The Role of Fcγ Receptor Polymorphisms in the Response to Anti-Tumor Necrosis Factor Therapy in Psoriasis
A Pharmacogenetic Study

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IMPORTANCE Variability in genes encoding proteins involved in the immunological pathways of biological therapy may account for the differences observed in outcomes of anti–tumor necrosis factor (TNF) treatment of psoriasis.

OBJECTIVE To assess the role of 2 Fcγ receptor (FcγR) polymorphisms in the response to anti-TNF therapy in psoriasis.

DESIGN Retrospective series of patients with psoriasis who received anti-TNF therapy (infliximab, adalimumab, or etanercept) from January 1, 2007, through December 31, 2010. Patients were followed up for 12 weeks.

SETTING Two psoriasis referral centers.

PARTICIPANTS Seventy treatment-naive patients with moderate to severe psoriasis who received anti-TNF agents.

INTERVENTION Patients underwent FcγRIIA-H131R and FcγRIIIA-V158F polymorphism genotyping.

MAIN OUTCOMES AND MEASURES The Psoriasis Area and Severity Index and the body surface area were assessed at baseline and at treatment weeks 6 to 8 and 12. The polymorphism genotypes were correlated with the treatment outcomes.

RESULTS Bivariate analysis showed a nonsignificant association between FcγR low-affinity genotypes and greater improvement in the Psoriasis Area and Severity Index and body surface area at the end of treatment. Conversely, patients harboring high-affinity alleles presented a greater reduction in body surface area at the intermediate point, which remained independent in the multivariate analysis. We also detected an additive effect of both polymorphisms in the multivariate analysis. High-affinity alleles may contribute to a quicker response owing to a more efficient removal of relevant cells expressing TNF.

CONCLUSIONS AND RELEVANCE Preliminary results of this pilot study on the pharmacogenetics of FcγR and biological therapy in psoriasis suggest a role with clinical implications for FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms in the outcome of anti-TNF treatment of psoriasis. These results might help dermatologists in guiding therapeutic decisions, especially in very severe cases where a quick response is needed.

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Psoriasis is a chronic, debilitating inflammatory disease caused by genetic and environmental factors. Although psoriatic manifestations vary widely, most patients show well-circumscribed red plaques with silvery white dry scale mainly in the extensor areas and the scalp. Histopathological findings in psoriasis are characterized by aberrant epidermal proliferation and the presence of neutrophils and lymphocytes in the skin. In psoriasis, individuals with a susceptible genetic background present an immune system imbalance leading to keratinocyte activation with eventual hyperproliferation of the epidermis. The innate and adaptive immune systems are impaired, with cutaneous overexpression of helper T cell 1 cytokines (interleukin 2 [IL-2], IL-6, IL-8, IL-12, interferon γ [IFN-γ], and tumor necrosis factor [TNF]) and helper T cell 17 cytokines (IL-17, IL-21, and IL-22). This cytokine network is believed to be responsible for the initiation, maintenance, and recurrence of skin lesions.

The emergence of biological therapies has significantly improved the prognosis of patients with psoriasis and other inflammatory disorders (eg, rheumatoid arthritis [RA] and Crohn disease) and neoplasms (eg, non–Hodgkin B-cell lymphoma) targeting proinflammatory cytokines and cell surface receptors. Biological agents are believed to exert their pharmacological effects through their variable portion (designed to block the target molecule) and their constant portion (the Fc fragment of IgG1, mostly IgG1), which specifically binds the human Fcγ receptors (FcγRs). The FcγRs are expressed on the surface of nearly all immune cells. On binding to the IgG Fc fragment, FcγRs can trigger different cell functions, such as cytolytic killing, induction of apoptosis, antibody-dependent cellular cytotoxicity (ADCC), and macrophage-mediated clearance of immunocomplexes. These actions can also be elicited by therapeutic antibodies and are partially responsible for the efficacy of these treatments.

Six types of human FcγR have been described (FcγRIA, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA, and FcγRIIIB) encompassing genes located in chromosome 1. Some of these genes present functional allelic single-nucleotide polymorphisms (SNPs) generating interindividual differences in the affinity for the Fc portion and, therefore, in the intensity of FcγR-mediated functions. The FcγRIIA type is expressed in platelets and most myeloid cells and presents an SNP resulting in an arginine (R) or histidine (H) at position 131 in the membrane ectodomain that affects receptor affinity for IgG immunocomplexes. The H131 allele presents higher affinity than the R131 allele for IgG1 and IgG2. The FcγRIIIA type is expressed predominantly on macrophages and natural killer cells and presents a functional SNP resulting in phenylalanine (F) or valine (V) at position 158. The V158 allele binds more avidly to IgG1, IgG2, and IgG3 subclasses than to the F158 allele. Besides being related to some autoimmune diseases, these polymorphisms have been shown to influence the clinical efficacy of biological therapies in neoplastic disorders, such as non–Hodgkin lymphoma (rituximab), colorectal cancer (cevuximab), and breast cancer (trastuzumab). These polymorphisms also have been found to influence the response of biological therapy in autoimmune diseases. The FcγRIIIA-V158F and FcγRIIA-H131R polymorphisms have been found to modify the outcome of patients with different types of autoimmune arthritis treated with anti-TNF agents. Finally, FcγRIIIA-V158F was related to the response to infliximab in patients with Crohn disease.

Various biological agents have been used to treat psoriasis, of which anti-TNF agents are the most widely used. Infliximab (a chimeric monoclonal antibody), adalimumab (a human monoclonal antibody), and etanercept (a fusion protein) are all TNF blockers containing the Fc fragment of human IgG1 in their structure. Despite their differences in the Fc fragments (eg, etanercept does not have the CH1 domain), the 3 TNF blockers can bind the FcγR system, especially in the presence of TNF. In addition, all TNF blockers are able to induce ADCC in vitro. Despite the proven therapeutic value of these treatments, they vary considerably in clinical efficacy, with 20% to 50% of patients not responding satisfactorily. Few pharmacogenetic studies have assessed this issue, and no well-established clinical, laboratory, or genetic predictive markers are available. Nonetheless, variability in genes encoding proteins involved in the immunological pathways of biological therapy may account for the differences observed in outcomes of anti-TNF therapy of psoriasis. The aim of this study was to assess the potential role of the FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms in the clinical response to anti-TNF therapy in psoriasis to determine a potential pharmacogenetic marker of treatment response.

**Methods**

**Patients**

From January 1, 2007, through December 31, 2010, we included 70 white patients with psoriasis who attended 2 dermatology services in tertiary hospitals in Barcelona and received anti-TNF therapy (infliximab, adalimumab, or etanercept). All patients were 18 years or older and had moderate to severe psoriasis, defined as a Psoriasis Area and Severity Index (PASI) greater than 10 and a body surface area (BSA) greater than 10%. All patients had psoriasis refractory (or with contraindications) to at least 1 conventional systemic therapy, including methotrexate sodium, cyclosporine, acitretin, or phototherapy. Patients received anti-TNF monotherapy, were naive of any biological therapy, and completed at least 12 weeks of treatment. The dose for each biological agent consisted of the standard regimen for induction therapy (infliximab, 5 mg/kg for weeks 0, 2, and 6; etanercept, 50 mg twice a week; and adalimumab, 80 mg for the first week, 40 mg for the following week, and then 40 mg biweekly). Although patients were recruited in different hospitals and the outcomes measures were evaluated by different physicians, all the evaluating physicians (J.M.C., C.F., and M.A.-G.) were members of the Spanish Psoriasis Group of the Spanish Academy of Dermatology and Venereology and followed the same clinical criteria. The ethics committees of the 2 hospitals approved the study protocol, and written informed consent was obtained from all participants before blood extraction for FcγR genotyping.
Clinical Assessment
Clinical and epidemiological data were retrospectively obtained from clinical medical records. The PASI and BSA were assessed at baseline and at treatment weeks 6 to 8 and 12 in all patients. Patients with an improvement in PASI of at least 75% at week 12 (PASI-75) were considered good responders. Treatment failure was defined as worsening of PASI or an improvement of less than 50% of the baseline PASI at week 12.

FcγR Polymorphism Genotyping
Peripheral blood was extracted and leukocyte genomic DNA was purified using a commercially available DNA blood kit (Qiagen). The biallelic polymorphism FcγRIIA-H131R was assessed using a polymerase chain reaction (PCR) sequencing-based typing method. Briefly, a 367-basepair genomic DNA fragment was PCR amplified using specific primer pairs. The PCR reaction mix included 50 to 200 ng of DNA, 10 pmol of primers, 1 U of Taq DNA polymerase (Expand 20-kb PLUS; Roche Diagnostics GmbH), and 0.5 mM deoxycytidinucleotide tripophosphates diluted in buffer (Expand 20-kb PLUS) at a final volume of 20 mL. The cycling conditions included 1 cycle at 94°C for 5 minutes; 10 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 60 seconds; 25 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds; and 1 cycle at 72°C for 7 minutes. Five microliters of the resulting amplicons were treated with reagent (ExoSAP-IT; USB Corporation) and directly sequenced using a cycle sequencing kit (BigDye Terminator, version 1.1; Applied Biosystems Inc) according to the manufacturer's instructions, with sense gene–specific primers. Sequencing reactions were analyzed by capillary electrophoresis in an automated single-capillary DNA sequencer (ABI PRISM 3100; Applied Biosystems Inc). A previously reported allele-specific PCR method was used to genotype the biallelic functional FcγRIIA-V158F polymorphism with some modifications.23 Amplicons were visualized by electrophoresis on agarose gel, ethidium bromide staining, and UV illumination.

Statistical Analysis
Outcome measures were correlated with the FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms. Differences in the frequencies of genotypes in the groups of response were analyzed using the 2-tailed t test, χ2 test, and Pearson product moment correlation test. The potential influence of clinical and epidemiological factors on the clinical outcome was evaluated by regression models. P ≤ .05 was considered significant. The statistical analysis was performed using commercially available software (SPSS, version 17.0; SPSS, Inc).

Results
Descriptive Study
The clinical, epidemiological, and genetic characteristics of our cohort are provided in Table 1. Sixty-two patients were men (74%), and the mean age of the patients was 45.8 years. Mean disease duration was 17.4 years. Etanercept was the most widely used anti-TNF therapy (38 patients [54%]), followed by adalimumab (18 [26%]), and infliximab (14 [20%]) (Table 2). A PASI-75 was achieved by 53 patients (76%) at week 12 (13 patients receiving infliximab [93%], 14 receiving adalimumab [78%]), and 26 receiving etanercept [68%]). Frequencies of the FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms are also shown in Table 1. The frequencies of the genotypes of these polymorphisms were similar to those observed in the healthy Spanish population and were in Hardy-Weinberg equilibrium.24-28 Figure 1 illustrates the evolution of PASI and BSA over time. At the end of treatment, most patients experienced a notable improvement in PASI and BSA.

Correlation Between Genotypes of FcγR Polymorphisms and Outcome Measures
The bivariate analysis is shown in Table 3. Outcome measures were correlated with genotypes, which were pooled with

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patients (n = 70)</th>
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<tbody>
<tr>
<td>Sex, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52 (74)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (26)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>45.8 (12.7)</td>
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<tr>
<td>Age at disease onset, mean (SD), y</td>
<td>26.2 (13.7)</td>
</tr>
<tr>
<td>Disease duration, mean (SD), y</td>
<td>17.4 (8.3)</td>
</tr>
<tr>
<td>Baseline PASI, mean (SD)</td>
<td>17.7 (11.1)</td>
</tr>
<tr>
<td>Baseline BSA, mean (SD), %</td>
<td>35.6 (19.4)</td>
</tr>
<tr>
<td>Genotypes, No. (%)</td>
<td></td>
</tr>
<tr>
<td>FcγRIIA</td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>16 (23)</td>
</tr>
<tr>
<td>HR</td>
<td>35 (50)</td>
</tr>
<tr>
<td>RR</td>
<td>19 (27)</td>
</tr>
<tr>
<td>FcγRIIIA</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>9 (13)</td>
</tr>
<tr>
<td>VF</td>
<td>42 (60)</td>
</tr>
<tr>
<td>FF</td>
<td>19 (27)</td>
</tr>
</tbody>
</table>

Abbreviations: BSA, body surface area; F, phenylalanine; FcγR, Fcγ receptor; H, histidine; PASI, Psoriasis Area and Severity Index; R, arginine; V, valine.

Table 2. Anti-TNF Agents Used

<table>
<thead>
<tr>
<th>Agent</th>
<th>All Patients, No. (%)</th>
<th>PASI at Week 12</th>
</tr>
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<tbody>
<tr>
<td>Etanercept</td>
<td>38 (54)</td>
<td>PASI-75 PASI &lt;50</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>18 (26)</td>
<td>26 (68) 4 (11)</td>
</tr>
<tr>
<td>Infliximab</td>
<td>14 (20)</td>
<td>14 (78) 2 (11)</td>
</tr>
<tr>
<td>Total</td>
<td>70 (100)</td>
<td>13 (93) 6 (9)</td>
</tr>
</tbody>
</table>

Abbreviations: PASI <50, PASI with improvement of less than 50%; PASI-75, Psoriasis Area and Severity Index with improvement of at least 75%; TNF, tumor necrosis factor.
regard to the presence of high-affinity alleles. At week 12, we observed a higher likelihood of achieving PASI-75 and a greater improvement in PASI and BSA in patients homozygous for low-affinity alleles of both SNPs, although these differences were not significant. We also analyzed the response to treatment over time for each polymorphism (Figure 2). Significant differences were found in the intermediate BSA (6-8 weeks) with regard to the studied polymorphisms. Patients harboring high-affinity alleles (ie, $HH_{131} + HR_{131}$ and $VV_{158} + VF_{158}$) had a lower mean intermediate BSA than did patients presenting only low-affinity alleles. These differences were not observed in the intermediate PASI values.

**Multivariate Analysis**

We performed a multivariate analysis to determine whether the significant association found between the presence of high-affinity alleles in the genotypes of the 2 SNPs and lower intermediate BSA was independent or was influenced by other confounding factors that could affect the outcome of treatment. The regression models provided in Table 4 demonstrate that the presence of high-affinity alleles in the genotype remained significantly associated with a better BSA intermediate response regardless of age, sex, the efficacy of the anti-TNF agent used (measured as the percentage of PASI improvement), and initial BSA. To analyze the potential combined effect of the 2 SNPs, we also analyzed the haplotype by calculating the number of high-affinity alleles, ranging from 0 to 4, where 0 indicates the presence of non-high-affinity alleles (RRFF) and 4, the presence of 4 high-affinity alleles in the haplotype (HHVV). We analyzed the number of high-affinity alleles in the haplotype in a regression model that showed that the higher the number of high-affinity alleles, the better the intermediate response to therapy, suggesting that these SNPs act additively in the same immunological pathway of the mechanism of action of anti-TNF therapy. Finally, we performed multivariate analysis for final PASI and final BSA, but we found no significant associations (Table 4).

**Discussion**

Considering that the rationale for the efficacy of anti-TNF agents relies partly on the actions exerted by the Fc portion of their structure, we studied the possible association between 2 functional SNPs of FcγRs and the response to these therapies. We determined the response to treatment using validated outcome measures at different time points in 70 patients with moderate to severe psoriasis who underwent anti-TNF therapy for the first time and correlated the responses with the genotypes of the FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms.
First, we found a nonsignificant association between achievement of PASI-75 and BSA and PASI improvement and the low-affinity genotypes -RR131 and -FF158 at week 12. Second, we analyzed the influence of FcγRII polymorphisms on the response to therapy over time and found that the presence of high-affinity alleles of FcγRII-H131R and FcγRIIIA-V158F were significantly associated with a lower BSA in the intermediate point of treatment. These associations retained significance in the multivariate study regardless of sex, age, the efficacy of the anti-TNF agent used, and the initial BSA. Because both polymorphisms seemed to act in the same direction, we decided to analyze them together. To gain statistical power and solve the problem of having few examples of some haplotypes, we analyzed the total number of high-affinity alleles in the haplotype. A linear regression model showed a significant inverse association between the number of high-affinity alleles in the haplotype and intermediate BSA values, suggesting that both polymorphisms may act through the same immunological pathways. The simultaneous action of high-affinity alleles of FcγRII-H131R and FcγRIIIA-V158F might lead to enhanced ADCC of pathogenetically relevant cells expressing TNF on their membranes, producing a more rapid clinical response.

Table 4. Multivariate Linear Regression Models

<table>
<thead>
<tr>
<th>Explanatory Variablea</th>
<th>Intermediate BSA</th>
<th>Final BSA</th>
<th>Final PASI</th>
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<tr>
<td></td>
<td>β Coefficient</td>
<td>P Value</td>
<td>β Coefficient</td>
</tr>
<tr>
<td>FcγRII, HR+RR vs HH</td>
<td>0.372</td>
<td>.03</td>
<td>0.025</td>
</tr>
<tr>
<td>FcγRIIIA, VV+VF vs FF</td>
<td>0.425</td>
<td>.02</td>
<td>0.012</td>
</tr>
<tr>
<td>No. of high-affinity alleles in the haplotype</td>
<td>-0.391</td>
<td>.04</td>
<td>-0.024</td>
</tr>
</tbody>
</table>

Abbreviations: See Table 1.

* Adjusted by age, sex, initial BSA, and the efficacy of the anti-tumor necrosis factor agent (percentage of PASI improvement).

Intermediate indicates treatment weeks 6 to 8; final, treatment week 12.

* Differences were statistically significant. The initial BSA and percentage of PASI improvement were significant in all models.
response. In fact, ADCC-mediated apoptosis of TNF-bearing cells by natural killer cells and macrophages has been pointed out as a relevant mechanism of action of TNF blockers in psoriasis.\textsuperscript{39} Our results therefore suggest a pharmacodynamic effect of the FcγR polymorphisms studied in the early phases of treatment. However, we could only detect such association with the intermediate BSA values and not with the PASI. This enhanced ADCC-driven mechanism might induce a faster clearance of milder lesions than those with higher scores of erythema, infiltration, and desquamation, leading to a more significant improvement in the BSA than in the PASI at this point of treatment.

Although we found no significant differences, patients with low-affinity genotypes achieved a better response than those with high-affinity genotypes at the end of the treatment, suggesting that low-affinity alleles, which may be producing lower levels of FcγR-mediated drug clearance, may be pharmacokinetically relevant in further stages of the treatment. In fact, a pharmacokinetic study has shown that patients with RA with low-affinity alleles in their genotypes (ie, -131RR and -158FF) presented low-level clearance of infliximab and disease control with low-dose infliximab.\textsuperscript{30}

The FcγR polymorphisms analyzed in our study have been linked to the response to anti-TNF therapy in rheumatologic diseases in clinical studies.\textsuperscript{13,14,31} Tutuncu et al\textsuperscript{18} observed a better response to 3 months of infliximab, adalimumab, or etanercept treatment in 35 patients with RA or psoriatic arthritis who presented the low-affinity genotype FcyRIIA-FF158. The authors suggested that FcγR-mediated clearance of biological agents by phagocytes would be impaired owing to the presence of low-affinity receptors, thus increasing the half-life of the agent and its efficacy. More recently, Cañete et al\textsuperscript{30} detected an association between the low-affinity genotypes of the FcyRIIA-H131R and FcyRIIIA-V158F polymorphisms and a better response to infliximab in patients with RA at different time points. Patients with the -FF158 genotype presented better values for joint involvement at week 6, and those with the -RR131 genotype at week 20. Finally, Morales-Lara et al\textsuperscript{31} studied the role of FcγRIIIA-V158F in the response to infliximab in patients with RA, psoriatic arthritis, and ankylosing spondylitis. The results confirmed those of Cañete et al\textsuperscript{31} with regard to RA but also found that the high-affinity -V158 allele was associated with a better response to infliximab in patients with ankylosing spondylitis. These data, taken together, suggest that the influence of FcγR polymorphisms in the response to anti-TNF may depend not only on the time point of treatment but also on immunopathological factors specific for each disease.

The limitations of our study include the retrospective recording of data, the small sample size, and the lack of analysis of some factors that may have influenced the outcome, such as weight, drug blood levels, or the presence of anti-drug antibodies. Further studies taking these factors into account and using a longer follow-up and larger cohorts are needed to confirm our preliminary results to establish robust pharmacogenetic markers that could help physicians to improve the management of psoriasis in patients treated with anti-TNF agents. In particular, a study with a follow-up of at least 1 year may shed light on how low-affinity alleles affect plasma levels of the drug and thus the clinical efficacy.

Despite the limitations mentioned, we have tried to analyze the potential relationship between FcγRIIA-H131R and FcγRIIIA-V158F polymorphisms and the response to anti-TNF therapy in psoriasis. Our results seem to point to an initial pharmacodynamic effect powered by the simultaneous effect of the high-affinity alleles of these polymorphisms. These results might help dermatologists in guiding therapeutic decisions, especially in very severe cases where a quick response is needed.