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EXTENDED REPORT

A new classification of HLA-DRB1 alleles based on acid–base properties of the amino acids located at positions 13, 70 and 71: impact on ACPA status or structural progression, and meta-analysis on 1235 patients with rheumatoid from two cohorts (ESPOIR and EAC cohort)

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ABSTRACT

Objective: To group *HLA-DRB1* alleles based on acid–base properties of amino acids at positions 13, 70 and 71 and analyse their association with the presence of anticitrullinated peptide antibodies (ACPA) and structural progression in 2 cohorts of early rheumatoid arthritis (RA).

Methods: Patients with RA (N=612) from ESPOIR cohort and from EAC cohort (n=624) were genotyped for *HLA-DRB1* alleles. The alleles containing the RAA sequence at positions 72–74 were classified into 3 groups according to the amino acid at positions 13, 70 and 71: BB encoding basic amino acids at positions 13, 70 and 71; A encoding acidic amino acids at positions 70 and 71; and BN encoding either neutral amino acids at position 13 and basic amino acids at positions 70 and 71, or basic amino acid at position 13 and neutral amino acids at positions 70 and 71. The associations between the different alleles and (1) the ACPA presence, and (2) the structural progression were assessed by χ^2 test; a meta-analysis was performed on the 2 cohorts using the Mantel-Haenszel method.

Results: After meta-analysis, BB alleles were significantly associated with ACPA presence (OR (95% CI) 4.08 (3.14 to 5.31)) and structural progression (OR (95% CI) 2.33 (1.76 to 3.09)). The alleles protected significantly against ACPA presence (OR (95% CI) 0.37 (0.28 to 0.50)) and structural progression (OR (95% CI) 0.34 (0.23 to 0.50)). This acid–base classification allowed to separate another group BN with an intermediate risk of ACPA production (OR (95% CI) 1.14 (0.91 to 1.44)) and structural progression (OR (95% CI) 1.01 (0.77 to 1.33)).

Conclusions: This new classification permitted to make a hierarchy of *HLA-DRB1* alleles in terms of association with ACPA presence or structural progression in early RA.

Key messages

What is already known about this subject?

- HLA-DRB1 shared epitope alleles are associated with rheumatoid arthritis susceptibility, ACPA presence and structural progression.
- Some alleles have also protective effects on ACPA presence or structural progression.
- Several classifications of *HLA-DRB1* alleles based on the amino acids located at positions 67, 70–74 were previously proposed to classify the alleles according to their risk of ACPA production and/or structural progression.

What does this study add?

- A new comprehensive classification is proposed based on acid–basic properties and charges of the amino acids located on key points at positions 13 and 70–74 on the HLA-DRB1 molecule.

How might this impact on clinical practice?

- This classification permitted the making of a hierarchy of four groups of alleles in terms of risk of ACPA production and structural progression.

Numerous reports have indicated that rheumatoid arthritis (RA) is associated with *HLA-DRB1* alleles, but their exact role in the pathogenesis is unknown. In 1987, Gregersen

et al[1] observed that *HLA-DRB1* alleles reported to be associated with RA share the RAA (arginine, alanine and alanine) amino acid motif at positions 72–74 of their third hypervariable region, which they hypothesised to act as a functional unit. However, this hypothesis does not fully explain the influence of *HLA-DRB1* alleles on disease susceptibility[2 3] because the amino acids at positions 13, 67, 70 and 71 also influence RA susceptibility. In terms of RA susceptibility, the alleles that greatly increase the risk encode the following sequences from position 70 to 74 of the molecules Q-K-RAA (*HLA-DRB1**04:01), Q-R-RAA (*HLA-DRB1**01:01, *04:04, *04:05, *04:08) and R-R-RAA (*HLA-DRB1**10:01), which are commonly called the *shared epitope (SE)* in most of the *HLA-DRB1* classifications; Q and K stand for glutamine and lysine, respectively. The *SE* also increases the risk of presence of anticitrullinated peptide antibodies (ACPA) and structural progression. Most of studies demonstrated that the association of *HLA-DRB1-SE* and structural progression was dependent on ACPA presence.[4 5] Furthermore, several studies identified some *HLA-DRB1* alleles as protective against RA susceptibility or ACPA production. Thus, the protective effects of specific *HLA-DRB1* alleles[6 7] or the presence of an isoleucine (I) at position 67 or an aspartic acid (D) at position 70 or the D-E-RAA motif at positions 70–74 have been suggested.[8–10] Several classifications have been proposed to classify the non-*SE* alleles into protective or neutral alleles for the risk of RA susceptibility or ACPA production.[11] A recent paper published by Raychaudhuri *et al*[12] showed that the main association of *HLA* region with RA susceptibility and ACPA presence was mediated by the presence of three amino acids on *HLA-DRB1* gene at positions 11/13, 71 and 74, and by two other amino acids outside HLA-DRB1 region on *HLA-B* and *HLA-DBPB1* genes. Further studies also raised the importance of the amino acid at position 13 and in particular, the presence of a histidine (H).[13]

Considering that *HLA-DRB1*SE* alleles are characterised by basic (and consequently positively charged) amino acids at positions 70 and 71, and that histidine at position 13 has also basic properties and positive charges while the D-E-RAA motif is characterised by acidic (and hence negatively charged amino acids) at positions 70 and 71, we hypothesised that the acid–base properties and the charges of the amino acids at positions 13, 70 and 71 may have an impact on the interactions between HLA-DRB1 molecules and citrullinated peptides. We have proposed an alternative classification of *HLA-DRB1* alleles based on the acid–base properties of the amino acids at positions 13, 70 and 71, combining the different classifications previously developed. We investigated the association between this alternative classification of *HLA-DRB1* alleles and the ACPA status or the structural progression and compared it with the previous *HLA-DRB1* allele classifications in a cohort of early RA.

PATIENTS AND METHODS

Patients

ESPOIR cohort

This work is derived from a large national, multicentre, longitudinal, prospective cohort of 813 French patients with early arthritis, the ESPOIR (Etude et Suivi des POlyarthrites Indifférentiées Récentes) cohort. The characteristics of the cohort have been described previously elsewhere.[14] Briefly, 813 patients with early arthritis recruited in 14 centres in France with arthritis duration <6 months and no prior treatment with disease-modifying antirheumatic drugs were included between 2002 and 2005. Patients underwent clinical, biological and radiological assessments at baseline and at each subsequent visit.

For the present study, we selected individuals who fulfilled the 2010 American College of Rheumatology (ACR)/ European League Against Rheumatisms (EULAR) criteria[15] for RA at baseline with available genotyping data for the *HLA-DRB1*.

Local institutional review boards approved the study, and written informed consent was obtained from all participants in the study.[14]

Anti-CCP2 antibodies (ACPA, ELISA, DiaSorin, France, Positive >50 U/mL) were quantified at baseline in a central laboratory.

Baseline and 1-year radiographs of the hands, wrists and feet were read and assessed using the Sharp-van der Heijde score (SHS).[16] The reader was blinded with regard to patient identity, characteristics and treatment. The results were expressed in a modified total Sharp score (mTSS). In order to evaluate the reproducibility of the radiographic scoring, radiographs from 30 patients representing the entire range of status and change were scored blindly once more by the same reader.[17] Intraclass correlation coefficients (ICCs) were 0.99 for both radiographic status and radiographic change scores. The smallest detectable change was calculated at 1.0 mTSS unit.

The rate of progression in ESPOIR cohort within the first year was low. Thus, since the smallest detectable change (SDC) was 1, a patient was considered as experiencing radiographic progression if the change within the first year was about one point of mTSS or more.

EAC cohort

The Leiden Early Arthritis cohort had 597 early Dutch patients with RA who were included from 1993 to 2009 as they had fulfilled the 1987 ACR criteria; 416 of these patients had X-ray at baseline and 1 year. The details of this cohort has already been described elsewhere.[4]

Radiographs of hand and feet were made at baseline and during the yearly follow-up visits and were chronologically scored by one experienced reader who was blinded to any clinical or genetic data using the SHS (within reader correlation coefficient (ICC) 0.91).

Patients in EAC cohort had higher rate of progression within the first year; thus, we considered a progression of at least five points of mTSS to be clinically significant

and this could be regarded in the EAC cohort as the threshold of progression.

HLA-DRB1 genotyping, allele classification and allele pooling

Genomic DNA was extracted from EDTA anticoagulated peripheral blood, using a standard proteinase K digestion and phenol–chloroform extraction method. *HLA-DRB1* four-digit typing and subtyping were performed in a single laboratory (Immunology laboratory, CHU Montpellier, France) by a polymerase chain reaction-based method, using a panel of sequence-specific oligonucleotide probes in ESPOIR cohort.

The *HLA-DRB1* (sub)typing for EAC was performed by PCR using specific primers and hybridisation with sequence-specific oligonucleotides.

The *HLA-DRB1* alleles were first divided into two groups according to the presence or absence (X alleles) of the RAA sequence at positions 72–74 as proposed by du Montcel *et al*.[18]

The non-X alleles were subsequently divided into three groups according to the acid–base properties of the amino acids at positions 13, 70 and 71:

The high-risk group of allele (BB): This group included the alleles with a basic amino acid at position 13 (histidine) and either two basic amino acids with two positive charges at positions 70 and 71 (R-R-RAA motif) or one basic amino acid at position 71 and one neutral amino acid at position 70 (Q-R-RAA and Q-K-RAA motifs).

The intermediate-risk allele group (BN) pooled first the alleles with no basic amino acid at position 13 and either two basic amino acids with two positive charges at positions 70 and 71 (R-R-RAA motif) or one basic amino acid at position 71 and one neutral amino acid at position 70 (Q-R-RAA and Q-K-RAA motifs); second the alleles with a basic amino acid at position 13 (arginine) and either one acidic amino acid at position 70 and one basic amino acid at position 71 (D-K-RAA or D-R-RAA motifs) or two neutral amino acid at positions 70 and 71 (Q-A-RAA).

The acidic group (A) included two amino acids with a negative charge (D-E-RAA motif).

The neutral group (NN) included the alleles with no basic amino acid at position 13 and either one acidic amino acid at position 70 and one basic amino acid at position 71 (D-K-RAA or D-R-RAA motifs) or two neutral amino acids at positions 70 and 71 (Q-A-RAA). This group of alleles was also pooled with the X group described previously (NN and X group).

Previous reports identified an association between the amino acid at position 67 and the risk of RA.[8] However, the amino acid at position 67 can be a leucine, an isoleucine or a phenyalanine, three amino acids without charge and with a very similar structure. We thus assumed that the polymorphism in this region could not influence the acid–base properties of HLA-DRB1 molecules and decided to not include this polymorphic region in this alternative classification.

This new classification and its relationships with previous ones are summarised in online supplementary appendix 1 table S1.

Statistical analysis

Owing to the non-Gaussian distribution of the total radiographic damage score and given the low structural progression of patients within 1 year, the median and the 10th and 90th centile range (p10–p90) were used to describe its distribution.

The associations between *HLA-DRB1* allele carriage and first the presence of ACPA, second the progression were tested by comparing the distribution of ACPA-positive patients then patients with progression among carriers and non-carriers of each *HLA-DRB1* allele pooled in the acid–base classification using a χ^2 test or a Fisher's exact test when appropriate. ORs with 95% CIs were also calculated. The same analyses were also performed with previous *HLA-DRB1* allele classifications of de Vries, Reviron, Mattey and Tezenas du Montcel.[8 9 18 19]

All the analyses were repeated for each allele classification system and by a genotype analysis, taking the supposed protective genotype as reference.

All the analyses were performed on each cohort separately; then a meta-analysis using Mantel-Haenszel method was performed to calculate ORs and 95% CIs.

To assess whether the relationship was independent of ACPA presence, we performed a multivariate analysis with logistic regression modelling of the OR of progression within 1 year depending on *HLA-DRB1* alleles carriage and ACPA presence.

All analyses were performed using the SAS V.9.3 software, RevMan V.5.3 software and SPSS V.20.

RESULTS

Characteristics of patients with RA

Among the 813 patients with early arthritis included in the ESPOIR cohort, 641 fulfilled the 2010 ACR/EULAR criteria for RA at baseline; genotyping data for *HLA-DRB1* were available in 612 patients with RA who constituted the sample of patients used to assess the association between *HLA-DRB1* and ACPA presence. Among these patients, 516 had sets of hand and foot radiographs at baseline and at 1 year follow-up available. This sample of 516 patients was used to assess the association between *HLA-DRB1* and structural progression within 1 year in the ESPOIR cohort.

Among EAC patients, 597 fulfilled the 1987 ACR criteria for RA and among these, 416 had radiographs.

The characteristics of the patients of ESPOIR and EAC are summarised in table 1.

Relationship between *HLA-DRB1* allele classifications and ACPA status

The different classifications of *HLA-DRB1* alleles were first compared in terms of association with ACPA

Table 1 Demographic and disease characteristics of patients with RA at baseline and at 1 year

	ESPOIR		EAC	
	All patients with RA (n=612)	Patients with RA with available radiographs (n=516)	All patients with RA (n=597)	Patients with RA with available radiographs (n=416)
Baseline characteristics				
Age, years, median (IQR)	50.3 (40.0–57.1)	50.6 (40.6–57.1)	57.3 (45.8–68.3)	56.0 (46.2–66.1)
Gender, female, n (%)	476 (78)	403 (78)	387 (65)	277 (67)
Smoking status, n (%)	289 (47)	244 (47)		
Symptom duration, months, median (IQR)	4.9 (3.0–7.1)	5 (3.1–7.4)	4.4 (2.2–8.3)	4.3 (2.1–8.7)
RF+, n (%)	349 (57)	301 (58)	345 (58)	248 (60)
ACPA+, n (%)	297 (49)	260 (50)	314 (53)	228 (56)
Total Sharp score, median (p10–p90)	–	3 (0–15)		5 (0–20)
One-year characteristics				
Total Sharp score, median (p10-p90)	–	4 (0–18)		10 (1–32)
Structural progression,* n (%)	–	152 (29)		164 (39)

Structural progression: variation of at least one point in the total Sharp score within 1 year according to the smallest detectable change of the radiographic scoring.
*Structural progression is defined by an increase of one point of mTSS for ESPOIR cohort within 1 year and five points of mTSS for EAC cohort within 1 year.
ACPA+, anticitrullinated peptide antibody presence; DAS28, disease Activity Score on 28 joints; DMARD, disease-modifying antirheumatic drugs; med, median; n, number of patients; p10–p90, 10th–90th range; RA, rheumatoid arthritis; RF+, rheumatoid factor presence.

presence by allele analysis (table 2, online supplementary appendix 1 table S2 for the number of patients).

When applying the acid–base classification, BB allele carriage was positively associated with ACPA positivity, while A and NN pooled with X allele carriage protects against presence of ACPA; BN allele carriage was not associated with ACPA positivity, allowing a hierarchical classification of *HLA-DRB1* alleles carrying the RAA sequence at positions 72–74.

According to de Vries classification, Reviron or Mattey classification, *HLA-DRB1*SE*+ alleles were positively associated with ACPA positivity, while other allele groups were negatively associated with ACPA positivity, allowing a binary classification of *HLA-DRB1* alleles, but no difference between either *HLA-DRB1**P and *HLA-DRB1**N alleles nor *HLA-DRB1* *D70+ and *HLA-DRB1** D70− alleles.

Using du Montcel classification, the S1, S3D and X groups were significantly associated with ACPA absence whereas the S2 and S3P groups were significantly associated with ACPA presence as previously demonstrated.[20]

To assess whether the association observed by allele analysis between 'protective' alleles and ACPA production was independent of *HLA-DRB1*SE* carriage, a genotype analysis in which the most protective genotype was taken as reference group for de Vries (P/P), Reviron (XP4n/XP4n) and Mattey (D70+D70+) classifications, and X/X genotype for Tezenas du Montcel classification was performed (table 3, online supplementary appendix 1 table S3 for the number of patients), as it had already been performed.[21] For acid–base classification, X/X, X/NN and NN/NN genotypes were taken as the reference group.

The results showed that the higher effect of the alleles on ACPA presence was supported by the *HLA-DRB1*SE* carriage and that the risk increased with the number of copies of the *HLA-DRB1*SE* carried. In acid–base classification, a significant difference was observed in non-*SE* patients between A allele carriers compared with A allele non-carriers with a significant protective effect of A allele carriage. Furthermore, another group of allele (BN group) could be separated with an intermediate risk of ACPA production allowing a hierarchy of the alleles in terms of risk of ACPA presence, with A group being the most protective group of alleles followed by X and NN groups, then BN group with an intermediate risk, and then BB with a high risk of ACPA presence. The other allele classifications could not permit such distinction in terms of risk of ACPA presence in non-SE alleles

Relationships between HLA-DRB1 allele classifications and structural progression

First, the association between the risk of progression according to *HLA-DRB1* alleles was studied by an allele analysis. A and X pooled with NN alleles had protective effects against progression, whereas BN alleles were not significantly associated with progression (table 4, online supplementary appendix 1 table S4 for the number of patients).

Table 2 Comparison of the association of the *HLA-DRB1* alleles and ACPA presence between the classifications of *HLA-DRB1* alleles

	ESPOIR		EAC		Meta-analysis	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Acid–base						
BB	**4.62 (3.16 to 6.75)**	**<0.001**	**3.63 (2.52 to 5.23)**	**<0.001**	**4.08 (3.14 to 5.31)**	**<0.001**
BN	1.14 (0.83 to 1.57)	0.4	1.15 (0.83 to 1.59)	0.4	1.14 (0.91 to 1.44)	0.2
A	**0.37 (0.25 to 0.56)**	**<0.001**	**0.37 (0.24 to 0.57)**	**<0.001**	**0.37 (0.28 to 0.50)**	**<0.001**
NN and X	**0.39 (0.27 to 0.55)**	**<0.001**	**0.49 (0.34 to 0.70)**	**<0.001**	**0.43 (0.34 to 0.56)**	**<0.001**
De Vries						
SE	**4.42 (3.10 to 6.33)**	**<0.001**	**5.00 (3.50 to 7.14)**	**<0.001**	**4.69 (3.66 to 6.01)**	**<0.001**
N	**0.36 (0.26 to 0.51)**	**<0.001**	**0.49 (0.36 to 0.69)**	**<0.001**	**0.42 (0.34 to 0.53)**	**<0.001**
P	**0.61 (0.44 to 0.85)**	**0.003**	**0.45 (0.32 to 0.63)**	**<0.001**	**0.53 (0.42 to 0.67)**	**<0.001**
Mattey						
SE	**4.42 (3.10 to 6.33)**	**<0.001**	**5.00 (3.50 to 7.14)**	**<0.001**	**4.69 (3.66 to 6.01)**	**<0.001**
D70–	**0.46 (0.32 to 0.65)**	**<0.001**	**0.59 (0.43 to 0.82)**	**0002**	**0.55 (0.43 to 0.69)**	**<0.001**
D70+	**0.51 (0.36 to 0.71)**	**<0.001**	**0.43 (0.31 to 0.60)**	**<0001**	**0.44 (0.35 to 0.56)**	**<0.001**
Du Montcel						
S1	**0.53 (0.38 to 0.75)**	**<0.001**	**0.55 (0.39 to 0.76)**	**<0.001**	**0.56 (0.44 to 0.72)**	**<0.001**
S2	**3.42 (2.29 to 5.14)**	**<0.001**	**3.09 (2.15 to 4.45)**	**<0.001**	**3.24 (2.49 to 4.23)**	**<0.001**
S3D	**0.66 (0.45 to 0.95)**	**0.02**	**0.55 (0.35 to 0.84)**	**0.006**	**0.61 (0.46 to 0.80)**	**<0.001**
S3P	**2.67 (1.89 to 3.80)**	**<0.001**	**3.21 (2.26 to 4.58)**	**<0.001**	**2.88 (2.26 to 3.66)**	**<0.001**
X	**0.37 (0.27 to 0.53)**	**<0.001**	**0.55 (0.40 to 0.76)**	**<0.001**	**0.45 (0.36 to 0.57)**	**<0.001**
Reviron						
SE	**4.42 (3.10 to 6.33)**	**<0.001**	**5.00 (3.50 to 7.14)**	**<0.001**	**4.69 (3.66 to 6.01)**	**<0.001**
XP4P	**0.57 (0.41 to 0.79)**	**<0.001**	**0.59 (0.43 to 0.83)**	**0.002**	**0.58 (0.46 to 0.73)**	**<0.001**
XP4N	**0.44 (0.31 to 0.61)**	**<0.001**	**0.43 (0.31 to 0.60)**	**<0.001**	**0.43 (0.34 to 0.55)**	**<0.001**

95% CI.
ACPA, anticitrullinated peptide antibody; BB, allele group containing basic AA at positions 13, 70 and 71; BN, allele group containing either a basic amino acid at position 13 and a neutral or acid and basic amino acid at positions 70 and 71 or a neutral amino acid at position 13 and a basic amino acid at positions 70 and 71; A, allele group containing acid amino acids at positions 70 and 71; NN, allele group with a neutral amino acid at position 13 and either a basic and an acidic amino acid at positions 70 and 71 or two neutral amino acids at positions 70 and 71; X, allele group not containing RAA sequence at positions 72–74; N, allele group not containing an isoleucine at position 67; P, allele group containing an isoleucine at position 67; SE, shared epitope; D70–, allele group not containing an aspartic acid at position 70; D70+, allele group containing an aspartic acid at position 70; XP4p, fourth pocket positively charged; XP4n, fourth pocket negatively or non-charged.

A hierarchy could be observed in terms of progression with a higher protection with A alleles as compared with NN and X alleles (OR=0.34 (0.23 to 0.50) for A alleles, compared with OR=0.71 (0.53 to 0.94) for NN and X alleles).

In Mattey's allele classification, *SE* alleles were significantly associated with progression, while *D70+* alleles were significantly associated with a protection against progression. *P* and *N* alleles of de Vries' classification were not independently associated with the risk of progression. In du Montcel's classification, only *S1* and *S3D* alleles were associated with protection against structural progression.

To assess whether the association with progression was only explained by ACPA production or if *HLA* alleles had independent effect on progression, we performed a logistic regression, including age, sex and ACPA presence as covariables of *HLA-DRB1* alleles to explain progression. In ESPOIR cohort, the association between *SE* alleles and progression remained significant after adjustment for ACPA presence (OR=2.20 (1.34 to 3.59), p=0.002) while it was not significant in EAC cohort (OR=1.05 (0.64 to 1.73), p=0.84). However, A alleles remained significantly associated with protection against progression even after adjusting on ACPA presence in both cohorts (ESPOIR cohort: OR=0.51 (0.28 to 0.92), p=0.02; EAC cohort: OR=0.49 (0.26 to 0.91), p=0.03) implying that the protection against progression given by A alleles was independent of ACPA presence. Interestingly, in patients without structural progression, the proportion of ACPA-positive patients was higher if they were carrying at least one BB allele compared with the other alleles (see online supplementary appendix 1 table S5).

Finally, to assess whether the association observed by allele analysis of the alleles as protective against progression was independent of *HLA-DRB1*SE* carriage, a genotype analysis where the most supposed to be protective genotype was taken as reference group was performed (table 5, online supplementary appendix table S6 for the number of patients).

The results showed that the higher effect of the alleles on structural progression was supported by the *HLA-DRB1*SE* carriage and that the risk increased with the number of copies of the *HLA-DRB1*SE* carried. Furthermore, in acid–base classification, a significant

Table 3 Comparison of hierarchy of risk of ACPA presence according to each classification by genotype analysis on ESPOIR and EAC cohorts

	ESPOIR OR (95% CI)	EAC OR (95% CI)	Meta-analysis OR (95% CI)	p Value
Acid–base				
NN/NN, X/NN or XX referent	1	1	1	
A/A, A/NN or A/X	0.59 (0.30 to 1.15)	0.49 (0.23 to 1.05)	**0.54 (0.33 to 0.90)**	**0.02**
BN/BN, BN/NN or BN/X	1.69 (0.99 to 2.89)	**2.09 (1.21 to 3.59)**	**1.88 (1.28 to 2.75)**	**0.001**
A/BB	**4.39 (1.62 to 11.88)**	**3.44 (1.32 to 8.98)**	**3.88 (1.95 to 7.73)**	**<0.001**
BN/BB	**6.89 (3.07 to 15.43)**	**4.21 (2.09 to 8.49)**	**5.27 (3.12 to 8.92)**	**<0.001**
BB/BB	**13.64 (4.12 to 45.13)**	**28.06 (4.84 to 162.69)**	**18.05 (6.74 to 48.34)**	**<0.001**
De Vries				
P/P referent	1	1	1	–
P/N and N/N	0.61 (0.33 to 1.12)	0.77 (0.43 to 1.37)	0.69 (0.45 to 1.05)	0.08
SE/P	**2.86 (1.53 to 5.35)**	**3.67 (2.09 to 6.45)**	**3.28 (2.16 to 4.99)**	**<0.001**
SE/N	**2.17 (1.14 to 4.11)**	**3.24 (1.82 to 5.74)**	**2.71 (1.14 to 4.11)**	**<0.001**
SE/SE	**11.86 (4.67 to 30.10)**	**14.22 (5.61 to 36.09)**	**12.99 (6.73 to 25.09)**	**<0.001**
Mattey				
D70+/D70+ referent	1	1	1	–
D70+/D70− and D70−/D70−	0.70 (0.41 to 1.20)	1.57 (0.80 to 3.10)	0.97 (0.64 to 1.46)	0.88
D70+/SE	**2.94 (1.77 to 4.88)**	**5.77 (2.86 to 11.63)**	**3.73 (2.48 to 5.62)**	**<0.001**
D70−/SE	**3.08 (1.74 to 5.43)**	**5.79 (2.94 to 11.41)**	**4.04 (2.62 to 6.22)**	**<0.001**
SE/SE	**11.86 (4.67 to 30.10)**	**14.22 (5.61 to 36.09)**	**12.99 (6.73 to 25.09)**	**<0.001**
Du Montcel				
XX referent	1	1	1	–
S1/X and S1/S1	0.92 (0.33 to 2.56)	0.98 (0.48 to 2.01)	0.96 (0.53 to 1.73)	0.9
S3D/X and S3D/S3D	1.18 (0.47 to 2.96)	0.79 (0.31 to 2.04)	0.97 (0.51 to 1.87)	0.9
S1/S2 and S1/S3P	**2.48 (1.07 to 5.76)**	**3.66 (1.77 to 7.60)**	**3.08 (1.77 to 5.35)**	**<0.001**
S3D/S2 and S3D/S3P	**4.32 (1.65 to 11.34)**	**3.14 (1.32 to 7.48)**	**3.63 (1.90 to 6.91)**	**<0.001**
S2/S2, S3P/S2 and S3P/S3P	**8.21 (3.53 to 19.08)**	**13.23 (5.34 to 32.75)**	**10.19 (5.50 to 18.89)**	**<0.001**
Reviron				
XP4n/XP4n referent				
XP4n/XP4p and XP4p/XP4p	0.93 (0.53 to 1.63)	0.94 (0.58 to 1.54)	0.94 (0.65 to 1.35)	0.7
XP4n/SE	**3.23 (1.85 to 5.65)**	**5.36 (2.64 to 10.87)**	**3.94 (2.55 to 6.09)**	**<0.001**
XP4p/SE	**3.91 (2.13 to 7.17)**	**5.66 (2.87 to 11.16)**	**4.63 (2.95 to 7.27)**	**<0.001**
SE/SE	**8.94 (4.37 to 18.32)**	**20.95 (8.32 to 52.75)**	**12.32 (7.02 to 21.62)**	**<0.001**

95% CI.
ACPA, anticitrullinated peptide antibody; BB, allele group containing basic AA at positions 13, 70 and 71; BN, allele group containing either a basic amino acid at position 13 and a neutral or acid and basic amino acid at positions 70 and 71 or a neutral amino acid at position 13 and a basic amino acid at positions 70 and 71; A, allele group containing acid amino acids at positions 70 and 71; NN, allele group with a neutral amino acid at position 13 and either a basic and an acidic amino acid at positions 70 and 71 or two neutral amino acids at positions 70 and 71; X, allele group not containing RAA sequence at positions 72–74; N, allele group not containing an isoleucine at position 67; P, allele group containing an isoleucine at position 67; SE, shared epitope; D70−, allele group not containing an aspartic acid at position 70; D70+, allele group containing an aspartic acid at position 70; XP4p, fourth pocket positively charged; XP4n, fourth pocket negatively or non-charged

difference was observed in non-BB patients between A allele carriers and A allele non-carriers, with a high protective effect of A allele carriage. Furthermore, no significant difference could be demonstrated between BN allele carriers compared with N or X allele carriers whereas BB/BB and BB/BN genotypes increased significantly the risk of progression compared with X/X, X/NN or NN/NN genotypes, allowing a hierarchy of the alleles in terms of risk of progression with A group being the most protective group of alleles followed by X pooled with NN groups and BN group with an intermediate risk, and then BB group with a high risk of progression. The other allele classifications could not permit such distinction in terms of risk of progression in non-SE alleles.

DISCUSSION

In two cohorts of patients with early RA, we have investigated the association between an alternative classification of *HLA-DRB1* alleles based on acid–basic properties of the amino acids at positions 13, 70 and 71, and the ACPA status or structural progression. This classification allowed for a hierarchical classification of *HLA-DRB1* alleles carrying the RAA sequence at positions 72–74 unlike the previous published classifications. While BB alleles were positively associated with ACPA positivity and structural progression, A alleles were negatively associated with these parameters while BN alleles had an intermediate risk of ACPA positivity and structural progression.

In this study, we found in ESPOIR an independent of ACPA presence effect of *HLA-DRB1*SE* on increased risk

Table 4 Comparison of the association of *HLA-DRB1* alleles and structural progression according to the classification of *HLA-DRB1* alleles on ESPOIR and EAC cohorts

	ESPOIR		EAC		Meta-analysis	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Acid–base						
BB	**3.05 (2.03 to 4.57)**	**<0.001**	**1.78 (1.19 to 2.66)**	**0.005**	**2.33 (1.76 to 3.09)**	**<0.001**
BN	0.94 (0.64 to 1.38)	0.76	1.10 (0.74 to 1.63)	0.65	1.01 (0.77 to 1.33)	0.92
A	**0.35 (0.21 to 0.59)**	**<0.001**	**0.32 (0.17 to 0.58)**	**<0.001**	**0.34 (0.23 to 0.50)**	**<0.001**
NN and X	**0.65 (0.43 to 0.96)**	**0.03**	**0.79 (0.52 to 1.19)**	**0.25**	**0.71 (0.53 to 0.94)**	**0.018**
De Vries						
SE	**3.73 (2.39 to 5.91)**	**<0.001**	**2.26 (1.49 to 3.44)**	**<0.001**	**2.91 (2.16 to 3.92)**	**<0.001**
N	**0.60 (0.41 to 0.87)**	**0.008**	**0.67 (0.45 to 0.99)**	**0.04**	**0.63 (0.48 to 0.83)**	**<0.001**
P	**0.55 (0.38 to 0.81)**	**0.002**	**0.61** (0.41 to 0.91)	**0.02**	**0.58 (0.44 to 0.76)**	**<0.001**
Mattey						
SE	**3.73 (2.39 to 5.91)**	**<0.001**	**2.26 (1.49 to 3.44)**	**<0.001**	**2.91 (2.16 to 3.92)**	**<0.001**
D70–	0.88 (0.60 to 1.29)	0.5	**0.65 (0.43 to 0.96)**	**0.03**	**0.76 (0.58 to 1.00)**	**0.05**
D70+	**0.44 (0.29 to 0.66)**	**<0.001**	0.77 (0.52 to 1.15)	0.2	**0.58 (0.44 to 0.77)**	**<0.001**
Du Montcel						
S1	**0.53 (0.34 to 0.82)**	**<0.001**	**0.47 (0.31 to 0.70)**	**<0.001**	**0.50 (0.34 to 0.67)**	**<0.001**
S2	1.26 (0.85 to 1.86)	0.2	**1.65 (1.10 to 2.49)**	**0.02**	**1.43 (1.08 to 1.90)**	**0.01**
S3D	**0.40 (0.24 to 0.66)**	**<0.001**	1.19 (0.71 to 2.00)	0.5	**0.65 (0.46 to 0.92)**	**0.02**
S3P	**2.18 (1.49 to 3.21)**	**<0.001**	**2.18 (1.45 to 3.29)**	**<0.001**	**2.18 (1.65 to 3.21)**	**<0.001**
X	0.78 (0.52 to 1.16)	0.2	0.79 (0.53 to 1.16)	0.2	0.78 (0.60 to 1.03)	0.08
Reviron						
SE	**3.73 (2.39 to 5.91)**	**<0.001**	**2.26 (1.49 to 3.44)**	**<0.001**	**2.91 (2.16 to 3.92)**	**<0.001**
XP4P	0.89 (0.61 to 1.30)	0.5	0.68 (0.46 to 1.02)	0.5	0.78 (0.60 to 1.03)	0.08
XP4N	**0.51 (0.35 to 0.76)**	**<0.001**	0.77 (0.52 to 1.14)	0.5	**0.63 (0.48 to 0.82)**	**<0.001**

95% CI.
BB, allele group containing basic AA at positions 13, 70 and 71; BN, allele group containing either a basic amino acid at position 13 and a neutral or acid and basic amino acid at positions 70 and 71 or a neutral amino acid at position 13 and a basic amino acid at positions 70 and 71; A, allele group containing acid amino acids at positions 70 and 71; NN, allele group with a neutral amino acid at position 13 and either a basic and an acidic amino acid at positions 70 and 71 or two neutral amino acids at positions 70 and 71; X, allele group not containing RAA sequence at positions 72–74; N, allele group not containing an isoleucine at position 67; P, allele group containing an isoleucine at position 67; SE, shared epitope; D70–, allele group not containing an aspartic acid at position 70; D70+, allele group containing an aspartic acid at position 70; XP4p, fourth pocket positively charged; XP4n, fourth pocket negatively or non-charged.

of structural progression, inconsistent with previous recent studies.[4 5] However, we also found a protective effect of alleles coding for D-E-RAA sequence pooled in A group and alleles encoding a D at position 70 according to Mattey's classification against structural progression independently of ACPA presence. To our knowledge, this is the first study reporting an independent association between protection against progression and these alleles.

A classification based on the charges was previously proposed by Reviron *et al* in 2001.[19] Our classification differs from Reviron's classification which did not pool the alleles encoding another sequence than RAA at positions 72–74 in a separate group, and did not take into account the acid–base and charge properties of the amino acid at position 71.

This allele classification can be applied to the Caucasian population but should be adapted to Asian populations since other amino acids at position 57 seem to be associated with RA susceptibility.[22]

ACPA-positive and ACPA-negative RA are two distinct diseases with different outcomes. The association between *HLA-DRB1 SE* alleles and ACPA-positive RA is stronger than with ACPA-negative RA. The polymorphic region of amino acid at positions 13, 70 and 71 may play an important role in the interaction between HLA-DRB1 molecules and peptides presented to T cell receptors. As the mechanism of citrullination implies the transformation of an arginine (positive charge) into a citrulline (no charge), some positively charged amino acids in the HLA-DRB1 molecule might hinder the presentation of arginine-containing peptides and facilitate the presentation of citrullinated peptides.

Freed *et al*[23] suggested that Q70 and K71 would form hydrogen bonds with the peptide, playing a critical role in peptide binding. In contrast, the presence of D70 and E71 results in a more acidic pocket and would repeal the weakly negative citrulline dipoles and greatly reduce peptide binding.[24] Thus, we assume that in HLA-DRB1 molecules expressing a RAA sequence at positions 72–74, a sequence of neutral–basic or basic–basic amino acid at positions 70–71 (SB alleles) could increase the peptide binding and might contribute to ACPA production, whereas a combination of acid–basic amino acid at positions 70–71 (SN alleles) neutralises this effect. Raychaudhuri *et al*[12] showed that the main association of *HLA* region with RA susceptibility and ACPA presence was mediated by the presence of three amino acids on *HLA-DRB1* gene at positions 11/13, 71 and 74. In his

Table 5 Comparison of hierarchy of risk of structural progression according to each classification by genotype analysis on ESPOIR and EAC cohorts

	ESPOIR OR (95% CI)	EAC OR (95% CI)	Meta-analysis OR (95% CI)	p Value
Acid–base				
NN/NN, X/NN or XX referent	1	1	1	
A/A, A/NN or A/X	0.30 (0.11 to 1.79)	0.44 (0.18 to 1.10)	**0.37 (0.19 to 0.71)**	**0.002**
BN/BN, BN/NN or BN/X	1.10 (0.57 to 2.10)	1.09 (0.57 to 2.04)	1.09 (0.69 to 1.72)	0.7
A/BB	1.78 (0.63 to 4.98)	0.48 (0.14 to 1.65)	0.99 (0.46 to 2.09)	0.9
BN/BB	**2.24 (1.03 to 4.83)**	**1.80 (0.87 to 3.75)**	**2.00 (1.17 to 3.39)**	**0.009**
BB/BB	**3.50 (1.42 to 8.59)**	**3.15 (1.12 to 8.80)**	**3.34 (1.69 to 6.57)**	**<0.001**
De Vries				
P/P referent	1	1	1	–
P/N and N/N	1.15 (0.47 to 2.83)	1.33 (0.65 to 2.71)	1.26 (0.72 to 2.20)	0.4
SE/P	**3.87 (1.61 to 9.33)**	**2.74 (1.36 to 5.49)**	**3.16 (1.84 to 5.45)**	**<0.0001**
SE/N	**4.00 (1.64 to 9.74)**	1.97 (0.96 to 4.04)	**2.67 (1.54 to 4.63)**	**0.0005**
SE/SE	**5.12 (2.01 to 13.03)**	**4.75 (2.12 to 10.68)**	**4.92 (2.67 to 9.07)**	**<0.0001**
Mattey				
D70+/D70+ referent	1	1	1	–
D70+/D70– and D70–/D70–	1.08 (0.52 to 2.28)	1.54 (0.67 to 3.53)	1.27 (0.73 to 2.21)	0.4
D70+/SE	**2.92 (1.47 to 5.80)**	**3.23 (1.38 to 7.60)**	**3.04 (1.78 to 5.19)**	**<0.0001**
D70–/SE	**5.26 (2.55 to 10.85)**	**2.41 (1.05 to 5.51)**	**3.72 (2.15 to 6.41)**	**<0.0001**
SE/SE	**4.81 (2.24 to 10.31)**	**5.51 (2.18 to 13.95)**	**5.08 (2.82 to 9.16)**	**<0.0001**
Du Montcel				
XX referent	1	1	1	–
S1/X and S1/S1	0.34 (0.10 to 1.20)	0.47 (0.20 to 1.14)	**0.42 (0.21 to 0.87)**	**0.02**
S3D/X and S3D/S3D	**0.22 (0.06 to 0.75)**	1.28 (0.45 to 3.68)	0.62 (0.29 to 1.35)	0.2
S1/S2 and S1/S3P	0.69 (0.26 to 1.83)	0.78 (0.34 to 1.77)	0.74 (0.40 to 1.39)	0.4
S3D/S2 and S3D/S3P	0.68 (0.22 to 2.06)	1.75 (0.64 to 4.79)	1.14 (0.55 to 2.40)	0.7
S2/S2, S3P/S2 and S3P/S3P	1.60 (0.63 to 4.07)	2.89 (1.17 to 7.11)	**2.16 (1.13 to 4.13)**	**0.02**
Reviron				
XP4n/XP4n referent	1	1	1	–
XP4n/XP4p and XP4p/XP4p	1.74 (0.75 to 4.05)	1.77 (0.75 to 4.20)	1.76 (0.96 to 3.21)	0.07
XP4n/SE	**4.14 (1.83 to 9.38)**	**3.53 (1.45 to 8.60)**	**3.86 (2.12 to 7.05)**	**<0.001**
XP4p/SE	**2.78 (1.18 to 6.56)**	**6.69 (2.88 to 15.57)**	**4.38 (2.41 to 7.96)**	**<0.001**
SE/SE	**6.67 (2.76 to 16.08)**	**6.20 (2.38 to 16.15)**	**6.45 (3.38 to 12.34)**	**<0.001**

95% CI.
BB, allele group containing basic AA at positions 13, 70 and 71; BN, allele group containing either a basic amino acid at position 13 and a neutral or acid and basic amino acid at positions 70 and 71 or a neutral amino acid at position 13 and a basic amino acid at positions 70 and 71; A, allele group containing acid amino acids at positions 70 and 71; NN, allele group with a neutral amino acid at position 13 and either a basic and an acidic amino acid at positions 70 and 71 or two neutral amino acids at positions 70 and 71; X, allele group not containing RAA sequence at positions 72–74; N, allele group not containing an isoleucine at position 67; P, allele group containing an isoleucine at position 67; SE, shared epitope; D70–, allele group not containing an aspartic acid at position 70; D70+, allele group containing an aspartic acid at position 70; XP4p, fourth pocket positively charged; XP4n, fourth pocket negatively or non-charged.

paper, the positions 11 and 13 were in very high linkage disequilibrium but the position 11 seemed to support the higher association with RA susceptibility. Because of the linkage disequilibrium, our study could not show what position would be the most important in the association with ACPA production. However, regarding the charges, the polymorphism of position 13 may substitute positively charged amino acids, such as histidine or arginine, whereas the polymorphism on position 11 do not change the charges. We assumed in this study that the association of this region with ACPA-positive RA is almost supported by position 13 as suggested by Scally *et al*.[13]

This study based on acid–base properties permitted us to make a clearer hierarchy of *HLA-DRB1* alleles in terms of association with ACPA and structural progression compared with the previous classification. This classification system could be used for further works when assessing the association between HLA-DRB1 alleles and ACPA presence or structural progression.

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