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Particular Association of Clinical and Genetic Features With Autoimmunity to Citrullinated α-Enolase in Rheumatoid Arthritis

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Objective. **To confirm that the presence of anti–citrullinated α-enolase peptide 1 (anti–CEP-1) antibodies identifies a subgroup of patients with rheumatoid arthritis (RA).**

Methods. **DNA and serum samples were obtained from 451 patients with RA and 279 healthy control subjects, all of whom were of Spanish ancestry. Antibodies to cyclic citrullinated peptide (CCP) and CEP-1 were measured by enzyme-linked immunosorbent assay. HLA–DRB1 and the R620W single-nucleotide polymorphism of PTPN22 were genotyped.**

Results. **Anti–CEP-1 and anti-CCP antibodies were observed in 26.8% and 71.2% of the patients with RA, respectively. Most of the patients (86.6%) with anti–CEP-1 antibodies also had anti-CCP antibodies. Erosive arthritis, rheumatoid factor (RF) positivity, and the presence of the HLA shared epitope (especially the DRB1*04 alleles) were disproportionately associated with the group of patients with both antibodies. In addition, evidence of a significant interaction between the shared epitope and the risk allele of PTPN22 was observed only in these patients. In contrast, the association with these clinical and genetic features was weaker in patients with anti-CCP antibodies but lacking anti–CEP-1 antibodies. These results were obtained in patients in whom the prevalence of RA risk factors differed from that in other previously studied patients.**

Conclusion. **We observed that autoimmunity against citrullinated α-enolase may identify a subset of patients with a higher frequency of joint erosions and RF positivity. In addition, we confirmed the disproportionately large effect of the susceptibility alleles of HLA–DRB1 and their interaction with PTPN22 in this subset of patients. These results extend, confirm, and generalize the evidence supporting the specificity of the anti–CEP-1 antibody–positive subgroup of patients with RA among anti-CCP antibody–positive patients with RA.**

One of the most fruitful findings in recent rheumatoid arthritis (RA) research has been the identification of anti–cyclic citrullinated peptide (anti-CCP) antibodies (1,2). They are very specific to RA, and they appear in 60–70% of patients. They are useful for clinical diagnostics and have been included in the new criteria for RA in patients with early arthritis (3). In addition, anti-CCP antibody status is important from both the clinical and pathogenic sides of RA (4). Antibody-positive patients have a more erosive disease (5,6) that progresses even during treatment with disease-modifying antirheumatic drugs (7–10). Such patients have a worse response to tumor necrosis factor blockers (11) but a better response to B cell depletion (12). In addition, many genetic susceptibility factors are restricted to the anti-CCP antibody–positive subset of RA patients, with no or weak effects in antibody-negative patients. Clear examples of such factors include HLA (13,14), PTPN22 (15), and tumor necrosis factor

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receptor–associated factor 1/C5 (16). In contrast, no genetic factors have been shown conclusively to be specific to anti-CCP antibody–negative patients (17), and only a few factors, such as STAT-4 (18), seem to be shared by the 2 subpopulations of patients with RA. Also of interest is the specific association of smoking with the anti-CCP antibody–positive subset of RA patients (19–21), although this association has not been observed in all patient cohorts (22–24).

All of these data and other experimental results have led to a very attractive hypothesis for the etiology of anti-CCP antibody–positive RA that combines genetic predisposition and citrullination of proteins in the lungs of smokers (4,19,25). This combination of genetic and environmental risk factors leads to the development of autoantibodies against the citrullinated proteins, which could precede overt joint inflammation by several years. There are still some areas of uncertainty in this model. For example, it is unknown whether or not all citrullinated proteins recognized by antibodies in the plasma of patients with RA are equally relevant to pathogenesis.

A recent study showed strong support for a particular role of citrullinated α-enolase (26). This protein is a widely expressed glycolytic enzyme that is up-regulated in RA synovium beginning early in the disease course (27,28). Antibodies against its citrullinated form are specific to RA (28–30). Many of them recognize the immunodominant citrullinated α-enolase peptide 1 (CEP-1) (29). The particular role of these antibodies in RA pathogenesis was supported by their stronger association with HLA, PTPN22, and smoking than was observed with other anti-CCP antibody–positive patients (26). Interestingly, α-enolase shows strong similarity and antibody cross-reactivity with enolase from *Porphyromonas gingivalis*, which is involved in periodontitis and perhaps in RA etiology (29). In addition, CEP-1 will be an excellent target for the design of very specific treatments (31).

In this study, we confirmed the particular association of the presence of anti–CEP-1 antibodies in patients with RA and the risk alleles of HLA–DRB1 and PTPN22. In addition, we identified a particular association with erosive arthritis and the presence of rheumatoid factor (RF).

PATIENTS AND METHODS

Acquisition of DNA and serum samples. DNA and serum samples from 451 patients with RA and 279 healthy adult (age ≥55 years) control subjects of Spanish ancestry were obtained at a single hospital. Patients with RA were classified according to the 1987 American College of Rheumatology criteria (32). The Ethical Committee for Clinical Research of Galicia approved this study, and all participants gave their written informed consent. The smoking history of the patients with RA was recorded as either never smoker or smoker (past or current).

Serologic assessment. The anti-CCP antibody status of the patients was determined using the EDIA Anti-CCP ELISA (Euro-Diagnostica). The cutoff level was set at 5 units/ml, according to the manufacturer's instructions. Anti–CEP-1α antibodies were assayed following the previously described enzyme-linked immunosorbent assay protocol (26). The CEP-1 peptide (CKIHA-X-EIFDS-X-GNPTVEC) (29) was synthesized at Abcam. A standard curve based on serial dilutions from pooled antibody-positive sera was used to measure antibody titers in arbitrary units (AUs). The cutoff for positivity was set at the mean plus 3 SD of the AU values from 173 of the healthy control subjects.

HLA–DRB1 and PTPN22 genotyping. HLA–DRB1 alleles were determined by a sequencing-based typing method, using the AlleleSEQR HLA–DRB1 Typing Kit (Abbott Diagnostics), which includes a single polymerase chain reaction (PCR) amplification for all alleles of the second exon of DRB1 and bidirectional sequencing. Alleles were assigned using Assign-SBT version 3.2.7 software (Abbott Diagnostics). Ambiguous samples were additionally sequenced with group-specific primers (AlleleSEQR HLA–DRB1 GSSP; Abbott). The PTPN22 rs2476601 single-nucleotide polymorphism (SNP) was genotyped with a TaqMan SNP genotyping assay (Applied Biosystems). A Chromo4 real-time PCR system (MJ Research) was used to run this assay.

Statistical analysis. Dichotomous variables were analyzed as 2×2 contingency tables. Genotypes were analyzed by logistic regression according to an additive model with codes 0 for AA, 1 for Aa, and 2 for aa genotypes. For a comparison of the effects between HLA–DRB1*01 and HLA–DRB1*04, subjects with no shared epitope (SE) were used as the common reference group. These analyses were performed using Statistica version 7.0 (StatSoft). Interaction analysis was carried out in R, implementing the formulas for departure from additivity developed by Hosmer and Lemeshow (33). The group of antibody-negative patients was used as reference, and the presence of either of the genetic factors, DRB1 and PTPN22, was considered only as carrier or no-carrier. A logistic regression model incorporating a multiplicative interaction term was also tested.

RESULTS

Stratification of patients with RA according to anti–CEP-1 and anti-CCP status. Serum samples from 451 Spanish patients with RA were analyzed for the presence of anti-CCP and anti–CEP-1 antibodies (the clinical characteristics of the patients are available from the corresponding author). Anti-CCP antibodies were present in 71.2% of the patients, and anti–CEP-1 antibodies were present in 26.8% of the patients. None of

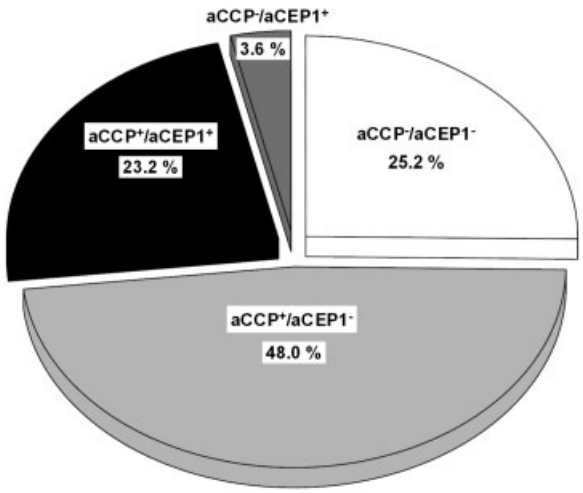

Figure 1. Stratification of the patients with rheumatoid arthritis according to their anti–cyclic citrullinated peptide (anti-CCP) and anti–citrullinated α-enolase peptide 1 (anti–CEP-1) antibody status. The anti-CCP−/anti–CEP-1− subgroup comprised 112 patients, the anti-CCP+/anti–CEP-1− subgroup comprised 213 patients, the anti-CCP+/anti–CEP-1+ subgroup comprised 103 patients, and the anti-CCP−/anti–CEP-1+ subgroup comprised 16 patients. For 7 patients, no information on anti-CCP status was available.

the 173 healthy control subjects was positive for either of the antibodies. Figure 1 shows the stratification of patients with RA into 4 subsets. Only 16 patients were both anti-CCP antibody negative and anti–CEP-1 antibody positive, and they were not analyzed further. The other 3 subsets of patients were designated as anti-CCP−/anti–CEP-1−, anti-CCP+/anti–CEP-1−, and anti-CCP+/anti–CEP-1+. Although 86.6% of the patients with anti–CEP-1 antibodies also had anti-CCP antibodies, these patients represented fewer than one-third of all anti-CCP antibody–positive patients. This distribution is different from that previously described in other RA patient cohorts (Table 1) (26), with the exception of the UK Norfolk Arthritis Register collection, which showed a similar fraction of anti–CEP-1 antibody–positive patients relative to either the whole set of patients with RA or anti-CCP antibody–positive patients.

Titers of anti-CCP antibodies were not higher in the anti-CCP+/anti–CEP-1+ subgroup of patients compared with the anti-CCP+/anti–CEP-1− subgroup ($P = 0.6$ by Mann-Whitney U test). In addition, titers of anti–CEP-1 and anti-CCP antibodies did not correlate ($r_s = 0.03$ in the anti-CCP+/anti–CEP-1+ subgroup). This lack of correlation means that particular associations with anti–CEP-1 antibodies cannot be ascribed to higher titers of anti-CCP antibodies.

Higher prevalence of joint erosion and RF in the anti-CCP+/anti–CEP-1+ subgroup of patients with RA. Very important differences were observed between the anti-CCP−/anti–CEP-1− subgroup and both the anti-CCP+/anti–CEP-1− and anti-CCP+/anti–CEP-1+ subgroups in the prevalence of joint erosion (44.4%, 74.5%, and 87.1%, respectively) (Figure 2). There were also differences between the anti-CCP+/anti–CEP-1− and anti-CCP+/anti–CEP-1+ subgroups, with a greater prevalence of erosion in the group with both antibodies ($P = 0.01$). Also, the prevalence of RF was much higher in patients with anti-CCP antibodies compared with that in patients in the anti-CCP−/anti–CEP-1− subgroup (78.6% versus 16.3%). Within the group of anti-CCP antibody–positive patients, the prevalence of RF positivity was higher in the anti-CCP+/anti–CEP-1+ subgroup than in the anti-CCP+/anti–CEP-1− (85.3% versus 74.1%) (Figure 2). The prevalence of sicca syndrome

Table 1. Characteristics of the RA patients in the current study compared with those in 3 previously described RA patient cohorts*

	Frequency, %				*P*, versus current study		
	Current study	EIRA	NR	NOAR	EIRA	NR	NOAR
Anti–CEP-1+	26.8	43	41	27	4.9×10^{-9}	8.2×10^{-5}	NS
Anti-CCP+	71.2	60	92	51	4.8×10^{-5}	1.4×10^{-11}	3.1×10^{-7}
Anti–CEP-1+/anti-CCP+	23.2	38	40	23	3.8×10^{-8}	1.2×10^{-6}	NS
SE carrier†	53.7	79.1	86.3	64.5	9.3×10^{-21}	3.8×10^{-19}	0.01
HLA–DRB1*01	30.6	15.3	9.4	21.2	1.8×10^{-10}	3.7×10^{-11}	0.013
HLA–DRB1*04	27.6	62.1	60.6	34.0	3.5×10^{-31}	2.4×10^{-18}	0.10
620W carrier†	26.1	30.3	–	–	0.12	–	–
Ever smoker†	19.2	69.9	–	–	4.7×10^{-62}	–	–

* The 3 cohorts previously described by Mahdi et al (26) are as follows: 1,000 patients from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA), a Swedish population-based case–control study; 279 patients from the UK National Repository (NR); and 218 patients from the UK Norfolk Arthritis Register (NOAR). NS = not significant; SE = shared epitope.

† Data on the percentages of anti–cyclic citrullinated peptide antibody–negative/anti–citrullinated α-enolase peptide 1–positive (anti-CCP−/anti–CEP-1+) patients with these features in the cohorts described by Mahdi et al were not available; therefore, the data were calculated excluding this group.

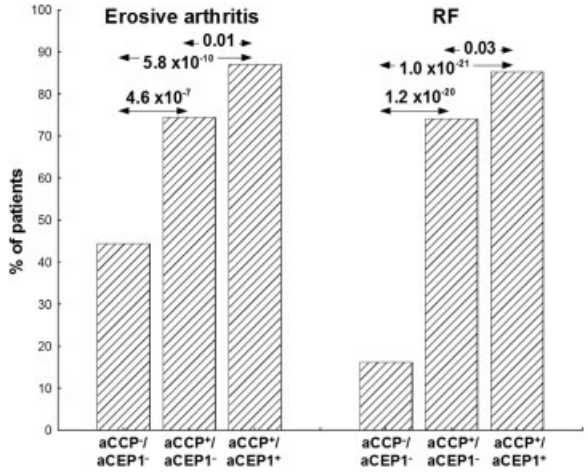

Figure 2. Relationship between erosive arthritis and rheumatoid factor (RF) positivity in rheumatoid arthritis, patients in the anti-CCP+/anti–CEP-1+ subgroup. The prevalence of these clinical features in each subgroup is shown, as well as the *P* values for all pairwise comparisons between subgroups. The minor subgroups comprised 12 anti-CCP+/anti–CEP-1+ patients without erosions, and 14 anti-CCP+/anti–CEP-1+ patients without RF. See Figure 1 for other definitions.

showed a trend in the same direction, but the differences were not significant (data not shown). These results are the first showing a particular association of autoimmunity against α-enolase with clinical features of RA.

Preferential association of the HLA–DRB1 SE with the anti-CCP+/anti–CEP-1+ subgroup of patients. The HLA–DRB1 SE was more frequent in patients with RA than in control subjects (53.7% versus 29.0%; $P = 1.2 \times 10^{-9}$), and this association was allele dose dependent; that is, the association was stronger in SE homozygotes than in SE heterozygotes. The difference was much more marked between control subjects and patients with anti-CCP antibodies (58.9%; $P = 3.35 \times 10^{-12}$) than between control subjects and anti-CCP antibody–negative patients (39.3%; $P = 0.06$). It was even more exaggerated between control subjects and patients positive for both antibodies (Figure 3); almost 70% of patients in the anti-CCP+/anti–CEP-1+ subgroup were carriers of the SE (69.9%; $P = 9.0 \times 10^{-14}$ versus control). The most interesting comparison was between this subgroup of anti-CCP+/anti–CEP-1+ patients and the subgroup of anti-CCP+/anti–CEP-1− patients (SE frequency 53.5%; $P = 0.0009$). The significant excess of association of the SE with patients who were positive for both antibodies confirmed the already described particular association of autoimmunity against α-enolase with the HLA–DRB1 risk alleles (26). In addition, the correlation between the number of SE alleles and anti–CEP-1 antibody titers was confirmed ($r_s = 0.13$, $P = 0.006$).

Previous studies have shown that the association between the different SE alleles and anti–CEP-1 antibody–positive RA is not uniform (26,30,34). We also observed a stronger association of anti–CEP-1 antibodies with HLA–DRB1*04 alleles than with HLA–DRB1*01 alleles (additional information is available from the corresponding author). Our confirmatory results were obtained in spite of the lower percentage of RA patients bearing SE alleles, the higher contribution of the HLA–DRB1*01 alleles, and the lower percentage of HLA—DRB1*04 alleles in our Spanish patients than in patients from Sweden or the UK in the study by Mahdi et al (26) (Table 1).

Synergism between the 2 main RA genetic risk factors in the anti-CCP+/anti–CEP-1+ subgroup of patients. The PTPN22 620W allele is the second major genetic risk factor for RA. In our patients, it was associated with RA, but only in those with anti-CCP antibodies (16.6% of control subjects were carriers, compared with 18.0% of patients in the anti-CCP−/anti–CEP-1− subgroup; *P* not significant). The 620W allele was more common in patients who were positive for both antibodies (34.0% of patients in the anti-CCP+/anti–CEP-1+ subgroup were carriers, compared with

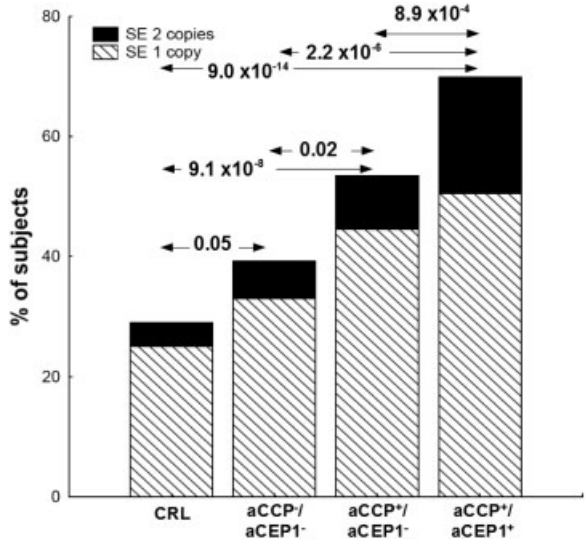

Figure 3. Preferential association of the shared epitope (SE) in the anti-CCP+/anti–CEP-1+ subgroup of patients with rheumatoid arthritis. The percentages of subjects carrying the specified number of SE alleles are shown, as well as the *P* values for all pairwise comparisons between each group of subjects, as obtained in an additive logistic regression model. CRL = control (see Figure 1 for other definitions).

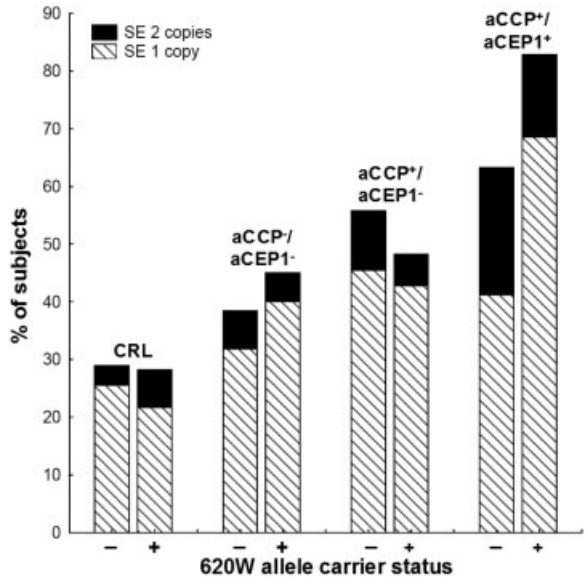

Figure 4. Representation of the interaction between shared epitope (SE) alleles and the 620W allele of PTPN22 in subgroups of patients with rheumatoid arthritis (RA) and control (CRL) subjects. Although the number of SE alleles is shown, the analyses were performed using carrier status. Significant evidence of deviation from additivity in the interaction between the 2 genetic factors was observed only in RA patients in the anti-CCP+/anti–CEP-1+ subgroup ($P = 0.002$). The minor subgroup comprised 6 patients in the anti-CCP+/anti–CEP-1+ subgroup who were carriers of 620W but were not carriers of the SE. See Figure 1 for other definitions.

26.4% of patients in the anti-CCP+/anti–CEP-1− subgroup), but the difference between the anti-CCP+/anti–CEP-1− and the anti-CCP+/anti–CEP-1+ subgroups was not significant ($P = 0.2$) (additional information is available from the corresponding author).

Analysis of interactions between the SE and the 620W allele of PTPN22 showed a significant deviation from additivity only in the subset of patients positive for both antibodies. In this group, the deviation from additivity is reflected by the difference between the percentage of patients who had the SE but did not carry the 620W risk allele (63.2%), and the percentage of patients who carried both risk alleles (82.9%) (Figure 4). The attributable proportion (AP) of disease due to this interaction was 0.59 (95% confidence interval 0.19–0.98; $P = 0.002$). In contrast, the AP in the anti-CCP+/anti–CEP-1− subgroup was not significantly different from 0. No significant multiplicative interaction was observed using a logistic regression model. These interaction results confirm previous findings by Mahdi et al (26).

Impact of smoking. The prevalence of smoking in our patients was low (18.7%), especially in women (8.2% versus 53.7% in men). These data are similar to those reported in our area for the general population in the patients' age range (35–37). We did not have information about smoking for the control subjects, and a direct comparison was therefore not possible. Smoking was equally prevalent in our patients with RA independently of the antibody status (22.6% in the anti-CCP−/anti–CEP-1− subgroup, 18.5% in the anti-CCP+/anti–CEP-1− subgroup, and 17.0% in the anti-CCP+/anti–CEP-1+ subgroup). The small number of smokers prevented meaningful comparisons, but a trend toward a positive interaction with the SE in anti-CCP antibody–positive patients was detected (AP = 0.43; $P = 0.07$).

DISCUSSION

Our analysis contributes to defining the role of autoimmunity against citrullinated α-enolase in RA. Our specific contribution includes the finding of new preferential associations with joint erosion and RF positivity. These associations need to be replicated, but they suggest that this type of autoimmunity is able to differentiate a particular subset among anti-CCP antibody–positive patients. In addition, we replicated the particular association of anti–CEP-1 antibodies with the SE and the 620W allele of PTPN22 but highlighted that smoking is a dispensable factor. Our results add generality to the results reported by Mahdi et al (26) because of the important differences between Spanish patients with RA and Northern European patients with RA in terms of the prevalence of RA risk factors (38–40).

It has previously been demonstrated that patients with anti–CEP-1 antibodies show a stronger association with RA susceptibility factors compared with patients who have anti-CCP reactivity but lack anti–CEP-1 antibodies (26). We have added the first evidence of a predominant association with erosive arthritis and RF positivity. The stronger association with these 2 features could suggest that autoimmunity against different citrullinated proteins determines not only different levels of disease susceptibility but also different disease phenotypes. An alternative suggestion is that these clinical associations could be secondary to a stronger relationship between anti–CEP-1 antibodies and other factors, such as DRB1*04 alleles, which themselves are more strongly associated with erosion and RF positivity. Additional studies are required to confirm the new preferential associations and to examine the possible effect of subclassification by the anti–CEP-1 antibodies in other facets of the phenotype and progression of RA.

Our results confirm the particular association of

the SE in patients positive for both anti-CCP and anti–CEP-1 antibodies (26). This independent confirmation was obtained in a population in whom the HLA–DRB1 allele frequencies were different from those previously reported (38,39), with fewer SE and DRB1*04 alleles and more DRB1*01 alleles. Nevertheless, the association was stronger with the DRB1*04 alleles than with the DRB1*01 alleles, as described in other populations (26,30). There was also a significant interaction between the SE and the risk allele of PTPN22, and this was observed only in patients in the anti-CCP+/anti–CEP-1+ subgroup. This interaction was previously demonstrated in the Epidemiological Investigation of RA cohort of patients, and it likely represents biologic interaction between the 2 susceptibility factors (26,41). Possible molecular mechanisms include recognition of CEP-1 in the context of the SE alleles of HLA–DR by autoreactive T cells, with a lower threshold for activation due to the risk allele of PTPN22.

Replication of the SE and PTPN22 associations with anti–CEP-1 reactivity in a population with a much lower exposure to tobacco than in most previous studies is of relevance. It is also notable because no association was detected between smoking and anti-CCP antibody–positive status in our patients. This lack of association has already been reported for some patient collections (22–24) and shows that the genetic associations with anti-CCP antibody–positive RA are not completely dependent on smoking. This was already noticeable in studies showing significant association between the SE and nonsmoker anti-CCP antibody–positive patients (22,23,26,41), but the dispensability of smoking was more evident in our patients. In addition, our results highlight that a fraction of nonsmoker patients with RA have anti-CCP antibodies. Disease-triggering factors that could be involved in these patients include air pollution and subclinical lung inflammation (22,42) or inflammation in other tissues such as joint inflammation triggered by trauma or infection (28,42–44) and periodontitis (45). All of these stimuli are known to increase protein citrullination. In addition, periodontitis can contribute by molecular mimicry between *P gingivalis*, the major agent of periodontitis, and human α-enolases (29,46). Any of these alternative triggering factors could have been at play in our anti-CCP antibody–positive patients.

In summary, our results confirm the particular role of autoimmunity against citrullinated α-enolase in RA by showing that patients with antibodies against its immunodominant peptide are particularly associated with erosive arthritis, RF positivity, and the presence of the SE. They also show a specific interaction between the SE and the PTPN22 risk allele. These results extend and confirm previous findings and give them more generality in populations of European ancestry with differences in the prevalence of RA risk factors. However, these results do not exclude the possibility that antibodies to other citrullinated antigens could also have particular association with RA features, as has been shown by the association of SE alleles with antibodies against a citrullinated vimentin peptide but not with antibodies against a citrullinated fibrinogen peptide (47). Research in autoimmunity against specific citrullinated autoantigens to establish their relative participation in the spectrum of RA features and genetic associations is warranted.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Gonzalez had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Montes, Gonzalez.

Acquisition of data. Montes, Dieguez-Gonzalez, Perez-Pampin, Mera-Varela, Gomez-Reino.

Analysis and interpretation of data. Montes, Calaza, Gonzalez.

REFERENCES

1. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van 't Hof M, et al. The prognostic value of anti–cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831–5.
2. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155–63.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569–81.
4. Klareskog L, Widhe M, Hermansson M, Ronnelid J. Antibodies to citrullinated proteins in arthritis: pathology and promise. Curr Opin Rheumatol 2008;20:300–5.
5. Van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. Arthritis Res Ther 2005;7:R949–58.
6. Bukhari M, Thomson W, Naseem H, Bunn D, Silman A, Symmons D, et al. The performance of anti–cyclic citrullinated peptide

antibodies in predicting the severity of radiologic damage in inflammatory polyarthritis: results from the Norfolk Arthritis Register. Arthritis Rheum 2007;56:2929–35.
7. Machold KP, Stamm TA, Nell VP, Pflugbeil S, Aletaha D, Steiner G, et al. Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. Rheumatology (Oxford) 2007;46:342–9.
8. Sanmarti R, Gomez-Centeno A, Ercilla G, Larrosa M, Vinas O, Vazquez I, et al. Prognostic factors of radiographic progression in early rheumatoid arthritis: a two year prospective study after a structured therapeutic strategy using DMARDs and very low doses of glucocorticoids. Clin Rheumatol 2007;26:1111–8.
9. De Vries-Bouwstra JK, Goekoop-Ruiterman YP, Verpoort KN, Schreuder GM, Ewals JA, Terwiel JP, et al. Progression of joint damage in early rheumatoid arthritis: association with HLA–DRB1, rheumatoid factor, and anti–citrullinated protein antibodies in relation to different treatment strategies. Arthritis Rheum 2008;58:1293–8.
10. Farragher TM, Lunt M, Plant D, Bunn DK, Barton A, Symmons DP. Benefit of early treatment in inflammatory polyarthritis patients with anti-cyclic citrullinated peptide antibodies versus those without antibodies. Arthritis Care Res (Hoboken) 2010;62: 664–75.
11. Potter C, Hyrich KL, Tracey A, Lunt M, Plant D, Symmons DP, et al. Association of rheumatoid factor and anti-cyclic citrullinated peptide positivity, but not carriage of shared epitope or PTPN22 susceptibility variants, with anti-tumour necrosis factor response in rheumatoid arthritis. Ann Rheum Dis 2009;68:69–74.
12. Dorner T, Kinnman N, Tak PP. Targeting B cells in immune-mediated inflammatory disease: a comprehensive review of mechanisms of action and identification of biomarkers. Pharmacol Ther 2010;125:464–75.
13. Van Gaalen FA, van Aken J, Huizinga TW, Schreuder GM, Breedveld FC, Zanelli E, et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004;50:2113–21.
14. Irigoyen P, Lee AT, Wener MH, Li W, Kern M, Batliwalla F, et al. Regulation of anti–cyclic citrullinated peptide antibodies in rheumatoid arthritis: contrasting effects of HLA–DR3 and the shared epitope alleles. Arthritis Rheum 2005;52:3813–8.
15. Kokkonen H, Johansson M, Innala L, Jidell E, Rantapaa-Dahlqvist S. The PTPN22 1858C/T polymorphism is associated with anti-cyclic citrullinated peptide antibody-positive early rheumatoid arthritis in northern Sweden. Arthritis Res Ther 2007;9: R56.
16. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med 2007;4:e278.
17. Dieguez-Gonzalez R, Calaza M, Perez-Pampin E, de la Serna AR, Fernandez-Gutierrez B, Castaneda S, et al. Association of interferon regulatory factor 5 haplotypes, similar to that found in systemic lupus erythematosus, in a large subgroup of patients with rheumatoid arthritis. Arthritis Rheum 2008;58:1264–74.
18. Orozco G, Alizadeh BZ, Delgado-Vega AM, Gonzalez-Gay MA, Balsa A, Pascual-Salcedo D, et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. Arthritis Rheum 2008;58:1974–80.
19. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, et al, for the Epidemiological Investigation of Rheumatoid Arthritis Study Group. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA–DR (shared epitope)–restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54:38–46.
20. Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S, et al. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis 2006;65:366–71.
21. Pedersen M, Jacobsen S, Klarlund M, Pedersen BV, Wiik A, Wohlfahrt J, et al. Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. Arthritis Res Ther 2006;8:R133.
22. Lee HS, Irigoyen P, Kern M, Lee A, Batliwalla F, Khalili H, et al. Interaction between smoking, the shared epitope, and anti–cyclic citrullinated peptide: a mixed picture in three large North American rheumatoid arthritis cohorts. Arthritis Rheum 2007;56: 1745–53.
23. Bang SY, Lee KH, Cho SK, Lee HS, Lee KW, Bae SC. Smoking increases rheumatoid arthritis susceptibility in individuals carrying the HLA–DRB1 shared epitope, regardless of rheumatoid factor or anti–cyclic citrullinated peptide antibody status. Arthritis Rheum 2010;62:369–77.
24. Mikuls TR, Hughes LB, Westfall AO, Holers VM, Parrish L, van der Heijde D, et al. Cigarette smoking, disease severity and autoantibody expression in African Americans with recent-onset rheumatoid arthritis. Ann Rheum Dis 2008;67:1529–34.
25. Klareskog L, Ronnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol 2008;26:651–75.
26. Mahdi H, Fisher BA, Kallberg H, Plant D, Malmstrom V, Ronnelid J, et al. Specific interaction between genotype, smoking and autoimmunity to citrullinated α-enolase in the etiology of rheumatoid arthritis. Nat Genet 2009;41:1319–24.
27. Kinloch A, Tatzer V, Wait R, Peston D, Lundberg K, Donatien P, et al. Identification of citrullinated α-enolase as a candidate autoantigen in rheumatoid arthritis. Arthritis Res Ther 2005;7: R1421–9.
28. Kinloch A, Lundberg K, Wait R, Wegner N, Lim NH, Zendman AJ, et al. Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. Arthritis Rheum 2008;58:2287–95.
29. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated α-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum 2008;58:3009–19.
30. Snir O, Widhe M, von Spee C, Lindberg J, Padyukov L, Lundberg K, et al. Multiple antibody reactivities to citrullinated antigens in sera from patients with rheumatoid arthritis: association with HLA-DRB1 alleles. Ann Rheum Dis 2009;68:736–43.
31. Satpute SR, Durai M, Moudgil KD. Antigen-specific tolerogenic and immunomodulatory strategies for the treatment of autoimmune arthritis. Semin Arthritis Rheum 2008;38:195–207.
32. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
33. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452–6.
34. Van der Helm-van Mil AH, Verpoort KN, le Cessie S, Huizinga TW, de Vries RR, Toes RE. The HLA–DRB1 shared epitope alleles differ in the interaction with smoking and predisposition to antibodies to cyclic citrullinated peptide. Arthritis Rheum 2007; 56:425–32.
35. Fernandez E, Schiaffino A, Garcia M, Salto E, Villalbi JR, Borras JM. Smoking in Spain, 1945-1995: a retrospective analysis based on the Spanish National Health Interview Surveys. Med Clin (Barc) 2003;120:14–6. In Spanish.
36. Perez-Rios M, Santiago-Perez MI, Carames SC, Alonso de la Iglesia B, Pintos AM, Vidal XH. Mortality associated to tobacco consumption in Galicia, Spain, 2001-2006. Rev Esp Salud Publica 2009;83:557–65. In Spanish.
37. Hernandez-Garcia I, Saenz-Gonzalez MC, Gonzalez-Celador R.

Mortality attributable to smoking in Spain in 2006. An Sist Sanit Navar 2010;33:23–33. In Spanish.
38. Balsa A, Minaur NJ, Pascual-Salcedo D, McCabe C, Balas A, Fiddament B, et al. Class II MHC antigens in early rheumatoid arthritis in Bath (UK) and Madrid (Spain). Rheumatology (Oxford) 2000;39:844–9.
39. Pascual M, Nieto A, Lopez-Nevot MA, Ramal L, Mataran L, Caballero A, et al. Rheumatoid arthritis in southern Spain: toward elucidation of a unifying role of the HLA class II region in disease predisposition. Arthritis Rheum 2001;44:307–14.
40. Gregersen PK, Lee HS, Batliwalla F, Begovich AB. PTPN22: setting thresholds for autoimmunity. Semin Immunol 2006;18: 214–23.
41. Kallberg H, Padyukov L, Plenge RM, Ronnelid J, Gregersen PK, van der Helm-van Mil AH, et al. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. Am J Hum Genet 2007;80: 867–75.
42. Bongartz T, Cantaert T, Atkins SR, Harle P, Myers JL, Turesson C, et al. Citrullination in extra-articular manifestations of rheumatoid arthritis. Rheumatology (Oxford) 2007;46:70–5.
43. Makrygiannakis D, af Klint E, Lundberg IE, Lofberg R, Ulfgren AK, Klareskog L, et al. Citrullination is an inflammation-dependent process. Ann Rheum Dis 2006;65:1219–22.
44. Foulquier C, Sebbag M, Clavel C, Chapuy-Regaud S, Al Badine R, Mechin MC, et al. Peptidyl arginine deiminase type 2 (PAD-2) and PAD-4 but not PAD-1, PAD-3, and PAD-6 are expressed in rheumatoid arthritis synovium in close association with tissue inflammation. Arthritis Rheum 2007;56:3541–53.
45. Liao F, Li Z, Wang Y, Shi B, Gong Z, Cheng X. Porphyromonas gingivalis may play an important role in the pathogenesis of periodontitis-associated rheumatoid arthritis. Med Hypotheses 2009;72:732–5.
46. Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, van der Woude D, et al. Antibodies to porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. J Rheumatol 2010;37:1105–12.
47. Verpoort KN, Cheung K, Ioan-Facsinay A, van der Helm-van Mil AH, de Vries-Bouwstra JK, Allaart CF, et al. Fine specificity of the anti–citrullinated protein antibody response is influenced by the shared epitope alleles. Arthritis Rheum 2007;56:3949–52.