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NEW POLYMORPHISMS ASSOCIATED WITH RESPONSE TO ANTI-TNF DRUGS IN PATIENTS WITH MODERATE-TO-SEVERE PLAQUE PSORIASIS

RUNNING TITLE: PHARMACOGENETICS OF ANTI-TNFs IN PSORIASIS

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ABSTRACT

Anti-TNF drugs are effective against psoriasis, although 20-30% of patients are non-responders. Few pharmacogenomic studies have been performed to predict the response to anti-TNF drugs in psoriasis. We studied 173 polymorphisms to establish an association with the response to anti-TNF drugs in patients with moderate-to-severe plaque psoriasis (N=144). We evaluated the response using PASI75 at 3, 6, and 12 months. The results of the multivariate analysis showed an association between polymorphisms in *PGLYR4*, *ZNF816A*, *CTNNA2*, *IL12B*, *MAP3K1*, and *HLA-C* genes and the response at 3 months. Besides, the results for polymorphisms in *IL12B* and *MAP3K1* were replicated at 6 months. We also obtained significant results for *IL12B* polymorphism at 1 year. Moreover, polymorphisms in *FCGR2A*, *HTR2A*, and *CDKAL1* were significant at 6 months. This is the first study to show an association with these polymorphisms. However, these biomarkers should be validated in large-scale studies before implementation in clinical practice.

INTRODUCTION

Psoriasis is a complex skin disease. Its etiology is unknown, but genetics and the immune system play a key role in its development [1]. Tumor necrosis factor (*TNF*), interleukin *(IL)-12, IL-23*, and the HLA-C*0602 allele are the main genetic risk factors for psoriasis [2]. Genome-wide association studies (GWAS) and candidate gene studies have provided initial data on these risk factors. TNFα and the p40 subunit of IL-12 and IL-23 are the current therapeutic targets in moderate-to-severe psoriasis (anti-TNF drugs and ustekinumab, respectively) [3]. Therefore, genetic studies can reveal new therapeutic targets and increase our knowledge of the etiology and pathogenesis of psoriasis.

Moderate-to-severe psoriasis can be treated using several anti-TNF drugs, including etanercept, adalimumab, and infliximab. Adalimumab and infliximab are antibodies, and etanercept is a dimeric fusion protein that acts against the proinflammatory cytokine, TNFα, which is involved in the development of psoriasis [3]. Anti-TNF drugs are usually safe and well tolerated, but patients can develop adverse effects or not respond to treatment [3]. Genetics can explain interindividual differences in the response to therapy. Few pharmacogenomic studies have been performed in psoriasis, and the results of the few studies performed have not been replicated [4-12]. Therefore, we performed a candidate gene study to investigate single-nucleotide polymorphisms (SNPs) that can predict the response to anti-TNF drugs in Caucasian patients with moderate-to-severe plaque psoriasis.

MATERIAL AND METHODS

Study population

Our study included 144 patients recruited between 16/10/2007 and 17/12/2012 from four hospitals in Madrid, Spain (Hospital Universitario de la Princesa, Hospital Universitario Gregorio Marañón, Hospital Universitario Fundación Alcorcón, and Hospital Universitario Infanta Leonor). All patients were Caucasians aged ≥ 18 years with moderate-to-severe plaque psoriasis (defined according to the European consensus published by Mrowietz et al. [2011] [13]) requiring treatment with biological drugs. The patients were treated with anti-TNF agents according to the Summary of Product Characteristics. Only patients who were naïve for anti-TNF treatment were included in the analysis. The parameter used to evaluate the effectiveness of treatment was the Psoriasis Area and Severity Index (PASI). Patients who achieved a 75% improvement over their baseline PASI (PASI75) were considered responders to treatment. We collected PASI75 data at 3, 6, and 12 months. All patients signed a written informed consent document to participate in this study. The protocol and informed consent document complied with Spanish legislation on biomedical research and were approved by the Ethics Committee for Clinical Investigation of Hospital Universitario de la Princesa.

Sample processing and genotyping

DNA was extracted from peripheral blood samples (EDTA 3 mL) using the MagNa Pure® System (Roche Applied Science, USA) and quantified in a NanoDrop® ND-1000 Spectrophotometer (Wilmington, USA). Samples were stored at –80ºC in the Clinical Pharmacology Service.

A total of 173 polymorphisms were evaluated using the IlluminaVeracode genotyping platform (Human Genotyping Unit-CeGen, Madrid, Spain). A description of the SNPs studied was shown in Supplementary Table S1 published by Prieto-Pérez et al. (2015) [14].

Statistical analysis

Allele and genotype frequencies, Hardy-Weinberg equilibrium, and linkage disequilibrium were analyzed using SNPStats [15]. The univariate analysis to evaluate the SNPs and the treatment response was performed using R 3.0.2. (SNPassoc package) [16] and SNPStats. We tested different logistic regression models (codominant, dominant, recessive, and additive). The optimal model was selected using the lower Akaike Information Criterion (AIC). SNPs with $p \leq 0.05$ in the univariate analysis were included in the multivariate analysis. Moreover, when several SNPs were in linkage disequilibrium and were significant in the univariate analysis, we analyzed the association between haplotype and response using SNPstats. Statistical significance was set at $p \leq 0.05$. The results were expressed as the odds ratio (OR), 95% confidence interval (CI), and p-value. Efficacy data were also analyzed using ITT-LOCF (Intention-To-Treat Last Observation Carried Forward) method [17].

The clinical variables compared between responders and non-responders included age at onset of psoriasis, type of psoriasis (I or II), weight, gender, presence or absence of psoriatic arthritis, and age at prescription of the first anti-TNF drug.

RESULTS

Population

The study population included 144 patients (84 men and 60 women) with moderate-to-severe plaque psoriasis treated with anti-TNF drugs. The phenotypic characteristics of the patients are shown in Table 1. The mean age at onset of psoriasis was 28.51 ± 14.04 years and the mean age when the first anti-TNF agent was prescribed was 43.61 ± 15.02 years.

The first anti-TNF drug was etanercept in 74 patients (51.39%), adalimumab in 42 (29.17%), and infliximab in 28 (19.44%). Throughout treatment, almost all patients (97%) were treated as monotherapy. A total of 106 patients achieved a PASI75 response at 3 months of treatment (73.61%). Data for the evaluation of the clinical response are missing in some patients owing to loss of follow-up, side effects, remission, intermittent therapy, and/or patient's wish (Table 1). Before 6 months, 6 patients decided not to continue with the treatment and we missed PASI evaluation at 6 months owing to lack of follow-up. In addition, 3 patients stopped anti-TNF treatment at 3-4 months because of lack of efficacy and 2 patients because of side effects. Before 1 year, other 8 patients did not wish to continue with the therapy. Moreover, the treatment was withdrawn because of lack of efficacy (N=8, 7-9 months), side effects (N=3, 6-8 months) or remission (N=1, 8 months). Therefore, 11 and 31 patients did not reach the visits at 6 and 12 months, respectively, and we included 133 and 113 patients to evaluate effectiveness at 6 months and 1 year of treatment. Of the 133 patients evaluated at 6 months, 102 achieved PASI75 (76.69%); of the 113 patients evaluated at 1 year, 92 achieved PASI75 (81.42%).

We did not obtain significant results for the following clinical variables evaluated for response to the treatment: age at onset of psoriasis (p=0.620), type I or II psoriasis

(p=0.740), weight (p=0.830), gender (p=0.950), presence or absence of psoriatic arthritis (p=0.920), age at prescription of the first anti-TNF drug (p=0.380), presence of comorbidities (p=0.530 for dyslipemia, p=0.300 for hypertension, p=0.510 for diabetes, p=0.190 for obesity), and prior therapies (p=0.390 for methotrexate, p=0.200 for cyclosporine, p=0.980 for acitretin, p=0.055 for phototherapy, p=0.200 for efalizumab).

Effectiveness

All significant results in the univariate analysis at 3 months (PASI75) are shown in Table S1 (22 significant SNPs). Only 6 SNPs were significant in the multivariate analysis (Table 2). Of these, 4 were associated with a lack of achievement of PASI75: AG/GG in rs2916205 (*PGLYRP4-24*; OR=3.62), CC in rs9304742 (*ZNF816A*; OR=7.66), AA in rs11126740 (*CTNNA2*; OR=20.56), and AG/GG in rs2546890 (*IL12B*; OR=3.22). However, patients with the CT/CC allele in rs96844 (*MAP3K1*; OR=0.17) and the CT/TT allele in rs12191877 (*HLA-C*; OR=0.30) responded better to anti-TNF drugs (Table 2).

All significant results in the univariate analysis at 6 months (PASI75) are shown in Table S2 (17 significant SNPs). Only 5 SNPs were significant in the multivariate analysis (Table 3): carriers of the CT/CC genotype for rs1801274 (*FCGR2A*; OR=13.32), CT/TT for rs6311 (*HTR2A*; OR=5.60), and AG/GG for rs2546890 (*IL12B*; OR=4.14) were poor responders. Nevertheless, carriers of the CT/CC genotype of rs96844 (*MAP3K1*; OR=0.24) and carriers of CT/TT in rs6908425 (*CDKAL1*; OR=0.14) achieved a better therapeutic response (Table 3).

We also performed a multivariate logistic regression analysis for PASI75 at 1 year of treatment (N=113; Table 4 and Table S3). We only obtained significant results for rs2546890 in *IL12B*: patients with the AG/GG genotype were more likely to be non-responders (Table 4). Moreover, rs2546890 is in linkage disequilibrium with rs6887695 in *IL12B* (which was also significant in the univariate analysis for PASI75 at 1 year of treatment, p=0.014; Table S3). The haplotype results showed an association between the GC haplotype (rs2546890 and rs6887695, respectively) and non-response to anti-TNF drugs at 1 year of treatment (Table S4).

The haplotype analysis (Table S4) showed an association between non-responders (PASI75) and the GACCT haplotype (rs2916205, rs821421, rs3006448, rs3006452, and rs3006457, respectively) in *PGLYRP* gene (p=0.028), TT haplotype (rs12191877 and rs10484554, respectively) in *HLA-C* (p=0.018), and TA haplotype (rs2073048 and rs2022544, respectively) in *C6orf10* (p=0.032) at 3 months of treatment. None of these haplotypes were significant at 6 months or 1 year of treatment. However, analysis of 2 SNPs in *FOXP3* (rs2280883 and rs3761548) revealed significant results in men at 6 months (TC haplotype; p=0.025; Table S4).

The results with ITT-LOCF method showed 6 significant SNPs in rs1801274 (*FCGR2A*; OR=6.53), rs6311 (*HTR2A*; OR=11.36), rs2546890 (*IL12B*; OR=3.27), rs6908425 (*CDKAL1*; OR=0.12), rs6028945 (*MAFB*; OR=0.12), and rs10945919 (QK1; OR=5.13) at 6 months of treatment (supplementary Table S5). Furthermore, we obtained 2 significant SNPs in rs96844 (*MAP3K1*; OR=0.20) and rs191190 (*TNFRSF1A*; OR=3.30) at 1 year of treatment (supplementary Table S6).

DISCUSSION AND CONCLUSIONS

Polymorphisms in *PGLYRP4-24* (rs2916205), *ZNF816* (rs9304742), and *CTNNA2* (rs11126740) have been associated with psoriasis [18-21]. However, the association between these SNPs and response to treatment has not yet been studied. Therefore, our study is the first to show an association between several SNPs and response to anti-TNF drugs in patients with moderate-to-severe plaque psoriasis.

PGLYRP4 encodes a protein involved in immune responses which binds to peptidoglycan and bacterial lipopolysaccharide [22]. This protein is found at the PSORS4 locus, which is within the epidermal differentiation complex and is expressed in epithelial cells in the skin [18, 22, 23]. Several polymorphisms in *PGLYRP4* seem to increase the risk of developing Crohn's disease [22], which is associated with psoriasis [2]. In addition, Sun et al. (2006) did not find an association between this gene and moderate-to-severe psoriasis in single SNP-based or haplotype-based tests in a case-control study [18]. We confirmed these results in a recent publication [14]. However, when we studied SNPs in the *PGLYRP4* gene, we found that carriers of the G allele of rs2916205 were 3.62 times more likely not to respond to anti-TNF drugs (Table 2). Moreover, we found a significant GACCT haplotype in the *PGLYRP* gene (rs2916205, rs821421, rs3006448, rs3006452, and rs3006457, respectively) that was associated with no response to anti-TNF drugs at 3 months (PASI75).

In addition, we describe for the first time that C allele of rs9304742 in *ZNF816A* gene were 7.66 times more likely not to respond to treatment (Table 2). This gene encodes a zinc-finger protein that could play a role in the recognition of specific proteins and have several regulatory functions [19]. Likewise, the SNP rs11126740 is found in *CTNNA2* and considerably affected the response to treatment (PASI75) at 3 months in the study

population. A allele carriers were 20.56 times more likely not to respond to treatment (Table 2). This gene encodes a catenin alpha 2 (cadherin-associated protein) involved in cellular adhesion. Overexpression of basal layer cadherins results in abnormal keratinocyte differentiation, a typical feature of psoriasis [24]. El Wahed Gaber et al. (2015) performed a study of gene expression of β-catenin (protein that interacts with α-catenin during cell adhesion) and demonstrated deregulation of β-catenin in psoriatic skin [24]. Therefore, these proteins may play a key role in the maintenance of normal tissue architecture.

Furthermore, the frequency of T allele carriers for rs12191877 was 70.8% for responders and 52.6% for non-responders at 3 month of treatment (p=0.027; Table 2). This SNP has also been associated with psoriasis and is in linkage disequilibrium with HLA-C*0602 [25, 26]. *HLA-C* plays a role in the immune system by presenting peptides derived from the lumen of the endoplasmic reticulum. HLA-C*0602 and late-cornified envelope (*LCE*; I carriers) genotypes have been associated with better response to anti-TNF drugs at 24 weeks (PASI75; N=116) [7]. In this sense, we found that carriers of the T allele for rs12191877 (*HLA-C*) were 3.33 times more likely to respond to anti-TNFs (Table 2). We also found a significant TT haplotype for rs12191877 and rs10484554, respectively, in *HLA-C* that was associated with response to anti-TNF agents at 3 months (PASI75). In contrast, the HLA-C*0602 allele has been associated with poor response to anti-TNF drugs [6]. Moreover, the HLA-C*0602 allele has been associated with an increased and faster response to ustekinumab [27]. Therefore, the *HLA-C* gene may play a relevant role in the development of the disease, as well as in the response to biological drugs. Ryan et al. (2014) compared the frequencies of *HLA-C* genotypes between responders and non-responders and found no significant results [5].

The results obtained for rs2546890 (*IL12B*) and rs96844 (*MAP3K1*) were replicated in our study at 3 and 6 months of anti-TNF treatment. Moreover, we replicated the association for rs2546890 at 1 year. Thus these SNPs could be predictors of anti-TNF response in the short and long terms. IL12 is a proinflammatory cytokine involved in the lymphocyte T helper 1 (Th1) pathway. This cytokine has been widely associated with psoriasis [28-30]. Moreover, rs2546890 in *IL12* has been associated with psoriasis [26]. We previously evaluated the influence of polymorphisms in *IL12B* (rs6887695 and rs3212227) on the response to anti-TNF drugs [6] but found no association. However, our results did indicate that carriers of the G allele of rs2546890 (*IL12B*) were 3.22, 4.11, and 2.79 times more likely not to respond to treatment at 3 months, 6 months, and 1 year, respectively, than patients carrying the AA genotype (Tables 2, 3, and 4). In addition, we found an association between the GC haplotype (rs2546890 and rs6887695, respectively) and response at 1 year of treatment (Table S4). Furthermore, we selected a series of SNPs in our previous study (see Table 2 by Prieto-Pérez et al. [2013]) [2]. Among the SNPs [2], we found an association between carriers of the C allele of rs96844 (*MAP3K1*) and better response to anti-TNF drugs at 3 and 6 months of treatment (5.88-fold and 4.17-fold, respectively; Table 2 and 3). Bowes et al. (2009) also found an association between the minor allele (G) of rs96844 (*MAP3K1*) and a good response to anti-TNF drugs in patients with RA (N=428) [31]. However, the authors did not validate their results in an independent cohort [31].

In addition, we obtained significant results for rs1801274 in *FCGR2A* at 6 months of treatment. Prieto-Pérez et al. (2013) [2] showed this protein to be associated with the response to anti-TNF therapy in RA [32, 33] (see Table 2 of Prieto-Pérez et al. [2013]). *FCGR2A* encodes Fc-γ receptor on the cell surface of macrophages and neutrophils. In

our study, carriers of the C allele of rs1801274 were 13.32 times more likely to be non-responders to anti-TNF drugs. Our results were consistent with those of Ramírez et al. (2012), who showed an association between the T allele (rs1801274) and a better response to anti-TNF drugs in patients with psoriatic arthritis [34], which is also associated with psoriasis [2]. In contrast, Dávila-Fajardo et al. (2015) found an association between the C allele and a better response to adalimumab in patients with RA at 14 weeks [35]. Julià et al. (2013) analyzed rs1801274 (*FCGR2A*) but obtained no significant results for PASI75 at 12 weeks of treatment with anti-TNF drugs in psoriatic patients [12].

Other SNPs associated with the response to anti-TNF drugs at 6 months of treatment in our population were rs6311 (*HTR2A*) and rs6908425 (*CDKAL1*). rs6311 in *HTR2A* (which encodes a serotonin receptor) was associated with late-onset psoriasis in a Thai population [36]. Psoriasis is characterized by high production of cytokines that could be inhibited in the presence of 2,5-dimethoxy-4-iodoamphetamine (selective serotonin receptor agonist) [37]. This inhibition was more pronounced in carriers of the T and C alleles of rs6314 and rs1328674, respectively, in patients with RA [37]. Moreover, we found an association between psoriatic carriers of the T allele of rs6311 (*HTR2A*) and a worse response to anti-TNF drugs (5.60-fold). In addition, a gene-gene interaction was described for *HTR2A* and the major genetic risk factor for RA, *HLA-DRB1* [38]. These results suggest that *HTR2A* may be associated with the immune system and could be a risk factor for psoriasis. Besides, rs6908425 (*CDKAL1*) has been described as a risk factor for psoriasis [39]. Our results showed an association between carriers of the T allele of rs6908425 (*CDKAL1*), which has an unknown function, and better response to anti-TNF drugs (7.14-fold) at 6 months. Nevertheless, the influence of this SNP on the response of anti-TNFs has not been studied yet.

Other haplotypes associated with response to anti-TNF drugs were TA for rs2073048 and rs2022544, respectively, in *C6orf10* (PASI75 at 3 months) and TC for rs2280883 and rs3761548, respectively, in *FOXP3* (PASI75 at 6 months in men). SNPs in *C6orf10* and *FOXP3* genes were previously associated with psoriasis, but not with response to anti-TNF drugs [25, 40-42]. *FOXP3* is expressed by regulatory T cells and has been associated with some autoimmune disorders [41]. While the function of the protein C6orf10 is not known, it has been described as a potential downstream effector of TNFα [25].

Our sample size was limited by the number of patients treated in the dermatology department. However, the limited sample size was counterbalanced by an exhaustive follow-up of patients and analysis of their data. Otherwise, we did not have enough statistical power to analyze each drug independently. We lost patients during the study at 6 and 12 months, but it is not clear how the problem that represents missing data may be addressed [43]. Our data showed patients non-responders at 3 months that were responders at 6 months and vice versa. For this reason, the main methodological approach used to analyze long-term efficacy data was "as-treated analysis". The problem with using ITT-LOCF for analyzing long-term efficacy results is that this analysis method assumes that efficacy will remain constant (consistent with the last known value) [17]. So, the results showed at 6 and 12 months of treatment should be considered as preliminary results due to missing data.

The SNPs in *FCGR2A*, *HTR2A*, *IL12B* and *CDKAL1* were replicated with ITT-LOCF analysis. In addition, SNPs in *IL12B* (rs2546890) and *MAK3K1* (rs96844) were significant at 6 and 1 year of treatment. So these SNP remain being long-term biomarkers. Moreover, ITT-LOCF analysis showed 2 new SNPs in *MAFB* (rs6028945)

and *QK1* (rs10945919) (Table S5; PASI75 at 6 months) and another one (rs191190) in *TNFRSF1A* (Table S6; PASI75 at 1 year) associated with response to anti-TNFs. The SNP in *TNFRSF1A* has been previously associated with psoriasis [14, 44], but not with response to the treatment. Furthermore, the SNPs in *MAFB* and *QKI* have been shown as possible predictors of response to anti-TNFs in patients with rheumatoid arthritis [45].

In conclusion, ours is the first study to show an association between several SNPs—rs2916205 (*PGLYR4*), rs9304742 (*ZNF816A*), rs11126740 (*CTNNA2*), rs2546890 (*IL12B*), rs96844 (*MAP3K1*), and rs12191877 (*HLA-C*)—and response to anti-TNFs in psoriatic patients (PASI75 at 3 months of treatment). All of the SNPs have regulatory functions and are involved in immune responses or differentiation of keratinocytes in the skin. Therefore, they affect the development of psoriasis and the response to anti-TNF drugs during the initial stages of treatment. The only SNPs replicated at 6 months were rs2546890 (*IL12B*) and rs96844 (*MAP3K1*). We also obtained significant results for rs2546890 (*IL12B*) at 1 year of treatment (PASI75). Finally, rs1801274 (*FCGR2A*), rs6311 (*HTR2A*), and rs6908425 (*CDKAL1*) were associated with response to anti-TNFs at 6 months and could be biomarkers of longer-term response. The results at 6 and 12 months of treatment should be considered carefully due to missing data.

Few studies have evaluated the effect of polymorphisms on the response to anti-TNF drugs [4-12]. Consequently, our findings add to current knowledge on the pharmacogenomics of moderate-to-severe plaque psoriasis. However, large studies continue to be necessary to validate biomarkers before they are routinely applied in clinical practice.

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CONFLICT OF INTEREST

F Abad-Santos has been a consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Farmalíder, Ferrer, GlaxoSmithKline, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Teva, and Zambon. E Daudén has potential conflicts of interest (advisory board member, consultant, grants, research support, participation in clinical trials, honoraria for speaking, and research support) with the following pharmaceutical companies: AbbVie (Abbott), Amgen, Janssen-Cilag, Leo Pharma, Novartis, Pfizer, MSD, and Celgene. P de la Cueva has conflicts of interest (advisory board member, consultant, grants, research support, participation in clinical trials, honoraria for speaking, and/or research support) with the following pharmaceutical companies: AbbVie (Abbott), Astellas, Janssen-Cilag, Leo Pharma, Novartis, Pfizer, MSD, Gebro, Isdin, and Lilly. JL López-Estebaranz has conflicts of interest (advisory board member, speaker, or participation in clinical trials) with AbbVie, Amgen, Pfizer, MSD, Janssen-Cilag, Lilly, Celgene. O Baniandrés has conflicts of interest (participation in clinical trials and honoraria for speaking) with the following pharmaceutical companies: AbbVie (Abbott), Janssen-

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REFERENCES

1. Prieto-Pérez R, Cabaleiro T, Dauden E, Ochoa D, Roman M, Abad-Santos F. Genetics of psoriasis and pharmacogenetics of biological drugs. *Autoimmune Dis* 2013b; **2013:** 613086.

2. Prieto-Pérez R, Cabaleiro T, Dauden E, Abad-Santos F. Gene polymorphisms that can predict response to anti-TNF therapy in patients with psoriasis and related autoimmune diseases. *Pharmacogenomics J* 2013a; **13**(4)**:** 297-305.

3. Prieto-Pérez R, Cabaleiro T, Dauden E, Ochoa D, Roman M, Abad-Santos F. Pharmacogenetics of topical and systemic treatment of psoriasis. *Pharmacogenomics* 2013c; **14**(13)**:** 1623-1634.

4. Julià A, Ferrándiz C, Daudén E, Fonseca E, Fernández-López E, Sanchez-Carazo JL*, et al*. Association of the PDE3A-SLCO1C1 locus with the response to anti-TNF agents in psoriasis. *Pharmacogenomics J* 2014; **15**(4)**:** 322-325.

5. Ryan C, Kelleher J, Fagan MF, Rogers S, Collins P, Barker JN*, et al*. Genetic markers of treatment response to tumour necrosis factor-alpha inhibitors in the treatment of psoriasis. *Clin Exp Dermatol* 2014; **39**(4)**:** 519-524.

6. Gallo E, Cabaleiro T, Roman M, Solano-Lopez G, Abad-Santos F, Garcia-Diez A*, et al*. The relationship between TNF-alpha promoter and IL12B/IL23R gene polymorphisms and the efficacy of anti-TNF-alpha therapy in psoriasis: a case control study. *Br J Dermatol* 2013; **169**(4)**:** 819-829.

7. Batalla A, Coto E, Gonzalez-Fernandez D, Gonzalez-Lara L, Gomez J, Santos-Juanes J*, et al*. The Cw6 and late-cornified envelope genotype plays a significant role in anti-tumor necrosis factor response among psoriatic patients. *Pharmacogenet Genomics* 2015; **25**(6)**:** 313-316.

8. Vasilopoulos Y, Manolika M, Zafiriou E, Sarafidou T, Bagiatis V. Pharmacogenetic analysis of TNF, TNFRSF1A, and TNFRSF1B gene polymorphisms and prediction of response to anti-TNF therapy in psoriasis patients in the Greek population. *Mol Diagn Ther* 2012; **16**(1)**:** 29-34.

9. Tejasvi T, Stuart PE, Chandran V, Voorhees JJ, Gladman DD, Rahman P. TNFAIP3 gene polymorphisms are associated with response to TNF blockade in psoriasis. *J Invest Dermatol* 2012; **132:** 596-600.

10. Di Renzo L, Bianchi A, Saraceno R, Calabrese V, Cornelius C, Iacopino L*, et al*. -174G/C IL-6 gene promoter polymorphism predicts therapeutic response to TNF-alpha blockers. *Pharmacogenet Genomics* 2012; **22**(2)**:** 134-142.

11. Gonzalez-Lara L, Batalla A, Coto E, Gomez J, Eiris N, Santos-Juanes J*, et al*. The TNFRSF1B rs1061622 polymorphism (p.M196R) is associated with biological drug outcome in Psoriasis patients. *Arch Dermatol Res* 2014.

12. Julià M, Guilabert A, Lozano F, Suarez-Casasus B, Moreno N, Carrascosa JM*, et al*. The role of Fcgamma receptor polymorphisms in the response to anti-tumor necrosis factor therapy in psoriasis A pharmacogenetic study. *JAMA Dermatol* 2013; **149**(9)**:** 1033-1039.

13. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths C, Nast A*, et al*. Definition of treatment goals for moderate to severe psoriasis: a European consensus. *Arch Dermatol Res* 2011; **303**(1)**:** 1-10.

14. Prieto-Pérez R, Solano-Lopez G, Cabaleiro T, Roman M, Ochoa D, Talegon M*, et al*. New immune system genetic polymorphisms associated with moderate-to-severe plaque psoriasis: a case-control study. *Br J Dermatol* 2015; **172**(5)**:** 1432-1435.

15. Solé X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 2006; **22**(15)**:** 1928-1929.

16. González JR, Armengol L, Sole X, Guino E, Mercader JM, Estivill X*, et al*. SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* 2007; **23**(5)**:** 644-645.

17. van Lumig PP, Driessen RJ, Kievit W, Boezeman JB, van de Kerkhof PC, de Jong EM. Results of three analytical approaches on long-term efficacy of etanercept for psoriasis in daily practice. *J Am Acad Dermatol* 2013; **68**(1)**:** 57-63.

18. Sun C, Mathur P, Dupuis J, Tizard R, Ticho B, Crowell T*, et al*. Peptidoglycan recognition proteins Pglyrp3 and Pglyrp4 are encoded from the epidermal differentiation complex and are candidate genes for the Psors4 locus on chromosome 1q21. *Hum Genet* 2006; **119**(1-2)**:** 113-125.

19. Sun LD, Cheng H, Wang ZX, Zhang AP, Wang PG, Xu JH*, et al*. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. *Nat Genet* 2010; **42**(11)**:** 1005-1009.

20. Tang H, Jin X, Li Y, Jiang H, Tang X, Yang X*, et al*. A large-scale screen for coding variants predisposing to psoriasis. *Nat Genet* 2014; **46**(1)**:** 45-50.

21. Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J*, et al*. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* 2008; **4**(3)**:** e1000041.

22. Zulfiqar F, Hozo I, Rangarajan S, Mariuzza RA, Dziarski R, Gupta D. Genetic Association of Peptidoglycan Recognition Protein Variants with Inflammatory Bowel Disease. *PLoS One* 2013; **8**(6)**:** e67393.

23. Kainu K, Kivinen K, Zucchelli M, Suomela S, Kere J, Inerot A*, et al*. Association of psoriasis to PGLYRP and SPRR genes at PSORS4 locus on 1q shows heterogeneity between Finnish, Swedish and Irish families. *Exp Dermatol* 2009; **18**(2)**:** 109-115.

24. El-Wahed Gaber MA, El-Halim Kandil MA, El-Farargy SM, Galbet DA. Beta-catenin expression in psoriasis. *Indian Dermatol Online J* 2015; **6**(1)**:** 13-16.

25. Feng BJ, Sun LD, Soltani-Arabshahi R, Bowcock AM, Nair RP, Stuart P*, et al*. Multiple Loci within the major histocompatibility complex confer risk of psoriasis. *PLoS Genet* 2009; **5**(8)**:** e1000606.

26. Ellinghaus E, Ellinghaus D, Stuart PE, Nair RP, Debrus S, Raelson JV*, et al*. Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat Genet* 2010; **42**(11)**:** 991-995.

27. Talamonti M, Botti E, Galluzzo M, Teoli M, Spallone G, Bavetta M*, et al*. Pharmacogenetics of Psoriasis: HLACw6 but not LCE3B/3C deletion nor TNFAIP3 polymorphism predisposes to clinical response to IL12/23 blocker ustekinumab. *Br J Dermatol* 2013; **169**(2)**:** 458-463.

28. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP*, et al*. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; **80**(2)**:** 273-290.

29. Lee YH, Song GG. Associations between interleukin-23R and interleukin-12B polymorphisms and psoriasis susceptibility: a meta-analysis. *Immunol Invest* 2013; **42**(8)**:** 726-736.

30. Zhu KJ, Zhu CY, Shi G, Fan YM. Meta-analysis of IL12B polymorphisms (rs3212227, rs6887695) with psoriasis and psoriatic arthritis. *Rheumatol Int* 2013; **33**(7)**:** 1785-1790.

31. Bowes JD, Potter C, Gibbons LJ, Hyrich K, Plant D, Morgan AW*, et al*. Investigation of genetic variants within candidate genes of the TNFRSF1B signalling pathway on the response to anti-TNF agents in a UK cohort of rheumatoid arthritis patients. *Pharmacogenet Genomics* 2009; **19**(4)**:** 319-323.

32. Cañete JD, Suarez B, Hernandez MV, Sanmarti R, Rego I, Celis R*, et al*. Influence of variants of Fc gamma receptors IIA and IIIA on the American College of Rheumatology and European League Against Rheumatism responses to anti-tumour necrosis factor alpha therapy in rheumatoid arthritis. *Ann Rheum Dis* 2009; **68**(10)**:** 1547-1552.

33. Nishio S, Yamamoto T, Kaneko K, Tanaka-Matsumoto N, Muraoka S, Kaburaki M*, et al*. Pharmacokinetic study and Fcgamma receptor gene analysis in two patients with rheumatoid arthritis controlled by low-dose infliximab. *Mod Rheumatol* 2009; **19**(3)**:** 329-333.

34. Ramírez J, Fernandez-Sueiro JL, Lopez-Mejias R, Montilla C, Arias M, Moll C*, et al*. FCGR2A/CD32A and FCGR3A/CD16A variants and EULAR response to tumor necrosis factor-alpha blockers in psoriatic arthritis: a longitudinal study with 6 months of followup. *J Rheumatol* 2012; **39**(5)**:** 1035-1041.

35. Dávila-Fajardo CL, van der Straaten T, Baak-Pablo R, Medarde Caballero C, Cabeza Barrera J, Huizinga TW*, et al*. FcGR genetic polymorphisms and the response to adalimumab in patients with rheumatoid arthritis. *Pharmacogenomics* 2015; **16**(4)**:** 373-381.

36. Ronpirin C, Tencomnao T, Wongpiyabovorn J. Association between the -1438A/G polymorphism of the serotonin 2A receptor gene and late-onset psoriasis in a Thai population. *Genet Mol Res* 2010; **9**(1)**:** 208-214.

37. Snir O, Hesselberg E, Amoudruz P, Klareskog L, Zarea-Ganji I, Catrina AI*, et al*. Genetic variation in the serotonin receptor gene affects immune responses in rheumatoid arthritis. *Genes Immun* 2013; **14**(2)**:** 83-89.

38. Seddighzadeh M, Korotkova M, Källberg H, Ding B, Daha N, Kurreeman FAS*, et al*. Evidence for interaction between 5-hydroxytryptamine (serotonin) receptor 2A and MHC type II molecules in the development of rheumatoid arthritis
Eur J Hum Genet 2010; **18**(7)**:** 821-826.

39. Li Y, Liao W, Chang M, Schrodi SJ, Bui N, Catanese JJ*, et al*. Further genetic evidence for three psoriasis-risk genes: ADAM33, CDKAL1, and PTPN22. *J Invest Dermatol* 2009; **129**(3)**:** 629-634.

40. Elder JT. Genome-wide association scan yields new insights into the immunopathogenesis of psoriasis. *Genes Immun* 2009; **10**(3)**:** 201-209.

41. Gao L, Li K, Li F, Li H, Liu L, Wang L*, et al*. Polymorphisms in the FOXP3 gene in Han Chinese psoriasis patients. *J Dermatol Sci* 2010; **57**(1)**:** 51-56.

42. Shen Z, Chen L, Hao F, Wang G, Liu Y. Intron-1 rs3761548 is related to the defective transcription of Foxp3 in psoriasis through abrogating E47/c-Myb binding. *J Cell Mol Med* 2010; **14**(1-2)**:** 226-241.

43. O'Neill RT, Temple R. The prevention and treatment of missing data in clinical trials: an FDA perspective on the importance of dealing with it. *Clin Pharmacol Ther* 2012; **91**(3)**:** 550-554.

44. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D*, et al*. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. *Nature* 2005; **437**(7057)**:** 369-375.

45. Liu C, Batliwalla F, Li W, Lee A, Roubenoff R, Beckman E*, et al*. Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. *Mol Med* 2008; **14**(9-10)**:** 575-581.

Table 1. Phenotypic characteristics of psoriatic patients.

	Naïve patients (N=144)
Age at onset of psoriasis (years)	28.51 ± 14.04
Men (%)	84 (58.33)
Women (%)	60 (41.67)
Weight (kg)	75.91 ± 13.74
Psoriasis type I (%)[1]	120 (83.33)
Psoriasis type II (%)[2]	24 (16.67)
Patients with PsA (%)	35 (24.31)
Presence of other comorbidities N (%):	
a. Dyslipidemia	53 (36.80)
b. Hypertension	23 (16.00)
c. Diabetes	15 (10.42)
d. Obesity (BMI ≥ 30.0)	44 (30.56)
Prior therapies N (%):	
a. Methotrexate	56 (38.89)
b. Cyclosporine	81 (56.25)
c. Acitretin	49 (34.03)
d. Phototherapy	76 (52.78)
e. Efalizumab	31 (21.53)
Number of prior therapies before anti-TNF treatment	1 (1-4)*
Wash-out periods (days)	28.18 ± 7.17
Age at first anti-TNF agent (years)	43.61 ± 15.02
Etanercept (%)	74 (51.39)
Adalimumab (%)	42 (29.17)
Infliximab (%)	28 (19.44)
Baseline PASI	22.56 ± 10.97
Clinical response at 3 months of treatment	
PASI at 3 months	3.93 ± 4.68
PASI 75 (%)	106 (73.61)
PASI 90 (%)	66 (45.83)
% improvement in PASI	81.83 ± 19.97
Clinical response at 6 months of treatment	
Missing data at 6 months[#]	11
Patients with effectiveness data	133
PASI at 6 months	3.57 ± 6.47
PASI 75 (%)	102 (76.69)
PASI 90 (%)	71 (49.31)
% improvement in PASI	84.76 ± 21.30
Clinical response at 1 year of treatment	
Missing data at 1 year[#]	31
Patients with effectiveness data	113
PASI at 1 year	2.92 ± 4.65
PASI 75 (%)	92 (81.42)
PASI 90 (%)	69 (61.06)
% improvement in PASI	86.77 ± 18.85

Data are shown as mean ± SD, number (%) or *median (range); PsA: psoriatic arthritis; BMI: Body Mass Index; PASI: Psoriasis Area and Severity Index; #: data missing for the evaluation of the clinical response owing to lack of follow-up, side effects, remission, intermittent therapy, and/or patient's wish; [1]: early-onset psoriasis (<40 years); [2]: late-onset psoriasis (≥40 years).

Table 2. Summary of the results of univariate and multivariate logistic regression analyses for PASI75 at 3 months of treatment (N=144). SNPs with p≤0.05 in the univariate analysis were included in the multivariate analysis (Table S1). Only polymorphisms that were significant for anti-TNF drugs in the multivariate analysis are shown.

SNP	Gene	Model	Risk genotype (%Responders/%Non-responders)	UNIVARIATE ANALYSIS ANTI-TNFs		MULTIVARIATE ANALYSIS ANTI-TNFs	
				OR* (95% CI)	p-value	OR* (95% CI)	p-value
rs2916205	*PGLYRP4-24*	D	AG/GG (20.8/39.5)	2.49 (1.12-5.55)	0.027	3.62 (1.00-13.07)	0.050
rs9304742	*ZNF816A*	R	CC (9.4/23.7)	2.98 (1.11-8.03)	0.034	7.66 (1.37-42.70)	0.020
rs11126740	*CTNNA2*	R	AA (4.8/23.7)	6.14 (1.91-19.78)	0.0018	20.56 (2.75-153.69)	0.003
rs2546890	*IL12B*	A	AG/GG (72.4/84.2)	1.91 (1.098-3.38)	0.024	3.22 (1.23-8.40)	0.017
rs96844	*MAP3K1*	A	CT/CC (55.7/31.6)	0.41 (0.21-0.83)	0.0081	0.17 (0.05-0.56)	0.004
rs12191877	*HLA-C*	A	CT/TT (70.8/52.6)	0.46 (0.24-0.87)	0.013	0.30 (0.11-0.88)	0.027

Abbreviations: PGLYRP4-24: peptidoglycan recognition protein 4, 24; ZNF816A: zinc finger protein 816; CTNNA2: catenin (cadherin-associated protein), alpha 2; IL12B: interleukin 12B; MAP3K1: mitogen-activated protein 3 kinase 1; HLA-C: major histocompatibility complex, class I, C; SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; A: additive; R: recessive; D: dominant; *: odds ratio of non-response.

Table 3. Summary of results of univariate and multivariate logistic regression analyses for PASI75 at 6 months of treatment (N=133). SNPs with p≤0.05 in the univariate analysis were included in the multivariate analysis (Table S2). Only polymorphisms that were significant in the multivariate analysis for anti-TNF drugs are shown.

SNP	Gene	Model	Risk genotype (%Responders/%Non-responders)	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
				OR* (95% CI)	p-value	OR* (95% CI)	p-value
rs1801274	*FCGR2A*	D	CT/CC (73.5/93.5)	5.22 (1.17-23.37)	0.009	13.32 (1.67-106.50)	0.015
rs6311	*HTR2A*	D	CT/TT (65.7/89.7)	4.53 (1.28-16.06)	0.007	5.60 (1.10-28.63)	0.038
rs2546890	***IL12B***	A	AG/GG (71.6/90.3)	2.16 (1.14-4.08)	0.014	4.14 (1.23-13.81)	0.022
rs96844	***MAP3K1***	D	CT/CC (54.9/22.6)	0.24 (0.09-0.61)	0.001	0.24 (0.06-0.97)	0.045
rs6908425	*CDKAL1*	A	CT/TT (30.4/12.9)	0.35 (0.12-1.00)	0.026	0.14 (0.03-0.66)	0.013

Abbreviations: FCGR2A: Fc fragment of IgG, low affinity IIa, receptor (CD32); HTR2A: 5-hydroxytryptamine (serotonin) receptor 2A; IL12B: interleukin 12B; MAP3K1: mitogen-activated protein 3 kinase 1; CDKAL1: CDK5 regulatory subunit associated protein 1-like 1; SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; A: additive; D: dominant; *: odds ratio of non-response.

Table 4. Summary of results of univariate and multivariate logistic regression analyses for PASI75 at 1 year of treatment (N=113). SNPs with p≤0.05 in the univariate analysis were included in the multivariate analysis (Table S3). Only polymorphisms that were significant in the multivariate analysis for anti-TNF drugs are shown.

SNP	Gene	Model	Risk genotype (%Responders/%Non-responders)	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
				OR* (95% CI)	p-value	OR* (95% CI)	p-value
rs2546890	***IL12B***	A	AG/GG (73.9/90.5)	2.99 (1.34-6.66)	0.005	2.79 (1.02-7.64)	0.046

Abbreviations: IL12B: interleukin 12B; SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; A: additive; *: odds ratio of non-response.