Influence of *IL6R* rs8192284 Polymorphism Status in Disease Activity in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To analyze the influence of *IL6R* rs8192284 polymorphism on the disease activity of rheumatoid arthritis (RA).

Methods. Patients with RA (n = 281) were followed for a median of 4.2 years. A total of 1143 disease activity measurements using the 28-joint count Disease Activity Score (DAS28) were performed. A mixed-effect model was used to analyze the measurements.

Results. A statistically significant interaction was observed between *IL6R* rs8192284 polymorphism and the presence of anticyclic citrullinated peptide (anti-CCP) antibodies (p = 0.008). An inverse relationship between the polymorphism and DAS28 was observed depending on anti-CCP status.

Conclusion. The anti-CCP status in patients with RA determines the association between the *IL6R* rs8192284 polymorphism and disease activity. (First Release June 15 2010; J Rheumatol 2010; 37:1579–81; doi:10.3899/jrheum.091455)

Key Indexing Terms:
RHEUMATOID ARTHRITIS INTERLEUKIN 6 INTERLEUKIN 6 RECEPTOR

Rheumatoid arthritis (RA) is an immune-mediated disease characterized by the presence of inflammatory cytokines[1]. The interleukin 6 (IL-6) receptor is formed by 2 different membrane glycoproteins: IL-6Rα and gp130[2]. IL-6Rα can be released as a soluble receptor (sIL-6R), bind to IL-6, and interact with the ubiquitous gp130[2]. This process is called transsignaling and may represent the most prominent mechanism of IL-6 signaling[1].

Cleavage of the membrane-bound form (shedding) is the main mechanism for the generation of sIL-6R[3]. The scleavage site is located at Gln357/Asp358[2], where a single-nucleotide polymorphism (SNP; rs8192284 A/C) has been identified[4]. Carriers of the minor allele are associated with higher serum levels of sIL-6R[5,6].

RA with or without anticyclic citrullinated peptide antibodies (anti-CCP) are considered as 2 phenotypically different entities[7] with different risk factors[8] and outcomes[9].

Our aim was to analyze the influence of the *IL6R* rs8192284 polymorphism on the disease activity of patients with RA.

MATERIALS AND METHODS

Patients with RA (n = 281) were randomly selected from a cohort of the Hospital Clinico San Carlos (Madrid, Spain). RA diagnosis was established based on the 1987 American College of Rheumatology criteria[10]. Written informed consent was obtained from all subjects.

Patients were recruited from March 2000 to November 2006 and followed up until April 2009. Disease activity measures (n = 1143) were performed by a trained rheumatologist. There were 4 determinations (interquartile range 3–4, range 3–9) per patient. The 28-joint count Disease Activity Score (DAS28) was calculated as described[11]. Each determination was performed at least 2 years after the onset of RA.

Sociodemographic and clinical data including anti-CCP status (considered positive if at least 1 determination yielded a level > 25 IU; Immunoscan CCPlus, Euro-Diagnostica, Malmö, Sweden) were obtained from OBDAR, a personal prospective RA database.

Genotyping. All subjects were genotyped to determine the *IL6R* SNP rs8192284 status using TaqMan Assays-on-Demand (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol, and analyzed using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems).

Statistical analysis. We constructed a mixed-effect model to analyze the influence of *IL6R* rs8192284 polymorphism in repeated DAS28 measures. We used the Bayesian information criterion (BIC) to choose the best effect pattern of the polymorphism over disease activity. To ascertain the influence of anti-CCP status in the association between *IL6R* polymorphism and disease activity, we considered an interaction between these 2 variables. The fittest model was further adjusted by sex, age, time elapsed from the onset of RA symptoms to the time of each DAS28 determination, education level, and treatment regimen [defined as a categorical variable, in which each value was a different combination of disease-modifying antirheumatic drug (DMARD) received by a patient at each DAS28 determination]. Statistical analyses were performed using Stata 10.

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RESULTS

Influence of IL6R rs8192284 polymorphism status on disease activity. Sociodemographic, clinical, and genetic characteristics of patients with RA are shown in Table 1.

The BIC results for the dominant, recessive, codominant, and additive effect patterns were 3585.04, 3577.75, 3594.16, and 3583.84, respectively; therefore we chose the recessive model. The interaction between *IL6R* polymorphism and anti-CCP status was significant (ß = –0.87, p = 0.008), therefore the effect of *IL6R* polymorphism in DAS28 was dependent on the anti-CCP status. Anti-CCP-negative patients with RA who were homozygous for the minor allele had less disease activity [0.52 DAS28 units less (IQR 0.03–1.00); p = 0.032] than those who were nonhomozygous. Anti-CCP-positive patients did not show significant differences in disease activity between homozygous and nonhomozygous patients for the minor allele (homozygous patients had 0.36 DAS28 units more than nonhomozygous patients; p = 0.097).

The mixed-effect model was adjusted further (Table 2). Interaction between *IL6R* polymorphism and anti-CCP status remained significant (ß = –0.82, p = 0.007). Anti-CCP-negative patients showed a significant influence of *IL6R* polymorphism status on DAS28 measures; patients homozygous for the minor allele had lower disease activity compared to those who were nonhomozygous for the minor allele [0.52 units less (IQR 0.07–0.97); p = 0.024]. Anti-CCP-positive patients showed a trend toward a positive adjusted influence of the *IL6R* polymorphism on DAS28 (ß = 0.30, p = 0.132).

Table 1. Clinical and sociodemographic characteristics of patients with RA.

Characteristic	n (%)
Sex (female)	221 (78.65)
Education level	
No schooling	11 (3.91)
Primary studies	185 (65.84)
Secondary studies	53 (18.86)
Superior studies	32 (11.39)
Anti-CCP-positive	168 (59.79)
Age at time of symptoms onset, yrs, median (IQR)	52.94 (43.72–61.17)
Age at time of recruitment, yrs, median (IQR)	61.95 (53.82–70.18)
Elapsed time between symptom onset and recruitment to study, yrs, median (IQR)	7.42 (3.61–12.95)
Followup period, yrs, median (IQR)	4.20 (3.35–5.04)
rs8192284 *IL6R* polymorphism	
AA	110 (39.15)
AC	121 (43.06)
CC	50 (17.79)

RA: rheumatoid arthritis; IQR: interquartile range; anti-CCP: anticyclic citrullinated peptide antibody.

Table 2. Mixed-effects model estimating the DAS28 based on *IL6R* rs8192284 polymorphism status, anti-CCP status, and interaction. The model was adjusted for sex, age, time elapsed from onset of RA until DAS28 determination, education level, and treatment regimen. Only data referring to rs8192284 status, anti-CCP status, and the interaction between them are shown.

Variables	Coefficient	p	95% CI
rs8192284 status	–0.52	0.024	–0.97, –0.07
Anti-CCP status	–0.08	0.550	–0.33, 0.16
Anti-CCP: rs8192284 interaction	0.82	0.007	0.22, 1.41

DAS28: 28-joint-count Disease Activity Score; anti-CCP: anticyclic citrullinated peptide antibody; RA: rheumatoid arthritis.

DISCUSSION

We have demonstrated that anti-CCP antibodies determine the influence that the *IL6R* rs8192284 polymorphism exerts on RA disease activity. We constructed a mixed-effect model with an interaction between anti-CCP antibodies and polymorphism status. This model was further adjusted by other variables associated with disease outcome[12]. According to our model, among anti-CCP-negative patients, patients who were homozygous for the minor allele showed a lower DAS28 compared to nonhomozygous patients, regardless of sex, education status, age, time elapsed since the onset of RA, or DMARD treatment. Among anti-CCP-positive patients, this polymorphism does not seem to influence disease activity.

Genetic studies, particularly genetic association studies, usually include large sample sizes. Our sample size (281 patients) is relatively modest. However, we have included longitudinal measures of disease activity along with a complete clinical and sociodemographic dataset, so the sample size seems reasonable. In any event, replication studies are necessary to validate these results.

The interaction between anti-CCP antibodies and the polymorphism could be a reflection of a different physiopathology behind the presence or absence of these autoantibodies[7]. Considering the pleiotropic effects of IL-6, we hypothesize that the balance between the proinflammatory[13] and antiinflammatory[14] effects could be tilted toward the antiinflammatory side in patients who are anti-CCP-negative.

Tocilizumab[15], a monoclonal antibody against IL-6Rα, is a novel biologic therapy used in the treatment of RA. Because more copies of the minor allele of rs8192284 polymorphism are associated to a higher plasma level of sIL-6R in both healthy subjects[5,6] and patients with RA (Rodriguez-Rodriguez, *et al*, unpublished data), we hypothesize that anti-CCP-negative patients with RA who are homozygous for the C allele of rs8192284 polymorphism would not be the ideal candidates for this treatment because they are likely to have higher plasma levels of sIL-6R but lower disease activity scores.

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