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# Protection Against Anti–Citrullinated Protein Antibody–Positive Rheumatoid Arthritis Is Predominantly Associated With HLA–DRB1*1301

## A Meta-Analysis of HLA–DRB1 Associations With Anti–Citrullinated Protein Antibody–Positive and Anti–Citrullinated Protein Antibody–Negative Rheumatoid Arthritis in Four European Populations

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**Objective.** **The protective effect of HLA–DRB1 alleles on the development of rheumatoid arthritis (RA) is poorly understood. The aim of this study was to perform a meta-analysis of 4 European populations to investigate which HLA–DRB1 alleles are associated with protection in anti–citrullinated protein antibody (ACPA)–positive RA and ACPA-negative RA.**

***Methods.*** **Data for >2,800 patients and >3,000 control subjects for whom information on HLA–DRB1 typing and ACPA status was available were collected from 4 European countries: Norway, Sweden, The Netherlands, and Spain. The odds ratios (ORs) and 95% confidence intervals (95% CIs) associated with the different HLA–DRB1 alleles were analyzed in a combined meta-analysis focused on protective alleles and classifications. The analysis of ACPA-positive RA was stratified for the shared epitope (SE) alleles, to correct for skewing due to this association.**

***Results.*** **In ACPA-positive RA, the only alleles that conveyed protection after stratification for SE were HLA–DRB1*13 alleles (OR 0.54 [95% CI 0.38–0.77]). The protective effect of the allele classifications based on the DERAA and D70 sequences was no longer present after exclusion of DRB1*13 (for D70, OR 0.97**

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**[95% CI 0.75–1.25]), indicating that DRB1*13, rather than the DERAA or D70 sequence as such, is associated with protection. Among the DRB1*13 alleles, only DRB1*1301 was associated with protection (OR 0.24 [95% CI 0.09–0.59]). Protection appeared to follow a north-to-south gradient, with the strongest association in northern European countries. In ACPA-negative RA, there were no robust associations with HLA–DRB1 alleles.**

***Conclusion.*** **Our data do not support any of the classifications of protective alleles and indicate that protection against ACPA-positive RA is predominantly associated with HLA–DRB1*1301.**

The HLA region contains the most prominent genetic risk factors for rheumatoid arthritis (RA). The association between RA and the HLA region was originally discovered based on the observation that lymphocytes from patients with RA were not reactive against cells from other patients with RA in mixed lymphocyte cultures (1,2). This meant that patients with RA had certain HLA alleles in common that were less prevalent in control populations. Serologic testing subsequently revealed that the HLA–Dw4 alloantigen, but not HLA–A, HLA–B, or HLA–C antigen, was associated with RA (3). Later studies demonstrated that several of the HLA–DR alleles were associated with RA, which led to formulation of the shared epitope (SE) hypothesis in 1987 (4). This hypothesis provided a theoretical background for the observed associations between the HLA region and RA based on the fact that all HLA–DR alleles that predispose to RA have the same or a similar amino acid sequence (the SE) at positions 70–74 of the HLA–DRB1 molecule. This sequence is located in the peptide-binding groove of the HLA alleles and may therefore be directly involved in the presentation of peptides to arthritogenic T cells. However, due to the complexity of the HLA region, the association between the HLA region and RA is multifaceted, and it is now known that not all HLA SE alleles contribute to RA to the same extent (5,6). Nonetheless, the formulation of the HLA SE hypothesis has provided a rationale for combining several HLA molecules in analyses and has thereby enabled further well-powered investigations of the contribution of the HLA region to the risk of RA (7).

The discovery of anti–citrullinated protein antibodies (ACPAs) led to a paradigm shift in the investigation of genetic risk factors for RA. The HLA SE alleles were shown to predispose not to RA as such, but rather to ACPA-positive disease, which is present in approximately two-thirds of patients with RA (8). These observations are very important for the pathophysiologic concept of disease development, because they indicate that the HLA SE alleles may be involved in the induction of the ACPA response (9). Several single-nucleotide polymorphisms known to be associated with RA, such as PTPN22, were also discovered to be specifically associated with ACPA-positive RA (10,11). In contrast, DRB1*03 has been reported to be associated with ACPA-negative disease (12,13), although not all studies have confirmed this association (14). These differences in underlying risk factors indicate that ACPA-positive and ACPA-negative RA may constitute distinct disease entities with a different underlying pathogenetic background.

In addition to the HLA–DRB1 alleles that contribute to RA susceptibility, other HLA–DRB1 alleles confer protection against disease (15–17). These protective HLA–DRB1 alleles have been categorized according to several different classifications, analogous to the SE classification of susceptibility alleles. The 3 best known classifications postulate that HLA–DRB1 alleles with a protective effect harbor the "shared sequence" DERAA at positions 70–74 (18), an aspartic acid at position 70 (D70 allele) (16), or an isoleucine at position 67 (I67 allele) of the HLA–DRB1 molecule (15). The DERAA and D70 alleles have been shown to be protective in both the presence and absence of SE alleles (19,20), which demonstrates that their protective effect is not solely attributable to the absence of SE alleles (21,22). A new classification of HLA–DRB1 alleles has recently been put forward that incorporates both predisposing and protective effects (23,24). Although this has provided some interesting nuances with regard to the predisposing effect of the different SE alleles, it is unclear whether this classification accurately describes the protective HLA effects (7).

The multitude of classifications of protective HLA alleles illustrates that a protective effect of HLA alleles in RA is now well accepted. However, it is still unclear exactly which HLA alleles are protective. Geographic differences in the prevalence of HLA alleles have led to conflicting results, which have been further complicated by the use of different classifications. Furthermore, it is as yet unclear whether protective effects are present in ACPA-positive as well as ACPA-negative disease.

For these reasons, we sought to determine the contribution of individual HLA–DRB1 alleles to RA, with respect to both susceptibility and protection, in a meta-analysis across European populations. Using data from 4 different populations (from Norway, Sweden,

The Netherlands, and Spain), we investigated the HLA–DRB1 associations with ACPA-positive and ACPA-negative RA in >2,800 patients and 3,000 control subjects. A significant protective effect of HLA–DRB1*13 was observed, which remained present after stratification for the effect of the SE alleles. Moreover, the protective effect of HLA–DRB1*13 was observed only for ACPA-positive RA and not ACPA-negative disease. An in-depth analysis of the protective classifications revealed that the protective effect of the DERAA and D70 alleles was limited to the HLA–DRB1*13 alleles and was in fact observed only for DRB1*1301. Taken together, our data do not support any of the classifications described above and indicate that protection is mainly associated with DRB1*1301.

## PATIENTS AND METHODS

**Populations.** Data on patients and control subjects were contributed by cohorts from 4 different European countries: Norway, Sweden, The Netherlands, and Spain. The protocol of each cohort was approved by the relevant local ethics committee, and all participants provided informed consent. More than 97% of patients and control subjects in all 4 cohorts were of Caucasian descent.

The Norwegian data set comprised patients with RA who participated in the Oslo RA Registry (ORAR) or the European Research on Incapacitating Disease and Social Support (EURIDISS) cohort. For the ORAR, which was initiated in 1992, inclusion criteria were a diagnosis of RA according to the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for the classification of RA (25) and a residential address in Oslo. The EURIDISS, which commenced in 1991, enrolled consecutive patients with RA in whom the maximum duration of disease at baseline was 4 years. Both RA cohorts have been described in detail elsewhere (26,27). Control subjects were randomly selected from the Norwegian Bone Marrow Registry, and patients and control subjects were matched for sex at a group level.

The Swedish Epidemiological Investigation of RA (EIRA) cohort recruited patients and control subjects aged 18–70 years, from May 1996 to December 2003, from a geographically defined area in the south and central regions of Sweden. Patients were seen by rheumatologists at private as well as general health care units and were eligible for inclusion if they fulfilled the ACR 1987 criteria for the classification of RA. Control subjects were randomly selected from a national population register and were matched to the patients for age, sex, and residential area. More details on the EIRA have been described previously (28).

Data on Dutch cases were provided by 2 inception cohorts of patients with early arthritis: the Leiden Early Arthritis Clinic (EAC) and the BehandelStrategieën (BeSt) trial. The Leiden EAC was initiated in 1993 and included patients with recent-onset arthritis (<2 years of symptoms) who were treated at the Leiden University Medical Center (LUMC). For the BeSt study, patients with arthritis with maximum disease duration of 2 years and active disease were recruited at 20 centers in the western part of The Netherlands, from 2000 to 2002. Only patients with a diagnosis of RA were included in the present study. These cohorts are described in further detail elsewhere (29,30). Dutch control subjects were randomly selected from the collection of the section of Immunogenetics and Transplantation Immunology of the Department of Immunohematology and Blood Transfusion, LUMC.

The Spanish data set comprised patients with RA fulfilling the ACR criteria for RA who were recruited from 4 Spanish hospitals in Granada, Seville, Lugo, and Madrid, respectively. Blood donors and bone marrow donors from the same cities were included as healthy control subjects. Control subjects were matched to patients for age and sex. More characteristics of the Spanish data set have been described previously (31).

**Genotyping.** The genotyping procedures for the HLA–DRB1 alleles have been previously described (14,20,31,32). High-resolution, 4-digit typing was available for the entire Norwegian and Spanish cohorts. For the Dutch cohort, low-resolution typing was complemented by 4-digit typing of the DRB1*04 alleles and by use of specific probes to detect the presence of the SE or DERAA sequence in individuals carrying the DRB1*01, DRB1*10, DRB1*11, or DRB1*13 alleles. DRB1*1301 and DRB1*1302 were differentiated in part by 4-digit typing and in part on the basis of their known specific associations with HLA–DRB3 and HLA–DQB1 alleles, which were determined in the entire data set.

Similarly, for the Swedish data set, high-resolution typing of all DRB1*04 alleles was performed. The identification of all alleles containing a DERAA sequence in the Swedish cohort was facilitated by using an interpretation table for the HLA–DRB1 low-resolution kit. This allowed ascertainment of the following allelic groups in this cohort: DRB1*0103, DRB1*0402, DRB1*11–DERAA (*1102 or *1103), and DRB1*13–DERAA (*1301 or *1302). Unfortunately, this did not allow the differentiation of DRB1*1301 from DRB1*1302. The SE alleles and the protective classifications were defined as shown in Table 1.

**Serologic measurements.** ACPAs were determined by measurement of anti–cyclic citrullinated peptide antibodies with a second-generation enzyme-linked immunosorbent assay (anti-CCP2) (for the ORAR, Diastat [Axis-Shield]; for the EURIDISS, Quanta Lite [Inova Diagnostics]; for the Swedish, Dutch, and Spanish cohorts, Immunoscan RA Mark 2 [Euro-Diagnostica]). These different anti-CCP2 assays have been shown to provide very similar results (33). Samples with a value above the cutoff, as specified by the manufacturer, were considered positive.

**Statistical analysis.** For each of the cohorts, we used logistic regression analysis to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for the development of ACPA-positive RA and ACPA-negative RA in association with the different HLA–DRB1 alleles. To take into account the matching of patients and control subjects in the study design, the analyses in the Swedish cohort were corrected for residential area, because age and sex have been shown to have no effect on the distribution of HLA alleles (34). A dominant

genetic model that estimates the effect of the presence of a certain allele, irrespective of the presence of 1 or 2 copies, was used for all analyses. This model provided a better fit of the data compared with an additive allele model that assumes the effect in homozygotes to be considerably larger than the effect in heterozygotes.

Due to the strong predominance of SE alleles among ACPA-positive patients, all other alleles are inherently less prevalent in patients than in control subjects. This results in seemingly protective effects of these alleles, which are in fact merely caused by skewing due to the large difference in the prevalence of SE alleles. In order to obtain an accurate estimate of the effect of the non-SE alleles, the analyses for these alleles in ACPA-positive patients were therefore stratified for the presence of SE alleles in the following manner. For each non-SE allele (e.g., DRB1*03), the 6 possible combinations of this allele with SE alleles were investigated: group A, DRB1*03/DRB1*03; group B, DRB1*03/x; group C, x/x; group D, SE/SE; group E, SE/x; and group F, SE/DRB1*03.

For the SE-negative stratum, the presence of DRB1*03 was compared with the absence of DRB1*03; hence, the effect in groups A and B was investigated using group C as the reference category. This corresponds to the dominant genetic model as described above. For the SE-positive stratum, the risk associated with the group F genotype (SE/DRB1*03) was analyzed using group E (SE/x) as the reference category, to adjust for the risk associated with the presence of 1 SE allele.

Subsequently, we performed a meta-analysis using the effect sizes ($\beta$) and standard errors of the different cohorts. To account for the fact that there was significant statistical heterogeneity (Q statistic, $P < 0.10$) in a small number of the analyses, a random-effects approach (35) was applied for all comparisons. This method allows between-study heterogeneity and incorporates it in the calculations. The data were analyzed per cohort, using SPSS version 16.0 software. For the meta-analysis, we used the freely available R software environment for statistical computing.

**Table 1.** Frequencies and classifications of HLA–DRB1 alleles according to predisposition and protection in rheumatoid arthritis*

| | | | | | Frequency in controls, % | | | |
|---|---|---|---|---|---|---|---|---|
| Allele | SE† | DERAA‡ | D70§ | I67¶ | Norway | Sweden | The Netherlands | Spain |
| *0101 | x | | | | 20 | 19 | 21 | 13 |
| *0102 | x | | | | 0.9 | | | 8.8 |
| *0103 | | x | x | x | 2.1 | 1.2 | 0.5 | 2.1 |
| *03 | | | | | 25 | 24 | 22 | 25 |
| *0401 | x | | | | 22 | 24 | 16 | 4.3 |
| *0402 | | x | x | x | 0.3 | 0.8 | 0.2 | 2.8 |
| *0403 | | | | | 2.0 | 1.5 | 2.5 | 5.8 |
| *0404 | x | | | | 13 | 7.6 | 6.5 | 5.8 |
| *0405 | x | | | | 0.4 | 0.9 | 0.7 | 3.9 |
| *0407 | | | | | 0.6 | 1.3 | 1.3 | 1.2 |
| *0408 | x | | | | 1.9 | 1.3 | 0.6 | 0.4 |
| *07 | | | x | x | 17 | 16 | 20 | 27 |
| *08 | | | x | | 8.5 | 9.0 | 7.0 | 5.4 |
| *09 | | | | | 2.7 | 3.2 | 2.0 | 2.1 |
| *1001 | x | | | | 2.0 | 2.1 | 3.0 | 4.1 |
| *1101 | | | x | | 6.3 | 7.4 | 14 | 20 |
| *1102 | | x | x | | 0.3 | | | 2.9 |
| *1103 | | x | x | | 0.4 | 1.2 | 1.0 | 1.2 |
| *1104 | | | x | | 0.9 | | | 3.6 |
| *12 | | | x | x | 5.2 | 4.3 | 5.0 | 1.9 |
| *1301 | | x | x | x | 15 | | 14 | 13 |
| *1302 | | x | x | x | 9.2 | 24 | 12 | 6.6 |
| *1303 | | | x | x | 0.4 | 1.0 | 1.4 | 4.4 |
| *1454# | | | | | 3.1 | 4.1 | 7.0 | 4.1 |
| *15 | | | | x | 29 | 29 | 27 | 19 |
| *16 | | | x | | 0.6 | 1.2 | 2.0 | 2.5 |

* Rare alleles with a median prevalence in controls of <0.5% are not listed. In the Swedish and Dutch cohorts, 4-digit typing was not available for all alleles. The frequencies were therefore listed in the following manner: sum of DRB1*0101 and DRB1*0102 (row showing *0101), sum of all DRB1*10 alleles (row showing *1001), sum of DRB1*1101 and DRB1*1104 (row showing *1101), sum of DRB1*1102 and DRB1*1103 (row showing *1103), for the Swedish cohort, sum of DRB1*1301 and DRB1*1302 (row showing *1302), and sum of all DRB1*14 alleles (row showing *1454). x = present.

† The frequency of the shared epitope (SE) in controls was as follows: Norway, 53%; Sweden, 49%; The Netherlands, 44%; Spain, 37%.

‡ The frequency of DERAA in controls was as follows: Norway, 26%; Sweden, 30%; The Netherlands, 26%; Spain, 27%.

§ The frequency of D70 in controls was as follows: Norway, 56%; Sweden, 60%; The Netherlands, 66%; Spain, 75%.

¶ The frequency of I67 in controls was as follows: Norway, 67%; Sweden, 67%; The Netherlands, 66%; Spain, 63%.

# It was recently shown that the majority of individuals previously genotyped as carrying DRB1*1401 in fact carry the genotype DRB1*1454 (see ref. 44). Thus, in anticipation of probable genotyping revisions, DRB1*1454 is listed as the most common DRB1*14 allele.

## RESULTS

The study cohort consisted of 2,806 patients with RA and 3,772 control subjects from 4 different European populations. The distribution of patients and control subjects across the cohorts was as follows: Norway, 788 patients and 898 control subjects; Sweden, 827 patients and 934 control subjects; The Netherlands, 844 patients and 1,213 control subjects; Spain, 347 patients and 727 control subjects. All patients fulfilled the ACR 1987 criteria for the classification of RA. The proportion of patients who were ACPA positive was very similar in all cohorts and ranged from 58% to 62%.

The classifications of predisposing and protective HLA–DRB1 alleles that have been described to be associated with RA are listed in Table 1. The SE classification incorporates several HLA–DRB1 alleles that confer a high risk of ACPA-positive disease, with reported ORs ranging from 4.6 to 11.3 (31,36).

The DERAA, D70, and I67 alleles have been claimed to be associated with protection against RA, with ORs of 0.50, 0.23, and 0.14 for DERAA presence (20), D70 homozygosity (16), and I67 homozygosity, respectively (15). As shown in Table 1, there is considerable overlap between the protective classifications. The frequencies of the separate HLA–DRB1 alleles in the 4 control populations are also presented in Table 1, as well as the frequencies of the allele classifications.

**Associations between HLA–DRB1 alleles and ACPA-negative RA.** Table 2 shows the results of the meta-analysis for ACPA-negative RA. Although the data show a predisposing effect of DRB1*03 and DRB1*04 as well as a possible protective effect of DRB1*07 and DRB1*15 on ACPA-negative RA, these associations were only weakly significant.

**Table 2.** HLA–DRB1 associations with ACPA-negative RA according to meta-analysis*

| HLA–DRB1 allele | OR (95% CI) | *P* |
|---|---|---|
| *01 | 1.06 (0.90–1.25) | 0.46 |
| *03 | 1.39 (1.01–1.93) | 0.05 |
| *04 | 1.17 (1.00–1.37) | 0.05 |
| *07 | 0.67 (0.48–0.95) | 0.03 |
| *08 | 0.97 (0.59–1.60) | 0.90 |
| *09 | 0.90 (0.54–1.50) | 0.68 |
| *10 | 1.04 (0.67–1.60) | 0.86 |
| *11 | 1.01 (0.79–1.29) | 0.95 |
| *12 | 0.84 (0.59–1.20) | 0.34 |
| *13 | 0.87 (0.73–1.03) | 0.10 |
| *14 | 1.09 (0.80–1.48) | 0.58 |
| *15 | 0.78 (0.65–0.94) | 0.01 |
| *16 | 1.04 (0.42–2.58) | 0.94 |

* ACPA = anti–citrullinated protein antibody; RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval.

With regard to the effects of DRB1*03 and DRB1*07, there were marked geographic differences. The previously reported association between DRB1*03 and susceptibility was present in the 2 Scandinavian cohorts and in the Dutch cohort but was absent in the Spanish cohort. However, a recent extensive study in Sweden (14) did not reveal a predisposing effect of DRB1*03 on ACPA-negative RA, indicating that more studies will be required in order to draw definitive conclusions about this association. With regard to the protection conferred by DRB1*07, there appeared to be a north-to-south gradient, with the strongest protective effect in Norway and no observable effect in Spain.

In order to perform a more detailed analysis of possible protective alleles, we also investigated the effects of the 3 classifications proposed to be associated with protection: the DERAA, D70, and I67 alleles. The DERAA alleles did not convey a protective effect for ACPA-negative RA, while both the D70 and I67 alleles showed a modest protective effect (OR 0.75 [95% CI 0.59–0.96) and OR 0.70 [0.53–0.94], respectively).

**Associations between HLA–DRB1 alleles and ACPA-positive RA.** Table 3 displays the results of the meta-analysis for ACPA-positive RA. Because our aim was to specifically investigate protective effects, Table 3 lists only the 4-digit subtype analysis of alleles that have been reported to be associated with protection. Due to the preponderance of SE alleles among ACPA-positive patients, all other alleles are inherently less prevalent in patients than in control subjects. This leads to seemingly protective effects of these alleles, which are in fact merely the result of skewing caused by the large difference in the prevalence of SE alleles. In order to obtain an accurate estimate of the effect of the non-SE alleles, the analyses for these alleles were therefore stratified for the presence of the SE.

The well known association between SE alleles and susceptibility to ACPA-positive RA was confirmed by our data. Our results also confirmed that the hierarchy in the strength of this association is DRB1*04 > DRB1*10 > DRB1*01. We also observed predisposing effects of DRB1*09, *15, and *16, although the effect of the *09 and *16 alleles was limited to SE-negative individuals. Despite the fact that some of these associations were relatively weak, they nonetheless suggest that the effect of HLA–DRB1 alleles on susceptibility to ACPA-positive RA may extend beyond the SE alleles.

Intriguingly, the DRB1*13 alleles appeared to be the only alleles associated with protection (Table 3). In both the SE-negative and SE-positive strata, the protective effect of DRB1*13 not only remained present but

**Table 3.** HLA–DRB1 associations with ACPA-positive RA according to meta-analysis*

| HLA–DRB1 allele | OR (95% CI) | *P* |
|---|---|---|
| *01 | **1.35 (1.10–1.66)** | **0.004** |
| *0101 and *0102 | **1.44 (1.18–1.74)** | **<0.001** |
| *0103 | 0.31 (0.13–0.75)† | 0.009 |
| *03 | 0.64 (0.55–0.75) | <0.001 |
| SE negative | 1.05 (0.80–1.38) | 0.71 |
| SE positive | 1.18 (0.95–1.46) | 0.14 |
| *04 | **3.76 (2.93–4.84)** | **<0.001** |
| *0402 | 1.48 (0.81–2.71) | 0.21 |
| Other *04 | **3.74 (2.86–4.91)** | **<0.001** |
| *07 | 0.56 (0.47–0.67) | <0.001 |
| SE negative | 1.04 (0.79–1.38) | 0.76 |
| SE positive | 0.89 (0.69–1.14) | 0.35 |
| *08 | 0.50 (0.36–0.70) | <0.001 |
| SE negative | 0.73 (0.46–1.14) | 0.17 |
| SE positive | 1.00 (0.50–1.98) | 0.99 |
| *09 | 1.43 (0.94–2.16) | 0.10 |
| SE negative | **3.25 (2.06–5.12)** | **<0.001** |
| SE positive | 1.76 (1.00–3.11) | 0.05 |
| *10 | **2.37 (1.56–3.60)** | **<0.001** |
| *11 | 0.56 (0.46–0.68) | <0.001 |
| SE negative | 0.93 (0.64–1.36) | 0.72 |
| SE positive | 0.90 (0.67–1.19) | 0.45 |
| *1102 and *1103 | 1.01 (0.61–1.67) | 0.97 |
| SE negative | 1.46 (0.63–3.37)† | 0.38 |
| SE positive | 1.53 (0.75–3.12) | 0.24 |
| Other *11 | 0.49 (0.39–0.62) | <0.001 |
| SE negative | 0.80 (0.49–1.29) | 0.36 |
| SE positive | 0.70 (0.51–0.97) | 0.03 |
| *12 | 0.60 (0.42–0.84) | 0.003 |
| SE negative | 0.76 (0.37–1.54) | 0.44 |
| SE positive | 1.10 (0.69–1.77) | 0.68 |
| *13 | 0.33 (0.25–0.45) | <0.001 |
| SE negative | **0.54 (0.38–0.77)** | **<0.001** |
| SE positive | **0.57 (0.41–0.81)** | **0.002** |
| *1301 and *1302 | 0.31 (0.22–0.45) | <0.001 |
| SE negative | **0.45 (0.27–0.74)** | **<0.001** |
| SE positive | **0.54 (0.34–0.83)** | **0.006** |
| Other *13 | 1.01 (0.48–2.10) | 0.99 |
| SE negative | 2.35 (0.63–8.72) | 0.20 |
| SE positive | 1.56 (0.65–3.71)‡ | 0.32 |
| *14 | 0.48 (0.34–0.68) | <0.001 |
| SE negative | 0.52 (0.28–0.98)§ | 0.04 |
| SE positive | 1.02 (0.46–2.24)§ | 0.96 |
| *15 | 0.69 (0.57–0.82) | <0.001 |
| SE negative | **1.51 (1.17–1.95)** | **0.001** |
| SE positive | 1.25 (1.02–1.55) | 0.04 |
| *16 | 1.21 (0.73–1.99) | 0.46 |
| SE negative | **2.91 (1.12–7.56)** | **0.03** |
| SE positive | 1.51 (0.69–3.27) | 0.30 |

* Stratification for the shared epitope (SE) was not performed, because in 2 of the 4 cohorts, such stratification led to the absence of cases or controls. Odds ratios (ORs) depicting a negative association with a *P* value of <0.01 after stratification were considered to represent a protective effect and are printed in bold; ORs depicting a positive association with a *P* value of <0.01 after stratification were considered to represent a predisposing effect and are printed in bold. ACPA = anti–citrullinated protein antibody; RA = rheumatoid arthritis; 95% CI = 95% confidence interval.
† No cases in the Leiden cohort.
‡ No cases in the Norwegian cohort.
§ HLA–DRB1*1402 alleles have an SE motif and were exceedingly rare in all cohorts (prevalence <0.3% in controls). Therefore, the HLA–DRB1*14 effect was stratified for the SE after exclusion of individuals with DRB1*1402 alleles.

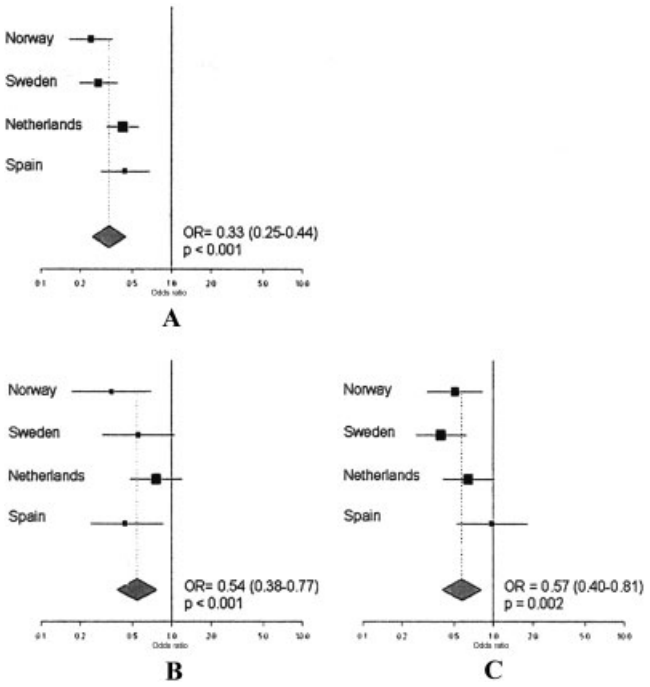


**Figure 1.** Effect of DRB1*13 in anti–citrullinated protein antibody–positive rheumatoid arthritis. Forest plots depict the odds ratios (ORs) and 95% confidence intervals of the 4 separate cohorts and the combined estimate of the random-effects meta-analysis. **A,** Unstratified analysis. **B,** Shared epitope–negative stratum. **C,** Shared epitope–positive stratum.

also was associated with a considerable effect size (OR 0.54 and OR 0.57, respectively) (Figure 1).

In the subtype analysis of some of the protective alleles, e.g., in the case of DRB1*0103 and DRB1*0402, stratification resulted in a lack of patients or controls in several cohorts, rendering a meta-analysis ineffective. We therefore cannot formally exclude the possibility that these alleles may be associated with a protective effect, although this is unlikely in the case of *0402, based on the results of the unstratified analysis.

**The protective effect of DRB1*13 and protective classifications on ACPA-positive RA.** All protective classifications described for RA include DRB1*13 or some of the DRB1*13 suballeles (Table 1). When analyzed according to the different protective classifications, the protective effect of the I67 alleles did not remain significant after stratification for the SE. Both the D70 and the DERAA alleles were associated with a protective effect, which remained present after stratification for the SE (Figures 2A and B), yet it was remarkable that, apart from DRB1*13, none of the alleles with a D at position 70 or a DERAA motif appeared to confer protection by themselves (Table 3). We therefore inves-

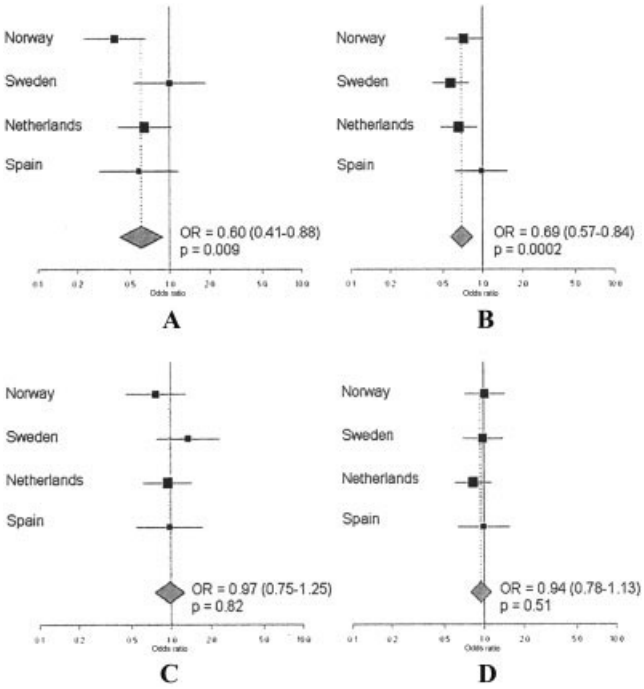


**Figure 2.** Effect of D70 alleles, with and without DRB1*13 alleles, in anti–citrullinated protein antibody–positive rheumatoid arthritis. Forest plots depict the odds ratios (ORs) and 95% confidence intervals of the 4 separate cohorts and the combined estimate of the random-effects meta-analysis. **A,** Effect of all D70 alleles in the shared epitope (SE)–negative stratum. **B,** Effect of all D70 alleles in the SE-positive stratum. **C,** Effect of D70 alleles, after exclusion of DRB1*13, in the SE-negative stratum. **D,** Effect of D70 alleles, after exclusion of DRB1*13, in the SE-positive stratum.

tigated whether the protection associated with the group of D70 or DERAA alleles as a whole could be explained solely by the protective effect of the DRB1*13 alleles. To this end, we excluded the DRB1*13 alleles from the analysis and reanalyzed the effect of all alleles with a D at position 70 on ACPA-positive RA. As can be seen in Figures 2C and D, these alleles were not protective, despite the fact that they had a D at position 70. The same was the case for the DERAA alleles (data not shown). These data indicate that the presence of a D at position 70 or of the DERAA sequence as such does not result in protection against ACPA-positive RA, but rather that DRB1*13 appears to be associated with protection.

In light of the strong protective effect conveyed by DRB1*13, we investigated whether the presence of a DRB1*13 allele could annul the predisposition associated with an SE allele. The risk in heterozygous individuals carrying both an SE and a DRB1*13 allele compared with the risk in individuals carrying neither the SE nor DRB1*13 alleles, was, however, still increased (OR 2.14 [95% CI 1.64–2.80]). Although this effect will vary according to the difference in risk associated with the different SE alleles and in different cohorts, the presence of 1 DRB1*13 allele does not compensate for the risk associated with the presence of 1 SE allele in meta-analysis.

Next, we analyzed whether the DRB1*13 association was confined to DRB1*13 alleles that contain a DERAA sequence, i.e., DRB1*1301 and*1302 (DRB1*1304 was not present in the study populations). As shown in Table 3, the protective effect was limited to the DRB1*1301 and *1302 alleles, although the analysis for the other DRB1*13 alleles was possibly hampered by relatively small numbers of patients and control subjects. Complete 4-digit typing of DRB1*13 was available for 3 of the 4 populations included in this meta-analysis (Norway, The Netherlands, and Spain). Subtype analysis revealed that the protective effect of DRB1*1302 was no longer present after stratification for the SE (Figure 3).

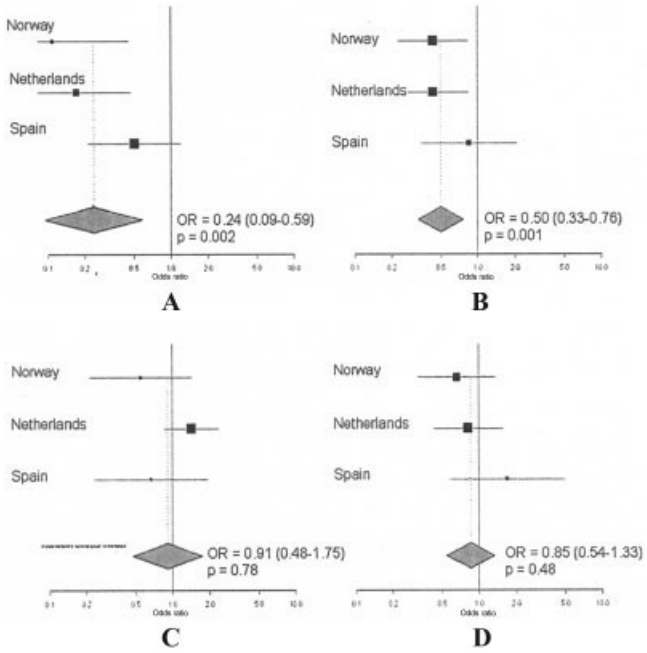


**Figure 3.** Effect of DRB1*1301 and *1302 in anti–citrullinated protein antibody–positive rheumatoid arthritis. Forest plots depict the ORs and 95% confidence intervals of the 3 separate cohorts with high-resolution typing of the DRB1*13 alleles, and the combined estimate of the random-effects meta-analysis. **A,** Effect of DRB1*1301 in the SE-negative stratum. **B,** Effect of DRB1*1301 in the SE-positive stratum. **C,** Effect of DRB1*1302 in the SE-negative stratum. **D,** Effect of DRB1*1302 in the SE-positive stratum. See Figure 2 for definitions.

Therefore, DRB1*1301 was the only allele that was consistently associated with protection against ACPA-positive RA.

## DISCUSSION

HLA alleles contribute to susceptibility to RA in various ways. As a consequence of the highly polymorphic nature of the HLA region, it has been difficult to dissect the contribution of the various HLA alleles to RA susceptibility. Previously, several different classifications have been developed in order to summarize the predisposing and protective effects of the HLA alleles with regard to RA. Our data confirm the predisposing effect of the SE alleles and also corroborate the differential effect sizes with which different HLA SE alleles predispose to ACPA-positive disease (5,6). Furthermore, our results indicate a contribution of DRB1*09 and DRB1*15 to ACPA-positive disease. The finding of a relatively modest effect of DRB1*15 requires replication in further studies, before any firm conclusions can be drawn. In contrast, the predisposing effect of DRB1*09 to ACPA-positive RA has been described in other populations as well (37). Therefore, it may be appropriate to include DRB1*09 in the list of susceptibility genes for ACPA-positive RA.

More importantly, however, our results confirm the association of HLA–DRB1 alleles with protection and considerably refine the definition of protective alleles. Our data indicate that the protective effect is apparent only for the DRB1*13 allelic group. Analysis of the different allele classifications that have been developed to capture the protective effects of HLA–DRB1 alleles in RA (15,16,18) revealed that the protective effect of the DERAA and D70 classification could largely be attributed to the DRB1*13 alleles. This underscores the relative importance of the protective effect mediated by DRB1*13 in comparison with other alleles and also raises the question of whether the classifications of protective effects may need to be reconsidered.

Further analysis of the DRB1*13 alleles showed that protection against RA was apparent only for DRB1*1301. Although our study included >2,700 patients and >3,000 control subjects from 4 large data sets from 4 different European populations, we cannot exclude the possibility that smaller protective effects may also be present for alleles other than DRB1*1301 that could not be detected in the present investigation. Previous studies in individuals of other ethnicities have also shown the demonstrated protective effects of other HLA alleles such as DRB1*1302 and DRB1*14 for RA, although not all of these results were stratified for ACPA status or corrected for the effect of the HLA SE alleles (38,39). It would be interesting to know to what extent the protective effects differ among different populations.

The present study clearly confirms that the SE alleles are associated only with ACPA-positive RA. The same is true for the association between DRB1*13 or *1301 and protection against RA. The present study thus demonstrates once more that the association between HLA–DRB1 alleles and ACPA-positive versus ACPA-negative RA are very different, both quantitatively and qualitatively. Regarding the protective effects of HLA–DRB1 alleles in ACPA-negative RA, a recent study in a limited number of patients demonstrated that the DERAA alleles may be protective in this subset of patients as well (40). We could not confirm this finding in the present study, although the weak protective effects we observed for DRB1*07 and DRB1*15 in ACPA-negative RA do not exclude the presence of HLA-mediated protection in ACPA-negative disease.

In the current investigation, the effects of the presence of the different HLA–DRB1 alleles were investigated separately. The risk of ACPA-positive RA in individuals heterozygous for the SE was assessed as part of the stratified analysis, but we cannot make conclusions about the risk associated with heterozygosity for the various other HLA–DRB1 alleles. It is conceivable that combinations of certain alleles may confer susceptibility or protection, as was described in a recent report, in which the combination of DRB1*03 and DRB1*13 alleles was found to be associated with an increased risk of ACPA-negative disease (14). A meta-analysis of heterozygosity effects may therefore yield very interesting results in the future.

For the statistical analysis in the present study, a dominant allele model was applied. An alternative approach would have been to use an additive allele model, which assumes substantially larger effects in homozygous versus heterozygous individuals. Although the dominant allele model provided the best fit to the data in the current analysis, other studies have favored an additive model (7). Discrepancies between reports may therefore be partly attributable to differences in statistical methods. A meta-analysis such as that presented here is helpful in this respect, because it overcomes these statistical differences and provides an overview of the results of 4 cohorts analyzed in the same manner.

As can be seen from Figures 1A, 3A, and 3B, there was a tendency toward a north-to-south gradient in

the strength of the associations of several HLA–DRB1 alleles. Associations were often the strongest in individuals in Norway and Sweden, slightly less strong in those in The Netherlands, and weakest or sometimes even absent in Spanish patients. This was the case for both predisposing and protective alleles in both ACPA-positive and ACPA-negative disease. For HLA-associated susceptibility, these same geographic differences can be observed in previous studies (41,42), but they have not been described for the protective effects of HLA in RA. If the existence of this gradient proves to be real, it may be a factor that needs to be taken into account when comparing data from different populations. It may also serve to reconcile some of the seemingly conflicting data that have been reported in different populations. Furthermore, and perhaps most importantly, it may provide clues to candidate environmental factors that may be involved in the pathogenesis of RA.

The main reason to perform studies such as the current meta-analysis is to obtain insight into the contribution of the HLA region to RA. This has provided important results in the past, such as the realization that the HLA SE alleles do not contribute to RA as such but rather to ACPA-positive disease (8). More recently, it was also shown that the presence of the HLA SE alleles influences both the magnitude and specificity of the ACPA response (9,43). Taken together, these observations indicate that the HLA SE alleles are primarily involved in shaping the ACPA response, presumably by facilitating T cell help to ACPA-producing B cells. Intriguingly, our data show that the protective effects associated with the presence of DRB1*13 are also most prominent in the ACPA-positive group of patients with RA. These observations would be consistent with the notion that the predisposing effect of the HLA SE alleles and the protective effect of DRB1*13 act within the same biologic pathway. Indeed, the presence of DRB1*13 considerably lowered the predisposing effects of the HLA SE alleles in individuals heterozygous for both, although the predisposing effect of the HLA SE alleles was not annulled. In case these effects target the same biologic pathway, the presence of DRB1*13 may perhaps also influence the specificity and magnitude of the ACPA response.

## AUTHOR CONTRIBUTIONS

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

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**Acquisition of data.** van der Woude, Lie, Lundström, Balsa, Verduijn, Nordang, Alfredsson, Klareskog, Gonzalez-Gay, Lopez-Nevot, Valero, Roep, Kvien, Martín, Padyukov, Toes.

**Analysis and interpretation of data.** van der Woude, Lundström, Feitsma, Houwing-Duistermaat, Klareskog, Gonzalez-Gay, Roep, Huizinga, Martín, Padyukov, de Vries, Toes.

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