Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis

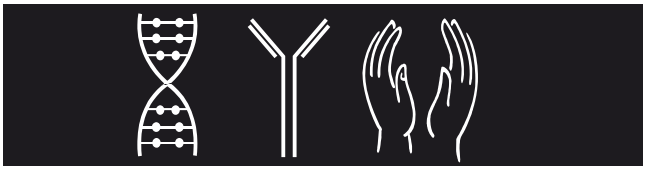
Helm-van Mil, A.H.M. van der

Chapter 3

No association between Tumor Necrosis Factor Receptor Type 2 gene polymorphism and rheumatoid arthritis severity: a comparison of the extremes of phenotypes

A.H.M. van der Helm - van Mil
P. Dieudé
J.J.M. Schonkeren
F. Cornélis
T.W.J. Huizinga

Rheumatology (Oxford) 2004;43(10):1232-4.



ABSTRACT

Objectives. To assess the association between the tumor necrosis factor receptor 2 (*TNFR2)* 196 M/R single nucleotide polymorphism and rheumatoid arthritis (RA) severity by taking advantage of the extremes of phenotypes that exist in arthritis.

Methods. Out of the Leiden Early Arthritis Cohort (1700 patients), we selected patients that initially had the diagnosis of definite or probable RA according to ACR criteria and developed complete remission (71 patients) or had the worst progression to destructive disease (72 patients). A group of 135 healthy controls was included. In these groups the *TNFR2* genotype was determined.

Results. The extremes of phenotypes did not differ significantly in genotype distribution. No difference in genotype distribution between rheumatoid arthritis patients and healthy controls was observed.

Conclusions. Our study demonstrates that even by comparing the extremes of phenotypes no association between the *TNFR2* genotype and disease severity can be detected in Caucasian patients with sporadic RA.

INTRODUCTION

Recent reports have suggested an association between the tumor necrosis factor receptor 2 (*TNFR2)* 196 M/R single nucleotide polymorphism (SNP) and susceptibility to rheumatoid arthritis (RA), restricted to familial RA (1,2). Two recent studies have reported conflicting results concerning the involvement of *TNFR2* 196 M/R SNP as a genetic factor of RA severity (3,4). Whereas Glossop et al. reports no association with this SNP and radiological and functional RA severity (3), Constantin et al. recently found a worse Health Assessment Questionnaire score in patients carrying the *TNFR2* 196R allele (4). However, in both studies the distribution of severity of RA in the relatively small number of patients is limited. An association between *TNFR2* 196M/R and severity would emerge best comparing patients with the best and the worst course, e.g. comparing the extreme phenotypes. Therefore, out of a cohort of 1700 patients we selected RA patients who developed complete remission and those patients of this cohort that had the worst progression to destructive disease. In these two unique groups of patients the *TNFR2* genotype was determined.

PATIENTS AND METHODS

The Leiden Early Arthritis Cohort consists of all patients that were referred to the Department of Rheumatology of the Leiden University Medical Center with recent onset arthritis. From 1993 till 2003 over 1700 patients have been included and followed. Follow-up included Health Assessment Questionnaire, Disease Activity Score, and radiographs of hands and feet at 0, 6 and 12 months and yearly thereafter (5). To detect the patients with the best course we selected those patients that at the enter of the cohort had 1) a symmetric arthritis of the small joints, 2) morning stiffness of at least one hour and 3) the diagnosis of definite or probable RA according to ACR criteria who 4) achieved long-term and complete remission (no signs of arthritis in the absence of disease modifying drugs) and were therefore discharged. Patients were not discharged when less than one year in remission without the use of disease modifying drugs. In total 78 patients fulfilled the criteria; of 71 patients DNA was available for *TNFR2* genotyping. To select the patients with the most progressive disease, the RA patients with the highest rate of joint destruction as measured by the Sharp/van der Heijde method at radiographs of hands and feet after one year of follow-up were selected. 79 Patients had a total score > 15; of 72 patients was DNA available. All patients included reported to have Caucasian grandparents. A group of 135 healthy controls was included; all these persons were Caucasian and had no family history of RA. Informed patient consent was obtained and the study was approved by the local Medical Ethical Committee under code P237-94.

Genotyping of *TNFR2* 196 M/R polymorphism was performed blinded, by PCR-restriction fragment polymorphism (N1a III) as previously described (6). Two samples of 71 and 72 patients provides a power of 82% to detect differences with 95% confidence and an odds ratio of 3 based on a 196R allele frequency of 22%. The X^2-test was used to compare the genotype distribution between the two groups of RA patients. All allele and genotype frequencies were in Hardy Weinberg equilibrium.

RESULTS

The frequencies of the 196M and 196R alleles among the 143 RA patients were 78% and 22% respectively. Table 1 depicts patients' characteristics and the genotype distributions of both groups of RA patients and of the healthy controls. In the remission group, 49 patients (69%) had fulfilled the ACR-criteria for definite RA, and 22 patients (31%) the ACR criteria for probable RA. The extremes of phenotypes did not differ significantly in genotype distribution, as well as determined in the total group of patients as when assessed in the first half of patients and replicated in the second half. In addition, there was no significant difference in Health Assessment Questionnaire and Disease Activity Score when the 196R and 196M alleles were compared (data not shown). No difference in genotype distribution between rheumatoid arthritis patients and healthy controls was

Table 1. Characteristics of and *TNFR2* 196 M/R gene polymorphism in rheumatoid arthritis patients with complete remission and severe progression.

	Complete Remission (No 71)	Severe Progression (No 72)
Age (mean SD)	57.6 ± 16.9	55.6 ± 16.8
Male/Female, No (%)	30 (42.3) / 41 (57.7)	25 (34.7) / 47 (65.3)
RF positive, No (%)	7 (9.9)	30 (41.7)
Total Sharp/van der Heijde score at inclusion	3.1 ± 7.2	11.0 ± 15.9
Total erosion score	0.8 ± 2.7	4.7 ± 9.2
Total joint space narrowing score	2.3 ± 5.4	6.1 ± 8.2
Total Sharp/van der Heijde score after 1 year follow-up	5.3 ± 8.3	36.1 ± 24.5
Total erosion score	1.9 ± 2.7	21.3 ± 17.0
Total joint space narrowing score	3.4 ± 6.5	14.7 ± 11.0
TNFR2 196 M/R genotype		
MM No (%)	43 (60.5)	50 (69.4)
MR No (%)	23 (32.4)	19 (26.4)
RR No (%)	5 (7.0)	3 (4.2)
Shared Epitope positive No, (%)	30 (42)	57 (79)

The *TNFR2* 196 M/R genotype distributions in the 135 healthy controls was: MM 81 (60.0%), MR 44 (32.6%), RR 10 (7.4%).

observed as well (Table 1). Patients in the severe progression group were more frequent shared epitope positive than patients in the remission group (odds ratio 5.2, 95% confidence interval 2.3-11.7) (Table 1).

DISCUSSION

In this study no association between the *TNFR2* 196 M/R gene polymorphism and severity in Caucasian patients with sporadic RA was observed. By comparing the genotypes of the patients with the worst and the best course out of a cohort of more than 1700 patients an association between *TNFR2* and severity, if present, very likely would have been detected. With the sample sizes used we have a power of 82% to detect differences with an odds ratio of 3. In general the numbers used in a power calculation are based on the difference between a 'normal' population of rheumatoid arthritis patient that contains many phenotypes and healthy controls that contain some phenocopies. In this circumstance a power of 80% to detect differences with an odds ratio of 2 is usually accepted. In the present study the power was enhanced by selection of extremes of the phenotypes. This method is supposed to enlarge the difference and can make a study more powerful. Therefore in this study we accept the magnitude of the odds ratio to be at least 3. The odds ratio for the association between HLA- Class II alleles and RA severity is about 3 in inception cohorts (7). In our design we observed an odds ratio for shared epitope positivity and RA severity of 5, indicating the power of the present approach.

The allele and genotype distributions in this Dutch study are similar to those in French (2,4), British (3), Italian (8) and Swedish (9) studies, in which allele frequencies of 75-79% are reported for the 196M allele and allele frequencies of 20-25% for the 196R allele. The fact that in the present study the gene distribution of patients and healthy controls were comparable confirms previous findings of an absent association between *TNFR2* and susceptibility to sporadic RA (1,2).

The *TNFR2* gene is located on chromosome 1p36 and consists of 10 exons and 9 introns. A SNP at codon 196 in exon 6 (ATG → AGG) results in a nonconservative amino acid substitution: methionine (M) → arginine (R). Little is known on the functionality of this amino acid substitution. In Japanese patients with systemic lupus erythematosus (SLE) it is demonstrated that the 196 *TNFR2* SNP has no influence on receptor binding of TNF-α or on receptor shedding, but that the 196R allele more effectively transduces signals for IL-6 production than does 196 M allele (10). However also in these Japanese SLE patients the 196R allele was not associated with disease severity (10).

In summary, our study demonstrates that even by comparing the extremes of phenotypes no association between the *TNFR2* genotype and disease severity can be detected in Caucasian patients with sporadic RA.

REFERENCES

1. Barton A, John S, Ollier WER, 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;44:61-65.
2. Dieudé P, Petit E, Cailleau-Moindrault S, Osorio J, Pierlot C, Martinez M, et al. Association between tumor necrosis factor receptor II and familial but not sporadic rheumatoid arthritis, Evidence for Genetic Heterogenity. Arthritis Rheum 2002;46:2039-2044.
3. Glossop JR, Nixon NB, Dawes PT, Hassell AB, Mattey DL. No association of polymorphisms in the tumor necrosis factor receptor I and receptor II genes with disease severity in rheumatoid arthritis. J Rheumatol 2003;30:1406-1409.
4. Constantin A, Dieude P, Lauwers-Cances V, Jamard B, Mazieres B, Cambon-Thomson A, et al. Tumor necrosis factor receptor 2 gene polymorphism and severity of rheumatoid arthritis. Arthritis Rheum 2004: 50:742-747.
5. Aken J, Bilsen JAM, Allaart CF, Huizinga TWJ, Breedveld FC. The Leiden Early Arthritis Clinic, Clin Exp Rheum 2003;21(Suppl 31):S100-S105.
6. Al-Ansari AS, Ollier WE, Villarreal J, Ordi J, Teh LS, Hajeer AH. Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus. Tissue Antigens 2000;55:97-99.
7. Kaltenhäuser S, Wagner U, Schuster E, Wassmuth R, Seidel W, Trözlsch M, et al. Immunogenetic markers and seropositivity predict radiological progression in early rheumatoid arthritis independent of disease activity. J Rheumatol 2001;28:735-744.
8. Fabris M, Tolusso B, Di Pol E, Assaloni R, Sinigaglia L, Ferraccioli G. Tumor necrosis factor-alpha receptor two polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. J Rheumatol 2002;29:1847-1850.
9. Dahlqvist SR, rlestig L, Sikström C, Linghult S. Tumor necrosis factor receptor type II (exon 6) and interleukin-6 (-174) gene polymorphisms are not associated with family history but tumor necrosis factor receptor type II is associated with hypertension in patients with rheumatoid arthritis in patients from northern Sweden. Arthritis Rheum 2002;46:3096-3098.
10. Morita C, Horiuchi T, Hatta N, Kikuchi Y, Arinobu Y, Otsuka T, et al. Association of tumor necrosis factor receptor type II polymorphism 196R with systemic lupus erythematosus in the Japanese. Arthritis Rheum 2001;44:2819-2827.