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EXTENDED REPORT

Identification of secreted phosphoprotein 1 gene as a new rheumatoid arthritis susceptibility gene

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ABSTRACT

Objective To evaluate the contribution of the *SPP1* rs11439060 and rs9138 polymorphisms, previously reported as autoimmune risk variants, in the rheumatoid arthritis (RA) genetic background according to anti-citrullinated protein antibodies (ACPAs) status of RA individuals.

Methods We analysed a total of 11 715 RA cases and 26 493 controls from nine independent cohorts; all individuals were genotyped or had imputed genotypes for *SPP1* rs11439060 and rs9138. The effect of the *SPP1* rs11439060 and rs9138 risk-allele combination on osteopontin (OPN) expression in macrophages and OPN serum levels was investigated.

Results We provide evidence for a distinct contribution of *SPP1* to RA susceptibility according to ACPA status: the combination of ≥3 *SPP1* rs11439060 and rs9138 common alleles was associated mainly with ACPA negativity ($p=1.29\times10^{-5}$, $OR_{ACPA\text{-}negative}$ 1.257 (1.135 to 1.394)) and less with ACPA positivity (p=0.0148, $OR_{ACPA\text{-}positive}$ 1.072 (1.014 to 1.134)). The ORs between these subgroups (ie, ACPA-positive and ACPA-negative) significantly differed ($p=7.33\times10^{-3}$). Expression quantitative trait locus analysis revealed an association of the *SPP1* risk-allele combination with decreased *SPP1* expression in peripheral macrophages from 599 individuals. To corroborate these findings, we found an association of the *SPP1* risk-allele combination and low serum level of secreted OPN (p=0.0157), as well as serum level of secreted OPN correlated positively with ACPA production (p=0.005; r=0.483).

Conclusions We demonstrate a significant contribution of the combination of *SPP1* rs11439060 and rs9138 frequent alleles to risk of RA, the magnitude of the association being greater in patients negative for ACPAs.

INTRODUCTION

Rheumatoid arthritis (RA) is a common, complex disease affecting 0.5–1% of the population. It can be subdivided clinically by the presence or absence of autoantibodies directed against the Fc portion of immunoglobulins (rheumatoid factor (RF)) and against citrullinated peptides (anti-citrullinated protein antibodies (ACPAs)).[1] Both genome-wide association studies (GWAS) and custom single-nucleotide polymorphism (SNP) immunochip arrays have identified 46 risk loci among subjects of European ancestry; some of these loci share autoimmune associations.[2–4] To date, most RA risk alleles have been identified and validated in ACPA-positive patients[5–7] or by pooling both ACPA-negative and ACPA-positive patients,[4 8 9] but little is known about the genetic contribution to ACPA-negative RA. The heritability of ACPA-positive and ACPA-negative disease is comparable,[10] and recent association studies provided further support for distinct genetic aetiologies of ACPA-positive and ACPA-negative RA subsets.[11 12]

Type I interferons (IFNs), a family of cytokines essential for antiviral immunity, have a prominent role in both autoimmunity and pathophysiological aspects of RA. A subgroup of RA patients with high ACPA level showed increased expression of type I IFN-inducible genes.[13 14] Several GWAS of RA have identified hits for molecules such as tyrosine kinase 2 and IFN regulatory factor 8 or 5, which participate in type I IFN signalling.[4] Recently, to further substantiate the involvement of type I IFN in the development of autoimmune phenotypes, recessive mutations in the *ACP5* gene (encoding tartrate-resistant acid phosphatase (TRAP)) were identified to cause spondyloenchondrodysplasia (OMIM 271550), a rare disease associated with systemic lupus erythematous (SLE)-related autoimmunity.[15 16] A major substrate of TRAP is osteopontin (OPN), an extracellular-matrix–glycosylated phosphoprotein with multiple functions including bone formation and remodelling,[17] T-cell and B-cell activation[18] and type I IFN

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production.[19] Elevated plasma levels of secreted OPN (s-OPN) were found in RA[20] and at sites of bone erosion in a murine experimental model of collagen-induced arthritis.[21]

To further support the role of OPN in autoimmunity, studies have suggested an association of variants of secreted phosphoprotein 1 (*SPP1*), which encodes OPN, and several autoimmune conditions.[22–25] In addition, several studies convincingly identified *SPP1* as an SLE susceptibility gene.[26–28] Among the *SPP1* autoimmune risk variants previously identified, rs11439060, rs3841116 and rs9138 are of interest. The rs9138 minor allele [C] (+1239A>C), located in the 3′ untranslated region (3′ UTR), was found to be associated with high serum OPN level[25 27 29] and high serum IFNα activity in SLE.[29] Interindividual differences in OPN expression may be influenced by variations in the promoter region: a G insertion at position −156 generates a runt-related transcription factor 2 (RUNX-2) binding site, which was found to increase *SPP1* transcriptional activity[30] and contribute to several autoimmune diseases.[22 25 27] Both rs11439060 (−155 −/G) and rs3841116/rs7687316 (−156 −/G/T) create the −156 insG risk allele; these two variants were previously genotyped and found to be in complete linkage disequilibrium (LD) in 1245 controls of French Caucasian origin (D′=1, r^2=0.99) (data not shown). LD between rs9138 and rs11439060 was reported to be low (r^2=0.23), and logistic regression models supported an independent genetic association of each of these SNPs and SLE.[27] Of interest, *SPP1* rs11439060-rs9138 haplotypes were found to confer susceptibility to SLE, systemic sclerosis and Crohn's disease[22 27 31] and to modulate the progression of multiple sclerosis.[32]

These findings prompted us to test a possible association of the *SPP1* rs11439060-rs9138 haplotype and RA in a case–control study involving a large number of samples.

Table 1 Samples used for analysis

	Cases				Controls	
	All	Female (%)	ACPA+ (*n* (%))	ACPA− (*n* (%))	All	Female (%)
France (FRAGC)	1584	78	753 (54)	649 (46)	1211	62
Spain	1775	73	856 (60)	572 (40])	1504	54
Sweden (EIRA)	3074	72	1975 (64)	1095 (36)	2201	72
UK (WTCCC)	1448	76	1448 (100)	0 (0)	10 053	48
US (BRASS)	474	81	474 (100)	0 (0)	1605	46
US (NARAC1)	853	74	853 (100)	0 (0)	1172	71
US (NARAC2)	869	49	869 (100)	0 (0)	6419	49
Canada	570	76	570 (100)	0 (0)	1517	54
Japan	1099	80	735 (89)	91 (11)	811	71
Total	11 715	73	8533 (78)	2407 (22)	26 493	53

Rheumatoid arthritis (RA) cases were from nine samples with available genotypes for both rs11439060 and rs9138 markers. Samples from France, Spain, Sweden and Japan underwent genotyping for markers. Markers in other samples were imputed from data from Stahl *et al.*[2] RA cases were classified as anti-citrullinated protein antibodies (ACPA)-positive (ACPA+) or ACPA-negative (ACPA−).
FRAGC, French Rheumatoid Arthritis Genetic Consortium; EIRA, Epidemiological Investigation of Rheumatoid Arthritis; WTCCC, Wellcome Trust Case Control Consortium; BRASS, Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study; NARAC, North American Rheumatoid Arthritis Consortium.

MATERIALS AND METHODS

Study design and sample collection

This case–control association study consisted of 11 715 RA cases and 26 493 controls and included a replication step. The discovery sample included 1584 RA patients and 1211 controls of European Caucasian ancestry from the French RA network and the ESPOIR cohort.[33] Our replication sample consisted of eight independent collections from six countries (Spain, Sweden, the UK, Canada, USA and Japan) for 10 131 RA cases and 25 282 controls (table 1). All patients fulfilled the 1987 American College of Rheumatology revised criteria for RA.[34] All subjects provided informed written consent as approved by the recruiting site review board at each of the affiliate institutions.

Genotyping and data processing

The French (French Rheumatoid Arthritis Genetic Consortium (FRAGC)), Spanish, Swedish (Epidemiological Investigation of Rheumatoid Arthritis (EIRA)) and Japanese collections, which include ACPA-positive and ACPA-negative patients, were genotyped for rs11439060 and rs9138. The genotypes were imputed for ACPA-positive RA collections (Wellcome Trust Case Control Consortium (WTCCC), Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study (BRASS), North American Rheumatoid Arthritis Consortium 1 (NARAC1), NARAC2 and CANADA) (see table 1 and online supplementary note).

Statistical analysis

Statistical analysis involved use of R V.2.14.0 (http://www.R-project.org, the R Foundation for Statistical Computing, Vienna, Austria). All association analyses compared controls with RA cases and with subgroups by ACPA status. ORs, 95% CIs and associated p values were assessed by standard logistic regression. All tested models were adjusted by sex because other studies reported a sex-specific association of *SPP1* and SLE.[28 29]

Cases and controls were compared by haplotype-based association analysis of each *SPP1* rs11439060-rs9138 haplotype using PLINK[35] (table 2) (for details, see online supplementary text).

To identify which model best explained the haplotype-based association signal for the ACPA-negative RA subgroup in the discovery sample, we used the Akaike information criterion

Table 2 *SPP1* rs11439060-rs9138 haplotype frequencies

	LD		Haplotype frequencies			
	R^2	D′	-A	-C	GA	GC
France (FRAGC)	0.158	0.892	0.394	0.299	0.296	0.010
Spain	0.193	0.885	0.348	0.298	0.342	0.013
Sweden (EIRA)	0.095	0.772	0.447	0.243	0.292	0.018
UK (WTCCC)	0.120	0.943	0.466	0.250	0.280	0.004
US (BRASS)	0.113	0.839	0.441	0.267	0.279	0.013
US (NARAC1)	0.125	0.902	0.443	0.255	0.294	0.008
US (NARAC2)	0.124	0.923	0.453	0.266	0.275	0.006
Canada	0.127	0.924	0.450	0.270	0.275	0.006
Japan	0.143	0.986	0.328	0.441	0.001	0.229

BRASS, Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study; EIRA, Epidemiological Investigation of Rheumatoid Arthritis; FRAGC, French Rheumatoid Arthritis Genetic Consortium; LD, linkage disequilibrium; NARAC, North American Rheumatoid Arthritis Consortium; WTCCC, Wellcome Trust Case Control Consortium.
R^2 and D′ scores and haplotype frequencies were calculated from controls with use of PLINK software.

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(AIC)[36] to evaluate the fit of eight distinct models (for details, see online supplementary text).

The best-fit model was replicated in a large trans-ethnic sample of eight independent samples. We next performed a combined analysis of the nine samples; heterogeneity was tested by the I^2, T^2 and Q statistics by use of the R package *rmeta* (http://CRAN.R-project.org/package=rmeta). Because we did not observe heterogeneity, we considered fixed-effects models with logistic regression adjusted on sex and sample.

To compare the magnitude of the association of ACPA-positive ($OR_{ACPA\text{-}positive}$) and ACPA-negative ($OR_{ACPA\text{-}negative}$) subgroups, we performed multinomial logistic regression using the *mlogit* function of STATA V.11 (StataCorp, College Station, Texas, USA).

OPN expression and eQTL data

We investigated the effect of the *SPP1* rs11439060 and rs9138 risk-allele combination (ILMN_1651354 and ILMN_2374449 probes) on OPN expression in macrophages, which are well known to express OPN,[37] using data for 599 individuals from the Cardiogenics Transcriptomic Study.[38] Genotyping data for *SPP1* rs10516798, rs12641001, rs6840362, rs7685225, rs6818927, rs7675246 and rs6838095 were used to calculate allele dosage for both rs11439060 and rs9138 genotypes using IMPUTE V.2.3.0[39], with the genotype data for the 379 European founders from the 1000 Genome Project as a reference.[40] The adjusted expression was analysed by linear regression for the corresponding p values.

s-OPN and ACPA serum levels

s-OPN level was measured in baseline serum samples from 60 RA patients of representative age, sex and genotype in the ESPOIR cohort by use of the Assay Designs human OPN ELISA kit (R&D Systems Europe, Lille, France). Samples from French Caucasian healthy unrelated donors (n=29) and SLE patients (n=32), taken from the Rheumatology Department of the Bichat hospital, were tested. Anticyclic citrullinated peptide 2 (anti-CCP2) level was assessed in baseline serum samples of anti-CCP2 RA patients (n=37) from the ESPOIR cohort by use of a commercial ELISA kit (Immunoscan, Eurodiagnostica, Arnheim, The Netherlands).

Non-parametric Mann–Whitney U or Kruskal–Wallis test was used to compare serum s-OPN level in two allelic combination subgroups (ie, presence or absence of the *SPP1* risk-allele combination) or rs11439060 and rs9138 genotype distribution, respectively. Pearson linear regression analysis with GraphPad Prism V.6.0 (http://www.graphpad.com/scientific-software/prism/) was used for correlation analysis of OPN and anti-CCP2 serum levels.

RESULTS

Genetic association study

Given the association of the *SPP1* rs11439060-rs9138 haplotype and various autoimmune conditions, we assessed its association with RA. In a discovery sample of 1585 RA patients and 1211 healthy controls of French Caucasian origin, we found an association of two *SPP1* haplotypes and RA restricted to the ACPA-negative subset: the rs11439060–rs9138A haplotype (hereafter termed '-A') showed a risk effect (p=0.012, $OR_{ACPA\text{-}negative}$ 1.197 (95% CI 1.040 to 1.378)) and the GA haplotype a protective effect (p=0.012, $OR_{ACPA\text{-}negative}$ 0.818 (95% CI 0.698 to 0.957)) (see online supplementary table S1). The lowest AIC value, which identifies the best-fit model, was the recessive model with allelic heterogeneity (see online supplementary table S2): rare alleles had a protective effect; individuals carrying at least three rs11439060 and rs9138 common alleles had an increased risk of ACPA-negative RA ($p=4.19\times10^{-3}$, $p_{adj}=8.38\times10^{-3}$, $OR_{ACPA\text{-}negative}$ 1.350 (95% CI 1.100 to 1.660); figure 1A). In good agreement with the haplotype analysis, we found no association of this *SPP1* risk-allele combination and ACPA-positive RA (p=0.445, p_{adj}=0.890, $OR_{ACPA\text{-}positive}$ 1.077 (95% CI 0.891 to 1.303; figure 1B).

Heterogeneity tests allowed us to combine all the populations investigated (figure 1A–C). Therefore, we tested the *SPP1* rs11439060 and rs9138 risk-allele combination (hereafter shortened to '*SPP1* risk-allele combination') for replication in three samples of ACPA-negative RA patients and replicated the association with the ACPA-negative subgroup ($p=5.52\times10^{-4}$, $OR_{ACPA\text{-}negative}$ 1.234 (95% CI 1.095 to 1.390); figure 1A). The combined analysis established *SPP1* as an ACPA-negative RA risk factor ($p=1.29\times10^{-5}$, $OR_{ACPA\text{-}negative}$ 1.257 (95% CI 1.135 to 1.394); figure 1A). The AIC for the combined population confirmed that the best-fit model was the *SPP1* risk-allele combination (see online supplementary table S2).

Next, to further validate whether the *SPP1* contribution was restricted to ACPA-negative RA, we tested an association in ACPA-positive disease using additional samples and found a weak association (p=0.015, $OR_{ACPA\text{-}positive}$ 1.072 (95% CI 1.014 to 1.134); figure 1B), which led to an association with overall RA ($p=8.38\times10^{-4}$, OR 1.094 (95% CI 1.038 to 1.153); figure 1C). The SPP1 risk-allele combination had a differential effect on risk of ACPA-negative and ACPA-positive disease (non-overlapping OR intervals) (see online supplementary figure S1). In testing the heterogeneity between ACPA-positive and ACPA-negative RA by multinomial logistic regression, we found a distinct contribution of the *SPP1* risk-allele combination and risk of both ACPA-negative and ACPA-positive disease ($p=7.33\times10^{-3}$).

OPN expression profile and eQTL data

We searched for biological evidence of the *SPP1* risk-allele combination to support a true positive association with RA. We used the data from the Cardiogenics Transcriptomic Study for 599 individuals[38] to search for an expression quantitative trait locus (eQTL) in macrophages. The *SPP1* risk-allele combination was associated with significantly decreased expression of *SPP1* with both ILMN_1651354 and ILMN_2374449 probes ($p=3.45\times10^{-3}$ and $p=5.38\times10^{-4}$, respectively; figure 2). Reinforcing the putative synergism between rs11439060 and rs9138, single-marker analysis revealed an effect of rs11439060 on *SPP1* expression, although at a lower level than with the risk-allele combination (p=0.011 for ILMN_1651354; $p=9.99\times10^{-4}$ for ILMN_2374449), while rs9138 had no effect (p=0.607 for ILMN_1651354; p=0.749 for ILMN_2374449).

Association with s-OPN serum level

To further corroborate the *SPP1* eQTL findings, we investigated the possible functional consequence of the *SPP1* risk-allele combination on s-OPN serum levels in RA patients with no biologic therapy (ESPOIR cohort) who were previously genotyped for rs11439060 and rs9138 (n=60). In addition, we assessed s-OPN serum level in SLE patients (n=32) and controls (n=29), considered positive and negative control populations, respectively. Mean serum s-OPN protein level was higher in SLE subjects than in RA subjects and controls (65.02±38.71 ng/mL, 34.64±19.95 ng/mL and 12.99±16.26 ng/mL, respectively; figure 3A). In agreement with the eQTL results, mean serum s-OPN protein level was lower in RA patients with than without the *SPP1* risk-allele combination (29.66±15.99 vs 44.62 ±23.54 mg/mL, p=0.0157; figure 3B). Of interest, rs11439060

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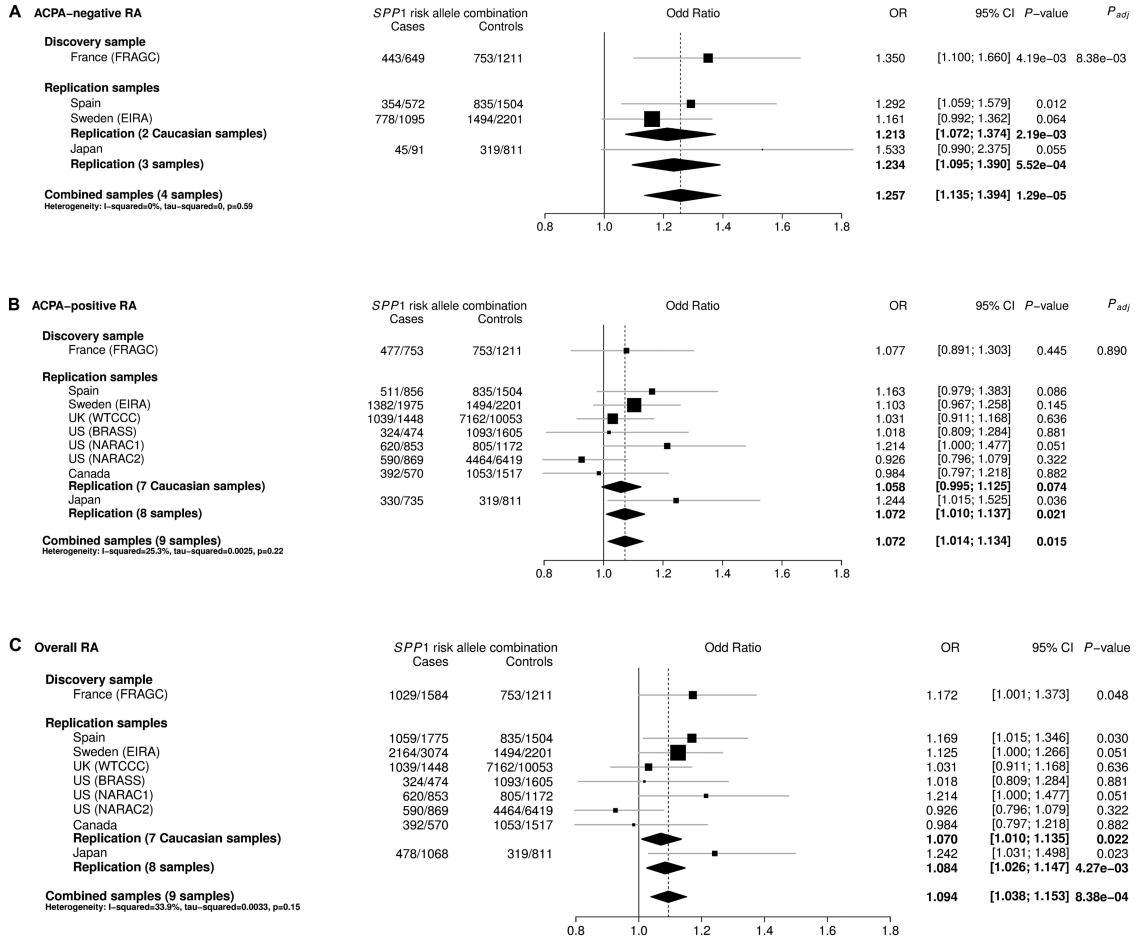

Figure 1 Association of secreted phosphoprotein 1 gene (*SPP1*) rs11439060 and rs9138 risk-allele combination and rheumatoid arthritis (RA). (A) Anti-citrullinated protein antibody (ACPA)-negative RA, (B) ACPA-positive RA and (C) overall RA. Data are proportion of *SPP1* risk-allele combination, ORs, 95% CIs and p values. Forest plot shows ORs with symbol size proportional to case sample size. Results for several samples are shown in bold, with diamond widths indicating 95% CIs.

was not associated with s-OPN serum level, whereas rs9138 slightly modulated s-OPN serum level (see online supplementary figure S2).

Correlation between s-OPN and ACPA serum level

Because our genetic data demonstrated that *SPP1* had a differential effect on risk of ACPA-negative and ACPA-positive RA and the risk-allele combination was associated with low *SPP1* expression and serum s-OPN level, we wondered whether s-OPN serum level was associated with ACPA production. We found a positive correlation between anti-CCP2 and s-OPN serum levels in 37 anti-CCP2-positive RA patients with no biologic therapy (ESPOIR cohort) ($p=0.005$, $r=0.483$; figure 4).

DISCUSSION

We provide the first evidence of a distinct genetic contribution of *SPP1* to risk of RA by ACPA status. Previous studies of RA have suggested that associated loci predispose to specific subsets of the disease characterised by ACPA status.[12 41 42] Furthermore, non-genetic data suggested that ACPA-positive disease behaves differently from ACPA-negative disease.[43] Therefore, these theoretically different forms of RA should be analysed separately. Our results indicate that the combination of ≥3 frequent alleles of two *SPP1* variants, rs11439060 and rs9138, is associated mainly with ACPA-negative disease ($p=1.29\times10^{-5}$) and less with ACPA-positive disease as compared with healthy controls ($p=0.0148$). The ORs between these subgroups (ie, ACPA-positive and ACPA-negative) significantly differed ($p=7.33\times10^{-3}$), which provides evidence for a distinct contribution of *SPP1* to risk of both ACPA-negative and ACPA-positive RA.

Of interest, in both our discovery sample (ie, French Caucasian population) and the combined sample, the best-fit model, provided by the AIC, was the recessive model with allelic heterogeneity, which led to the hypothesis of a true synergism of rs11439060 and rs9138 in RA susceptibility. This hypothesis is consistent with a growing number of other observations.[44] In our study, as in the 1000 Genome Project Phase I, the *SPP1* rs11439060-rs9138 haplotype structure differed for European and East Asian populations (table 2). Even with a different haplotype structure, rs11439060 and rs9138 showed a similar contribution, with an association of comparable magnitude and direction. Therefore, accounting for allelic combinations may be necessary to identify genetic effects that may otherwise be missed (see online supplementary table S3). Indeed, the univariate approach considering only a single marker at a time, commonly used in GWAS, could overlook the complex interactions that often occur in biological systems.[45]

Multiple genetic association studies have found an association of *SPP1* and several autoimmune disorders, reporting an effect of the rs9138 variant opposite to that observed in RA, the C rare allele being the autoimmune risk allele.[22 24–28 31] These

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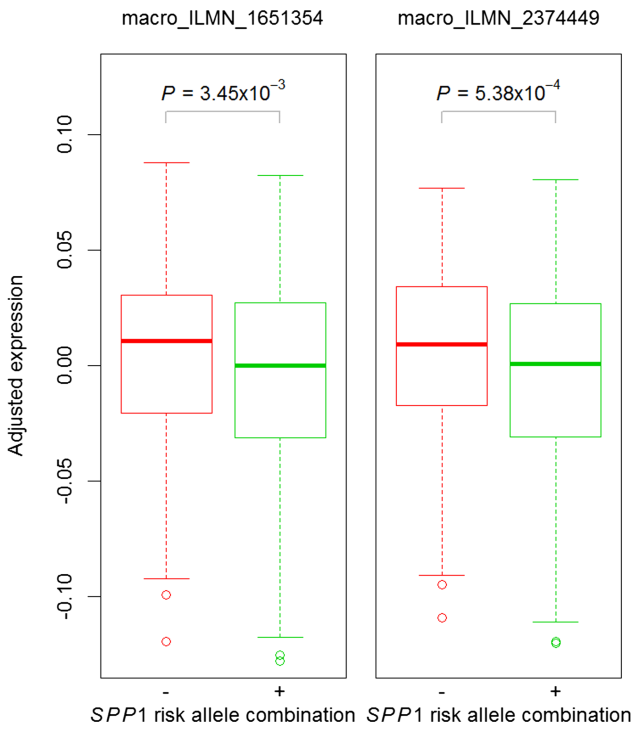

Figure 2 Association of *SPP1* risk-allele combination and *SPP1* expression in macrophages of 599 subjects from the Cardiogenics Transcriptomic Study. Top box plot shows results of *SPP1* ILMN_1651354 probe in macrophages. Bottom box plot shows results of *SPP1* ILMN_2374449 probe in macrophages. Genotypes of rs11439060 and rs9138 were imputed. Best-called genotypes were used to stratify subjects for box plots (n=599; in red, n=247 subjects without the risk-allele combination; in green, n=352 subjects with the combination). p Values were derived from the probabilities of having the *SPP1* risk-allele combination.

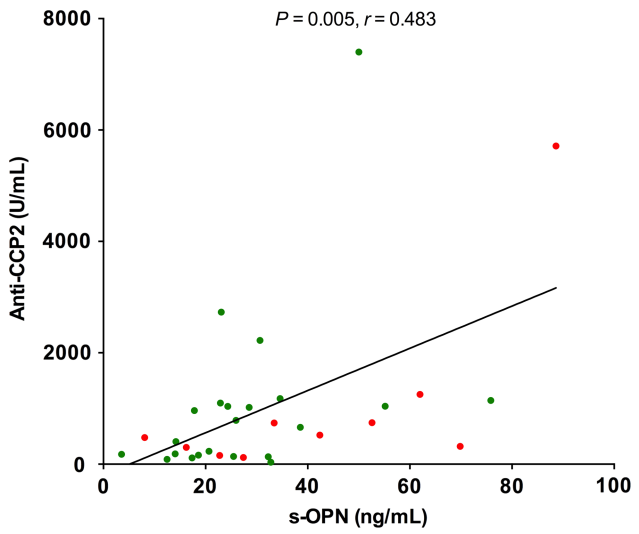

Figure 4 Correlation between secreted osteopontin (s-OPN) and anti-citrullinated protein antibody (ACPA) serum level. The line is the best-fit regression line. Each dot represents the anti-CCP2 serum level for one anti-CCP2-positive rheumatoid arthritis patient according to s-OPN serum level. In red, n=10 subjects without the risk allele combination; in green, n=27 subjects with the combination. The r values were evaluated by Pearson correlation analysis.

findings support that common genetic factors in autoimmune diseases may be associated with a given marker but differ in the direction of the association.[46 47] We identified no predicted functional exonic variant in subjects carrying the *SPP1* risk-allele combination (see online supplementary text), so, in addition to the functional data, both rs11439060 and rs9138 polymorphisms may indeed be the causal variants. Of note, the G insertion at position −156 generates a RUNX-2 binding site, which was found to increase *SPP1* transcriptional activity.[30] As well, rs9138, which is located in the 3′ UTR, was found to be significantly associated with OPN serum levels in controls[27 48] and males with SLE.[29] However, direct re-sequencing of the *SPP1* exons would be necessary to definitely exclude the existence of

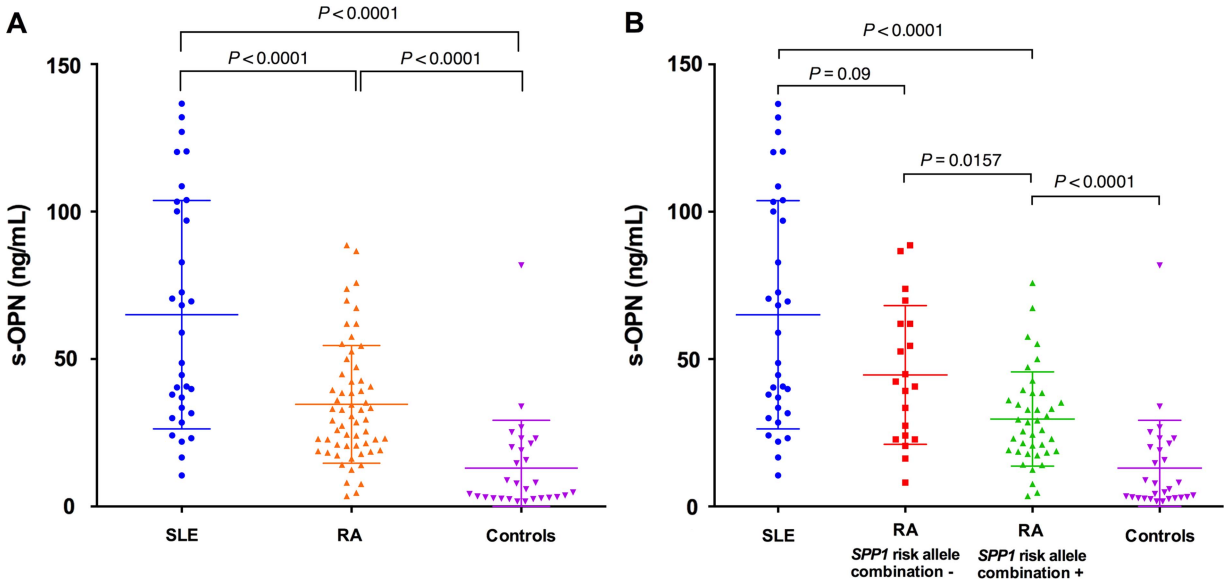

Figure 3 Association of *SPP1* risk-allele combination and secreted osteopontin (s-OPN) serum level. (A) s-OPN serum level in systemic lupus erythematous (SLE) patients (n=32), rheumatoid arthritis (RA) patients (n=60) and controls (n=29). SLE and controls were considered positive and negative controls. Both SLE patients and controls were not genotyped for *SPP1*. (B) s-OPN serum level in RA patients stratified by presence of the *SPP1* risk-allele combination: in red, 20 RA patients without the combination; in green, 40 RA patients with the combination. Each dot represents the s-OPN serum level for one patient. Lines and outer bars are mean±SD. p Values were calculated by non-parametric Mann–Whitney U test.

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a very rare coding variant. Of interest, a recent large genetic association study of 25 genes from 20 GWAS-identified risk loci showing overlap among six common autoimmune disorders found little support for a significant impact of rare coding variants in known risk genes for the autoimmune phenotypes investigated.[49]

In addition to the novel finding of *SPP1* as an RA risk gene, our study suggests that the *SPP1* risk-allele combination has a functional consequence. The risk-allele combination was associated with decreased *SPP1* expression in macrophages in a large sample. However, at this step, we could not establish a definitive association of the *SPP1* risk-allele combination because the data were from imputed genotypes, which is a limitation of our study. We next provide clear evidence of an association between the *SPP1* risk-allele combination and low s-OPN serum level. Our results are consistent with a reported effect of rs9138 on s-OPN serum level.[25 27 29] Conversely, no association of rs11439060 and s-OPN serum levels has been detected.[22 27] The rs11439060 variant may have a synergistic effect in regulating both s-OPN and i-OPN production: by regulating *SPP1* expression, rs11439060, located in the promoter, may cooperate with rs9138, located in the 3′ UTR, to regulate s-OPN serum level by altering its mRNA polyadenylation or stability. In agreement with (1) the greater effect of SPP1 on ACPA-negative than ACPA-positive RA and (2) the association of the SPP1 risk-allele combination and low serum s-OPN levels, we observed a weak correlation between ACPA levels and s-OPN serum levels. Thus, OPN may have an important role in regulating ACPA production.

Through the properties of both its isoforms, OPN has multiple contributions to the humoral immune response: in T cells, OPN potentiates proliferation, IFN-γ production and CD40 L expression, which in turn favours B-cell proliferation and antibody production.[50] In plasmacytoid dendritic cells, i-OPN promotes type I IFN production, which may also enhance antibody responses.[51] In several RA samples, the presence of a type I IFN signature in peripheral blood mononuclear cells was associated with the presence and titres of autoantibodies, which is similar to findings in other autoantibody-associated diseases.[14 52–54] The type I IFN signature was also identified in a subset of arthralgia patients positive for autoantibodies in whom RA developed later.[55] In addition to our findings, several lines of evidence support the hypothesis of a pivotal role of OPN in autoantibody production: rs11730582, a *SPP1* promoter variant in high LD with rs11439060, was found to be associated with autoantibody-mediated cytopenia in SLE,[56] and serum OPN level was associated with IgG serum level in DALD patients.[25] Finally, rs9138, which modulates s-OPN serum level, was also reported to affect IFN-α serum activity in SLE.[29]

In conclusion, our study, involving a large number of samples, demonstrates a significant contribution of *SPP1* to risk of RA, the magnitude of the association most important in ACPA-negative RA. The *SPP1* risk-allele combination of rs11439060 and rs9138 was associated with decreased expression of s-OPN serum level, which was correlated with ACPA production. Our study illustrates that accounting for allelic combinations could be of interest to identify genetic effects that may otherwise be ignored and could contribute to a better understanding of the genetic architecture and pathogenesis of complex diseases such as RA.

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Contributors PD, SG, KS carried out the primary data analyses. PD, LP, JM and NT conducted genotyping and replication study. SG and KS conducted the eQTL analysis. PN, MH-N, JE-B, SC-M, CR and LK conducted OPN and ACPA serum level analysis. All other authors contributed to additional analyses and genotype and clinical data enrolments. SG and PD designed the study and wrote the manuscript, with contributions from all authors on the final version of the manuscript.

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