Collagenase-1 (MMP-1) and HLA-DRB1 Gene Polymorphisms in Rheumatoid Arthritis: A Prospective Longitudinal Study

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ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) is characterized by chronic synovitis leading to permanent damage of the joints. Collagenase-1 (MMP-1) is a matrix metalloproteinase involved in articular cartilage degradation. We investigated the association between a biallelic polymorphism in the MMP-1 gene promoter and the susceptibility to, and severity of, RA. We also investigated the association between HLA-DRB1 gene polymorphism and severity of RA.

Methods. One hundred and three patients with early RA were included in this prospective longitudinal study. A radiographic damage score was used to quantify disease severity at baseline and after 4 years of followup. MMP-1 polymorphism genotyping was analyzed using a fluorescent-based polymerase chain reaction (PCR). HLA-DRB1 genotypes were determined by PCR sequence-specific oligonucleotide probes. One hundred and thirty-three healthy individuals were used as controls.

Results. MMP-1 allele and genotype frequencies did not differ between RA patients and controls. The radiographic damage or its progression over the 4 years of followup did not differ across MMP-1 genotypes. The radiographic damage score and its progression over the 4 years of followup differed across HLA-DRB1 genotypes. The HLA-DRB1 shared epitope +/+ genotype was associated with the highest radiographic damage score and the highest progression, while the shared epitope -/- genotype was associated with the lowest.

Conclusion. Our results do not support the hypothesis of an association between this particular polymorphism in the MMP-1 gene promoter and susceptibility to, or severity of, RA. This study confirms the previous reports of an association between the HLA-DRB1 gene polymorphism and severity of RA. (J Rheumatol 2002;29:15–20)

Key Indexing Terms:

COLLAGENASE-1 GENE | MMP-1 GENE | HLA-DRB1 GENE
RHEUMATOID ARTHRITIS | DISEASE SEVERITY | GENETICS

Rheumatoid arthritis (RA) is an autoimmune disease affecting about 1% of the world's adult population[1], characterized by chronic inflammation of the joints. The resulting joint pain and stiffness cause impaired function, and for the majority of cases the synovitis will lead to permanent damage of the articular cartilage and bone[2]. The identification of sensitive and specific prognostic factors in early RA appears essential to focus on the therapeutic agents that have been shown to slow down the progression of joint destruction and resulting disability in those patients most likely to undergo poor outcomes[3].

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HLA and non-HLA genes contribute to the pathogenesis of RA. The presence and dose effect of HLA-DRB1 alleles encoding the shared epitope (SE) affect the course and the outcome of RA[4]. Most estimates of the HLA component of the overall genetic risk for RA are < 50%[5]. Thus, the identification of new RA susceptibility and severity genes is currently an important challenge, using genome-wide screening or the candidate gene approach[6,7].

Matrix metalloproteinases (MMP) can degrade a range of extracellular matrix proteins and have been implicated in connective tissue destruction and remodeling associated with cancer invasion and metastasis, atherosclerotic plaque rupture, development of aneurysms and cartilage destruction in arthritis[8]. The expression of most metalloproteinases is regulated at the transcription level by growth factors, hormones, and cytokines. The proteolytic activities of metalloproteinases are precisely controlled during activation from their precursors and inhibition by endogenous inhibitors, α-macroglobulins and tissue inhibitors of metalloproteinases[9]. Collagenase-1 (MMP-1) can degrade the interstitial collagens, types I, II and III. It is produced by synoviocytes and chondrocytes. MMP-1 level is elevated in patients with RA, both in plasma[10] and in

synovial fluid where it correlates with the degree of synovial inflammation[11]. It is commonly observed in both synovium and cartilage, especially prominent at cartilage erosion sites in rheumatoid lesions[12]. A guanine (G) insertion/deletion polymorphism was recently reported in the promoter sequence of the MMP-1 gene. This polymorphism influences the transcription of this gene: the 2G (insertion-type) promoter possesses greater transcriptional activity than the 1G (deletion-type) promoter[13]. This polymorphism may be involved in pathological conditions such as cancer development and progression[14-16].

In the present prospective longitudinal study, we used a candidate gene approach to test the hypothesis of an association between this MMP-1 gene polymorphism and susceptibility to, and severity of RA in a cohort of 103 early patients with RA followed for 4 years. We also investigated the association between HLA-DRB1 gene polymorphism and severity of RA.

MATERIALS AND METHODS

Patients and controls. One hundred and three Caucasian outpatients who attended the Rangueil Hospital Department of Rheumatology between November 1992 and December 1994 were included in this prospective longitudinal study. All patients met the following criteria: (1) the American College of Rheumatology 1987 criteria for RA[17], (2) disease duration < 1 year, (3) age > 16 years. The following clinical, functional, and biological variables were evaluated yearly for 4 years in RA patients: Ritchie articular index, number of swollen joints, French version of the Health Assessment Questionnaire[18] (HAQ), erythrocyte sedimentation rate, C-reactive protein level, IgM rheumatoid factor positivity (IgM RF, ELISA, < 40 IU/ml negative) and disease activity score (including 3 variables: Ritchie articular index, number of swollen joints, and erythrocyte sedimentation rate)[19].

Informed consent was obtained from each individual and the committee for protection of persons participating in biomedical research approved this protocol (French law 88-1138, 20 December 1988). One hundred and thirty-three unrelated healthy Caucasian individuals living in the same geographic area were used as controls.

Radiographic assessments. Radiographs of the hands and feet were obtained in RA patients at the start of the study and every year thereafter for 4 years. All radiographs were scored by the same investigator, according to the Sharp/van der Heijde method[20]. Each patient's radiographs were scored in chronological order. The joint erosion score (JES: range 0–280) and joint narrowing score (JNS: range 0–168) were added to produce the total radiographic damage score (TDS: range 0–448). The TDS was used to quantify RA severity at the start of the study and after 4 years of followup. To determine the intraobserver reliability, 30 randomly chosen pairs of radiographs of the hands and feet were scored twice by the investigator. The intraclass correlation coefficient (proportion of the total variance in the measurements attributable to true difference between subjects) was 0.98 for TDS and 0.97 for both JES and JNS.

DNA extraction and HLA-DRB1 genotyping. Genomic DNA was extracted from EDTA anticoagulated peripheral blood using a standard proteinase K digestion and phenol/chloroform extraction method. HLA-DRB1 genotyping was performed by polymerase chain reaction (PCR) amplification with primers specific for framework sequences flanking the polymorphic region of exon 2. The reagents and procedures were those recommended by the XIIth International Histocompatibility Workshop[21]. Amplified DNA was subsequently blotted onto nylon membrane (Hybond, Amersham, Arlington Heights, IL, USA) and hybridized to labeled sequence-specific oligonucleotide probes. After washing the membranes in stringent conditions, the chemoluminescent signal was detected.

After HLA-DRB1 genotyping, RA patients were classified into 3 groups according to the absence or presence of allele(s) encoding the shared epitope (DRB1*0101, DRB1*0102, DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*1001). These groups called SE+/+, SE+/- and SE-/- included patients carrying 2, 1 or 0 allele(s) encoding the shared epitope (SE+ allele), respectively.

MMP-1 polymorphism genotyping. We analyzed a biallelic polymorphism in the MMP-1 gene (gene map locus 11q22-q23), defined by a guanine insertion or deletion at –1607 bp in the MMP-1 promoter sequence, respectively designated 2G (insertion-type) or 1G (deletion-type) alleles[13,14]. The MMP-1 1G/2G polymorphism was amplified using a fluorescent-based PCR method with the following primers: 5′-(Hex)GTT ATG CCA CTT AGA TGA GG-3′ and 5′-TTC CTC CCC TTA TGG ATT CC-3′ (Genset SA, Paris, France). Amplification was performed in an Amplitron II Thermolyne apparatus (Bioblock Scientific, Illkirch, France) using the following PCR cycling conditions: denaturation at 95˚C for 4 minutes, followed by 20 cycles at 95˚C for 45 seconds, 55˚C for 45 seconds, 72˚C for 90 seconds, and a final extension at 72˚C for 5 minutes. Each amplified and labeled PCR sample was run on a 6% denaturing polyacrylamide gel with a fluorescent size marker (Prism Genescan-2500 Rox, Applied Biosystems, Foster City, CA), in an automatic DNA sequencer (373A DNA Sequencer, Applied Biosystems). Data were processed on 672 Genescan Collection software (1991 Applied Biosystems). The size of each band of amplified and labeled product was assessed by Genescan PCR analysis software (1993 Applied Biosystems). The expected sizes were 148 bp for the 1G allele and 149 bp for the 2G allele[14].

Statistical analysis. The hypothesis of an association between the MMP-1 gene polymorphism and susceptibility to RA was tested by comparing allele carrier frequency (number of individuals with at least one copy of a particular allele divided by the total number of individuals in the group), allele frequency (number of copies of a particular allele divided by the total number of alleles within the group) and genotype frequency (number of copies of a particular genotype divided by the total number of individuals within the group) between RA patients and healthy controls (chi-square test).

The hypothesis of an association between MMP-1 and HLA-DRB1 gene polymorphisms and severity of RA was first investigated in the 103 patients with early RA included in this study by comparing the baseline TDS, JES, and JNS of carriers of the different genotypes. Then this association was investigated in the 96 patients with RA who completed the 4 year followup period by comparing the fourth year TDS, JES, and JNS of carriers of the different genotypes. We tested the TDS, JES, and JNS variables for normality (skewness and kurtosis tests) at baseline and after 4 years of followup. As the TDS variable was not normally distributed, non-parametric tests were used to compare the TDS of carriers of the different genotypes (Kruskal-Wallis test) and to test for a trend of increasing TDS across the different genotypes (Cuzick test)[22].

The hypothesis of an association between MMP-1 and HLA-DRB1 gene polymorphisms and progression of the radiographic damage score was investigated in the 96 patients with RA who completed the 4 year followup period. The progression of the radiographic damage score was calculated by subtracting the baseline radiographic damage score from the 4 year followup radiographic damage score in each RA patient. We used the Kruskal-Wallis test to compare the progression of the radiographic damage score of carriers of the different genotypes and the Cuzick test to look for a trend of increasing radiographic damage score progression across the different genotypes.

Statistical analyses were performed using Stata Statistical Software: Release 6.0. (Stata Corporation, College Station, Texas, USA). A p value < 0.05 was considered statistically significant.

RESULTS

RA patient characteristics at baseline and after 4 years of followup. One hundred and three patients with early RA were included; 96 completed the study. Characteristics of the 103 RA patients at inclusion were as follows: 80 females, 23 males, mean (SD) age 50.5 (15.0) years, mean (SD) disease

duration 6.9 (3.6) months, mean (SD) disease activity score 2.8 (1.1), median (range) of the Health Assessment Questionnaire 0.75 (0–2.375), rheumatoid factor positive (ELISA method) 71/103, shared epitope positive 70/103. At baseline, the medians (range) of the TDS, JES, and JNS were 1 (0–58), 0 (0–35) and 0 (0–29), respectively. After 4 years of followup, the characteristics of the 96 patients who completed the study were as follows: mean (SD) disease activity score 1.7 (1.0), median (range) of the HAQ 0.56 (0–2.750), median (range) of the TDS 11.5 (0–190), JES 7.5 (0–106) and JNS 3.5 (0–84).

At the start of the study, 80% of the patients were treated with nonsteroidal antiinflammatory drugs (NSAID), 27% with glucocorticoids and 41% with disease modifying antirheumatic drugs (DMARD: methotrexate, sulfasalazine, gold thiomalate injection, D-penicillamine, or hydroxychloroquine). Over the 4 year followup period, 50% of the patients required treatment with glucocorticoids > 1 year and the mean (SD) number of DMARD per patient was 2.06 (1.14). At the end of the 4 year followup period, 62% of the patients were treated with NSAID, 35% with glucocorticoids, and 76% with DMARD.

MMP-1 gene polymorphism and susceptibility to RA. Table 1 shows the distribution of the MMP-1 gene polymorphism in patients with RA and healthy controls. MMP-1 polymorphism genotyping was obtained in all patients but one (a technical problem on genomic DNA). The comparison of allele carrier frequency, allele frequency, and genotype frequency between RA patients and healthy controls did not reveal any statistically significant difference (chi-square test). These results do not support the hypothesis of an association between this MMP-1 gene polymorphism and susceptibility to RA.

MMP-1 and HLA-DRB1 gene polymorphisms and severity of early RA. Table 2 shows the baseline radiographic damage score of carriers of the different MMP-1 and HLA-DRB1 genotypes in the 103 early RA patients included in the study. TDS, JNS, and JES distributions at baseline did not differ across all 3 MMP-1 genotypes (Kruskal-Wallis test). These results do not support the hypothesis of an association between this MMP-1 gene polymorphism and severity of early RA. The comparison of the baseline TDS, JES, and JNS of carriers of the different HLA-DRB1 genotypes did not show any statistically significant difference.

MMP-1 and HLA-DRB1 gene polymorphisms and severity of RA after 4 years of followup. Table 3 shows the radiographic damage score of carriers of the different MMP-1 and HLA-DRB1 genotypes in the 96 patients with RA who completed the 4 year followup period. TDS, JES, and JNS distributions did not differ across all 3 MMP-1 genotypes (Kruskal-Wallis test). These results do not support the hypothesis of an association between this MMP-1 gene polymorphism and severity of RA after 4 years of followup.

TDS and JES distributions differed across all 3 HLA-DRB1 genotypes ($p = 0.03$), while the JNS distribution did not. The Cuzick test revealed a trend of increasing TDS and JES across these 3 genotypes ($p = 0.01$). These results confirm the previous reports of an association between the HLA-DRB1 gene polymorphism and severity of RA. The HLA-DRB1 SE+/+ genotype is associated with the highest radiographic damage score while the SE-/- genotype is associated with the lowest.

MMP-1 and HLA-DRB1 gene polymorphisms and progression of the radiographic damage score over the 4 year followup period. Table 4 shows the progression of the radiographic damage score of carriers of the different MMP-1 and HLA-DRB1 genotypes in the 96 patients with RA who completed the 4 year followup period. Over the 4 years of followup, TDS, JES, and JNS increased in all patients but 3. In the first patient, TDS decreased by 8 points (JES decreased by 7 points and JNS by 1 point). In the second patient, TDS decreased by 1 point (JES increased by 2 points while JNS decreased by 3 points). In the third patient, TDS increased by 1 point (JES decreased by 1 point while JNS increased by 2 points). The median (range) of TDS, JES, and JNS progression was, respectively, 9.5 (-8–180), 5.5 (-7–99) and 3.0 (-3–48). The distributions of TDS, JES, and JNS progression did not differ across all 3 MMP-1 genotypes (Kruskal-Wallis test). These results do not support the hypothesis of an association between this MMP-1 gene polymorphism and progression of the radiographic damage score in RA.

The distributions of TDS and JES progression differed across all 3 HLA-DRB1 genotypes ($p = 0.02$), while the dis-

Table 1. MMP-1 gene polymorphism and susceptibility to RA. MMP-1 genotypes: 1G/1G, 2G/2G, 1G/2G = homozygosity for the 1G allele, homozygosity for the 2G allele, heterozygosity for the 1G and 2G alleles. Comparison of allele carrier frequency, allele frequency and genotype frequency between patients with RA and healthy controls did not reveal any statistically significant difference (chi-square test).

	Allele Carrier Frequency (%)		Allele Frequency (%)			Genotype Frequency (%)	
Allele	RA Patients (n = 102)	Controls (n = 133)	RA Patients (N = 204)	Controls (N = 266)	Genotype	RA Patients (n = 102)	Controls (n = 133)
1G	71.6	75.9	51.5	49.6	1G/1G	31.4	23.3
					1G/2G	40.2	52.6
2G	68.6	76.7	48.5	50.4	2G/2G	28.4	24.1

n = number of individuals; N = number of alleles.

Table 2. MMP-1 and HLA-DRB1 genotypes and severity of early RA.

Radiographic Damage Score at Baseline	MMP-1 Genotypes 1G/1G (n = 31)	1G/2G (n = 42)	2G/2G (n = 29)	p value
TDS, median (range)	0 (0–9)	1(0–58)	2 (0–11)	0.61
JES, median (range)	0 (0–9)	1 (0–35)	0 (0–7)	0.16
JNS, median (range)	0 (0–6)	0 (0– 29)	0 (0–11)	0.07
	HLA-DRB1 Genotypes SE–/– (n = 33)	SE+/– (n = 48)	SE +/+ (n = 22)	p value
TDS, median (range)	0 (0–58)	2 (0–54)	1 (0–8)	0.10
JES, median (range)	0 (0–31)	0.5 (0–35)	0.5 (0–6)	0.32
JNS, median (range)	0 (0–27)	0 (0–29)	0 (0–6)	0.38

TDS = total radiographic damage score; JES = joint erosion score; JNS = joint narrowing score. MMP-1 genotypes: 1G/1G, 2G/2G, 1G/2G = homozygosity for the 1G allele, homozygosity for the 2G allele, heterozygosity for the 1G and 2G alleles. HLA-DRB1 genotypes: SE–/–, SE+/–, SE+/+ = patients carrying no, one, or two allele(s) encoding the shared epitope. The TDS, JES, and JNS of carriers of the different MMP-1 and HLA-DRB1 genotypes were compared using a non-parametric test (Kruskal-Wallis test). A p value < 0.05 was considered statistically significant.

Table 3. MMP-1 and HLA-DRB1 genotypes and severity of RA after 4 years of followup.

Radiographic Damage Score After 4 Years of Followup	MMP-1 Genotypes 1G/1G (n = 30)	1G/2G (n = 38)	2G/2G (n = 27)	p value
TDS, median (range)	10 (0–109)	10 (0–134)	14 (0–190)	0.76
JES, median (range)	3.5 (0–62)	8.5 (0–86)	8 (0–106)	0.76
JNS, median (range)	2.5 (0–47)	3 (0–60)	6 (0–84)	0.50
	HLA-DRB1 Genotypes SE–/– (n = 29)	SE+/– (n = 46)	SE+/+ (n = 21)	p value
TDS, median (range)	3 (0–190)	11.5 (0–134)	26 (0–84)	0.03
JES, median (range)	3 (0–106)	8 (0–86)	13 (0–55)	0.03
JNS, median (range)	1 (0–84)	3.5 (0–60)	10 (0–37)	0.15

TDS = total radiographic damage score; JES = joint erosion score; JNS = joint narrowing score. MMP-1 genotypes: 1G/1G, 2G/2G, 1G/2G = homozygosity for the 1G allele, homozygosity for the 2G allele, heterozygosity for the 1G and 2G alleles. HLA-DRB1 genotypes: SE–/–, SE +/–, SE+/+ = patients carrying no, one or 2 allele(s) encoding the shared epitope. The TDS, JES and JNS of carriers of the different MMP-1 and HLA-DRB1 genotypes were compared using a non-parametric test (Kruskal-Wallis test). A p value < 0.05 was considered statistically significant.

tribution of JNS progression did not. The Cuzick test revealed a trend of increasing TDS and JES progression across these 3 genotypes (p = 0.01). These results indicate an association between the HLA-DRB1 gene polymorphism and progression of radiographic damage in RA. The HLA-DRB1 SE+/+ genotype is associated with the highest progression of the radiographic damage score while the SE-/- genotype is associated with the lowest.

DISCUSSION

The results of the present prospective longitudinal study of patients with early RA do not support the hypothesis of an association between a particular biallelic polymorphism in the MMP-1 gene promoter and susceptibility to, and severity of, RA. Allele and genotype frequencies did not differ between RA patients and controls. Allele and genotype frequencies reported in healthy controls in the present study are in accor-

Table 4. MMP-1 and HLA-DRB1 genotypes and progression of the radiographic damage score over the 4 years of followup.

Progression of the Radiographic Damage Score over the 4 years of Followup	MMP-1 Genotypes			
	1G/1G (n = 30)	1G/2G (n = 38)	2G/2G (n = 27)	p value
TDS progression, median (range)	6 (0–107)	10 (–8–110)	12 (–1–180)	0.80
JES progression, median (range)	3 (0–60)	7 (–7–62)	8 (–1–99)	0.83
JNS progression, median (range)	2 (0–47)	2(–1–48)	5(–3–81)	0.64
	HLA-DRB1 Genotypes			
	SE–/– (n = 29)	SE+/– (n = 46)	SE+/+ (n = 21)	p value
TDS progression, median (range)	3(0–180)	9.5(–8–110)	26 (0–76)	0.02
JES progression, median (range)	2(–1–99)	6.5 (–7–62)	12 (0–49)	0.02
JNS progression, median (range)	1 (0–81)	3.5 (–3–48)	10 (0–33)	0.09

The progression of the radiographic damage score was calculated in the 96 RA patients who completed the 4 year followup period by subtracting the baseline radiographic damage score from the 4 year followup radiographic damage score. TDS = total radiographic damage score; JES = joint erosion score; JNS = joint narrowing score. MMP-1 genotypes: 1G/1G, 2G/2G, 1G/2G = homozygosity for the 1G allele, homozygosity for the 2G allele, heterozygosity for the 1G and 2G alleles. HLA-DRB1 genotypes: SE–/–, SE+/–, SE +/+ = patients carrying no, one or 2 allele(s) encoding the shared epitope. The TDS, JES and JNS progression of carriers of the different MMP-1 and HLA-DRB1 genotypes were compared using a non-parametric test (Kruskal-Wallis test). A p value < 0.05 was considered statistically significant.

dance with those previously reported in a sample of 142 healthy Caucasian subjects from the United Kingdom[15]. TDS, JNS, and JES distributions at baseline and after 4 years of followup did not differ across all 3 MMP-1 genotypes. The distributions of TDS, JES, and JNS progression over the 4 year followup period did not differ across all 3 MMP-1 genotypes. A retrospective statistical power analysis was done. The powers of the study were 99, 92 and 21% respectively for a large, medium, and small effect size[23]. The absence of statistical difference shown in Tables 2, 3 and 4 enables us to conclude that this MMP-1 gene polymorphism has no large or medium effect on the radiographic damage score or its progression over the 4 years of followup.

Our results confirm the association between the HLA-DRB1 gene polymorphism and severity of RA. After 4 years of followup, the HLA-DRB1 SE+/+ genotype is associated with the highest radiographic damage score while the SE-/- genotype is associated with the lowest. Such an association was previously reported in prospective longitudinal studies[24,25]. Moreover, the HLA-DRB1 SE+/+ genotype is associated with the highest progression of the radiographic damage score over the 4 year followup period, while the SE-/- genotype is associated with the lowest. Taken together, these results support the interest of HLA-DRB1 genotyping in patients with early RA to predict the progression of disease[26].

In conclusion, although our study does not support the hypothesis of an association between this particular MMP-1 gene polymorphism and RA, the search for RA susceptibility and severity genes remains an important issue to better understand RA pathophysiology and to optimize the strategies for treatment of early RA.

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