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The association of degeneration of the intervertebral disc with 5a/6a polymorphism in the promoter of the human matrix metalloproteinase-3 gene

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It has been suggested that matrix metalloproteinase-3 (MMP-3, stromelysin-1) has an important role in the degeneration of intervertebral discs (IVDs). A human MMP-3 promoter 5A/6A polymorphism was reported to be involved in the regulation of MMP-3 gene expression. We suggest that IVD degeneration is associated with 5A/6A polymorphism.

We studied 54 young and 49 elderly Japanese subjects. Degeneration of the lumbar discs was graded using MRI in the younger group and by radiography in the elderly. 5A/6A polymorphism was determined by polymerase-chain reaction-based assays. We found that the 5A5A and 5A6A genotype in the elderly was associated with a significantly larger number of degenerative IVDs than the 6A6A ($p < 0.05$), but there was no significant difference in the young. In the elderly, the IVD degenerative scores were also distributed more highly in the 5A5A and 5A6A genotypes ($p = 0.0029$).

Our findings indicate that the 5A allele is a possible risk factor for the acceleration of degenerative changes in the lumbar disc in the elderly.

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Low back pain (LBP) is one of the most common musculoskeletal disabilities, affecting from 70% to 80% of all people at some time. The annual prevalence ranges from 15% to 45%, with point prevalence averaging 30%.[1]

Degenerative changes in IVDs contribute to the development of LBP and the acute lumbar radiculopathy associated with herniation of a disc.[2,3] These changes are a part of the normal ageing process. In a study of lumbar IVDs using MRI, the prevalence of degenerative IVDs was shown to increase linearly with age and 80% of all lumbar discs were abnormal at 70 years of age.[4] The exact physiopathological mechanism is, however, still unclear. Heavy physical loading, injury, vibration, infection and smoking have been reported to be risk factors.[5] Genetic factors also affect the degeneration and herniation of IVDs.[6] Recently, the relationship between IVD degeneration and polymorphism of genes such as the vitamin-D receptor,[7] type-IX collagen,[8] and aggrecan[9] has been investigated and the identification of such genetic factors may aid in the prediction and prevention of disc degeneration and LBP.

One of the important steps in IVD degeneration is the degradation of the disc matrix by enzymes such as matrix metalloproteinases (MMPs). MMP-3 (stromelysin-1, EC#3.4.24.17) is a potent proteoglycan-degrading enzyme. Recent studies suggest that it has an important role in the degeneration of IVDs.[10] MMP-3 expression is induced in response to local conditions such as mechanical loading,[11,12] inflammation,[13] etc. The degeneration of IVDs resulting from MMP-3 expression may therefore increase with time. In addition, a common polymorphism in the promoter region of the human MMP-3 gene has been identified in which one allele has a run of six adenosines (6A) and the other five (5A).[14] This polymorphism was reported to be involved in the regulation of MMP-3 gene expression with the 5A allele having twice as much promoter activity as the 6A allele.[15] The accumulative effects resulting from differences in MMP-3 expression when the genotypes are compared may be significant in ageing.

We suggest that IVD degeneration in the elderly is associated with this polymorphism in the promoter region of the MMP-3 gene. Our aim in this study was to investigate the possible relationship between MMP-3 promoter, 5A/6A polymorphism and IVD degeneration in young and elderly subjects.

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Subjects and Methods

Genomic DNA was extracted from 2ml of whole blood which had been obtained from 54 young and 55 elderly Japanese volunteers with their informed consent.

In the younger group, we performed T2-weighted fast spin-echo (2700/110, TR/TE) MRI (MRT-50A; Toshiba Medical, Tokyo, Japan) of the lumbar spine using a 0.5T scanner. All 54 subjects were women with a mean age of 21.4 years (18 to 28).

Lumbar spinal radiographs were obtained from the 55 subjects in the elderly group. Six were excluded because they had sustained lumbar spinal fractures, leaving 49 in the study. There were 11 men and 38 women with a mean age of 74.3 years (64 to 94).

Grading of IVD degeneration. In the younger group we defined a degenerative IVD as one with low intensity on T2-weighted MRI. In the elderly group, each IVD was graded using a semiquantitative score from 0 (normal) to 4 (severe) according to the radiological classification system of IVD degeneration described by Kellgren and Lawrence.[16] All assessments of IVD degeneration were performed by a single observer in a blinded fashion.

Genotyping of 5A/6A polymorphism. The 5A/6A polymorphism in the promoter of the human MMP-3 gene was determined by both single-strand conformation polymorphism (SSCP) and the polymerase chain reaction (PCR) with allele-specific primers (AS-PCR) (Fig. 1). The promoter sequences of the human MMP-3 gene were obtained by reference to GenBank entries HSU43511 and HSU56422.

A 179 or 180 bp region of the MMP-3 promoter containing the 5A/6A polymorphism was amplified by PCR with specific primers (5'-GAT TAC AGA CAT GGG TCA CG-3' and 5'-AAT TCA CAT CAC TGC CAC CA-3'). The PCR products were treated in an equal volume of denaturing solution at 95°C for five minutes, and then placed immediately on ice before loading on to a gel. Electrophoresis was carried out at 10°C for 100 minutes.

The allele-specific primers were used as follows: for the forward primer, 5'-GAT TAC AGA CAT GGG TCA CGG CAC-3', and for the reverse primer, 5'-AAT CAG GAC AAG ACA TGG TTT TTC-3' for the 5A allele or 5'-AAT CAG GAC AAG ACA TGG TTT TTT-3' for the 6A allele. Hot-start PCR was performed. The annealing temperature was 65°C for the 5A allele and 62°C for the 6A allele, and 30 cycles of amplification were carried out.

Statistical analysis. The subjects were divided into two groups according to the MMP-3 promoter genotype. One had a 5A allele (5A5A and 5A6A genotype; 5A+ group), the other had no 5A allele (6A6A genotype, 5A-group). The difference in the number of degenerative IVDs was analysed between genotypes using Student's *t*-test. In the elderly group, the difference in the distribution of IVD degenerative scores was assessed between genotypes by the Mann-Whitney U test. Any difference in age or gender between genotypes was also determined by Student's *t*-test or Fisher's exact test.

Results

Tables I and II give the promoter genotypes and allele frequency. These data conform to the Hardy-Weinberg equilibrium in that the allele frequencies will remain unchanged generation after generation in a hypothetical situation. The frequency of the 5A allele was 0.18 in the younger group and 0.20 in the elderly group. This is similar to that in a healthy Japanese population reported by Terashima et al[17] although different from those in the UK and The Netherlands.[14,18,19] The genotype was independent of age and gender.

MRI is a more efficient method of assessing IVD degeneration than radiography and was therefore the method of choice in our younger subjects. In the elderly

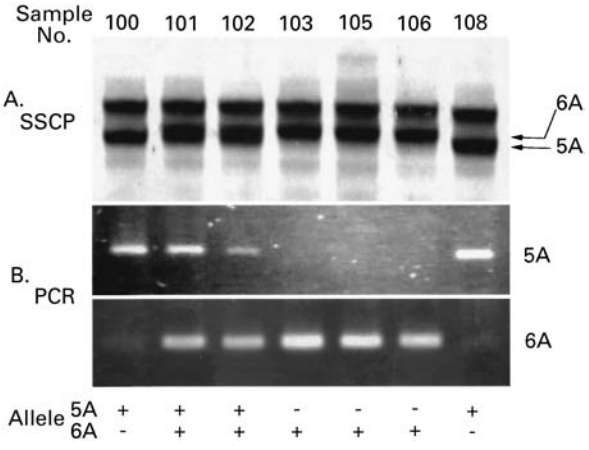

Fig. 1

Detection of the MMP-3 promoter genotype by PCR-SSCP analysis (A) and allele-specific PCR (B). The results of both methods were the same.

Table I. Prevalence of MMP-3 promoter genotypes and 5A allele frequency in the younger group of 54 subjects

	Age (yr)	Total
Genotype		
5A+ (5A5A+5A6A)	22.3 ± 2.9	2+15 (*31%*)
5A- (6A6A)	21.0 ± 2.8	37 (*69%*)
5A allele frequency		0.18

Table II. Prevalence of MMP-3 promoter genotypes and 5A allele frequency in the elderly group of 49 subjects

	Age (yr)	Male	Female	Total
Genotype				
5A+ (5A5A+5A6A)	74.1 ± 6.2	0+5	2+11	2+16 (*37%*)
5A- (6A6A)	74.4 ± 6.7	6	25	31 (*63%*)
5A allele frequency		0.23	0.20	0.20

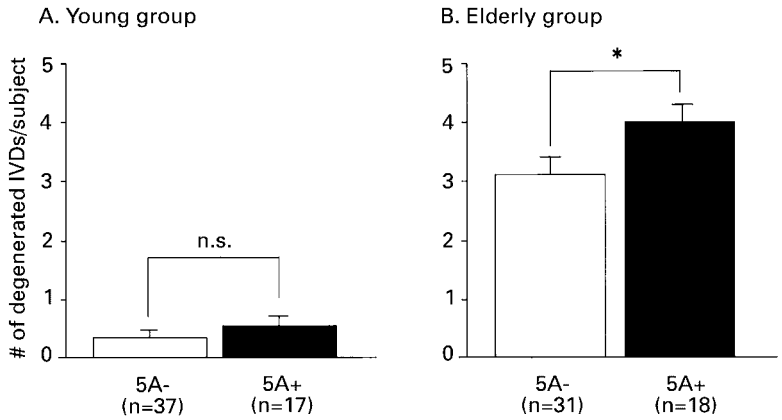

Fig. 2

The number of degenerative IVDs in the 5A- and 5A+ groups in A) the young and B) the elderly. Although there is no significant difference between the 5A- and 5A+ group in younger subjects, the 5A+ group had a significantly higher number of degenerative IVDs than the 5A- group in the elderly (Student's *t*-test, $p < 0.05$).

group, however, most IVDs already show abnormal signal intensity on MRI.[4] We therefore used a radiographical grading system and assessed indirect changes of degeneration, e.g. osteophytes, narrowing of the disc height and endplate sclerosis.[16]

Although the younger group had no significant differences in the number of degenerative IVDs between the 5A+ and 5A- group (Fig. 2A), in the elderly group there was a significantly larger number of degenerative IVDs graded 2 and higher in the 5A+ genotype than in the 5A- genotype ($p < 0.05$, Fig. 2B). In this group also there was a significant difference in the distribution of IVD degenerative scores between the 5A+ and 5A- genotypes ($p = 0.0029$, Fig. 3). The 5A+ group showed a higher incidence of severely degenerative lumber discs than the 5A- group.

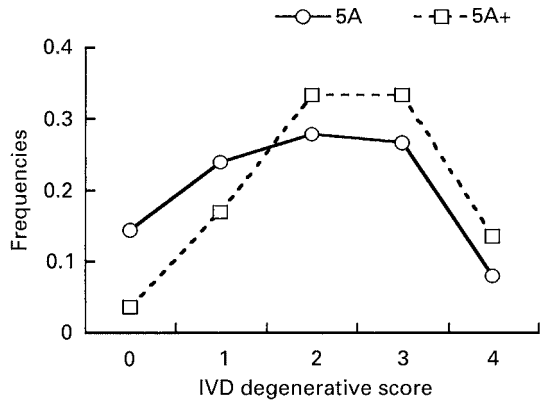

Fig. 3

The frequencies of the degenerative scores of the lumbar discs in the 5A- and 5A+ groups in the elderly. Their distributions were significantly different between the 5A- and 5A+ genotypes (Mann-Whitney U test, $p = 0.0029$). As a whole, the distribution of IVD degenerative scores in the 5A+ group moved into a higher grade compared with the 5A- group.

Discussion

The precise biological mechanism of IVD degeneration remains unclear, but biochemical mediators of tissue degradation, especially MMPs, have been identified as significant factors.[10] Our study has shown that the MMP-3 promoter genotype was associated with IVD degeneration in the elderly group aged more than 64 years, but not in subjects of less than 28 years of age.

MMP-3 is a potentially key enzyme which directly degrades components of the extracellular matrix including proteoglycans, laminin, fibronectin, gelatins, and collagens.[20] MMP-3 also indirectly affects degradation of the extracellular matrix by activating other latent MMPs.[10,21]

An immunohistological study has shown that there is an increased expression of MMP-3 than of tissue inhibitor of metalloproteinases-1 (TIMP-1) in degenerative IVDs.[22] MMP-3 production in IVD can be induced by pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α).[23] Mechanical loading, such as that induced by a high hydrostatic pressure[11] or an acute shear stress,[12] also induces production of MMP-3.

Degradation of the disc matrix is one of the important steps in IVD degeneration. Haro et al[23] reported that the induction of chondrocyte MMP-3 by co-culture with macrophages was necessary for degradation of the matrix in a model of herniated IVD resorption. The physical degradation does not occur with MMP-3-null disc tissue in that co-culture model.[23] These reports suggest that MMP-3 plays an important role in IVD degeneration.

In a transient transfection experiment, Ye et al[15] reported that an MMP-3 promoter construct with 5A had an expression which was twice that of the chloramphenicol acetyltransferase (CAT) reporter gene compared with a construct with 6A. This suggests that IVDs in the 5A+ group may express a larger amount of MMP-3 than those in the 5A-

group. Our study showed that having a 5A allele could accelerate IVD degeneration in the elderly. Under these conditions, an imbalance of MMP-3 and TIMP-1 may accelerate degeneration.

Some studies have addressed the relationship between MMP-3 promoter polymorphism and coronary atherosclerosis[14,18,19] or acute myocardial infarction (AMI).[17] The 6A allele was associated with a more rapid progression of coronary stenosis due to atherosclerosis,[15,18,19] and the 5A allele was more common in patients with AMI than in control subjects.[17] These findings have led to the speculation that MMP-3 transcriptional regulation in an allele-specific manner could affect continuous connective tissue remodelling in arterial walls and in rupture of atherosclerotic plaques. Kauppila[24] has suggested that LBP and IVD degeneration may be due to disease of the lumbar artery. The association of MMP-3 promoter polymorphism and IVD degeneration may therefore be related to arterial disease.

It is possible that genetic factors may have a specific role in herniation of the lumbar disc in the younger population. Varlotta et al[25] showed a familial basis for herniation of the lumbar disc in young patients. Since young lumbar discs are exposed to environmental factors for only a short period, any disease could be strongly associated with genetic factors. Our data indicate, however, that there was no significant relationship between the MMP-3 promoter polymorphism and IVD degeneration in the younger group.

Since MMP-3 promoter polymorphism affects the expression of MMP-3 in response to environmental factors, a different amount of MMP-3 expression and activity between genotypes may accumulate with advancing age. Haro et al[23] reported that IVD chondrocytes and macrophages in combination may induce prominent MMP-3 expression, resulting in IVD degradation. Although the IVD is avascular after adolescence, vascularisation commonly occurs again after the fifth decade in association with degenerative changes and in response to trauma.[26]

Vascularisation may expose IVD chondrocytes to macrophages contributing to the induction of MMP-3 expression. MMP-3 promoter polymorphism is therefore associated with disc degeneration in the elderly rather than in the younger population.

The genetic factors associated with degeneration of cartilage have been studied using the genetic analysis of hereditary skeletal dysplasias and models of animal disease. Mutations of some structural proteins, which are elements of the cartilage matrix such as collagen or proteoglycan, have been identified. Mutation in type-IX collagen or aggrecan can cause age-related degeneration of IVD and herniation in mice.[27,28] Recent studies have reported that an allele of the *COL9A2* gene, which codes for the alpha-2 chain of type-IX collagen, or a variable number of tandem repeat polymorphism in the coding region of the GAG-attachment domain of aggrecan is associated with IVD disease in man.[8,9] Polymorphism of a structural protein could lead to microstructural changes in the matrix of cartilage resulting in a fragile matrix, or it may affect cell function causing accelerated degradation of the matrix.

Our study is the first report to indicate a relationship between polymorphism of the gene coding for a matrix-degrading enzyme and IVD degeneration. Polymorphism of structural proteins and degrading enzymes should be considered as potential risk factors for degeneration of IVDs.

The 5A allele could enhance the degeneration of IVDs associated with environmental conditions resulting from the induction of a higher level of MMP-3 expression in response to such conditions. Thus, we conclude that the 5A allele of the human MMP-3 promoter is a crucial risk factor for the acceleration of IVD degeneration especially in the older population.

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