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Genetic polymorphisms of the matrix metalloproteinase-3 (*MMP-3*) and tissue inhibitors of matrix metalloproteinases-1 (*TIMP-1*) modulate the development of ankylosing spondylitis

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ABSTRACT

Background: The aetiology of ankylosing spondylitis (AS) remains unclear. Inflammation progresses to fibrosis and calcification of the spine and sacroiliac joints in AS development. Fibrosis results from excessive accumulations of the extracellular matrix (ECM). ECM turnover depends on the balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

Objective: To evaluate the effects of the *MMP-3* -1171 and *TIMP-1* 372 T>C polymorphisms on the modified risk of AS.

Methods: Genotypes of 241 patients with AS and 241 controls were identified by PCR. Disease activity and functional status were assessed by the Bath Ankylosing Spondylitis Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BASFI) and the Bath Ankylosing Spondylitis Global (BAS-G) Score.

Results: *MMP-3 6A/6*A carriers had a 2.41-fold (95% confidence interval (CI) 1.55 to 3.74) increased risk of AS compared with 6A/5A and 5A/5A carriers. *TIMP-1* C alleles had a greater risk of AS, but this was not significant (odds ratio (OR) = 1.28, 95% CI 0.92 to 1.77). Pairwise analysis of the *MMP-3/TIMP-1* alleles showed that 6A/C (OR = 3.23, 95% CI 1.50 to 6.95) and 6A/T (OR = 2.55, 95% CI 1.17 to 5.54) had a significantly greater risk of AS than the 5A/T alleles. After adjustment for the effects of age, gender and disease duration, the *MMP-3/TIMP-1* 5A/T alleles had the lowest BASDAI (p = 0.02), BASFI (p = 0.05) and BAS-G (p = 0.02) among all *MMP-3/TIMP-1* alleles.

Conclusion: The findings highlight the importance of the *MMP-3* and *TIMP-1* genes as crucial elements in AS development.

Ankylosing spondylitis (AS) is characterised by chronic inflammation of the sacroiliac joints and the vertebral column.[1] AS ultimately limits the mobility of the spine and other joints, leading to increasing functional impairment.[2] Men are affected more frequently than women,[1] and the aetiology of AS remains unclear, though heritability has a major role. Twin studies suggest that up to 97% of AS susceptibility is attributable to genetic factors.[3] Some have suggested an association between AS and the major histocompatibility complex (MHC), including HLA-B27.[4] However, non-MHC genes may account for at least 50% of the genetic variability seen in AS.[5]

Inflammatory processes gradually lead to fibrosis and calcification of the spine and sacroiliac joints during the development of AS.[6 7] The fibrosis is caused by an excessive accumulation of extracellular matrix (ECM). AS pathogenesis is additionally mediated by cytokines, chemokines and factors that degrade ECM and its components.[8] Matrix metalloproteinases (MMPs) are the major enzymes that degrade ECM. They play a part in arthritis through ECM degradation and activation of highly proinflammatory factors.[9] Matrix metalloproteinase 3 (MMP-3), also known as human fibroblast stromelysin, is a secreted metalloprotease predominantly produced by connective tissue cells.[10] MMP-3 and other metalloproteases can synergistically degrade major ECM components. MMP-3 also degrades proteoglycans, fibronectins, laminins and basement membrane (type IV) collagens.[11] Further, MMP-3 serum levels are correlated with parameters of inflammation including the erythrocyte sedimentation rate, C-reactive protein and interleukin 6 levels.[12 13] Patients with rheumatoid arthritis have increased serum MMP-3 levels, which probably originated in synovial tissue.[14] An *MMP-3* gene promoter polymorphism exists at position -1171 with a 6-adenosine run in one allele (6A) and a 5-adenosine run in the other allele (5A).[15] In vitro assays have shown that the 5A allele has twofold higher promoter activity than the 6A allele.[15] Therefore, homozygosity in the 6A allele should result in lower stromelysin levels than other genotypes owing to reduced gene transcription, and this lower level of proteolytic activity should favour ECM deposition.

Turnover of the ECM depends on the balance between MMPs and the specific tissue inhibitors of matrix metalloproteinases (TIMPs).[16] Previous studies have observed that TIMPs block aortic wall destruction and prevent aneurysm development through MMP inhibition,[17] and also inhibit type IV collagenolysis.[18] Enhanced MMP activity and aneurysm formation was shown in a mouse model of TIMP-1 deficiency.[19] The gene encoding *TIMP-1* maps to the short arm of the X chromosome (Xp11.3-p11.23),[20] and three polymorphisms have been described.[21] The first polymorphism, *TIMP-1* -19 C>T, is located in the 5′-UTR of the gene while the second, *TIMP-1* 261 C>T, is in exon 4 with no effects on amino acid sequence and the third, *TIMP-1* 372 T>C, is in the exon 5. The *TIMP-1* 372 T>C polymorphism account for the great majority of polymorphisms in the gene, while the other two polymorphisms have a very low frequency of occurrence.[21] The 372 T>C polymorphism might serve as a marker in association studies.

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Therefore, we examined the association between the occurrence of the *MMP-3* -1171 and *TIMP-1* 372 T>C polymorphisms and the modified risk for AS through a case–control, hospital-based association study. In addition, disease severity has also been shown to be largely genetically determined as noted by the Bath Ankylosing Spondylitis Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI).[22] Therefore, we also evaluated the relationship of AS severity and genetic polymorphisms of *MMP-3* and *TIMP-1*.

PATIENTS AND METHODS

Study subjects

Patients were recruited sequentially from the AS clinic at Chung Shan Medical University Hospital in Taichung, Taiwan from November 2002 to December 2007. The study protocol conformed to the Declaration of Helsinki and had been approved by the relevant ethics committees of Chung Shan Medical University Hospital. Patients with AS who met selection criteria were asked to participate in the study. Informed consent was obtained before any data was collected from the respondents. Four selection criteria were used to recruit subjects: (*a*) patients aged 16–65 years; (*b*) AS diagnosis by the modified New York criteria[23]; (*c*) fluent Chinese language speakers; (*d*) cognitive performance not influenced by other diseases such as dementia. Sacroiliitis was confirmed by a qualified radiologist and AS diagnosis was confirmed by a qualified rheumatologist. A total of 241 unrelated patients with AS were included in the study as cases. A detailed clinical history was recorded by the doctor at enrolment, including details of age of disease onset, family history of AS and extraspinal manifestations.

General health questionnaires were completed at the time of sample collection to determine whether or not a person could serve as a healthy control. Potential controls were randomly selected from sequentially admitted patients with no significant medical histories or abnormal laboratory results. These people were admitted to the same medical centre for general physical examinations and were selected from the same geographical areas as the cases. A 1:1 ratio of case to control subjects was used in this study. The cases were matched for age ($\pm$5 years) and gender with control subjects. A total of 241 patients with AS and 241 controls were included in the statistical analyses. Further, a second cohort of 99 patients with AS and 99 controls were collected and then tested again.

Clinical evaluations

Disease activity and functional status were assessed by the Chinese versions of the BASDAI, the BASFI and the Bath Ankylosing Spondylitis Global (BAS-G) Score. Good reliability (0.87–0.94) and validity (0.92–0.94) of these Chinese methods have been documented.[24]

Laboratory analyses

Venous blood was collected during medical surveillance, stored at 4°C and processed on the same day. The blood was centrifuged to separate the serum and the cells. All specimens were stored under −70°C until analysis. HLA-B27 carriage had previously been assessed by flow cytometry.[25]

The *MMP-3* -1171 polymorphism was genotyped by polymerase chain reaction (PCR) amplification with mutagenic primers, followed by restriction fragment length polymorphisms (RFLP).[26] Primers used for the amplification of the *MMP-3* gene were 5′-GGT TCT CCA TTC CTT TGA TGG GGG GAA AGA-3′ and 5′-CTT CCT GGA ATT CAC ATC ACT GCC ACC ACT-3′. DNA (0.5 µl) was added to the PCR buffer containing a 200 ng mix of primers, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 0.1% of bovine serum albumin in a final volume of 50 µl. Amplification was achieved by the following: denaturation (94°C for 5 min), followed by 30 cycles at 94°C for 30 s, annealing (65°C for 30 s) and extension (72°C for 1 min). The PCR products were digested with *Tth*111I for 3 h at 65°C. The 6A/6A subjects had a unique undigested 130 bp fragment, the 5A/5A subjects had two fragments sized 97 bp and 33 bp and the heterozygous 6A/5A subjects had all three fragments.

*TIMP-1 Bss*SI polymorphisms were also determined using a PCR-RFLP technique.[27] A synonymous C>T exchange at amino acid position 124 was amplified to form a 175 bp undigested fragment with the primer pair 5′-GCA CAT CAT CAC TAC CTG CAG T C-3′ (*mismatched base*) and 5′-GAA ACA AGC CCA CGA TTT AG-3′. The amplification was carried out as follows: denaturation (94°C for 5 min), followed by 30 cycles at 94°C for 30 s, annealing (50°C for 45 s) and extension (72°C for 45 s). The PCR products were digested with *Bss*SI. Homozygous T/T subjects had a single 175 bp fragment, homozygous C/C subjects had both 152 bp and 23 bp fragments and heterozygous C/T subjects had all three fragments.

Genotyping was also accomplished with blinding to disease status of subjects. Rigorous quality control procedures were applied throughout the genotyping process. To avoid PCR contamination, reagents for the PCR reaction were carefully aliquoted and each aliquot was used no more than three times. For each assay, a negative control (no DNA template) was added to monitor PCR contamination. Pilot experiments were always conducted to optimise the restriction digestion conditions. After genotyping each genetic polymorphism, ~ 20– 25% of the samples in each genotype group were randomly selected for repeated assays to validate the results.

Statistical analysis

A multiple conditional logistic model was used for the matched odds ratios (ORs) and 95% confidence intervals (CIs) for *MMP-3* and *TIMP-1* genotypes and alleles in AS case and control groups. The differences among the genotypes and alleles for continuous variables (BASDI, BASFI and BAS-G) were calculated using analysis of variance or the general linear model to compare the mean values. All p values were calculated using two-tailed statistical tests. SAS 9.1 for Windows was used for the analysis.

RESULTS

Basic and clinical characteristic of the subjects

Table 1 shows the basic and clinical characteristics of the subjects. The mean (SD) age of cases and controls was 34.5 (11.6) and 34.4 (12.4) years, respectively. About 74.3% of all subjects were male. Mean (SD) age at onset was 25.0 (9.2) years, mean disease duration was 9.7 (9.2) years and mean duration from initial symptoms to diagnosis was 55.0 (70.7) months. All AS subjects were HLA-B27 positive and their mean BASDAI, mean BASFI and mean BAS-G scores were 3.9 (2.4), 2.2 (2.3) and 4.2 (3.0), respectively.

Association of *MMP-3* and *TIMP-1* alleles with AS susceptibility

Table 2 shows the genotypic prevalence of *MMP-3* and *TIMP-1* among the study subjects. In controls, *MMP-3* alleles were measured at 84.2% (6A) and 15.8% (5A), while *TIMP-1* alleles were measured at 55.8% (C) and 44.2% (T). The number of

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Table 1 Basal characteristics and clinical features of patients with ankylosing spondylitis (AS) and of normal controls

Characteristics	Patients with AS	Normal controls
Number of subjects	241	241
Gender: male, No (%)	179 (74.3)	179 (74.3)
Age (years)	34.5 (11.6)	34.4 (12.4)
Range	16–65	19–61
Clinical features		
Age on initial symptom (years)	25.0 (9.2)	
Disease duration (years)	9.7 (9.2)	
Delayed diagnosis (months)	55.0 (70.7)	
BASDAI (0–10)	3.9 (2.4)	
BASFI (0–10)	2.2 (2.3)	
BAS-G (0–10)	4.2 (3.0)	

Data are given as mean (SD) unless stated otherwise.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index, BASFI, Bath Ankylosing Spondylitis Functional Index; BAS-G, Bath Ankylosing Spondylitis Global.

MMP-3 5A/5A alleles was small and therefore those with at least one *MMP-3* 5A allele were grouped together. The matched ORs of AS were calculated to investigate the effect of *MMP-3* and *TIMP-1* using a conditional logistic regression model. Subjects with the *MMP-3* homozygous 6A/6A genotype had a 2.41-fold (95% CI 1.55 to 3.74; p<0.01) increased risk of AS compared with those with the 6A/5A and 5A/5A genotypes. The presence of the 6A allele frequency in the *MMP-3* -1171 promoter genotype among patients with AS was higher than in normal controls (91.3% vs 84.2%; OR = 1.96, 95% CI 1.31 to 2.92; p = 0.001). The *TIMP-1* C allele was associated with a higher AS risk (OR = 1.28, 95% CI 0.92 to 1.77) than the *TIMP-1* T allele. Since the *TIMP-1* gene is on the X chromosome,[20] we also analysed the results segregated by sex. The C allele of the *TIMP-1* 372T>C polymorphism was not over-represented in female controls. The *TIMP-1* C allele was associated with a higher AS risk in men (OR = 1.09, 95% CI 0.72 to1.66) and women (OR = 1.63, 95% CI 0.97 to 2.75; p = 0.07), but this was not significant. Further, the *MMP-3/TIMP-1* pairwise allele analysis found that 6A/C (OR = 3.23, 95% CI 1.50 to 6.95; p = 0.003) and 6A/T (OR = 2.55, 95% CI 1.17 to 5.54; p = 0.02) had an association with significantly higher AS risks in comparison with the 5A/T alleles.

The validation of these finding in our second study group (99 male patients with AS and 99 male controls) showed that the frequency of *MMP-3* 6A/6A genotype differs between patients and controls (OR = 2.15; 95% CI 1.08 to 4.26, table 3).

Association of *MMP-3* and *TIMP-1* alleles with the severity of AS disease

We analysed the relationship between disease activity and *MMP-3* and *TIMP-1* alleles among patients with AS (table 4). Higher BASDAI (p = 0.70), BASFI (p = 0.85) and BAS-G (p = 0.68) scores were seen in *MMP-3* 6A/6A and *MMP-3* 6A/5A carriers than in *MMP-3* 5A/5A carriers, respectively, although crude differences were not significant. Lower BASDAI (p = 0.12), BASFI (p = 0.10) and BAS-G (p = 0.01; Bonferroni correction p = 0.01) scores were seen in *TIMP-1* C carriers than in T carriers. Further, after adjustment for the effects of age, gender and disease duration, the *MMP-3/TIMP-1* 5A/T alleles had the lowest BASDAI (p = 0.02), BASFI (p = 0.05) and BAS-G (p = 0.02) in different *MMP-3/TIMP-1* alleles and these values were statistically significant. When multiple testing (Bonferroni correction) was taken into consideration, our results for the *MMP-3/TIMP-1* 5A/T alleles became non-significant.

DISCUSSION

Our study suggests that *MMP-3* genetic polymorphism at position -1171 in the promoter region and the *TIMP-1* 372 polymorphism in exon 5 were associated with AS development.

Table 2 Genotyping and allele frequency of *MMP-3* and *TIMP-1* single nucleotide polymorphism (SNP) in patients with ankylosing spondylitis (AS) and in controls

SNP	Alleles	Patients with AS No (%)	Normal controls No (%)	Odds ratio (95% CI)*
MMP-3	6A/6A	203 (84.2)	166 (68.9)	2.41 (1.55 to 3.74)
	6A/5A	34 (14.1)	74 (30.7)	Reference†
	5A/5A	4 (1.7)	1 (0.4)	
	6A	440 (91.3)	406 (84.2)	1.96 (1.31 to 2.92)
	5A	42 (8.7)	76 (15.8)	Reference
TIMP-1	C	187 (61.7)	169 (55.8)	1.28 (0.92 to 1.77)
	T	116 (38.3)	134 (44.2)	Reference
Men	C	101 (56.4)	97 (54.2)	1.09 (0.72 to 1.66)
	T	78 (43.6)	82 (45.8)	Reference
Women	C/C	32 (51.6)	24 (38.7)	2.32 (0.84 to 6.38)
	C/T	22 (35.5)	24 (38.7)	1.60 (0.57 to 4.52)
	T/T	8 (12.9)	14 (22.6)	Reference
	C	86 (69.4)	72 (58.1)	1.63 (0.97 to 2.75)
	T	38 (30.7)	52 (41.9)	Reference
MMP-3/TIMP-1	6A/C	157 (51.8)	126 (41.6)	3.23 (1.50 to 6.95)
	6A/T	106 (34.9)	108 (35.6)	2.55 (1.17 to 5.54)
	5A/C	30 (9.9)	43 (14.2)	1.81 (0.76 to 4.30)
	5A/T	10 (3.4)	26 (8.6)	Reference

*Data were matched by age and gender and calculated using conditional logistic regression.
†*MMP-3* 5A/5A and 6A/5A genotypes were grouped together as reference.

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Table 3 Genotyping and allele frequency of *MMP-3* and *TIMP-1* single nucleotide polymorphism (SNP) in a second sample of 99 male patients with ankylosing spondylitis (AS) and 99 male controls

SNP	Alleles	Patients with AS No (%)	Normal controls No (%)	Odds ratio (95% CI)*
MMP-3	6A/6A	83 (83.8)	70 (70.7)	2.15 (1.08 to 4.26)
	6A/5A	16 (16.2)	28 (28.3)	Reference†
	5A/5A	0 (0)	1 (1.0)	
	6A	182 (91.9)	168 (84.8)	1.78 (0.98 to 3.31)
	5A	16 (8.1)	30 (15.2)	Reference
TIMP-1	C	59 (61.7)	54 (54.6)	1.22 (0.69 to 2.14)
	T	40 (38.3)	45 (45.4)	Reference

*Data were matched by age and calculated using conditional logistic regression.
†*MMP-3* 5A/5A and 6A/5A genotypes were grouped together as reference.

The 6A and 5A alleles have been identified in the polymorphism at position -1171 in the *MMP-3* promoter.[15] In this study, the *MMP-3* 5A allele frequency in our normal controls was 15.8%. This was comparable with corresponding values among controls (18.3%) in an acute coronary atherosclerotic study in the Taiwanese population,[28] and validated our genotyping techniques. Further, we observed an association between an *MMP-3* gene promoter polymorphism and AS susceptibility, but not AS severity. In 97 patients of the Outcome Assessments in Ankylosing Spondylitis International Study (OASIS) it was shown that MMP-3 levels were independently predictive of structural damage over 2 years in patients with AS.[29] Recently, serum MMP-3 levels were observed to be significantly elevated compared with healthy controls in a group of 23 French patients with active AS chiefly involving the axial skeleton.[30] Ribbens *et al*[31] had previously reported that 11% of patients with AS had abnormal serum MMP-3 levels. Yang *et al*[32] also found no significant differences in serum MMP-3 levels between patients with AS and healthy subjects.

It may be necessary to examine the effects of genotype-related expression in cell subsets involving AS pathology in the post-translational modification of MMP-3 proteins. The relationship between structural variations in the *MMP-3* promoter gene and AS development is still unknown. Previously, Jin *et al*[33] investigated the role of *MMP-3* in AS susceptibility using a large cohort collected by the North American Spondylitis Consortium. None of the eight *MMP-3* single nucleotide polymorphisms (SNPs) were reported to be significantly associated with AS. However, 5A/6A was not genotyped in their study. We further found the association between the occurrence of the *MMP-3* -1171 and polymorphisms and the modified risk for AS in our cohort. However, it is possible that the *MMP-3* gene may only be a susceptibility gene for AS in certain ethnic populations. Additional study including more subjects from different populations may shed light on this question.

Homozygosity of the 6A allele may have a greater association with lower stromelysin levels than other genotypes owing to reduced gene transcription.[15] This lower level of proteolytic activity could favour ECM deposition. A recent study found that the presence of the 6A allele had an additive effect on increasing levels of serum MMP-3, but no significant associations were noted between the presence of the *MMP-3* -1171 polymorphism and disease activity or rheumatoid arthritis severity in Japanese patients.[34]

In addition, MMP-3 serum levels have been correlated with measures of inflammation including erythrocyte sedimentation rate, C-reactive protein and interleukin 6 levels.[12 13] Therefore,

Table 4 Difference in the scores of BASDAI, BASFI and BAS-G among patients with AS stratified by different *MMP-3* and *TIMP-1* alleles

SNP	Alleles	BASDAI	BASFI	BAS-G
MMP-3	6A/6A	3.9 (2.4)*	2.2 (2.3)	4.2 (3.0)
	6A/5A	3.8 (2.4)	2.4 (2.3)	4.6 (2.7)
	5A/5A	2.9 (1.7)	1.8 (1.3)	3.6 (3.0)
	Unadjusted p value	0.70	0.85	0.68
	Adjusted p value†	0.46	0.89	0.89
TIMP-1	C	3.7 (2.3)	2.0 (2.2)	3.8 (3.0)
	T	4.1 (2.5)	2.4 (2.4)	4.7 (3.0)
	Unadjusted p value	0.12	0.10	0.01
	Adjusted p value†	0.17	0.09	0.04
MMP-3/TIMP-1	6A/C	3.6 (2.3)	1.9 (2.2)	3.7 (3.1)
	6A/T	4.3 (2.5)	2.5 (2.5)	4.8 (3.0)
	5A/C	4.1 (2.3)	2.6 (2.3)	4.9 (2.7)
	5A/T	2.6 (1.8)	1.6 (1.3)	3.6 (2.3)
	Unadjusted p value	0.04	0.07	0.01
	Adjusted p value†	0.02	0.05	0.02

*Data represent means (SD).
†Adjusted for the effects of age, gender and disease duration.
AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index, BASFI, Bath Ankylosing Spondylitis Functional Index; BAS-G, Bath Ankylosing Spondylitis Global; SNP, single nucleotide polymorphism.

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our observations suggest that the 5A/6A polymorphism in the *MMP-3* gene promoter is functionally relevant in the pathophysiology of AS.[15] However, the control of MMP-3 expression in vivo is complex and modulated by other transcription or post-transcription factors, such as cytokines.[35 36] Functional studies are required to test these hypotheses.

The process of ECM degradation depends on the balance between the reduction of MMPs and/or the increase of TIMPs[16 17] and may result in joint damage. Our data further emphasise this concept. SNPs of the *TIMP-1* gene are of particular interest since *TIMP-1* is located on the X chromosome[20] and there is a sex difference in AS.[1] Importantly, the T allele at SNP +372 accompanied lower TIMP-1 protein expression in inflamed tissues in men but not in women.[37] Higher TIMP-1 protein levels relative to MMP in susceptible subjects might shift the balance to a disease phenotype favouring AS development. In our AS subjects, 43.6% of men had the *TIMP-1* T allele. Female patients with AS with *TIMP-1* T allele comprised only 30.7%. Therefore, the protective effect of the *TIMP-1* T allele on AS susceptibility is not as obvious in women as in men. In addition, the proportion of *TIMP-1* heterozygous C/T genotypes in female patients with AS was 35.5% and polymorphic X chromosome inactivation of the *TIMP-1* gene has been suggested.[38] Therefore, our results might also reflect X chromosome inactivation in female patients with AS. *TIMP-1* heterozygous C/T female carriers would have restricted TIMP-1 expression and reduced AS development of AS in this model, as a consequence of the *TIMP-1* homozygous T/T genotype. Variable TIMP-1 expression from the inactive X chromosome may be expected. Further, this also explains the association in our study between the presence of the *TIMP-1* C allele and female AS susceptibility, although it did not reach statistical significance (OR = 1.63, 95% CI 0.97 to 2.75, table 2). In addition, the *TIMP-1* T allele in men had a higher AS risk, but this was not significant. This illustrates that *TIMP-1* may have dual roles in the fibrotic processes generated in AS: (*a*) it may act as a growth factor for fibroblasts, which are the major producers of type I collagen or (*b*) it may reduce ECM turnover, thereby inhibiting the activity of MMPs. Interestingly, subjects who had susceptible *MMP-3* 6A and *TIMP-1* C alleles were more likely to experience AS development. This indicates that each susceptible genotype may generate increased risks for AS development; however, when combined together, a stronger risk may result.

The possible effect of the *MMP-3* and *TIMP-1* genes on AS severity is unknown. In our study, differences in BASDAI, BASFI and BAS-G were shown in our patients with AS with different *MMP-3/TIMP-1* alleles, even after adjusting for the effects of age, gender and disease duration. Interestingly, *MMP-3/TIMP-1* 5A/T alleles had the lowest BASDAI, BASFI and BAS-G scores in different *MMP-3/TIMP-1* alleles, although the significance did not remain from multiple statistical testing. Subjects with *MMP-3/TIMP-1* 6A/T or 5A/C had obviously increased AS severity. However, patients with AS with the *MMP-3/TIMP-1* 6A/C alleles only had moderately increased BASDAI, BASFI and BAS-G scores. Several sex- and immune-related genes, crucial in the maintenance of physiological levels of sex hormones and of immune tolerance, have been localised to the X chromosome.[39 40] Non-MHC genes may also have decisive roles in determining tolerance and arthritis susceptibilities.[41] However, we observed that *MMP-3/TIMP-1* 6A/C carriers are more likely to develop AS than other allele carriers. It is also possible that subjects with the *MMP-3/TIMP-1* 6A/C alleles might experience increased immune tolerance and as a result might experience less severe forms of AS.

There are some limitations in the interpretation of our data. Selection bias might have occurred when patients with AS with either active or inactive disease were enrolled as our study subjects. In addition, our study may have included referral bias since the data were collected from a single medical centre. Although recall bias could be a potential problem, the administration of three different instruments made it less likely. Our Chinese versions of the BASDAI, BASFI and BAS-G have shown adequate reliability and validity.[24] Thus, disease activity and functional status in Chinese-speaking patients with AS may be adequately evaluated with these adapted versions of the original instruments.

In summary, the balance between MMPs and TIMPs might act as crucial determinants in AS pathophysiology. We observed that, among patients with AS, *MMP-3* promoter polymorphisms at position -1171 and *TIMP-1* 372 in exon 5 were associated with susceptibility and severity in patients with AS.

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REFERENCES

1. **Braun J,** Sieper J. Ankylosing spondylitis. *Lancet* 2007;**369**:1379–90.
2. **Van der Heijde D,** Calin A, Dougados M, *et al*. Selection of instruments in the core set for DC-ART, SMARD, physical therapy, and clinical record keeping in ankylosing spondylitis. Progress report of the ASAS Working Group. Assessments in Ankylosing Spondylitis. *J Rheumatol* 1999;**26**:951–4.
3. **Brown MA,** Kennedy LG, MacGregor AJ, *et al*. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997;**40**:1823–8.
4. **Brewerton DA,** Hart FD, Nicholls A, *et al*. Ankylosing spondylitis and HL-A 27. *Lancet* 1973;**1**:904–7.
5. **Brown MA,** Laval SH, Brophy S, *et al*. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis* 2000;**59**:883–6.
6. **LeBlanc CM,** Inman RD, Dent P, *et al*. Retroperitoneal fibrosis: an extraarticular manifestation of ankylosing spondylitis. *Arthritis Rheum* 2002;**47**:210–4.
7. **Afeltra A,** Gentilucci UV, Rabitti C, *et al*. Retroperitoneal fibrosis and ankylosing spondylitis: which links? *Semin Arthritis Rheum* 2005;**35**:43–8.
8. **Zhu J,** Yu DT. Matrix metalloproteinase expression in the spondyloarthropathies. *Curr Opin Rheumatol* 2006;**18**:364–8.
9. **Murphy G,** Knauper V, Atkinson S, *et al*. Matrix metalloproteinases in arthritic disease. *Arthritis Res* 2002;**4**(Suppl 3):S39–49.
10. **Sellers A,** Murphy G. Collagenolytic enzymes and their naturally occurring inhibitors. *Int Rev Connect Tissue Res* 1981;**9**:151–90.
11. **Chin JR,** Murphy G, Werb Z. Stromelysin, a connective tissue-degrading metalloendopeptidase secreted by stimulated rabbit synovial fibroblasts in parallel with collagenase. Biosynthesis, isolation, characterization, and substrates. *J Biol Chem* 1985;**260**:12367–76.
12. **Ribbens C,** Andre B, Kaye O, *et al*. Synovial fluid matrix metalloproteinase-3 levels are increased in inflammatory arthritides whether erosive or not. *Rheumatology (Oxford)* 2000;**39**:1357–65.
13. **Ates A,** Turkcapar N, Olmez U, *et al*. Serum pro-matrix metalloproteinase-3 as an indicator of disease activity and severity in rheumatoid arthritis: comparison with traditional markers. *Rheumatol Int* 2007;**27**:715–22.
14. **Sasaki S,** Iwata H, Ishiguro N, *et al*. Detection of stromelysin in synovial fluid and serum from patients with rheumatoid arthritis and osteoarthritis. *Clin Rheumatol* 1994;**13**:228–33.
15. **Ye S,** Eriksson P, Hamsten A, *et al*. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 1996;**271**:13055–60.
16. **Woessner JF Jr.** Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 1991;**5**:2145–54.
17. **Nakamura M,** Tachieda R, Niinuma H, *et al*. Circulating biochemical marker levels of collagen metabolism are abnormal in patients with abdominal aortic aneurysm. *Angiology* 2000;**51**:385–92.
18. **Keeling WB,** Armstrong PA, Stone PA, *et al*. An overview of matrix metalloproteinases in the pathogenesis and treatment of abdominal aortic aneurysms. *Vasc Endovascular Surg* 2005;**39**:457–64.
19. **Silence J,** Collen D, Lijnen HR. Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (*TIMP-1*) gene. *Circ Res* 2002;**90**:897–903.
20. **Docherty AJ,** Lyons A, Smith BJ, *et al*. Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. *Nature* 1985;**318**:66–9.
21. **Hinterseher I,** Krex D, Kuhlisch E, *et al*. Tissue inhibitor of metalloproteinase-1 (*TIMP-1*) polymorphisms in a Caucasian population with abdominal aortic aneurysm. *World J Surg* 2007;**31**:2248–54.

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22. **Hamersma J,** Cardon LR, Bradbury L, *et al*. Is disease severity in ankylosing spondylitis genetically determined? *Arthritis Rheum* 2001;**44**:1396–400.
23. **Van der Linden S,** Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984;**27**:361–8.
24. **Wei JC,** Wong RH, Huang JH, *et al*. Evaluation of internal consistency and re-test reliability of Bath ankylosing spondylitis indices in a large cohort of adult and juvenile spondylitis patients in Taiwan. *Clin Rheumatol* 2007;**26**:1685–91.
25. **Chou CT,** Tsai YF, Liu J, *et al*. The detection of the HLA-B27 antigen by immunomagnetic separation and enzyme-linked immunosorbent assay-comparison with a flow cytometric procedure. *J Immunol Methods* 2001;**255**:15–22.
26. **Gnasso A,** Motti C, Irace C, *et al*. Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects. *Arterioscler Thromb Vasc Biol* 2000;**20**:1600–5.
27. **Wollmer MA,** Papassotiropoulos A, Streffer JR, *et al*. Genetic polymorphisms and cerebrospinal fluid levels of tissue inhibitor of metalloproteinases 1 in sporadic Alzheimer's disease. *Psychiatr Genet* 2002;**12**:155–60.
28. **Liu PY,** Li YH, Chan SH, *et al*. Genotype-phenotype association of matrix metalloproteinase-3 polymorphism and its synergistic effect with smoking on the occurrence of acute coronary syndrome. *Am J Cardiol* 2006;**98**:1012–7.
29. **Maksymowych WP,** Landewé R, Conner-Spady B, *et al*. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis Rheum* 2007;**56**:1846–53.
30. **Wendling D,** Cedoz JP, Racadot E. Serum levels of MMP-3 and cathepsin K in patients with ankylosing spondylitis: effect of TNFalpha antagonist therapy. *Joint Bone Spine* 2008;**75**:559–62.
31. **Ribbens C,** Martin y Porras M, Franchimont N, *et al*. Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment. *Ann Rheum Dis* 2002;**61**:161–6.
32. **Yang C,** Gu J, Rihl M, *et al*. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. *Arthritis Rheum* 2004;**51**:691–9.
33. **Jin L,** Weisman M, Zhang G, *et al*. Lack of association of matrix metalloproteinase 3 (MMP3) genotypes with ankylosing spondylitis susceptibility and severity. *Rheumatology (Oxford)* 2005;**44**:55–60.
34. **Tsukahara S,** Shinozaki M, Ikari K, *et al*. Effect of matrix metalloproteinase-3 functional SNP on serum matrix metalloproteinase-3 level and outcome measures in Japanese RA patients. *Rheumatology (Oxford)* 2008;**47**:41–4.
35. **Mauviel A.** Cytokine regulation of metalloproteinase gene expression. *J Cell Biochem* 1993;**53**:288–95.
36. **Millward-Sadler SJ,** Wright MO, Davies LW, *et al*. Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. *Arthritis Rheum* 2000;**43**:2091–9.
37. **Meijer MJ,** Mieremet-Ooms MA, van Hogezand RA, *et al*. Role of matrix metalloproteinase, tissue inhibitor of metalloproteinase and tumor necrosis factor-α single nucleotide gene polymorphisms in inflammatory bowel disease. *World J Gastroenterol* 2007;**13**:2960–6.
38. **Anderson CL,** Brown CJ. Polymorphic X-chromosome inactivation of the human *TIMP1* gene. *Am J Hum Genet* 1999;**65**:699–708.
39. **Ranke MB,** Saenger P. Turner's syndrome. *Lancet* 2001;**358**:309–14.
40. **Davis CJ,** Davison RM, Payne NN, *et al*. Female sex preponderance for idiopathic familial premature ovarian failure suggests an X chromosome defect: opinion. *Hum Reprod* 2000;**15**:2418–22.
41. **Delgado M,** Robledo G, Rueda B, *et al*. Genetic association of vasoactive intestinal peptide receptor with rheumatoid arthritis: altered expression and signal in immune cells. *Arthritis Rheum* 2008;**58**:1010–19.

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