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The influence of a polymorphism at position −857 of the tumour necrosis factor α gene on clinical response to etanercept therapy in rheumatoid arthritis

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***Objectives*. We aimed to test whether polymorphisms in the etanercept target genes *TNFA* and *LTA* are associated with clinical responses to etanercept therapy in RA patients.**

***Methods*. Clinical responses of 70 patients treated with etanercept were determined according to the ACR criteria. We genotyped 13 single-nucleotide polymorphisms (SNPs) within *TNFA* and *LTA* and tested whether they influenced the responses to 12 weeks of etanercept therapy. Univariate and multivariate analyses were performed to compare allele, genotype and haplotype distributions between responders and non-responders.**

***Results*. Association of the −857C/T SNP at the *TNFA* promoter was marginally significant when patients were divided into responders and non-responders according to improvement criteria ACR20 or ACR70. When ACR70 responders (the best responders) were compared with ACR20 non-responders (the worst responders), however, the association was prominent [odds ratio (OR) = 12, 95% confidence interval (CI) = 1.4–105, $P = 0.0077$, $P_{corrected} = 0.054$], as the frequency of the T allele was 5% in the ACR20 non-responders but 39% in the ACR70 responders. Moreover, the ratio of ACR70 responder number to ACR20 non-responder number among T-allele carriers was >10-fold higher than in the C-allele homozygotes (OR = 12, 95% CI = 1.2–120, $P = 0.033$).**

***Conclusions*. RA patients with the T allele of *TNFA* −857C/T SNP respond better to etanercept therapy than homozygotes for the C allele, indicating that, when the results have been confirmed, this SNP could become a useful genetic marker for predicting responses.**

KEY WORDS: Tumour necrosis factor, Rheumatoid arthritis, Etanercept, Single-nucleotide polymorphism.

Tumour necrosis factors (TNF) α (TNFA) and β (or lymphotoxin α, LTA) are proinflammatory cytokines that play pivotal roles in regulating the inflammatory response in rheumatoid arthritis (RA) [1]. Although it is controversial whether *TNF* genes are associated with RA susceptibility [2, 3], they are well known to mediate RA pathogenesis [1]. Elevated levels of TNFA are found in the synovium and synovial fluid of RA patients [4]. The TNF functions are mediated by two distinct TNF receptors, TNFRSF1A and TNFRSF1B, which exist as monomers on cell surfaces and in soluble forms [5].

Intensive studies on TNF-driven inflammation processes have led to the development of TNF blockers for RA treatment. They are derived from a recombinant TNF receptor, TNFRSF1B (for etanercept), or an anti-TNFA monoclonal antibody (for infliximab and adalimumab). The molecular mechanisms of these TNF blockers are similar: they inhibit binding of TNFs to cell-surface TNF receptors and thereby block signal transduction pathways that are induced or regulated by TNFs. However, etanercept binds to both TNFA and LTA, whereas infliximab and adalimumab bind to TNFA only.

TNF blocker therapies have been widely used and demonstrated to be very effective in treating RA. However, they are very expensive and can cause side events, and not all RA patients respond well to the therapy [6, 7]. Genetic factors may affect the response and toxicity in individual patients, hence maximizing the response and minimizing toxicity requires individualized drug therapy. The targets of TNF blockers may be responsible for the response variations and include TNFA, LTA and the proteins involved in TNF-driven inflammation processes.

Pharmacogenetic studies on the effectiveness of etanercept and infliximab in treating RA have been performed with Caucasian patients. The −308 G/A single-nucleotide polymorphism (SNP) in the *TNFA* promoter [8] and the major histocompatibility complex (MHC) region [9] influence the response to infliximab. Extended haplotypes spanning from *HLA-DRB1* to the *TNF* region influence the etanercept response [10]. It is unclear, however, how these genetic variants influence the response to TNF blockers.

In the present study, we recruited 70 Korean RA patients in the context of a clinical trial of etanercept and genotyped them for suspiciously functional polymorphisms in the *TNFA*, *LTA* and *HLA-DRB1* regions to test their influence on etanercept response. We found an SNP in the *TNFA* promoter associated with

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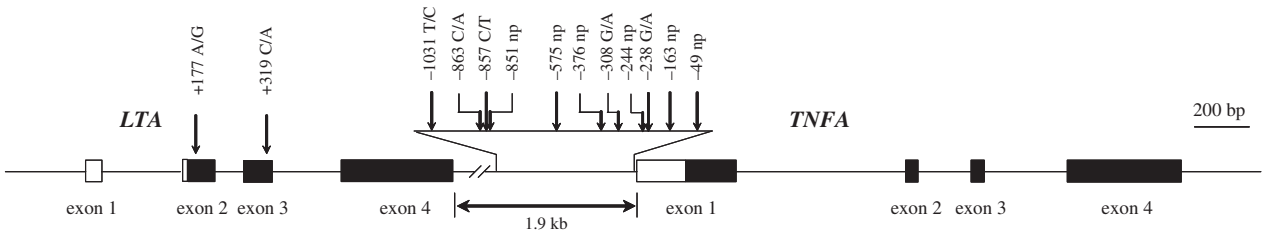

FIG. 1. Physical map of the *TNF* locus. A genomic region from *LTA* exon 1 to *TNFA* exon 4 is shown with exons marked by filled blocks and the 5′ UTR by blank blocks. The two exonic SNP sites in *LTA* are numbered as their positions in mature mRNA relative to the first nucleotide (+1) of the *LTA* reference sequence, NM_000595. Numbering of the *TNFA* promoter sites descends from the transcription start site (+1) in the genomic reference sequence, Z15026. Six sites, indicated as np, were not polymorphic in our study subjects.

etanercept response and identified genotypes responding better to etanercept therapy.

Patients and methods

Patients

In the context of a 12-week clinical trial evaluating etanercept efficiency, 70 Korean RA patients were recruited from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Republic of Korea, and did not withdraw from the trial. The study was approved by Institutional Review Board of Hanyang University Medical Center. The patients provided written informed consent, and their genomic DNA was extracted from peripheral blood using standard protocols. All the patients satisfied the ACR 1987 revised criteria for RA [11] and were in functional class I, II or III. The inclusion criteria included (i) showing an unsatisfactory response to one or more conventional DMARDs, including methotrexate (MTX), (ii) having more than six swollen and painful joints, and (iii) having at least one of the following characteristics: an ESR of 28 mm/h or greater, CRP of 2.0 mg/dl or greater, and morning stiffness lasting 45 min or longer. None of the patients had previously received cyclophosphamide or prednisone at 10 mg/day or greater or TNF antagonists at any dosage.

The patients received subcutaneous injections of etanercept (25 mg) twice a week for 12 weeks while continuing to receive MTX at a stable dosage of 7.5–25 mg a week. DMARDs other than MTX were discontinued at least 4 weeks prior to etanercept injection in the present study. The patients were allowed to receive stable dosages of corticosteroid and non-steroidal anti-inflammatory drugs throughout the study period.

Efficacy measurements

The clinical efficacy variables were the percentage improvements (20, 50 or 70%) in disease activity according to the ACR criteria [12] at 12 weeks. The ACR criteria assess 68 joints for tenderness and 66 joints for swelling. An ACR20 response, for example, indicates a decrease of at least 20% in both the number of tender joints and the number of swollen joints, as well as an improvement of at least 20% in at least three of the following: the patient's assessment of pain, the patient's global assessment of disease status, the physician's global assessment of disease status, the patient's assessment of physical function measured using the Korea Health Assessment Questionnaire (KHAQ) [13], and CRP.

Van Vollenhoven *et al.* [14] recently provided evidence that clinical responses to anti-TNF therapies (including etanercept) display a normal or skewed distribution, but not a bimodal distribution. Therefore, we primarily compared patients in two extreme groups: ACR20 non-responders and ACR70 responders.

Genotyping for polymorphisms

Eleven SNPs in the *TNFA* promoter region, at positions −1031, −863, −857, −851, −575, −376, −308, −244, −238, −163 and −49, and two non-synonymous SNPs in *LTA*, at positions +177 (Arg13Cys) and +319 (Tyr60Asn), shown in Fig. 1, were genotyped using the MassARRAY^{TM} system (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions, and SpectroTYPER software was used to characterize the alleles. The genotyping accuracy was controlled by blind testing with 12 samples for each SNP. *HLA-DRB1* alleles were genotyped using the polymerase chain reaction and sequence-specific oligonucleotide probe hybridization, according to the reference protocol of the 12th International Histocompatibility Workshop, followed by direct DNA sequencing [15].

Statistical analysis

Baseline characteristics of all patients were measured before the etanercept treatment and a two-tailed Fisher's exact test was used in comparison between two groups when a 2×2 contingency table was available; otherwise Student's *t*-test was used. Association between each SNP and the etanercept response was assessed by univariate and multivariate analyses using the SPSS program, and the effects of KHAQ and patient's global assessment at the start of the study were adjusted in multivariate analysis. Structures and frequencies of haplotypes, consisting of five SNPs in the *TNFA* promoter (−1031, −863, −857, −308 and −238), were estimated using the Haplotyper program [16], based on a Bayesian algorithm.

Results

Characteristics of patients

The baseline characteristics of the 70 patients are summarized in Table 1. They were 45 ± 10 (mean ± S.D.) yr old, their disease duration was 11.8 ± 6.8 years, 93% were women, 70% were RF-positive, and 73% were carriers of the shared epitope (SE). Before the etanercept treatment, patients had been using a mean of 4.5 DMARDs and receiving prednisone equivalents at 4.0 mg/day.

Also in Table 1, the baseline characteristics of the patients carrying T allele in *TNFA* −857C/T SNP (TT-CT group) are compared with those of C allele homozygotes (CC group). The characteristics were similar ($P > 0.05$) between the two groups, except for KHAQ ($P = 0.045$) and patient's global assessment ($P = 0.028$) at the start of the study, and the compounding effects of the two dissimilar characteristics were therefore adjusted in further multivariate association analyses.

TABLE 1. Baseline characteristics of the patients

Characteristic	All patients ($n=70$)	CC group[c] ($n=43$)	TT-CT group[c] ($n=27$)	P[d]
Age (yr)	45 ± 10	45 ± 11	45 ± 9	0.99
Women: no. (%)	65 (93)	41 (95)	24 (89)	0.37
SE[a]-positive: no (%)	48 (73)	26 (65)	22 (85)	0.097
RF-positive: no. (%)	49 (70)	32 (74)	17 (63)	0.42
Disease duration (yr)	11.8 ± 6.8	12.8 ± 7.8	10.2 ± 4.4	0.081
Steroids (mg/day)	4.0 ± 2.2	4.0 ± 2.2	3.9 ± 2.2	0.78
Previous DMARDs	4.5 ± 1.5	4.4 ± 1.4	4.6 ± 1.8	0.63
Tender joints (no.)	16 ± 12	17 ± 13	15 ± 9	0.48
Swollen joints (no.)	11 ± 5.2	11 ± 5.4	11 ± 4.9	0.70
KHAQ[b]	1.4 ± 0.64	1.6 ± 0.63	1.3 ± 0.62	0.045
Duration of morning stiffness (min)	211 ± 155	213 ± 155	208 ± 158	0.90
Physician's global assessment	5.8 ± 1.6	6.1 ± 1.4	5.4 ± 1.7	0.070
Patient's global assessment	6.8 ± 1.7	7.1 ± 1.4	6.2 ± 1.9	0.028
ESR (mm/h)	60 ± 31	63 ± 32	56 ± 30	0.34
Serum CRP level (mg/dl)	2.5 ± 2.4	2.6 ± 2.6	2.3 ± 2.0	0.68

Data are mean ± S.D. except where indicated otherwise.

[a]Includes *HLA-DRB1* *0101, *0401, *0404, *0405, *0410, *1001 and *1406 alleles.

[b]Korea Health Assessment Questionnaire (KHAQ) score at the start of the study is shown.

[c]Patients in the CC and TT-CT groups carried the C allele homozygote and the T allele in the *TNFA* −857C/T SNP respectively.

[d]*P* values were calculated using Fisher's exact test (for dichotomous variables) or Student's *t*-test (for continuous variables).

Individual genotyping

All 70 patient samples were genotyped successfully for the total of 13 SNPs in *TNFA* and *LTA* (Fig. 1). The results were confirmed by blind testing with 12 randomly selected samples for each SNP. Among the 13 genotyped SNPs, six SNPs in the *TNFA* promoter region at positions −851, −575, −376, −244, −163 and −49 were not polymorphic in our study subjects. The other SNPs, five (−1031T/C, −863C/A, −857C/T, −308G/A, and −238G/A) in *TNFA* and two (+177A/G, +319C/A) in *LTA*, were polymorphic and in Hardy–Weinberg equilibrium, as revealed by χ^2 statistics ($P > 0.05$). Thus, data for the seven SNPs were used for association analyses.

Influence of genetic variants on etanercept response

The clinical responses to etanercept were analysed by comparing ACR responses before the first injection and after the last injection at 12 weeks: 86% of the patients achieved an ACR20 response and 20% achieved an ACR70 response. When allelic associations with etanercept response were analysed with the ACR20 criteria alone (those who achieved ACR20 were regarded as responders and those who did not achieve ACR20 as non-responders) for the seven *TNF* SNPs and *HLA-DRB1* SE, only the −857C/T SNP in the *TNFA* promoter was marginally associated with etanercept efficacy [odds ratio (OR) = 6.6, 95% confidence interval (CI) = 0.85–51], showing T allele frequencies of 5% in non-responders and 26% in responders (Table 2). In the ACR70 response criteria, the association of the −857C/T SNP was significant (OR = 2.8, 95% CI = 1.2–6.9). The significance of the association was greater (OR = 12, 95% CI = 1.4–105), however, when ACR20 non-responders (who showed the worst etanercept response) were compared with ACR70 responders (who showed the best response), with T allele frequencies of 5% in non-responders and 39% in responders (Table 2).

Next, genotypic associations were tested. The patients could be divided into two groups according to the genotype for the *TNFA* −857C/T SNP: 61% were C-allele homozygotes (CC group) and 39% carried the T allele (TT-CT group). Almost all patients (96%) in the TT-CT group, but a smaller percentage of patients (79%) in the CC group, were ACR20 responders (Table 3). Also, the percentage of ACR70 responders in the TT-CT group (30%) was higher than that in the CC group (13%). Thus, the percentage of responders was higher in TT-CT group than in the CC group, although the genotypic associations were not significant separately for the ACR20 ($P = 0.076$) and ACR70 ($P = 0.11$) criteria. In contrast, when the ACR20 non-responders were compared only with the ACR70 responders, the genotypic association was significant ($P = 0.033$, $P_{corrected} = 0.23$, OR = 12). In addition, this significant association was sustained when multivariate logistic regression was performed to adjust the effects of KHAQ and patient's global assessment at the start of the study ($P = 0.049$, $P_{corrected} = 0.34$).

The *TNFA* −857C/T SNP was not in linkage disequilibrium ($r^2 < 0.023$) with any SE allele, except for moderate linkage disequilibrium between the −857C and *0405 alleles ($r^2 = 0.30$). However, the SE allele *0405 was not associated with etanercept response even in comparison between the ACR20 non-responders and the ACR70 responders ($P = 1.0$). When multivariate logistic regression was performed to adjust the effect of *0405 allele, the significant association of the −857 SNP was still sustained ($P = 0.028$, $P_{corrected} = 0.20$).

Haplotype structures and frequencies

Eight haplotypes were constructed with five SNPs in the *TNFA* promoter, at positions −1031, −863, −857, −308 and −238 (e.g. the haplotype TCCGG consisted of −1031T, −863C, −857C, −308G and −238G). The five most frequent haplotypes (TCCGG, TCTGG, CACGG, CCCGA and CATGG) accounted for 98% of the total, with frequencies of 61, 21, 10, 4 and 2% respectively, and these data are consistent with a previous report on a Korean population [17]. When the genetic effects of *TNFA* haplotypes on the etanercept response were tested (Table 4), the TCTGG haplotype containing the *TNFA* −857T allele showed a frequency of 32% in the ACR70 responders but only 5% in the ACR20 non-responders ($P = 0.031$).

Discussion

In the present study, we followed up the clinical results of 70 RA patients who received 12 weeks of etanercept treatment. We found that a −857C/T SNP (rs1799724) in the promoter of *TNFA* was positively associated with the etanercept response, and that T-allele carriers were more frequent in responders of ACR20 or ACR70 than the C allele homozygotes. Moreover, the allelic and genotypic associations were prominent when the ACR20 non-responders were compared with the ACR70 responders. The results are consistent with the clinical outcomes of etanercept therapy in that they do not exhibit a sharp bimodal distinction between responders and non-responders, as suggested by van Vollenhoven *et al.* [14]. This illustrates that genetic factors with minor effects may be detected only when the best and worst responders are compared.

It is highly conceivable that *TNFA* −857C/T SNP can functionally influence the etanercept response, as van Heel *et al.* [18] suggested that the SNP directly affects the transcription efficiency of *TNFA*, although several previous studies on the functional effects of the −857 SNP on the cellular level of TNFA [18–21] have been controversial. Recently, van Heel *et al.* [18] reported that *ex vivo* lipopolysaccharide-stimulated production of whole-blood TNFA was higher in healthy −857C-allele homozygotes than

TABLE 2. Allele frequencies of *TNFA* and *LTA* minor alleles and *HLA-DRB1* SE in etanercept responders and non-responders

Polymorphism	Minor allele	ACR20 non-responders *vs* responders			ACR70 non-responders *vs* responders			ACR20 non-responders *vs* ACR70 responders	
		Non-responders ($n=10$)	Responders ($n=60$)	Odds ratio[a] (95% CI)	Non-responders ($n=56$)	Responders ($n=14$)	Odds ratio[a] (95% CI)	Odds ratio[a] (95% CI)	$P_{corrected}$[b]
TNFA									
−1031T/C	C	5 (25%)	18 (15%)	0.53 (0.17–1.64)	18 (16%)	5 (18%)	1.1 (0.38–3.4)	0.65 (0.16– 2.6)	0.72
−863C/A	A	4 (20%)	14 (11%)	0.53 (0.15–1.81)	14 (12%)	4 (14%)	1.2 (0.35–3.9)	0.67 (0.15–3.1)	0.70
−857C/T	T	1 (5%)	31 (26%)	6.6 (0.85–51)	21 (19%)	11 (39%)	2.8 (1.2–6.9)	12 (1.4–105)	0.0077 (0.054)
−308G/A	A	0 (0%)	1 (1%)	–	0 (0%)	1 (4%)	–	–	1.0
−238G/A	A	1 (5%)	4 (3%)	0.66 (0.07–6.2)	4 (4%)	1 (4%)	1.0 (0.11–9.3)	0.70 (0.04–12)	1.0
LTA									
+177A/G	G	5 (25%)	21 (17%)	0.64 (0.21–1.9)	21 (19%)	5 (18%)	0.94 (0.32–2.8)	0.65 (0.16–2.6)	0.72
+319C/A	A	9 (45%)	49 (41%)	0.84 (0.33–2.2)	46 (41%)	12 (43%)	1.1 (0.47–2.5)	0.92 (0.29–2.9)	1.0
HLA-DRB1									
SE		10 (56%)	47 (41%)	1.8 (0.65–4.8)	49 (47%)	8 (29%)	2.2 (0.90–5.5)	3.1 (0.90–10.8)	0.15

Numbers of the minor alleles are shown with their frequencies in parentheses, except where indicated otherwise.
[a]Odds ratio is larger than 1 where the minor allele is associated with good response. The odds ratio of the −308G/A SNP cannot be obtained because the frequency of the A allele is too low.
[b]*P* values were corrected by multiplying *P* by the number (7) of SNPs tested.

T-allele carriers. The −857T allele variant of *TNFA* promoter contains a transcription factor OCT1 binding site (ATGAAGAC) from position −858 to position −851 (the −857 SNP is underlined). OCT1 binds to the sequence only with the −857T allele but not with the −857C allele, and inhibits *TNFA* promoter activity [18, 22]. Thus, the −857C-allele homozygotes would have too much TNFA to be sufficiently inhibited by the standard dose of etanercept, responding poorly to the treatment.

OCT1 binding inhibits the transcription activator NFκB, which binds to a nearby site from position −873 to position −863C/A SNP. NFκB is produced in two forms, a canonical p65–p50 heterodimer and a p50–p50 homodimer. The latter binds to the sequence with the major −863C allele rather than with the minor −863A allele, while the former binds to both variants [23]. Since only the p65 subunit has a transcription activation domain [24, 25], p50–p50 homodimer exerts competitive inhibition as binding only to the −863C allele variant [23].

Thus, the p65–p50 NFκB activation of the *TNFA* promoter may be subject to two mechanisms of inhibition, by OCT1 and homodimer NFκB, which can be affected by the −857C/T SNP and the −863C/A SNP respectively. The −863A/−857C variant, not being affected by the two inhibitors, would produce the highest level of TNFA. In contrast, the −863C/−857T variant would be inhibited by both mechanisms and produce the lowest level. Carriers of the latter haplotype constituted 32% of ACR70 responders but only 5% of ACR20 non-responders in our study, and the difference is statistically significant ($P=0.031$). However, the −863C/A SNP alone or the −1031T/C SNP, which is in high linkage disequilibrium ($r^2=0.65$, $|D'|=0.86$) with the −863 SNP, was not associated with the etanercept response in our study.

The cellular TNFA level has been reported to be affected also by other SNPs in the TNFA promoter. Although the −238A allele was shown to reduce the TNFA level in psoriasis patients [26], the reduction was not observed in various cell lines [27] and the SNP was not associated with the etanercept response in our study. The −308G/A SNP has been shown to affect the TNFA level in some, but not all, previous studies [27, 28] and to affect the response to another TNF blocker, infliximab [8], but all our subjects carried only the G allele, except for one heterozygous carrier. The A allele of −376G/A SNP, located in another OCT1 binding site, was suggested to reduce the TNFA level and increase susceptibility to cerebral malaria [29], but position −376 was not polymorphic in our study subjects.

In a recent study with a mostly Caucasian population, early RA patients with two copies of SE in *HLA-DRB1* exhibited a better response to standard-dose treatment with etanercept than those with no copies or one copy [10]. The results were not replicated in our study with Korean patients (Tables 2 and 3), however, possibly due to an ethnic difference in the frequencies of SE alleles. For example, the *DRB1* *0401 and *0404 are the most frequent among Caucasian RA patients but are rare (< 2%) in Korean patients.

When Bonferroni correction (multiplying by the number of SNPs tested, i.e. 7) was applied, the allelic association of the *TNFA* −857C/T SNP for the ACR20 non-responders ($n=10$) and the ACR70 responders ($n=14$) became marginal ($P_{corrected}=0.054$). This could be attributed to the small sample size. In our comparison between the ACR20 non-responders and the ACR70 responders, the maximum statistical power to detect an association at $P=0.05$ is 77%, was calculated according to the genetic-model-free method using the PAWE (Power for Association With Error) program [30]. Therefore, the sample size needs to be increased to at least 53 (22 ACR20 non-responders and 31 ACR70 responders) to provide the same statistical power of 77% at $P_{corrected}=0.05$.

In conclusion, RA patients with the T allele at position −857 of the *TNFA* promoter respond better to etanercept therapy than C-allele homozygotes, suggesting that this SNP could become

TABLE 3. Influence of the genotype distribution of the *TNFA* −857C/T SNP and SE homozygosity on the etanercept response

	All patients ($n=70$)				ACR20 non-responders and ACR70 responders ($n=24$)	
	ACR20 non-responders	ACR20 responders	ACR70 non-responders	ACR70 responders	ACR20 non-responders	ACR70 responders
TNFA −857 SNP						
CC	9 (21%)	34 (79%)	37 (86%)	6 (14%)	9 (60%)	6 (40%)
CT	1 (5%)	21 (95%)	17 (77%)	5 (23%)	1 (17%)	5 (83%)
TT	0 (0%)	5 (100%)	2 (40%)	3 (60%)	0 (0%)	3 (100%)
OR[a] (95% CI)	6.9 (0.82–58)		2.6 (0.79–8.6)		12 (1.2–120)	
P_{before}[b]	0.076		0.11		0.033 ($P_{corrected}=0.23$)	
P_{after}[b]	0.075		0.22		0.049 ($P_{corrected}=0.34$)	
HLA-DRB1 SE						
2 copies	3 (33%)	6 (67%)	8 (89%)	1 (11%)	3 (75%)	1 (25%)
1 copy	4 (10%)	35 (90%)	33 (85%)	6 (15%)	4 (40%)	6 (60%)
0 copy	2 (11%)	16 (89%)	11 (61%)	7 (39%)	2 (22%)	7 (78%)
OR[a] (95% CI)	0.24 (0.046–1.2)		0.42 (0.048–3.7)		0.15 (0.013–1.8)	
P	0.098		0.67		0.26	

Numbers in parentheses indicate genotype distributions between non-responders and responders, except where indicated otherwise.
[a]ORs were calculated by comparing T allele carriers with CC homozygotes of the *TNFA* −857C/T SNP or by comparing carriers of 0 or 1 copy of the SE with carriers of 2 copies.
[b]P values were calculated by logistic regression before (P_{before}) and after (P_{after}) adjusting for the effects of both KHAQ and patient's global assessment at the start of the study as covariates.

TABLE 4. Haplotype structures and frequencies in the *TNFA* promoter and their influence on the etanercept response

	ACR20 non-responders *vs* responders			ACR70 non-responders *vs* responders			ACR20 non-responders *vs* ACR70 responders	
Haplotype[a]	Non-responders ($n=10$)	Responders ($n=60$)	P	Non-responders ($n=56$)	Responders ($n=14$)	P	Odds ratio (95% CI)	P
TCCGG	14 (70%)	72 (60%)	0.46	73 (65%)	13 (46%)	0.084	2.7 (0.80–9.0)	0.14
TCTGG	1 (5%)	28 (23%)	0.075	20 (18%)	9 (32%)	0.12	9.0 (1.0–98)	0.031
CACGG	4 (20%)	10 (8%)	0.12	12 (11%)	2 (7%)	0.74	0.31 (0.050–1.9)	0.22
CCCGA	1 (5%)	4 (3%)	0.54	4 (4%)	1 (4%)	1.0	0.70 (0.041–12)	1.0
CATGG	0 (0%)	3 (3%)	1.0	1 (1%)	2 (7%)	0.10	–	0.50
TACGG	0 (0%)	1 (0.8%)	1.0	1 (0.9%)	0 (0%)	1.0	–	1.0
CCCGG	0 (0%)	1 (0.8%)	1.0	1 (0.9%)	0 (0%)	1.0	–	1.0
TCCAG	0 (0%)	1 (0.8%)	1.0	0 (0%)	1 (4%)	0.20	–	1.0

Numbers of haplotypes are shown with their frequencies in parentheses.
[a]Constituents of the haplotypes are written in the order −1031, −863, −857, −308 and −238 SNPs of *TNFA*.

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Key messages

- *TNFA* −857C/T SNP influences the etanercept response in RA patients.

a useful genetic marker for predicting responses after the results are further confirmed.

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