

IL10 GGC Haplotype Is Positively and HLA-DQA1*05-DQB1*02 Is Negatively Associated with Radiographic Progression in Undifferentiated Arthritis

JENNIE URSUM, MIGNON A.C. van der WEIJDEN, DIRKJAN van SCHAARDENBURG, AREND P.A. PRINS, BEN A.C. DIJKMANS, JOS W.R. TWISK, JAKOB B.A. CRUSIUS, and IRENE E. van der HORST-BRUINSMA

ABSTRACT. Objective. In rheumatoid arthritis (RA), many genetic markers, such as the shared-epitope (SE) alleles, are described in association with radiographic progression, but limited data are available on undifferentiated arthritis (UA). We investigated whether single-nucleotide polymorphisms (SNP) and haplotypes in immune response genes and HLA class II alleles are associated with radiographic progression in patients with early UA.

Methods. Progression of radiographic damage was determined in white Dutch patients with early UA after 2 years of followup. Severe progression was defined as an increase in Sharp/van der Heijde Score ≥ 5 points after 2 years of followup. The remainder was classified as mild. These SNP were genotyped by Taqman technology: tumor necrosis factor (TNF) -1031, -863, -857, -308, -238; lymphotoxin- α (LTA) +368, +252; interleukin 10 (IL10) -2849, -1082, -819; IL1A -889, IL1B -31, +3953; and IL1RN +2018. Carriage of SE alleles and HLA-DQA1*05-DQB1*02 haplotype was established. These markers were analyzed in relation to radiographic progression.

Results. Forty-eight out of 151 patients with early UA had severe radiographic progression. Severe radiographic progression was associated with an increased carrier frequency of SE alleles (OR 5.12, 95% CI 2.0–13.1, $p < 0.001$) and IL10 GGC haplotype (OR 2.8, 95% CI 1.4–5.8, $p = 0.003$). Mild radiographic progression was associated with the HLA-DQA1*05-DQB1*02 haplotype (OR 0.3, 95% CI, 0.1–0.8, $p = 0.013$) and with allele TNF -308A (OR 0.4, 95% CI, 0.2–0.9, $p = 0.02$).

Conclusion. The SE and the IL10 GGC haplotype are associated with severe progression of radiographic damage, in contrast to the DQA1*05-DQB1*02 haplotype and the TNF -308A allele, which are associated with mild radiographic progression in early UA. (J Rheumatol First Release May 15 2010; doi:10.3899/jrheum.090913)

Key Indexing Terms:

UNDIFFERENTIATED ARTHRITIS
SINGLE-NUCLEOTIDE POLYMORPHISM

RADIOGRAPHIC DAMAGE
HLA CLASS II ALLELES

The progress of rheumatoid arthritis (RA) is characterized by a large variation in severity of inflammation and joint destruction. Joint damage often begins in the early stages of

the disease¹. Among patients with early arthritis there is a large group that cannot be classified into any established disease category^{2,3}. The term “undifferentiated arthritis” (UA) was introduced to distinguish these unclassifiable arthritides from RA and other inflammatory rheumatic diseases. The frequency of UA in early arthritis cohorts ranges from 20% to 45%³ and UA can result in severe radiographic damage^{4,5}. Of the patients with UA studied in Jansen, *et al*, 42% showed radiographic progression with an increase in Sharp-van der Heijde score (SHS) of at least 10 points within 1 year⁴. In contrast to RA, in which associations with single-nucleotide polymorphisms (SNP) have been extensively studied, fewer data are available on the genetic characteristics of patients with UA⁶⁻⁹.

The major histocompatibility complex (MHC) class II alleles, referred to as the shared-epitope (SE) alleles, contribute to 30%–50% of the genetic predisposition to RA^{10,11}: HLA-DRB1*0401, *0404, *0405, *0408, *0101, *1001,

From the Jan van Breemen Institute; Departments of Rheumatology and Clinical Epidemiology; and the Laboratory of Immunogenetics, Department of Pathology, VU University Medical Centre, Amsterdam, The Netherlands.

J. Ursum, MSc; A.P.A. Prins, MD, PhD, Jan van Breemen Institute; M.A.C. van der Weijden, MD; I.E. van der Horst-Bruinsma, MD, PhD, Department of Rheumatology, VU University Medical Centre; D. van Schaardenburg, MD, PhD; B.A.C. Dijkmans, MD, PhD, Jan van Breemen Institute and Department of Rheumatology, VU University Medical Centre; J.W.R. Twisk, PhD, Department of Clinical Epidemiology, VU University Medical Centre; J.B.A. Crusius, PhD, Laboratory of Immunogenetics at the Department of Pathology, VU University Medical Centre.

Address correspondence to Dr. I.E. van der Horst-Bruinsma, VU University Medical Centre, Department of Rheumatology, Room 3A-64, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

E-mail: ie.vanderhorst@vumc.nl

Accepted for publication February 9, 2010.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2010. All rights reserved.

and *1402. Moreover, these SE alleles contribute to the development of antibodies against cyclic citrullinated peptide (ACPA)⁷ and are associated with a worse radiographic outcome in RA^{12,13}. In contrast with SE, HLA-DRB1*03 is more frequently present in the ACPA-negative patients with RA compared to ACPA-positive patients (OR 1.6, 95% CI 1.2–2.1) and is associated with a less severe disease¹⁴.

Apart from MHC class II alleles, SNP in genes coding for cytokines such as tumor necrosis factor- α (TNF), lymphotoxin- α (LTA), interleukin 1 α (IL1A), IL1B, and the natural IL-1 receptor antagonist IL1RN play a pivotal role in the regulation of inflammation and seem to be important in the onset and progression of RA as well as UA.

TNF- α is a proinflammatory cytokine with a key function in the pathogenesis of RA¹⁵. Excessive production of TNF- α can incite synovial inflammation and proliferation as well as degradation of articular cartilage and bone, which causes joint damage. Patients with RA receiving anti-TNF treatment have a large decrease in disease activity and have significantly less radiographic progression compared to a placebo group¹⁶. The carrier frequency of the TNF -308A allele is associated with an increase in TNF- α concentrations in peripheral blood mononuclear cells cultured with T cell activators¹⁷.

LTA (formerly TNF- β), a homolog of TNF- α , has similar biological activity as TNF- α and shares one of its receptors¹⁸. Therefore LTA also might be a susceptibility gene for RA and for UA. The *TNF* and *LTA* genes are arranged in tandem and map within the MHC centromeric to HLA-B and telomeric to the MHC class III genes.

Other potential candidates in the onset of UA are polymorphisms at the IL1 family loci IL1A, IL1B, and IL1RN. The activity of IL-1, which initiates and perpetuates inflammatory and destructive processes in the rheumatoid joint¹⁹, is normally balanced by endogenous inhibitors, e.g., IL-1ra^{20,21}. It is known that an imbalance between IL-1 and IL-1ra may be a predisposing factor for RA²⁰, most likely reflected by homozygosity for the allele 2 of the IL1RN 86-bp variable number of tandem repeats, which is in strong linkage disequilibrium with IL1RN +2018C allele²². A relation between radiographic progression and SNP at the IL1 cluster in RA was also demonstrated^{23,24}.

The antiinflammatory cytokine IL-10 is negatively correlated with the progression of joint destruction in RA²⁵. The IL10 -2849 promoter polymorphism is associated with autoantibody production and subsequent joint damage in RA²⁶. Also, IL10 -592CC and IL10 -1082GG have been associated with more severe radiographic damage only in patients who are ACPA-negative²⁷, while another study showed opposite results²⁸.

We investigated whether SNP in cytokine genes associated with arthritis as well as the presence of SE alleles and the DQA1*05-DQB1*02 haplotype were associated with the progression of radiographic damage in patients with UA.

MATERIALS AND METHODS

The study population consisted of patients with early UA who were included in the Early Arthritis Cohort (EAC) of the Jan van Breemen Institute, a large rheumatology clinic in Amsterdam, The Netherlands. Since 1995, newly referred patients are included and followed prospectively in the EAC if they are aged ≥ 18 years, have peripheral arthritis in ≥ 2 joints, and a symptom duration of ≤ 3 years. Patients were excluded if they had been treated with a disease-modifying antirheumatic drug (DMARD) or if they had spondyloarthropathy, reactive arthritis, crystal-induced arthropathy, systemic lupus erythematosus, Sjögren's syndrome, or osteoarthritis. The choice of treatment after inclusion was made by the rheumatologist. All included patients were Dutch and white, defined as patients with at least 3 Dutch white grandparents. The local Ethics Committee approved the study protocol, and all patients gave written informed consent.

Measurements. Data were collected on demographics, symptom duration, and disease activity in 28 joints (DAS28)²⁹. The laboratory assessments included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IgM-rheumatoid factor (IgM-RF), and ACPA. Radiographs of the hands and feet were also made. Data on demographics and symptom duration were collected at baseline and data on the other variables were collected annually (Table 1).

Dependent variable. Radiographic damage was assessed with the SHS³⁰ by 2 experienced rheumatologists who were blinded to the other variables and had a good intraclass correlation coefficient of 0.95. Severe progression was defined as an increase of the SHS of at least 5 points between baseline and 2 years of followup³¹. The remainder was classified as mild progression. After 2 years of followup, patients who fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA³² were classified as having RA, and the remainder were classified as UA.

Genotyping. Genomic DNA was extracted from EDTA blood samples using a standard method. Genotyping of promoter SNP was performed at positions -1031 (db SNP ID: rs1799964), -863 (rs1800630), -857 (rs1799724),

Table 1. Characteristics of 151 Dutch white patients with undifferentiated arthritis. Data are mean (SD), median with interquartile range, or frequencies.

Characteristics	Radiographic Progression		p
	Severe	Mild	
n	48	103	
Women, %	79	62	0.03
Age at baseline, yrs	53.1 (12.3)	53.9 (0.72)	0.72
Duration of symptoms before baseline, yrs	0.45 (1.9)	0.4 (0.8)	0.77
No. of DMARD use	2 (1–3)	1 (1–2)	< 0.001
DAS28 at baseline	5.2 (1.2)	4.6 (1.2)	0.006
DAS28 at 2-yr followup	3.7 (1.3)	2.9 (1.3)	< 0.001
ESR baseline, mm/h	31.7 (1.9)	19.1 (2.3)	< 0.001
CRP baseline, mg/l	20.7 (3.4)	12.3 (3.7)	0.025
IgM-RF-positive, %	79	32	< 0.001
ACPA-positive*, %	84	34	< 0.001
Shared-epitope copies, %			
0	19	47	< 0.001
1	48	41	
2	33	13	
SHS at baseline	2 (0–10)	0 (0–2)	< 0.001
SHS at 2-yr followup	19.5 (10.5–31.5)	1 (0–3)	< 0.001

DMARD: disease-modifying antirheumatic drug; DAS28: disease activity score based on 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IgM-RF: IgM rheumatoid factor; ACPA: antibodies to anticyclic citrullinated peptide/protein; SHS: Sharp-van der Heijde score. * Only available for 124 patients.

–308 (rs1800629), and –238 (rs361525) in the *TNF* gene and of intronic SNP at positions +252 (rs909253) and +368 (rs746868) in the *LTA* gene. Further, we studied *IL1* family genes at position –889 (rs1800587) within the promoter of the *IL1A* gene, at position –31 in the promoter region (rs1143627), and another at position +3953 in exon 5 (rs1143634) of the *IL1B* gene and intron 2 SNP IL1RN +2018 (rs419598). Additionally, we studied IL10 promoter SNP IL10 –2849 (rs6703630), IL10 –1082 (rs1800896), and IL10 –819 (rs1800871).

SNP were genotyped with Taqman 5' allelic discrimination technology (Applied Biosystems Inc., Foster City, CA, USA)³³. Sequences of primers, fluorescent reporter dye-labeled probes, and reaction conditions were recorded.

Carriage of 1 or 2 HLA-DRB1 SE copies (i.e., SE+) was inferred from HLA-DQA1-DQB1 haplotypes in very strong linkage disequilibrium with HLA-DRB1 alleles in whites^{34,35}. Polymerase chain reaction-amplified exon 2 DQA1 and DQB1 amplicons were generated for genotyping in a combined single-stranded conformation polymorphism/heteroduplex assay by a semiautomated electrophoresis and gel staining method on the Phastsystem (Amersham Pharmacia Biotech, Uppsala, Sweden). The method has been validated using a panel of reference DNA against the Dynal Allset+ high-resolution typing kit (Dynal A.S., Oslo, Norway)³³.

These haplotypes could be reliably characterized in homozygous and heterozygous states³³: HLA-DQA1*03-DQB1*0301 and DQA1*03-DQB1*0302 (both DR4-related SE), DQA1*0101-DQB1*0501 (DR1 and DR10-related SE), and DQA1*0501-DQB1*02 (strictly DRB1*0301-related).

The SE+ status as established by the DQA1 and DQB1 PCR-SSCP/HD assay was validated in 87 established patients with RA by high-resolution sequence-based typing of HLA-DRB1 exon 2. To contain the SE, the HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, and *1001 alleles were taken. The technique was used to correctly classify SE carriage in 99% of the patients. Independent confirmation in a second cohort of DRB1-typed Dutch patients with RA showed that with DQA1 and DQB1 typing, only 2 out of 167 patients (1.2%) would have been incorrectly classified³⁶.

Statistical analysis. Haplotypes for SNP in the TNF-LTA region and the *IL10* gene were inferred by use of PHASE 2.0, which implements a Bayesian algorithm to estimate the haplotype frequencies (<http://www.stat.washington.edu/stephens/software.html>).

Differences in baseline characteristics between patients with severe and mild radiographic progression were analyzed with Student's *t* test, Mann-Whitney *U* test, and chi-squared test, as appropriate. Logistic regression analyses were used to determine associations between radiographic severity and the carrier frequency of alleles and haplotypes. Results are presented as OR with 95% CI, and the *p* value. If a significant effect was found for a single SNP, logistic regression was used to correct for linkage disequilibrium between distinct alleles. Logistic regression was also used for the determination of confounding of baseline variables. *P* values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS 15.0 Software (SPSS, Chicago, IL, USA).

RESULTS

A total of 151 patients with UA were eligible for our study. A minority of the patients (32%) were classified as having severe radiographic progression and the remainder (68%) as mild radiographic progression. At baseline, the mean age and symptom duration were comparable for both groups (Table 1). The mean DAS28, median CRP, mean ESR, percentage IgM-RF positivity, and ACPA positivity were higher in the severe disease group. The severe group had a higher SHS at baseline. After 2 years of followup, the number of DMARD used was significantly lower in the mild group compared to the severe group, while 50% of the patients in

the severe group and 18% in the mild group fulfilled the 1987 ACR criteria for RA at that time.

In a univariate analysis, the carrier frequency of TNF –308A allele was significantly lower in the severe group (OR 0.43, 95% CI 0.20–0.90, *p* = 0.02). Carrier frequencies of other TNF or LTA minor alleles did not differ between patients with severe and mild radiographic progression (Table 2). The SE frequency was significantly lower in carriers of the TNF –308A allele (*p* < 0.001). Focusing on the TNF-LTA haplotypes, only the carrier frequency of haplotype E, tagged by TNF –308A allele, was significantly lower in the severe group (Table 3). A strong linkage disequilibrium was found between the TNF –308A allele and HLA-DQA1*05-DQB1*02 haplotype: 72.1% of the TNF –308A carriers were carriers of the HLA-DQA1*05-DQB1*02 haplotype. After stratification for radiographic progression, similar percentages were found (mild 53.8% vs progressive 77.1%).

Table 4 shows the carrier frequencies of (combinations of) HLA-DQA1*-DQB1* haplotypes. The numbers of HLA-DR4-related SE copies (OR 4.24, 95% CI 2.05–8.79, *p* = 0.001) and DR1-, DR4-, or DR10-related SE copies (OR 3.78, 95% CI 1.66–8.60, *p* = 0.001) were positively associated with an increased risk of severe progression of radiographic damage. Carrier frequency of HLA-DQA1*05-DQB1*02 was significantly lower in the severe disease group (OR 0.30, 95% CI 0.13–0.71, *p* = 0.013).

SNP in *IL1* family genes and IL10 were similarly distributed among the groups (Table 5). The IL10 –1082A allele showed a trend toward a lower carrier frequency in the severe disease group compared to the mild group. Focusing on the IL10 haplotypes, only the carrier frequency of the GGC haplotype of the IL10 –2849G/A, IL10 –1082G/A, and IL10 –819C/T SNP was increased significantly in the severe group compared to the mild group (OR 2.84, 95% CI 1.40–5.75, *p* = 0.003; Table 6).

In order to detect additive or synergistic effects or interaction between HLA-DQA1*05-DQB1*02 and the IL10 GGC haplotype, logistic regression was performed. The DQA1*05-DQB1*02 haplotype and the IL10 GGC haplotype were both independently associated with progression of radiographic damage. The IL10 GGC haplotype was positively associated, while the DQA1*05-DQB1*02 haplotype was negatively associated.

To explore whether variables measured at baseline act as confounders of the SNP association, multivariate logistic regression was performed. The number of SE, SHS at baseline, CRP at baseline, and the number of DMARD used were not confounders. But ACPA positivity, IgM-RF positivity, DAS28 at baseline, and sex were marked as confounders. In a multivariate logistic model with IL10 GGC haplotype and HLA-DQA1*05-DQB1*02 haplotype, adjusted for these 4 confounders, HLA-DQA1*05-DQB1*02 haplotype was no longer a significant contribu-

Table 2. TNF and LTA SNP carrier frequency and radiographic progression.

SNP Genotype	Severe, n = 48	Mild, n = 103	p	Minor Allele Carrier	Severe, n = 48	Mild, n = 103	OR (95% CI)	p
LTA +252	GG	4	0.12	GG + GA	31	67	0.98 (0.48–2.0)	0.96
	GA	27			17	36		
	AA	17			36			
LTA +368	CC	7	0.64	CC + CG	30	56	1.40 (0.69–2.82)	0.35
	GC	23			18	47		
	GG	18			47			
TNF –1031	CC	3	0.38	CC + CT	17	35	1.07 (0.52–2.19)	0.86
	CT	14			31	68		
	TT	31			68			
TNF –863	AA	3	0.11	AA + CA	13	31	0.86 (0.40–1.85)	0.70
	CA	10			35	72		
	CC	35			2			
TNF –857	TT	0	0.70	TT + CT	5	13	0.81 (0.27–2.40)	0.70
	CT	5			43	90		
	CC	43			90			
TNF –308	AA	1	0.07	AA + GA	13	48	0.43 (0.20–0.90)	0.02*
	GA	12			35	55		
	GG	35			55			
TNF –238	AA	0	0.67	AA + GA	2	6	0.70 (0.14–3.62)	0.67
	GA	2			46	97		
	GG	46			97			

TNF: tumor necrosis factor; LTA: lymphotoxin alpha; A: adenine; C: cytosine; G: guanine; T: thymine; SNP: single-nucleotide polymorphism. * Significant association.

Table 3. TNF-LTA haplotype carrier frequency and radiographic progression in undifferentiated arthritis.

Haplotype	TNF –238	TNF –308	TNF –857	TNF –863	TNF –1031	LTA +368	LTA +252	Severe, n = 48 (%)	Mild, n = 103 (%)	OR (95% CI)	p
B	G	G	C	C	T	G	A	4 (8.3)	11 (10.7)	0.76 (0.23–2.25)	0.65
C	G	G	C	C	T	G	G	18 (37.5)	33 (32.0)	1.27 (0.62–2.60)	0.51
E	G	A	C	C	T	G	G	13 (27.1)	48 (46.6)	0.43 (0.20–0.90)	0.02*
G	G	G	C	C	C	G	A	2 (4.2)	0 (0.0)	∞	1.0
H	A	G	C	C	C	G	A	2 (4.2)	5 (4.9)	0.85 (0.16–4.56)	1.0
K	G	G	C	A	C	G	A	13 (27.1)	31 (30.1)	0.86 (0.40–1.85)	0.7
L	G	G	C	C	T	C	A	27 (56.3)	48 (46.6)	1.47 (0.74–2.94)	0.27
O	G	G	T	C	T	C	A	5 (10.4)	13 (12.6)	0.81 (0.27–2.40)	0.79

TNF: tumor necrosis factor; LTA: lymphotoxin alpha; A: adenine; C: cytosine; G: guanine; T: thymine. OR were calculated comparing the carriers vs the non-carriers. * Significant association. One patient carried a recombinant haplotype (not shown).

tion in the model (IL10 GGC haplotype: OR 7.79, 95% CI 2.19–29.01, $p = 0.002$; and HLA-DQA1*05-DQB1*02: OR 0.33, 95% CI 0.09–1.19, $p = 0.091$).

DISCUSSION

In patients with early UA, we have shown the IL10 GGC haplotype to be related to severe radiographic progression, in contrast with the DQA1*05-DQB1*02 haplotype [strictly DRB1*0301 (DR3) related]^{34,35}, which is associated with mild progression of radiographic damage after 2 years. The TNF –308A allele was also associated with mild progression, but was not independent from the HLA-DQA1*05-DQB1*02 haplotype. The higher carrier frequency of the TNF –308A allele in the mild disease group was rather

unexpected because carriers of the TNF –308A were described as having an increased TNF- α production¹⁷.

Contradictory results are reported of association between the TNF –308 SNP and erosive outcome in RA^{37,38}. One study in only IgM-RF-seropositive patients with early RA described an association between the TNF –308 AA+GA genotypes and a higher radiographic progression rate over 5 years, adjusted for the presence of SE³⁹, while our result is in accord with a reported association between the TNF –308GG genotype and erosions in RA⁴⁰. Other studies found no association between TNF –308 polymorphisms and radiographic progression, probably because of use of the Larsen score, which is a less sensitive method in an early stage of the disease^{38,41}. Similarly, in a recent prospective

Table 4. Haplotype HLA-DQA1* – DQB1* Carrier frequency in undifferentiated arthritis.

HLA-DQA1* –DQB1* Haplotype	No. of Haplotypes	Severe, n = 48 (%)	Mild, n = 103 (%)	Carrier	Severe, n = 48	Mild, n = 103	OR (95% CI)	p
DQA1*0101–DQB1*0501 (DR1 or DR10, SE+)	2	1 (2)	3 (3)	Yes	14	28	1.10 (0.52–2.36)	0.8
	1	13 (27)	25 (24)	No	34	75		
	0	34 (71)	75 (73)					
DQA1*03–DQB1*0301/DQA1*03–DQB1*0302 (DR4, SE+)	2	8 (17)	4 (4)	Yes	32	33	4.24 (2.05–8.79)	0.001
	1	24 (50)	29 (28)	No	16	70		
	0	16 (33)	70 (68)					
DQA1*03–DQB1*0301/DQA1*03/DQB1*0302/DQA1*0101–DQB1*0501 (DR1, DR4 or DR10, SE+)	2	16 (33)	13 (13)	Yes	39	55	3.78 (1.66–8.60)	0.001
	1	23 (48)	42 (41)	No	9	48		
	0	9 (19)	48 (47)					
DQA1*05–DQB1*02 (DR3)	2	1 (2)	4 (4)	Yes	8	41	0.30 (0.13–0.71)	0.005
	1	7 (15)	37 (36)	No	40	62		
	0	40 (83)	62 (60)					
DQA1*0102–DQB1*0502	1	3 (6)	0 (3)	Yes	3	0	0 ∞	1.0
	0	45 (98)	103 (97)	No	45	103		
DQA1*0102–DQB1*0602	2	0 (0)	1 (1)	Yes	9	16	1.26 (0.51–3.09)	0.62
	1	9 (19)	15 (15)	No	39	87		
	0	39 (81)	87 (84)					
DQA1*0102–DQB1*0604	1	1 (2)	12 (12)	Yes	1	12	0.16 (0.02–1.28)	0.06
	0	47 (98)	91 (88)	No	47	91		
DQA1*0103–DQB1*0603	2	0 (0)	1 (1)	Yes	2	13		
	1	2 (4)	12 (12)	No	46	90	0.30 (0.07–1.39)	0.11
	0	46 (96)	90 (87)					
DQA1*0201–DQB1*02	1	2 (4)	13 (13)	Yes	2	13	0.30 (0.07–1.39)	0.11
	0	46 (96)	90 (87)	No	46	90		
DQA1*0201–DQB1*0303	1	2 (4)	3 (3)	Yes	2	3	1.45 (0.23–8.97)	0.69
	0	46 (96)	100 (97)	No	46	100		
DQA1*0401–DQB1*0402	1	1 (2)	7 (7)	Yes	1	7	0.29 (0.04–2.44)	0.44
	0	47 (98)	96 (93)	No	47	96		
DQA1*0501–DQB1*0301	2	1 (2)	1 (1)	Yes	7	18	0.81 (0.31–2.08)	0.66
	1	6 (13)	17 (17)	No	41	85		
	0	41 (85)	85 (83)					

SE: shared epitope. DR1 or DR10: HLA-DQA1–DQB1 haplotype in very strong linkage disequilibrium with SE alleles HLA-DR1 or HLA-DR10. DR4: HLA-DQA1–DQB1 haplotypes in very strong linkage disequilibrium with SE allele HLA-DR4. DR1, DR4 or DR10: HLA-DQA1–DQB1 haplotypes in very strong linkage disequilibrium with SE alleles HLA-DR1, DR4, or DR10. DR3: HLA-DQA1–DQB1 haplotype in very strong linkage disequilibrium with HLA-DR3. For clarity we present only haplotype carrier frequencies with > 3% in one of the groups.

study of patients with early-onset RA, the erosive damage at 1 year was not influenced by this polymorphism⁴².

In our study, baseline characteristics such as tender and swollen joints and the number of DMARD taken during the 2 years of followup were similar between carriers and non-carriers of the TNF –308A allele. It therefore seems unlikely that a difference in treatment may account for the reported association between TNF –308A carriage and mild radiographic progression. Therefore, it is hard to explain why patients with this TNF –308A allele, which is known for its association with a high production of proinflammatory TNF- α and a higher radiographic progression in RA, would have milder progression in our UA group. The fact that the TNF –308A carrier frequency was increased in patients with mild UA might be explained by the linkage disequilibrium

with the DQA1*05–DQB1*02 haplotype, which is associated with a mild disease and might overrule the effect of the TNF –308A allele.

The carrier frequency of the HLA-DR4-related SE copies and DR1-, DR4-, or DR10-related SE copies was positively associated with severe progression, which is well known, and was independent from ACPA status at baseline. The lower carrier frequency of the DQA1*0501–DQB1*02 haplotype (in very strong linkage disequilibrium with the HLA-DRB1*03 allele) in the severe disease group compared to the mild group might be explained by the absence of HLA alleles carrying the SE, which are known to be associated with a more severe disease, or by the presence of protective HLA-DQ haplotypes^{9,43}. In contrast, it was reported that the frequency of HLA-DRB1*03 in 88 Finnish patients with

Table 5. Carrier frequencies of *IL1* family and *IL10* minor alleles and radiographic progression in undifferentiated arthritis.

Genotype		Severe, n = 48	Mild, n = 103	p	Minor Allele Carrier	Severe, n = 48	Mild, n = 103	OR (95% CI)	p
<i>IL1A</i> -889	CC	8	16	0.96	CC + CT TT	26	57	0.95 (0.48–1.90)	0.89
	CT	18	41			22	46		
	TT	22	46			22	46		
<i>IL1B</i> -31	CC	6	11	0.50	C/C + CT TT	31	56	1.53 (0.76–3.10)	0.24
	CT	25	45			17	47		
	TT	17	47			17	47		
<i>IL1B</i> +3953	TT	3	10	0.74	TT + CT CC	21	50	0.82 (0.41–1.64)	0.58
	CT	19	40			27	53		
	CC	27	53			27	53		
<i>IL1RN</i> +2018	CC	4	9	0.37	CC + CT TT	24	40	1.58 (0.80–3.14)	0.20
	CT	20	31			24	63		
	TT	24	63			24	63		
<i>IL10</i> -2849	AA	3	8	0.63	AA + GA GG	23	58	0.71 (0.36–1.12)	0.34
	GA	20	50			25	45		
	GG	25	45			25	45		
<i>IL10</i> -1082	AA	9	24	0.24	AA + GA GG	31	80	0.52 (0.25–1.11)	0.09
	GA	22	56			17	23		
	GG	17	23			17	23		
<i>IL10</i> -819	TT	0	4	0.30	TT + CT CC	16	43	0.70 (0.34–1.43)	0.32
	CT	16	39			32	60		
	CC	32	60			32	60		

IL1: interleukin-1; *IL10*: interleukin-10; *IL1A*: interleukin-1 α ; *IL1B*: interleukin-1 β ; *IL1RN*: interleukin-1 receptor antagonist; A: adenine; C: cytosine; G: guanine; T: thymine.

Table 6. *IL10* haplotype carriers and radiographic progression in undifferentiated arthritis.

<i>IL10</i> Haplotype	Severe, n = 48 (%)	Mild, n = 103 (%)	OR (95% CI)	p
AGC	23 (47.9)	58 (56.3)	0.71 (0.36–1.42)	0.34
GAC	20 (41.7)	52 (50.5)	0.70 (0.35–1.40)	0.31
GAT	16 (33.3)	43 (41.7)	0.70 (0.34–1.43)	0.32
GGC	28 (58.3)	34 (33.0)	2.84 (1.40–5.75)	0.003

IL10: interleukin-10; A: adenine; C: cytosine; G: guanine; T: thymine. Haplotype based on *IL10* -2849, *IL10* -1082, *IL10* -819. OR were calculated comparing the carriers vs the noncarriers.

RA was associated with features of severe disease, such as extraarticular disease ($p < 0.01$) and prostheses in large joints ($p < 0.05$), but this was only a small group with a different genetic background⁴⁴.

The TNF -308A allele and DQA1*0501-DQB1*02 were not independently associated with radiographic progression; this linkage disequilibrium was described before in RA³⁹. Further, HLA-DRB1*0301 was significantly associated with ACPA-negative RA, while the absence of ACPA is associated with less radiographic progression in early RA^{14,45}. The results in our patients with early arthritis are different from an early RA population, but the association between carriage of the TNF -308A allele, supported by the linkage disequilibrium with the DQA1*0501-DQB1*02 haplotype (DRB1*0301-related), are in accord with other reports in RA.

The carrier frequencies of other TNF alleles at -238,

-857, -863, and -1031 and the LTA alleles at +368 and +252 were comparable for both radiographic groups. Some studies reported higher frequencies of the TNF -238GG genotype in patients with RA with severe disease^{46,47}, others reported no effect of the TNF -238 polymorphism on radiographic progression⁴⁸. No associations were found for the SNP TNF -238, -857, -863, and RA, although in a Taiwanese population, ethnic differences may have accounted for these discrepancies⁴⁹.

The SNP in other cytokine genes (*IL1A* -889, *IL1B* -31, *IL1B* +3953, *IL1RN* +2018, *IL10* -2849, *IL10* -1082, and *IL10* -819) were not associated with radiographic progression in UA, which is in agreement with earlier results on *IL1B* -31, *IL1B* +3953, *IL1RN* +2018, and *IL10* -1082 observed in patients with early RA⁵⁰. Of note, Cantagrel, *et al*⁵⁰ reported that patients who were SE-positive and also carried the minor allele *IL1B* +3953T showed an increased risk of erosive disease, while patients carrying this allele and who were SE-negative did not. We did not find that result in our study. Although these observations are very interesting, they have to be considered preliminary and will need to be confirmed.

Our results showed a significant association of the promoter *IL10* GGC haplotype with severe radiographic damage, but showed only a nonsignificant association with the *IL10* -1082 SNP. This association was independent of the other associations found with progression of radiographic damage and therefore the influence of the *IL10* haplotype at an early stage of the disease might be high. A recent report described that the *IL10* -1082A/G polymorphism was asso-

ciated with the production of rheumatoid factor²⁸ but not with radiographic progression in early RA. Others described an association between the IL10 -2849 A/G promoter polymorphism and autoantibody production and joint damage in RA²⁶. The fact that in our study on radiographic progression in early UA the association with the IL10 promoter GGC haplotype reached significance but these polymorphisms individually did not might point to the presence of another allele with influence on the GGC haplotype in the haplotype block encompassing the *IL10* gene⁵¹.

Our study has some limitations. First, the population size was limited, which with stratification in the analyses may cause a lack of power to detect differences between the groups, resulting in a type II error. Larger studies might detect the effects of the described SNP and haplotypes better. Second, we are aware that the associations found in this study have to be viewed with caution, because bias due to multiple testing might exist.

Radiographic progression in early UA seems to be enhanced in patients carrying the GGC haplotype of the IL10 -2849G/A, IL10 -1082G/A, or IL10 -819C/T SNP, as well as the SE, and less severe in patients carrying the DQA1*05-DQB1*02 haplotype or the TNF -308A allele.

ACKNOWLEDGMENT

The authors thank E. de Wit-Taen and V. van der Lugt, clinical research assistants, for collecting patient data, R. Heijmans for genotyping the SNP, and W. Bos for valuable comments on the manuscript.

REFERENCES

- van der Horst-Bruinsma IE, Speyer I, Visser H, Breedveld FC, Hazes JM. Diagnosis and course of early-onset arthritis: results of a special early arthritis clinic compared to routine patient care. *Br J Rheumatol* 1998;37:1084-8.
- Mau W, Raspe HH, Mersjann H. Early arthritides: nosography, nosology, and diagnostic criteria. *Scand J Rheumatol* 1989;Suppl 79:3-12.
- Wolfe F, Ross K, Hawley DJ, Roberts FK, Cathey MA. The prognosis of rheumatoid arthritis and undifferentiated polyarthritis syndrome in the clinic: a study of 1141 patients. *J Rheumatol* 1993;20:2005-9.
- Jansen LM, van Schaardenburg D, van der Horst-Bruinsma IE, Dijkmans BA. One year outcome of undifferentiated polyarthritis. *Ann Rheum Dis* 2002;61:700-3.
- Hitchon CA, Peschken CA, Shaikh S, El Gabalawy HS. Early undifferentiated arthritis. *Rheum Dis Clin North Am* 2005;31:605-26.
- Morel J, Legouffe MC, Bozonat MC, Sany J, Eliaou JF, Daures JP, et al. Outcomes in patients with incipient undifferentiated arthritis. *Joint Bone Spine* 2000;67:49-53.
- van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum* 2006;54:1117-21.
- Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008;40:1216-23.
- Carrier N, Cossette P, Daniel C, Brum-Fernandes A, Liang P, Menard HA, et al. The DERA HLA-DR alleles in patients with early polyarthritis: protection against severe disease and lack of association with rheumatoid arthritis autoantibodies. *Arthritis Rheum* 2009;60:698-707.
- Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. *Clin Genet* 1989;36:178-82.
- Dieude P, Cornelis F. Genetic basis of rheumatoid arthritis. *Joint Bone Spine* 2005;72:520-6.
- Emery P, Salmon M, Bradley H, Wordsworth P, Tunn E, Bacon PA, et al. Genetically determined factors as predictors of radiological change in patients with early symmetrical arthritis. *BMJ* 1992;305:1387-9.
- Mattey DL, Hassell AB, Dawes PT, Cheung NT, Poulton KV, Thomson W, et al. Independent association of rheumatoid factor and the HLA-DRB1 shared epitope with radiographic outcome in rheumatoid arthritis. *Arthritis Rheum* 2001;44:1529-33.
- Irigoyen P, Lee AT, Wener MH, Li W, Kern M, Batliwalla F, et al. Regulation of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis: contrasting effects of HLA-DR3 and the shared epitope alleles. *Arthritis Rheum* 2005;52:3813-8.
- Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996;14:397-440.
- Lipsky PE, van der Heijde DM, St. Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000;343:1594-602.
- Bouma G, Crusius JB, Oudkerk PM, Kolkman JJ, von Blomberg BM, Kostense PJ, et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996;43:456-63.
- Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996;334:1717-25.
- Dayer JM. The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. *Rheumatology* 2003;42 Suppl 2:ii3-10.
- Arend WP. Cytokine imbalance in the pathogenesis of rheumatoid arthritis: the role of interleukin-1 receptor antagonist. *Semin Arthritis Rheum* 2001;30(5 Suppl 2):1-6.
- Dayer JM. Interleukin 1 or tumor necrosis factor-alpha: which is the real target in rheumatoid arthritis? *J Rheumatol* 2002;29 Suppl 65:10-5.
- Carreira PE, Gonzalez-Crespo MR, Ciruelo E, Pablos JL, Santiago B, Gomez-Camara A, et al. Polymorphism of the interleukin-1 receptor antagonist gene: a factor in susceptibility to rheumatoid arthritis in a Spanish population. *Arthritis Rheum* 2005;52:3015-9.
- Cox A, Camp NJ, Cannings C, di Giovine FS, Dale M, Worthington J, et al. Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis. *Hum Mol Genet* 1999;8:1707-13.
- Buchs N, di Giovine FS, Silvestri T, Vannier E, Duff GW, Miossec P. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. *Genes Immun* 2001;2:222-8.
- Verhoef CM, van Roon JA, Vianen ME, Bijlsma JW, Lafeber FP. Interleukin 10 (IL-10), not IL-4 or interferon-gamma production, correlates with progression of joint destruction in rheumatoid arthritis. *J Rheumatol* 2001;28:1960-6.
- Lard LR, van Gaalen FA, Schonkeren JJ, Pieterman EJ, Stoeken G, Vos K, et al. Association of the -2849 interleukin-10 promoter polymorphism with autoantibody production and joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2003;48:1841-8.
- Marinou I, Healy J, Mewar D, Moore DJ, Dickson MC, Binks MH, et al. Association of interleukin-6 and interleukin-10 genotypes

- with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. *Arthritis Rheum* 2007;56:2549-56.
28. Nemeč P, Pavkova-Goldbergova M, Gatterova J, Fojtik Z, Vasku A, Soucek M. Association of the -1082 G/A promoter polymorphism of interleukin-10 gene with the autoantibodies production in patients with rheumatoid arthritis. *Clin Rheumatol* 2009;28:899-905.
 29. Prevoost ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44-8.
 30. van der Heijde DM. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 1999;26:743-5.
 31. Bruynsteyn K, van der Heijde DM, Boers M, Saudan A, Peloso P, Paulus H, et al. Determination of the minimal clinically important difference in rheumatoid arthritis joint damage of the Sharp/van der Heijde and Larsen/Scott scoring methods by clinical experts and comparison with the smallest detectable difference. *Arthritis Rheum* 2002;46:913-20.
 32. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
 33. Crusius JB. The immunogenetics of chronic inflammatory and autoimmune disease (PhD dissertation). Amsterdam: Vrije Universiteit; 2002.
 34. Doherty DG, Vaughan RW, Donaldson PT, Mowat AP. HLA DQA, DQB, and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British caucasoids. *Hum Immunol* 1992;34:53-63.
 35. Knipper AJ, Hakenberg P, Enczmann J, Kuhrober A, Kiesel U, Kogler G, et al. HLA-DRB1,3,4,5 and -DQB1 allele frequencies and HLA-DR/DQ linkage disequilibrium of 231 German caucasoid patients and their corresponding 821 potential unrelated stem cell transplants. *Hum Immunol* 2000;61:605-14.
 36. de Vries N, Tijssen H, van Riel PL, van de Putte LB. Reshaping the shared epitope hypothesis: HLA-associated risk for rheumatoid arthritis is encoded by amino acid substitutions at positions 67-74 of the HLA-DRB1 molecule. *Arthritis Rheum* 2002;46:921-8.
 37. Brinkman BM, Huizinga TW, Kurban SS, van der Velde EA, Schreuder GM, Hazes JM, et al. Tumour necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? *Br J Rheumatol* 1997;36:516-21.
 38. Lacki JK, Moser R, Korczowska I, Mackiewicz S, Muller W. TNF-alpha gene polymorphism does not affect the clinical and radiological outcome of rheumatoid arthritis. *Rheumatol Int* 2000;19:137-40.
 39. Khanna D, Wu H, Park G, Gersuk V, Gold RH, Nepom GT, et al. Association of tumor necrosis factor alpha polymorphism, but not the shared epitope, with increased radiographic progression in a seropositive rheumatoid arthritis inception cohort. *Arthritis Rheum* 2006;54:1105-16.
 40. Nemeč P, Pavkova-Goldbergova M, Stouracova M, Vasku A, Soucek M, Gatterova J. Polymorphism in the tumor necrosis factor-alpha gene promoter is associated with severity of rheumatoid arthritis in the Czech population. *Clin Rheumatol* 2008;27:59-65.
 41. Barton A, Platt H, Salway F, Symmons D, Barrett E, Bukhari M, et al. Polymorphisms in the tumour necrosis factor gene are not associated with severity of inflammatory polyarthritis. *Ann Rheum Dis* 2004;63:280-4.
 42. Reneses S, Gonzalez-Escribano MF, Fernandez-Suarez A, Pestana L, Davila B, Wichmann I, et al. The value of HLA-DRB1 shared epitope, -308 tumor necrosis factor-alpha gene promoter polymorphism, rheumatoid factor, anti-citrullinated peptide antibodies, and early erosions for predicting radiological outcome in recent-onset rheumatoid arthritis. *J Rheumatol* 2009;36:1143-9.
 43. van der Horst-Bruinsma IE, Visser H, Hazes JM, Breedveld FC, Verduyn W, Schreuder GM, et al. HLA-DQ-associated predisposition to and dominant HLA-DR-associated protection against rheumatoid arthritis. *Hum Immunol* 1999;60:152-8.
 44. Hakala M, Silvennoinen-Kassinen S, Ikaheimo I, Isosomppi J, Tiilikainen A. HLA markers in a community-based rheumatoid arthritis series. *Ann Med* 1997;29:291-6.
 45. Verpoort KN, van Gaalen FA, van der Helm-van Mil AH, Schreuder GM, Breedveld FC, Huizinga TW, et al. Association of HLA-DR3 with anti-cyclic citrullinated peptide antibody-negative rheumatoid arthritis. *Arthritis Rheum* 2005;52:3058-62.
 46. Kaijzel EL, van Krugten MV, Brinkman BM, Huizinga TW, van der Straaten T, Hazes JM, et al. Functional analysis of a human tumor necrosis factor alpha (TNF-alpha) promoter polymorphism related to joint damage in rheumatoid arthritis. *Mol Med* 1998;4:724-33.
 47. Fabris M, Di Poi E, D'Elia A, Damante G, Sinigaglia L, Ferraccioli G. Tumor necrosis factor-alpha gene polymorphism in severe and mild-moderate rheumatoid arthritis. *J Rheumatol* 2002;29:29-33.
 48. Fonseca JE, Cavaleiro J, Teles J, Sousa E, Andreozzi VL, Antunes M, et al. Contribution for new genetic markers of rheumatoid arthritis activity and severity: sequencing of the tumor necrosis factor-alpha gene promoter. *Arthritis Res Ther* 2007;9:R37.
 49. Yen JH, Chen CJ, Tsai WC, Lin CH, Ou TT, Wu CC, et al. Tumor necrosis factor promoter polymorphisms in patients with rheumatoid arthritis in Taiwan. *J Rheumatol* 2001;28:1788-92.
 50. Cantagrel A, Navaux F, Loubet-Lescoulie P, Nourhashemi F, Enault G, Abbal M, et al. Interleukin-1 beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum* 1999;42:1093-100.
 51. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature* 2007;449:851-61.