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Association of Anti–Citrullinated Vimentin and Anti–Citrullinated α-Enolase Antibodies With Subsets of Rheumatoid Arthritis

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Objective. **To determine whether the anti–citrullinated vimentin peptide 60–75 (anti–Cit-vimentin) and the immunodominant anti–citrullinated α-enolase peptide 1 (anti–CEP-1) antibodies are associated with subsets of patients with rheumatoid arthritis (RA) independently of the associations between anti–cyclic citrullinated peptide (anti-CCP) antibodies and clinical features of RA.**

Methods. **The 3 antibody types were quantified by enzyme-linked immunosorbent assay (ELISA) in serum samples from 521 patients with RA and 173 healthy controls of Spanish ancestry. Genotypes for HLA–DRB1 alleles and rs2476601 in *PTPN22* were available for these patients and controls plus an additional 106 healthy controls. A combined analysis of the 3 antibodies was conducted using stratified contingency tables and logistic regression models.**

Results. **A differential, particularly strong, and independent association was observed between the presence of anti–Cit-vimentin antibodies and the presence of shared epitope (SE) alleles, specifically in patients carrying 2 SE alleles, and between the presence of anti–Cit-vimentin antibodies and the prevalence of joint erosion. Associations were observed between anti–CEP-1 positivity and the presence of HLA–DRB1 and *PTPN22* risk alleles and their additive interaction. These associations were not accounted for by the anti-CCP status.**

Conclusion. **Our results indicate that the 2 antibodies against citrullinated peptides analyzed in this study add specific information beyond that obtained with the anti-CCP status. They define subgroups of patients with RA in which genetic factors have different weight and there is an observed difference in the prevalence of erosions.**

Progress in the understanding of rheumatoid arthritis (RA) has been greatly advanced by the discovery of anti–cyclic citrullinated peptide (anti-CCP) antibodies. These antibodies are specific for RA and allow us to distinguish 2 large groups of patients with RA (1,2). These 2 groups have different etiologic features, clinical courses, and responses to treatments (3–9). For example, joint erosions are more common in anti-CCP–positive patients than in anti-CCP–negative patients (3,4,7,9). In addition, anti-CCP antibodies seem to play an important pathogenic role (10,11).

A first step in the pathogenic process is the citrullination of specific endogenous proteins, which is potentiated in the lungs of smokers and in other locations as a consequence of tissue inflammation (12–15). This first step is not specific for RA, but it causes some self-peptides that, in their native form, cannot bind the shared epitope (SE) alleles to be able to bind them (16,17). The citrullinated self-peptides bound to the antigen pocket of the SE alleles are then presented to T cells (16). This action, together with flaws in im-

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mune regulation mechanisms, leads to a chronic autoimmune response against citrullinated peptides and to RA. Among the defects in immune regulation, a notable effect of the *PTPN22* RA susceptibility locus has been identified; the 620W allele determines T and B cell signaling abnormalities that facilitate loss of tolerance to autoantigens (18–20). This allele shows an additive interaction with the SE and smoking in predisposing to anti-CCP–positive RA, but not to anti-CCP–negative RA (12,21,22). This interaction is interpreted as meaning that the 2 genetic factors and smoking contribute to anti-CCP–positive RA by acting in successive steps along the described pathway.

Anti-CCP antibodies are detected clinically with an enzyme-linked immunosorbent assay (ELISA) that uses a mixture of synthetic cyclic citrullinated peptides. This test is both sensitive and specific for RA, but it does not reveal which citrullinated endogenous proteins triggered the immune response in the patient. Investigation of this question has shown that there is large variability in the pattern of citrullinated proteins recognized by anti-CCP antibodies in different patients (23,24). The fine specificity of anti-CCP antibodies could help to delineate RA disease mechanisms and their relationship with etiologic factors and with some clinical features (25–29). In particular, antibodies against 2 of the citrullinated peptides seem to be particularly informative. They are the antibodies against the immunodominant citrullinated α-enolase peptide 1 (CEP-1) and the citrullinated vimentin peptide 60–75 (Cit-vimentin). These antibodies have been shown to identify patients with a marked association with SE alleles (25–28,30) and with a particularly strong interaction between the SE, the 620W *PTPN22* allele, and smoking, but with some differences between studies (25,26,28). Patients with anti–CEP-1 antibodies also had an increased prevalence of joint erosions compared to other anti-CCP–positive patients in our previous study (26), and a similar finding has been reported for patients positive for anti-Sa antibodies (29), which detect citrullinated vimentin (31). However, some recent studies show conflicting results regarding this clinical association (32,33).

In the present study, we addressed some of these questions by analyzing the role of anti–Cit-vimentin in 521 Spanish patients with RA and combining this information with the data on anti–CEP-1 that we reported previously (26). The 2 antibodies showed a particularly strong and independent association with characteristic features of anti-CCP–positive RA that were not completely accounted for by the anti-CCP status. In addition, the 2 antibodies are not interchangeable, as shown by the differential association of anti–Cit-vimentin with joint erosions and with the presence of 2 SE alleles and the association of anti–CEP-1 antibodies with the *PTPN22*–SE interaction.

PATIENTS AND METHODS

Acquisition of DNA and serum samples. DNA and serum samples from 521 patients with RA and 279 healthy adult controls (ages 55 years or older) of Spanish ancestry were obtained from a single hospital. RA patients were classified according to the American College of Rheumatology 1987 criteria (34). Clinical characteristics of the patients are shown in Table 1. The Ethics Committee for Clinical Research of Galicia approved this study, and written informed consent was obtained from all participants. History of smoking for each of the RA patients was recorded as never smoked or ever smoked (past or current).

Serologic assessment. Some of the anti-CCP and anti–CEP-1 antibody results have been reported previously (26). The anti-CCP status of the patients was determined using the EDIA anti-CCP test kit (Euro-Diagnostica). Quantification was performed according to the manufacturer's instructions, and the cutoff level was set at 5 units/ml as recommended by the manufacturer. Anti–CEP-1 antibodies were assayed following the previously described ELISA protocol (25,26). To evaluate the anti–Cit-vimentin 60–75 antibodies, we followed a published protocol (27,28), which was similar to the anti–CEP-1 ELISA except for the use of streptavidin-coated high binding capacity 96-well plates (Pierce) bound to the biotinylated and citrullinated vimentin 60–75 linear peptide (final concentration 10 μg/ml): VYATcitSSAVcitLcitSSVP-OH. This peptide was synthesized by Schafer-N. A standard curve made with serial dilutions from pooled positive sera was used to measure antibody titers of anti–CEP-1 and anti–Cit-

Table 1. Clinical characteristics of the RA patients*

Female	77.5
Age at disease onset, median (IQR) years	46 (35–56)
Duration of followup, median (IQR) years	18 (10–25)
Morning stiffness†	99.5
Arthritis of ≥3 joint areas†	100.0
Arthritis of the hand joints†	100.0
Symmetric arthritis†	100.0
Rheumatoid nodules†	12.5
Rheumatoid factor positive	60.5
Erosions	65.6
Sicca syndrome	10.2
Interstitial pneumonitis†	3.0
ANA positive†	15.3
Shared epitope carrier	53.9
PTPN22 carrier	26.0
Anti-CCP positive	64.3

* Except where indicated otherwise, values are the percent of patients. RA = rheumatoid arthritis; IQR = interquartile range; ANA = antinuclear antibody; anti-CCP = anti–cyclic citrullinated peptide.
† Data were available for fewer than 500 of the 521 patients included in the study.

vimentin in arbitrary units. The cutoff for positivity was set at the 98% specificity level obtained in the 173 healthy controls.

HLA–DRB1 and *PTPN22* genotyping. HLA–DBR1 alleles were determined by a sequencing-based typing method using an AlleleSEQR HLA–DRB1 typing kit (Abbott Diagnostics), which includes bidirectional sequencing of the second exon of DRB1. Ambiguous samples were also 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).

Statistical analysis. Spearman's rank correlation (r_s) was used for correlation analyses. Dichotomous variables were analyzed using 2×2 contingency tables. Analysis of independence was performed using a chi-square test applied to a 4×2 table of observed versus expected frequencies. Multivariate logistic regression analysis was used to evaluate the effect of each antibody type conditional on the other types. A simplified model without interaction parameters was used for this analysis with codes 0 and 1 for the absence and presence, respectively, of each antibody type. For conditional evaluation of the antibody status, an ordered logistic regression model was used with codes 0, 1, and 2 for the number of SE alleles. These multivariate analyses also included age at disease onset, time since disease onset, and sex as covariates.

Interaction analysis was carried out implementing the formulas for departure from additivity developed by Hosmer and Lemeshow (35). The healthy control group was used as a reference and the two genetic factors, DRB1 and *PTPN22*, were considered as separate carrier status variables. A logistic regression model incorporating a multiplicative interaction term was also tested. *P* values less than 0.05 were considered significant. All analyses were performed with Statistica 7.0 software (StatSoft).

RESULTS

Characteristics of the anti–Cit-vimentin 60–75–positive patients. The fraction of patients with anti–Cit-vimentin antibodies was 21.5% (Figure 1A). Most of these patients (85.7%) were anti-CCP positive. The few who were anti-CCP negative (16 [3.1% of the total]) were excluded from further analysis. The percentage of anti–Cit-vimentin–positive patients was lower than in previous studies of other European Caucasian populations (Table 2). This difference could be related to the low rate of smokers in our population and a significantly different frequency of SE alleles in the present study compared to the previous studies (Table 2).

Titers of anti-CCP were not higher in the patients with anti–Cit-vimentin antibodies than in the anti–Cit-vimentin–negative patients (data not shown), and there was no correlation between titers of anti-CCP and anti–Cit-vimentin antibodies in the patients who had both antibodies ($r_s = 0.16$, $P > 0.1$). Therefore, any particular

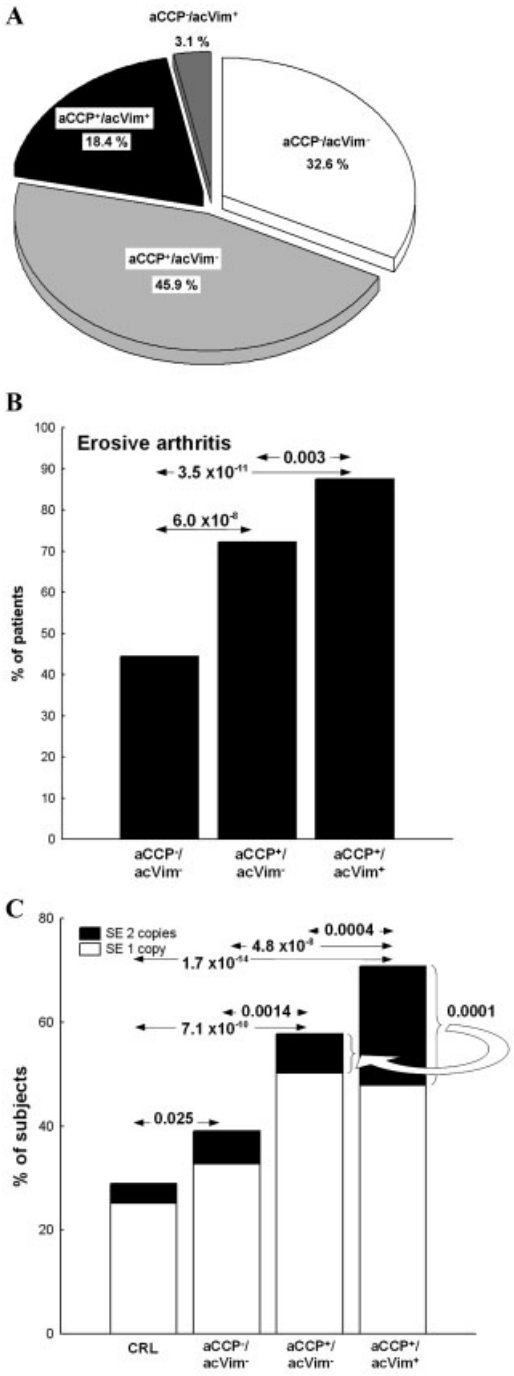

Figure 1. A, Stratification of the patients with rheumatoid arthritis by anti–cyclic citrullinated peptide (aCCP) and anti–citrullinated vimentin peptide (acVim) antibody status. **B,** Percent of patients with erosive arthritis in the subgroups of patients stratified by antibody status. **C,** Percent of healthy control (CRL) subjects and of patients, stratified by antibody status, with 1 or 2 shared epitope (SE) alleles. Values in **B** and **C** are *P* values for pairwise comparisons.

Table 2. Comparison between the RA patients in this study and those in other studies that included analysis of anti–Cit-vimentin antibodies*

	Present study (n = 521)		KUH (n = 290)		EAC (n = 701)		BeSt (n = 141)	
	%	P†	%	P†	%	P†	%	P†
Anti–Cit-vimentin+/anti-CCP+	28.6	–	50.9	1.5×10^{-7}	49.1	5.0×10^{-8}	57.7	1.0×10^{-8}
Anti-CCP+	64.3	–	73.1	0.01	48.5	4.0×10^{-8}	100	NA
SE carrier	53.9	–	84	1.4×10^{-14}	64.2	0.0004	–	–
SE carrier/anti-CCP+	61.6	–	–	–	78.8	9.6×10^{-7}	74.8	0.008
Ever smoked	17.0	–	69.9‡	NA	41.9	$<2.2 \times 10^{-16}$	–	–

* The other studies included patients attending the Rheumatology Clinic at Karolinska University Hospital (KUH) (24), the Leiden Early Arthritis Clinic (EAC) cohort, an inception cohort of patients with recent-onset arthritis (<2 years of symptoms) established in Leiden, The Netherlands (28), and anti–cyclic citrullinated peptide (anti-CCP)–positive patients from the Behandelstrategieën voor Reumatoide Artritis (BeSt; Treatment strategies for rheumatoid arthritis [RA]) study, a multicenter, randomized controlled trial designed to compare different treatment strategies in patients with early-onset RA (27). Anti–Cit-vimentin = anti–citrullinated vimentin peptide; NA = not applicable; SE = shared epitope.
† Versus the present study.
‡ Percentage of a larger group of patients from the Epidemiological Investigation of RA study (n = 1,000).

association with anti–Cit-vimentin could not be ascribed to high anti-CCP titers.

The anti–Cit-vimentin–positive patients were characterized by an increased prevalence of erosions (Figure 1B). The difference was very marked when this subgroup of patients was compared to the anti-CCP–negative patients (87.2% versus 44.4%; $P = 3.5 \times 10^{-11}$). There was also a 15% increase in erosions in the subgroup of patients who were anti-CCP positive and anti–Cit-vimentin positive compared to the subgroup of patients who were anti-CCP positive and anti–Cit-vimentin negative (87.2% versus 72.2%; $P = 0.003$).

The 2 major RA susceptibility loci, HLA–DRB1 and *PTPN22*, were explored for their association with anti–Cit-vimentin antibodies. There was an association between the presence of the SE alleles and anti–Cit-vimentin positivity (Figure 1C), as has previously been described by others (27,28). Of particular relevance was the increased number of patients with SE alleles in the anti-CCP–positive/anti-Cit-vimentin–positive subgroup (70.8%) compared to the anti-CCP–positive/anti-Cit-vimentin–negative subgroup (57.8%; $P = 0.0004$ for genotype frequency comparison). Notably, when the subgroups of patients with 1 SE allele and those with 2 SE alleles were considered separately, all of the increase in the percentage of patients with SE alleles was due to the patients with 2 SE alleles (22.9% in the anti-CCP–positive/anti–Cit-vimentin–positive subgroup versus 7.6% in the anti–CCP–positive/anti–Cit-vimentin–negative subgroup; $P = 0.0001$). The percentage of patients with 1 SE allele was similar between the 2 groups (47.9% in the anti-CCP–positive/anti–Cit-vimentin–positive subgroup versus 50.2% in the anti-CCP–positive/anti–Cit-vimentin–negative subgroup; $P = 0.7$). This exclusive association of anti–Cit-vimentin positivity with the presence of 2 SE alleles has not been previously described.

No particular association was observed between anti–Cit-vimentin antibody positivity and the 620W allele of *PTPN22*, history of smoking, or rheumatoid factor (RF) (data not shown). The 16 RA patients who had anti–Cit-vimentin antibodies but were negative for anti-CCP antibodies were more similar to the anti-CCP–negative patients than to the other anti–Cit-vimentin–positive patients. Compared to the other anti–Cit–vimentin–positive patients, these patients had reduced frequency of SE alleles (46.7% versus 70.8%), prevalence of erosions (50.0% versus 87.6%), frequency of RF (35.7% versus 82.4%), and frequency of anti–CEP-1 antibodies (13.3% versus 47.9%). Therefore, these patients most likely had false-positive results of the anti–Cit-vimentin assay.

Relationship between anti–Cit-vimentin 60–75 and anti–CEP-1 reactivity. Stratification of all of the patients with RA according to the presence or absence of anti-CCP, anti–Cit-vimentin, and anti–CEP-1 antibodies showed that approximately one-third of the patients were negative for all 3 antibodies, one-third were positive for anti-CCP antibodies only, and one-third were positive for anti-CCP and at least 1 other antibody. This last third of patients was distributed into 3 subgroups of similar size: those positive for anti–Cit-vimentin but not for anti–CEP-1, those positive for anti–CEP-1 but not for anti–Cit-vimentin, and those positive for both antibodies (Figure 2). The frequencies of anti–Cit-vimentin positivity and anti–CEP-1 positivity

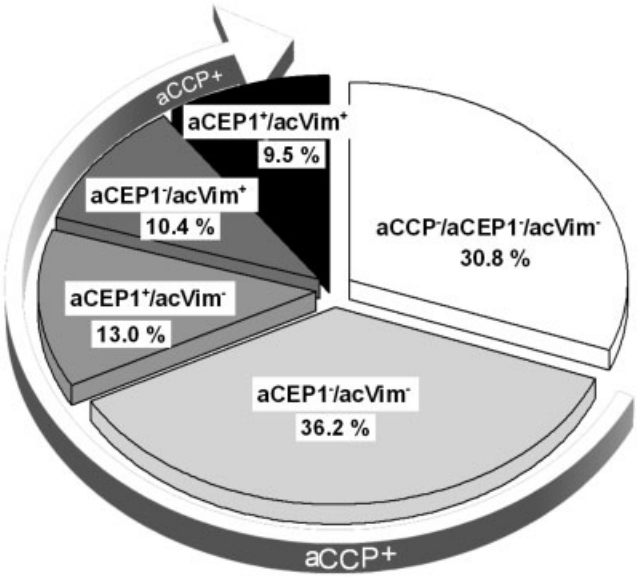

Figure 2. Stratification of the patients with RA by anti–cyclic citrullinated peptide (aCCP), anti–citrullinated α-enolase peptide 1 (aCEP-1), and anti–citrullinated vimentin peptide (acVim) antibody status. Patients who were positive for either anti–Cit-vimentin peptide antibody or anti–CEP-1 antibody but negative for anti-CCP antibodies were not included (7.3%).

were not independent of each other ($P = 1.5 \times 10^{-9}$ by test of independence). Patients who were positive for anti–Cit-vimentin antibodies showed an increased probability of being positive for anti–CEP-1 antibodies (47.9% observed versus 22.6% expected if independent). As a consequence of the lack of independence, the specificity of the associations with anti–Cit-vimentin demonstrated above or of the associations with anti–CEP-1 antibodies previously reported (26) needed to be re-evaluated. To address this question, we used conditional logistic regression analysis. This analysis distinguishes between independent associations and those due to the presence of both antibodies in a fraction of the patients.

First, we conducted a conditional analysis to assess the association of the individual antibodies with erosions. A gradation of the prevalence of erosions was observed, with the largest change in prevalence associated with anti-CCP status, followed by anti–Cit-vimentin status and then anti–CEP-1 status (Figure 3A). Only the first 2 antibodies showed a significant association with erosions in the conditional analysis (P for conditional analysis [P_{cond}] $= 2.2 \times 10^{-5}$ for anti-CCP, $P_{cond} = 0.016$ for anti–Cit-vimentin, and $P_{cond} = 0.10$ for anti–CEP-1). These results indicate that anti–CEP-1 status did not add to the association when the patients were anti–Cit-vimentin positive (compare the last two columns in Figure 3A). Therefore, the previously reported association of erosions with anti–CEP-1 status (26) seemed to be largely due to the subgroup of patients

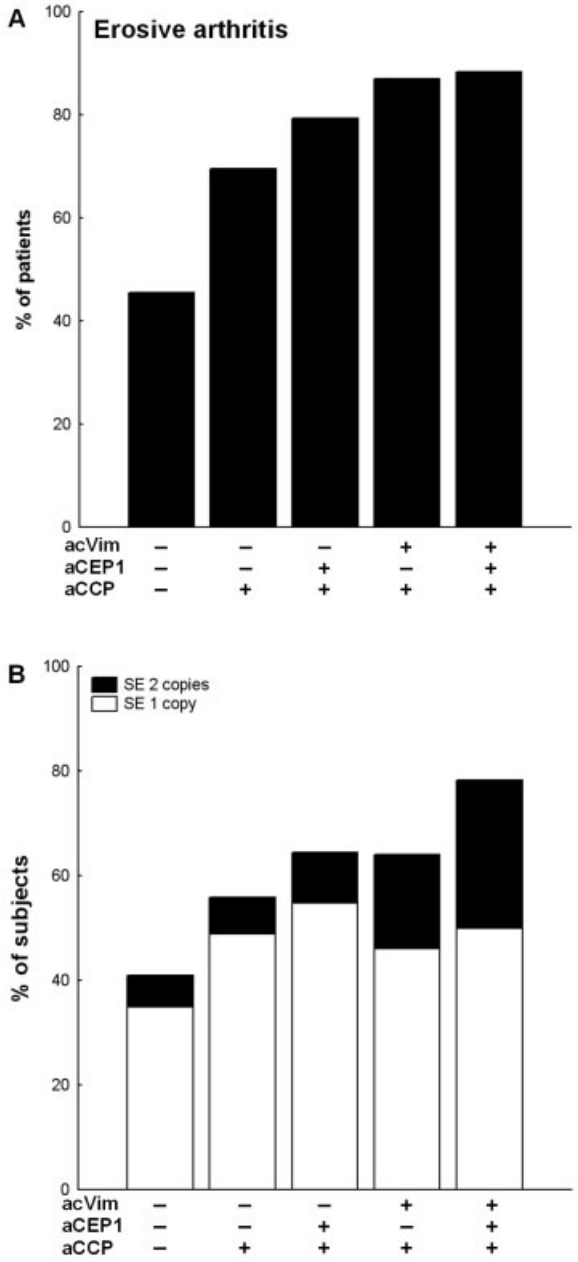

Figure 3. A, Percent of patients with erosive arthritis in the subgroups of patients defined by antibody status. **B,** Percent of patients with 1 or 2 shared epitope (SE) alleles in the subgroups of patients defined by antibody status. See Figure 2 for other definitions.

who were positive for both anti–CEP-1 and anti–Cit-vimentin. It should be noted that one of the covariates included in this analysis, duration of followup, was also associated with the prevalence of erosions (P_{cond} = 3.3×10^{-6}).

A second conditional analysis showed a significant association of the 3 antibodies with the SE genotypes. The strongest conditional association was found with anti–Cit-vimentin (P_{cond} = 0.002), followed by anti-CCP and anti–CEP-1 antibodies (P_{cond} = 0.02 and P_{cond} = 0.03, respectively). These associations correspond to the following 2 effects: a positive correlation between SE frequency and the number of antibodies, and the association of anti–Cit-vimentin with the presence of 2 SE alleles (Figure 3B). In this analysis, the subgroup of patients who were positive for both anti–CEP-1 and anti–Cit-vimentin showed an increased percentage of patients with SE alleles relative to the subgroups of patients who were positive for only 1 of the antibodies, reflecting their independent contribution to the association. We also found that the correlation between anti–CEP-1 antibody titers and the number of SE alleles that we previously reported (26) did not remain after conditioning on the anti-CCP and anti–Cit-vimentin status (P = 0.12).

Specificity of the interaction between the 2 major RA genetic loci. Previous studies have shown a positive additive interaction between the presence of SE alleles and the presence of the 620W allele of *PTPN22* on disease predisposition among anti-CCP–positive patients (12,21,22) and, more specifically, among the anti–CEP-1–positive subgroup (25,26). A similar additive interaction was not present in the anti–Cit-vimentin–positive patients in this study; the attributable proportion due to interaction was not different from 0. The attributable proportion was 0.27 (95% confidence interval [95% CI] −0.3, 0.9). This parameter is significant when the 95% CI does not include 0. Therefore, the interaction between the risk alleles of *PTPN22* and HLA–DRB1 did not extend to all subgroups of anti-CCP–positive patients.

To examine this issue in more detail, we stratified patients according to their anti-CCP, anti–Cit-vimentin, and anti–CEP-1 status and repeated the analysis for interaction in each of the subgroups (Figure 4). We found that an additive interaction between the presence of the 620W allele of *PTPN22* and the presence of SE alleles was limited to patients who were positive for anti–CEP-1 antibodies. Within this subgroup, an additive interaction was more marked in the anti-CEP-1–positive patients lacking anti–Cit-vimentin antibodies

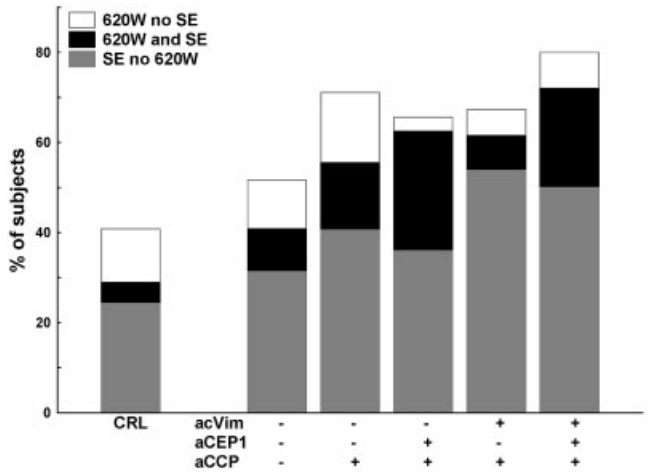

Figure 4. Interaction between shared epitope (SE) alleles and the 620W allele of *PTPN22* in healthy control (CRL) subjects and in the subgroups of patients with rheumatoid arthritis stratified by antibody status. Departure from additivity appears as a large fraction of patients carrying both the 620W allele and SE alleles. See Figure 2 for other definitions.

than in the anti–CEP-1–positive/anti–Cit-vimentin–positive subset, although both were significant (attributable proportion 0.79 [95% CI 0.6, 1.0] and attributable proportion 0.49 [95% CI 0.04, 0.97], respectively). No significant additive interaction between the 2 loci was detected in the other subgroups of patients. Notably, the anti-CCP–positive/anti–CEP-1–negative/anti–Cit-vimentin–negative patients did not show evidence of residual departure from additivity in association with the presence of SE and *PTPN22* (attributable proportion 0.22 [95% CI −0.4, 0.8]). In addition, no multiplicative interaction with the logistic model and no interaction with history of smoking were detected (data not shown). However, the low prevalence of smoking in our patients (17.0%) must be taken into account when interpreting this negative result.

DISCUSSION

Delineation of the particular effect of each anti–citrullinated protein antibody (ACPA) in RA is difficult because these antibodies are present in only a fraction of patients and because some patients have more than 1 such antibody. The results of the present study provide examples of the effect of this overlap in the analysis. In a previous study (26), we found an association between anti–CEP-1 and erosions and replicated the previously described positive correlation of anti–CEP-1 titers with the number of SE alleles (23). In the present study, we

demonstrated that none of these results was independent of the presence of anti–Cit-vimentin antibodies. In addition, the weight of the etiologic factors for RA can vary between populations, as shown by the differences in SE frequency and smoking prevalence in Table 2. These differences can modify the strength and prominence of the associations.

In spite of these problems, we found evidence of special and specific associations with the 2 ACPAs studied. These 2 antibodies, anti–CEP-1 and anti–Cit-vimentin, were the most promising to have detectable differential associations (25–30). Our findings include confirmation of the special association of anti–Cit-vimentin antibodies with SE genotypes (27,28,30) and with the prevalence of joint erosions (with a different antigen format) (29). In addition, we demonstrated that anti–Cit-vimentin–positive patients and anti–CEP-1–positive patients exhibit differential features. The anti–Cit-vimentin–positive patients were more commonly carriers of 2 SE alleles, whereas the anti–CEP-1–positive patients did not show a high frequency of the presence of 2 SE alleles but were characterized by a positive interaction between the risk allele of *PTPN22* and the SE alleles, which was absent in anti–Cit-vimentin–positive patients. Also, our analyses showed that when the anti–CEP-1 and anti–Cit-vimentin antibodies were considered together, only the anti–Cit-vimentin antibodies were independently associated with the prevalence of joint erosions.

Previous studies have shown that the anti–Cit-vimentin antibodies have the strongest association with the SE among the ACPAs (27,28,30). We confirmed this point and made the important observation that all of the excess association was due to patients carrying 2 SE alleles. This is a new finding that requires replication, but it is supported by the only previous study in which an association with anti–Cit-vimentin positivity was analyzed separately in patients with 1 SE allele and those with 2 SE alleles (32). These results raise the possibility that the anti–Cit-vimentin antibodies could be involved in the increased risk and severity that has been described in patients with RA who are carriers of a homozygous or compound heterozygous SE genotype (36–39).

The differential characteristics of the genetic associations of anti–Cit-vimentin and anti–CEP-1 antibodies are of particular interest. The above-mentioned association of anti–Cit-vimentin with the presence of 2 SE alleles was not observed with anti–CEP-1 antibodies. In addition, the association with *PTPN22* and the additive interaction that was found between SE and 620W *PTPN22* alleles were limited to the anti–CEP-1–positive patients. The specificity of the interaction for anti–CEP-1 antibodies in relation to other ACPAs is also supported by the findings of other studies, although anti–Cit-vimentin antibodies were not analyzed (25). This result seems to indicate that anti–CEP-1 antibodies are very particularly favored by concurrent antigen presentation in the SE alleles and lymphocyte abnormalities due to the risk allele of *PTPN22*.

We also found that the anti–Cit-vimentin antibodies were associated with the prevalence of erosions. This association was significantly more marked than that observed in anti-CCP–positive patients in general and was also significant in conditional analysis. These results should be considered in the context of recent studies that did not find an association beyond that observed with anti-CCP status (32,33). One of those studies considered only anti–CEP-1 versus anti-CCP (33), but the other study analyzed multiple ACPAs, including vimentin and enolase peptides (32). We think that there are 2 main factors that could explain the contradictory outcomes. Our results are from a cross-sectional study conducted in patients with longstanding disease, whereas Fisher et al (33) and Scherer et al (32) studied patients with early arthritis and determined antibody status at baseline. Therefore, those 2 studies are more valid than ours for assessing the predictive value of ACPAs. Another difference between the studies concerns the genetic and environmental risk factors affecting diverse European populations that could also result in a different pattern of association.

We interpret our results as supporting the idea that all ACPAs are not equal (25–28). Therefore, investigations of the different ACPAs could give us new clues to understand the pathogenesis of RA or to better characterize subgroups of patients. This idea contrasts with some recent reports that stress the commonalities between the different antibodies. In some studies, this was due to the lack of independent association, as for joint erosions (32,33). But in a recent study, the similarities were highlighted despite findings indicating significant differences in the association with SE alleles (30). Additional studies have stressed the common features of the various ACPAs without directly analyzing their differences (23). From our point of view, the many commonalities between the different ACPAs are undeniable, but we have focused on the differences because they may give us a more accurate picture of RA pathogenesis.

Our study has some limitations, including the small size of strata in some of the comparisons. Therefore, we restricted our subanalyses to a cautious level and to findings that were supported by data from

previous studies. In addition, we used conditional logistic regression, in which all information across strata is considered. In this way, we decreased the incidence of sample partition problems. An additional limitation is the retrospective nature of our patient collection, which restricts analysis and interpretation.

In summary, we found new evidence of differences between patients with 2 different ACPAs, those with anti–Cit-vimentin 60–75 and those with anti–CEP-1, and between these groups of patients and the overall group of anti-CCP–positive patients. The anti–CEP-1–positive patients showed an interaction between the risk alleles of *PTPN22* and HLA–DRB1, and the anti-Cit-vimentin–positive patients showed specific association with the presence of 2 SE alleles. This second group of Spanish patients with longstanding disease also showed a specific increase in the prevalence of erosions. These associations are very likely of pathogenic relevance.

Addendum. In a recently published study (40), Harre et al identified a biologic mechanism that could explain the association we found between the presence of anti–Cit-vimentin antibodies and bone erosions in RA patients in the present study. Those authors showed specific N-terminal citrullination of vimentin during osteoclast differentiation and robust induction of osteoclastogenesis and bone-resorptive activity in mice that received affinity-purified antibodies against this antigen from RA patients.

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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, Perez-Pampin, Calaza, Gomez-Reino, Gonzalez.

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

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