Expression of QK/QR/RRRAA or DERAA Motifs at the Third Hypervariable Region of HLA-DRB1 and Disease Severity in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To examine the relationship between disease severity in patients with confirmed rheumatoid arthritis (RA) and the carriage of alleles expressing the high risk epitope (HRE) QK/QR/RRRAA or the low risk epitope (LRE) DERAA at positions 70–74 of the third hypervariable region of HLA-DRB1.

Methods. A case-control design to compare allele carriage rates in 204 Caucasian subjects with severe RA and mild RA and healthy controls. Patients had a mean disease duration of 12–18 years and severity of RA was defined using clinical and therapeutic criteria. Molecular typing at the HLA-DRB1 locus was performed using a polymerase chain reaction method.

Results. Eighty-seven percent of patients (52/60) with severe RA had one or more of the alleles bearing the QK/QR/RRRAA motif or HRE, compared with 54% (21/39) with mild RA (OR 5.57, $p = 0.0007$) and 39% (41/105) of controls (OR 10.15, $p < 0.0001$). Twenty-five percent of patients (15/60) with severe disease expressed 2 disease associated HRE DRB1 alleles, compared with 13% of patients (5/39) with mild disease (OR 2.3, p = NS) and 5% (5/105) of controls (OR 6.67, $p = 0.0003$). In contrast, only 5% of patients (3/60) with severe RA expressed one of the LRE alleles that carry the DERAA motif at positions 70–74, compared with 31% of patients (12/39) with mild RA (OR 0.12, $p = 0.0013$) and 22% of controls (23/105) (OR 0.19, $p = 0.0082$). No patient or control was homozygous for LRE alleles. Eighty-three percent (50/60) of patients with severe RA expressed the HRE without the LRE, compared with 44% (17/39) of those with mild disease (OR 6.47, $p < 0.0001$) and 35% (37/105) of controls (OR 9.19, $p < 0.0001$). In contrast, only one patient (2%) with severe disease expressed the LRE without the HRE, compared with 20% (8/39) of those with mild disease (OR 0.07, $p = 0.0047$) and 16% (17/105) of controls (OR 0.09, $p = 0.009$). There was no significant difference between the 3 groups in the frequency of patients who expressed both or neither epitope. Logistic regression showed that age at disease onset ($p = 0.0009$), duration of disease ($p = 0.007$), positive rheumatoid factor status ($p = 0.003$), and presence of the HRE or LRE ($p = 0.00005$) were significantly associated with the presence of severe disease.

Conclusion. HLA-DRB1 alleles appear to confer an important bidirectional influence on the risk of disease severity in RA, with 20-fold difference in OR between those associated with the highest (HLA-DRB1*0401) and lowest (HLA-DRB1*1301/02) risk. The HRE and LRE exhibit diametrically opposed effects, which may be mutually antagonistic. These data support a multistep pathogenesis in which MHC class II genes are one component of a coordinate genetic and environmental interaction leading to immunological injury and joint destruction. (J Rheumatol 2002;29:1358–65)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
HLA
SEVERITY
EPITOPE

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Supported by the Immunology Laboratory Research Program and The Arthritis Society, BC and Yukon Division. Dr. Khani-Hanjani was the recipient of a Research Fellowship from Novartis Pharmaceuticals, Canada. Dr. Lacaille was the recipient of a Research Fellowship from The Arthritis Society.

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Submitted January 29, 2001; revision accepted January 11, 2002.

Rheumatoid arthritis (RA) is a heterogeneous disorder with a variable and protracted course and fluctuating disease activity. Clinical expression ranges from a mild, nondeforming arthropathy with little longterm disability to severe, incapacitating erosive articular disease that may be refractory to conventional disease modifying agents[1-3]. Prediction of disease course is imprecise, and is often based on demographic, clinical, and laboratory measures[4-7]. Prognostic factors associated with severe forms of disease include low socioeconomic status and education levels, sex, age at onset, severe initial disease presentation, systemic manifestations and extraarticular features, early appearance of joint erosions, elevated erythrocyte sedimentation rate and C-reactive protein, the presence of rheumatoid factor (RF), and a family history of RA.

Epidemiological studies suggest that RA has an important genetic component, and that alleles within the major histocompatibility complex (MHC) class II region play an important role in determining disease susceptibility and/or severity[8-13]. The associations vary depending upon the population under study, suggesting that susceptibility alleles may differ according to ethnic origin and disease status. Studies show that certain subtypes of HLA-DRB1*01 (0101, 0102) and HLA-DRB1*04 (0401, 0404, 0405, 0408) are preferentially associated with RA in Caucasian patients. In contrast, RA has been associated with HLA-DRB1*03 in Arabs in Kuwait, with HLA-DRB1*04 in Muslim and HLA-DRB1*10 in Tamil and Hindi Indians in South Africa, and with HLA-DRB1*1402 in Tlingit Indians in Alaska[14-16]. There is a low frequency of HLA-DRB1*04 in patients from northern Italy, while Greek and Israeli Jewish patients commonly type as HLA-DR10 and HLA-DRB1*01, respectively[17,18]. Not all HLA-DRB1 alleles are associated with increased propensity to or severity of RA, however, and HLA-DR2 and phenotypes DRB1*03 + DRB1*07 and DR5 + DRB1*07 have all been reported to be decreased in frequency in this disease[9,10,19,20].

Many of the alleles associated with susceptibility to and progression of RA exhibit a shared sequence motif consisting of the amino acid sequences QKRAA, QRRAA, or RRRAA at positions 70–74 in the third hypervariable region of HLA-DRB1[21,22]. This high risk epitope (HRE), which forms an integral component of the RA pocket[23], is contiguous with both the T cell receptor and the expressed peptide, and thus is a functional component of the trimolecular antigen recognition complex[24]. Recently, it was observed that alleles expressing the DERAA motif at positions 70–74 in the third hypervariable region (HLA-DRB1*0103, 0402, 1102, 1103, 1301, 1302) were reduced in frequency in patients with severe disease, suggesting the existence of a low risk epitope (LRE) that may confer protection against disease progression[25-29]. These findings suggest a spectrum of genetically encoded risk that is not explicable on the basis of a single high risk shared epitope alone, but in which HLA-DRB1 alleles confer a bidirectional effect on disease outcome.

To explore these associations, we examined the relationship between HLA-DRB1 alleles and disease severity in a case-control study of 2 homogeneous groups of patients at polar extremes of the clinical spectrum. Our findings strongly indicate that HLA-DRB1 alleles exert a bidirectional risk. Certain alleles expressing the high risk epitope QK/QR/RRRAA are highly associated with severe RA, and others carrying the low risk epitope DERAA are significantly reduced in frequency in patients with severe RA, while patients expressing both or indifferent alleles are intermediate in risk. Genotyping for these motifs at the onset of RA may therefore be valuable for predicting the disease course and selecting appropriate therapy.

MATERIALS AND METHODS

Study design and patient selection. The study employed a case-control design to compare 3 groups of Caucasian subjects with severe RA and mild RA and healthy controls at the University of British Columbia. Patients were selected to constitute 2 homogeneous cohorts with clearly defined disease of at least 4 years' duration who were at extremes of the clinical spectrum. Patients in the severe group were drawn from the Mary Pack Arthritis Centre at Vancouver General Hospital. All had severe deforming RA and had failed treatment with at least 3 conventional disease modifying antirheumatic drugs (DMARD). Patients with mild disease were drawn from the antimalarial followup program at the Eye Care Centre at Vancouver General Hospital. All had mild RA with less than 4 swollen joints and no severe deformities, and disease had been controlled with antimalarials for at least 3 years without current or prior use of other DMARD. All patients had been extensively characterized by the treating team, and were reviewed by a rheumatologist (DL, AC) to confirm that they fulfilled the selection criteria. Data collected included age, sex, duration of RA, previous and current therapy, American College of Rheumatology classification of functional status[30], presence or history of extraarticular features, active joint count, swollen joint count, and presence of RA joint deformities. Medical records were reviewed to obtain the results of RF analyses, for which a titer > 1:80 was considered positive. A comparator group of Caucasian subjects selected sequentially from the donor list of the provincial bone marrow transplant registry served as healthy controls. The study was conducted with the approval of the Research Ethics Board of the University of British Columbia and in accord with the Helsinki Declaration.

Genotyping. Peripheral blood was obtained from patients and controls, and genomic DNA extracted by proteinase K digestion and by salting out. Molecular typing at the DRB1 locus was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[31]. Locus or group-specific amplification was performed using 5' and 3' oligonucleotide amplification primers. Genomic DNA (100 ng) was amplified using 50 pmole each of oligonucleotide primers, 100 μM each of dNTP, 1.5 mM $MgCl_2$, and 0.8 units of Taq polymerase in a Perkin-Elmer PCR cycler. The amplified product was digested using the restriction enzymes and subjected to polyacrylamide gel electrophoresis. Gels were stained with ethidium bromide and photographed and alleles assigned on the basis of the RFLP pattern observed. This routine typing process did not distinguish between HLA-DRB1*0401 and the rare allele HLA-DRB1*0416. A group of 25 subjects (5 patients, 25 controls) with HLA-DRB1*0401/0416 were therefore either sequenced through the third hypervariable region or checked using the Bsrl restriction enzyme; all were found to be DRB1*0401. For this study, it was therefore presumed that the majority of individuals with HLA-DRB1*0401/0416 designations were actually DRB1*0401, and they were treated as a homogeneous group.

Statistical methods. Sample size calculation was based upon the detection of a minimum absolute difference in allele prevalence of 35% between severe and mild disease groups. Power analysis based on the chi-square test indicated that a sample size of 50 patients per group was sufficient to provide power between 92% and 95% for detection of such a difference, assuming a prevalence in the comparator population from 0% to 40%, which encompassed the reported range of differences in relevant HLA-DRB1 allele frequencies. Power was enhanced through the use of logistic regression models allowing adjustment for other patient characteristics. Patients with severe RA were enrolled in 2 sequential blocks. No differences in carriage rates were observed between these groups, which were then pooled for this analysis.

Comparison between groups of normally distributed continuous variables was performed using a 2 tailed t test, while the Wilcoxon test was used for non-normally distributed variables. The frequency of HLA-DRB1 alleles in the 2 study groups and controls was compared using the chi-square test with Yates' correction. The increased risk of severe versus mild disease associated with the presence of each allele was estimated by calculation of the odds ratio (OR) and 95% confidence interval (CI). Univariate statistical tests were used to select variables for the logistic regression model, and were not adjusted for the effect of multiple tests. A forward stepwise multiple logistic regression model employing the likelihood ratio was used to calculate the OR of severe versus mild disease associated with the presence of each variable after controlling for other prognostic variables of RA. Covariates evaluated were age, sex, age at disease onset, disease duration, and RF. Significance values associated with the multiple regression were adjusted for multiple testing by multiplying by the number of comparisons made (i.e., 2 comparisons, severe RA vs control, and severe RA vs mild RA).

RESULTS

Patient and disease characteristics. A total of 204 subjects were included in the study, 60 patients with severe RA, 39 with mild RA, and 105 healthy controls. Demographic and clinical features of patients are shown in Table 1. There was no difference in the mean age of patients with severe or mild RA (58 vs 61 years). As expected, the 2 groups differed in prognostic factors of disease severity and in clinical markers of disease activity and severity. Patients in the severe group were more frequently male, and had a younger age of onset of RA and longer duration of disease (median 16 yrs, range 4–51, compared with 10 yrs, range 4–35) than those with mild disease. They also had higher active and swollen joint counts, greater frequency of deformities and extraarticular features, and poorer functional classification. Of the patients with severe RA who were tested, 87% (42/48) were RF positive compared to 36% (10/28) with mild RA. Patients with severe RA had been taking cyclosporine for an average of 26 months, while those with mild disease had been taking antimalarials for an average of 90 months.

Table 1. Demographic and clinical characteristics of patients with severe and mild RA. Values represent mean ± standard deviation or number of subjects (%). Where specified, denominator represents the number of subjects with available data.

Variable	Controls, n = 105	Severe RA,* n = 60	Mild RA*, n = 39
Age, yrs	41 ± 2	58 ± 11	61 ± 13
Female, n (%)	48 (46)	39 (65)	30 (77)
Age of onset, yrs	NA	39 ± 14	49 ± 13
Disease duration, yrs	NA	18 ± 12	12 ± 7
RF positive (%)	NA	42/48 (87)	10/ 28 (36)
Active joint count	NA	14 ± 12	5 ± 5
Swollen joint count	NA	8 ± 8	1 ± 2
Joint deformities (%)	NA	60 (100)	10 (26)
Extraarticular features† (%)	NA	38 (63)	14 (36)
Functional class (%)			
I	NA	0	18/39 (46)
II	NA	21/41 (51)	19/39 (49)
III	NA	16/41 (39)	2/39 (5)
IV	NA	4/41 (10)	0
Duration of therapy, mo	NA	26 ± 22	90 ± 47

* All differences between the 2 groups are significant ($p < 0.05$), except age and sex. † Presence of an extraarticular feature other than sicca syndrome. NA: not applicable.

HLA-DRB1 genotype. The individual frequencies of HLA-DRB1 alleles in the 3 study groups are shown in Table 2. Eighty-seven percent of patients (52/60) with severe RA had one or more of the alleles HLA-DRB1*0101, 0102, 0401, 0404, 0405, 0408, or 1001 bearing the QK/QR/RRRAA motif or HRE, compared with 54% (21/39) with mild RA (OR 5.57, $p = 0.0007$) and 39% (41/105) of controls (OR 10.15, $p < 0.0001$). Fifty percent of patients with severe RA were positive for HLA-DRB1*0401, compared with 23% of those with mild disease (OR 3.3, $p = 0.0136$) and 16% of controls (OR 5.2, $p < 0.0001$). HLA-DRB1*0404 was present in 17% of patients with severe RA compared to 13% with mild RA (OR 1.36, p = NS) and 6% of controls (OR 3.3, $p = 0.044$). HLA-DRB1*0101 and/or 0102 were present in 30% of patients with severe RA compared with 21% with mild RA (OR 1.1, p = NS) and 18% of controls, a difference that became significant only in those subjects who did not express HLA-DRB1*0401. There was no significant difference in the other alleles expressing the HRE. HLA-DRB1*0405 and 0408 were indifferent between the 3 study groups, each being present in less than 3% of patients with severe RA, mild RA, or controls, and HLA-DRB1*1001 was marginally more prevalent in patients with mild disease (p = NS). Twenty-five percent of patients (15/60) with severe disease expressed 2 disease associated HRE DRB1 alleles, compared with 13% of patients (5/39) with mild disease (OR 2.3, p = NS) and 5% (5/105) of controls (OR 6.67, $p = 0.0003$). Seven percent (4/60) of patients with severe disease were homozygous for DRB1*0401/0416, compared with 3% (1/39) of patients with mild disease (OR 2.7, p = NS). No controls were homozygous for these alleles (OR 16.81, $p = 0.0314$).

In contrast, only 5% of patients (3/60) with severe RA expressed one of the LRE alleles HLA-DRB1*0103, 0402, 1102/1103, 1301/1302, which carry the DERAA motif at positions 70–74, compared with 12 subjects (31%) with mild RA (OR 0.12, $p = 0.0013$) and 23 controls (22%) (OR 0.19, $p = 0.0082$). While several of these individual HLA-DRB1* alleles were less common in patients with severe

Table 2. Frequencies of patients and controls expressing HLA-DRB1 alleles.

	Controls, n = 105	Severe RA, n = 60			Mild RA, n = 39			Severe vs Mild RA	
DRB1	%	%	OR*	p*	%	OR*	p*	OR	p
0101	15	27	2.02	NS	18	1.2	NS	1.7	NS
0102	3	8	3.09	NS	3	0.90	NS	3.4	NS
0103	1	0	0.58	NS	8	8.7	NS	0.09	NS
0301	32	25	0.70	NS	21	0.54	NS	1.29	NS
0401	16	50	5.2	< 0.0001	23	1.5	NS	3.33	0.0136
0402	1	2	1.77	NS	3	2.7	NS	0.64	NS
0403/06	3	0	0.24	NS	0	0.37	NS	—	—
0404	6	17	3.3	0.044	13	2.4	NS	1.36	NS
0405	3	2	0.58	NS	0	0.37	NS	1.99	NS
0407	6	2	0.28	NS	0	0.19	NS	1.99	NS
0408	2	0	0.34	NS	0	0.52	NS	—	—
0701	19	19	0.95	NS	21	1.1	NS	0.87	NS
0801/02/03	8	2	0.21	NS	8	1.2	NS	0.20	NS
0901	1	2	1.77	NS	0	0.88	NS	1.99	NS
1001	1	2	1.77	NS	5	5.62	NS	0.31	NS
1102/1103	1	0	0.58	NS	0	0.88	NS	—	—
1101/04	10	8	0.86	NS	10	0.98	NS	0.79	NS
1201	4	0	0.19	NS	0	0.29	NS	—	—
1301/02	19	3	0.15	0.0088	20	1.1	NS	0.14	0.0151
1303	2	0	0.34	NS	3	1.3	NS	0.21	NS
1401	8	0	0.10	NS	8	1.0	NS	0.09	NS
1501/02/03	30	20	0.60	NS	26	0.79	NS	0.72	NS
1601/02/03	5	3	0.69	NS	3	0.52	NS	1.3	NS

* Compared with controls.

RA, this difference was significant only for HLA-DRB1*1301/1302, which were expressed by only 3% of patients with severe RA compared with 20% of those with mild RA (OR 0.14, p = 0.015) and 19% of controls (OR 0.15, p = 0.0088). No patient or control was homozygous for alleles that expressed the LRE.

Epitope analysis. To explore this relationship, patients and controls were divided into 4 categories according to whether they carried alleles that expressed the HRE (QK/QR/RRRAA), the LRE (DERAA), both, or neither. As shown in Table 3, the frequencies of these high and low risk epitopes differed significantly between the patients with severe RA and those with mild RA and controls. Eighty-three percent (50/60) of patients with severe RA expressed the HRE without the LRE, compared with 44% (17/39) of those with mild disease (OR 6.47, p < 0.0001) and 35% (37/105) of controls (OR 9.19, p < 0.0001). In contrast, only one patient (2%) with severe disease expressed the LRE without the HRE, compared with 20% (8/39) of those with mild disease (OR 0.07, p = 0.0047) and 16% (17/105) of controls (OR 0.09, p = 0.009). There was no significant difference between the 3 groups in the frequency of patients who expressed both or neither risk epitope.

Logistic regression analysis was used to examine the relationship between HLA-DR1 genotype and the presence of severe or mild disease after controlling for other prognostic factors. HLA-DRB1 genotype was treated as a categorical variable with 4 groups as identified in Table 3. This model showed that younger age at disease onset (p = 0.0009), longer duration of disease (p = 0.007), male sex (p = 0.07), RF positive status (p = 0.003), and HLA-DRB1 genotype measured by presence of the HRE or LRE (p = 0.00005) were associated with the presence of severe disease. After controlling for other factors, the odds of

Table 3. Frequencies of patients and controls expressing high risk epitope (HRE), low risk eptiope (LRE), both, or neither at HLA-DRB1 locus.

	Controls, n = 105	Severe RA, n = 60			Mild RA, n = 39			Severe vs Mild RA	
Category	%	%	OR*	p*	%	OR*	p*	OR†	p†
HRE	35	83	9.19	< 0.0001	44	1.42	NS	6.47	< 0.0001
Both	6	3	0.57	NS	10	1.89	NS	0.30	NS
Neither	43	12	0.18	< 0.0001	26	0.46	NS	0.38	NS
LRE	16	2	0.09	0.009	20	1.34	NS	0.07	0.0047

* Compared with controls. † Severe compared with mild RA.

Table 4. Logistic regression analysis using clinical data and HLA-DRB1 genotype to predict clinical severity of RA.

Factor	df	Chi-square	p
Age at onset	1	11.07	0.000877
Duration	1	7.36	0.006688
Sex	1	3.30	0.069178
RF	2	11.79	0.002755
HLA-DR	3	22.74	0.000046

Factor	Basis of comparison	OR (95% CI)
Age at onset	per year	0.91 (0.86, 0.97)
Duration of RA	per year	1.00 (0.92, 1.09)
Sex	male/female	3.66 (0.84, 15.82)
RF	pos/neg	10.66 (2.29, 49.56)
RF	NA/neg	2.07 (0.38, 11.19)
HLA-DRB1		
HRE/LRE		27.30 (2.21, 337.72)
Both/LRE		1.90 (0.06, 58.45)
Neither/LRE		0.89 (0.04, 19.40)

Patients with severe RA (n = 60), patients with mild RA (n = 39).
Odds ratios and chi-square statistics are marginal (i.e., have been adjusted for all other factors). Confidence intervals based on Wald approximation to the log-likelihood.
Odds ratios here reflect the distribution of patients observed rather than underlying prevalence of mild and severe RA.
RF: Because results were not available for all patients, subjects were divided into 3 categories consisting of those with a positive RF (pos), negative RF (neg), and those for whom this result was not available (NA). Subjects with missing RF data were not significantly different from those with negative results.
HLA-DRB1: HRE: high risk epitope, LRE: low risk epitope, Both: both high and low risk epitopes, Neither: neither epitope.

having severe disease were roughly 27 times higher for patients with the HRE than for those with the LRE (OR 27.30, CI 2.21 to 337.72) (Table 4). Patients who carried both the HRE and LRE (OR 1.99, CI 0.06–58.45) or neither epitope (OR 0.89, CI 0.04–19.40) had odds of severe disease similar to those expressing the LRE only. The logistic regression model showed good predictive ability in discriminating between subjects with severe and mild disease, with sensitivity of 88% and specificity of 87% when using a cut-point of predicted probability ≥ 0.5 (Figure 1).

DISCUSSION

Immunogenetic studies have produced conflicting results regarding the role of MHC class II genes in RA. Such discrepancies may be related to heterogeneity within the study populations examined in terms of patient ethnicity or disease severity, or to the absence of healthy controls for comparison. In designing this study, special attention was addressed to these factors to allow accurate evaluation of the role of these genes in disease susceptibility and severity. Patients in this study were of Caucasian origin and were deliberately selected to consist of 2 stable cohorts at opposite poles of the disease spectrum defined according to a combination of clinical and therapeutic response criteria. Patients with severe RA had significantly higher numbers of active and swollen joints, joint deformities, and extraarticular features than those with mild disease, and almost 50% were in functional class III-IV despite the use of immune suppressive therapy. Carriage rates in both patient groups were compared with an ethnically comparable matched normal population from the same geographic region.

The results revealed no important allelic differences between patients with mild disease and controls. This may provide an explanation for the paucity of association between HLA-DRB1 alleles and RA reported in the community setting, and diminishes the importance of these antigens as a measure of overall disease susceptibility in clinical practice[32-35]. In contrast, the data show that HLA-DRB1 alleles are closely associated with severe RA, and indicate a spectrum of allelic risk. HLA-DRB1*0401 was most prevalent in subjects with severe disease compared to those with mild disease or controls, followed by HLA-DRB1*0404 and 0101, while HLA-DRB1*0405/0408 and HLA-DRB1*0101/0102 had low carriage rates, which were indifferent between the study groups. Of the alleles negatively associated with severe disease, the most powerful link was with HLA-DRB1*1301/1302, followed by HLA-DRB1*0103 and HLA-DRB1*0402. These data are consistent with reports that show a variable strength of positive association of high risk alleles in Caucasian subjects and a preferential expression in patients with severe disease[9,10,12,13]. They

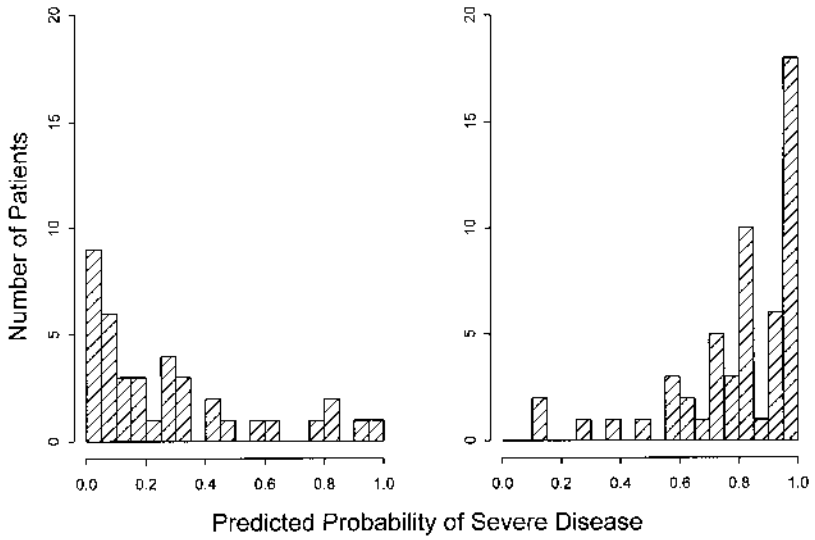

Figure 1. Goodness-of-fit analysis of the logistic regression model using clinical data and HLA-DRB1 genotype to predict clinical severity of RA.

confirm the negative association recently reported with HLA-DRB1*1301/1302, but do not support a putative protective influence of HLA-DR2, DR3, DR5, or DR7[19,20,24,26]. The data suggest, but do not confirm, that homozygosity or compound heterozygosity may potentiate the clinical effect[36-38]. The proportion of individuals homozygous for high-risk alleles was 2 to 5-fold higher in patients with severe disease than in those with mild RA or controls, while no patient in either group was homozygous for low risk alleles. However, the limited number of patients in any of these groups rendered detailed analysis of gene dose impractical.

It has been postulated that the effect of HLA-DRB1 alleles in RA is related to the expression of individual amino acid motifs at positions 70–74 within the third hypervariable region. Roughly 90% of patients with severe disease expressed the HRE (QK/QR/RRAA), a rate almost twice that observed among patients with mild disease or healthy controls. In contrast, 36% of patients with mild disease expressed the LRE (DERAA), a rate 5-fold higher than in patients with severe disease. Whether this LRE confers protection against the development of severe disease cannot be established conclusively from these data[39]. However, the probability of severe disease in patients expressing both HRE (QK/QR/RRRAA) and LRE (DERAA) was comparable to that in subjects expressing neither (null) motif, suggesting that the influence of the HRE is modified by inheritance of the LRE.

Despite the lack of standardized patient selection criteria for immunogenetic studies, the data shown here are remarkably consistent with reports using clinical or radiological measures of disease severity[27,36-38,40-45]. These studies show HLA-DRB1*0401 to be the most prevalent high risk allele in European Caucasians, while HLA-DRB1*0101 and other high risk alleles appear to confer a similar risk in patients from Central America and other geographic regions. They indicate that the HRE (QK/QR/RRRAA) is associated with an elevated Ritchie score, extraarticular manifestations, radiological damage, joint surgery, and progression to disability. Finally, they provide variable evidence for a relationship between disease severity and allele dose or compound heterozygosity, and suggest that the LRE (DERAA) may decrease susceptibility to or progression of RA. In a recent cross sectional investigation, Mattey, *et al* have shown the HRE to be associated with more severe and the LRE with less severe radiological damage[27]. Patients with 2 high risk alleles had significantly higher Larsen scores, while patients who were homozygous for HLA-DRB1*0401/0401 exhibited the most severe damage. The lowest Larsen score was observed in patients carrying the LRE without an accompanying HRE, although in contrast to the results reported here, possession of the LRE did not reduce the severity of radiological damage in patients carrying the HRE[27].

The molecular function of the HRE and LRE in determining disease outcome remains uncertain. These motifs differ by 2 amino acids at positions 70 and 71, the HRE encoding glutamine/arginine[70] and lysine/arginine[71], compared with aspartate[70] and glutamate[71] in the LRE[46]. These amino acids differ in electrostatic charge, those in the HRE expressing a neutral or positive charge, while those in the LRE are both negatively charged. It is possible that these electrostatic charges may influence the disease by determining the binding pattern of antigenic peptides within the peptide groove. Studies using high performance liquid chromatography have recently shown that RA associated HLA-DRB1 molecules bind more avidly to negatively charged amino acids such as aspartic acid and glutamic acid than to

those positively charged, like arginine and lysine[46]. The risk modifying effect of these alleles is not determined solely by the electrostatic charge of the 2 amino acids, since HLA-DRB1*03 alleles also carry QK at positions 70–71 but differ from the HRE motif at positions 72–74, and are not associated with a high risk of disease progression. Other amino acids outside the 70–74 motif may therefore be critical in determining peptide binding or receptor structure. Alternatively, the importance of the HLA-DRB1 sequence motif may lie more in its structural similarity to certain viral proteins (EBV gp110) and DNAJ heat shock proteins of bacteria like *Escherichia coli*, *Lactobacillus lactis*, and *Brucella ovis*[47]. Based upon a series of elegant murine studies, Zanelli, *et al* recently proposed that HLA-DQ is the critical molecule for presentation of the arthritogenic antigen in RA, and that the HRE and LRE trigger opposing effects when presented by HLA-DQ[39,48]. Studies in transgenic mice provide support for this theory and show that DQ binding and T cell receptor recognition are both determined by the DERAA motif[49,50].

In summary, HLA-DRB1 alleles appear to confer an important bidirectional influence on the risk of disease severity in RA, with 20-fold difference in odds ratios between those associated with the highest (HLA-DRB1*0401) and lowest (HLA-DRB1*1301/1302) risk. The HRE and LRE exhibit diametrically opposed effects that may be mutually antagonistic, but expression of these motifs does not fully explain disease susceptibility or severity. In this study, 14% of patients with severe RA did not possess the HRE, while almost two-thirds of those with mild disease did not carry the LRE, indicating that other genetic or environmental factors are important in determining the progression of RA. These observations reinforce the concept of a multistep pathogenesis in which MHC class II genes are one component of a coordinate genetic and environmental interaction leading to immunological injury and joint destruction[51-56]. Recent population based data suggest that the HLA-DRB1 shared epitope and RF are independently associated with radiographic outcome in RA, and the effect of HLA-DRB1 is expressed preferentially in patients who are RF negative[57]. We did not directly examine this issue in this study, which was constructed using a case comparison design and was not intended to parallel normal clinical practice, and RF results were not available from all patients, limiting the power to translate these observations to routine care. In addition, because the patient groups were highly selected and differed fundamentally in their treatment, it was impossible to determine whether the relationship reflected primarily an alteration in disease expression or in therapeutic response[58]. Nonetheless, the goodness-of-fit model suggests that HLA-DRB1 typing may be a valuable tool for predicting disease outcome and/or therapeutic response, although whether this is superior or complementary to RF must now be tested in formal and appropriately designed clinical studies.

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