 U Taq DNA polymerase with 1 $\times$ reaction buffer and 2% dimethylsulfoxide. The reaction was carried out under the following conditions: an initial melting step of 2 min at 94°C, followed by 35 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 45 s, with a final elongation of 7 min at 72°C. PCR products were purified and directly analyzed with an ABI PRISM 377 automatic sequencer using a dye terminator sequencing kit.

Statistical analysis

Continuous data were expressed as mean $\pm$ SD. The difference in the degenerative scores between genotypes was analyzed by Student's *t* test. Chi-square analysis was used to assess differences in genotype frequencies between cases and controls. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS10.0 software package (SPSS Company, Chicago, IL, USA).

Results

Disc degeneration and herniation were present in 131 (81%) and 93 (57%) patients, respectively. Disc degeneration of a single level, two levels and more than three levels was found in 89, 25 and 17 patients, respectively. The overall severity of the disc degeneration was determined by the total score at each level of the five

intervertebral discs, with a range from 0 to 15. In 26 participants disc herniation was present at more than one level.

The allele frequencies for MMP-2 -1306C and -1306T were 0.83 and 0.17, respectively, in controls, compared with 0.93 and 0.07 in LDD patients. The frequencies of three MMP-2 genotypes among controls were CC, 68.6%, CT, 28.9%, and TT, 2.5%, whereas the genotype distribution in patients was 87.0% for CC, 12.4% for CT, and 0.6% for TT, with the CC genotype being more prevalent in LDD group than in controls ($P < 0.001$) (Table 1). The genotype frequencies fit in with the Hardy–Weinberg equilibrium law. Because the TT homozygotes were rare in this study, this genotype was combined with the CT genotype as the reference group for statistical analysis. Subjects carrying the CC genotype had nearly threefold increased risk for LDD (odds ratio 3.08; 95% confidence interval 1.84–5.16) compared with subjects carrying at least one variant T allele.

We also examined the relationship between MMP-2 genotype and severity of disc degeneration in LDD group. In 131 patients with disc degeneration proven by MRI, the total degenerative disc score in individuals with the CC genotype (mean $\pm$ SD 3.1 ± 0.7) was significantly greater than that in those with the CT/TT genotype (1.9 ± 0.5 ; $P < 0.001$). The patients with the CC genotype had more severe disc degeneration.

Discussion

Disc degeneration is a process that begins early in life and is a consequence of a variety of genetic and environmental factors as well as of normal aging. Epidemiologic studies suggest that heredity is the largest single determinant of disc degeneration [16]. According to the hypothesis that persons with earlier age of disease onset are more likely to have a genetic predisposition, the association of the MMP-2 -1306C/T polymorphism with the susceptibility to LDD was investigated in young adults. The frequency of the MMP-2 -1306CC genotype was significantly higher in patients with LDD than in the healthy population. Subjects with the CC genotype had a nearly threefold increased risk for LDD. In addition, this genotype was found to correlate with more severe grades of disc degeneration observed on

MRI. Moreover, in a Japanese study, a common 5A/6A polymorphism of the MMP-3 gene promoter was associated with disc degeneration in the elderly group aged more than 64 years, but not in subjects of less than 28 years of age [20].

Biochemically, disc degeneration is characterized by enhanced breakdown of the matrix. The main components of disc ECM, collagens and proteoglycans, are degraded by a specific class of proteolytic enzymes known as the MMPs. Several lines of evidence show that MMPs play a pivotal role in regulation of intervertebral disc homeostasis. Among the MMP family members, MMP-2 is of particular importance due to its broad spectrum of proteolytic activity toward ECM components including gelatin, proteoglycans, fibronectin, elastin and laminin et al. [24]. MMP-2 was expressed in cultured disc tissue at both cervical and lumbar levels [6–8]. The presence of MMP-2 within normal and degenerative nucleus pulposus and annulus fibrosus cells was also demonstrated in several immunohistochemical studies [15, 23]. Enhanced expression of MMP-2 has been found in aging and degenerative discs [3, 15, 23], and MMP-2 production appears to increase when disc cells are exposed to abnormal physical stresses [5]. Furthermore, in situ zymography confirmed that gelatinolytic activity was localized in area close to tissue clefts where MMP-2 was expressed at elevated levels [23]. In addition, Kozaci et al. reported that Pro-MMP-2 levels were higher at early stages of the degenerative disc disease, which were negatively correlated with the collagen content in herniated disc material [10].

Although the activity of MMP-2 is known to be regulated by posttranscriptional mechanisms, inclusion of the activation of proenzyme and inhibition of TIMPs and transcriptional regulation also play a major role. Recently, Price et al. reported a functional SNP of the MMP-2 gene [14]. The -1306C→T transition in the promoter region of MMP-2 disrupts an Sp1-binding site (CCACC box), leading to a strikingly lower promoter activity with the T allele. In contrast, the presence of the Sp1 promoter site in the MMP-2 -1306C allele may enhance transcription. Therefore, MMP-2 protein expression would be higher in individuals who carry the CC genotype than those who carry the TT or CT genotype. This polymorphism has been associated with a variety of diseases, including rheumatoid arthritis [11].

Table 1 MMP-2 genotype frequencies in patients with LDD and controls and their association with the risk of LDD

Genotypes	LDD (%) $n = 162$	Controls (%) $n = 318$	P	OR(95%CI)
CC	141(87.0)	218(68.6)	$P < 0.001^a$	3.08(1.84–5.16)
CT	20(12.4)	92(28.9)		
TT	1(0.6)	8(2.5)		

MMP-2 matrix metalloproteinase-2; LDD lumbar disc disease; OR odds ratio; CI confidence interval

^a Calculation for the genotypes was performed CC versus CT + TT

How the functional polymorphism of the MMP-2 gene contributes to the clinical syndrome is unclear, but numerous studies have shown that MMP-2 is crucially involved in the pathophysiology of LDD. As mentioned above, MMP-2 was expressed commonly in intervertebral disc tissue, and its expression and activity was up-regulated as disc degeneration progressed. Increased expression of MMP-2 may contribute to the formation of tissue clefts and the resorption of disc material, leading to accelerated disc degeneration. In addition, MMP-2 has been shown to interact with and activate other MMPs and cytokines. Therefore, we hypothesize that individuals carrying the CC genotype might have higher MMP-2 levels, which may predispose to the development of LDD at an early age.

The studied polymorphism might not be the direct cause of LDD, but could instead be a genetic marker that is in linkage disequilibrium with a true disease predisposing locus nearby. Due to the small sample sizes, the results should be interpreted with caution and need to be confirmed in larger and ethnically divergent population samples.

In conclusion, we found that MMP-2 -1306C/T polymorphism may be a genetic risk factor associated with the susceptibility to LDD in young individuals. The relation between the mutation of this gene and LDD warrants further investigation.

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