

Susceptibility for and Clinical Manifestations of Rheumatoid Arthritis Are Associated with Polymorphisms of the TNF- α , IL-1 β , and IL-1Ra Genes

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ABSTRACT. *Objective.* To analyze the association of genetic polymorphisms of pro-inflammatory cytokines with rheumatoid arthritis (RA) in comparison with healthy controls from Northern Sweden and the potential contribution of these genetic variants for disease severity and development of cardiovascular complications.

Methods. Polymerase chain reaction amplification was used for analysis of TaqI restriction fragment length polymorphism (RFLP) of interleukin-1 beta (IL-1 β), variable tandem repeat polymorphism of IL-1 receptor antagonist (IL-1Ra) gene and NcoI RFLP at position -308 of tumor necrosis factor-alpha (TNF- α) gene. One hundred and fifty-four patients with RA, 42 men and 112 women, were consecutively recruited into the study through the Department of Rheumatology.

Results. The allele A1 of TNF- α was more common in the patient group ($p < 0.01$; OR = 1.62). Patients having the genotype A1A2 seemed to develop more severe disease compared with patients with A1A1 genotype: they were younger at disease onset ($p < 0.05$), had a higher accumulated disease activity ($p < 0.05$) and worse functional class ($p < 0.05$). Patients with genotype A2A2 of IL-1 β had higher accumulated disease activity score than patients with A1A1 and A1A2 ($p < 0.05$). The allelic combination A1 IL-1 β /A2 IL-1Ra was less prevalent in RA patients who developed cardiovascular complications ($p < 0.005$; OR = 0.20).

Conclusions. The A1 allele of TNF- α associates with RA. Genotypes A1A2 of TNF- α and A2A2 of IL-1 β are associated with more severe disease. The allelic combination A1 IL-1 β /A2 IL-1Ra is less often present in RA patients who developed cardiovascular complications. (J Rheumatol 2002;29:212-9)

Key Indexing Terms:

INTERLEUKIN-1 β

TUMOR NECROSIS FACTOR ALPHA

INTERLEUKIN-1 RECEPTOR ANTAGONIST
POLYMORPHISM RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is recognized as a multigenic disorder, with genetic polymorphisms contributing to both the susceptibility and the severity of the disease. Several genes from the HLA-DRB1 locus have been associated with the disease^{1,2}. Reported associations of HLA genes to RA led to further investigations of the tumor necrosis factor (TNF) gene,

which is located within the MHC class-III region on chromosome 6 (6p21.3). The interest in polymorphisms of pro-inflammatory cytokines, such as interleukin 1 (IL-1)³, TNF- α ^{2,4-6} and IL-6^{7,8} developed, because certain alleles have been correlated to higher secretion of biologically active products⁹⁻¹¹.

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Supported by the Swedish Medical Research Council, the Swedish Heart-Lung Foundation, the King Gustaf Vth 80-years Foundation, the Serafimerlasarettet Foundation, the Börje Dahlin Foundation, and the Swedish Rheumatism Association.

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Submitted February 22, 2001; revision accepted July 27, 2001.

Genetic studies have also been performed on the anti-inflammatory cytokines IL-4 and IL-10¹² and the IL-1 antagonist IL-1Ra¹³. Allele RP1 of IL-4 is associated with enhanced IL-4 activity and with RA, while polymorphisms of the IL-10 gene are not. An increased frequency of high secretory allele A2 of IL-1Ra variable number of tandem repeats (VNTR) has been reported in inflammatory diseases¹⁴⁻¹⁶ and investigated in RA^{12,13}.

The systemic effects of pro-inflammatory cytokines are mediated by stimulating the synthesis of acute phase proteins¹⁷ and up-regulating the expression of adhesion molecules on endothelial cells, e.g., E-selectin and VCAM-1, thus providing a link to vascular damage¹⁸. The secretion of acute phase proteins, such as C-reactive protein and serum amyloid A, and their influence in RA patients have been studied¹⁹, as well as their actions in combination with chemokines, such as

IL-8, in stimulation of leukocyte infiltration²⁰. Cytokines, chemokines, and acute phase proteins constitute a network of communication between fibroblasts, macrophages, lymphocytes, and hepatocytes. Pro-inflammatory cytokines, as well as a wide spectrum of chemokines²¹, are present in the inflamed joints of patients with RA. The synergistic action of TNF- α and IL-1 β results in progressive destruction of joints via activation of matrix metalloproteinases²², including collagenase²³, and causes the systemic symptoms characteristic of RA.

Patients with RA have a shortened life span and the most common cause of death is cardiovascular diseases²⁴⁻²⁷. Although the etiopathology of atherosclerosis is still incompletely known, a role of pro-inflammatory cytokines for the development of the atherosclerotic plaque has been suggested in several reports^{28,29}. There are several investigations of atherogenic factors in RA³⁰⁻³². Endothelial dysfunction or sub-clinical vasculitis has been suggested to have a major underlying role for inducing cardiovascular diseases³³. Inflammatory activity, measured as increase of erythrocyte sedimentation rate and serum haptoglobin, was predictive for cardiovascular disease^{34,35}.

Our aim was to investigate the prevalence of genetic polymorphisms of IL-1 β , TNF- α and IL-1Ra in patients with RA from Northern Sweden and their potential association with associated cardiovascular diseases.

MATERIALS AND METHODS

Study groups. One hundred and fifty-four patients (42 men and 112 women) with RA³⁶, from Northern Sweden and attending the outpatient clinic at the Department of Rheumatology were consecutively recruited into the study during a 4 month period. The Department was at that time the only referral center for rheumatology patients in the county, and there was thus no selection of patients for more severe cases. Demographic and clinical data for the patients are presented in Table 1. All but 9 patients were rheumatoid factor (RF) positive according to the Waaler-Rose test, with titers ranging from 1/40 to 1/40,000. The accumulated disease activity score was estimated on the basis of erythrocyte sedimentation rate, number of swollen and tender joints, and the clinician's global assessment of the disease activity calculated every

second year of the patient's disease and divided by the number of observations³⁷. Cardiovascular and/or cerebrovascular events, myocardial infarction (MI, n = 19), stroke/transient ischemic attacks (n = 12) or deep vein thrombosis/pulmonary embolism (n = 5) (as defined by Wällberg-Jonsson, *et al*²⁷) occurred in 28 patients after the onset of RA. Forty-four patients had been treated for hypertension at the time of the study. Patients with hypertension, and/or MI, stroke/transient ischemic attacks and deep vein thrombosis/pulmonary embolism were grouped as patients with cardiovascular complications (n = 57).

Since 1985 the 2 Northern counties in Sweden, the geographic area of the RA patients, with a total population of 510,000, have constituted one of 39 collaborating centers in the WHO MONICA study (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease)³⁸. As a control group, we used 226 individuals who were part of a prospective, nested case-referent MONICA study and 131 individuals randomly selected from a population register of the county. They had the same ethnic and geographical background (based on the place of birth) as the patients and were selected for age and gender.

Due to a lack of DNA material, numbers of controls included in analysis of each gene polymorphism were different. An effort was made to balance the number of female and male controls. For the analysis of TNF- α polymorphism, the control group included 324 individuals (172 females and 152 males). In the analysis of IL-1 β gene polymorphism, 207 controls were used (96 females and 111 males), and for IL-1Ra gene polymorphism, 202 controls were used (99 females and 103 males). We used a higher number of controls for the analysis of TNF- α polymorphisms because our initial study showed that the allelic distribution was not in concordance with the Hardy-Weinberg law. The use of a larger sample did not change the results.

This study was approved by the Research Ethics Committee of Umeå University.

DNA extraction and PCR reactions. Genomic DNA was extracted either from EDTA-preserved blood or from buffy coats using standard proteinase K digestion and phenol/chloroform extraction method. Polymerase chain reaction (PCR) amplification was performed using a thermal cycler (PHC-3 Dri-Block Techné, Cambridge, UK).

IL-1 β TaqI RFLP, IL-1Ra VNTR and TNF- α NcoI RFLP polymorphisms. Amplification of the region which contains the TaqI polymorphic site within exon 5 of the IL-1 β gene was performed as described previously¹⁰. TaqI digestion of the 249-bp PCR product resulted in 2 fragments of 135 and 114 bp (allele 1) or remained intact (allele 2). Amplification of the fragment containing variable numbers of an identical tandem repeat of 86 bp of IL-1Ra gene was performed according to Mansfield, *et al*³⁹. Alleles 1 to 5 were detected according to their different PCR fragment lengths. Genotyping for bi-allelic polymorphism of TNF- α at position -308 in the promotor region

Table 1. Demographic and clinical variables in 154 patients with RA.

	All Patients n = 154	Women n = 112	Men n = 42
Age, yrs, mean \pm SD	64.0 \pm 20.3	44.1 \pm 14.1	52.8 \pm 10.7
Disease duration, yrs, mean \pm SD	15.5 \pm 9.7	16.9 \pm 10.0	11.7 \pm 7.8
Accumulated disease activity score ³⁷ (median, Q1-Q3)	4.8 (4.2-5.4)	4.8 (4.2-5.6)	4.8 (4.3-5.3)
Functional class I-II (%)	81 (53.0)	54 (48.2)	27 (64.3)
Extraarticular manifestations* (%)	21 (13.6)	54 (48.2)	15 (35.7)
Cardiovascular and/or cerebrovascular complications (%)	28 (18.2)	14 (12.5)	14 (33.3)
Treatment for hypertension (%)	44 (28.6)	29 (25.9)	15 (35.7)
Oral prednisolone \leq 7.5 mg (%)	83 (53.9)	55 (49.1)	28 (66.7)
DMARD (%)	107 (69.5)	75 (67.0)	32 (76.2)

*Vasculitic ulcers/neuropathy, pericarditis, pleuritis, scleritis, and Sjögren's syndrome.

was performed by PCR amplification, using primers and NcoI digestion conditions as described⁴⁰. Digestion by NcoI restriction enzyme confirmed 2 alleles: allele A1 is split into 2 fragments, 87 bp and 20 bp long; allele A2 is an intact fragment of 107 bp.

Statistical analysis. Fisher's exact test and chi-squared distribution were used. Probability (p value) was considered significant when $p < 0.05$ and was corrected for the number of comparisons at each locus (p_c) according to Bonferroni. The chi-squared test was used for the calculations of the Hardy-Weinberg law. Odds ratios (OR), and 95% confidence intervals (CI) were calculated as estimates of the relative risks. Continuous data were compared with Kruskal-Wallis H for one-way analysis of variance by ranks (3 groups) and Mann-Whitney U test (2 groups).

RESULTS

TaqI RFLP of the IL-1 β gene. TaqI RFLP of IL-1 β gene was investigated in 154 RA patients and 207 controls. There were 85 patients with genotype A1A1, 54 with genotype A1A2, and 15 with genotype A2A2 compared to 117 with A1A1, 68 with A1A2, and 22 with A2A2 in the control group. There was no difference between genotypes or alleles in patients and controls.

Accumulated disease activity score was significantly different between the genotypes. Medians and interquartile range (Q1-Q3) for patients with A1A1 (n = 85) was 4.7 (3.9-5.2), for patients with A1A2 (n = 54) 4.9 (4.5-5.4), and for patients with A2A2 (n = 14) 5.3 (4.9-5.4) ($p = 0.02$).

There was no association of this polymorphism to cardiovascular complications. The frequency distribution of the alleles was in accordance with the Hardy-Weinberg law ($p = 0.24$).

IL-1Ra VNTR polymorphism. IL-1Ra VNTR (variable number of tandem repeats) polymorphism was investigated in 154 patients and 202 controls. The distribution of genotypes and alleles is presented in Table 2. Genotype A1A2 was decreased in RA patients (OR = 0.52), but the genotype A2A3 was

increased (OR = 12.11). The allele A3 showed an increased frequency in patients (OR = 4.49), and accordingly, carriage of A3 was more common in patients (OR = 4.61). All these significant differences were lost after correction. There was no association of genotypes or alleles with clinical manifestations or disease severity. Allelic distribution of IL-1Ra gene polymorphism was in concordance with the Hardy-Weinberg law ($p = 0.05$).

RA patients with cardiovascular complications. Comparisons of IL-1 β and IL-1Ra genotypes, alleles and carriers between RA patients with and without cardiovascular complications did not reveal any differences between these 2 groups of patients. The allelic associations between alleles of IL-1 β and IL-1Ra were investigated (Table 3). Allelic combinations A1⁺ IL-1 β / A2⁺ IL-1Ra and A2⁺ IL-1 β / A2⁺ IL-1Ra were decreased in patients with cardiovascular complications compared with those without complications (OR = 0.20 and 0.13, respectively). The latter significance was lost after correction.

After exclusion of patients diagnosed with only deep vein thrombosis (n = 2) from the group of patients with cardiovascular complications, there was no association between any allele combination and cardiovascular complication.

TNF- α NcoI RFLP. The TNF- α NcoI RFLP was investigated in 154 patients and 324 controls. The genotype A1A2 was increased (OR = 1.63), although the statistical significance was lost after correction. Genotype A2A2 was decreased (OR = 0.04), and the frequency and carriage of A1 increased (OR = 1.62 and 23.41, respectively) (Table 4). Of the patients, 99.4% carried allele A1, compared with 86.7% of controls. The distributions of genotypes, carriers and allele frequencies in females and males are presented in Table 5 A and B, respectively. In both females and males, genotype A2A2 was decreased in patients (OR = 0.06 and 0.07, respectively).

Table 2. Distribution of genotypes, alleles, and carriers of alleles of IL-1Ra VNTR gene polymorphism in RA patients and controls. Allele frequencies are given in brackets. P values are corrected for the number of comparisons made at each locus.

	Patients n = 154	Controls n = 202	p	p_c	OR	95% CI
A1A1	109	138	NS			
A1A2	19	43	0.0339	0.1695	0.52	0.29-0.94
A2A2	16	18	NS			
A1A3	6	3	NS			
A2A3	4	0	0.0342	0.1710	12.11	0.65-226.8
Alleles						
A1	243 (0.79)	322 (0.80)	NS			
A2	55 (0.18)	79 (0.19)	NS			
A3	10 (0.03)	3 (0.01)	0.0206	0.0618	4.49	1.22-16.45
Carriers						
A1	134	184	NS			
A2	39	61	NS			
A3	10	3	0.0195	0.0585	4.61	1.25-17.04

Table 3. Allelic association between alleles of IL-1 β and IL-1Ra genes in RA patients with and without cardiovascular complications. P values are corrected for the number of comparisons at each locus.

IL-1 β / IL-1Ra	Patients with Complications (n = 57)	Patients without Complications (n = 97)	p	p _c	OR	95% CI
A1 ⁺ /A1 ⁺	41	78	NS			
A1 ⁺ /A2 ⁺	4	27	0.0016	0.0064	0.20	0.06–0.59
A2 ⁺ /A1 ⁺	24	40	NS			
A2 ⁺ /A2 ⁺	1	12	0.0320	0.1280	0.13	0.02–1.00

Carriage of A1 was increased in both male and female patients (OR = 16.3 and 13.9, respectively). These collected data lead us to conclude that allele A1 of TNF- α is associated with RA (OR = 1.62) and that carriage of allele A1 could be considered a risk factor for RA in patients from Northern Sweden (OR = 23.41; Tables 4 and 5).

Patients with genotype A1A2 were significantly younger ($p < 0.05$) at disease onset, the median and interquartile range (Q1-Q3) being 44.0 years (33.8-52.0) compared with those with genotype A1A1, who had a median of 49.0 years (36.0-56.8). Patients with A1A2 genotype compared with patients with A1A1 also had a higher accumulated disease activity score with median and interquartile range (Q1-Q3) being 5.0 (4.5-5.7) and 4.7 (4.0-5.2) ($p < 0.05$) and worse functional class ($p < 0.05$). Fifty-nine per cent of patients with A1A2 genotype were in functional class III-IV compared with 41% of those with A1A1, as determined in patients after the same disease duration. There were no differences concerning extraarticular disease, corticosteroid therapy, or frequency or number of disease modifying antirheumatic drugs used (data not shown), or in the distribution of genotypes, allele frequency, or carriers between patients with and without cardiovascular complications.

Allelic distribution for this polymorphism was not in concordance with the Hardy-Weinberg law ($p < 0.01$).

Combinations of alleles of the genes for IL-1 β , IL-1Ra, and

TNF- α . Results of genotyping for all 3 cytokine genes were available for 142 controls and 154 patients. Two combinations of alleles were decreased in RA patients. Carriage of A1 of IL-1 β , A1 of IL-1Ra, and A2 of TNF- α was more common in the referent group ($p = 0.0435$; $p_c = 0.348$; OR = 0.59; 95% CI = 0.36-0.97). Also carriage of A1 of IL-1 β , A2 of IL-1Ra, and A2 of TNF- α was more common in controls ($p = 0.0077$; $p_c = 0.0616$; OR = 0.34; 95% CI = 0.15-0.76). The significance of these differences was lost after correction. In both combinations, the high secretory allele A2 of TNF- α was present, implying that this allele decreases the susceptibility to develop RA. There were no associations between these allelic combinations and cardiovascular events.

DISCUSSION

Certain polymorphisms of the genes of pro- and antiinflammatory cytokines are associated with autoimmune and inflammatory diseases. The TNF- α gene is located on chromosome 6, between HLA-B and HLA-DR genes⁴¹. The locus HLA-DRB1 is associated with susceptibility for RA⁴², suggesting that also the polymorphisms of the TNF- α gene might be of importance. Recently, a study by Cox, *et al*⁴³ showed that the products of the IL-1 gene cluster, located on the long arm of chromosome 2, could contribute to the development and/or severity of RA without correlation to the HLA complex.

Table 4. Distribution of genotypes, alleles and carriers of alleles A1 and A2 of TNF- α NcoI RFLP in RA patients and controls. Allele frequencies are given in brackets. P values are corrected for the number of comparisons made at each locus.

	Patients n = 154	Controls n = 324	p	p _c	OR	95% CI
A1A1	104	209	NS	NS		
A1A2	49	72	0.0321	0.0963	1.63	1.06–2.51
A2A2	1	43	< 0.0001	< 0.0003	0.04	0.01–0.31
Alleles						
A1	257 (0.83)	490 (0.76)	0.0080	0.016	1.62	1.15–2.31
A2	51 (0.16)	158 (0.24)	0.0080	0.016	0.62	0.43–0.87
Carriers						
A1 carriers	153	281	< 0.0001	< 0.0002	23.41	3.19–171.77
A2 carriers	50	115	NS	NS		

Table 5. Distribution of genotypes, alleles and carriers of alleles A1 and A2 of TNF- α NcoI -308 RFLP in female and male RA patients and controls. Allele frequencies are given in brackets. P values are corrected for the number of comparisons made at each locus.

(A) Females						
	Patients n = 112	Controls n = 172	p	p _c	OR	95% CI
A1A1	73	115	NS			
A1A2	38	35	0.0155	0.0465	2.01	1.17–3.45
A2A2	1	22	0.0002	0.0006	0.06	0.01–0.46
Alleles						
A1	184 (0.82)	265 (0.77)	NS			
A2	40 (0.18)	79 (0.23)				
Carriers						
A1	111	150	0.0002	0.0004	16.30	2.16–122.7
A2	39	57	NS			
(B) Males						
	Patients n = 42	Controls n = 152	p	p _c	OR	95% CI
A1A1	31	94	NS			
A1A2	11	37	NS			
A2A2	0	21	0.0089	0.0267	0.07	0.004–1.13
Alleles						
A1	73 (0.87)	225 (0.74)	0.0129	0.0258	2.33	1.18–4.62
A2	11 (0.13)	79 (0.26)				
Carriers						
A1	42	131	0.0089	0.0178	13.90	0.82–234.5
A2	11	58	NS			

The cytokine TNF- α functions synergistically with IL-1 β , suggesting that polymorphisms of the IL-1 β gene could be associated with RA, similar to its association with diabetes in individuals homozygous for allele A2⁹ and with myasthenia gravis⁴⁴. Allele A2 is related to higher secretion of IL-1 β ¹⁰ and the severity of autoimmune myasthenia gravis is associated with this allele and its dosage effect⁴⁴. Allele A2 of IL-1Ra VNTR polymorphism is also a high secretory allele¹⁰ that is associated with ulcerative colitis and inflammatory bowel disease³⁹, systemic lupus erythematosus with skin disease¹⁴, Grave's disease¹⁵, and multiple sclerosis¹⁶. The balance between IL-1 β and IL-1Ra is of importance in inflammatory bowel disease⁴⁵ and also in myasthenia gravis⁴⁴.

There are several polymorphisms of the promoter region of TNF- α gene and for some of them (e.g., position -863), functional correlates exist⁴⁶. There are, however, controversial reports regarding other polymorphic sites. Some reports consider certain polymorphisms to be independent markers of susceptibility for RA⁴⁷, while others have shown associations of polymorphisms to the severity of RA⁴⁸. However, other reports have not confirmed such associations⁴⁹. We found a strong association between allele A1 of NcoI TNF- α RFLP and RA, whereas A2 of TNF- α was related to decreased risk for RA. In patients with RA, however, it seemed to be associated with a more severe disease.

Biological actions of IL-1 β and TNF- α in RA and their influence on the activation of matrix metalloproteinases have been extensively studied^{21,50}. Local high secretion and high concentrations of cytokines, caused by high secretory alleles could exert a strong effect on metalloproteinases, as well as on the degradative processes in joints, which are caused by interplay of metalloproteinases²¹ and their tissue inhibitors. Likewise, local high secretion of cytokines from lymphocytes and macrophages, which adhere to the inflammatory site, can also damage the epithelium of blood vessels and surrounding tissues. Thus, carriage of alleles associated with higher secretion of cytokines might constitute an increased risk for the disease manifestations in RA and/or its cardiovascular complications. Our findings of a more severe disease in patients with the TNF- α genotype A1A2 are consistent with reports from other groups. Wilson, *et al*⁵¹ showed a tendency for patients with genotype A1A2 (or GA) to have an increased number of erosions. Brinkman, *et al*⁵ failed to find an association with -308, but found that genotype -238GG is associated with a more severe course of RA and conclude that there is some linkage disequilibrium between -238G and -308A (A2) alleles. Similarly, Vinasco, *et al*⁵² could not find an association of the -308 polymorphism with susceptibility to RA, but could associate certain polymorphisms at TNF- α locus with the clinical manifestation of the disease. They found significantly

increased frequency of nodular disease in heterozygous patients expressing A1A2 ($p = 0.03$). Seki, *et al*⁵³ studied the polymorphisms in the 5'-flanking promoter/enhancer region of the TNF- α gene in Japanese RA patients. They found lower frequency of allele -308 A2 than in controls and suggested that this could be due to its linkage disequilibrium with the HLA-DRB*1302 allele in Japanese patients since this allele has been reported to be inversely associated with RA. Their conclusion was that these polymorphisms might be markers for extended HLA haplotypes.

In the controls from Northern Sweden, the allelic distribution for the TNF- α gene polymorphism was not in concordance with the Hardy-Weinberg law. This can be explained by confounding effects, since Northern Sweden has been sparsely populated and the population to a large extent inbred, leading to skewed allelic distribution.

The distribution of genotypes and allele frequencies of IL-1 β TaqI were similar to those found in other Caucasian populations^{10,44}. As in the study of Cantagrel, *et al*¹², we could not demonstrate any association with RA. However, patients with genotype A2A2 have the highest accumulated disease activity score compared with those with A1A2 or A1A1 genotypes. Several studies had proven that IL-1 β does play an important role in pathogenesis of RA⁵⁴ and the high-secretory allele A2 might thus be important for the severity of the disease.

In concordance with previous results^{12,13}, we confirmed that there is no association of allele A2 of IL-1Ra VNTR polymorphism with RA. In fact, there are indications that this allele is associated with less risk for cardiovascular complications. Recently, Dewberry, *et al*⁵⁵ showed that expression of IL-1Ra in endothelial cells is intracellular and that allele A2 of IL-1Ra VNTR is associated with significantly reduced levels of IL-1Ra in human umbilical vein endothelial cells. It was suggested that in contrast to monocytes, intra-cellular IL-1Ra splice variant is the exclusive isoform present in endothelial cells. Thus, the same polymorphism influences secretion of IL-1Ra differently in various tissues. In our study, genotype A1A2 was more prevalent in controls than in patients and although the p value lost its significance when corrected, this result is nonetheless notable. Allele A3 of IL-1Ra was associated with RA, although these data are based on a small number of patients. The functional implications of this allele are not known and remain to be investigated.

There was no association between alleles A1 and A2 of IL-1 β and alleles A1 and A2 of IL-1Ra with RA. However, our data showed allele A2 of IL-1Ra together with allele A1 of IL-1 β to be associated with protection against cardiovascular complications (OR = 0.20).

The controversial reports about TNF- α loci, together with our present results, lead us to the following speculative conclusion: certain combinations of alleles, from several polymorphic sites, build up a final form of the TNF- α molecule, which in some cases increases susceptibility to RA, while in others it could be associated with more severe forms of dis-

ease. It is probable that not only the combination of alleles of the TNF- α gene, but also the combination of a certain haplotype from other neighboring genes is of importance.

Anti-cytokine therapy offers a new era for treatment of RA. The anti-TNF- α therapies have already shown promising results⁵⁶⁻⁵⁸, although not all patients respond to this treatment. Based on our collected data, we speculate that certain combinations of alleles of pro-inflammatory cytokines could have a major effect on the success of anti-TNF- α therapy. From a clinical point of view it would be of great interest to analyze the severity of RA in relation to polymorphisms of cytokine genes, as well as the effect of anti-TNF- α treatment in patients with different genetic background. It is probable that future treatment of RA patients will include combinations with cytokine therapy. Treatment with IL-1Ra has already been tested⁵⁹, and the combination of IL-1Ra treatment with anti-TNF- α therapy is currently a subject of investigation in animal models⁶⁰.

In summary, we found a positive association of allele A1 of TNF- α with RA. The genotypes A1A2 of TNF- α and A2A2 of IL-1 β are associated with more severe disease. From the clinical point of view, identification of phenotypic/genotypic variants of RA might be of importance for the evaluation of new therapeutic approaches.

REFERENCES

1. Weyand CM, Klimiuk PA, Goronzy JJ. Heterogeneity of rheumatoid arthritis: from phenotypes to genotypes. *Springer Semin Immunopathol* 1998;20:5-22.
2. Gomolka M, Menninger H, Saal JE, et al. Immunoprinting: various genes are associated with increased risk to develop rheumatoid arthritis in different groups of adult patients. *J Mol Med* 1995;73:19-29.
3. McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. *Arthritis Rheum* 1995;38:221-8.
4. Hajeer AH, Worthington J, Silman AJ, Ollier WE. Association of tumor necrosis factor microsatellite polymorphisms with HLA-DRB1*04-bearing haplotypes in rheumatoid arthritis patients. *Arthritis Rheum* 1996;39:1109-14.
5. Brinkman BM, Huizinga TW, Kurban SS, et al. Tumor necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? *Br J Rheumatol* 1997;36:516-21.
6. Mathey DL, Hassell AB, Dawes PT, Ollier WE, Hajeer A. Interaction between tumor necrosis factor microsatellite polymorphisms and the HLA-DRB1 shared epitope in rheumatoid arthritis: influence on disease outcome. *Arthritis Rheum* 1999;42:2698-704.
7. Fugger L, Morling N, Bendtzen K, et al. IL-6 gene polymorphism in rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, and in healthy Danes. *J Immunogenet* 1989;16:461-5.
8. Fugger L, Morling N, Bendtzen K, et al. MspI polymorphism in the human interleukin 6 (IL 6) gene. *Nucleic Acids Res* 1989;17:4419.
9. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992;22:396-402.
10. Bioque G, Crusius JB, Koutroubakis I, et al. Allelic polymorphism

- in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin Exp Immunol* 1995;102:379-83.
11. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998;102:1369-76.
 12. Cantagrel A, Navaux F, Loubet-Lescoulier P, et al. Interleukin-1beta, 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.
 13. Perrier S, Coussediere C, Dubost JJ, Albuissou E, Sauvezie B. IL-1 receptor antagonist (IL-1Ra) gene polymorphism in Sjogren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopathol* 1998;87:309-13.
 14. Blakemore AI, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* 1994;37:1380-5.
 15. Blakemore AI, Watson PF, Weetman AP, Duff GW. Association of Graves' disease with an allele of the interleukin-1 receptor antagonist gene. *J Clin Endocrinol Metab* 1995;80:111-5.
 16. Crusius JB, Pena AS, Van Oosten BW, et al. Interleukin-1 receptor antagonist gene polymorphism and multiple sclerosis [letter]. *Lancet* 1995;346:979.
 17. Lentsch AB, Ward PA. Regulation of inflammatory vascular damage. *J Pathol* 2000;190:343-8.
 18. Sfikakis PP, Tsokos GC. Lymphocyte adhesion molecules in autoimmune rheumatic diseases: basic issues and clinical expectations. *Clin Exp Rheumatol* 1995;13:763-77.
 19. Badolato R, Oppenheim JJ. Role of cytokines, acute-phase proteins, and chemokines in the progression of rheumatoid arthritis. *Semin Arthritis Rheum* 1996;26:526-38.
 20. Loetscher P, Dewald B, Baggiolini M, Seitz M. Monocyte chemoattractant protein 1 and interleukin 8 production by rheumatoid synoviocytes. Effects of anti-rheumatic drugs. *Cytokine* 1994;6:162-70.
 21. Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996;14:397-440.
 22. Migita K, Eguchi K, Kawabe Y, et al. TNF-alpha-mediated expression of membrane-type matrix metalloproteinase in rheumatoid synovial fibroblasts. *Immunology* 1996;89:553-7.
 23. Kontinen YT, Salo T, Hanemaaijer R, et al. Collagenase-3 (MMP-13) and its activators in rheumatoid arthritis: localization in the pannus-hard tissue junction and inhibition by alendronate. *Matrix Biol* 1999;18:401-12.
 24. Allebeck P. Increased mortality in rheumatoid arthritis. *J Rheumatol* 1982;11:81-6.
 25. Jacobsson LT, Knowler WC, Pillemer S, Hanson RL, et al. Rheumatoid arthritis and mortality. A longitudinal study in Pima Indians. *Arthritis Rheum* 1993;36:1045-53.
 26. Myllykangas-Luosujärvi R, Aho K, Kautiainen H, Isomäki H. Cardiovascular mortality in women with rheumatoid arthritis. *J Rheumatol* 1995;22:1065-7.
 27. Wällberg-Jonsson S, Ohman ML, Dahlqvist SR. Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *J Rheumatol* 1997;24:445-51.
 28. Frostegård J, Ulfgrén AK, Nyberg P, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis* 1999;145:33-43.
 29. Fiotti N, Giansante C, Ponte E, et al. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. *Atherosclerosis* 1999;145:51-60.
 30. Rantapää-Dahlqvist S, Wallberg-Jonsson S, Dahlen G. Lipoprotein (a), lipids, and lipoproteins in patients with rheumatoid arthritis. *Ann Rheum Dis* 1991;50:366-8.
 31. Wällberg-Jonsson S, Dahlén GH, Nilsson TK, Ranby M, Rantapää-Dahlqvist S. Tissue plasminogen activator, plasminogen activator inhibitor-1 and von Willebrand factor in rheumatoid arthritis. *Clin Rheumatol* 1993;12:318-24.
 32. Wällberg-Jonsson S, Dahlén G, Johnson O, Olivecrona G, Rantapää-Dahlqvist S. Lipoprotein lipase in relation to inflammatory activity in rheumatoid arthritis. *J Intern Med* 1996;240:373-80.
 33. Bacons PA, Kitas GD. The significance of vascular inflammation in rheumatoid arthritis. *Ann Rheum Dis* 1994;53:621-3.
 34. Wällberg-Jonsson S, Johansson H, Ohman ML, Rantapää-Dahlqvist S. Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. *J Rheumatol* 1999;26:2562-71.
 35. Wällberg-Jonsson S, Cederfelt M, Rantapää-Dahlqvist S. Hemostatic factors and cardiovascular disease in active rheumatoid arthritis: an 8 year followup study. *J Rheumatol* 2000;27:71-5.
 36. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
 37. Baecklund E, Ekblom A, Sparen P, Feltelius N, Klareskog L. Disease activity and risk of lymphoma in patients with rheumatoid arthritis: nested case-control study. *BMJ* 1998;317:180-1.
 38. Ahmed E, Trifunovic J, Stegmayr B, Hallmans G, Lefvert AK. Autoantibodies against oxidatively modified LDL do not constitute a risk factor for stroke: a nested case-control study. *Stroke* 1999;30:2541-6.
 39. Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994;106:637-42.
 40. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1992;1:353.
 41. Campbell RD, Trowsdale J. Map of the human MHC. *Immunol Today* 1993;14:349-52.
 42. Cornelis F, Faure S, Martinez M, et al. New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. *Proc Natl Acad Sci USA* 1998;95:10746-50.
 43. Cox A, Camp NJ, Cannings C, 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.
 44. Huang D, Pirskanen R, Hjelmstrom P, Lefvert AK. Polymorphisms in IL-1beta and IL-1 receptor antagonist genes are associated with myasthenia gravis. *J Neuroimmunol* 1998;81:76-81.
 45. Casini-Raggi V, Kam L, Chong YJ, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. *J Immunol* 1995;154:2434-40.
 46. Skoog T, van't Hooft FM, Kallin B, et al. A common functional polymorphism (C->A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Hum Mol Genet* 1999;8:1443-9.
 47. Martinez A, Fernandez-Arquero M, Pascual-Salcedo D, et al. Primary association of tumor necrosis factor-region genetic markers with susceptibility to rheumatoid arthritis. *Arthritis Rheum* 2000;43:1366-70.
 48. Verweij CL. Tumour necrosis factor gene polymorphisms as severity markers in rheumatoid arthritis. *Ann Rheum Dis* 1999;58 Suppl 1:120-6.
 49. Lacki JK, Moser R, Korcowska 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.
50. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. *Arthritis Rheum* 1995;38:151-60.
 51. Wilson AG, de Vries N, van de Putte LB, Duff GW. A tumour necrosis factor alpha polymorphism is not associated with rheumatoid arthritis. *Ann Rheum Dis* 1995;54:601-3.
 52. Vinasco J, Beraun Y, Nieto A, et al. Polymorphism at the TNF loci in rheumatoid arthritis. *Tissue Antigens* 1997;49:74-8.
 53. Seki N, Kamizono S, Yamada A, et al. Polymorphisms in the 5'-flanking region of tumor necrosis factor-alpha gene in patients with rheumatoid arthritis. *Tissue Antigens* 1999;54:194-7.
 54. Poutsika DD, Clark BD, Vannier E, Dinarello CA. Production of interleukin-1 receptor antagonist and interleukin-1 beta by peripheral blood mononuclear cells is differentially regulated. *Blood* 1991;78:1275-81.
 55. Dewberry R, Holden H, Crossman D, Francis S. Interleukin-1 receptor antagonist expression in human endothelial cells and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:2394-400.
 56. Kavanaugh A, St. Clair EW, McCune WJ, Braakman T, Lipsky P. Chimeric anti-tumor necrosis factor-alpha monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. *J Rheumatol* 2000;27:841-50.
 57. Maini R, St Clair EW, Breedveld F, et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999;354:1932-9.
 58. Moreland LW, Baumgartner SW, Schiff MH, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* 1997;337:141-7.
 59. Bresnihan B. Treatment of rheumatoid arthritis with interleukin 1 receptor antagonist. *Ann Rheum Dis* 1999;58 Suppl 1:196-8.
 60. Joosten LA, Helsen MM, Saxne T, van De Loo FA, Heinegard D, van Den Berg WB. IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. *J Immunol* 1999;163:5049-55.