Concise report

Genotype at the sIL-6R A358C polymorphism does not influence response to anti-TNF therapy in patients with rheumatoid arthritis

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Abstract

Objectives. To investigate the association between genotype at the soluble interleukin 6 receptor (sIL-6R) A358C single nucleotide polymorphism (SNP, rs8192284), previously reported to correlate with soluble receptor levels, and response to anti-TNF therapy in subjects with RA.

Methods. In a large cohort of Caucasian RA patients treated with anti-TNF medications (total, n = 1050; etanercept, n = 455; infliximab, n = 450; and adalimumab, n = 142), the sIL-6R A358C polymorphism was genotyped using a Taqman 5′-allelic discrimination assay. Linear regression analysis adjusted for baseline 28 joint disease activity score (DAS28), baseline HAQ score, gender and use of concurrent DMARDs was used to assess the association of genotype at this polymorphism with response to anti-TNF therapy, defined by change in DAS28 after 6 months of treatment. Analyses were performed in the entire cohort, and also stratified by an anti-TNF agent. Additional analysis according to the EULAR response criteria was also performed, with the chi-squared test used to compare genotype groups.

Results. No association between genotype at sIL-6R A358C and response to anti-TNF treatment was detected either in the cohort as a whole or after stratification by anti-TNF agent, in either the linear regression analysis or with response segregated according to EULAR criteria.

Conclusions. This study shows that genotype at the functional sIL-6R A358C SNP is not associated with response to anti-TNF treatment in patients with RA.

Key words: Infliximab, Etanercept, Adalimumab, Anti-TNF, Rheumatoid, IL-6 receptor, Pharmacogenetics, Biomarkers.

Introduction

Anti-TNF therapy has made a significant contribution to the treatment of patients with RA. However, these agents are expensive and have significant side effects. In addition, only 70% of the treated patients achieve ACR20 response and 40% achieve ACR50 [1, 2]. Therefore, it would be advantageous if it were possible to predict the treatment outcome so that these agents could be targeted to patients most likely to benefit and least likely to suffer adverse effects.

A number of pharmacogenetic studies have investigated the association of single nucleotide polymorphisms (SNPs) with response to anti-TNF treatment in RA, and to date these studies have focused predominantly on genetic variation within the TNF gene. The TNF-308 polymorphism has been best studied, and in a well-powered study, our group has demonstrated that the genotype at this marker associates with a differential response to etanercept and infliximab [3].

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IL-6 is a pleiotropic cytokine produced by a variety of cells and has been shown to be a key cytokine in the inflammatory and immunological manifestations of RA [4]. IL-6 signalling occurs via a receptor (IL-6R) that exists in membrane-bound (cis-signalling) and soluble (trans-signalling) forms, and both forms of the receptor require association with a membrane-bound protein known as gp130 for signal transduction [5]. Crucially in RA, key cell types in the synovial joint including chondrocytes, fibroblasts and synoviocytes do not express membrane-bound IL-6R, and therefore key actions of IL-6 are mediated by the soluble receptor (sIL-6R) [6]. The importance of signalling via this pathway has been demonstrated by the clinical effects of tocilizumab [7], a humanized anti-IL-6R antibody, and studies have demonstrated a correlation between the expression of sIL-6R in SF and extent of joint destruction [8].

A non-synonymous SNP (A→C) at position 358 in the sIL-6R gene (rs8192284) has been identified, which results in an amino acid change from alanine to aspartate [9]. Carriage of the C allele at this SNP has been shown to correlate with higher levels of sIL-6R in Japanese [9] and Caucasian [10] populations, and our group has recently demonstrated that the frequency of the C allele is lower in patients with RA than controls [11]. In this study, we hypothesised that patients with RA carrying the CC genotype at A358C would potentially have more severe disease, driven by increased IL-6 signalling and therefore respond less well to anti-TNF therapy than patients with the AA genotype.

Methods

Details of the patients included in this study and the methods by which they were recruited are outlined elsewhere [3], but in brief patients who met the ACR criteria for a diagnosis of RA [12], were naïve to anti-TNF treatment and had a 28 joint disease activity score (DAS28) of >5.1 despite prior treatment with two or more DMARDs before commencing anti-TNF therapy were enrolled. Blood samples were obtained from consenting patients, and DNA was isolated using a standard phenol/chloroform extraction method. Ethical approval was obtained from the UK Central Office of Research Ethics Committees (04/Q1403/37) and all participants gave informed consent. In addition ESR and CRP measurements were available at baseline and after 6 months of anti-TNF treatment for a proportion of the cohort.

Genotyping of sIL-6R (rs8192284) was performed using Taqman assays designed by Applied Biosystems (Foster City, CA, USA). Positive (sequenced templates) and negative controls (ultrapure water) were included in all genotyping plates and 10% of the assays were repeated for quality control purposes. The total reaction volume was 5μl, containing 10ng of genomic DNA. Thermal cycling in a 384-well plate was performed using a PTC-225 DNA Engine Tetrad thermal cycler (MJ Research, San Francisco, CA, USA), and rs8192284 genotypes were determined using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK).

Deviation from the Hardy–Weinberg equilibrium (HWE) was tested using a chi-squared test with one degree of freedom and a threshold of \( P < 0.05 \). SPSS pack version 14 was used for all analyses.

Response to the anti-TNF treatment according to genotype at sIL-6R A358C was investigated using linear regression analysis adjusted for gender, concurrent DMARD treatment, baseline DAS28 and HAQ score, with change in DAS28 score between baseline and 6 months as the dependent variable as has been described previously. Change in ESR and CRP after 6 months of treatment was also separately investigated by a linear regression model, adjusted for gender and DMARD and prednisolone use. All analyses were performed in the cohort as a whole and then stratified by anti-TNF agent. Additional analysis was performed according to the EULAR response criteria [13] with the chi-squared test used to compare genotype groups. A power calculation [14] suggests that for our sample size of 1050, and a minor allele frequency of 0.38, this study has 99.9% power to detect a change in the DAS28 of 0.6 at the 5% significance level. In the stratified analysis there was 98.3% power to detect the same difference in a sample size of 455, but only 62% power for the sample size of 142 in the adalimumab-treated group.

Results

A total of 1050 patients were included in the study, of whom 455 (43.3%) received etanercept, 453 (43.1%) infliximab and 142 (13.5%) adalimumab. The detailed baseline characteristics of the patients have been shown previously [3], but 76.9% were females, and 88 and 82% were RF- and anti-cyclic citrullinated peptide (anti-CCP) antibody positive, respectively; and mean disease duration was 14.2 years. Baseline and 6-month ESR and CRP measurements were available for 95 and 40% of the cohort, respectively. Genotyping was successful in all samples.

In the linear regression model, there was no association between genotype at rs8192284 and either baseline DAS28 or response to anti-TNF treatment in the cohort as a whole \((P = 0.45 \text{ and } 0.87, \text{ respectively})\), or after stratification by an anti-TNF agent (Table 1). Genotype at rs8192284 showed no association with change in ESR or CRP in isolation (Table 2). Treatment outcome was also analysed according to the EULAR response criteria, with no significant association between genotype at rs8192284 and response to treatment in the whole cohort (Table 3), or by anti-TNF agent (data not shown).

Discussion

The present study was undertaken to address the potential association of genotype at the sIL-6R A358C polymorphism and response to anti-TNF therapy in subjects with RA. We hypothesized that subjects with
the CC genotype, known to associate with higher levels of sIL-6R and hence increased IL-6 signal transduction, would have a poorer response to treatment. Our results, however, showed no association between genotype at A358C and response to anti-TNF therapy, or indeed with baseline disease activity, suggesting that variation in sIL-6R levels may not on their own be important in influencing disease activity or response to anti-TNF therapy in RA.

To our knowledge, this is the first study examining the association between the sIL-6R A358C polymorphism and response to anti-TNF therapy in RA. IL-6 is important in the pathogenesis of RA, evidenced by the IL-6 knockout mouse models demonstrating absent or greatly reduced manifestations of arthritis [15, 16], and the recent success of IL-6R blockade in humans with RA [7]. IL-6 levels are significantly reduced after anti-TNF treatment [17], and it is likely therefore that the reduction in inflammatory cytokine signalling downstream of TNF plays a significant role in the observed benefits of the anti-TNF treatment. Due to lack of expression of membrane-bound IL-6R, IL-6 signalling in rheumatoid joints is largely dependent on sIL-6R, and we therefore hypothesized that increased expression of this receptor in carriers of the C allele at rs8192284 would result in increased inflammatory joint activity, and a poorer response to anti-TNF treatment. Our results, however, showed no difference in either disease severity assessed by DAS28 or in response to treatment according to genotype at this marker, suggesting that the variability in response to these agents is not mediated by inter-individual variability in levels of sIL-6R.

There are some potential shortcomings in this study. First, signal transduction after binding of sIL-6R and IL-6 requires interaction with the gp130 receptor for signal transduction and can also be influenced by a soluble form of this receptor, which effectively functions as an antagonist [18]. Genetically mediated variation in expression of both of these molecules was not examined, but clearly has the potential to influence the results that we have demonstrated. Secondly, the extended disease

<table>
<thead>
<tr>
<th>Anti-TNF agent</th>
<th>rs8192284 genotype</th>
<th>n</th>
<th>Baseline DAS28, mean (s.d.)</th>
<th>Change DAS28, mean (s.d.)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etanercept (n = 455)</td>
<td>AA</td>
<td>168</td>
<td>6.64 (1.02)</td>
<td>-2.53 (1.36)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>212</td>
<td>6.77 (1.01)</td>
<td>-2.61 (1.57)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>55</td>
<td>6.69 (0.94)</td>
<td>-2.43 (1.44)</td>
<td>0.81</td>
</tr>
<tr>
<td>Infliximab (n = 453)</td>
<td>AA</td>
<td>159</td>
<td>6.69 (0.98)</td>
<td>-2.32 (1.57)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>222</td>
<td>6.78 (0.94)</td>
<td>-2.44 (1.60)</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>56</td>
<td>6.83 (1.03)</td>
<td>-2.27 (1.40)</td>
<td>0.66</td>
</tr>
<tr>
<td>Adalimumab (n = 142)</td>
<td>AA</td>
<td>49</td>
<td>6.53 (0.95)</td>
<td>-2.78 (1.61)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>61</td>
<td>6.55 (1.00)</td>
<td>-2.59 (1.61)</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>23</td>
<td>6.25 (0.98)</td>
<td>-2.71 (1.66)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

P-values stated are by linear regression analysis, adjusted for current DMARD treatment, gender, baseline HAQ score and baseline DAS28.

<table>
<thead>
<tr>
<th>rs8192284 genotype</th>
<th>Baseline, mean (s.d.)</th>
<th>Change, mean (s.d.)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>47.1 (29.0)</td>
<td>-17.7 (25.2)</td>
<td>–</td>
</tr>
<tr>
<td>AC</td>
<td>47.1 (29.5)</td>
<td>-18.3 (25.0)</td>
<td>0.7</td>
</tr>
<tr>
<td>CC</td>
<td>45.8 (29.1)</td>
<td>-15.7 (22.4)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

P-values stated are for linear regression analysis, adjusted for gender and use of DMARDs and oral steroids.

<table>
<thead>
<tr>
<th>rs8192284 genotype</th>
<th>EULAR response</th>
<th>Poor (35.2)</th>
<th>Moderate (38.5)</th>
<th>Good (35.8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>70</td>
<td>209 (38.5)</td>
<td>92 (35.8)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>102 (51.3)</td>
<td>260 (47.9)</td>
<td>132 (51.4)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>27 (13.5)</td>
<td>74 (13.6)</td>
<td>33 (12.8)</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

P-value stated is for the chi-squared test.
duration of the participants could result in a lack of sensitivity of the DAS28 score to detect change in inflammatory activity on a background of considerable joint damage, although the lack of association with change in ESR or CRP taken in isolation makes it unlikely that the joint damage is masking an important association between genotype at rs8192284 and treatment effect. Thirdly, there is potential for lack of standardization in the recording of DAS28 scores among the large numbers of clinicians who contributed to this study, although this is balanced by the high power to detect a clinically significant change in DAS28.

In conclusion, we have shown that genetic variation at the functional sIL-6R A358C SNP does not influence disease activity or response to anti-TNF therapy in patients with RA. The substantial variation in sIL-6R levels as a result of genetic variation at this marker does, however, suggest that it may be a promising pharmacogenetic target in patients treated with the anti-IL-6R monoclonal antibody tocilizumab.

Rheumatology key messages

- Genotype at the functional sIL-6R A358C SNP is not associated with baseline DAS28 in patients with RA.
- Genotype at sIL-6R A358C does not influence response to anti-TNF treatment in patients with RA.

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Appendix

Members of the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS) are as follows.

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