EXTENDED REPORT

Systematic approach demonstrates enrichment of multiple interactions between non-*HLA* risk variants and *HLA-DRB1* risk alleles in rheumatoid arthritis

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ABSTRACT

Objective In anti-citrullinated protein antibody positive rheumatoid arthritis (ACPA-positive RA), a particular subset of *HLA-DRB1* alleles, called shared epitope (SE) alleles, is a highly influential genetic risk factor. Here, we investigated whether non-*HLA* single nucleotide polymorphisms (SNP), conferring low disease risk on their own, interact with SE alleles more frequently than expected by chance and if such genetic interactions influence the *HLA-DRB1* SE effect concerning risk to ACPA-positive RA.

Methods We computed the attributable proportion (AP) due to additive interaction at genome-wide level for two independent ACPA-positive RA cohorts: the Swedish epidemiological investigation of rheumatoid arthritis (EIRA) and the North American rheumatoid arthritis consortium (NARAC). Then, we tested for differences in the AP p value distributions observed for two groups of SNPs, non-associated and associated with disease. We also evaluated whether the SNPs in interaction with *HLA-DRB1* were cis-eQTLs in the SE alleles context in peripheral blood mononuclear cells from patients with ACPA-positive RA (SE-eQTLs).

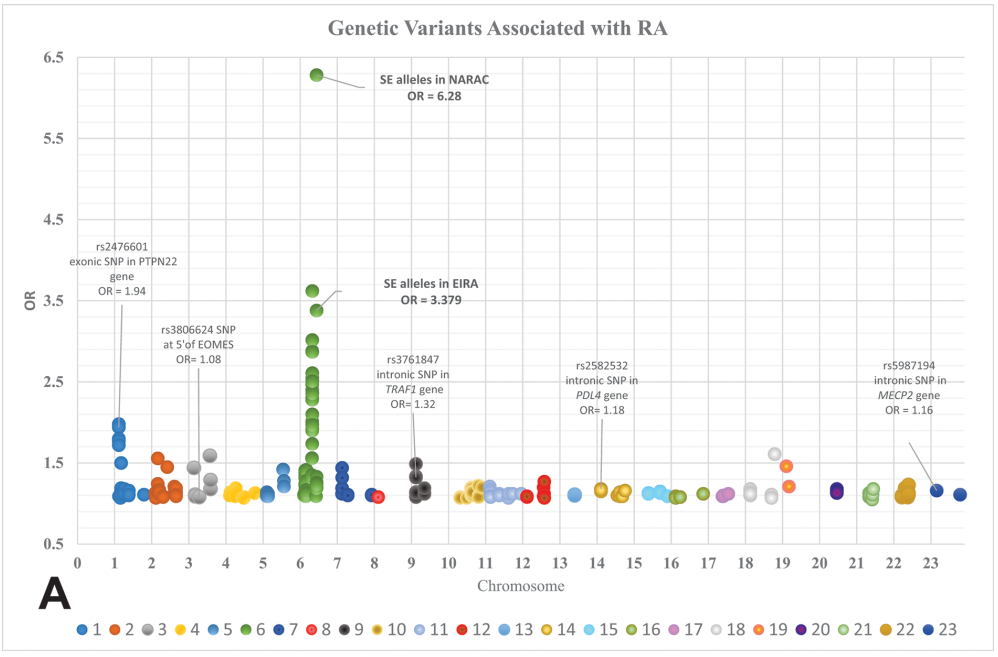
Results We found a strong enrichment of significant interactions (AP p<0.05) between the *HLA-DRB1* SE alleles and the group of SNPs associated with ACPA-positive RA in both cohorts (Kolmogorov-Smirnov test D=0.35 for EIRA and D=0.25 for NARAC, p<2.2e-16 for both). Interestingly, 564 out of 1492 SNPs in consistent interaction for both cohorts were significant SE-eQTLs. Finally, we observed that the effect size of *HLA-DRB1* SE alleles for disease decreases from 5.2 to 2.5 after removal of the risk alleles of the two top interacting SNPs (rs2476601 and rs10739581).

Conclusion Our data demonstrate that there are massive genetic interactions between the *HLA-DRB1* SE alleles and non-*HLA* genetic variants in ACPA-positive RA.

INTRODUCTION

In rheumatoid arthritis (RA (OMIM: 180300)), a particular subset of *HLA-DRB1* gene variants (major alleles at *01, *04 and *10 groups), commonly called shared epitope (SE) alleles, is the most important genetic contributor for the risk of developing anti-citrullinated protein antibody (ACPA)-positive RA.[1–3] It is noteworthy that the strength of the association between non-*HLA* genetic variants and ACPA-positive RA risk is, in general, very moderate in comparison to that of the *HLA-DRB1* SE alleles[4–7] (figure 1A). This prompted us to investigate whether the *HLA-DRB1* SE alleles could be a genetic hub[8] that captures multiple interactions. Indeed, previous studies have demonstrated interactions between the *HLA-DRB1* SE alleles and several single nucleotide polymorphisms (SNP), including variations in *PTPN22*, *HTR2A* and *MAP2K4* with regard to the risk of developing ACPA-positive RA,[9–12] where the combination of both risk factors shows significantly higher risk (measured as OR) than the sum of their separate effects. Departure from additivity is a way to demonstrate interaction between risk factors regarding the risk of disease. The additive scale, defined by attributable proportion (AP) due to interaction, has the advantage of a straightforward interpretation in the sufficient-component cause model framework.[9 13–16]

In our current study, we aimed to investigate whether there is an enrichment of genetic interactions between non-*HLA* SNPs, conferring low disease risk on their own, and the major *HLA-DRB1* related disease risk to develop ACPA-positive RA. We also explored to what extent the top interactions influence the association between *HLA-DRB1* and risk to ACPA-positive RA. First, we assessed departure from additivity regarding the interaction between the *HLA-DRB1* SE alleles and SNPs at the genome-wide level. The outcome of this analysis was used to investigate the potential enrichment of significant interactions among certain variants by comparing the distribution of the p value of interaction between two defined groups of SNPs: the pool of variants which exhibited a significant nominal association with ACPA-positive RA in comparison to SNPs that are not associated with disease risk. We thereafter performed a replication study with the same approach in an independent ACPA-positive RA cohort. Second, we performed an expression quantitative trait loci (eQTL) analysis for non-*HLA* SNPs in interaction with *HLA-DRB1*, stratified by the *HLA-DRB1* SE alleles in order to identify genes influenced by the interactions. Finally, we analysed the effect size from the *HLA-DRB1* SE alleles concerning the risk of developing ACPA-positive RA before and after step-by-step removal of the effect of the risk alleles of the strongest SNPs in interacting with *HLA-DRB1* SE. Our observations



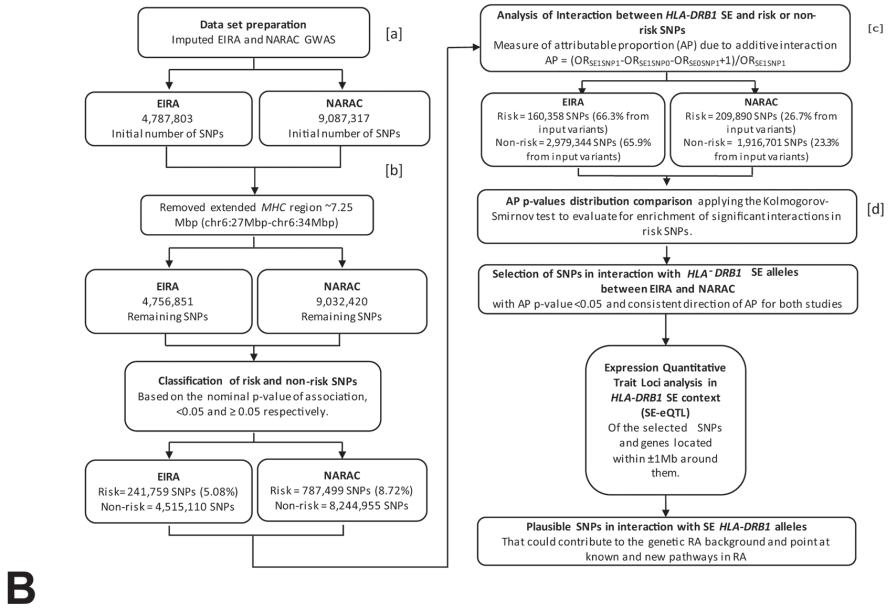


Figure 1 (A) Genetic variants associated with ACPA-positive RA. This plot represents the association signals (p<1.0e-05) from different GWAS in ACPA-positive RA, taken from the NHGRI-EBI GWAS catalogue (https://www.ebi.ac.uk/gwas/home).[46–48] X-axis: genomic positions, including chromosome X (marked as 23). Y-axis: the OR value observed for each SNP in different studies. Some examples are pointed. (B) Methodology workflow. (a) The workflow was also applied with non-imputed genotyping data (online supplementary table S2). (b) An alternative step excluding the PTPN22 locus was included at this point. (c) The AP value, its respective p value and CI (95% CI) were assessed using logistic regression implemented in GEISA (https://github.com/menzzana/geisa).[13 27 28] (d) The classification of risk and non-risk SNPs was permuted 10 000 times and each time the KS test was applied. The workflow was implemented until this step for each of the 1000 SE permuted variables, a lower number of permutations due to computational constrains. Both types of permutations showed that less than 5% of the KS test will exhibit a p value less than 2.2e-16, strongly indicating that differences in the AP p value distribution detected by the KS test from the original data are unlikely to be by chance. ACPA-positive RA, anti-citrullinated protein antibody positive rheumatoid arthritis; EBI, European Bioinformatics Institute; EIRA, epidemiological investigation of rheumatoid arthritis; GWAS, genome-wide association study; KS, Kolmogorov-Smirnov test; LD, linkage disequilibrium; MAF, minor allele frequency; MHC, major histocompatibility locus; NARAC, North American rheumatoid arthritis consortium; NHGRI, National Human Genome Research Institute; PCA, principal component analysis; SE, shared epitope; SE0SNP1, absence of the HLA-DRB1 SE alleles and presence of the risk allele from the SNP; SE1SNP0: presence of the HLA-DRB1 SE alleles and absence of the risk allele from the SNP; SE1SNP1, presence of the HLA-DRB1 SE alleles and the risk allele from the SNP; SNP, single nucleotide polymorphism. *PTPN22* is abbreviation for the gene.

indicated that the *HLA-DRB1* SE alleles act as a hub of cumulative additive interactions with multiple genetic variants in the development of ACPA-positive RA. We proposed that the analytic approach used here provides a novel way to study the impact of gene–gene interactions and the role of *HLA* in other autoimmune diseases.

Table 1 Description of studied populations

Study	Number of individuals	Female:male ratio	Frequency of *HLA-DRB1* SE alleles	rs4507692 MAF and nominal p value of association*	Number of SNPs in GWAS †	Number of SNPs in GWAS after imputation †
EIRA			0.45	MAF=0.45 P=0.57	282 527	4 756 851
Cases ‡	1151	2.4:1	0.59			
Controls	1079	2.6:1	0.30			
NARAC			0.43	MAF=0.43 P=0.67	398 551	9 032 420
Cases ‡	867	2.8:1	0.68			
Controls	1194	2.5:1	0.26			

*The rs4507692 SNP (chromosome 7) was used as a negative control instead of *HLA-DRB1* SE alleles, since the rs4507692 SNP is not associated with RA but has the same minor allele frequency (MAF) as the *HLA-DRB1* SE alleles.
†After removing the extended major histocompatibility (MHC) region.
‡Patients with anti-citrullinated protein antibody positive rheumatoid arthritis (ACPA-positive RA).
EIRA, epidemiological investigation of rheumatoid arthritis; GWAS, genome-wide association study; NARAC, North American rheumatoid arthritis consortium; RA, rheumatoid arthritis; SE, shared epitope; SNP, single nucleotide polymorphism.

MATERIALS AND METHODS

The methodology workflow is represented in figure 1B.

Studied populations

This project was based on genome-wide association study (GWAS) data from two independent case–control studies of RA, epidemiological investigation of rheumatoid arthritis (EIRA)[6 14 17–20] and North American rheumatoid arthritis consortium (NARAC).[6 18 21 22] A total of 4291 individuals were included in this study, where 2018 are ACPA-positive RA cases and 2273 are healthy controls (table 1). A detailed description is found in the online supplementary information.

Genotyping and data filtering

HLA typing was performed by sequence-specific primer PCR assay as described elsewhere.[23 24] A standard quality control for GWAS was performed before and after imputation for both cohorts (see the description in the online supplementary information). The extended *MHC* region (chr6:27339429-34586722, GRCh37/hg19) was removed from the analyses, to exclude influence of the high linkage disequilibrium (LD) and independent signals of association with ACPA-positive RA in the locus. Association tests for EIRA and NARAC were applied using logistic regression model in plink (V.1.07).[25] Based on the nominal p values of association, the SNPs were grouped into risk ($p<0.05$) or non-risk SNPs ($p\geq0.05$) (figure 1B and table 2).

Interaction analysis

The additive model was used to test for interactions between the *HLA-DRB1* SE alleles and each SNP from the EIRA and NARAC GWAS. The null hypothesis of the additive model assumes that there is additivity between the different sufficient causes for a phenotype, while the alternative hypothesis is assumed when departure from additivity is observed. The departure from additivity is estimated by the AP due to interaction using OR as measure of association,[26] with the following equation:

$$AP = (OR_{SE1\,SNP1} - OR_{SE1\,SNP0} - OR_{SE0\,SNP1} + 1) / OR_{SE1\,SNP1}$$

where 1 and 0 refer to presence or absence of the risk factor/allele, respectively, the SE0SNP0 was used as a reference group. A cut-off of five observations for each of the cell frequencies was applied. The gender and the first 10 principal components (online supplementary information) were included as covariates in the model. The AP, its respective p value and CI (95% CI) were assessed using logistic regression implemented in GEISA (V.0.1.12)[13 27 28] (table 2).

Comparison of the distribution of AP p values between the risk and non-risk groups of SNPs and quality controls

The distribution of AP p values observed in the interaction analysis from the ACPA-positive RA risk SNPs was compared with the distribution of AP p values observed from the non-risk SNPs using the Kolmogorov-Smirnov (KS) test, implemented in

Table 2 The Kolmogorov-Smirnov (KS) test for AP p value distributions of the interaction analysis with the *HLA-DRB1* SE alleles and risk or non-risk SNPs in EIRA and NARAC imputed data

Case–control group	SNP group	Number of initial input SNPs	Number of SNPs after cut-off *	% of SNPs analysed	Number of SNPs with AP p<0.05	% of analysed SNPs with AP p<0.05	D^+value from KS test (risk vs no-risk)†	Group of SNPs with enrichment of significant interactions
EIRA	Risk	241 759	160 358	66.33	39 518	24.64	0.354	Risk
	No risk	4 515 110	2 979 344	65.99	83 287	2.80		
NARAC	Risk	787 499	209 890	26.65	31 992	15.24	0.247	Risk
	No risk	8 244 955	1 916 701	23.25	64 012	3.44		

*Interaction was estimated using sex and the 10 first eigenvectors as covariables. A minimum of five individuals in each of the four combinations that formed the basis for the OR calculations was required.
†The alternative hypothesis for KS test was that the empirical cumulative distribution function (ECDF) of AP p values for risk SNPs lies above that of non-risk SNPs (figure 2). KS test p<2.2e-16 for both EIRA and NARAC. As mentioned in the Materials and methods section, these KS test p values are lower than the machine precision, meaning that when the precise p value was calculated the result was 0.
AP, attributable proportion; EIRA, epidemiological investigation of rheumatoid arthritis; NARAC, North American rheumatoid arthritis consortium; SE, shared epitope; SNP, single nucleotide polymorphism.

the *stats* package of R software (V.3.3.2).[29] The KS test statistic quantifies the maximum distance (D) between the two empirical cumulative distribution functions of the AP p values from the risk and non-risk SNP groups.

The SNP category of risk and non-risk was permuted 10 000 times to evaluate whether the observed AP p value distribution with the original SNP classification remains with random SNP classification. Similarly, the *HLA-DRB1* SE allele variable was iterated 1000 times with the same purpose. Both types of permutations showed that less than 5% of the KS tests will exhibit a p value under 2.2e-16 (online supplementary information). The rs4507692 SNP (chromosome 7) was used as a negative control instead of *HLA-DRB1* SE alleles, since the rs4507692 SNP is not associated with RA but has the same minor allele frequency as the *HLA-DRB1* SE alleles (table 1).

The SNPs from the *PTPN22* locus (chr1:113679091-114679090, GRCh37/hg19) were removed to apply the same workflow, from step 1 to 9 in figure 1B, to determine the influence of this locus in the enrichment analysis, due to the known gene–gene interaction between the rs2476601variant (or SNPs in LD with this variant) and the *HLA-DRB1* SE alleles.[9] Additionally, to address possible inflation in the results due to imputation and LD, the same steps as described in figure 1B were implemented with (1) raw (non-imputed) GWAS data, (2) with the exclusion of all variants from chromosome 6, and (3) LD pruned GWAS data.

SNPs in interaction with SE between EIRA and NARAC

We selected those variants with AP p<0.05 and same AP direction from both the EIRA and NARAC studies to evaluate their distribution across the genome. False discovery rate (FDR) correction was applied to these SNPs with a 5% threshold (online supplementary table S3)

Conditional eQTL analysis in the context of the *HLA-DRB1* SE alleles (SE-eQTL)

The selected SNPs were tested for eQTLs in the carrier and non-carriers of *HLA-DRB1* SE alleles (SE-eQTLs) for genes±1 Mbp around the SNPs. Data from peripheral blood mononuclear cells (PBMC) of 97 patients with ACPA-positive RA (69% female) from the COMBINE study[30] were included. The mixed-linear model function in the nlme 3.1 package from R/Bioconductor (V.3.3.2)[31] was used for the analysis (details in the online supplementary information).

RESULTS

Interaction between the ACPA-positive RA-associated SNPs and *HLA-DRB1* SE alleles is more common than with non-associated SNPs

EIRA study was the discovery cohort to test for enrichment of significant interactions between the *HLA-DRB1* SE alleles and the predefined risk SNPs from this study. The risk SNPs represent 5% of the variants analysed for interaction in EIRA. Out of these risk SNPs, 24.5% exhibited an AP p value less than 0.05 (table 2, figure 2A). On the other hand, among the non-risk variants (nominal p values of association ≥0.05) representing the remaining 95% of analysed SNPs, only 2.8% displayed a significant interaction (AP p<0.05) with the *HLA-DRB1* SE alleles (table 2, figure 2B). This striking difference in the frequency of significant interactions is reflected in the KS test (D=0.35, p<2.2e-16) (table 2, figure 2C). The enrichment was also observed when only the segments of the AP p values below 0.05 for risk and non-risk SNPs were compared (KS test D=0.25, p<2.2e-16, figure 2D–F), suggesting that the enrichment of significant interactions corresponds mainly to the low AP p values of the risk group of SNPs.

Importantly, this enrichment of low AP p values in the risk variants is totally absent when the rs4507692 SNP was tested instead of the *HLA-DRB1* SE variable as negative control. The proportion of interacting risk SNPs with the rs4507692 variant dropped to 2.8% (table 1 and online supplementary table S1 and figure S1a–f). Since the same group of risk variants was tested for interaction with the *HLA-DRB1* SE alleles and rs4507692 SNP, both AP p value distributions were comparable (KS test D value=0.35, p<2.2e-16) (online supplementary figure S2a). This comparison confirmed that the enrichment of significant interactions is only present when the risk SNPs and the *HLA-DRB1* SE alleles are tested.

The enrichment of significant interactions was not remarkably affected after removing the *PTPN22* locus (KS test D=0.353, p<2.2e-16), highlighting that the strong increment of significant interactions is due to multiple ACPA-positive RA risk variants.

Consistent enrichment of significant interactions was observed when the workflow was applied to non-imputed genotyping data for EIRA, before (KS test D=0.33, p<2.2e-16) and after (KS test D=0.33, p<2.2e-16) removing all the variants from chromosome 6 and LD pruning (online supplementary table S2). The enrichment of significant interactions in the risk group of SNPs was detected also when more stringent thresholds of nominal p value for association (0.005 and 0.0005) were used to classify associated SNPs (online supplementary figure S3).

Altogether, the results from EIRA study indicate that there is a strong enrichment of significant interactions between non-*HLA* risk variants and *HLA-DRB1* SE alleles in ACPA-positive RA.

An independent replication supports the observed enrichment of significant interactions between the ACPA-positive RA-associated SNPs and the *HLA-DRB1* SE alleles

To confirm the results, the independent NARAC study was used. Consistently, a significant proportion of interactions with AP p values below 0.05 was detected between the *HLA-DRB1* SE alleles and the risk SNPs (15.2%), compared with the ones found with the non-risk SNPs (3.3%) (KS test D=0.25, p<2.2e-16, table 2, figure 2G–I). This observation was corroborated when only the fraction of AP p values below 0.05 was compared between the risk and non-risk groups of variants in the NARAC study (KS test D=0.17, p<2.2e-16, figure 2J–L). As in EIRA, there was no enrichment of significant interactions in the risk group (2.6%) compared with the non-risk group (3%) of SNPs when the negative control (rs4507694) was implemented (online supplementary table S1 and figure S1g–l). Consequently, the proportion of relevant interactions remained higher between the risk SNPs and *HLA-DRB1* SE alleles, when compared with the proportion of such interactions with the risk SNPs and the rs4507692 variant (KS test D=0.26, p<2.2e-16; online supplementary figure S2b). Analogous results to EIRA were observed when the workflow was applied to non-imputed GWAS in the NARAC study (online supplementary table S2).

Chromosomes 1 and 9 were highlighted by the top interactions with the *HLA-DRB1* SE alleles

The comparison of results from EIRA and NARAC studies identified 1492 SNPs in interaction with the *HLA-DRB1* SE alleles, with AP p<0.05 and the same direction of AP for both studies (figure 3A,B, online supplementary table S3). The signals within chromosomes were ranked based on the minimum AP p value,

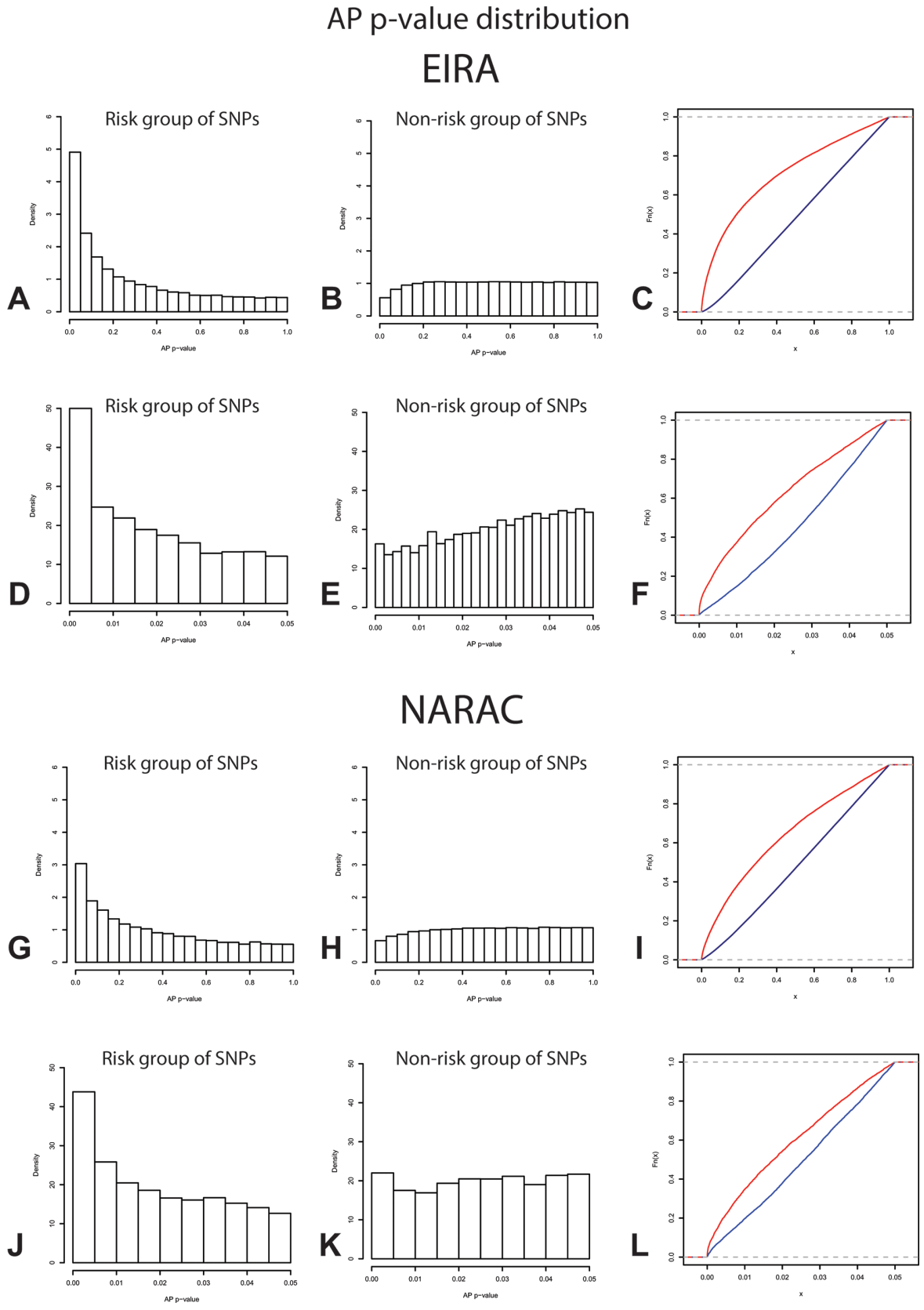


Figure 2 AP p value distributions for interaction between the *HLA-DRB1* shared epitope (SE) alleles and two groups of genetic variants. (A, G) Density plots of AP p values for the interaction between the *HLA-DRB1* SE alleles and the risk group of SNPs (nominal p value of association <0.05) or (B, H) non-risk group of SNPs (nominal p value of association ≥0.05) in the EIRA or NARAC studies, respectively. (C, I) The respective ECDF plots of the AP p values from risk (red line) or non-risk (blue line) SNPs in interaction with the *HLA-DRB1* SE alleles in the respective studies (table 2). The AP p value distribution and the KS tests on the fraction of AP p values less than 0.05 are represented in panels (D)–(F) for EIRA study and (J)–(L) for NARAC study. AP, attributable proportion due to interaction; ECDF, empirical cumulative distribution function; EIRA, epidemiological investigation of rheumatoid arthritis; KS, Kolmogorov-Smirnov test; NARAC, North American rheumatoid arthritis consortium; SNP, single nucleotide polymorphism.

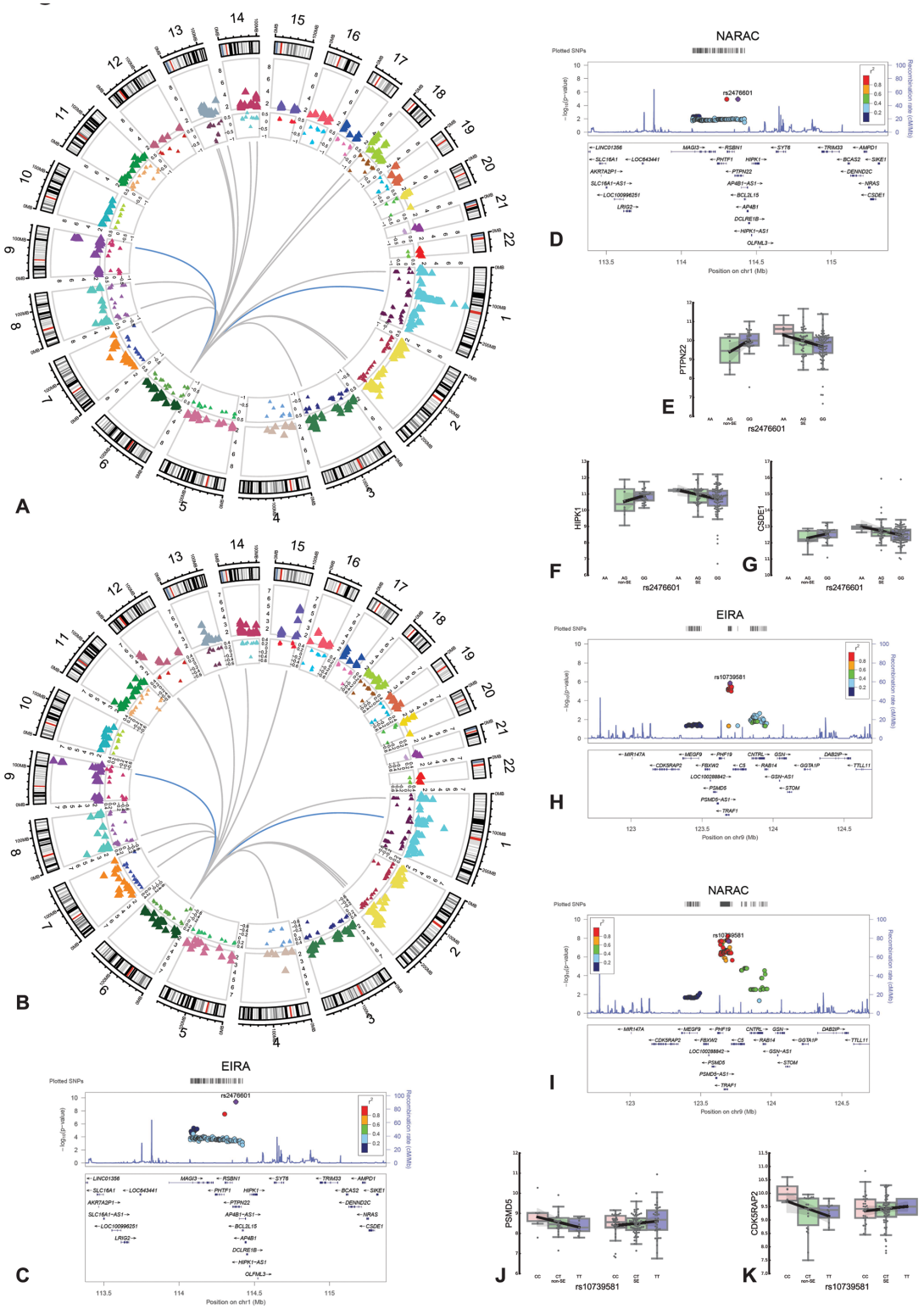


Figure 3 Selected SNPs from both studied cohorts with AP p<0.05 and the same direction of AP. The circos plots for (A) the EIRA study and (B) the NARAC study represent the 1492 selected SNPs in additive interaction with the *HLA-DRB1* SE alleles. The outermost track of the circos plots is the cytoband for 22 human chromosomes. The y-axis of the second track is the negative logarithm of the AP p values (additive interaction with the *HLA-DRB1* SE alleles). In the third track, the y-axis corresponds to the AP value. The internal connector lines highlight the interactions that exhibited an AP p<1e-03. (C) and (D) panels represent the 1p13 locus centred on the rs2476601 SNP. This variant is a conditional eQTL in the *HLA-DRB1* SE allele context for (E) *PTPN22*, (F) *HIPK1* and (G) *CSDE1* genes in PBMCs from the patients with ACPA-positive RA. (H) and (I) panels represent the 9q33 locus centred on the rs10739581 SNP. This variant is a conditional eQTL in the *HLA-DRB1* SE allele context for (J) *PSMD5* and (I) *CDK5RAP2* genes. Panels (C), (D), (H) and (I) were done using LocusZoom (V.0.4.8) (http://locuszoom.org/genform.php?type=yourdata).[49] AP, attributable proportion due to interaction; EIRA, epidemiological investigation of rheumatoid arthritis; FDR, false discovery rate; LD, linkage disequilibrium; NARAC, North American rheumatoid arthritis consortium; PBMC, peripheral blood mononuclear cell; SE, shared epitope; SE-eQTL, expression quantitative trait loci in shared epitope context; SNP, single nucleotide polymorphism. *PTPN22*, *HIPK1*, *CSDE1*, *PSMD5* and *CDK5RAP2* are abbreviations for the genes.

the maximum AP value and the percentage of these 1492 SNPs in interaction with the *HLA-DRB1* SE alleles (online supplementary table S4). Chromosomes 1 and 9 reach the highest position for both studied cohorts (minimum AP p=4.3e-10 in EIRA and p=1.6e-08 in NARAC; online supplementary table S4). Specifically, the loci 9q33 and 1p13 contain the top SNPs in interaction with the *HLA-DRB1* SE alleles (figure 3C,D,H,I and online supplementary table S3). Chromosomes 2, 7, 8 and 13 followed in the ranking when the results from both EIRA and NARAC were considered. The majority (84.6%) of these SNPs in interaction with the *HLA-DRB1* SE alleles exhibited positive AP values, and most of them were under 0.5 (figure 3A,B).

SNPs in interaction with the *HLA-DRB1* SE alleles show functional features

The genotypes of 564 variants out of 1492 (37.7%) were considered to be SE-eQTLs in patients with ACPA-positive RA (online supplementary tables S5 and S6). The four top SE-eQTL pairs are graphically represented in the online supplementary figure S4.

The non-synonymous variant rs2476601 in the *PTPN22* gene (1p13) exhibited the most significant interaction with the *HLA-DRB1* SE alleles (figure 3C,D). In turn, this polymorphism is an SE-eQTL for *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), *HIPK1* (homeodomain interacting protein kinase 1) and *CSDE1* (cold shock domain containing E1) genes (figure 3E–G, online supplementary table S5).

The rs10739581 SNP (9q33) is the second top replicated variant in interaction with *HLA-DRB1* SE alleles (EIRA: AP=0.4, 95% CI 0.24 to 0.57, AP p=1.4e-6, FDR q=0.04; NARAC: AP=0.43, 95% CI 0.28 to 0.59, AP p=2.1e-8, FDR q=2.5e-4, online supplementary table S3). This variant is in high LD (r^2>0.9) with the rs3761847 SNP that has previously been associated with RA.[4 6 19 23 24] The rs10739581 SNP is an SE-eQTL for the *CDK5RAP2* (CDK5 regulatory subunit associated protein 2) and *PSMD5* (proteasome 26S subunit, non-ATPase 5) genes (figure 3J,K, online supplementary table S5).

These results suggest a plausible involvement of the 1492 selected SNPs in the pathogenesis of ACPA-positive RA through interaction with the *HLA-DRB1* SE alleles.

The top interacting SNPs explain a considerable part of the influence from *HLA-DRB1* SE on ACPA-positive RA

Finally, we observed that the step-by-step removal of the effect from the risk alleles of the rs2476601 and rs10739581, the two top replicated SNPs in interaction with the *HLA-DRB1* SE alleles, decreases the effect size of SE alleles for ACPA-positive RA in the studied cohorts (figure 4). This observation clearly suggests that there is a strong mutual influence, reflected in the additive interactions detected, between non-*HLA* genetic variants and the *HLA-DRB1* SE alleles in the development of ACPA-positive RA.

DISCUSSION

Our study demonstrates that there is an important enrichment of RA-associated SNPs interacting with *HLA-DRB1* SE alleles concerning the risk to develop ACPA-positive RA. Importantly, there is a gradual decrease in the effect size of the *HLA-DRB1* SE alleles in the risk of ACPA-positive RA after adjusting for top interacting SNPs. Based on these findings, we propose a concept called the *dominion hypothesis*, which suggests that the

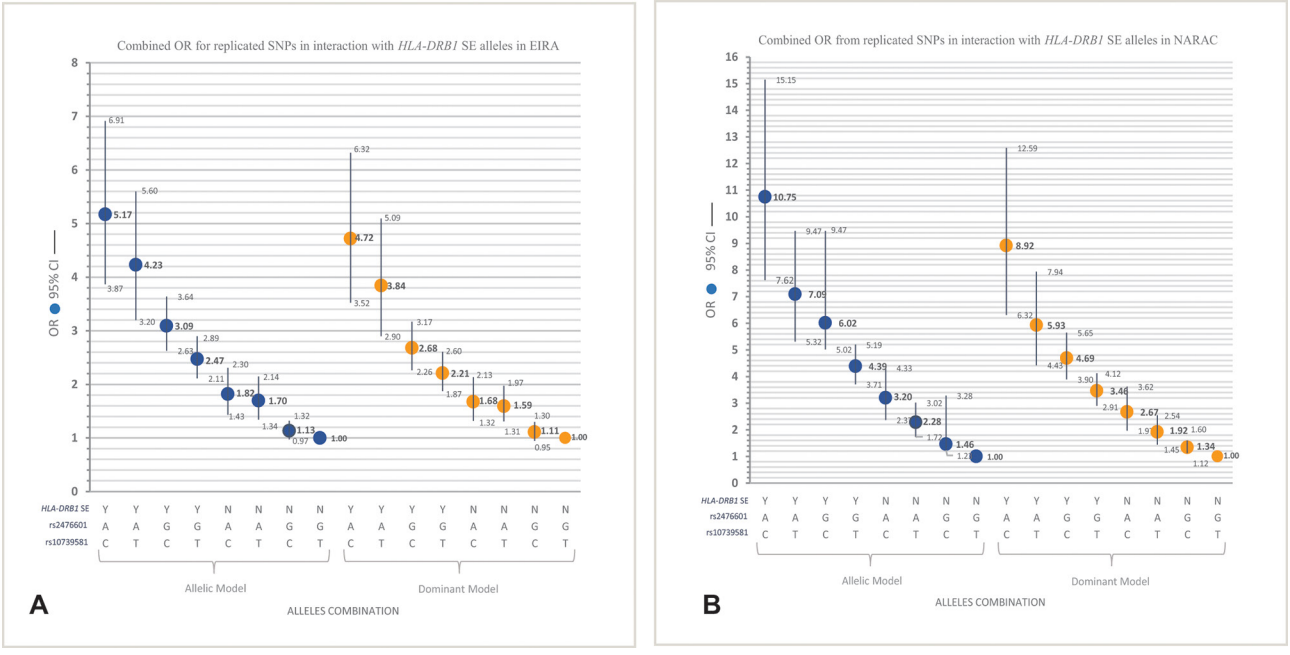


Figure 4 Three-factor OR calculation, the *HLA-DRB1* SE alleles and the top two SNPs in significant interaction. Panel (A) shows data from EIRA study while panel (B) shows data from NARAC study. The x-axis corresponds to the combinations of presence or absence of risk alleles from three factors in two models. The factors are the *HLA-DRB1* SE alleles (presence—Y, or absence—N), the rs2476601 SNP (G>A, 1p13 locus) and the rs10739581 SNP (T>C, 9q33 locus). The rs2476601 and rs10739581 SNPs are in significant interaction with the *HLA-DRB1* SE alleles after FDR correction in both EIRA (AP=0.45, 95% CI 0.31 to 0.60, p=4.3e-10, FDR q=5.2e-5 and AP=0.40, 95% CI 0.24 to 0.57, p=1.4e-6, FDR q=0.04, respectively) and NARAC (AP=0.41, 95% CI 0.23 to 0.6, p=1.1e-5, FDR q=0.04 and AP=0.43, 95% CI 0.28 to 0.6, p=2.1e-8, FDR q=2.5e-4, respectively). The y-axis represents the combined OR with 95% CI. AP, attributable proportion due to interaction; EIRA, epidemiological investigation of rheumatoid arthritis; FDR, false discovery rate; NARAC, North American rheumatoid arthritis consortium; SE, shared epitope; SNP, single nucleotide polymorphism.

HLA-DRB1 SE alleles function as a genetic hub that is involved in multiple interactions with non-*HLA* genetic variants that by themselves have a modest effect size in RA. These interacting non-*HLA* variants do cumulatively contribute to the high effect size of the *HLA-DRB1* SE alleles concerning risk to develop ACPA-positive RA. The *dominion hypothesis* has its foundations in the sufficient-component cause model,[16] which suggests that several diverse components are part of a sufficient cause for a disease in a given affected individual, where each sufficient cause can include one or more component causes. Together, these component causes can form a minimal set of conditions that drive disease.[32] Our hypothesis integrates the *HLA* alleles with other genetic variations across the human genome thereby potentially providing knowledge about the mechanisms behind this autoimmune disease.

The statistical approach in our study resulted in a list of 1492 SNPs (~270 independent loci, online supplementary table S7) as good candidates interacting with the *HLA-DRB1* SE alleles in conferring risk for developing of ACPA-positive RA. Out of these SNPs 37.7% are SE-eQTLs that define genes that could be involved in the development of ACPA-positive RA. These findings suggest that the now identified additive interactions may reflect biological processes involved in the pathogenesis of ACPA-positive RA. For instance, the rs2476601 SNP appears to be an SE-eQTL for *PTPN22*, *HIPK1* and *CSDE1* genes in PBMCs of patients with ACPA-positive RA (figure 3 and online supplementary table S5). Furthermore, Capture Hi-C studies showed that the rs2476601 is in physical contact with the *HIPK1* and *CSDE1* genes in CD4+ and CD8+ T cells (https://www.chicp.org, data accessed: February 2018),[33–35] which are relevant in RA.[36] Another illustration is the rs1506691, rs6804917 and rs12630663 SNPs (online supplementary table S6) that are in physical contact in T cells with the *EOMES* gene (https://www.chicp.org),[33 35] important in CD4+ T cell differentiation in the context of RA.[37] Moreover, the *HLA-DRB1* genotype appears to have a key role in shaping the T cell receptor repertoire.[38] Our findings reinforce previous observations that have suggested that the *HLA* locus plays a special role in transgenomic regulatory mechanisms.[39–41] The observed interactions might in some cases also reflect altered protein interactions in a given molecular pathway. For instance, it has been shown that the minor allele of rs2476601 SNP affects the LYP (protein encoded by *PTPN22* gene)-CSK protein complex on the TCR signalling context,[42] which in turn could have consequences for functions that involve TCR-peptide-MHC complexes. Additionally, one of the top interacting SNPs, the rs10739581, seems to be an SE-eQTL for the *PSMD5* gene that is involved in antigen presentation and proteasome regulation.[43] Interestingly, *H3F3A* and *TNC* genes that encode proteins that are known citrullinated autoantigens in RA[44 45] are in the proximity of SNPs in interaction with *HLA-DRB1* SE alleles (online supplementary table S6). Further interpretation and study of these interactions are required to understand their probable mechanisms in the RA pathogenesis.

When calculating the narrow heritability for ACPA-positive RA that could be explained by the current interaction analysis and the confirmed RA-associated loci,[6] we obtained values of 54% for EIRA and 64% for NARAC (online supplementary information and supplementary table S8). However, these values should be considered cautiously since the calculation does not completely integrate interaction effects.

In conclusion, we used a systematic approach to investigate interactions at the genome-wide level between non-*HLA* and *HLA-DRB1* alleles in ACPA-positive RA and we were able to identify a significant enrichment of interacting with *HLA-DRB1* SE alleles among disease-associated non-*HLA* SNPs. We suggest that the *dominion hypothesis* might be used to explore the next level of complexity in this disease as well as in other multifactorial immune-mediated diseases that involve *HLA*.

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