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Interleukin-10 promoter microsatellite polymorphisms are associated with response to long-term treatment with etanercept in patients with rheumatoid arthritis

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Running title: IL-10 polymorphisms and etanercept response in RA

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Abstract

Objectives: To analyze the association of interleukin-10 (IL-10) promoter polymorphisms, that have been shown to be related to IL-10 secretion capacity, with the response to long-term treatment with etanercept in patients with rheumatoid arthritis (RA).

Methods: Fifty (50) patients with active RA were treated up to 4 years (median 39 months, range 3-52) with stable doses of etanercept as monotherapy. Therapy response was assessed as defined by the EULAR criteria in an intention-to-treat analysis with the last observation carried forward. IL-10 promoter microsatellite polymorphisms IL10.R and IL10.G were genotyped by fragment length analysis in patients and 189 ethnically, age- and sex-matched healthy controls. Haplotypes were reconstructed using a Bayesian, coalescent theory-based method with the PHASE software.

Results: The IL-10 microsatellite polymorphisms were not associated with susceptibility to RA. Upon comparison of patients with good treatment response (n=25) to patients with moderate (n=17) or no response (n=8) a significantly different distribution of the prevailing alleles R2, R3 and G9, G13, respectively, became evident. A good treatment response was associated with carriage of the R3 allele or the R3-G9 haplotype, whereas the allele G13 and the haplotype R2-G13 predominated in patients with moderate or no response.

Conclusion: Genotyping of the IL-10 promoter microsatellites may be useful in the prognostic estimation of the clinical response to etanercept in patients with RA. The high prevalence of the presumptive IL-10 low-producer allele R3 in patients with a favorable response suggests that IL-10 promotes disease activity in RA under the specific condition of TNF antagonization.

Keywords: Arthritis, rheumatoid – Etanercept response - Interleukin-10 – Promoter microsatellite polymorphisms
Introduction

Tumor necrosis factor (TNF)-α is a dominant regulator of the inflammatory cascade in rheumatoid arthritis (RA). Today antagonization of TNF is a well-established therapeutical concept in RA. However, not all patients respond equally well to it. Lack of efficacy in some patients may be ascribable by the broad genetic heterogeneity of RA that results in a multitude of different phenotypes. Synovial tissue cytokine production in RA patients correlates with different patterns of lymphocyte infiltration, suggesting that several pathomechanisms can modulate the expression of the immune response in the synovial membrane. Interleukin-10 (IL-10) is a major immunoregulatory cytokine that usually is considered to mediate downregulation of the inflammatory response, as it inhibits the activation and effector functions of T cells, macrophages, and monocytes. In particular, it acts as a negative autocrine regulator of TNF-α and further pro-inflammatory cytokines. Some other effects are not anti-inflammatory, as it stimulates B cell survival, proliferation, differentiation, and antibody isotype switching. Elevated levels of IL-10 have been found in the serum and synovial fluid of RA patients, possibly contributing to the diminished T cell function and increased antibody and rheumatoid factor production. In fact, IL-10 has been reported to activate B cells in vitro to promote autoantibody production like rheumatoid factor or antibodies against cyclic citrullinated peptide.

Interindividual variability in IL-10 secretion is determined for about 75% by genetic differences. The IL-10 gene maps to chromosome 1q31-q32. In the IL-10 promoter, multiple single-nucleotide polymorphisms and 2 microsatellite polymorphisms have been identified. They combine to form 4 major haplotype families. Approximately 1.1 kilobasepairs (kb) and 4.0 kb upstream of the transcription initiation site the CA dinucleotide repeat microsatellites designated IL10.G and IL10.R, respectively, are located. The lipopolysaccharide-induced IL-10 secretion in vitro varied according to the haplotypic composition of the microsatellite alleles. Susceptibility to RA has been suggested to be influenced by different IL10.R alleles. More importantly, in RA patients associations of cytokine gene polymorphisms have also been described with the response to biologic agents. We hypothesized that genetically determined interindividual differences in IL-10 production may influence the therapy response to TNF blocking agents in RA. Thus, in the present study we analyzed the association of functionally relevant IL-10 promoter microsatellite polymorphisms with the response to long-term treatment with etanercept, a soluble TNF receptor fusion protein that binds and inactivates TNF, in patients with long-standing and active RA.
Patients and Methods

Study subjects. The study protocol was approved by the local independent ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität Münster). Prior to study admission, patients and controls consented in written form. All 50 patients were German Caucasians and met the diagnostic criteria for rheumatoid arthritis as established by the American College of Rheumatology (ACR). Thirty-one (31) patients were recruited from the outpatient clinic for rheumatology, Department of Medicine B, Münster University Hospital, Germany, and 19 from the Clinic of Rheumatology, University of Magdeburg, Vogelsang / Gommern, Germany. Thirty-eight (38) patients were females, 12 males. Their median age was 55 years, range 21-80. Thirty-eight (38) patients were positive for rheumatoid factor. Median disease duration at study admission was 6 years, range 1-39. The patients had been pretreated either consecutively or simultaneously with in the median 3 disease modifying antirheumatic drugs (range 0-7). Forty-one (41) patients had received methotrexate, 28 patients sulfasalazine, 28 patients gold salts, 25 patients antimalarials, 15 patients azathioprine, 12 patients leflunomide, 9 patients cyclosporine A, 3 patients cyclophosphamide, and, one patient each, infliximab, D-penicillamine or interferon-γ. Ethnically, age- and sex-matched healthy controls (n = 189) derived from the Prospective Cardiovascular Münster (PROCAM) study. Participants of this study were employees of Westphalian companies, with significant cardiovascular, pulmonary, metabolic, rheumatic, and renal disease being excluded before study admission by review of medical history and physical examination.

Therapy. Recombinant human TNF receptor (p75)-Fc fusion protein (etanercept) was obtained from Wyeth-Ayerst Research, Münster, Germany. The patients received 25 mg etanercept as monotherapy twice a week subcutaneously for up to four years. Clinical evaluations comprised the calculation of a disease activity score (DAS28), a combined index based on a 28 tender and swollen joint count, erythrocyte sedimentation rate, and the patients global disease activity measured on a visual analog scale. In Münster, follow-ups were performed before the start of therapy and then every three months. In Magdeburg, patients were examined at baseline, after 3 and 6 months and than every six months. Treatment response was assessed as defined by the EULAR criteria in an intention-to-treat analysis, with the last observation carried forward. The EULAR response criteria are based on differences of the DAS28 at two different points in time and the current DAS28 reached under therapy. The median treatment period was 39 months, ranging from 3 to 52 months, only 3 patients were treated for less than one year. We thereby surveyed a total of 140 patient-years under etanercept.

Genotyping. DNA was extracted from EDTA-anticoagulated blood of all 50 patients according to standard protocols. The multiallelic IL-10 microsatellites IL10.R and IL10.G were genotyped by fragment length analysis. Flanking primers were constructed as follows: IL10.1 5´-GTC.CTT.CCC.CAG.GTA.GAG.CAA.CAC.TCC-3´ (5´-labelled with 6-FAM fluorescent dye, PE Applied Biosystems, Weiterstadt, Germany), IL10.2 5´- CTC.CCA.AAG.AAG.CCT.TAG.TAG.TGT.TG-3´, IL10.3 5´- CCC.TCC.AAA.ATC.TAT.TTG.CAT.AAG-3´ (5´-labelled with HEX fluorescent dye, PE Applied Biosystems), and IL10.4 5´-CTC.CGC.CCA.CCA.GTA.AGT.TTC.ATC.AC-3´. IL10.1 and IL10.2 amplified the IL10.G microsatellite, IL10.3 and IL10.4 the IL10.R microsatellite. A multiplex PCR reaction was carried out in a thermal cycler (GeneAmp® PCR System 9700, PE Applied Biosystems) under the following conditions: hot start at 94°C for 10 min, 30 cycles of 30 sec at 95°C, 45 sec at 65°C, 45 sec at 72°C, final extension for 7 min at 72°C. The PCR reaction mixture contained 0.6 U AmpliTaq Gold™, primers IL10.1, IL10.2,
IL10.3, and IL10.4 at 0.2 µM, dNTP at 50 µM, MgCl₂ at 1 mM, and approximately 50 ng DNA in a total volume of 20 µl. A DNA size standard (20 µl of a 1:40 dilution of Genescan-ROX 500, PE Applied Biosystems) was added to 1 µl of PCR product in a 96 well MicroAmp Optical Reaction Plate (PE Applied Biosystems). The samples were denatured after heating at 90 °C for 2 min and then subjected to fragment length analysis on a four color-laser-induced fluorescence capillary electrophoresis system (ABI Prism 3700 Genetic Analyser, PE Applied Biosystems) using POP6 as polymer. The size of microsatellite containing DNA fragments was measured by comparison to the DNA size standard using GeneScan™ Software. The analysis of the IL10.R microsatellite revealed 4 alleles which ranged in size between 111 bp (IL10.R2) and 117 bp (IL10.R5). The 10 alleles of the IL10.G microsatellite ranged in size between 130 bp (IL10.G7) and 148 bp (IL10.G16). Each electrophoresis run included a negative control and a DNA sample with known IL10.R and IL10.G genotypes as positive control. Specificity of genotyping was confirmed in selected cases (N = 20 chromosomes) by direct sequencing of PCR products (data not shown).

**Haplotype reconstruction and statistical analysis.** Haplotypes were reconstructed using a Bayesian, coalescent theory-based method with the PHASE software (Version 2.0.2 for DOS). The software incorporates a model that allows for recombination and decay of linkage disequilibrium with physical distance of alleles. Moreover, the type of polymorphism (SNP or multiallelic with/without stepwise mutation mechanism, respectively) is taken into account. Allele and haplotype frequencies in RA patients and healthy controls as well as in subgroups of patients with different therapy response were compared by Monte Carlo simulation. The T4 statistics was applied to test for significance which was assumed in case of a P-value ≤ 0.05. Phenotype frequencies were compared using Chi-square analysis or Fisher’s exact test where appropriate. The strength of association was calculated as the odds ratio (OR) and is presented with 95% confidence intervals (CI). For calculation of linkage disequilibria Δ expected haplotype frequencies were estimated by multiplication of allele frequencies and subtracted from observed haplotype frequencies. Chi-square analysis was used to determine the significance of the deviation from 0 to the Δ value. Differences between quantitative data over time were analysed with the Wilcoxon’s signed rank test. All statistical calculations were performed on a personal computer using the CLUMP (Version 1.6 for DOS) or the MedCalc software (Version 4.20.006 for Windows 95/NT).
Results

Clinical outcome. Within the first year of etanercept therapy, the median DAS28 was reduced from 6.2 (range 4.1-8.6) to 3.8 (1.2-8.6), the median glucocorticosteroid dosage from 7.5 mg prednisolone equivalent (0-23) to 5 mg (0-12.5), either highly significant at \( P < 0.0001 \). This reduction was stable, as no further significant changes were registered thereafter. With respect to the DAS28, before start of therapy 45 patients were at highly active disease. At their most recent visit, 18 patients were in remission, 7 patients at low, and 8 patients at high disease activity. As defined by the EULAR criteria, 25 patients responded well, 17 patients moderately well, and 8 patients failed treatment. Fourteen (14) patients stopped etanercept therapy within the study period. Reasons for discontinuation were ineffectiveness as assessed by the patients (n = 7), severe adverse events (n = 2), long distance to the study center (n = 2), scheduled surgery (n = 1), desire for pregnancy (n = 1), and non-compliance (n = 1). The severe adverse events that led to discontinuation of therapy were, each in one patient, a perforation of the sigmoid colon and thrombocytopenia.

Genotype distribution in RA patients and healthy controls. In our study population, we found 4 different alleles for the IL10.R, and 10 different alleles for the IL10.G microsatellite, respectively. The most frequent alleles were IL10.R2 (70% in RA patients vs. 73% in healthy controls), IL10.R3 (28% vs. 25%), IL10.G9 (34% vs. 39%), and IL10.G13 (25% vs. 29%). As shown in figures 1 and 2, there were no significant differences comparing the allele distribution between RA patients and healthy controls. Haplotype reconstruction with the PHASE software resulted in a total of 19 different haplotypes with an average phase probability of 98%. Ninety-five (95) percent of the reconstructed phases had a probability greater than 97%. Highest positive linkage was observed for R3-G9 and, less strong, for R2-G13, while R2-G9 and R3-G13 were negatively linked (Table 1). The prevailing haplotypes were R2-G9 (11% in RA patients vs. 16% in healthy controls), R2-G13 (24% vs. 28%), and R3-G9 (21% vs. 21%). Again, the haplotype distribution was not significantly different between patients and healthy controls (Figure 3). However, the heterozygous presence of the R2-G10 haplotype was associated with a significant risk for RA with an odds ratio of 3.08 (95% CI 1.17-8.14). This haplotype was not found in homozygous genotype. No other significant associations of individual alleles or haplotypes with RA susceptibility were detected.

Table 1: Linkage disequilibria between IL10.R and G alleles

<table>
<thead>
<tr>
<th>IL10.R Allele frequency</th>
<th>IL10.G Allele frequency</th>
<th>Haplotype frequency</th>
<th>( \Delta )</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2 0.724</td>
<td>G7 0.025</td>
<td>0.019</td>
<td>0.001</td>
<td>0.02</td>
<td>0.9004</td>
</tr>
<tr>
<td></td>
<td>G8 0.052</td>
<td>0.046</td>
<td>0.008</td>
<td>0.21</td>
<td>0.6533</td>
</tr>
<tr>
<td></td>
<td>G9 0.379</td>
<td>0.151</td>
<td>-0.123</td>
<td>20.88</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>G10 0.056</td>
<td>0.040</td>
<td>-0.001</td>
<td>0.01</td>
<td>0.9357</td>
</tr>
<tr>
<td></td>
<td>G11 0.079</td>
<td>0.065</td>
<td>0.007</td>
<td>0.10</td>
<td>0.7576</td>
</tr>
<tr>
<td></td>
<td>G12 0.052</td>
<td>0.052</td>
<td>0.014</td>
<td>0.79</td>
<td>0.3780</td>
</tr>
<tr>
<td></td>
<td>G13 0.278</td>
<td>0.274</td>
<td>0.073</td>
<td>6.64</td>
<td>0.0100</td>
</tr>
<tr>
<td></td>
<td>G14 0.067</td>
<td>0.067</td>
<td>0.018</td>
<td>1.26</td>
<td>0.2671</td>
</tr>
<tr>
<td></td>
<td>G15 0.006</td>
<td>0.006</td>
<td>0.002</td>
<td>0.05</td>
<td>0.8250</td>
</tr>
<tr>
<td></td>
<td>G16 0.004</td>
<td>0.004</td>
<td>0.001</td>
<td>0.08</td>
<td>0.7791</td>
</tr>
<tr>
<td>R3 0.253</td>
<td>G7 0.025</td>
<td>0.006</td>
<td>0.000</td>
<td>0.18</td>
<td>0.6784</td>
</tr>
<tr>
<td></td>
<td>G8 0.052</td>
<td>0.004</td>
<td>-0.009</td>
<td>1.35</td>
<td>0.2508</td>
</tr>
<tr>
<td></td>
<td>G9 0.379</td>
<td>0.207</td>
<td>0.111</td>
<td>22.05</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>G10 0.056</td>
<td>0.017</td>
<td>0.002</td>
<td>0.01</td>
<td>0.9122</td>
</tr>
</tbody>
</table>
Data represent the individual allele frequencies in the study population (n = 478), the haplotype frequencies as calculated with the PHASE software, differences $\Delta$ between the expected and the observed haplotype frequencies and levels of significance as determined by $\chi^2$ test.

**Genotype distribution and etanercept response.** Comparing patients with a good response to etanercept treatment as defined by the EULAR criteria to patients with a moderate or no response, significant differences in the allele and haplotype distribution of the IL-10 promoter microsatellite loci became evident (Figures 4-6). The alleles R3 (40% in patients with good response vs. 16% in patients with moderate or no response), G9 (46% vs. 22%), and the haplotype R3-G9 (32% vs. 10%) prevailed in patients responding well, whereas the alleles R2 (58% vs. 82%), G13 (12% vs. 38%), and the haplotype R2-G13 (10% vs. 38%) were more frequent in patients with moderate or no response to the etanercept therapy. The carrier status of the IL10.R3 allele and the R3-G9 haplotype was thus associated with a good response to etanercept therapy, whereas the presence of the allele IL10.G13 or the haplotype R2-G13 resulted in odds ratios indicative of moderate or no response (Table 2). The alleles IL10.R2 and IL10.G9 were associated with the therapy response without reaching significance. Comparison of the patients with moderate response to patients who failed treatment provided no further information as the latter group was too small to draw conclusions in the study of this highly polymorphic locus.

<table>
<thead>
<tr>
<th>Allele/haplotype</th>
<th>Good response (n)</th>
<th>Moderate/ no response (n)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>21 vs. 4</td>
<td>24 vs. 1</td>
<td>0.3487</td>
<td>0.22 (0.02-2.11)</td>
</tr>
<tr>
<td>R3</td>
<td>17 vs. 8</td>
<td>7 vs. 18</td>
<td>0.0113</td>
<td>5.46 (1.63-18.36)</td>
</tr>
<tr>
<td>G9</td>
<td>16 vs. 9</td>
<td>11 vs. 14</td>
<td>0.2623</td>
<td>2.26 (0.73-7.05)</td>
</tr>
<tr>
<td>G13</td>
<td>6 vs. 19</td>
<td>16 vs. 9</td>
<td>0.0106</td>
<td>0.18 (0.05-0.61)</td>
</tr>
<tr>
<td>R2-G13</td>
<td>5 vs. 20</td>
<td>16 vs. 9</td>
<td>0.0046</td>
<td>0.14 (0.04-0.50)</td>
</tr>
<tr>
<td>R3-G9</td>
<td>14 vs. 11</td>
<td>5 vs. 20</td>
<td>0.0212</td>
<td>5.09 (1.45-17.92)</td>
</tr>
</tbody>
</table>

Data represent the number of phenotype positive vs. negative patients, relative to therapy response as defined by the EULAR criteria. The strength of association is quoted as the odds ratio (OR) with a 95% confidence interval (CI).
Discussion

Etanercept has proven a sustained efficacy and an excellent safety profile when used for the treatment of patients with RA. However, therapy with etanercept is expensive, and a causal relationship with the rare incidence of severe adverse events under the therapy cannot be definitively ruled out. Thus, outcome predictors allowing for individual treatment decisions are clearly needed. The well-documented pleiotropic effects of IL-10 in RA suggest it as an important disease-modifying factor, and gene polymorphisms associated with IL-10 production capacity may in consequence function as prognostic markers.

First we characterized our RA study population in terms of the IL-10 promoter genotypes. In contrast to Eskdale et al., we found no associations of individual IL10.R or IL10.G alleles with the disease susceptibility. Our data corroborate results from a study performed in UK Caucasian and South African patients. These discrepant findings may arise from considerable genetic heterogeneity between the different ethnic groups, but also from phenotypic variability of RA. Additionally, the IL-10 microsatellites may be rather markers of disease susceptibility due to linkage disequilibrium than the primary causative genetic polymorphisms. For instance, the genotype of a single nucleotide polymorphism that is associated with high IL-10 production has been found more frequently in patients with a higher rate of joint destruction and higher autoantibody titers. Others, possibly not yet identified polymorphisms in the IL-10 promoter and their haplotypes may thus be more informative with respect to susceptibility to RA.

The low number of patients failing etanercept treatment in our study population raised the need to pool data from patients with moderate or no response into one group. In this way two equally-sized groups were generated for the purpose of comparability. The IL-10 promoter microsatellite allele IL10.R3 and the haplotype R3-G9 prevailed significantly in patients with a good response, whereas IL10.G13 and R2-G13 were more frequent among patients with a moderate or no response. These findings propose the IL-10 promoter microsatellite polymorphisms as predictors of etanercept response. The relevant alleles are frequent in all examined populations, thus enhancing the applicability of this genetic testing. Other than in previous studies, we did not investigate the association of genetic polymorphisms with the therapy response at a predefined point in time within the first months of therapy, but considered the most recent observation from clinical data assembled over 4 years. This approach may be more accurate as the response status varied to a considerable extent within the first year of therapy and gained stability thereafter.

The combination of polymorphic elements from the IL-10 promoter in form of haplotypes has previously been demonstrated. In general, the analysis of haplotypes may be more informative when trying to find a functionally relevant sequence variation than the study of single microsatellite allele associations. We therefore reconstructed haplotypes using a Bayesian, coalescent theory-based method. By means of this well established method the most frequent haplotypes were R2-G9, R2-G13 and R3-G9, matching previously published data of Eskdale et al. who deduced haplotypes from analysis of family genotyping data. Indeed, in contrast to the individual allele analysis the haplotype analysis revealed a significant association of the haplotype IL10.R2-G10 with susceptibility to RA. Admittedly, this association refers to a rare haplotype, and we do not believe that it constitutes a major risk factor for RA. In the response analysis, consideration of the haplotypic organization did not influence the strength of association observed. This is most likely due to the strong positive linkage disequilibria between R2-G13 and R3-G9. However, these data support the notion of
the IL-10 microsatellites being rather markers of therapy response due to linkage disequilibrium than being the primary causative genetic polymorphisms.

Functional studies revealed that haplotypes containing the IL10.R3 allele are associated with a low lipopolysaccharide-induced IL-10 secretion in vitro.\textsuperscript{20} Under the presumption that IL-10 is a predominant anti-inflammatory cytokine, our finding of IL10.R3 being associated with a favorable response to etanercept is at a first glance surprising. However, in another study the allele of a single nucleotide polymorphism within the IL-10 promoter that has been linked with low IL-10 production capacity in vitro tended to be more prevalent among etanercept responders.\textsuperscript{22} IL-10 shares anti- and pro-inflammatory properties, and there is evidence that quantitative synovial IL-10 mRNA correlates to joint damage progression.\textsuperscript{42} It has been suggested that intrinsically high IL-10 production is associated with severe, progressive RA.\textsuperscript{41} In addition, abundant transcription of IL-10 has been found in the synovium of patients with follicular synovitis who display higher serum TNF-\(\alpha\) levels than patients with diffuse synovitis.\textsuperscript{7,43} Thus, these patients with constitutively high IL-10 production may represent a subset with more aggressive disease.

In summary, a good response to long-term treatment with etanercept has been found to be associated with carriage of the IL10.R3 allele or the R3-G9 haplotype, whereas the allele IL10.G13 and the haplotype R2-G13 predominated in patients with moderate or no response. Our data suggest that genotyping of the IL-10 promoter microsatellites may be useful in the prognostic estimation of the clinical response to etanercept in patients with RA. The high prevalence of the presumptive IL-10 low-producer allele R3 in patients with a favorable etanercept response suggests that IL-10 promotes disease activity in RA under the specific condition of TNF antagonization.
Competing interest statement

M. Gaubitz is consultant for Wyeth-Ayerst Pharma, Germany, the manufacturer of etanercept, and for other producers of antirheumatic drugs.
Figure legends

Figure 1: IL10.R allele distribution in RA patients and healthy controls (HC)

Bars represent the allele frequencies in the respective population. Allele distribution was not significantly different between RA patients (n = 100 alleles) and healthy controls (n = 378 alleles) ($\chi^2$ (T4 statistics) = 0.62; $P = 0.2232$).

Figure 2: IL10.G allele distribution in RA patients and healthy controls (HC)

Bars represent the allele frequencies in the respective population. Allele distribution was not significantly different between RA patients (n = 100 alleles) and healthy controls (n = 378 alleles) ($\chi^2$ (T4 statistics) = 8.09; $P = 0.1684$).

Figure 3: IL10.R-G haplotype distribution in RA patients and healthy controls (HC)

Bars represent the haplotype frequencies in the respective population. Haplotype distribution was not significantly different between RA patients (n = 100 alleles) and healthy controls (n = 378 alleles) ($\chi^2$ (T4 statistics) = 12.08; $P = 0.2140$).

Figure 4: IL10.R allele distribution in RA patients as related to etanercept response

Bars represent the allele frequencies in the respective patient subgroup. Allele distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ (T4 statistics) = 8.21; $P = 0.0101$).

Figure 5: IL10.G allele distribution in RA patients as related to etanercept response

Bars represent the allele frequencies in the respective patient subgroup. Allele distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ (T4 statistics) = 10.93; $P = 0.0346$).

Figure 6: IL10.R-G haplotype distribution in RA patients as related to etanercept response

Bars represent the haplotype frequencies in the respective patient subgroup. Haplotype distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ (T4 statistics) = 17.83; $P = 0.0186$).
References


28 van Gestel AM, Prevoo ML, 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of


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Bars represent the allele frequencies in the respective population. Allele distribution was not significantly different between RA patients (n = 100 alleles) and healthy controls (n = 378 alleles) ($\chi^2$ (T4 statistics) = 8.09; $P = 0.1684$).
Bars represent the haplotype frequencies in the respective population. Haplotype distribution was not significantly different between RA patients (n = 100 alleles) and healthy controls (n = 378 alleles) (χ² (T4 statistics) = 12.08; P = 0.2140).
Bars represent the allele frequencies in the respective patient subgroup. Allele distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ (T4 statistics) = 8.21; $P = 0.0101$).
Bars represent the allele frequencies in the respective patient subgroup. Allele distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ (T4 statistics) = 10.93; $P = 0.0346$).
Bars represent the haplotype frequencies in the respective patient subgroup. Haplotype distribution was significantly different between patients responding well to etanercept treatment (n = 50 alleles) and patients responding moderately or not (n = 50 alleles) ($\chi^2$ test) = 17.83; $P = 0.0186$).