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ORIGINAL ARTICLE

Influence of IL6R gene polymorphisms in the effectiveness to treatment with tocilizumab in rheumatoid arthritis

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In the present study, we aimed to investigate the influence of clinical parameters and single-nucleotide polymorphisms of interleukin-6 receptor (rs12083537, rs2228145, rs4329505 and rs11265618) on response to tocilizumab, TCZ (European League Against Rheumatism (EULAR) response, remission, low disease activity (LDA) and improvement of DAS28). We performed a retrospective cohort study in patients with Rheumatoid Arthritis (RA) treated with TCZ for 12 months. Multivariable analysis showed that the only variable independently associated to satisfactory EULAR response (odds ratio (OR): 0.61; 95% of confidence interval $(CI)_{95\%}$: 0.42, 0.88; $P=0.008$), remission (OR: 0.51; $CI_{95\%}$: 0.35, 0.75; $P=0.001$), LDA (OR: 0.41; $CI_{95\%}$: 0.24, 0.72; $P=0.002$) and improvement in DAS28 ($B=-0.32$; $CI_{95\%}$): −0.47, −0.17; $P=7.5\times10^{-5}$) at 12 months was lower number of previous biological therapy (BT). High baseline DAS28 was also associated with a greater decrease in DAS28 at 12 months of treatment ($B=0.99$; $CI_{95\%}$: 0.79, 1.20; $P=1.5\times10^{-14}$). Those patients who were carriers of AA genotypes for rs12083537 (OR: 13.0; $CI_{95\%}$: 2.31, 72.91; $P=0.004$) and CC for rs11265618 (OR: 12.15; $CI_{95\%}$: 2.18, 67.81; $P=0.004$) had better LDA response at 12 months of treatment with TCZ. In conclusion, RA patients treated with TCZ showed better EULAR response, remission, LDA and DAS28 improvement rates when a lower number of BT were previously administered. The AA genotype for rs12083537 and CC for rs11265618 polymorphisms for may act as predictors of good response LDA.

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INTRODUCTION

Rheumatoid arthritis (RA) is the most prevalent autoimmune inflammatory disease (0.24%; 95% of confidence interval ($CI_{95\%}$): 0.23–0.25%).[1] Tocilizumab (TCZ) is a recombinant humanized antibody targeting soluble and membrane IL6 receptor relatively recently incorporated to the clinical practice in RA, which is showing response rates of 80% and remission rates up to 55%,[2–4] compared to 24% remission in patients treated with tumor necrosis factor inhibitors (TNFi).[5] Despite of this, other biological therapies (BT), mainly TNFi, are prescribed more frequently after disease-modifying antirheumatic drugs (DMARDs) failure. The interindividual variability in the response to TNFi therapy is caused by clinical, genetic and environmental factors, causing up to 40% of non-responders,[6,7] leading to increased disease activity and failure to reach the therapeutic target, which results in a decrease in the quality of life of RA patients. The identification of clinical and genetic markers as predictors of response can be a useful tool for selecting patients who will more likely respond to treatment. To date, there is no consensus among all studies to establish consistent clinical, biochemical and genetic parameters for the prediction of response to BT in RA.[2,3,8–12] Genetics is demonstrating to play a role of increasing importance in understanding the interindividual response to BT.[11,13,14] TCZ pharmacogenetics is still under development and to date there is little literature showing single-nucleotide polymorphisms (SNPs) as predictors of response to this drug.[12,15] A recent study in seven RA patients has showed a role for the AAC haplotype (rs12083537, rs2228145, rs4329505) on the poor response to TCZ treatment in terms of swollen joint count.[16] No other studies have explored the influence of interleukin-6 receptor (IL6R) polymorphisms on TCZ response, and the only information available is referred to increased soluble IL6R levels (IL6Rs)[17,18] or susceptibility to RA.[19]

In the present study, we aim to investigate the influence of clinical parameters and rs12083537, rs2228145, rs4329505, rs11265618 gene polymorphisms of IL6R on response to TCZ (European League Against Rheumatism (EULAR) response, remission, low disease activity (LDA) and DAS28 improvement). To achieve this, we conducted a retrospective cohort study in patients with RA treated with TCZ for 12 months.

MATERIALS AND METHODS

Study design

A cohort study was performed in 77 White Caucasian patients diagnosed with RA according to the ACR classification criteria[20] and ⩾18 years treated with TCZ (8 mg kg^{-1} intravenous or 162 mg subcutaneous administration, monthly) for 12 months at the Complejo Hospitalario Universitario de Granada (CHUG).

This study was approved at the CHUG by Ethics and Research Committee and was performed conform the declaration of Helsinki; prior patients signed an informed consent. All data were subjected to confidentiality.

Sociodemographic and clinical variables

The sociodemographic and clinical variables were collected from the medical records of patients, which were: sex, age at RA diagnosis, years with RA without TCZ, age at TCZ start, previous BT, duration of the previous

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BT (months), concomitant DMARDs (Leflunomide, Methotrexate), TCZ administration (intravenous, subcutaneous), concomitant corticosteroid, baseline DAS28[21] baseline C-reactive protein (CRP), baseline erythrocyte sedimentation rate (ESR), baseline Clinical Disease Activity Index (CDAI),[22] baseline Simplified Disease Activity Index (SDAI),[23] baseline Health Assessment Questionnaire (HAQ).

Genetic variables

DNA isolation. Genomic DNA was extracted from saliva samples using the QIAamp DNA Mini Kit (Qiagen GmBH, Hilden, Germany) according to the manufacturer's instructions for DNA purification from saliva and tissues.

Detection of gene polymorphisms. The gene polymorphisms of IL6R (rs12083537, rs2228145, rs4329505, rs11265618) were analysed by Real-Time PCR using TaqMan probes Genotyping, according to previously described methodology.[24]

Response variables

EULAR response was evaluated according to the guidelines given by the European League against Rheumatism and classified in satisfactory (present DAS28 < 3.2 and DAS28 improvement > 1.2) and unsatisfactory (present DAS28 $\geqslant 3.2$ and DAS28 improvement $\leqslant 1.2$)[25,26] at 12 months.

Remission and LDA were considered when patients achieved DAS28 < 2.4 and DAS28 < 3.6, respectively, at 12 months.[22]

Improvement in DAS28 was calculated as the difference between baseline DAS28 and DAS28 values at 12 months.

Statistical analysis

Quantitative data were expressed as the mean ($\pm$ s.d.) for normally distributed variables or medians and percentiles (25 and 75) for non-normal distribution variables. Normality was assessed by Shapiro–Wilks test. The *t*-Student test was applied for normally distributed variables, and non-parametric Wilcoxon test otherwise. Bivariate association for qualitative dichotomous variables was analysed with the Pearson's χ^2 or Fisher's exact test. Bonferroni correction was applied for multiple comparisons.

ANOVA factor or Kruskal–Wallis tests were applied for qualitative variables with more than two categories. Pearson correlation or Spearman tests were used to compare quantitative variables, according to the normality of the data. Bonferroni correction was applied for multiple comparisons in normally distributed variables; non-parametric tests were manually corrected considering the number of degrees of freedom in each variable. Multivariable analysis (binomial logistic or linear regression) was performed to evaluate potential confounding variables on EULAR response, remission and LDA. Normality of the residuals in the linear regression model was tested using the Shapiro–Wilks test and was also checked graphically (Q–Q plots). Homoscedasticity of the residuals was tested using the Breusch–Pagan test.

A significance level of $P < 0.05$ was considered significant for all tests, and all tests were two-sided. Data analysis was performed using R 3.2.2.[27]

Hardy–Weinberg equilibrium and pairwise haplotype frequencies were estimated using the Hardy–Weinberg calculator[28] and CubeX[29] tools respectively, both provided by the Online Encyclopedia for Genetic Epidemiology studies.

RESULTS

Characteristics of patients

The study included 77 patients, whose sociodemographic and clinic characteristics are summarized in Table 1. The gender ratio was 1:5; the diagnosis of RA had been established at 46 (37.0–53.0) years and the disease course was 8 (3–15) years. All patients had been previously treated with DMARD, and 32.5% (25/77) were naïve for BT. Non-naïve patients had tried 2.2 (1.0–3.0) biological drugs during 43.5 (16.5–81.0) months. The age at TCZ start was 52.2 $\pm$ 11.6 years old, and the administration procedure was intravenous in 77.9% (60/79) patients. A concomitant DMARD was administered in 88.4% (68/77) cases and glucocorticoids in 92.2% (71/77).

Baseline rheumatoid factor and cyclic citrullinated peptide antibodies were positive in 64.9% (50/77) and 68.8% (53/77) patients. Other baseline clinical parameters (DAS28, HAQ, CDAI and SDAI) and acute phase reactants (CRP, ESR) are detailed in Table 1.

Clinical effectiveness of TCZ

After 12 months of TCZ treatment, EULAR response was satisfactory in 74.0% (57/77; Table 1). Remission was achieved in 59.7% (46/77; Table 1) and 80.5% (62/77; Table 1) showed LDA. Decrease in DAS28 was 3.1 $\pm$ 1.5 and decrease in ESR was 21 (7.5–35.0) after 12 months of treatment.

Genetic characteristics of patients

Genotype distributions are shown in Supplementary Table S1. All gene polymorphisms were in Hardy–Weinberg equilibrium. Linkage disequilibrium values (D' and r^2) are shown in Supplementary Table S2.

Predictors of response at 12 months

EULAR response. In the bivariate analysis, EULAR response was higher in naïve patients (relative risk (RR): 1.4; CI_{95}%: 1.12, 1.77; $P = 0.027$; Supplementary Table S3), treated with less number of previous BT ($P = 0.004$; Supplementary Table S3) during less time ($P = 0.011$; Supplementary Table S3). None of the gene polymorphisms were associated to EULAR response (Supplementary Table S4). Multivariable analysis showed that the only variable independently associated to satisfactory EULAR response at 12 months was lower number of previous BT (odds ratio (OR): 0.61; $CI_{95\%}$: 0.42, 0.88; $P = 0.008$; Table 2).

Remission. In the bivariate analysis, higher remission was shown when TCZ was administered subcutaneously (RR: 1.7; $CI_{95\%}$: 1.23, 2.31: $P = 0.015$; Supplementary Table S4), patients were naïve (RR: 2.1; $CI_{95\%}$: 1.51, 2.88: $P = 0.0001$; Supplementary Table S5), had a shorter course of disease in years (6.5 (2.0–12.3) vs 11.0 (6.0–16.0); $P = 0.032$), and had tried less BT alternatives (0.5 (0.0–2.0) vs 2.0 (1.0–3.0); $P = 5.5 \times 10^{-5}$; Supplementary Table S5), during less months (1.5 (0.0–39.5.) vs 48.0 (15.0–81.0); $P = 0.001$; Supplementary Table S5). None of the gene polymorphisms were associated to remission (Supplementary Table S6). After multivariate analysis, the factor independently associated to higher remission rates was lower number of previous BT (OR: 0.51; $CI_{95\%}$: 0.35, 0.75; $P = 0.001$; Table 2).

Low activity disease. In the bivariate analysis (Supplementary Table S7), higher LDA rates were found in naïve patients (RR: 0.7; $CI_{95\%}$: 0.58, 0.87; $P = 0.002$), previously treated with lesser number of BT (0.5 (0.0–2.0) vs 2.0 (1.0–3.0); $P = 0.001$) during a shorter period in months (9.0 (0.0–57.5) vs 54 (17.0–88.0); $P = 0.007$) and subcutaneous administration (RR: 1.3; $CI_{95\%}$: 1.22, 1.54; $P = 0.032$). Gene polymorphisms associated to higher LDA were rs12083537-AA genotype (RR: 1.4; $CI_{95\%}$: 1.13, 2.01; $P = 0.021$; Supplementary Table S7), and rs11265618-CC genotype (RR: 1.3; $CI_{95\%}$: 1.13, 1.77; $P = 0.031$; Supplementary Table S8). The multivariate analysis showed the rs12083537-AA genotype (OR: 13.0; $CI_{95\%}$: 2.31, 72.91; $P = 0.004$), rs11265618-CC genotype (OR: 12.15; $CI_{95\%}$: 2.18, 67.81; $P = 0.004$) and lower number of previous BT (OR: 0.41; $CI_{95\%}$: 0.24, 0.72; $P = 0.002$) were the independent factors capable to predict higher LDA rates at 12 months (Table 2).

Improvement in DAS28. In the bivariate analysis (Supplementary Table S9), there was a greater decrease of DAS28 in patients administered subcutaneously (3.9 $\pm$ 1.3 vs 2.9 $\pm$ 1.5; $P = 0.010$), naïve for BT (3.8 $\pm$ 1.3 vs 2.7 $\pm$ 1.5; $P = 0.003$), lesser number of BT (Rho Pearson $= -0.36$; $P = 0.002$), lower duration of the previous BT (Rho Spearman $= -0.34$; $P = 0.003$), higher baseline ESR (Rho Pearson $= 0.39$; $P = 0.001$), higher baseline DAS28

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Table 1. Demographic and clinical characteristics of patients treated with tocilizumab

Variable	*Baseline*			*12 months*		
	N	%		N	%	
Sex	77					
Male	14	18.2				
Female	63	81.8				
Disease duration (years)	77		8 (3.0–15.0)			
Age at RA diagnosis	77		46 (37.0–53.0)			
Previous BT	77					
TNFi	28	36.4				
TNFi and non-TNFi BT	24	31.2				
Naïve	25	32.5				
Number of previous BT	52		2.2 (1.0–3.0)			
Duration of the previous BT (months)	52		43.5 (16.5–81.0)			
Age at TCZ start	77		52.2 ± 11.6			
TCZ administration	77					
Intravenous	60	77.9				
Subcutaneous	17	22.1				
Concomitant DMARDs				77		
Monotherapy				9	11.7	
Methotrexate				33	42.9	
Leflunomide				35	45.5	
Concomitant corticosteroid	77					
Yes	71	92.2				
No	6	7.8				
Rheumatoid factor	77					
Positive	50	64.9				
Negative	27	35.1				
Anti-CCP antibodies	75					
Positive	53	68.8				
Negative	20	26.0				
HAQ	77		1.6 ± 0.6			
CDAI	77		27.1 ± 12.1			
SDAI	77		29.5 (20.4–37.8)			
DAS28-ESR	77		5.7 ± 1.2	75		2.5 (1.7–3.1)
TJC	77		9.0 (5.0–14.0)			
SJC	77		4.0 (2.0–7.0)			
CRP	77		1.0 (0.4–2.0)			0.1 (0.1–0.5)
ESR	77		30.3 (17.9–48.5)			7.0 (5.0–13.5)
EULAR response				77		
Satisfactory				57	74.0	
Unsatisfactory				20	26.0	
Remission (DAS28 < 2.4)				46	59.7	
LDA (DAS28 < 3.6)				62	80.5	
Decrease in DAS28				75		3.1 ± 1.5
Decrease in ESR				73		21 (7.5–35.0)

Abbreviations: BT, biological therapy; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28, 28-joint DAS; DMARDs, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; HAQ, Health Assessment Questionnaire; LDA, Low disease activity; RA, rheumatoid arthritis; SDAI, Simplified Disease Activity Index; SJC, swollen joint count; TCZ, tocilizumab; TJC, tender joint count; TNFi, Tumor necrosis factor inhibitor. Normally distributed variables: mean ± s.d. Non-normally distributed variables: median (P25–P75). Variables with normal distribution: mean ± s.d., Variables with non-normal distribution: median (interquartile range).

(Rho Pearson = 0.71; $P = 8.2 \times 10^{-13}$), higher baseline SDAI (Rho Pearson = 0.49; $P = 1.1 \times 10^{-5}$) and higher baseline CDAI (Rho Pearson = 0.60; $P = 1.9 \times 10^{-8}$). None of the gene polymorphisms were associated to DAS28 improvement (Supplementary Table S10). In multivariate analysis, the greater decrease in DAS28 was shown in patients with lower number of previous BT ($B = -0.32$; $CI_{95\%}$: −0.47, −0.17; $P = 7.5 \times 10^{-5}$; Table 2) and a higher baseline DAS28 ($B = 0.99$; $CI_{95\%}$: 0.79, 1.20; $P = 1.5 \times 10^{-14}$; Table 2).

DISCUSSION

BT has revolutionized the treatment of RA, showing excellent results and improving the quality of life of RA patients. Despite of this, inter-individual variability remains a challenge, causing up to 40% of non-responders, accelerating the symptoms of RA.[6,7,30] TCZ is usually prescribed after treatment failure to TNFi in current clinical practice. Recent cumulative evidence on TCZ effectiveness is undoubtedly positioning this inhibitor of IL6R as one of the most effective BT alternatives nowadays, with EULAR response rates over 80% and up to 55% remission rates.[2–4,31] These good results are confirmed in the present study, with 74% patients classified as satisfactory EULAR response and 59.7% remission rates after 12 months. TCZ therapy in our patients even achieved higher LDA rates after 12 months of treatment (80.5%), compared to other studies (48–66%).[3–5,8,32,33]

Clinical implementation of Pharmacogenetics, whose main objective is to optimize the treatment of diseases, through a more secure and efficient personalized therapy which allows to

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Table 2. Predictors of response at 12 months of treatment with tocilizumab in rheumatoid arthritis patients (multivariate analysis)

Response variable	*Independent variable*	*Reference*	B	*Odds ratio*	P	*Confidence interval 95%*	R^2	*Goodness of fit*	P-*value*[a] *(model)*
EULAR response	Number of previous BT			0.608	0.008	0.42, 0.88	R^2Cox–Snell = 0.096 R^2Nagelkerke = 0.140	$P = 0.154$ $\chi^2 = 5.250$	$P = 0.005$ $\chi^2 = 7.750$
Remission	Number of previous BT			0.514	0.001	0.35, 0.75	R^2Cox–Snell = 0175 R^2Nagelkerke = 0.236	$P = 0.023$ $\chi^2 = 9.530$	$P = 0.0001$ $\chi^2 = 14.770$
Low activity disease	Number of previous BT			0.412	0.002	0.24, 0.72	R^2Cox–Snell = 0.169 R^2Nagelkerke = 0.270	$P = 0.270$ $\chi^2 = 5.168$	$P = 0.003$ $\chi^2 = 14.292$
	IL6R- rs12083537-AA	GA/GG		13.0	0.004	2.31, 72.91			
	IL6R- rs11265618-CC	TC/TT		12.15	0.004	2.18, 67.81			
Decrease in DAS28	Number of previous BT		−0.320		7.5×10^{-5}	−0,47, −0.17	0.593	0.526	$P = 0.003$
	Baseline DAS28		0.997		1.5×10^{-14}	0,791, 1.20			

Abbreviations: BT, biologic therapy; DAS28, 28-joint DAS. [a]Likelihood ratio test.

select the right drug, at the right dose, for the right patient, requires the availability of reliable biomarkers capable to predict response to drugs. In RA, despite the investigation of predictive biomarkers for BT has been extensive, particularly regarding SNPs, most of the proposed SNPs are still inconclusive and inconsistent among different studies.[10,11] TCZ is a relatively new approved BT drug which is still underinvestigated regarding the potential pharmacogenetic implications, particularly in terms of effectiveness biomarkers.[12,15,16] Only one previous study has explored the influence of gene polymorphisms involved in the IL6 pathway on response to TCZ in 79 RA patients, showing no evidence in response variables, only in the percentage of swollen joint count.[16] In the present study, four SNPs in the IL6R were investigated as potential biomarkers of response to TCZ. Multivariate analysis showed that patients carrying the AA-genotype for rs12083537 (OR: 13.0; $CI_{95\%}$: 2.31, 72.91; $P = 0.004$) and CC for rs11265618 (OR: 12.15; $CI_{95\%}$: 2.18, 67.81; $P = 0.004$) (Table 2), had a better response in terms of LDA after 12 months of TCZ therapy. Curiously, the rs12083537-A allele was related to better response (LDA), oppositely to that previously described for swollen joint count improvement, particularly when analyzed as haplotype along with rs2228145 and rs4329505 polymorphisms (AAC).[16] Regarding the rs2228145 and rs4329505 polymorphisms, we did not find any association with response variables (EULAR response, remission, LDA and improvement DAS28). The C-allele of rs2228145 polymorphism has been associated with increased ectodomain shedding and consequently lower intracellular signaling through IL6 and higher plasma levels of the IL6Rs, which avoids downstream signaling due to most of IL6Rs-IL6 complexes are bound to their natural inhibitor (sgp130), a phenomenon consistent with its protective effect in RA which reflects the clinical effect of TCZ.[18,34] To our knowledge, no other studies have associated these SNPs with response to TCZ, although the A-alelle of rs12083537 has been associated with higher plasma levels of IL6Rs and higher risk of asthma.[17] Regarding the rs11265618 polymorphism, this is the first study showing a potential ability to predict response to TCZ. Patients carrying the CC-genotype showed better response (LDA), despite a previous study in 527 Spanish RA patients had associated the C-allele with the severity of joint destruction.[35] This polymorphism has also been associated to RA susceptibility in Asian population, according to a study of 162 RA patients and 188 healthy unrelated controls,[19] although no data are available for Caucasian population.

In our patients, no effect of the gene polymorphisms investigated was found for EULAR response, remission rates and improvement in DAS28 after 12 months of TCZ therapy. However, a lower number of previous BT alternatives showed to be a good predictor of response (EULAR response, remission, LDA and DAS28 improvement), indicating that an early use of TCZ may improve response in these patients, as demonstrated by higher continuation rates observed in naïve patients (81% vs 66%),[2] higher EULAR, LDA and remission rates[3,5,30,32] and lower SDAI/CDAI end points.[36] Altogether, these results support the use of TCZ as first line of BT, at the same level than the most widely used alternatives, the TNFi drugs. In fact, a large recent study in 1603 RA patients has shown better remission rates for TCZ compared to TNFi after 12 months (44% vs 29.6%; $P < 0.0001$);[30] better EULAR response (64.2% vs 33.3%; OR = 3.6; $CI_{95\%}$: 2.3 a 5.7; $P < 0.001$) and DAS28 remission rates (57.9% vs 23.8%; OR = 4.4; $CI_{95\%}$: 2.8–7; $P < 0.001$) have also been found for TCZ compared with TNFi in 524 RA patients.[5]

The main limitation of this study is the limited sample size, which may have caused an underpowered approach in any of the SNPs analyzed. However, this is a cohort of patients recruited from the same hospital and therefore treated according to the same protocols, by the same team of rheumatologists, which ensures the homogeneity of the response variables. Further analysis in larger cohorts will be necessary to corroborate the associated

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SNPs as potential biomarkers of therapy selection in RA and explore more thoroughly the effect of the SNPs which did not show any relation to outcomes.

In summary, the AA-genotype for rs12083537 and CC for rs11265618 showed better LDA rates in our patients, acting as predictors of good response in RA after 12 months of TCZ therapy.

CONCLUSIONS

RA patients treated with TCZ showed better EULAR response, remission, LDA and DAS28 improvement rates in patients when a lower number of BT was administered previously. IL6R polymorphisms rs12083537-AA and rs11265618-CC were associated to higher LDA rates.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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