Letters to the Editor

Rheumatology 2007;46:1034–1045

doi:10.1093/rheumatology/kem041
Advance Access publication 4 April 2007

Combined tumour necrosis factor-α and tumour necrosis factor receptor genotypes could predict rheumatoid arthritis patients’ response to anti-TNF-α therapy and explain controversies of studies based on a single polymorphism

Sir, Tumour necrosis factor-α (TNF-α) blocker therapies are widely used in the treatment of chronic inflammatory diseases. However, patients show large heterogeneity in their response to anti-TNF-α therapy. The genotypic background of TNF-α and TNF receptor (TNFR) genes could account for patients’ resistance to TNF-α blockers [1, 2]. HLA-DR haplotypes have been related with rheumatoid arthritis (RA) susceptibility, severity and course [3], but TNF and TNFR polymorphisms seem to have independent predictive value of patients’ response to anti-TNF-α therapy [2, 4, 5].

In a previous study, we have investigated the efficacy, toxicity and drug discontinuation in RA patients treated with infliximab [6]. In the present retrospective study, 58 unrelated patients (46 females, mean age: 58.4 yrs, disease duration: 15.4 yrs, seropositivity: 67%) were studied in order to investigate genetic factors that could account for the lack of efficacy to anti-TNF-α therapy. All patients gave informed written consent and approval had been granted by the local ethics committee.

Specifically, the polymorphism 36A > C (exon 1) of TNFR1 gene, the 676T > G (Met196Arg, exon 6) of TNFR2, and the polymorphisms −857C > T (promoter), −308G > A (promoter), −238G > A (promoter) and 489G > A (intron 1) of TNF-α gene have been studied by polymerase chain reaction-single strand conformation polymorphism method. Their selection was based on the fact that the results of previous studies are controversial for their role in RA pathogenesis, severity and course, and in patients’ response to anti-TNF-α treatment. Statistical analysis was performed by a population genetics data analysis software, the Arlequin, according to Raymond and Rousset [7].

Twenty-seven patients showed good response according to the disease activity score (DAS) for 28 joint indices (DAS-28 > 2.2) to infliximab, 18 moderate response (1.2 ≤ DAS-28 ≤ 2.2), and 13 lack of efficacy (DAS-28 < 1.2). Table 1 shows the distribution of genotypes of the studied polymorphisms in accordance to their response to TNF-α inhibitor. All patients were homozygous for the wild-type −238G allele. Very low heterogeneity was also revealed for polymorphism −308G > A (1 AA-homozygote and 14 GA-heterozygotes). Thus, these two polymorphisms were excluded from the statistical analysis, since they were not found in Hardy–Weinberg equilibrium.

Table 1. The distribution of 36A > C (TNFR1), 676T > G (TNFR2), −857C > T (TNF-α) and 489G > A (TNF-α) genotypes in RA patients (n = 58) according to their response to anti-TNF-α therapy (infliximab)

<table>
<thead>
<tr>
<th>DAS-28</th>
<th>36A &gt; C</th>
<th>676T &gt; G</th>
<th>−857 C &gt; T // 489G &gt; A</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>8 (0.8)</td>
<td>22 (3.2)</td>
<td>21 (3)</td>
</tr>
<tr>
<td>AG</td>
<td>14 (1.4)</td>
<td>5 (0.8)</td>
<td>3.4 (1.1)</td>
</tr>
<tr>
<td>GG</td>
<td>5 (0.5)</td>
<td>3 (0.4)</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Good response</td>
<td>3.2 (0.8)</td>
<td>3.2 (0.9)</td>
<td>3.2 (0.9)</td>
</tr>
<tr>
<td>Mean DAS-28, mean (s.d.)</td>
<td>3.4 (1.1)</td>
<td>3.3 (1.1)</td>
<td>3.3 (1.1)</td>
</tr>
<tr>
<td>Moderate response</td>
<td>3.2 (0.8)</td>
<td>3.2 (0.9)</td>
<td>3.2 (0.9)</td>
</tr>
<tr>
<td>Poor response</td>
<td>1.4 (0.3)</td>
<td>1.4 (0.3)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>Mean DAS-28, mean (s.d.)</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.3)</td>
<td>1.5 (0.3)</td>
</tr>
</tbody>
</table>

Statistical analysis revealed that polymorphisms −857C > T and 489G > A were in linkage disequilibrium (P = 0.000). Specifically, the C allele of −857C > T was linked with the G allele of 489G > A (only one RA patient carried the genotype 489G/489A together with the −857T/−857T). Therefore, polymorphisms −857C > T and 489G > A were studied as one.

No independent polymorphism could predict patients’ response to anti-TNF-α therapy. However, the complex genotypic analysis of both TNFR2 and TNF-α gene polymorphisms revealed statistical significant difference in the distribution of the genotypic association of 676T > G with −857C > T/489G > A between the good and poor responders to infliximab (P = 0.008). Good responders carried more frequently the TNFR2 allele 676T in homozygosity together with the homozygosity of TNF-α allele −857C/489G compared with poor responders.

Previously, the genotypic analysis of polymorphism 676T > G revealed conflicting results concerning the association of 676G allele with the pathogenesis of RA and disease severity. However, in transfected HeLa cells, the functional analysis of the polymorphism revealed increased toxicity in cells with the 676G allele which may explain the report by Fabris et al. about poor response to anti-TNF-α treatment in patients carrying the 676G allele [1, 8]. This may explain why the complex genotype in the majority of our patients who responded well to anti-TNF-α treatment carried the 676T allele.

Moreover, controversial results exist in the association of polymorphism −857C > T with RA pathogenesis, disease severity and its effect on TNF-α gene transcription. In our study, the complex genotype carried in homozygosity the −857C allele in good responders. However, according to Kang et al. the −857T allele is associated with good response to anti-TNF-α therapy. In contrast, Soga et al. [9] reported a tendency for high TNF-α productivity in the carriers of −857T allele and, therefore, increased toxicity. The later seems to be in accordance to our results for the association of −857C allele and the good response to anti-TNF-α therapy.

Finally, according to the literature, polymorphism 489G > A was not able to predict the predisposition to RA and patients’ response to anti-TNF-α treatment. Kaizel et al. [10] reported no correlation of the polymorphism 489G > A in TNF-α gene transcription. In our study, the wild type allele of polymorphism 489G > A was in strict linkage disequilibrium with the wild type allele of −857C > T and therefore, does not contribute to the variability of genotypic combinations found in good and poor RA responders. Consequently, the reported functional polymorphism −857C > T seems to have higher predictive value compared with 489G > A.

In conclusion, no independent polymorphism can predict the optimal therapy and especially in complex diseases such as chronic...
inflammatory disorders. Thus, we suggest that the combined study of polymorphisms 676T > G (TNFR2) and −857C > T (TNF-α) could be used in the prognosis of RA patients’ response to anti-TNF-α therapy. More studies in larger and of other ethnic groups could confirm the use of the suggested complex genotypic analysis.

The authors have declared no conflicts of interest.

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Accepted 24 January 2007

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Letters to the Editor

Use of the QuantiFERON TB Gold test as part of a screening programme in patients with RA under consideration for treatment with anti-TNF-α agents: the Newcastle (UK) experience

Sir, RA patients treated with anti-TNF-α agents have an increased risk of developing Mycobacterium tuberculosis (MTB) infection, the magnitude of which (compared with background risk) depends on the agent used, approaching 10-fold for infliximab-treated patients [1]. Such cases are likely predominately to represent reactivation of latent infection, as opposed to the de novo cases, which are thought to account for most etanercept-associated infections [2]. For the purposes of latent MTB infection screening the tuberculin skin test (TST) has well-recognized shortcomings [3], and the development of blood tests whose basis is the detection of IFN-γ released by T-cells in response to MTB-specific antigens, including the commercially available QuantiFERON-TB Gold (QTG) test (Cellestis, Carnegie, Australia), has been cautiously welcomed. These are more specific than the TST, especially amongst patients previously exposed to BCG, and may also be more sensitive, as well as having operational advantages over the TST [4]. Lack of a gold standard test for latent TB makes validation of such tests difficult though it would seem that their sensitivities and specificities vary according to the characteristics of the population studied [4, 5]. The same probably applies to the tests’ informativeness, and, of note, the rate of indeterminate QTG test results amongst cancer chemotherapy patients has been noted to be significantly higher than that seen amongst relatively immunocompetent individuals in an Italian study (36% vs 9%) [6]. This raises the question of whether the test might be similarly uninformative in RA patients under consideration for anti-TNF-α therapy, who are invariably immunosuppressed (albeit to a lesser extent). The QTG test has been employed by the Rheumatology department at The Freeman Hospital, Newcastle-upon-Tyne, UK, since March 2004 as part of the TB screening programme for such a patient group. We have audited its use amongst 101 consecutive RA patients, recruited over a 2 year period, who fulfilled published criteria permitting use of a biologic drug [3], and we present data addressing the feasibility, informativeness and, so far as possible, the prospective accuracy of the QTG test in this highly relevant clinical context.

Prior to treatment all patients were screened for MTB infection as per published guidelines [3]. In addition a QTG test was undertaken, employing Newcastle’s Public Health Laboratory Service (PHLS) and according to the test manufacturer’s instructions [7].

Mean age of recruited patients was 55 (s.d. 44–66), 77% were female and 99% were white Caucasian; mean disease activity score (DAS28) was 6.6 (s.d. 5.86–7.34). BCG status was known in 93 (92%) of the patients, and amongst this group 78.5% had undergone vaccination. Recruited patients were invariably exposed to DMARDs including methotrexate at maximum tolerated dose (up to 25 mg) at the time of QTG testing, and ~40% of them had been recipients of systemic corticosteroid therapy prior to recruitment. Test results, recorded as positive, negative or, in instances where no IFN-γ response was seen to a positive control mitogen, indeterminate, are summarized in Fig. 1.

Before treatment all patients were screened for MTB infection, using the QuantiFERON-TB Gold (QTG) test which targets MTB-specific antigens and has been shown to be more specific and sensitive than the standard tuberculin skin test (TST) [3]. Our audit of its use amongst 101 consecutive RA patients has highlighted its feasibility and informativeness, as well as the potential for optimal treatment in RA.

Rheumatology key messages

- Identification of factors that could predict patient response to anti-TNF-α therapy remains the key for optimal treatment in RA.
- It seems that the combined study of polymorphisms 676T>G (TNFR2) and −857C>T (TNF-α) could predict RA patients’ response to anti-TNF-α therapy.

9. Soga Y, Nishimura F, Ohyama H, Maeda H, Takahashi S, Murayama Y. Tumor necrosis factor-alpha gene (TNF-α) -1031G/A polymorphism and all but three went on to receive an anti-TNF-α agent. No cases of MTB reactivation or progression have been followed up for between 6 and 30 months (average 18.3 months) following initiation of an anti-TNF-α agent. Of the 98 patients subsequently administered a biologic, 68.3% received etanercept, 18.3% adalimumab and 13.3% infliximab. All of the patients have been followed up for between 6 and 30 months (average 18.3 months) following initiation of an anti-TNF-α agent. No cases of MTB reactivation or de novo infection have been observed in our cohort during this period regardless of the agent used.

An infrastructure within which routine use of the QTG test is established has been developed with relative ease in our centre, thanks to effective cooperation between the Rheumatology department and the PHLS. Blood samples must be processed in the laboratory within 12 h, and blood is therefore drawn on a week-day morning, with results available within 48 h. The laboratory charges £35.00 for the service. An excessive proportion of patients have been followed up for between 6 and 30 months (average 18.3 months) following initiation of an anti-TNF-α agent. No cases of MTB reactivation or de novo infection have been observed in our cohort during this period regardless of the agent used.