European Review for Medical and Pharmacological Sciences 2013; 17: 1830-1834

Association of the polymorphism of MMP2 with the risk and severity of lumbar disc degeneration in the Chinese Han population

Y. ZHANG[1,2], Z. GU[2], G. QIU[1]

[1]Department of Orthopaedics, Peking Union Medical College Hospital, Beijing, P. R. China
[2]Department of Orthopaedics, The First People's Hospital of Chengdu, Sichuan Province, P. R. China

Yu Zhang and *Zuchao Gu* contributed equally to this manuscript

Abstract. – OBJECTIVE: The present study aimed to determine whether the -735 C/T polymorphism of the Matrix Metalloproteinase 2 (MMP2) gene is associated with the risk and severity of lumbar disc degeneration (LDD) in the Chinese Han population.

PATIENTS AND METHODS: A total of 1008 patients with LDD and 906 healthy controls were enrolled in this study. The grade of disc degeneration was determined according to Schneiderman's classification for Magnetic Resonance Imaging (MRI). The -735 C/T polymorphism of MMP2 was genotyped using polymerase chain reaction and the restriction fragment length polymorphism method.

RESULTS: The genotype frequency of the -735 C/T polymorphism was in agreement with the Hardy-Weinberg equilibrium ($p = 0.087$). The frequencies of the -735CT and TT genotypes were significantly lower among LDD patients compared with normal controls ($p < 0.001$); CT and TT genotype were significantly associated with a decreased risk of LDD compared with the CC genotype (for TT genotype, $p = 0.031$; OR 0.413; 95% CI 0.184-0.924; for CT genotype, $p < 0.001$, OR 0.645, 95% CI 0.506-0.822). Patients with LDD showed significantly higher frequencies of the C allele than normal controls ($p < 0.001$), T allele was significantly associated with a decreased risk of LDD compared with the C allele ($p < 0.001$; OR 0.631; 95% CI 0.508-0.783). In addition, the -735TT and CT genotypes, as well as the T allele were associated with lower degenerative grades of LDD compared with CC genotype and the C allele, respectively (both $p < 0.001$).

CONCLUSIONS: The -735 C/T polymorphism of MMP2 may be associated with the risk and severity of LDD in the Chinese Han population.

Key Words:
Polymorphism, MMP2, -735 C/T, Lumbar disc degeneration.

Introduction

Lumbar disc degeneration (LDD) is the major cause of low back pain (LBP). LBP is a major cause of disability and it substantially contributes to healthcare cost[1,2]. The etiology and pathogenesis of LDD is still unclear. Various environmental risk factors, such as age, gender, occupation (lifting heavy loads), cigarette smoking, height, weight, and exposure to vehicular vibration are considered to be involved in the mechanism of LDD[3]. In addition, genetic factors may play an important role in the development of LDD. Polymorphisms in a number of genes, such as aggrecan[4,5], interleukin 1[6], vitamin D receptor[7], and matrix metalloproteinase[8,9], are reported to be associated with an increased risk of LDD.

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. MMP-2 (gelatinase A), an important member of the MMP family, is of particular importance to intervertebral discs homeostasis. Increased expression and activity of MMP-2 has been documented in disc tissue with degenerative lesions[10].

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 -735 disrupts Sp1-binding site and results in decreased transcriptional activity, whereas the presence of the Sp1 promoter site in the -735C allele may enhance transcription[11,12]. 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

Corresponding Author: Guixing Qiu, MD; e-mail: guixingqiu77@126.com

this study was, therefore, to investigate the possible association of MMP-2 gene -736C/T polymorphism with the occurrence and the clinical characteristics of LDD.

Patients and Methods

Patients

A total of 1008 patients with LDD were enrolled in this study. The cases were all symptomatic patients with surgically- or radiologically-proven LDD. Patients with synovial cysts, spondylolisthesis, spinal tumor, spondylosis, trauma and inflammatory disease were excluded. The grade of disc degeneration was determined according to Schneiderman's classification for Magnetic Resonance Imaging (MRI)[13]. The control sample consisted of 906 healthy check-up examinees who matched to the cases by age and gender. They had no history of back problems and had negative MRI findings. Individuals with familial relation to any of the cases and a history of seeking medical attention for back pain or sciatica were excluded. All the subjects were unrelated Han Chinese.

This study was approved by the Ethics Committee of Peking Union Hospital, and informed consent was obtained from all participants.

Genotyping

Genomic DNA from all the subjects was extracted from peripheral blood leukocytes using a DNA isolation kit following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The -735 C/T polymorphism of the MMP2 gene was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. The specific primer for detecting C(-735)T polymorphism in the MMP-2 gene was 5'-ATAGGGTAAACCTCCCCACATT-3' (forward) and 5'-GGTAAAATGACCCTGAGACCTG-3' (reverse), synthesized by Invitrogen (subgroup in Shanghai, China). PCR was performed in a 25 μl volume containing 0.1 μg of DNA, 2.5 μl of 10 × buffer, 1 μl of 25 mM $MgCl_2$, 0.5 μl of 10 mM dNTP, 0.5 μl of 20 μM primers (forward and reverse), 0.5 μl Taq DNA polymerase (Promega, Madison, WI, USA): 5 U/μl and an appropriate volume of sterile water. The parameter for amplification of the MMP-2 gene was pre-denatured at 95°C for 5 min followed by 35 cycles of 94°C for 45 s, 62°C for 45 s and 72°C for 1 min and a final extension of 72°C for 10 min. PCR was performed with a ABI 2720 PCR instrument. PCR products were purified (Agarose Gel DNA Fragment Recovery Kit; Takara, Osaka, Japan) and digested with the Hinf I restriction enzyme (Ferments, Glen Burnie, MD, USA) for 16 h at 37°C. The digested products were electrophoresed in 2% agarose gel. Parts of samples were for sequencing in order to validate the result of PCR-RFLP.

Positive and negative controls were used in each genotyping assay, and 5% of the samples were randomly selected and run in duplicates with 100% concordance. The results were reproducible with no discrepancy in genotyping.

Statistical Analysis

Statistical analyses were performed using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). The characteristics of LDD patients and controls were compared with chi square or Student's t test according to the variable types. Chi square test was performed to assess Hardy-Weinberg equilibrium. Comparison of genotype and allele frequencies between cases and controls was carried out in terms of the chi square test. The risk associated with individual alleles and genotypes was calculated as the Odds Ratio (OR) with 95% confidence intervals (CI) using unconditional logistic regression. The severity of disc degeneration of different genotypes among LDD patients was analyzed using chi square. All statistical tests were two-sided, and $p < 0.05$ for the differences was considered statistically significant.

Results

Baseline Parameters

No significant differences in age and gender were found between LDD patients and normal controls. The characteristics of the two groups are presented in Table I.

Table I. The characteristics between patients with LDD and normal controls.

Characteristics	Patients with LDD	Normal controls	*p* value
Age (years)	50.12 ± 8.65	49.54 ± 8.35	0.504
Gender (male/female)	652/356	555/351	0.469

Table II. The genotype and allele distributions of -735 C/T polymorphism in the LDD and control groups.

Genotype	LDD group		Control group		p^*	OR (95% CI)	$p^{\#}$
	n	%	n	%			
CC	860	85.3	710	78.4	< 0.001	1.00 (reference)	
CT	139	13.8	178	19.7		0.645 (0.506-0.822)	< 0.001
TT	9	0.9	18	2		0.413 (0.184-0.924)	0.031
C	1859	92.2	1598	88.2	< 0.001	1.00 (reference)	
T	157	7.8	214	11.8		0.631 (0.508-0.783)	< 0.001

*Note: *p* value was calculated by Chi test among all the different genotypes; #p value was calculated by unconditional logistic regression.

The Association Between -735 C/T Polymorphism of the MMP2 Gene and the Risk of LDD

The genotype and allele distributions of the -735 C/T polymorphism of the MMP2 gene in the LDD and control groups are shown in Table II. The genotype distribution of the -735 C/T polymorphism in controls was in with the Hardy-Weinberg equilibrium ($p = 0.087$). There were significantly lower frequencies of the -735CT and TT genotypes in patients with LDD compared with normal controls ($p < 0.001$). Patients with LDD showed a significantly lower frequency of the T allele than normal controls ($p < 0.001$). Unconditional logistic regression analysis revealed that the T allele was significantly associated with the decreased risk of LDD compared with the C allele ($p < 0.001$; OR 0.631; 95% CI 0.508-0.783), and CT and TT genotype were significantly associated with a decreased risk of LDD compared with the CC genotype (for TT genotype, $p = 0.031$; OR 0.413; 95%CI 0.184-0.924; for CT genotype, $p < 0.001$, OR 0.645, 95% CI 0.506-0.822).

The Association of -735 C/T Polymorphism with the Severity of LDD

The association between the genotype and allele distribution of the -735 C/T polymorphism and the severity of LDD are displayed in Table III. LDD patients with the -735CT and TT genotypes, as well as the T allele showed significantly lower grades of disc degeneration compared with those with the -735CC genotypes and the C allele, respectively (both $p < 0.001$).

Discussion

The exact mechanism of LDD is still unknown. Recent studies have focused on the role of genetic factors in the etiology of LDD, and epidemiologic studies suggest that heredity is the largest single determinant of disc degeneration[14]. Biochemical mediators of tissue degradation, especially MMPs, have been identified as significant factors[15,16]. Previous studies demonstrate that MMP family plays important roles in the pathology of LDD. Our study has shown that the MMP-2 promoter genotype was associated with LDD degeneration in the big population of Han Chinese. The frequency of the MMP-2 -735CC genotype was significantly higher in patients with LDD than in the healthy population. Subjects with the CC genotype had a nearly 2.5 folds increased risk for LDD compared to TT genotype. In addition, this genotype was found to correlate with more severe grades of disc degeneration observed on MRI.

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

Table III. The association of different genotypes and alleles of -735 C/T polymorphism with the severity of LDD.

Genotype	n	Grade 2	Grade 3	Grade 4	*p* value
CC	860	129 (15.0%)	216 (25.1%)	515 (59.9%)	< 0.001
CT	139	38 (27.3%)	36 (25.9%)	32 (46.8%)	
TT	9	7 (77.8%)	1 (11.1%)	1 (11.1%)	
C	1859	286 (15.4%)	467 (25.1%)	1106 (59.5%)	< 0.001
T	157	59 (37.6%)	36 (22.9%)	62 (39.5%)	

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[17]. MMP-2 was expressed in cultured disc tissue at both cervical and lumbar levels[18-20]. The presence of MMP-2 within normal and degenerative nucleus pulposus and annulus fibrosus cells was also demonstrated in several immunohistochemical studies[21-23]. Enhanced expression of MMP-2 has been found in aging and degenerative discs[21,24], and MMP-2 production appears to increase when disc cells are exposed to abnormal physical stresses[25,26]. Furthermore, in situ zymography confirmed that gelatinolytic activity was localized in area close to tissue clefts where MMP-2 was expressed at elevated levels[10]. In addition, Kozaci et al[27] 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.

Although the activity of MMP-2 is known to be regulated by posttranscriptional mechanisms, inclusion of the activation of proenzyme and inhibition of tissue inhibitor of metalloproteinases (TIMPs) and transcriptional regulation also play a major role. Recently, Price et al[28] reported a functional SNP of the MMP-2 gene. The -735C→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 -735C 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[29].

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[30-32]. 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 -735CC genotype might have higher MMP-2 levels, which may predispose to the development of LDD with high possibility. 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. There are also some criticisms in the present study, one of them is because all the subjects are Chinese individuals, the results should be interpreted with caution and need to be confirmed in larger and ethnically divergent population samples.

Conclusions

We found that MMP-2 -735C/T polymorphism may be a genetic risk factor associated with the susceptibility to LDD in Han Chinese individuals. The relation between the mutation of this gene and LDD warrants further investigation.

Declaration of Interest

The Authors report no conflict of interest.

References

1) Tan H, Zhao J, Jiang J, Ren Y. Association of the polymorphism of DR4 with the risk and severity of lumbar disc degeneration in the Chinese Han population. Scand J Clin Lab Invest 2012; 72: 576-579.
2) Katz JN. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am 2006; 88(Suppl 2): 21-24.
3) Higashino K, Matsui Y, Yagi S, Takata Y, Goto T, Sakai T, Katoh S, Yasui N. The alpha2 type IX collagen tryptophan polymorphism is associated with the severity of disc degeneration in younger patients with herniated nucleus pulposus of the lumbar spine. Int Orthop 2007; 31: 107-111.
4) Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Zinchenko N, Hanley EN, Jr. Variations in aggrecan localization and gene expression patterns characterize increasing stages of human intervertebral disk degeneration. Exp Mol Pathol 2011; 91: 534-539.
5) Mashayekhi F, Shafiee G, Kazemi M, Dolati P. Lumbar disk degeneration disease and aggrecan gene polymorphism in northern Iran. Biochem Genet 2010; 48(7-8): 684-689.
6) Lee JM, Song JY, Baek M, Jung HY, Kang H, Han IB, Kwon YD, Shin DE. Interleukin-1beta induces angiogenesis and innervation in human intervertebral disc degeneration. J Orthop Res 2011; 29: 265-269.

7) Xu G, Mei Q, Zhou D, Wu J, Han L. Vitamin D receptor gene and aggrecan gene polymorphisms and the risk of intervertebral disc degeneration–a meta-analysis. PLoS One 2012; 7: e50243.
8) Zigouris A, Alexiou GA, Batistatou A, Voulgaris S, Kyritsis AP. The role of matrix metalloproteinase 9 in intervertebral disc degeneration. J Clin Neurosci 2011; 18: 1424-1425.
9) Yuan HY, Tang Y, Liang YX, Lei L, Xiao GB, Wang S, Xia ZL. Matrix metalloproteinase-3 and vitamin d receptor genetic polymorphisms, and their interactions with occupational exposure in lumbar disc degeneration. J Occup Health 2010; 52: 23-30.
10) Dong DM, Yao M, Liu B, Sun CY, Jiang YQ, Wang YS. Association between the -1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults. Eur Spine J 2007; 16: 1958-1961.
11) Yu C, Zhou Y, Miao X, Xiong P, Tan W, Lin D. Functional haplotypes in the promoter of matrix metalloproteinase-2 predict risk of the occurrence and metastasis of esophageal cancer. Cancer Res 2004; 64: 7622-7628.
12) Zhou Y, Yu C, Miao X, Wang Y, Tan W, Sun T, Zhang X, Xiong P, Lin D. Functional haplotypes in the promoter of matrix metalloproteinase-2 and lung cancer susceptibility. Carcinogenesis 2005; 26: 1117-1121.
13) Schneiderman G, Flannigan B, Kingston S, Thomas J, Dillin WH, Watkins RG. Magnetic resonance imaging in the diagnosis of disc degeneration: correlation with discography. Spine (Phila Pa 1976) 1987; 12: 276-281.
14) Sambrook PN, MacGregor AJ, Spector TD. Genetic influences on cervical and lumbar disc degeneration: a magnetic resonance imaging study in twins. Arthritis Rheum 1999; 42: 366-372.
15) Goupille P, Jayson MI, Valat JP, Freemont AJ. Matrix metalloproteinases: the clue to intervertebral disc degeneration? Spine (Phila Pa 1976) 1998; 23: 1612-1626.
16) Takahashi M, Haro H, Wakabayashi Y, Kawa-uchi T, Komori H, Shinomiya K. The association of degeneration of the intervertebral disc with 5a/6a polymorphism in the promoter of the human matrix metalloproteinase-3 gene. J Bone Joint Surg Br 2001; 83: 491-495.
17) Tuysuz B, Mosig R, Altun G, Sancak S, Glucksman MJ, Martignetti JA. A novel matrix metalloproteinase 2 (MMP2) terminal hemopexin domain mutation in a family with multicentric osteolysis with nodulosis and arthritis with cardiac defects. Eur J Hum Genet 2009; 17: 565-572.
18) Kang JD, Georgescu HI, McIntyre-Larkin L, Stefanovic-Racic M, Donaldson WF, 3rd, Evans CH. Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. Spine (Phila Pa 1976) 1996; 21: 271-277.
19) Kang JD, Georgescu HI, McIntyre-Larkin L, Stefanovic-Racic M, Evans CH. Herniated cervical intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. Spine (Phila Pa 1976) 1995; 20: 2373-2378.
20) Kang JD, Stefanovic-Racic M, McIntyre LA, Georgescu HI, Evans CH. Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. Spine (Phila Pa 1976) 1997; 22: 1065-1073.
21) Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine (Phila Pa 1976) 2000; 25: 3005-3013.
22) Vaccaro AR, Carrino JA, Venger BH, Albert T, Kelleher PM, Hilibrand A, Singh K. Use of a bioabsorbable anterior cervical plate in the treatment of cervical degenerative and traumatic disc disruption. J Neurosurg 2002; 97(4 Suppl): 473-480.
23) Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. Eur Spine J 2002; 11: 308-320.
24) Roughley PJ, Mort JS. Analysis of aggrecan catabolism by immunoblotting and immunohistochemistry. Methods Mol Biol 2012; 836: 219-237.
25) Rutges JP, Kummer JA, Oner FC, Verbout AJ, Castelein RJ, Roestenburg HJ, Dhert WJ, Creemers LB. Increased MMP-2 activity during intervertebral disc degeneration is correlated to MMP-14 levels. J Pathol 2008; 214: 523-530.
26) Hsieh AH, Lotz JC. Prolonged spinal loading induces matrix metalloproteinase-2 activation in intervertebral discs. Spine (Phila Pa 1976) 2003; 28: 1781-1788.
27) Kozaci LD, Guner A, Oktay G, Guner G. Alterations in biochemical components of extracellular matrix in intervertebral disc herniation: role of MMP-2 and TIMP-2 in type II collagen loss. Cell Biochem Funct 2006; 24: 431-436.
28) Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 2001; 276: 7549-7558.
29) Nemec P, Goldbergova M, Swobodnik T, Polaskova D, Soucek M, Vasku A. [Polymorphism of gene promotor region for MMP-2 in rheumatoid arthritis]. Vnitr Lek 2006; 52: 348-354.
30) Dong DM, Yao M, Liu B, Sun CY, Jiang YQ, Wang YS. Association between the -1306C/T polymorphism of matrix metalloproteinase-2 gene and lumbar disc disease in Chinese young adults. Eur Spine J 2007; 16: 1958-1961.
31) Wu WP, Jiang JM, Qu DB, Wei QZ, Jiang H. [Expression of hypoxia-inducible factor-1alpha and matrix metalloproteinase-2 in degenerative lumbar intervertebral disc]. Nan Fang Yi Ke Da Xue Xue Bao. 2010; 30: 1152-1155.
32) Salo J, Mackiewicz Z, Indahl A, Konttinen YT, Holm AK, Sukura A, Holm S. Plasmin-matrix metalloproteinase cascades in spinal response to an experimental disc lesion in pig. Spine 2008; 33: 839-844.