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Original article

Anti-CCP antibodies are associated with early age at onset in patients with rheumatoid arthritis

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ABSTRACT

Objectives: To determine factors influencing the age at onset of rheumatoid arthritis (RA).
Methods: A sample of 152 Colombian patients was investigated. Hazard ratios (HRs) that measured the effect size of risk factors on the age at RA onset were computed by using Cox regression models.
Results: Positive anti-CCP antibodies were associated with an increased risk of early RA onset (HR = 1.60; 95% confidence interval, [1.06, 2.4]), whereas the presence of the protective ^{70}DERAA^{74} sequence was associated with a delayed onset (HR = 0.55; [0.33, 0.92]). After controlling for both anti-CCP antibodies and the ^{70}DERAA^{74} sequence, the following variables did not influence significantly the age at RA onset: gender, ever cigarette smoking, family RA history, the *TNF-308 A* polymorphism, HLA shared epitope, and the presence of rheumatoid factor.
Conclusion: Anti-CCP antibodies and the HLA-DRB1 ^{70}DERAA^{74} sequence influence the age at onset of RA.

1. Introduction

Age at onset of rheumatoid arthritis (RA) is influenced by genetic [1], hormonal [2], immunological and environmental factors [3]. Genetic risk factors are thought to be responsible for up to 50–60% of RA risk [4], and the *HLA-DRB1* gene has been unequivocally associated with RA susceptibility. Anti-cyclic citrullinated peptide (anti-CPP) antibodies have received special attention, since they may be helpful for RA diagnosis; they are moderately sensitive but highly specific to RA, with a specificity that is higher than that of RF [5]. Anti-CCP antibodies can be detected at very early disease stages, and may be used as indicators of RA progression and prognosis [6]. The objective of the current study was to investigate the effects of demographic, antibody profile and immunogenetic characteristics on the age at onset of RA (AORA).

2. Methods

2.1. Study population

A sample of Colombian patients with RA was analyzed. The sample included 152 consecutive patients attending the Clinical Immunology and Rheumatology Unit at the "Clínica Universitaria Bolivariana-Corporación para Investigaciones Biológicas", Medellin, the Center for Autoimmune Diseases Research at the School of Medicine in the Rosario University, and the Rheumatology Unit at the "Universidad Nacional de Colombia" School of Medicine, Bogota, Colombia. All patients met the American College of Rheumatology criteria for RA classification [7] and had an AORA ≥ 17 years. Only patients with a confirmed diagnosis of RA were included in the sample. Local institutional review boards and ethics committees approved the study, and all patients signed a written informed consent. A patients' description is in Table 1.

2.2. Variables

Data were recorded as previously reported [8,9] and collected with a specifically created standard data collection form, which included demographic, clinical and laboratory variables. The AORA

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Table 1
Demographic and clinical characteristics of 152 Colombian patients with RA.

Characteristics	Mean ± SD
Age at RA onset (years)	42 ± 14[a]
Disease duration (years)[b]	8.5 ± 7.3
	% (*n*)
Female gender	85 (129/152)
Ever smoking[c]	22 (33/152)
Autoantibodies	
Rheumatoid Factor	73 (103/141)
Positive anti-CCP titers	78 (119/152)
Genetic factors	
Family history of RA	7 (10/151)
Alleles encoding ^{70}DERAA^{74}	11 (17/152)
HLA-DRB1 SE[d]	51 (78/152)
TNF-308 *A* allele	26 (38/149)

RA: rheumatoid arthritis; CCP: cyclic citrullinated peptide; HLA: human leukocyte antigen; SE: shared epitope; TNF: tumor necrosis factor gene; SD: standard deviation.

[a] The median, 25th and 75th percentiles were 40, 32 and 52 years, respectively.

[b] Disease duration at moment of serological sampling.

[c] Ever smoking was assessed by a self-reported, validated questionnaire and was defined as having smoked at least one pack year in lifetime [7].

[d] Carrying at least one copy of any SE allele.

was defined as the age at which patients began to suffer from pain, morning stiffness, or a clinically confirmed inflammation of hand and/or foot joints in a symmetrical fashion. A family history of RA was defined as the clinically confirmed presence of the disease in at least one first-degree relative [6].

The *TNF*–308 A/G single nucleotide polymorphism (SNP) was genotyped by polymerase chain reaction (PCR) [10]. *HLA-DRB1* typing was done by reverse dot-blot hybridization (Inno-LiPA assay) [11]. *HLA-DRB1* status was categorized according to the presence of the shared epitope (SE) or ^{70}DERAA^{74} motifs, as described elsewhere [9,11].

Total rheumatoid factor (RF) was measured by turbidimetry. An Elisa kit was used to measure third generation anti-cyclic citrullinated peptide (anti-CCP3) antibodies according to the manufacturer's protocol (Quanta-Lite, Inova, San Diego, CA).

2.3. Statistical analysis

A Cox regression model of AORA was built by using the SAS software [12] and a backward selection procedure. The AORA was the survival time. A model including all potential independent variables was initially fitted. Then the less significant variable was eliminated and the model was fitted with the remaining variables. This elimination continued until all variables in the model were significant at the 0.05 level [13]. The following were considered potential independent variables: being a carrier of the *TNF-308 A* allele, carrying HLA-DRB1 SE alleles, the HLA ^{70}DERAA^{74} amino-acid sequence, positive RF titers (> 40 U/mL), positive anti-CCP titers (> 60 U), family history of RA, ever smoking, and female gender. Cox model goodness-of-fit was examined through log-cumulative-hazard plots and Kolmogorov-type supremum tests. The final model fitted well. The size of the effect of a significant independent variable on AORA was measured as the hazard ratio corresponding to the variable [13]. Ninety-nine percent CIs for regression coefficients and hazard ratios were computed. Results with *P*-values < 0.05 were considered significant.

3. Results

The mean ± SD AORA was 42 ± 14 years (Table 1). Table 2 shows the variables significantly associated with AORA according to the Cox model. Positive anti-CCP3 antibodies were significantly associated with a lowered AORA after controlling for the presence of the HLA ^{70}DERAA^{74} sequence ($P = 0.02$, Table 2). Having a ^{70}DERAA^{74} sequence was significantly associated with a raised AORA after controlling for positive anti-CCP3 antibodies ($P = 0.02$). In patients who had the ^{70}DERAA^{74} sequence and were anti-CCP3 negative, the mean ± SD AORA was 45.5 ± 19.5 years. In patients who had a ^{70}DERAA^{74} sequence but were anti-CCP3 positive, it was 51.4 ± 13.3 years. In patients who did not have a ^{70}DERAA^{74} sequence and were anti-CCP3 negative, it was 45.7 ± 15.6 years. In patients who did not have the ^{70}DERAA^{74} sequence and were anti-CCP3 positive, it was 39.5 ± 12.1 years.

The hazard ratio that compared the risk of RA onset in patients with positive versus patients of equal age with negative anti-CCP3 antibodies, adjusted for positive ^{70}DERAA^{74} sequence, was 1.60 (Table 2). Thus, at any particular age after 17 years, the hazard of RA onset for a patient with current positive anti-CCP3 antibodies was 60% higher than that for a patient with negative anti-CCP3 antibodies. Similarly, the hazard ratio that compared patients with the ^{70}DERAA^{74} sequence versus patients without it, adjusted by positive anti-CCP3 antibodies, was 0.55. Therefore, at any particular age after 17, the hazard of RA onset for a patient who had the ^{70}DERAA^{74} sequence was 45% lower than that for a patient who did not have it.

After controlling for both positive anti-CCP3 antibodies and ^{70}DERAA^{74} sequence, no other investigated variable was significantly associated with AORA. A trend between HLA-DRB1*0404 and positive anti-CCP3 antibodies was observed (Table 3). No significant differences in SE allele frequencies were observed between anti-CCP3 positive and negative patients (50% vs 55%, respectively). There was a statistically significant difference in the frequencies of positive RF between anti-CCP3 positive and negative patients (81% vs 41%, respectively; OR = 6.14; 95% CI, [2.55–14.77]; $P < 0.001$).

The mean ± SD disease duration at moment of serological sampling was 8.5 ± 7.3 years. An additional Cox regression showed that there were not significant interactions between disease duration and the significant variables reported in Table 2, and the hazard

Table 2
Variables significantly associated with the age at onset of rheumatoid arthritis (AORA) in Colombian patients with RA according to a Cox model.

Variables[a,b]	β[c]	95% CI[d]	*P*-value	Hazard ratio	95% CI[d]
Positive anti-CCP antibodies	0.47	(0.006, 0.88)	0.02	1.60[e]	(1.06, 2.4)
^{70}DERAA^{74} sequence	−0.59	(−1.10, −0.08)	0.02	0.55[f]	(0.33, 0.92)

RA: rheumatoid arthritis; CCP: cyclic citrullinated peptide; 95% CI: 95% confidence interval.

[a] The Cox model of AORA was $\log\{h(t)\} = \log\{h_0(t)\} + 0.47X_1 - 0.59X_2$, where X_1 = being anti-CCP positive, X_2 = having an HLA ^{70}DERAA^{74} sequence, h(t) is the hazard of RA onset at age *t* for a patient who will develop RA, and $h_0(t)$ is the hazard of RA onset at age *t* for a person who is anti-CCP negative, does not have a ^{70}DERAA^{74} sequence, and will develop RA (proportional hazards test for positive anti-CCP, $P = 0.91$; for ^{70}DERAA^{74}sequence, $P = 0.90$).

[b] Independent variables included in the Cox model were dichotomous (1 = positive, 0 = negative).

[c] The numbers in this column are Cox regression coefficients.

[d] 95% confidence interval for β or the hazard ratio.

[e] The hazard ratio and its 95% CI are adjusted for the presence of the ^{70}DERAA^{74} sequence.

[f] The hazard ratio and its 95% CI are adjusted for positive anti-CCP antibodies.

Table 3
Clinical and genetic characteristics of Colombian patients with RA stratified by the presence of anti-CCP antibodies status.

Characteristics	Anti-CCP3 positive (n = 119)	Anti-CCP3 negative (n = 33)
Female gender	86 (102/119)	82 (27/33)
Ever smoking	21 (25/119)	24 (8/33)
Family history of RA	8 (9/119)	3 (1/32)
Reumatoid factor (+)	81 (91/112)[a]	41 (12/29)
Having the ^{70}DERAA^{74} sequence	11 (13/119)	12 (4/33)
HLA-DRB1*0404	20 (24/119)	6 (2/33)
HLA-DRB1*0401/0404	0.8 (1/119)	0 (0/33)
HLA-DRB1 ^{70}QKRAA^{74} SE	0.9 (1/114)	3 (1/33)
HLA-DRB1 ^{70}RRRAA^{74} SE	0 (0/114)	3 (1/33)
HLA-DRB1 ^{70}QRRAA^{74} SE	47 (54/114)	48 (16/33)
HLA-DRB1 SE[b]	50 (60/119)	55 (18/33)
TNF-308 A allele	26 (30/117)	25 (8/32)

Data are in % (n). RA: rheumatoid arthritis; SE: shared epitope; CCP: cyclic citrullinated peptide.
[a] OR: 6.14 (95% CI = 2.55–14.77), $P < 0.0001$. No other significant differences were observed, although a trend was observed in HLA-DRB1*0404 patients ($P = 0.06$).
[b] Carrying at least a copy of any SE allele.

ratios reported in the Table 2 were not substantially affected after including disease duration in the model.

4. Discussion

The main result of this study was that RA patients who are currently positive for anti-CCP antibodies developped RA at an earlier age than anti-CCP negative patients. This observation was established in a sample of Colombian patients, an admixed population from tropical latitudes. However, an association of anti-CCP antibodies with early RA onset was also found in 468 Swedish patients in whom anti-CCP antibodies were present in 67.9% of them [14]. In a sample of 411 Spanish patients with RA in which 54% were anti-CCP positive, the trend for association observed between anti-CCP antibodies and age at onset disappeared when disease duration was taken into account [15].

Although important differences exist in the characteristics of patients reported in the literature as well as the cut-off value used to define a positive test, anti-CCP antibody testing is particularly useful in the diagnosis of RA, with high specificity [16]. Anti-CCP antibodies are usually present before the development of RA symptoms, suggesting that a break in tolerance to citrullination products occurs early in RA development [17]. Recent studies suggest that anti-CCP positive and negative RA might have different pathogenesis [18].

Having the ^{70}DERAA^{74} sequence protected against early RA onset in these Colombian patients. The ^{70}DERAA^{74} sequence alleles have been suggested to protect against RA onset and severity [19]. A model supported by some experimental and clinical studies, known as the RA-protection hypothesis, postulates that DQ molecules predispose to RA and that DR molecules (^{70}DERAA^{74}) could modulate disease severity by serving as a source of self-peptides presented in the context of DQ molecules [20]. The frequency of patients with RA carrying the ^{70}DERAA^{74} sequence ranges from 8 to 16% [20]; this variation is independent of the presence of HLA-DQ molecules or SE status. In the current study, this frequency was 11% (Table 1). Recently, the ^{70}DERAA^{74} sequence was reported to be a strong independent predictor of a better RA prognosis in a North-American early polyarthritis cohort [21].

A significant association between SE alleles and positive anti-CCP3 antibodies was not detected in Colombian patients (Table 3), despite the fact that significant associations have been reported in the published literature. This lack of association may be caused by an insufficient sample size or by a modest effect of HLA on the synthesis of anti-CCP antibodies in our population since the influence of genotype on phenotype varies across populations [6].

Although individuals with an RA family history or *HLA-DRB1* risk alleles are considered to be at high risk of developing RA [22,23], our study did not find an association between these variables and an early AORA. Our study did not find a significant effect of ever smoking on AORA after adjusting for significant variables, although an effect of current cigarette smoking on AORA has been previously reported [3].

In conclusion, anti-CCP antibodies and the HLA-DRB1 ^{70}DERAA^{74} sequence influence the age at onset of RA in Colombian patients. Prospective studies are needed to examine whether this finding may be useful to predict an early RA onset in subjects genetically prone to RA, and to design strategies for preventing early RA onset as well.

Conflict of interest statement

The authors report no conflicts of interest.

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