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Association of polymorphisms of the tumour necrosis factor receptors I and II and rheumatoid arthritis

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Objective. **To assess the role of polymorphisms of the tumour necrosis factor (TNF) receptors, TNF-RI (p55) and TNF-RII (p75) in the susceptibility to and severity of rheumatoid arthritis (RA) in Dutch patients.**
Methods. **A total of 319 consecutive RA patients, and a cohort of 90 female RA patients with detailed 12-yr follow-up were genotyped for the TNF-RI exon 1 (+36 A to G) and TNF-RII 3′ UTR (+1690 T to C) polymorphisms.**
Results. **The frequencies of the TNF-RI and TNF-RII polymorphisms were determined in both patient groups and healthy controls, but no significant differences were found. To determine the relationship of these polymorphisms to disease severity, the extent of joint damage in the cohort of 90 female RA patients was analysed. No differences in severity were observed.**
Conclusion. **These TNF-RI and TNF-RII polymorphisms were not found to be associated with susceptibility to or severity of RA in the Dutch population.**

KEY WORDS: TNF receptor, Rheumatoid arthritis, Polymorphism, Association study.

Rheumatoid arthritis (RA) is characterized by chronic inflammation that results in joint swelling, pain and progressive joint destruction. Both environmental and genetic components influence pathogenesis and genetic studies have noted both a familial clustering and high concordance of RA in monozygotic twins, with an estimated genetic component of around 50–60% [1]. Tumour necrosis factor-α (TNFα) plays a central role in inflammation, and has been directly implicated in the pathogenesis of RA [2]. The efficacy of reducing TNF levels in RA has been clearly demonstrated by the use of neutralizing antibodies (infliximab) [3], leading to significant clinical benefit in patients refractory to standard treatments. TNF interacts with the TNF receptors TNF-RI (p55) and TNF-RII (p75). TNF-Rs are active both in membrane-bound and soluble forms, and the soluble receptors act as physiological attenuators of TNF activity [4]. The TNF receptors play an important role in resistance to infection [5] and TNF-mediated pathologies [6]. The chromosomal location of the TNF-RI gene is 12p13 and the TNF-RII gene is located at 1p36.2. Genome-wide linkage studies in the mouse collagen-induced arthritis model [7] and the rat adjuvant-induced arthritis model [8] have revealed linkage to regions syntenic with 12p13. Linkage to this region was also found in an RA-affected sibpair study [9]. The 1p36 region has been identified as a further susceptibility locus for RA in two genome-wide linkage studies [9, 10]. The TNF-R genes are therefore very attractive candidates to explain the association of these chromosome regions with RA and associated animal models. Here we focus on two single nucleotide polymorphisms (SNPs) in the TNF receptor genes, at position +36 in exon 1 of the TNF-RI gene and at +1690 in the 3′ UTR region of TNF-RII.

A recent UK study of the +36 variant in RA patients found no significant association with RA [11], but the evidence from the genome-wide studies discussed above is sufficiently compelling to examine both of these polymorphisms in another population. In addition we assessed the possible role of these polymorphisms in

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disease severity, by examining a cohort of RA patients for whom extensive and long-term follow-up data were available.

Patients and methods

Patients and healthy control subjects

The frequency of TNF-R polymorphisms was determined in two groups of RA patients. RA group 1 consisted of 319 consecutive RA patients from the rheumatology out-patient clinic of the Leiden University Medical Center as described previously [12]. All patients fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA. RA group 2 was a cohort of 90 incident female RA patients who were enrolled between 1982 and 1986 and fulfilled the 1958 ACR criteria for RA [13]. Radiographs of the hands and feet were made at enrolment and subsequently at 3, 6 and 12 yr, and the progressive radiological damage described.

To determine the frequency of gene variants in a healthy population, a control group of 179 healthy Dutch Caucasian individuals was analysed. These studies were carried out with the approval of the Medisch-Ethische Commissie, LUMC.

Each of the association studies described here included sufficient participants to ensure a power of 90% to detect a gene conferring a relative risk of 2.0 at the 5% significance level.

DNA isolation and PCR analysis

DNA was isolated as described previously [12]. Polymerase chain reaction (PCR) amplification of 75 ng DNA was performed on a Perkin Elmer thermal cycler (PE 9700, Perkin-Elmer-Cetus, Norwalk, CT, USA) with 10× AmpliTaq Buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 20 pmol of each primer and 1 unit of *Taq* polymerase (Perkin-Elmer Cetus). The following cycling conditions were used: 95°C for 5 min; 35 cycles of 95°C, 1 min, 60°C, 1 min, 72°C, 1 min; followed by 72°C for 7 min. Primers for the TNF-RI gene were as follows; sense primer TNF-RI-1, 5′ GAGCCCAAATGGGGGAGTG AGAGG 3′, and antisense primer TNF-RI, 5′ ACCAGGCC CGGGCAGGAGAG 3′. Primers for the TNF-RII gene were as follows; sense primer TNF-RII-3, 5′ CGTCTGGGGAGC ACCGAAGA 3′, and antisense primer TNF-RII-4, 5′ CAG CCTTCCGAGAGGGAgAC 3′. (Lower case g indicates nucleotide change in relation to the cDNA sequence.)

Genotyping of the TNF-R gene polymorphisms

TNF-RI. Genotyping of the exon 1 A/G SNP at position +36 of TNF-RI gene, (rs767455) was carried out as described previously [14]. The restriction enzyme *Msp*A1I was obtained from New England Biolabs (Beverly, MA, USA).

TNF-RII. Genotyping of the +1690 T/C SNP (rs3397) in the 3′ UTR of the TNF-RII gene was carried out with an induced restriction digest, by changing a G to C at the third position of the 3′ end of the primer, which together with the T to C transition in the minor allele creates an *Esp*3I restriction site. *Esp*3I (Fermentas AB, St Leon-Rot Germany) (3 units) was used to digest 10 μl of a 40 μl PCR. PCR products were separated on agarose gels of the appropriate concentration or 5% polyacrylamide gels, and the polymorphism of individuals was noted.

Statistical analysis

SNP frequencies were analysed in 2×2 contingency tables using the Pearson χ^2 method, and the odds ratio (OR) and 95% confidence intervals (CI) quoted refer to RA group 1 *vs* the healthy controls. All groups were in Hardy–Weinberg equilibrium except for a minor deviation in the group RA1 for the +36 polymorphism. As there was no indication of technical problems, we assume that this is due to a chance sampling error. This deviation does not affect the conclusions of this study.

TABLE 1. Frequencies of the TNF-RI +36 polymorphism in RA patients and healthy controls

	TNF-RI +36			OR, genotype[a]	
	RA1	RA2	HC	(95% CI)	*P*
Genotype					
AA	91 (31.1%)	31 (35.2%)	50 (30.3%)	1.04 (0.69–1.57)	0.87
AG	163 (55.6%)	45 (51.1%)	83 (50.3%)	1.24 (0.85–1.82)	0.27
GG	39 (13.3%)	12 (13.6%)	32 (19.4%)	0.64 (0.38–1.07)	0.08
Total	293	88	165		

[a]OR and *P* refer to the RA1 *vs* HC groups.

Results

PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the frequency of the TNF-RI +36 polymorphism in the RA patient groups and healthy controls (HC) showed that the TNF-RI +36 GG genotype was decreased in RA patients compared with the healthy controls (13.3 and 13.6% *vs* 19.4%, $P=0.08$) (Table 1), though this difference was not significant. The heterogeneous nature of RA indicates that certain genetic factors may be playing a role in specific aspects of the disease. Therefore we analysed the association of the TNF-RI +36 polymorphism in subgroups stratified for the presence of erosions, the duration of disease, the number of erosions in the hands, rheumatoid factor positivity, duration of disease before the onset of erosions, Health Assessment Questionnaire score and gender. None of these criteria showed significant variation dependent on polymorphism (data not shown).

For the analysis of the TNF-RII +1690 polymorphism we developed a novel PCR-RFLP method. Genotyping of RA patient group 1 and healthy controls revealed no significant differences in the frequency of the TNF-RII +1690 polymorphism (Table 2). We also analysed the association of the +1690 polymorphism in subgroups stratified for the disease characteristics

TABLE 2. Frequencies of the TNF RII +1690 polymorphism in RA patients and healthy controls

	TNF-RII +1690			OR, genotype[a]	
	RA1	RA2	HC	(95% CI)	*P*
Genotype					
TT	132 (41.4%)	29 (32.2%)	71 (39.7%)	1.07 (0.74–1.56)	0.71
TC	146 (45.8%)	49 (54.4%)	82 (45.8%)	1.00 (0.69–1.44)	0.99
CC	41 (12.8%)	12 (13.3%)	26 (14.5%)	0.87 (0.51–1.47)	0.60
Total	319	90	179		

[a]OR and *P* refer to the RA1 *vs* HC groups.

described above. None of these criteria showed any significant variation in distribution dependent on polymorphism (data not shown).

To determine the influence of the TNF-RI and TNF-RII polymorphisms on disease severity, the extent of joint damage in a cohort of 90 RA patients followed for 12 yr was analysed. Joint damage was defined as cumulative erosive disease as seen on sequential radiographs of both hands and feet, taken at enrolment and after 3, 6 and 12 yr. No significant association of either TNF-R polymorphism with disease progression was found (data not shown).

Discussion

In this study we examined the frequency of two TNF-R polymorphisms in two groups of RA patients and in a healthy control group. The TNF-RI +36 polymorphism is a silent mutation in codon 12, so can have no functional significance for TNF-RI protein structure, but may act as a disease-associated marker. The TNF-RII +1690 polymorphism occurs in the 3′ UTR region of the transcript and may affect transcript stability or processing, though this is not known at present. These polymorphisms are promising disease markers in RA, being located in regions that have been associated with RA-like diseases in syntenic regions in several animal models, and having been identified in genome-wide scans in RA patients. However, we found no convincing evidence in this study that these polymorphisms are associated with either the occurrence of RA or specific disease manifestations.

A study of the frequency of the +36 variant in a UK Caucasian population of RA patients also showed no significant association of this polymorphism and RA [11]. However, this same UK study did find a significant association between a TNF-RII polymorphism and RA. These authors examined the codon 196 (+676) exon 6 polymorphism (rs1061622) in contrast to the +1690 polymorphism examined here. In addition these authors only found a significant association when they stratified patients according to a family history of RA. The patients examined in our study are assumed to be sporadic cases of RA. These contrasting findings may indicate that our study group was too heterogeneous to detect a relatively weak association of this polymorphism in the TNF-RII gene and RA. Alternatively, these polymorphisms may not be in linkage disequilibrium. The TNF-RII gene spans 43 kb and these two polymorphisms are separated by 14.3 kb. Another possibility is that the exon 6 polymorphism has a direct functional effect on the TNF-RII protein, explaining its increased prevalence in some RA patients, and is on a haplotype that does not include the +1690 polymorphism. Further studies are needed to clarify these issues.

In conclusion, the polymorphisms of the TNF-RI and TNF-RII genes studied here were not found to be associated with susceptibility to or severity of RA in the Dutch population.

References

1. MacGregor AJ, Snieder H, Rigby AS *et al.* Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43:30–7.
2. Feldmann M, Brennan FM, Chantry D *et al.* Cytokine production in the rheumatoid joint: implications for treatment. Ann Rheum Dis 1990;49:480–6.
3. Elliott MJ, Maini RN, Feldmann M *et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. Arthritis Rheum 1993; 36:1681–90.
4. Aderka D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. Cytokine Growth Factor Rev 1996;7:231–40.
5. Kollias G, Douni E, Kassiotis G, Kontoyiannis D. The function of tumour necrosis factor and receptors in models of multi-organ inflammation, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Ann Rheum Dis 1999;58(Suppl. 1):I32–9.
6. Rothe J, Lesslauer W, Lotscher H *et al.* Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. Nature 1993;364:798–802.
7. McIndoe RA, Bohlman B, Chi E *et al.* Localization of non-MHC collagen-induced arthritis susceptibility loci in DBA/1j mice. Proc Natl Acad Sci USA 1999;96:2210–4.
8. Lorentzen JC, Glaser A, Jacobsson L *et al.* Identification of rat susceptibility loci for adjuvant-oil-induced arthritis. Proc Natl Acad Sci USA 1999;95:6383–7.
9. 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.
10. Shiozawa S, Hayashi S, Tsukamoto Y *et al.* Identification of the gene loci that predispose to rheumatoid arthritis. Int Immunol 1998;10:1891–5.
11. Barton A, John S, Ollier WE, Silman A, Worthington J. Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians. Arthritis Rheum 2001;44:61–5.
12. Brinkman BM, Huizinga TW, Kurban SS *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.
13. Drossaers-Bakker KW, de Buck M, van Zeben D *et al.* Long-term course and outcome of functional capacity in rheumatoid arthritis: the effect of disease activity and radiologic damage over time. Arthritis Rheum 1999; 42:1854–60.
14. Pitts SA, Olomolaiye OO, Elson CJ, Westacott CI, Bidwell JL. An MspA1 I polymorphism in exon 1 of the human TNF receptor type I (p55) gene. Eur J Immunogenet 1998;25:269–70.