HLA-DR/DQ Haplotype in Rheumatoid Arthritis: Novel Allelic Associations in UK Caucasians

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ABSTRACT. ***Objective.*** To elucidate the relative importance of the HLA-DR and HLA-DQ loci in conferring genetic predisposition to rheumatoid arthritis (RA).

Methods. HLA-DRB1 and HLA-DQB1 alleles were typed in a set of 685 patients with RA using sequence-specific polymerase chain reaction. Allele and phenotype frequencies were compared with those in 2 large sets of historical, ethnically matched healthy controls, using the relative predispositional effect method.

Results. Positive association was confirmed with the shared epitope positive HLA-DRB1 alleles associated with RA in Caucasians. A significant susceptibility effect was observed with HLA-DRB1*09, described in other ethnically diverse populations but not in Caucasians. A significant underrepresentation of the HLA-DRB1*0103 variant was noted among the RA cases, supporting the proposed protective role of the DERAA motif at residues 70–74 of the DRß molecule. No HLA-DRB1 independent association of the HLA-DQB1 alleles, implicated in predisposing to RA, was evident.

Conclusion. These data corroborate the shared epitope hypothesis of susceptibility to RA and provide strong evidence for the DRB1 locus as the primary RA susceptibility factor in the HLA region. (J Rheumatol 2002;29:1821–6)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
HLA-DR
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ASSOCIATION

The genetic contribution to rheumatoid arthritis (RA) accounts for about 60% of the population variance[1] and around 30–40% of this is attributable to HLA linked genes[2]. The association between RA and certain alleles of the highly polymorphic HLA-DRB1 locus, in particular some HLA-DRB1*04 alleles[3], has been recognized for over 20 years[2,4]. Despite this, a convincing explanation of the mechanisms involved has proved elusive. The RA associated HLA-DRB1 variants all encode DRß chains with a conserved amino acid sequence encompassing residues 70–74, which contribute to the P4 antigen-binding pocket[5,6]. This sequence is QRRAA in HLA-DRB1*0101/02, *0404/05/08 and *1402, QKRAA in HLA-DRB1*0401, and RRRAA in HLA-DRB1*1001. In contrast, alleles with nonconservative amino acid substitutions within this segment (DRB1*0103, *0403, *0407, *0701, *0802, *1501, etc.) are neutral or negatively associated with RA[3,7,8]. The shared epitope (SE) hypothesis of susceptibility to RA provided a structural framework to account for the positive association of specific HLA-DRB1 alleles with RA[9]. It is possible that the conserved sequence at residues 70–74 allows the presentation of a specific immunogenic peptide to the relevant T cell populations and facilitates the development of RA.

However, an alternative hypothesis has recently emerged, involving both the HLA-DR and the HLA-DQ loci[10]. First, according to this hypothesis, the observed HLA-DR association with RA merely reflects the extreme linkage disequilibrium with HLA-DQ, and the autoimmune T cell proliferation arises from the presentation of immunogens by a subset of HLA-DQ molecules. Second, peptides derived from the HLA-DRß molecules serve as the immunogens when presented to the immune system by the RA associated HLA-DQ molecules. The concept that HLA-DQ may be the primary antigen-presenting element and that DRß derived peptides may be the critical immunogens originally came from the study of collagen induced arthritis in HLA-DQ8 (HLA-DQB1*0302) transgenic mice[11]. In this hypothesis, peptides derived from "permissive" (SE positive) HLA-DRß molecules are poorly presented by the predisposing HLA-DQ molecules and allow the development of potentially autoreactive T cell populations. In contrast, peptides derived from SE negative HLA-DRß chains have a higher affinity of binding to the relevant HLA-DQ molecules and lead to negative thymic selection of the potentially pathogenic T cells.

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It has been proposed that the HLA-DQ susceptibility alleles in RA are DQB1*0301, *0302 and *0303 (collectively DQ3), and HLA-DQB1*05 (collectively DQ5), but only when encoded on the same haplotypes as HLA-DQA1*0301 and HLA-DQA1*0101/04, respectively. In Caucasians, HLA-DRB1*04 (strongly associated with RA) is in extreme linkage disequilibrium with HLA-DQB1*0301 (DQ7) and *0302 (DQ8). However, HLA-DQB1*0301 is also found on the non-RA associated HLA-DRB1*11, -DRB1*12, and some -DRB1*13 haplotypes. HLA-DQB1*0303 is in linkage disequilibrium with HLA-DRB1*09 but is also found on some HLA-DRB1*07 haplotypes. HLA-DQB1*0501 (DQ5) is in linkage disequilibrium with a variety of HLA-DR variants (including DRB1*0101, some *0103, *10, *1401, and *16), some of which (DRB1*0101 and DRB1*10) encode the shared epitope.

Distinguishing the HLA loci that primarily confer predisposition to RA represents a major challenge because of the extensive linkage disequilibrium between HLA-DR and -DQ, and the multiplicity of potentially RA associated alleles. Generating sufficient power to dissect the RA associated haplotypes and to identify less strongly associated alleles requires a large data set. We performed an extensive case-control study in UK Caucasian patients and healthy ethnically matched controls to clarify the relative merits of the HLA-DQ predisposition model and the SE model of susceptibility to RA.

MATERIALS AND METHODS

Cases and controls. Genomic DNA samples were obtained by standard methods from peripheral blood of 685 UK Caucasian patients satisfying the 1987 revised American College of Rheumatology criteria for RA[12]. All patients had erosive disease for at least 2 years. HLA-DRB1 allele and phenotype frequencies, previously obtained from 13,914 healthy bone marrow donors from UK, were used as the first control data set[13]. Published data on 1798 ethnically matched healthy blood donors were used in subsequent comparisons[14].

Methods. Typing of the RA patients for the HLA-DRB1 and HLA-DQB1 alleles was carried out using the polymerase chain reaction-sequence specific primers (PCR-SSP) approach[15] and the products visualized on agarose gels stained with ethidium bromide.

Statistical analysis. Allele and haplotype frequencies were determined by direct counting. The chi-square values for the individual alleles (Table 1) were determined after stratifying the data using the relative predispositional effect (RPE) method[16]. This approach compares the allelic frequencies in patients and controls and allows the determination of predispositional, protective, or neutral effects of individual alleles relative to each other. Briefly, the overall frequency distribution of all alleles is compared between the patients and the controls using the chi-square test. The allele with the largest contribution to the overall chi-square is then removed from the analysis and the expected frequency distribution of the remaining alleles in the controls is normalized accordingly. This sequential process of stratifying the analysis on the associated alleles is continued until no significant deviation from the control frequencies is observed. This method has 2 main advantages over the standard odds ratio (OR) calculation. First, it maximizes the chance of finding relatively weak genetic effects, which is important in a multiallelic locus; second, the associated alleles are detected sequentially, according to their contribution to the overall chi-squared.

Table 1. Allelic frequencies of the HLA-DRB1 types among RA cases (n = 685) and controls (n = 13,634). The values in columns 2 and 3 are the absolute allele frequencies; the individual chi-square and p values represent the association of each allele after stratifying for the overrepresentation of the other associated variants, in the order of descending chi-square values.

HLA-DRB1 Allele	RA Cases (%)	Controls (%)	Chi-square	p
*0101,02	201 (14.7)	2472 (9.1)	180	$< 10^{-10}$
*02	105 (7.7)	4034 (14.8)	0.6	0.5
*03	125 (9.1)	4172 (15.3)	2.4	0.1
*04	654 (47.7)	5722 (21.0)	540	$< 10^{-10}$
*06	79 (5.8)	3343 (12.2)	0.05	0.8
*07	108 (7.9)	3916 (14.4)	0.9	0.3
*08	16 (1.1)	565 (2.1)	0.6	0.4
*09	20 (1.5)	349 (1.3)	12	5×10^{-4}
*10	12 (0.9)	163 (0.6)	12	4×10^{-4}
*11	34 (2.5)	1505 (5.5)	0.03	0.9
*12	9 (0.6)	446 (1.6)	0.2	0.7
*0103	7 (0.5)	581 (2.1)	4	0.03

Subsequent calculations (Tables 2, 3, and 4) were performed from 2 × 2 contingency tables, using the chi-square statistic. The corrected p values were 2 sided and set at 5% significance level. Statistical power of the study varied for the different observations, depending on the size of the samples analyzed (see table legends).

RESULTS

The frequencies of the common HLA-DRB1 alleles are shown in Table 1. Significant associations were observed, as expected, with HLA-DRB1*04, HLA-DRB1*0101/02, and HLA-DRB1*1001. A significant association following RPE analysis was also detected with HLA-DRB1*09 (chi-square = 12, $p < 5 \times 10^{-4}$), not previously noted among Caucasians with RA. A striking underrepresentation of HLA-DRB1*0103 was observed among the RA cases (chi-square = 17, $p < 4 \times 10^{-5}$), which remained significant following the exclusion of the effect of the positively associated variants, using the relative predispositional effect analysis (chi-square = 4, $p < 0.03$; Table 1).

Due to the strong linkage disequilibrium of some RA associated HLA alleles, it is relatively difficult to identify the primary allelic association on certain haplotypes, including HLA-DRB1*04/-DQB1*0301/02 and HLA-DRB1*09/-DQB1*0303. To investigate any independent effects of these HLA-DQ variants, we analyzed their distribution among subsets of RA cases carrying the same DR specificity (Table 2). The relative frequencies of the HLA-DQB1*0301 (DQ7) and *0302 (DQ8) alleles were very similar in HLA-DRB1*04 (DR4) homozygote RA patients and healthy controls (Table 2A). The distribution of the HLA-DQB1*0301 allele was also assessed among HLA-DRB1*13 positive patients and controls to test for a possible independent effect of DQB1*0301. This comparison was possible due to HLA-DRB1*13 being commonly found in haplotypes with either HLA-DQB1*06 or HLA-

Table 2. Testing for a possible independent association of DQ alleles linked to RA associated DR variants. The controls used for this analysis were specific subsets derived from 1798 individuals. (This analysis had 90% power to detect a statistically significant OR of 1.6 in Tables 2A and 2C and OR of 1.9 in 2B.)

A. Distribution of HLA-DQB1*0301 (DQ7) and HLA-DQB1*0302 (DQ8) among HLA-DRB1*04 (DR4) homozygote RA cases (n = 342) and controls (n = 172).

				OR	95% CI	Chi-square	p
DRB1*04/ DQB1*0301 RA cases	DRB1*04/ DQB1*0301 Controls	DRB1*04/ DQB1*0302 RA cases	DRB1*04/ DQB1*0302 Controls				
147 (43.0%)	78 (45.3%)	195 (57.0%)	94 (54.7%)	1.1	0.6–2.1	0.07	0.7

B. Distribution of HLA-DQB1*06 (DQ6) and HLA-DQB1*0301 (DQ7) among HLA-DRB1*13 (DR13) positive cases (n = 66) and controls (n = 352).

DRB1*13/ DQB1*06 RA cases	DRB1*13/ DQB1*06 Controls	DRB1*13/ DQB1*0301 RA cases	DRB1*13/ DQB1*0301 Controls				
60 (90.9%)	315 (89.5%)	6 (9.1%)	37 (10.5%)	0.8	0.07–1.00	0.02	0.7

C. Distribution of HLA-DQB1*02 (DQ2) and HLA-DQB1*0303 (DQ9) among HLA–DRB1*07 (DR7) positive cases (n= 109) and controls (n = 527).

DRB1*07/ DQB1*02 RA cases	DRB1*07/ DQB1*02 Controls	DRB1*07/ DQB1*0303 RA cases	DRB1*07/ DQB1*0303 Controls				
77 (70.6%)	382 (72.5%)	32 (29.4%)	145 (27.5%)	1.1	0.07–1.00	0.02	0.7

Table 3. Allelic distribution of the HLA-DRB1*04 (DR4) subtypes among the DR4 positive RA cases and controls. A set of 500 controls (subgroup of the 13,914 bone marrow donors) was used in this analysis. The subtypes are grouped according to the amino acid sequence in the shared epitope region. (Statistical power to detect a significant allelic association with OR = 2.0, for an average allele frequency of 0.5, was 90%.)

	RA Cases, n = 654 (%)	Controls, n = 207 (%)	OR	95% CI	p_{corr}
DRB1*0401 QKRAA	464 (71.0)	123 (59.4)	1.7	1.2–2.3	0.003
DRB1*0402 DERAA	5 (0.8)	2 (1.0)	—	—	NS
DRB1*0403/07 QRRAE	8 (1.2)	27 (13.0)	0.08	0.04–0.2	3×10^{-13}
DRB1*0404/05/08 QRRAA	176 (26.9)	55 (26.6)	1.0	0.7–1.4	NS
DRB1*0413 RKRAA	1 (0.1)	0(0)	–	–	NS

DQB1*0301. The frequency of the HLA-DRB1* 13/DQB1*0301 haplotype was actually slightly reduced among the RA cases, indicating that there was no evidence of an independent effect from HLA-DQB1*0301 (Table 2B). The HLA-DQB1*0303 variant, apart from being in strong linkage disequilibrium with HLA-DRB1*09, is also found on some HLA-DRB1*07 haplotypes that are not associated with RA. To test for any independent association of HLA-DQB1*0303, we analyzed its distribution among HLA-DRB1*07 positive RA cases and controls (the more common HLA-DQB1 allele found in linkage disequilibrium with HLA-DRB1*07 being HLA-DQB1*02). Once again, no significant difference was found, negating an independent association of HLA-DQB1*0303 with RA (Table 2C).

Several HLA-DRB1*04 alleles diverge from the SE pattern at positions 70–74 and are generally not associated with RA. The distribution of the common HLA-DRB1*04 alleles, grouped according to the amino acid alignment at residues 70–74, was compared between the RA cases and a subset of 500 controls for which HLA-DRB1*04 subtyping data were available (Table 3). A strong underrepresentation of alleles bearing QRRAE (DRB1*0403 and *0407) was evident (OR 0.08, 95% confidence interval 0.04–0.2, $p < 3 \times 10^{-13}$). HLA-DRB1*0401 (encoding the QKRAA motif) revealed a significant positive association (OR 1.7, 95% CI

Table 4. HLA-DRB1 genotype analysis in the 2 sets according to the presence of the shared epitope sequences ^{70}QKRRA^{74} and ^{70}(Q/R)RRAA^{74}. ^{70}QKRRA^{74} is found in DR4*0401. ^{70}QRRAA^{74} includes DR4*0404, 05, 08 and DRB1*0101, 02, 04. RRRAA is found in DRB1*1001. X = sequences other than ^{70}QKRRA^{74} and ^{70}(Q/R)RRAA^{74}. (Relative risks are expressed relative to the low risk genotype X/X, where X are all alleles that do not contain the ^{70}QKRRA^{74} and ^{70}(Q/R)RRAA^{74} SE motifs. Statistical power for detecting a significant genotypic association with OR = 2 was 90%.)

Motif at Residues 70–74	RA Cases, n = 685 (%)	Controls, n = 500 (%)	OR [95% CI]	p_{corr}	Relative Risk
QKRAA+/QKRAA+ 0401/0401	70 (10.2)	7 (1.4)	8.0 [4.1–16.7]	1×10^{-9}	23.5
QKRAA+/(QR)RRAA+	163 (23.8)	20 (4.0)	7.5 [4.9–11.5]	3×10^{-20}	19.2
0401/0404,05,08	89	6	12.5 [6.2–24.2]	10^{-13}	34.6
0401/0101,02	69	13			
0401/1001	5	1			
QKRAA+/(Q/R)RRAA– 0401/X	161 (23.5)	84 (16.8)	1.5 [1.0–2.0]	4×10^{-3}	4.5
QKRAA–/(Q/R)RRAA+	184 (26.9)	134 (26.8)	1.0 [0.8–1.1]	0.9	3.2
0404,05,08/0404,05,08	9	1	0.1 [0.02–0.9]	0.08	3.0
0404,05,08/X	53	39			
0101/0404,05,08	16	7			
0404,05,08/1001	0	1			
0101,02/0101,02	15	6			
1001/1001	0	0			
0101,02/X,	87	76			
0101,02/1001	2	1			
1001/X	2	3			
X/X	107 (15.6)	255 (51.0)	0.2 [0.1–0.3]	1×10^{-38}	1.0

1.2–2.3, $p < 0.003$), while subtypes with the QRRAA motif (DRB1*0404, *0405, *0408) showed a weaker, nonsignificant association. The DRB1*0402 subtype (DERAA) was too scarce for any meaningful analysis.

Table 4 shows the overall HLA-DRB1 genotype analysis of the RA patients and controls with respect to the presence of the SE susceptibility sequences QKRAA and (Q/R)RRAA. Absence of both of these sequences shows a strong protective effect (OR 0.2, 95% CI 0.1–0.3, $p < 10^{-38}$). The presence of both of these sequences confers a high susceptibility to RA (OR 7.5, 95% CI 4.9–11.5, $p < 3 \times 10^{-20}$), with the relative risk for developing the disease 19.2 times greater than if neither sequence is present. This association was primarily due to the homozygosity of alleles on the HLA-DRB1*04 background, with a combined relative risk of 34.6 (OR 12.5, 95% CI 6.2–24.2, $p < 10^{-13}$). As documented, homozygosity for the QKRAA sequence (HLA-DRB1*0401 homozygotes) carries the highest relative risk (RR 23.6) in predisposing to RA (OR 8.0, 95% CI 4.1–16.7, $p < 10^{-9}$). The distribution of genotypes bearing only one copy of the QKRAA motif, in the absence of (Q/R)RRAA, was still significantly increased among the patients (RR 4.5, $p < 4 \times 10^{-3}$). The total frequency of genotypes encoding one or 2 copies of (Q/R)RRAA (HLA-DRB1*0101/02, 0401/05/08, or 1001), in the absence of QKRAA (HLA-DRB1*0401), was similar in patients and controls, although again there was a trend for overrepresentation of 2 copies of the SE present on a HLA-DRB1*04 background ($p < 0.08$).

DISCUSSION

Analyzing the amino acid sequence of the antigen-binding pocket in variants positively and negatively associated with RA across diverse populations has proved valuable in revealing a common pattern, and led to the SE theory. HLA-DRB1 alleles that encode the (Q/R)RRAA/QKRAA motifs at residues 70–74 are associated with RA across different populations and our study supports these findings, with 84.4% of patients encoding these epitopes. It also shows a significant positive association with HLA-DRB1*09, which encodes an ^{70}RRRAE^{74} motif. This association was first described in Chilean patients with RA[17,18], and subsequently reported in Singaporean Chinese[19], Tlingit Indians[20], and Japanese[21]. A weak association in Northern European Caucasians can be inferred from a recent Dutch study[22].

Specific variants of HLA-DRB1*07 (*0701), DRB1*08 (*0802), and DRB1*13 (*1302) are found to be significantly underrepresented in Mexican Americans[23] and Japanese[21] with RA. At residues 70–74, their encoded molecules have DRRGQ, DRRAL, and DERAA motifs, respectively. The protective effect of DRB1*0402 (^{70}DERAA^{74}) has been well established in studies of RA in Israeli Jews[24] and Spanish[7]. HLA-DRB1*0103 is an uncommon variant

that shares the same ^{70}DERAA^{74} epitope as DRB1*0402. Our study had sufficient power to demonstrate that HLA-DRB1*0103 is also markedly reduced in RA, highlighting the protective role of the ^{70}DERAA^{74} motif.

Thus, the majority of the RA protective alleles contain aspartic acid (D), an acidic (negatively charged) residue with a short side chain, at position 70. This amino acid markedly differs from the 2 residues found at position 70 in the RA associated alleles: arginine (R), basic (positively charged) with a long side chain, or glutamine (Q), which is neutral, with a medium length side chain. Such nonconservative amino acid variation in the p4 peptide-binding pocket of the HLA-DRß molecule might have a strong influence on the specificity and affinity of antigen-binding and presentation.

Functional studies of the T cell clonal proliferations in response to antigen presentation by modified HLA-DRß molecules have shown that this indeed is the case[25-27]. In particular, alteration of the residue at position 71 and to a lesser extent at 67, 70, and 86 significantly affects antigen presentation[25,27]. Further, introduction of a negative charge in the p4-binding pocket [as is the case with aspartic acid (D) at position 70 in the RA protective HLA-DRB1 alleles] markedly decreases the affinity of peptide binding by the DR molecules[26], which could result in downregulation of the T cell clonal proliferation. Although variation in the HLA-DRß p4 pocket may crucially affect peptide binding and its recognition by T cells, these effects can be modified by substitutions elsewhere in the HLA-DRß chain, such as Gly/Val at position 86 and Ser/Asp at position 57[28,29]. Also, peptides have been generated that retain the capacity to be recognized by clones of T cells in the context of a variety of RA associated HLA molecules. From these observations it is conceivable that potentially arthritogenic peptides may exist with relatively promiscuous HLA restriction.

It was suggested that HLA-DRB1*0403 and *0407 (^{70}QRRAE^{74}) may be protective against RA in the Spanish[7] and British[3,30]. This study strongly supports the negative association of the ^{70}QRRAE^{74} motif with RA. However, there is mounting evidence that HLA-DRB1*09 (DR9), which encodes the similar ^{70}RRRAE^{74}, is positively associated with RA in different ethnic groups. Such different behavior of 2 alleles with similar antigen-binding domains could be accounted for by the finding that ^{70}Arg (R) is capable of forming several more hydrogen bonds with the bound peptide than ^{70}Gln (Q), thus increasing the avidity of peptide binding[29]. ^{74}Glu (E), which can hinder peptide binding, might be less critical in the context of ^{70}Arg (as in HLA-DRB1*09), and hence more easily accommodated without compromising the HLA peptide conformation. In addition, a distant effect from ^{67}Phe (F) in HLA-DRB1*09, compared to ^{67}Leu (L) in HLA-DRB1*0403/07, might have an influence on ^{74}Glu.

The SE hypothesis concentrates on the shared characteristics of the α helix forming one side of the antigen binding site, but there are also differences in the floor of the binding site among the HLA-DRB1*04, DRB1*0101/02, and DRB1*1001 variants. There are important differences in the degree of susceptibility to RA associated with each of these variants: the risk is substantially greater for DRB1*04 than DRB1*0101/02 or DRB1*1001. Perhaps the differences elsewhere in the DRß chain are responsible. It also remains unclear why the HLA-DR*0401/*0404, 05, 08 compound heterozygote state should exhibit the strongest association with RA (Table 4). Any comprehensive evaluation of the SE hypothesis must take these observations into account.

The results presented here are generally consistent with the SE hypothesis. No HLA-DR independent association of the HLA-DQ alleles previously implicated in susceptibility to RA was evident. The overrepresentation of HLA-DRB1*09 (DR9) in the RA patients and the lack of independent association with RA of the tightly linked DQB1*0303 (DQ9) variant indicate that the primary susceptibility allele is DRB1*09. In addition, we observed no independent association of DR4 linked DQB1*0301 (DQ7) and DQB1*0302 (DQ8) alleles, thus supporting the finding reported by de Vries, *et al* in a Dutch sample[31].

HLA-DRB1 alleles demonstrating positive and negative associations with susceptibility to RA are in accord with the findings of the effects of p4 residue modifications on antigen binding and presentation. However, it is not clear whether and how these residues affect the conformation of the bound peptide and its presentation to T cell receptors. Further functional studies are required to elucidate these complex interactions.

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