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ORIGINAL ARTICLE

Candidate's single-nucleotide polymorphism predictors of treatment nonresponse to the first anti-TNF inhibitor in ankylosing spondylitis

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Abstract The objective of this study is to identify single-nucleotide polymorphisms (SNPs) predictors of treatment nonresponse to the first anti-TNF-alpha agent in ankylosing spondylitis (AS). Patients were classified as "nonresponders" if they failed to achieve improvement ≥50 % of the initial BASDAI. We selected candidate SNPs previously reported, associated with susceptibility or pathogenesis of AS and with other spondylarthropaties (SpAs). The predictors of nonresponse were modeled with multiple logistic regression. The predictive power of the genetic model of nonresponse to treatment was tested with AUC-ROC. One hundred and twenty-one (121) AS patients fulfilled the inclusion criteria. Of the candidate SNPs tested for association with treatment effectiveness, five independent predictors were identified: rs917997, rs755622, rs1800896, rs3740691, and rs1061622. The genetic model of nonresponse to treatment had a predictive power of 0.77 (95 % CI 0.68–0.86). Our study identified several polymorphisms which could be the useful genetic biomarkers in predicting nonresponse to anti-TNF-alpha therapy.

Keywords Ankylosing spondylitis · Anti-TNF-alpha agents · SNPs · Treatment response

This study was carried out on behalf of the REGISPONSER Study Group.

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Introduction

Ankylosing spondylitis (AS) is an inflammatory rheumatic disease in which the inflammatory process mainly involves the spine and, to a lesser extent, the peripheral joints [1]. One of the most important clinical challenges is to control the inflammation and, therefore, to maintain AS patients symptom-free. Several studies have strongly confirmed the implication of the pro-inflammatory cytokine TNF-alpha in the pathogenesis of AS [2–4]. Hence, after a documented failure to previous nonsteroidal anti-inflammatory drugs (NSAIDs), anti-TNF-alpha agents are being administrated in patients with active disease, according to Assessment of SpondyloArthritis international Society (ASAS) recommendations.

Anti-TNF-alpha agents act by inhibiting the binding of TNF-alpha to its receptors and therefore interfere with TNF-alpha signaling transduction pathways. Placebo-controlled randomized trials (RTCs) revealed similar efficacy in controlling active disease for all four accepted TNF-alpha inhibitors (infliximab [5], etanercept [6], adalimumab [7], and golimumab [8]).

Clinical markers in response to anti-TNF-alpha agents [9–12] have been investigated in several RTCs. Nevertheless, patient selection for RTCs is not always representative

of patients to whom drugs are prescribed in clinical practice. Thus, data from observational registries provide valuable knowledge about daily patients. Recent evidence from observational studies has reported that raised erythrocyte sedimentation rate (ESR) level, higher C-reactive protein (CRP) level, lower BASFI, younger age at baseline, male gender, peripheral arthritis, and concurrent use of disease modifying antirheumatic drug (DMARDS), primly methotrexate (MTX) and sulphasalazine (SSZ), showed association with BASDAI 50 clinical response [13–15]. Still, data from meta-analysis do not support any benefit of MTX and only some benefit for SSZ in the treatment of AS [16, 17]. Moreover, there is a high percentage of AS patients who remained disease-active despite long-term anti-TNF-alpha treatment [18, 19], in which case genetic background may play an important role.

In contrast with rheumatoid arthritis (RA), few studies have analyzed the role of genetic markers in the response to anti-TNF-alpha treatment in AS patients and the results were contradictory. Most studies analyzed either HLA-B27 status of the patients or TNF-alpha-gene polymorphisms as potential predictor factors in response to biological treatment in AS [10–12, 20, 21].

Pharmacogenomic studies, focusing on genes involved in AS etiology and pathogenesis, in order to analyze the role of allelic polymorphisms in the individual difference in treatment response to TNF-alpha inhibitors, are lacking in AS. Taking into account the cost and the potential severe side effects of these agents, identification of genetic biomarkers of treatment inefficacy would be of major use for prospectively selecting patients that will most likely respond to such treatment.

Therefore, the aim of our study was to identify SNPs predictors of treatment nonresponse to the first TNF-alpha inhibitor in Spanish AS patients from daily clinical practice.

Patients and methods

Patients and study design

We performed a longitudinal multicenter study on AS patients enrolled in the REGISPONSER registry [22]. REGISPONSER commenced in 2004 and gathers over 2,000 patients from 31 rheumatology departments throughout Spain, followed up annually for 5 years. Out of all REGISPONSER patients, 529 patients [23] fulfilled the modified New York Criteria for AS [24]—(REGISPONSER-AS cohort). For this study, we only selected the patients with AS who started their first TNF-alpha inhibitor according to ASAS recommendation, during the interval between two REGISPONSER-AS scheduled visits. The decision to commence a particular agent depended on the decision of the attending rheumatologist together with the patient's specific preference. Until 2006, patients were treated with either infliximab or etanercept, since adalimumab has been approved for AS treatment in Spain in 2006. None of the patients had been treated with Golimumab. In this study, patients could receive concomitant medication with either NSAIDs or DMARDs (sulphasalazine, methotrexate) as prescribed by their rheumatologist.

Baseline characteristics of the disease, prior anti-TNF-alpha treatment, together with baseline and follow-up parameters of disease activity were registered and analyzed in our study. They included patient's clinical and demographic data such as gender, age, ethnicity, age at disease onset, family history of SpA, initial SpA symptoms (low back pain, enthesitis, dactylitis, coxitis, uveitis, peripheral arthritis, psoriasis, inflammatory bowel disease), and comorbidities. Disease activity parameters were assessed with (1) BASDAI (on a scale 0–10), (2) erythrocyte sedimentation rate (ESR), (3) C-reactive protein (CRP, mg/L). ASAS-endorsed disease activity score (ASDAS) was calculated using the accepted formula with CRP.

Definition of nonresponse

This is a multicenter evaluating response study, and in order to standardize, all centers annually assessment reports were required from all the doctors who participated in the study, but each patient was evaluated in its own center every 12–20 weeks, and only patients who maintain an adequate therapeutic response after 1 year remained enrolled in the study. Thus, we classified patients as "responders" if they achieved BASDAI 50 clinical response at the assessment visit and "nonresponders" if they failed to achieve BASDAI 50 clinical improvement at the assessment visit, according to ASAS guidelines (a 50 % improvement of the initial BASDAI).

The primary outcome was to identify genetic polymorphisms associated to patients who commenced TNF-alpha blockers and did not achieve BASDAI 50 improvement criteria at the assessment visit.

Genotyping

Genomic DNA was isolated from saliva samples using the Oragene™ DNA Self-Collection kit (DNA Genotek Inc., Ottawa, Canada), following the manufacturer's extraction protocol. After an extensive bibliographic search, we selected candidate SNPs previously reported to be associated with susceptibility or pathogenesis of AS and with other SpAs (psoriatic arthritis, juvenile idiopathic arthritis, reactive arthritis, undifferentiated arthritis and

inflammatory bowel disease-associated spondyloarthropathy), SNPs associated with autoimmune and bone-related diseases, and SNPs from the metabolic pathways of the IL-23 receptor (IL-23R) and endoplasmic reticulum aminopeptidase 1 (ERAP1) genes. In total, 384 candidate SNPs distributed in 190 genes were analyzed in this study. SNP genotyping was performed using the Illumina Golden gate genotyping platform (Illumina, Inc., San Diego, CA, USA) [25].

Statistical analysis

Statistical analysis was performed with SPSS v19.0 software (SPSS, Chicago, IL, USA) and SVS software v7.3.1 (Golden Helix Inc., Bozeman, Montana, USA). Patient population data in the study were shown as mean and standard deviation (±SD) for quantitative variables and as absolute number and relative frequencies (%) for qualitative variables. We compared clinical characteristics of treatment response groups with the χ^2 test for categorical variables and with the unpaired student *t* test for continuous variables.

A test for deviation from Hardy–Weinberg equilibrium (HWE) was performed for each SNP using the Helix Tree software version 7.3.1. Pruning of the initial genotype dataset with default parameters (exclusion of SNPs with poor genotype cloud clustering, of SNPs with call rate <85 %, of SNPs with severe deviation from HWE ($P < 0.0001$), and of samples with call rate <85 %) led to 456 samples and 345 SNPs being analyzed [26, 27]. Measures of pairwise linkage disequilibrium (LD) ($r^2 > 0.8$) were determined using Haploview version 4.1 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) [28]. All SNPs reported in this study had minor allele frequency (MAF) >10 %.

An association test between allele frequencies and the treatment response groups was performed with the χ^2 test. The magnitude of allele association was expressed as odd ratio (OR) with a 95 % confidence interval (CI) (OR >1 indicates a risk allele, and OR <1 indicates a protective allele). *P* values were adjusted using a single-value permutation test (1,000 permutations). The probability of nonresponse was modeled using logistic regression, considering as dependent variable the variable "nonresponder" (1 = yes, 0 = no) and as independent variables the polymorphisms identified statistically significant associated with nonresponse to treatment in the allele frequency χ^2 test after adjustment. Independent variables were introduced as genotypes groups (1—homozygote and heterozygote for risk allele, 0—homozygote without risk allele) after dominance or recessive behavior for each SNPs was tested. The genotypes which showed association with nonresponse to treatment in the univariate analysis ($P < 0.05$) were entered into the multiple backward logistic regression through the Wald statistic test. We used elimination until $P \geq 0.15$; the reduced model was compared with the initial model using the likelihood ratio test. We tested the discrimination of the final model via the Hosmer–Lemeshow statistic and the receiver operating characteristic (ROC) curve with 95 % CI. To analyze the predictive power of our genetic model of nonresponse to biological treatment, AUC-ROC was used (Analyse-it software v2.09, Leeds, UK). Contrasts were all bilateral and *P* values <0.05 were considered statistically significant.

Ethical approval

This study was approved by the Ethics Committee of Reina Sofia University Hospital and of Puerta de Hierro Majadahonda University Hospital, Madrid, Spain. Each patient signed an informed consent upon inclusion in the REGISPONSER-AS, according to the fundamental principles established in the Declaration of Human Rights in Helsinki.

Results

Response status to anti-TNF-alpha treatment

Among the 529 REGISPONSER-AS patients, 121 AS patients fulfilled the inclusion criteria. Sixty-eight (56.2 %) were responders and fifty-three (43.8 %) were nonresponders to anti-TNF-alpha treatment at the assessment visit after applying BASDAI 50 improvement criteria compared with baseline (initiation of TNF-alpha treatment). Baseline clinical and demographic characteristics are summarized in Table 1. The mean age of studied patients at treatment initiation visit was 47.7 ± 9.5 years, with mean age at onset of the disease of 26.6 ± 10.6 years and mean disease duration since first symptoms of 21.1 ± 8.9 years. There were no statistically significant differences in baseline clinical and demographic characteristics between the two treatment response groups, with the exception of those patients who did not respond to anti-TNF-alpha treatment which had a statistically significantly older age at disease onset (28.4 ± 8.7 vs. 24.2 ± 9.4; $P = 0.021$). Mean anti-TNF-alpha treatment duration between initiation and the assessment visit for all patients was 12 ± 3 months. After applying ASDAS formula, we did not find statistically significant differences in baseline disease activity between the two treatment response groups. Interestingly, at baseline, the nonresponders group had a trend of lower-inflammation biomarkers than patients which responded to biological treatment (Table 1), but at the assessment visit, they showed significantly higher ESR (mm/h) (22.8 ± 22.4 vs.

Table 1 Baseline characteristics and disease activity in AS patients at inclusion visit

Clinical variables and disease activity at baseline*	All patients $N = 121$	Responders $N = 68$	Nonresponders $N = 53$	P value
Age (±SD) (year)	47.7 ± 9.5	46.3 ± 10.2	49.6 ± 8	NS
Age at disease onset (±SD) (year)	26.6 ± 10.6	24.2 ± 9.4	28.4 ± 8.7	0.021
Males *n* (%)	89 (73.6)	48 (70.6)	41 (77.4)	NS
Race caucasian *n* (%)	112 (99.1)	64 (100)	48 (98)	NS
Disease duration (±SD) (year)	21.1 (±8.9)	21.5 (±10.4)	21.16 (±6.6)	NS
Inflammatory back pain *n* (%)	118 (97.5)	65 (95.6)	53 (100)	NS
Peripheral arthritis *n* (%)	55 (45.8)	31 (46.3)	24 (45.3)	NS
Coxitis *n* (%)	5 (4.1)	4 (5.9)	1 (1.9)	NS
Entesitis *n* (%)	46 (38.3)	25 (37.3)	21 (39.6)	NS
Uveitis *n* (%)	27 (22.7)	15 (22.4)	12 (23.1)	NS
Dactilitis *n* (%)	10 (8.8)	6 (8.8)	4 (7.8)	NS
Psoriasis *n* (%)	14 (11.7)	6 (8.8)	8 (15.4)	NS
Colitis *n* (%)	17 (14)	13 (19.1)	4 (7.5)	NS
Family history of SpA *n* (%)	21 (20.2)	11 (18.6)	10 (22.2)	NS
HLA-B27 positivity *n* (%)	94 (77.7)	54 (79.4)	40 (75.5)	NS
ESR (mm/h) (±SD)	32.3 ± 4.8	32.8 ± 25.7	31.7 ± 23.8	NS
CRP (mg/L) (±SD)	17.6 ± 17.2	20 ± 20.1	14.3 ± 12	NS
BASDA I (±SD)	5.68 ± 2.05	5.04 ± 2.1	6.3 ± 1.7	0.003
ASDAS (±SD)	3.6 ± 0.9	3.5 ± 0.9	3.84 ± 0.7	NS

ESR erythrocyte sedimentation rate, *HLA* human leukocyte antigen, *NS* not statistically significant, *SD* standard deviation, *y* years; * Baseline-at the inclusion in REGISPONSER, before anti-TNF-alpha therapy

Table 2 Allelic association test of nonresponse to anti-TNF-alpha treatment in AS patients *according to BASDAI50 clinical response*

Snp	Gene	Risk allele, OR (95 % CI)	X^2 test unadjusted P	X^2 test adjusted P*
rs755622	MIF	G, 2.92 (1.41–6.03)	0.002	0.003
rs917997	IL18RAP	A, 2 .4 (1.32–4.35)	0.003	0.005
rs1061622	TNFRSF1B	G, 2.12 (1.15–3.91)	0.014	0.009
rs4343	ACE	G, 1.82 (1.05–3.13)	0.029	0.016
rs4355801	TNFRSF11B	A, 1.79 (1.04–3.08)	0.031	0.051
rs6060369	UQCC	A, 1.77 (1.03–3.02)	0.035	0.032
rs3740691	ARFGAP2	A, 1.76 (1.03–3.02)	0.035	0.031
rs764481	CYP2D6	G, 1.77 (1.02–3.09)	0.040	0.077
rs331377	ASPN	G, 1.7 (1.01–2.84)	0.041	0.043
rs3213718	CALM1	A, 1.72 (1.01–2.92)	0.043	0.044
rs1800896	IL10	A, 1.69 (1.01–2.84)	0.044	0.029
rs2300496	CALM1	A, 1.68 (1.002–2.83)	0.048	0.055
rs2300500	CALM1	G, 1.68 (1.002-2.83)	0.048	0.055

* P values adjusted by a single-value permutation test

13.87 ± 13.1; $P = 0.016$) and CRP (mg/L) (9.27 ± 8.5 vs. 5.09 ± 4.9; $P = 0.003$) values than responders. Infliximab, etanercept, and adalimumab were used in 62 (51.2 %), 34 (28.1 %) and 25 (20.7 %) patients, respectively. There were no statistically significant differences between the response groups for concomitant DMARDs use(sulphasalazine, methotrexate) (data not shown). However, a statistically significant difference was observed between the two groups regarding NSAID use, as nonresponders were more frequent NSAIDs consumers (44 [83 %] vs. 40 [73.5 %]; $P = 0.048$).

SNPs association with treatment response status to the first TNF-alpha inhibitor agent

From the 345 SNPs tested for association with individual response to anti-TNF-alpha agents, 13 polymorphisms showed significant association with treatment response status assessed with BASDAI50 (Table 2). Three SNPs from the *CALM1* (*Calmoduline 1*) gene were in linkage disequilibrium (LD). After adjustment with a single-value permutation test, four SNPs did not remain significantly

Table 3 Logistic regression analysis to predict nonresponse to biological treatment in AS

SNP	Gene	Risk genotype	Univariate analysis OR (95 % CI), *P*	Multivariate analysis[a] OR(95 % CI), *P*
rs755622	*MIF*	GG+CG	3.002 (1.33–6.78), 0.008	3.14 (1.19–8.22), 0.019
rs917997	*IL18RAP*	AA+AG	3.15 (1.49–6.78), 0.003	3.35 (1.38–8.15), 0.007
rs1061622	*TNFRSF1B*	GG+TG	2.5 (1.19–5.28), 0.016	2.46 (1.00–6.04), 0.048
rs4343	*ACE*	GG	2.40 (1.09–5.27), 0.028	
rs6060369	*UQCC*	AA	2.15 (1.02–4.53), 0.043	
rs3740691	*ARFGAP2*	AA+AG	2.38 (1.08–5.26), 0.031	2.90 (1.12–7.51), 0.002
rs331377	*ASPN*	GG+AG	2,25 (1.005–5.04), 0.049	
rs3213718	*CALM1*	AA	2.15 (1.02–4.53), 0.043	
rs1800896	*IL10*	AA	3.02 (1.24–7.29), 0.014	3.09 (1.04–9.15), 0.041

[a] To asses the goodness to fit of the model, Hosmer–Lemeshow test and the receiver operating characteristic (ROC) curve with 95 % CI were used

associated with treatment response status, two of them were SNPs in the *CALM1* gene; therefore, nine independent associations were finally identified (Table 2).

All nine polymorphisms that showed significant association with nonresponse status to biological treatment, in the allele frequencies association test, were entered as genotypes in the multivariate model. We found that: rs917997 in the *IL18RAP* gene (OR 3.35, 95 % CI 1.38–8.15), rs755622 (OR 3.14, 95 % CI 1.19–8.22) in the *MIF* gene, rs1800896 in the *IL10* gene (OR 3.09, 95 % CI 1.04–9.15), rs3740691 (OR 2.90, 95 % CI 1.12–7.51) in the *ARFGAP2* gene, rs1061622 (OR: 2.46, 95 % CI 1.00–6.04) in the *TNFRSF1B* gene were independent predictors of nonresponse of the first anti-TNF-alpha agent (Table 3). The genetic model obtained in nonresponse to treatment has a predictive power, as indicated by the ROC AUC, of 0.77 (95 % CI 0.68–0.86) in Fig. 1. The distribution of the probabilities obtained with the prediction model for the responders and nonresponders patients is shown in Fig. 2. The median value for the probability in the responder group was 0.32, whereas higher value was obtained for nonresponders to biological treatment, reaching 0.57.

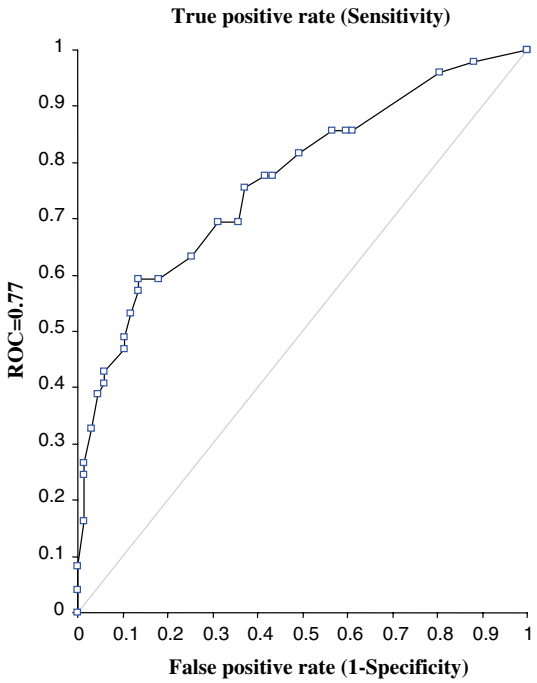

Fig. 1 ROC plot of the predictive genetic model of nonresponse to biological treatment

Discussion

In this study, we searched for pharmacogenomic markers responsible for nonresponse to anti-TNF-alpha agents in previously untreated AS patients. Our candidate gene study led to the identification of five SNPs in five different genes as being predictive factors of nonresponse to the first biological treatment. These genetic variants could alter the effectiveness of the anti-TNF-alpha drugs: rs755622 in the *macrophage migration inhibitory factor (MIF)* gene, rs917997 in the *interleukin 18 receptor accessory protein (IL18RAP) gene*, rs1800896 in the *IL10 gene,* rs1061622 in the *tumor necrosis factor receptor superfamily, member 1B (TNFRSFB1)* and *rs3740691* in *ADP-ribosylation factor GTPase-activating protein 2* (*ARFGAP2) gene.*

The percentage of BASDAI 50 responders to TNF-alpha agents in our study was 56.2 %, close to the previous findings [14, 19]; therefore, it supports the need to search for objective predictors of treatment response, beyond clinical and demographic factors. REGISPONSER-AS patients belong to daily clinical practice, but, interestingly, the clinical characteristics of the two treatment response status groups did not differ. Moreover, no difference was encountered in AS disease duration or in *HLA B27* status in the two treatment activity groups (responders 54 [79.4 %] vs. nonresponders 40 (75.5 %), *P* = NS). Nevertheless,

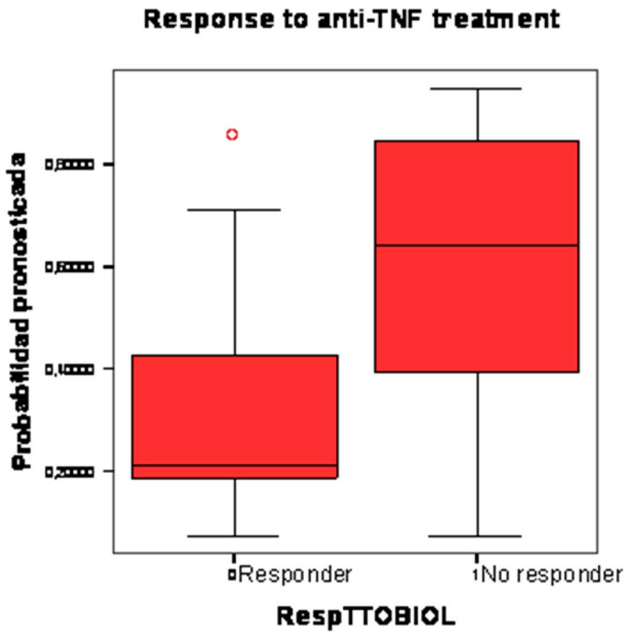

Fig. 2 Distribution of probabilities in the predictive genetic model (responders and nonresponders to biological treatment). For each *box*, it is indicated median value, first and third quartile, minimum and maximum value

patients with nonresponse to treatment had a statistically significant older age at disease onset (24 [±9.4] vs. 28 (±8.7), OR 1.048, CI 1.006–1.091, $P = 0.025$) and had lower levels of baseline inflammatory biomarkers, consistent with previous studies regarding clinical predictors of treatment response in AS [11, 14]. An interesting finding is that patients in nonresponder group had a significantly higher BASDAI ($P = 0.003$) which comes to support the results from studies that analyzed predictors of TNF-alpha treatment discontinuation and drug survival [14, 29].

Our study showed that polymorphism rs917997 risk allele A, in the *IL18RAP* gene, was the strongest predictor of nonresponse to biological treatment. *IL18RAP* gene encodes the ß chain of the heterodimeric receptor for IL-18 (IL18Rß), and it is responsible for signal transduction in response to IL-18. IL-18 is one of the most effective cytokines in regulating NK cell activity and Th1-mediated immune responses and, therefore, defense against infection with intracellular microbes through the induction of IFN-gamma [30]. Defective IL-18 receptor function has been reported in patients with systemic-onset juvenile idiopathic arthritis together with a nonfunctional IL-18/NK cell axis [31]. Allele A in rs917997 was strongly associated with celiac disease susceptibility [32], and it has also shown association with inflammatory bowel disease [33]. Risk allele A in rs917997 correlated with lower levels of mRNA IL18RAP expression, suggesting that individuals with risk allele A have reduced IL18Rß, leading to low IFN-gamma secretion [32]. Our study revealed a statistically significant likelihood of nonresponse to anti-TNF-alpha treatment for rs917997 risk allele A in *IL18RAP* gene (OR 3.35 [1.38–8.15], $P = 0.007$), suggesting a link between nonresponse to anti-TNF-alpha agents and impairment in the efficacy of IL-18 signaling that may generate low production of IFN-gamma and TNF-alpha.

Moreover, our findings showed that a polymorphism in the *MIF* gene located on chromosome 22q11.2 contributes to the absence of response to treatment. MIF cytokine is released by many immunologic effector cells after exposure to microbial products and pro-inflammatory cytokines and promotes the production of pro-inflammatory mediators, including TNF-alpha. The minor allele, allele G, of the SNP rs755622 in the *MIF* gene predicts nonresponse to treatment in our study (OR 3.14 [1.19–8.22], $P = 0.019$). This SNP, located in the gene's promoter region, was previously reported to be associated with treatment response. Specifically, rs755622 risk allele G has been previously associated with relapse to local steroid treatment in psoriasis arthritis patients [34]. Studies in RA patients demonstrated that carriers of the minor allele of rs755622 have higher levels of circulating MIF and higher levels of radiological joint damage [35]. Thus, we may speculate that in AS patients, rs755622 risk allele G reflects a more active disease associated with higher inflammatory activity because of increased MIF levels.

Previous studies demonstrated that polymorphism rs1800896 located on chromosome 1q31–q32, within the *IL10 gene* promoter region, influences IL-10 cytokine plasma levels, which were significantly higher in patients homozygous for the G allele [36]. IL-10 was demonstrated to inhibit the production of inflammatory mediators and can be considered as a natural immunosuppressant of TNF-α [37]. Low IL-10 producer genotype (AA) in RA patients was found to predispose to development of anticyclic citrullinated peptide antibodies (anti-CCP) positivity RA disease with reduced response to prednisone treatment [38]. Our study identified that AS patients carrying the risk allele A of rs1800896 were nonresponders to anti-TNF-alpha treatment (OR 3.09 [1.04–9.15], $P = 0.041$), sustaining the association of low-level producer IL-10 genotype with the risk of lack of response to treatment.

The actions of TNF-alpha are mediated by means of binding to two distinct cell surface receptors, namely tumor necrosis factor receptors I (TNF-RI) and II (TNF-RII, also known as TNFRSF1B or p75). The two receptors appear to promote distinct TNF-alpha-induced cellular responses, although both are capable of inducing the nuclear factor-κB (NF-κB) pathway. In addition to membrane-bound forms, both TNF receptors can exist as soluble proteins (sTNFRs) and can act as natural inhibitors of TNF-alpha by preventing soluble TNF-alpha from binding to membrane-bound

TNF receptors. TNF-RII is typically found on immune and endothelial cells [39] and binds preferentially to transmembrane TNF-alpha [40]. TNF-RII is necessary for antigen-driven differentiation and survival of T-cells, being an important co-stimulator for T cell activation and for optimal IL-2 and IFN-gamma induction [41]. The levels of both soluble and membrane-bound TNF-RII were high in patients with RA [42, 43]. In our analysis, we identified the SNP rs1061622 in the *TNFRSF1B (TNFRII)* gene, located on chromosome 1p36, which encodes the p75 receptor, strongly influenced on treatment response status. In RA patients, it was shown that rs1061622 affected the level and functions of soluble TNF receptor p75 and, consequently, influenced the response to anti-TNF-alpha treatment [44, 45]. Patients with *TNF-RII* GG genotype at rs1061622 expressed the lowest sTNF-RII levels and exhibited a poorer response to anti-TNF-alpha therapy [45, 46]. In this study, we identified the rs1061622 risk allele G to influence the of nonresponse likelihood to anti-TNF-alpha treatment (OR 2.46 [1.00–6.04], $P = 0.048$), consistent with the previous results in RA patients.

As with other previous reports on the pharmacogenomics of response to anti-TNF-alpha agents, our study is limited by a relatively small sample size lack and by a lack of an independent cohort to validate the observed genetic associations with biological treatment nonresponse. We chose to present our results based on BASDAI, as this represents the main tool in daily clinical practice to assess disease activity. Nevertheless, parameters that measure the disease's inflammatory activity more objectively may provide a more accurate means of assessing treatment response. In this context, the ASDAS scoring system has been shown to be a more powerful tool than BASDAI 50 in patients with high CRP and may reflect the inflammatory process and the efficacy of biological treatment better [11, 47]. Future studies will need to investigate whether ASDAS cutoffs facilitate the identification of the same genetic markers associated with lack of response to biological treatment.

In conclusion, the contribution of genetic factors accounting for treatment response to anti-TNF-alpha agents is not yet well known. Our findings suggest that there are certain genetic profiles for which TNF-alpha blockers are ineffective and validation of a genetic model that predict response status to the first anti-TNF-alpha treatment would be a changing point in facilitating individualized therapy. After AS patients fail their first TNF-alpha inhibitor, physicians are faced with trying a second TNF-alpha inhibitor as, so far they represent the only effective biological treatment for AS. It would be interesting to study in the next step whether polymorphisms associated with nonresponse to the second and/or the third TNF-alpha inhibitor are the same genetic markers responsible for nonresponse to the first inhibitor. The ability to determine whether a patient is genetically a nonresponder to a TNF-alpha inhibitor despite good clinical prognostic factors would make these treatment decisions more rational.

In our study of assessing efficacy to the first anti-TNF treatment in AS patients using a candidate SNPs approach, we developed a genetic model of nonresponse. Replication of these genetic biomarkers in other independent and much larger cohorts would be of major value to confirm the robustness of the results, especially because TNF-alpha blockers are the only currently available biological therapy for treating AS. Validation of our genetic model in prospective studies may lead to the design of a clinico-genetic algorithm to initiate biological treatment.

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Conflict of interest MS., N.B., M.A., A.M. and D.T. are currently employees of Progenika Biopharma, SA. A.S. is supported by an unrestricted Grant from Pfizer. This does not alter our compliance with all the policies on sharing data and materials. All the other authors have no conflicts of interest to declare.

References

1. Wright V (1978) Seronegative polyarthritis: a unified concept. Arthritis Rheum 21:619–633
2. Braun J, Bollow M, Neure L et al (1995) Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum 38:499–505
3. Braun J, Xiang J, Brandt J et al (2000) Treatment of spondyloarthropathies with antibodies against tumor necrosis factor α: first clinical and laboratory experiences. Ann Rheum Dis 59:i85–i89
4. Maksymowych W (2010) Disease modification in ankylosing spondylitis. Nat Rev Rheumatol 6:75–81

5. van der Heijde D, Dijkmans B, Geusens P et al (2005) Efficacy and safety of infliximab in patients with ankylosing spondylitis: results of a randomized, placebo-controlled trial (ASSERT). Arthritis Rheum 52:582–591
6. Davis JC Jr, van der Heijde D, Braun J et al (2003) Recombinant human tumor necrosis factor receptor (etanercept) for treating ankylosing spondylitis: a randomized, controlled trial. Arthritis Rheum 48:3230–3236
7. van der Heijde D, Kivitz A, Schiff MH et al (2006) Efficacy and safety of adalimumab in patients with ankylosing spondylitis: results of a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 54:2136–2146
8. Braun J, Deodhar A, Inman RD et al (2012) Golimumab administered subcutaneously every 4 weeks in ankylosing spondylitis: 104-week results of the GO-RAISE study. Ann Rheum Dis 71:661–667
9. Davis JC Jr, van der Heijde DM, Dougados M et al (2005) Baseline factors that influence ASAS 20 response in patients with ankylosing spondylitis treated with etanercept. J Rheumatol 32:1751–1754
10. Rudwaleit M, Listing J, Brandt J, Braun J, Sieper J (2004) Prediction of a major clinical response (BASDAI 50) to tumour necrosis factor alpha blockers in ankylosing spondylitis. Ann Rheum Dis 63:665–670
11. Vastesaeger N, van der Heijde D, Inman RD et al (2011) Predicting the outcome of ankylosing spondylitis therapy. Ann Rheum Dis 70:973–981
12. Rudwaleit M, Claudepierre P, Wordsworth P et al (2009) Effectiveness, safety, and predictors of good clinical response in 1,250 patients treated with adalimumab for active ankylosing spondylitis. J Rheumatol 36:801–810
13. Lord PA, Farragher TM, Lunt M, Watson KD, Symmons DP, Hyrich KL (2010) Predictors of response to anti-TNF therapy in ankylosing spondylitis: results from the British Society for Rheumatology Biologics Register. Rheumatology (Oxford) 49:563–570
14. Arends S, Brouwer E, van der Veer E et al (2011) Baseline predictors of response and discontinuation of tumor necrosis factor-alpha blocking therapy in ankylosing spondylitis: a prospective longitudinal observational cohort study. Arthritis Res Ther 20(13):R94
15. Can M, Aydın SZ, Niğdelioğlu A, Atagündüz P, Direskeneli H (2012) Conventional DMARD therapy (methotrexate–sulphasalazine) may decrease the requirement of biologics in routine practice of ankylosing spondylitis patients: a real-life experience. Int J Rheum Dis 15:526–530
16. Chen J, Veras MM, Liu C, Lin J (2013) Methotrexate for ankylosing spondylitis. Cochrane Database Syst Rev 28(2):CD004524
17. Chen J, Liu C (2005) Sulfasalazine for ankylosing spondylitis. Cochrane Database Syst Rev 18(2):CD004800
18. Baraliakos X, Brandt J, Listing J et al (2005) Outcome of patients with active ankylosing spondylitis after two years of therapy with etanercept: clinical and magnetic resonance imaging data. Arthritis Rheum 53:856–863
19. Baraliakos X, Listing J, Fritz C et al (2011) Persistent clinical efficacy and safety of infliximab in ankylosing spondylitis after 8 years-early clinical response predicts long-term outcome. J Rheumatol (Oxford) 50:1690–1699
20. Poddubnyy DA, Märker-Hermann E, Kaluza-Schilling W et al (2011) Relation of HLA-B27, tumor necrosis factor-α promoter gene polymorphisms, and T cell cytokine production in ankylosing spondylitis—a comprehensive genotype-phenotype analysis from an observational cohort. J Rheumatol 38:2436–2441
21. Seitz M, Wirthmu¨ ller U, Moller B, Villiger PM (2007) The −308 tumour necrosis factor-gene polymorphismpredicts therapeutic response to TNF-blockers in rheumatoid arthritis and spondyloarthritis patients. Rheumatology 46:93–96
22. Collantes E, Zarco P, Munoz E et al (2007) Disease pattern of spondyloarthropathies in Spain: description of the first national registry (REGISPONSER) extended report. Rheumatology (Oxford) 46:1309–1315
23. Schiotis R, Font P, Escudero E et al (2011) Usefulness of a centralized system of data collection for the development of an international multicentre registry of spondyloarthritis. Rheumatology (Oxford) 50:132–136
24. Van der Linden S, Valkenburg HA, Cats A (1984) Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 27:361–368
25. Fan JB, Oliphant A, Shen R et al (2003) Highly parallel SNP genotyping. Cold Spring Harb Symp Quant Biol 68:69–78
26. Paynter RA, Skibola DR, Skibola CF et al (2006) Accuracy of multiplexed Illumina platform-based single-nucleotide polymorphism genotyping compared between genomic and whole genome amplified DNA collected from multiple sources. Cancer Epidemiol Biomarkers Prev 15:2533–2536
27. Reich D, Patterson N, De Jager PL et al (2005) A whole-genome admixture scan finds a candidate locus for multiple sclerosis susceptibility. Nat Genet 37:1113–1118
28. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 15(21):263–265
29. Glintborg B, Østergaard M, Krogh NS et al (2013) Clinical response, drug survival and predictors thereof in 432 ankylosing spondylitis patients after switching tumour necrosis factor α inhibitor therapy: results from the Danish nationwide DANBIO registry. Ann Rheum Dis 72:1149–1155
30. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K (1998) Regulation of interferon-_ production by IL-12 and IL-18. Curr Opin Immunol 10:259–264
31. de Jager W, Vastert SJ, Beekman JM et al (2009) Defective phosphorylation of interleukin-18 receptor beta causes impaired natural killer cell function in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 60:2782–2793
32. Hunt KA, Zhernakova A, Turner G et al (2008) Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 40:395–402
33. Zhernakova A, Festen EM, Franke L et al (2008) Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. Am J Hum Genet 82:1202–1210
34. Eder L, Chandran V, Ueng J et al (2010) Predictors of response to intra-articular steroid injection in psoriatic arthritis. Rheumatology (Oxford) 49:1367–1373
35. Radstake TR, Sweep FC, Welsing P et al (2005) Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor. Arthritis Rheum 52:3020–3029
36. Lauten M, Matthias T, Stanulla M, Beger C, Welte K, Schrappe M (2002) Association of initial response to prednisone treatment in childhood acute lymphoblastic leukaemia and polymorphisms within the tumor necrosis factor and the interleukin-10 genes. Leukemia 16:1437–1442
37. Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM (1998) Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood 92:3943–3948
38. de Paz B, Alperi-López M, Ballina-García FJ, Prado C, Mozo L, Gutiérrez C, Suárez A (2010) Interleukin 10 and tumor necrosis factor-alpha genotypes in rheumatoid arthritis-association with clinical response to glucocorticoids. J Rheumatol 37:503–511

39. Wajant H, Pfizenmaier K, Scheurich P (2003) Tumor necrosis factor signaling. Cell Death Differ 10:45–65
40. Grell M, Douni E, Wajant H et al (1995) The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 83:793–802
41. Kim EY, Priatel JJ, Teh SJ, Teh HS (2006) TNF receptor type 2 (p75) Functions as a costimulator for antigen-driven T cell responses in vivo. J Immunol 176:1026–1035
42. Cope AP, Aderka D, Doherty M et al (1992) Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. Arthritis Rheum 35:1160–1169
43. Deleuran BW, Chu C-Q, Field M et al (1992) Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis: implications for local actions of tumor necrosis factor. Arthritis Rheum 35:1170–1178
44. Tolusso B, Sacco S, Gremese E, La Torre G, Tomietto P, Ferraccioli GF (2004) Relationship between the tumor necrosis factor receptor II (TNF-RII) gene polymorphism and sTNF-RII plasma levels in healthy controls and in rheumatoid arthritis. Hum Immunol 65:1420–1426
45. Glossop JR, Dawes PT, Nixon NB, Mattey DL (2005) Polymorphism in the tumour necrosis factor receptor II gene is associated with circulating levels of soluble tumour necrosis factor receptors in rheumatoid arthritis. Arthritis Res Ther 7:R1227–R1234
46. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G (2002) Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Rheumatology 29:1847–1850
47. Pedersen SJ, Sørensen IJ, Garnero P et al (2011) ASDAS, BASDAI and different treatment responses and their relation to biomarkers of inflammation, cartilage and bone turnover in patients with axial spondyloarthritis treated with TNFα inhibitors. Ann Rheum Dis 70:1375–1381