Association of anti-CCP positivity and carriage of TNFRII susceptibility variant with anti-TNF-α response in rheumatoid arthritis

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ABSTRACT

Objective. *To investigate the possible influence of tumour necrosis factor-alpha (TNF), TNF receptor I (TNFRI) and TNF receptor II (TNFRII) gene polymorphisms on anti-TNF treatment responsiveness, stratified by autoantibody status.*

Methods. *A Greek multi-centre collaboration was established to recruit a cohort of patients (n=100) with active RA treated with anti-TNF drugs. TNF g.-238G>A (rs361525), g.-308G>A (rs1800629), g.-857C>T (rs1799724), TNFRI c.36A>G (rs4149584) and TNFRII c.676T>G (rs1061622) polymorphisms were genotyped by PCR-RFLP assays. Serum RF and anti-CCP antibody status were determined using commercially available kits. Single-SNP, haplotype and stratification by autoantibody status analyses were performed in predicting response to treatment by 6 months, defined as the absolute change in DAS28.*

Results. *31 patients (31%) were defined as non-responders due to failure to fulfill the DAS28 criteria. 79% and 66% were RF and anti-CCP positive, respectively. None of the genotyped SNPs was alone associated with responsiveness to drug treatment. However, after stratification by autoantibody status, carriage of TNFRII c.676G allele was associated with poorer response to drug treatment in anti-CCP positive patients (p=0.03), after 6 months of anti-TNF therapy.*

Conclusion. *In concordance with previous studies, genetic polymorphisms alone cannot be used to safely predict clinical response to anti-TNF therapy however the combination of genetic factors and autoantibody status warrants further investigation in larger independent cohorts.*

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease affecting around 1% of the western world and characterised by progressive joint damage and significant disability that compromises quality of life. While first-line treatment for RA patients involves the use of non-steroidal anti-inflammatories to reduce pain and stiffness and disease modify agents such as methotrexate that modulate inflammation and tissue damage, during the last decade the introduction of the anti-TNF-α biological therapies (infliximab, etanercept, adalimumab) have revolutionised the treatment of RA with remarkable contribution to the alleviation of symptoms and improvement of the quality of life of patients (1). However, 40–60% of patients do not respond to therapy, others do not sustain a positive response, while several serious adverse effects have been reported including heart defects, increased autoimmunity and even oncogenesis (2).

A substantial number of studies have suggested that this heterogeneous response to treatment may reflect complex genetic heterogeneity (3) as well as heterogeneity in autoantibody production (4). Indeed several studies have been undertaken to associate genetic polymorphisms, mainly in the genes encoding TNF-α and their receptors as well as other cytokines (5-9), and/or autoantibody status, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) (10-13) with prediction to response to treatment but results were contradictory. Since a combination of serological and genetic factors may prove to be more useful in predicting drug response we sought to identify the possible influence of tumour necrosis factor-alpha (TNF-α), TNF receptor I (TNFRI) and TNF receptor II (TNFRII) gene polymorphisms, shown to be associated with increased TNF-α production (14), on anti-TNF treatment responsiveness, stratified by autoantibody status.

Materials and methods

Patients

One hundred patients (92 female, 8 male) with active RA diagnosed according to the 1987 revised American College of Rheumatology (ACR) criteria for classification of RA (15) underwent treatment with an anti-TNF biological agent for at least 6 months at the outpatient clinics of the Department of Rheumatology at the University General Hospital Larissa and the First Department of Internal Medicine, AHEPA University Hospital, Thessalo-

Table I. Clinical characteristics of patient population used in this study.

Variable	Value
Age in years, mean±SD	57.35 ± 10.759
Women, percentage	92%
Disease duration in years, mean±SD	13.61 ± 6.482
RF positive, percentage	79%
Anti-CCP positive, percentage	66%
DAS28 at baseline, mean±SD	5.62 ± 0.254
DAS28 at 6 months, mean±SD	3.96 ± 1.527
Responders, percentage	69%
Non-responders, percentage	31%
Anti-TNF-α	
Infliximab, number	24
Etanercept, number	26
Adalimumab, number	50

Table II. Association between SNP genotype and anti-TNF-α treatment outcome at 6 months.

Gene, polymorphism	Genotype	Responders n. (%)	Non-responders n. (%)	*p-value*
TNF-α g.-308G>A	GG	59 (85.5)	27 (87.1)	
	GA	10 (14.5)	4 (12.9)	
	AA	0 (0)	0 (0)	1
TNF-α g.-857C>T	CC	44 (63.8)	19 (61.3)	
	CT	21 (30.4)	12 (38.7)	
	TT	4 (5.8)	0 (0)	0.323
TNFRII c.676T>G	TT	34 (49.3)	12 (38.7)	
	TG	31 (44.9)	18 (58.1)	
	GG	4 (5.8)	1 (3.2)	0.459

niki, Greece. A total of 24 patients were treated with infliximab (3 mg/kg intravenously at 0, 2 and 6 weeks and then every 8 weeks) and methotrexate (15–20 mg/week), 26 patients were treated with etanercept (25 mg subcutaneously twice weekly) with or without methotrexate and 50 patients were treated with adalimumab (40 mg subcutaneously every other week) with or without methotrexate or leflunomide. The mean age of the patients was 57.4 years and the mean duration of the disease was 13.6 years. Patients had inadequate response to at least two DMARDs, including methotrexate, prior to entry in the study. Written informed consent was obtained from each individual and the research protocol was approved by the local ethics committee.

Clinical and laboratory assessments

The disease activity index (DAS28) was applied to assess disease activity, while response to treatment after 6 months was evaluated using the modified DAS28 index (16), as before (8). Blood samples were obtained from consenting patients when they required a blood test as part of routine care. At baseline, rheumatoid factor was measured by immunonephelometry assays using the quantitative N latex rheumatoid factor system (Dade Behring, Marburg, Germany), whereby concentrations ≥15 IU/ml were considered positive. Anti-CCP was tested using a commercially available ELISA kit (Axis-Shield, Dundee, UK), serum samples were evaluated in triplicate and the upper normal limit (5 U/ml) was set according to the manufacturer's recommendations.

Genotyping

Genomic DNA was isolated using a standard phenol/chloroform extraction method. Genotyping for the selected SNPs, TNF g.-238G>A (rs361525), g.-308G>A (rs1800629), g.-857C>T (rs1799724), TNFRI c.36A>G (rs4149584) and TNFRII c.676T>G (rs1061622) was performed using polymerase chain reaction-restriction fragment length polymorphism analysis, with primers and conditions described previously (6).

Statistical analysis

For single SNP analysis responders and non-reponders were compared non- parametrically by means of using two by two tables by grouping the heterozygous and homozygous for the rare allele and comparing the risk for the individuals grouped for the common allele, in each case. The chi-square test was used to test for Hardy-Weinberg equilibrium. In addition to analyses performed for each individual SNP, haplotypes were constructed using the HapStat software platform (17). The threshold haplotype frequency value for inclusion in the analysis was set at 3%. Fisher's exact test was used to compare genotype groups. All the analyses were performed using SPSS 14 (Inc, Chicago, IL, USA). A value of $p \leq 0.05$ was used to identify a significant result.

Results

The major clinical characteristics of the RA patients participated in this study are summarised in Table I. Based on the DAS28 criteria 31/100 patients were defined as non-responders to anti-TNF-α treatment. Also, 79% and 66% of the patients were RF and anti-CCP positive, respectively. All SNPs studied complied with Hardy-Weinberg equilibrium distribution, except from TNF g.-238G>A, g.-857C>T and TNFRI c.36A>G and thus were excluded from further analysis. Results of single SNP association analysis with response to treatment are shown in Table II. This analysis showed no significant difference in allele or genotype frequencies between responders and non-responders. The HapStat program was then used to estimate haplotype frequencies and identify haplotype association with clinical responsiveness (Supplementary Table I). The data showed that haplotype-based analysis appear to confirm the single-SNP analysis with respect to no association of TNF-α g.-308G>A, TNF-α g.-857C>T and TNFRII c.676T>G with response to drug treatment. We then stratified the cohort of patients by RF-positive and anti-CCP positive status, in order to examine polymorphism association as previous studies have shown that autoantibody positivity corresponds to a distinct type of RA associated with reduced response to anti-TNF-α treatment (13). Interestingly we found that carriage of TNFRII c.676G allele was associated with negative response to drug treatment in anti-CCP positive patients (p=0.03), a finding that was not seen in RF-positive patients (p=0.327) (Table III).

Discussion

The introduction of the anti-TNF-α biological agents has been a significant

Supplementary Table I. Results of haplotype association tests with response to anti-TNF-α therapy.

Haplotype	Responders	Non-responders	p_c
G-C-T	0.537	0.594	0.43
G-C-G	0.205	0.152	0.11
G-T-T	0.104	0.111	0.71
G-T-G	0.089	0.071	0.22
A-C-T	0.052	0.042	0.17
other	0.013	0.03	–

Tested haplotypes correspond to TNF-α (g.-308G>A)-TNF-α (g.-857C>T)-TNFR2 (c.676T>G). Haplotype frequencies for responders and non-responders are shown. The test was implemented using the software HapStat.

Table III. Association between SNP genotype and anti-TNF-α treatment outcome at 6 months stratified by autoantibody-positive status.

	RF -Positive			CCP-Positive		
Gene, polymorphism	Responders	Non-responders	*p*	Responders	Non-responders	*p*
TNF-α g.-308G>A						
GG	43 (81.1)	22 (84.6)		32 (82.1)	23 (85.2)	
GA	10 (18.9)	4 (15.4)		7 (17.9)	4 (14.8)	
AA	0 (0)	0 (0)	**1**	0 (0)	0 (0)	**1**
TNF-α g.-857C>T						
CC	33 (62.3)	16 (61.5)		28 (71.8)	17 (63)	
CT	16 (30.2)	10 (38.5)		10 (25.6)	10 (37)	
TT	4 (7.5)	0 (0)	**0.812**	1 (2.6)	0 (0)	**0.449**
TNFR2 c.676T>G						
TT	29 (54.7)	11 (42.3)		25 (64.1)	10 (37)	
TG	21 (39.6)	14 (53.8)		13 (33.3)	16 (59.3)	
GG	3 (5.7)	1 (3.8)	**0.327**	1 (2.6)	1 (3.7)	**0.03**

breakthrough in the treatment of RA. However, a large number of patients are treated at considerable expense and risk of serious toxicity without in many cases a significant clinical benefit, which merits the need of identifying good predictors for response to the anti-TNF treatments (2, 3). Previous findings have suggested that clinical and genetic factors alone are poor predictors of responsiveness to anti-TNF therapy (18). Therefore in this study we sought to examine the use of both genetic and serological markers in predicting response to therapy. Our results show that carriage of TNFRII c.676G allele is associated with negative response to clinical responsiveness in anti-CCP positive patients. To the best of our knowledge this is the first study that demonstrates the association of a TNF related polymorphism in an autoantibody-positive stratified population. Taken separately, our finding agrees with previous results of correlation of TNFRII c.676G allele with reduced response to therapy (19), a result partially explained by an increase in IL-6 production and an effect in membrane receptor shedding and/or ligand binding in individuals harboring the c.676G allele (20). In addition, anti-CCP positive RA patients have been associated with reduced response to anti-TNF-α therapy in several studies (12, 13), thus further validating its use as a serological marker of prognostic value for clinical responsiveness in RA. Notwithstanding the importance of our findings, our results should be regarded as preliminary due to the small size of our patient cohort and the inability to assign the suggested association to a specific anti-TNF drug. Further investigation in larger independent cohorts would be required to confidently claim that the combined use of genetic and serological markers could be used in the prognosis of RA patients' response to anti-TNF-α therapy.

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