Chapter 2

Haplotype *IL10* GGC is positively and HLA-*DQA1**05-*DQB1**02 is negatively associated with radiographic progression in undifferentiated arthritis

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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 (SNPs) 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 follow-up. Severe progression was defined as an increase in Sharp/van der Heijde Score ≥ 5 points after 2 years of follow-up. The remainder was classified as mild. These SNPs were genotyped by Taqman technology: tumor necrosis factor (TNF) -1031, -863, -857, -308, -238; lymphotoxin-α (LTA) +368, +252; interleukin (IL)10 -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.

Introduction

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 disease[1]. 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 year.[4] In contrast to RA, in which associations with single-nucleotide polymorphisms (SNPs) have been extensively studied, fewer data are available on the genetic characteristics of patients with UA.[6-9]

The major histocompatibility complex (MHC) class II alleles, referred to as the shared-epitope (SE) alleles, contribute 30%-50% of the genetic predisposition to RA:[10,11] HLA-DRB1*0401, *0404, *0405, *0408, *0101, *1001, 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 disease.[14]

Apart from MHC class II alleles, SNPs in genes coding for cytokines such as tumor necrosis factor-α (*TNF*), lymphotoxin-α (*LTA*), interleukin 1α (*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 RA.[15] 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 group.[16] 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 activators.[17]

LTA (formerly TNF-β), a homolog of TNF-α, has similar biological activity as TNF-α and shares one of its receptors.[18] 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 joint,[19] 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 allele.[22] A relation between radiographic progression and SNPs at the *IL1* cluster in RA was also demonstrated.[23,24]

The anti-inflammatory cytokine IL-10 is negatively correlated with the progression of joint destruction in RA.[25] The IL10 -2849 promoter polymorphism is associated with autoantibody production and subsequent joint damage in RA.[26] Also, *IL10* -592CC and *IL10* -1082GG have been associated with more severe radiographic damage only in patients who are ACPA-negative,[27] while another study showed opposite results.[28]

We investigated whether SNPs 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.

Patients 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 SHS[30] 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 followup.[31] The remainder was classified as mild progression. After 2 years of follow-up, patients who fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA[32] 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 SNPs was performed at positions -1031 (db SNP ID: rs1799964), -863 (rs1800630), -857 (rs1799724), -308 (rs1800629), and -238 (rs361525) in the *TNF* gene and of intronic SNPs 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 SNPs *IL10* -2849 (rs6703630), *IL10* -1082 (rs1800896), and *IL10* -819 (rs1800871).

SNPs 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).[33]

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 classified.[36]

Statistical analysis

Haplotypes for SNPs 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://stephenslab.uchicago.edu/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 follow-up, 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 1. Characteristics of 151 Dutch Caucasian patients with undifferentiated arthritis

Radiographic progression	Severe n=48	Mild n=103	p
Women, %	79	62	0.03
Age at baseline, years	53.1 (12.3)	53.9 (0.72)	0.72
Duration of symptoms before baseline, years	0.45 (1.9)	0.4 (0.8)	0.77
Number of DMARD use	2 (1-3)	1 (1-2)	<0.01
DAS28 at baseline	5.2 (1.2)	4.6 (1.2)	0.006
DAS28 at the 2-years follow-up	3.7 (1.3)	2.9 (1.3)	<0.01
ESR baseline, mm/1st hr	31.7 (1.9)	19.1 (2.3	<0.01
CRP baseline, mg/l	20.7 (3.4)	12.3 (3.7)	0.03
IgM-RF positive,%	79	32	<0.01
ACPA positive*,%	84	34	<0.01
Shared Epitope (SE) copies,%			
0	19	47	<0.01
1	48	41	
2	33	13	
SHS at baseline	2 (0-10)	0 (0-2)	<0.01
SHS at the 2-years follow-up	19.5 (10.5-31.5)	1 (0-3)	<0.01

Data are mean with standard deviation, median with inter-quartile range or frequencies. DMARD= disease modifying anti-rheumatic 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 anti-cyclic citrullinated peptide/protein; SHS= Sharp-Van der Heijde Score. *Only available of 124 patients

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).

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 *I*L10 GGC haplotype was positively associated, while the *DQA1*05-DQB1*02* haplotype was negatively associated.

Table 2. *TNF* and *LTA* SNPs carrier frequency and radiographic progression

SNPs	**Severe**	**Mild**	**p**	**Carrier**	**Severe**	**Mild**	**OR**	**(95% CI)**	**p**
Genotypes	n=48	n=103		**minor allele**	n=48	n=103			
LTA +252 GG	4	22		GG + GA	31	67			
GA	27	45		AA	17	36	0.98	0.48-2.00	0.96
AA	17	36	0.12						
LTA +368 CC	7	13		CC + CG	30	56			
GC	23	43		GG	18	47	1.40	0.69-2.82	0.35
GG	18	47	0.64						
TNF –1031 CC	3	2		CC + CT	17	35			
CT	14	33		TT	31	68	1.07	0.52-2.19	0.86
TT	31	68	0.38						
TNF –863 AA	3	1		AA + CA	13	31			
CA	10	30		CC	35	72	0.86	0.40-1.85	0.70
CC	35	72	0.11						
TNF –857 TT	0	0		TT + CT	5	13			
CT	5	13		CC	43	90	0.81	0.27-2.40	0.70
CC	43	90	0.70						
TNF –308 AA	1	6		AA + GA	13	48			
GA	12	42		GG	35	55	0.43	0.20-0.90	0.02*
GG	35	55	0.07						
TNF –238 AA	0	0		AA + GA	2	6			
GA	2	6		GG	46	97	0.70	0.14-3.62	0.67
GG	46	97	0.67						

TNF = tumour necrosis factor, *LTA* = lymphotoxin alpha, OR = odds ratio, CI = confidence interval.
A= adenine, C=cytosine, G=guanine, T=thymine * significant association

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 contribution 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).

Table 3. *TNF-LTA* haplotype carrier frequency and radiographic progression in UA

Haplo-type	*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)	∞		
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 = tumour necrosis factor, *LTA* = lymphotoxin alpha, OR = odds ratio, CI = confidence interval, A= adenine, C=cytosine, G=guanine, T=thymine. ORs were calculated comparing the carriers vs the non-carriers; * significant association. One patient carried a recombinant haplotype (not shown)

SNPs 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 SNPs 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).

Tabel 4. Haplotype HLA-*DQA1 - *DQB1** carrier frequency in UA**

HLA-*DQA1** - *DQB1** haplotype	No	Severe n=48 (%)	Mild n=103 (%)	Carrier	Severe n=48	Mild n=103	OR (95% CI)	p
*DQA1**0101 - *QB1**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 - *QB1**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-*B1**0302 / *DQA1**0101 - *B1**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 - *B1**0502	1	3 (6%)	0 (3%)	yes	3	0	0	1.0
	0	45 (98%)	103 (97%)	no	45	103	∞	
*DQA1**0102 - *B1**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 - *B1**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 - *B1**0603	2	0 (0%)	1 (1%)	yes	2	13	0.30 (0.07-1.39)	0.11
	1	2 (4%)	12 (12%)	no	46	90		
	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 - *B1**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 - *B1**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 - *B1**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 alleles 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 only present haplotype carrier frequencies with > 3% in one of the groups

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

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

IL1 = Interleukin-1, *IL10*= Interleukin-10, OR = odds ratio, CI = confidence interval, *IL1A* = Interleukin-1 alpha, *IL1B* = Interleukin-1 beta, *IL1RN* = Interleukin-1 receptor antagonist, A= adenine, C=cytosine, G=guanine, T=thymine

Table 6. *IL10* haplotype carriers and radiographic progression in UA

***IL10* 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	>0.01

IL10 = interleukin-10, OR = odds ratio, CI = confidence interval,

A= adenine, C=cytosine, G=guanine, T=thymine. Haplotype based on *IL10* -2849, *IL10* -1082, *IL10* -819. ORs were calculated comparing the carriers vs. the non-carriers

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 production.[17]

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 SE,[39] while our result is in accord with a reported association between the *TNF* -308GG genotype and erosions in RA.[40] 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 study of patients with early-onset RA, the erosive damage at 1 year was not influenced by this polymorphism.[42]

In our study, baseline characteristics such as tender and swollen joints and the number of DMARD taken during the 2 years of follow-up 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 pro-inflammatory 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 RA was associated with features of severe disease, such as extra-articular disease ($p < 0.01$) and prostheses in large joints ($p < 0.05$), but this was only a small group with a different genetic background.[44]

The *TNF* -308A allele and *DQA1*0501-DQB1*02* were not independently associated with radiographic progression; this linkage disequilibrium was described before in RA.[39] 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,[36,46,47] others reported no effect of the *TNF* -238 polymorphism on radiographic progression.[48] No associations were found for the SNPs TNF -238, -857, -863, and RA, although in a Taiwanese population, ethnic differences may have accounted for these discrepancies.[49]

The SNPs 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 RA.[50] 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 non-significant 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 associated with the production of rheumatoid factor[28] 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 RA.[26] 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* gene.[51]

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 SNPs 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 SNPs, as well as the SE, and less severe in patients carrying the *DQA1*05-DQB1*02* haplotype or the *TNF* -308A allele.

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