Polymorphism rs2275913 of Interleukin-17A is related to more intensive therapy with disease-modifying anti rheumatic drugs in Rheumatoid Arthritis Mexican patients

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ABSTRACT

Aim: This study has two aims: 1) to evaluate the association of IL-17 polymorphism rs2275913 with RA severity and 2) to evaluate if this particular single-nucleotide polymorphism (SNP) is associated with susceptibility for RA in Mexican patients.
Methods: Seventy-six RA patients and ninety-four healthy controls were included in the study. RA patients were evaluated according to DAS 28. Treatment with DMARDs was prescribed and radiological damage was evaluated according to the Larsen method. A case-control study was used. Oral epithelial cells were obtained as source for genetic material. DNA was amplified using PCR. Subsequently, a RFLP was carried out. Finally, in order to confirm the IL-17 SNP rs2275913 presence, direct sequencing of the DNA was performed.
Results: A significant difference was observed between the RA patients and controls when the prevalence of IL-17 SNP rs2275913 was compared. There was a statistically significant disparity among the two groups with an OR of 5.6 (95%CI 1.5 – 20.9, P=<0.01).

This study showed that the RA patients who were positive for the IL-17 polymorphism rs2275913 required 3 DMARDs to control the disease compared to 32% of the patients who were negative for the IL-17 polymorphism rs2275913, OR 6.6 (95%CI 1.6 – 27.0, P<0.01).
Conclusion: This study draws two main conclusions: 1) The presence of IL-17 polymorphism rs2275913 is closely related to a more severe form of the disease and as a result, a higher number of DMARDs required to control it, 2) The presence of IL-17 polymorphism rs2275913 may confer a risk of developing RA in Mexican carriers.

Keywords: Il-17 rs2275913; Rheumatoid arthritis; Interleukin-17.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease whose pathogenesis is a complex interplay between the patient's genetics and environmental factors. Its etiology is multifactorial and it is considered the prototype disease of T-cells[1]. At an immunological level, there is a dysregulation in cytokine-mediated signals that cause an infiltration of immune cells into the synovium, an unceasing phenomenon that is maintained by the activation of amplification mechanisms prompting chronic inflammation and progressive destruction of the joint[2].

It has been shown within cellular elements involved in the pathogenesis of the disease, that interleukin 17 (IL-17), a cytokine secreted exclusively by activated T-cells, is a major contributor to inflammation and joint destruction. IL-17 substantially alters cartilage homeostasis and stimulates synoviocytes, chondrocytes and macrophages to release matrix metalloproteinases (MMPs), the most important enzymes involved in cartilage destruction. In a mouse model, administration

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of recombinant IL-17 in a rodent's joint, induced cartilage destruction[3]. Furthermore, blocking of endogenous IL-17 had a protective effect in inhibiting bone destruction.

IL-17 was initially identified in herpes virus saimiri[4] and later in rodents, receiving the name of Cytotoxic T-Lymphocyte Antigen 8 (CTLA-8)[5]. It was described in humans in 1997[6]. It is encoded by a gene localized on the short arm of chromosome 6, in the 6p12 locus, consisting of 1874 bp[7]. The IL-17 A is the founding member of a new subclass of cytokines[8] and is mainly produced by the pro inflammatory cells Th1/Th0.

Now, with respect to the susceptibility to rheumatoid arthritis, it is accepted that genetic factors play a major role. It was found that the concordance rate in homozygous twins was higher than 50%[9]. Also differences were found in the prevalence in various countries, ranging between 0.5% and 1% of the population. However, it has to be considered that most of the information regarding rheumatic diseases comes from developed countries and data in Latin America (LA) is still scarce. Some reports in LA show epidemiological differences among which we find: a) the prevalence of RA is higher in LA than in the rest of the world[10] (1.6%), b) the male/female ratio is higher than in other parts of the world (7: 1 Vs 3: 1), and c) some surveys of LA suggest that the onset of RA occurs around age 40, almost a decade earlier than in Caucasians[11]. These differences may be the result of genetic susceptibility or represent exposure to different environmental factors.

It is known that the variations in the human genome range from a change in a simple pair of bases to several hundred pairs. These changes may confer biochemical and physiological variations, which can be directly linked to susceptibility or resistance to specific traits or certain diseases. The spectrum of the resulting effects caused by genetic variations in individual genes, functions or protein expression is very broad[12].

In this regard, in order to determine whether it is related to the severity of RA or to susceptibility, several studies had been done with the Polymorphisms IL-17 SNP rs2275913, which is located in the -197 position of initiation codon of the gene of IL-17A. However, the results were mixed.

In a study amidst the Algerian population, the presence of IL-17 SNP rs2275913 showed no relationship to the risk of developing RA but a negative association with the production of IgA of rheumatoid factor was found[13]. In an analysis carried out by Nordang *et al*. in Norwegian patients[14], the presence of IL-17 SNP rs2275913 was associated with an increased risk of rheumatoid arthritis with an OR of 1.28 (95% CI 1.01 to 1.54, p = 0.01). Contrarily, in another research carried out among the population of China, Le Shen y cols. found a lower frequency of IL-17 SNP rs2275913 in patients with RA compared to the healthy population, resulting in an odds ratio of 0.77 (95% CI 0.59 to 0.99, P = 0.043)[15].

Taking into account the varying results in different populations and considering that the relationship between IL-17 SNP rs2275913 and development of RA remains uncertain, and the fact that Mexico has not evaluated the association between these variables, we proposed this investigation to establish the potential clinical effects of this genetic variant.

MATERIAL AND METHODS

PATIENTS AND CONTROLS

We conducted a prospective case-control study in a general, secondary care hospital: 76 outpatients with RA, including men and women older than 18 years of age, were recruited who met the following criteria: 1) all patients satisfied the 2010 ACR/EULAR revised criteria for RA[16], 2) progression time of disease was inferior or equal to 5 years, 3) currently receiving a specific treatment by a rheumatologist, 4) Both, the cases and the controls were unrelated individuals and self-reported as having at least three generations of Mexican mestizo ancestry. Patients suffering from another autoimmune diseases or dubious cases were excluded from the study. All the patients signed an informed consent form. The research protocol received approval from the National Committee for Clinical Research at the Social Security Mexican Institute (No. R-2013--785-060).

The controls were 94 healthy subjects sharing the same demographic characteristics as the patients under the following criteria: 1) absence of illness, 2) no relatives suffering from RA or any other known autoimmune or inflammatory disease (at least three generations), 3) of Mexican descent and from the same geographical area where the study was conducted.

During the rheumatology consultation, buccal DNA samples were collected as described below, and the patients were evaluated according to: a) Disease Activity Score 28 (DAS 28), b) treatment with disease-modi-

fying anti rheumatic drugs (DMARDs), and c) radiographic damage. Joint damage was assessed on radiographs of hands and feet according to the Larsen method. The total score (range 0–160) was used to quantify RA structural severity. The radiological assessment was performed by an expert radiologist blinded to the results of the genetic analysis.

All patients enrolled in the study had previously been managed according to the Update of the Mexican College of Rheumatology Guidelines for the Pharmacologic Treatment of Rheumatoid Arthritis.

Lastly DAS-28, Larsen scores and DMARDs treatments were compared between the patients with and without the Polymorphism rs2275916 to relate their association with it.

GENOTYPING

Collection of buccal swab samples

Porous foam-tipped swabs (Epicentre Biotechnologies, Madison, WI) were used to collect buccal epithelial cells from patients and controls (hospital volunteers).

Buccal DNA samples were collected with Buccal Amp TM DNA Extraction Kits (Epicenter BioTechnologies).

Preparation of DNA from buccal cell swabs

BuccalAmp samples were processed according to the manufacturer's instructions. Buccal swabs were briefly swirled directly in tubes containing 500 l of Quick Extract DNA extraction solution. Samples were incubated at 65°C for 1 min, briefly vortexed (15 seconds), transferred to 98°C for 2 min and stored at -20°C.

Genotyping PCR of DNA samples prepared from buccal cells

PCR products were subjected to electrophoresis on 1.5% agarose gels and the bands corresponding to the specific amplicons (300pb) were cut off from the gels. DNA was extracted using the Wizard RSV Gel and PCR Clean-Up System. Subsequently, a Restriction Fragment Length Polymorphism (RFLP) analysis was performed using the XmnI enzyme (PdmI, by Thermo Scientific), which is a restriction endonuclease from bacterial strain *Xanthomonas manihotis* with nucleotide sequences for cleavage sites in 5'... G A A N N N N T T C ...3'. The samples were then run on an agarose gel in order to identify the fragments generated by the restriction enzyme. When there was a presence of polymorphism in the study, a cut was generated that produced two DNA fragments, a 56 bp (inferior) and an additional 244 bp (superior) (Figure 1).

Finally, in order to confirm the SNP presence by RFLP, each product was used as a template to conduct a sequencing reaction with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to manufacturer procedures. The sequences were analyzed using the ABI 3130xl Genetic analyzer by Applied Biosystems, (Figure 2).

STATISTICAL ANALYSIS

Sample size was calculated using the formula to compare two means for a quantitative variable and the variance found in Larsen score. A type I error risk of 5% and a type II error risk of 20% were assumed. A two-sided *P*-value <0.05 was considered significant for all the analyses.

The odds ratio (OR) between the SNP rs2275913 carriers among the healthy subjects and the patients was calculated in order to assess the risk of developing RA among positive subjects.

Similarly, the OR was calculated to assess the risk to have more than 2 DMARDs depending on the presence or absence of the SNP among the patients with RA. This was calculated using the Epidat V 3.1 statistical software.

The outcome variables were compared in a Chi-squared (χ^2) test for qualitative variables and Student's t for quantitative ones, using SPSS V.17.0 statistical soft-

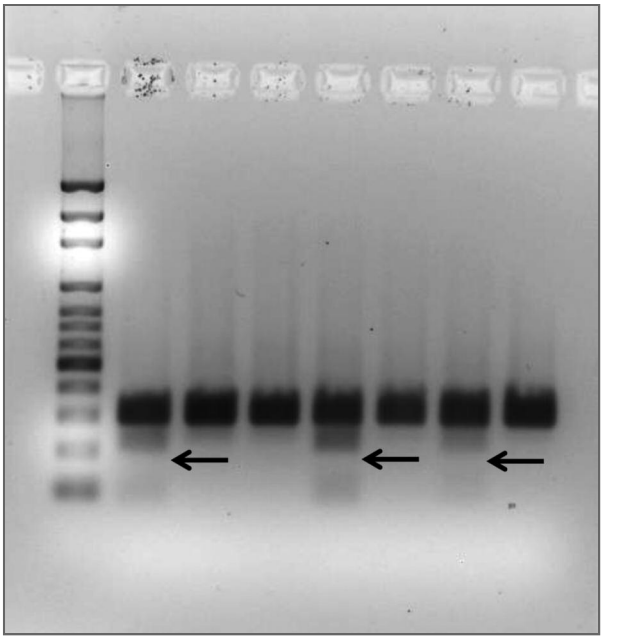

FIGURE 1. Agarose gel showing positive samples for IL-17 rs2275913 (arrows)

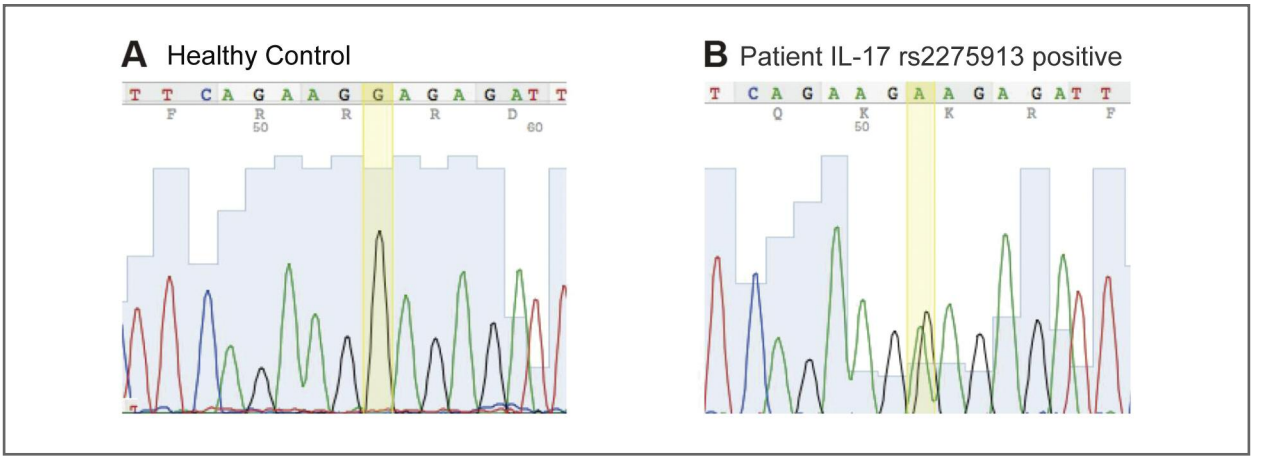

FIGURE 2. Difference in gene sequence. A. Healthy control B. Patient who tested positive for IL-17 rs2275913

ware. In addition, a descriptive analysis was run for demographic variables.

RESULTS

Seventy-six RA patients satisfying the above-detailed criteria and ninety-four healthy subjects were recruited for this study. All participants had the same demographic profile and ethnic background. Of the RA patients 87% (N=66) were women vs 85% (N=80) in the healthy group, with an average age of 49 ± 13 years and 45± 15 years respectively (P=0.8). No difference in age and sex distributions was found between the two groups (P= 0.8). Clinical and demographic characteristics of patients and controls are presented in Table I.

TABLE I. BASAL CHARACTERISTICS OF STUDY POPULATION

Characteristics	Patients n = 76	Controls N = 94	Value
Gender			
Female	66 (87%)	80 (85%)	p = 0.8
Male	10 (13%)	14 (15%)	P = 0.8
Age (years)	49 ± 13	45 ± 15	p = 0.6
Disease duration (years)	1.7 ± 0.6	–	
Rheumatoid Factor	265 ± 363	–	
Anti-CCP	385 ± 567	–	
HAQ†	1.7 ± 0.6	–	
DAS 28‡	1.9 ± 0.9		

† HAQ: Health Assessment Questionnaire
‡ DAS-28: Disease Activity Score 28
Anti-CCP, cyclic citrullinated peptides antibodies

The mean of disease duration of RA patients was 1.8 ± 1.2 years. Rheumatoid Factor and anti-cyclic citrullinated peptide antibody (anti-CCP) concentrations were 265 ± 363 U/ml and 363 ± 619 UI/ml, respectively. The average of HAQ score was 1.7 ± 0.6, and the radiological evaluation according to the Larsen method showed an overall mean of 43 ± 20 points. Mean disease activity (DAS28) was 1.97 ± 0.8. The complete clinical data and characteristics of the RA group are presented on Table II.

When we analyzed the prevalence of IL-17 rs2275913 on RA patients and healthy controls, we found a significant difference between these two groups. Of the 76 patients evaluated, 15% (N=12) were positive for the IL-17 rs2275913 vs 3% (N=3) for the control group. This results in an odds ratio of 5.6 (95%CI 1.5 – 20.9, P=<0.01) for risk of Rheumatoid Arthritis in subjects carrying the polymorphism (Table III).

The review of the therapeutic schemes administered to the patients with RA showed that one patient (1%) received methotrexate monotherapy, 44% (N = 33) had dual scheme DMARDs, 50% (N = 38) had triple scheme DMARDs and only 5% (N = 4) were in biologic therapy with infliximab.

When we compared the DMARDs regime according with SNP carrier status, 73% of the patients who tested positive for the IL-17 rs2275913 required 3 DMARDs to control the disease compared with 32% who tested negative, this results in an odds ratio of 6.6 (95% CI 1.6 – 27.0, P=<0.01) (Table IV).

TABLE II. CHARACTERISTICS OF PATIENTS ACCORDING TO THEIR CARRIER STATUS OF POLYMORPHISM

Characteristics	Patients with SNP rs2275913 N=12	Patients without SNP rs2275913 N=64	Value
Gender			
Female	9 (75%)	57 (89%)	p = 0.4
Male	3 (25%)	7 (11%)	p = 0.4
Age	49 ± 11	49 ± 14	p = 0.9
Disease duration (years)	1.4 ± 0.6	1.8 ± 1.3	p = 0.3
Rheumatoid Factor	181 ± 230	281 ± 383	p = 0.4
Anti-CCP	424 ± 766	352 ± 597	p = 0.7
HAQ†	2.0 ± 0.6	1.7 ± 0.6	p = 0.1
DAS‡	1.8 ± 0.9	2.0 ± 0.7	p = 0.2
Larsen score (0-180 range)	39 ± 30	29 ± 26	p = 0.7

† HAQ: Health Assessment Questionnaire
‡ DAS-28: Disease Activity Score 28
Anti-CCP: cyclic citrullinated peptides antibodies; SNP: single-nucleotide polymorphism

TABLE III. ASSOCIATION BETWEEN IL-17 RS2275913 AND RHEUMATOID ARTHRITIS

Carrier status	Cases 76 (100%)	Controls 94 (100%)	OR (95% CI)	P
IL-17 rs2275913 (+)	12 (15%)	3 (3%)	5.6 (1.5 - 20.9)	< 0.01
IL-17 rs2275913 (-)	64 (85 %)	91 (97%)		

P value <0.05 indicates a significant difference. CI= Confidence interval. OR = odds ratio.

TABLE IV. ASSOCIATION BETWEEN IL-17 RS2275913 AND DMARDs PROFILE

Patient group	≥ 3 DMARDs N (%)	≤2 DMARDs N (%)	OR (95% CI)	P
IL-17 rs2275913 (+)	9 (73%)	3 (27%)	6.6 (1.6 – 27.0)	< 0.01
IL-17 rs2275913 (-)	20 (32 %)	44 (68%)		

P value <0.05 indicates a significant difference. CI= Confidence interval. OR = odds ratio.

No significant difference was found between IL-17 rs2275913 carriers and non-carriers for the radiological evaluation of proximal interphalangeal, metacarpophalangeal, carpal and metatarsophalangeal joints using Larsen's method (39±30 vs 29±26, P=0.7), HAQ score (2.0±0.6 vs 1.7±0.6, P=0.1) or DAS-28 score (1.8±0.9 vs 2.0±0.7, P=0.7).

DISCUSSION

Rheumatoid arthritis is a chronic autoimmune disease with an important genetic predisposition. Although many analyses have shown that genetic factors play an important role in the pathogenesis of RA, only a few have identified an association with the susceptibility to RA.

The aim of this study was to determine whether IL-17 polymorphism rs2275913 is associated with more severe clinical presentations and to explore their influence on the risk of developing rheumatoid arthritis in Mexican patients.

In the current study, we found a modest but significantly higher prevalence of SNP IL-17 rs2275913 in patients with rheumatoid arthritis in relation to healthy

controls, resulting in an OR of 5.6 (95% CI 1.5 - 20.9, P = < 0.01) to develop the disease. These results are consistent with findings reported by Nordang *et al.*[14], although in our case the difference in the prevalence of SNP IL-17 rs2275913 between the control group and the patients group was much more noticeable, though it must be considered that the sample size reported by Nordang is certainly much higher.

In addition, we showed that patients carrying IL-17 SNP rs2275913, required a DMARDs scheme significantly more aggressive to achieve optimal disease control OR 6.6 (95% CI 1.6 - 27.0, P = <0.01). It should be considered that the treatment for RA is individually suited according to clinical, biochemical and radiological variables that determine disease activity such as: the number of painful and swollen joints, C-reactive protein concentrations, concentrations of anti-CCP and rheumatoid factor, among others. Overall, a higher activity level requires a more aggressive DMARDs scheme.

Our study shows that with the presence of IL-17 in patients with RA rs2275913, the probability of requiring more than two DMARDs increases by five times compared with patients without IL-17 SNP rs2275913. We observed that there was no greater radiographic damage in patients with polymorphism than in those with negative status, which is relevant considering that radiological progression is one of the most important variables in determining the aggressiveness of the disease[17-18]. These findings are concordant equally with those reported by Pawlik, whose investigation found no difference in the immune profile or erosive lesions among patients who were carriers of polymorphism rs2275913 IL-17 compared to non-carriers[19].

There are two factors to which we can attribute the fact that significant differences in Larsen scoring were found. First and foremost, those patients who are carriers of IL-17 rs2275913 received a more aggressive DMARDs regime, which could have altered the course of the disease.

In various randomized clinical trials it has been shown that patients who were treated with a combination of three DMARDs exhibit less radiographic progression compared to patients receiving a lesser amount of these drugs[20-21]. Secondly, our group of patients had an average disease progression of 1.8 years. This brief evolution probably had a significant impact on the minimal radiographic progression. The reported prevalence of radiographic lesions in patients with early RA[22] is 8% to 40%.

Our study has several limitations. First, although this primary approach allows us to formulate some conclusions regarding the frequency of the mutation in patients with RA, it should be noted that the size of the sample is small. It is necessary to conduct large-scale studies to determine the true prevalence of the SNP in both the RA and general population.

Moreover, with respect to the finding of an increased risk of RA in people who are carriers of SNP, one must be cautious in establishing an association of causality because as previously mentioned, the etiopathogenesis is multifactorial and there are many immunopathogenic mechanisms beyond the analysis of this study.

Finally, our observations are mainly based on frequency analysis but experimental studies are needed to accurately determine the biological or functional implications of change of bases of this polymorphism.

In short, to our knowledge, this is the first study conducted in Mexico that analyzes the relationship between IL-17 rs2275913 and the clinical presentation of RA. This research allows us to establish two main conclusions: 1) the presence of SNP IL-17 rs2275913 may confer a risk of developing the disease in patients who are carriers and, 2) the presence of IL-17 rs2275913 correlates closely with the number of DMARDs used for disease control.

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