Shared Epitope and Anti-Cyclic Citrullinated Peptide Antibodies: Relationship with Age at Onset and Duration of Disease in Rheumatoid Arthritis

To the Editor:

Rheumatoid arthritis (RA) is a complex disease with a strong immune inflammatory component. Recent progress in defining the role of genetic factors in RA has been remarkable. It has been proposed that the shared epitope (SE) is a primary genetic element of susceptibility. Citrullination is a post-translational protein modification that is accomplished by deimination of arginine by peptidyl-arginine deiminases. Interestingly, polymorphisms in one member of the family, PADI4, have been associated with RA in Asian populations[1], although this association could not be reproduced in Caucasian populations[2,3]. An association has been described between the presence of the SE and anti-cyclic citrullinated peptide antibody (anti-CCP) levels, and of each variable with disease severity[4,5]. Also, anti-CCP-positive has been reported to be immunogenetically distinct from anti-CCP-negative disease, with the former subgroup being primarily responsible for association and linkage to HLA-DRB1 SE[6].

In this cross-sectional study we aimed at analyzing whether the presence of the SE and anti-CCP caused an acceleration in disease development that could be reflected in an earlier age at onset. This acceleration might be considered a sign of a worse prognosis.

We analyzed 411 Spanish patients with RA (76% women) consecutively recruited from a single center, all meeting American College of Rheumatology criteria for RA. Patients' mean age at disease onset was 51 ± 15 years, and median disease duration was 8 years (range 3–15); 58% of patients carried the SE (Lifecodes HLA-SSO kit; Terpenel-Diagnostics Ltd., Abingdon, UK). The following alleles were identified: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001 or *1402. Fifty-four percent of patients presented anti-CCP antibodies (determined by ELISA with Immunoscan Euro-Diagnostica assay; Euro-Diagnostica, Malmoe, Sweden; single testing) and 69% were rheumatoid factor (RF)-positive (all patients who had been RF-positive in just one analysis irrespective of when the analysis had been performed). Statistical analysis was performed with standard statistical software (Stata v 9.0). Normality was tested by skewness and kurtosis tests.

There was a high association between anti-CCP and SE: 68% of 222 anti-CCP-positive individuals were also positive for SE, and only 47% of 189 anti-CCP antibody-negative patients were SE-positive (p = 0.00002; odds ratio 2.39, 95% CI 1.60–3.56; Table 1). In both groups of patients (anti-CCP-positive and anti-CCP-negative), SE positivity was statistically higher than in healthy Spanish controls (32% of 595 individuals; OR 4.53, 95% CI 3.22–6.40 for anti-CCP-positive and OR 1.90, 95% CI 1.34–2.69 for anti-CCP-negative patients). We analyzed whether age at onset is dependent on presence of SE or anti-CCP antibodies; in both cases an earlier age at onset was found in patients with positive SE or anti-CCP, although no statistical significance was reached (Table 1; p = 0.12 and p = 0.07, respectively). Positivity for both factors simultaneously was associated with an even earlier age at onset when compared with all other patients (48 ± 14 yrs vs 53 ± 18 yrs; p = 0.05), although this comparison did not withstand Bonferroni correction.

It seemed reasonable that patients with early age at onset will probably have a longer disease duration. We found that a clear and highly significant link existed between age at onset and disease duration ($p < 10^{-7}$; r = –0.43).

We analyzed whether disease duration was dependent on SE or presence of anti-CCP (positivity); only the presence of anti-CCP antibodies was associated with disease duration (p = 0.005). The presence of SE showed no statistically significant association with disease duration (p = 0.62; Table 2).

In order to ascertain the relationship between SE, anti-CCP antibodies, age at onset, and disease duration, we performed a univariate analysis of variance test considering all these variables. As Table 1 shows, the trend for association (p = 0.07) observed between anti-CCP antibodies and age at onset disappeared when disease duration was taken into account (p = 0.52). Positivity for both factors (SE and anti-CCP antibodies) simultaneously was not associated with earlier age at onset, when adjusted by disease duration, even when compared with double-negative patients (p = 0.15). Therefore, our data suggest that association of anti-CCP with age at onset is secondary to disease duration.

It can been argued that, as a result of an inflammatory process, anti-CCP antibodies may appear not only before the first symptoms, as has been occasionally described[7], but also during the course of the disease. According to this idea, we have shown in our study that it is more probable to find anti-CCP antibodies after a long disease history. Indeed, our data suggest that an association exists between anti-CCP antibodies and disease duration (Table 2), but not primarily with age at onset (Table 1). These results are supported by reports that have described the acquisition of anti-CCP antibodies, resulting from an immune response[8]. There are no studies analyzing the prevalence of anti-CCP antibodies during the whole course of the disease (anti-CCP antibodies have been a recent addition to the RA

Table 2. Statistical analysis of disease duration using Mann-Whitney U test.

		Disease Duration, Median yrs (interquartile range)	p
SE	+	9 (4–15)	0.62
	–	8 (3–15)	
CCP	+	10 (4–16)	0.005
	–	7 (2–12)	

SE: shared epitope; CCP: Citric citrullinated peptide.

Table 1. Statistical analysis of age at RA onset using parametric test.

		Age at Disease Onset N (%)	Age at Disease Onset Mean yrs (SD)	Bivariate p, β (95% CI)	Multivariate p, β (95% CI)
SE	+	240 (59)	50 (± 17)	0.12, –2.46 (–5.58; 0.65)	0.16, –2.07 (–4.95; 0.81)*†
	–	171 (41)	52 (± 14)		
CCP	+	222 (54)	50 (± 15)	0.07, –2.82 (–5.90; 0.26)	0.52, –0.93 (–3.81; 1.94)*†
	–	189 (46)	53 (± 16)		
SE/CCP double-positive		151 (37)	48 (± 14)	0.05, –4.01 (–8.12–0.10)	0.15, –2.7 (–6.35–0.95)*†
SE/CCP double-negative		100 (24)	53 (± 18)		
Duration of disease				$<10^{-3}$, –0.76 (–0.92; –0.61)	$<10^{-3}$, –0.75 (–0.90; –0.59)*

* Adjusted by sex. † Adjusted by duration of disease. SE: shared epitope; CCP: citric citrullinated peptide.

diagnostic arsenal), but Rönnelid, *et al*[9] showed that during the first 5 years for anti-CCP-positive individuals, antibody levels remained fairly constant. Nonetheless, it is remarkable that 3 out of 119 anti-CCP-negative patients (2.5%; analyzed with the same anti-CCP assay) became positive after only 1 year of followup.

The fact that anti-CCP-negative patients have higher SE positivity than that found in our healthy control population (49% vs 32%) suggests that the SE contributes independently to RA development, even in absence of anti-CCP antibodies. We are aware that our hypothesis contrasts with current thinking on the role of the SE in RA pathogenesis[10], and further studies will be necessary to fully address this issue.

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REFERENCES

1. Yamada R, Suzuki A, Chang X, Yamamoto K. Peptidylarginine deiminase type 4: identification of a rheumatoid arthritis-susceptible gene. Trends Mol Med 2003;9:503-8.
2. Barton A, Bowes J, Eyre S, Symmons D, Worthington J, Silman A. Investigation of polymorphisms in the PADI4 gene in determining severity of inflammatory polyarthritis. Ann Rheum Dis 2005;64:1311-5.
3. Martinez A, Valdivia A, Pascual-Salcedo D, et al. PADI4 polymorphisms are not associated with rheumatoid arthritis in the Spanish population. Rheumatology 2005;44:1263-6.
4. Forslind K, Ahlmen M, Eberhardt K, Hafstrom I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004;63:1090-5.
5. Wagner U, Kaltenhauser S, Sauer H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum 1997;40:341-51.
6. Deighton C, Criswell LA. Recent advances in the genetics of rheumatoid arthritis. Curr Rheumatol Rep 2006;8:394-400.
7. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.
8. Meyer O, Nicaise-Roland P, Santos MD, et al. Serial determination of cyclic citrullinated peptide autoantibodies predicted five-year radiological outcomes in a prospective cohort of patients with early rheumatoid arthritis. Arthritis Res Ther 2006;8:R40.
9. Ronnelid J, Wick MC, Lampa J, et al. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Ann Rheum Dis 2005;64:1744-9.
10. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006;54:1117-21.

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