EXTENDED REPORT

HLA-DR-DQ haplotypes and genotypes in Finnish patients with rheumatoid arthritis

S Laivoranta-Nyman, T Möttönen, R Hermann, J Tuokko, R Luukkainen, M Hakala, P Hannonen, M Korpela, U Yli-Kerttula, A Toivanen, J Ilonen, and the FIN-RACo Trial Group

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Objectives: To elucidate the contribution of HLA-DR-DQ haplotypes and their genotypic combinations to susceptibility to rheumatoid arthritis, and to evaluate the various models for HLA associated risk for the disease in a series of Finnish patients.

Methods: 322 Finnish patients with rheumatoid arthritis were typed for common north European HLA-DR-DQ haplotypes and compared with a series of 1244 artificial family based control haplotypes.

Results: The association of the so called shared epitope (SE) haplotypes (DRB1*0401, *0404, *0408, and *01) with rheumatoid arthritis was confirmed. The DRB1*0401 haplotypes carried a far stronger risk for the disease than the (DRB1*01/10)-(DQA1*01)-DQB1*0501 haplotypes. Seven protective HLA haplotypes—(DRB1*15)-(DQA1*01)-DQB1*0602; (DRB1*08)-(DQA1*04)-DQB1*04; (DRB1*11/12)-DQA1*05-DQB1*0301; (DRB1*1301)-(DQA1*01)-DQB1*0603; (DRB1*1302)-(DQA1*01)-DQB1*0604; (DRB1*07)-DQA1*0201-DQB1*0303; and (DRB1*16)-(DQA1*01)-DQB1*0502—were identified. In accordance with the reshaped shared epitope hypothesis, all the protective DRB1 alleles in these haplotypes share either isoleucine at position 67 or aspartic acid at position 70 in their third hypervariable region motif. However, differences in the disease risk of haplotypes carrying the same DR but different DQ alleles were also found: (DRB1*07)-DQA1*0201-DQB1*0303 was protective, while (DRB1*07)-DQA1*0201-DQB1*02 was neutral. The same haplotypes carried different risks for rheumatoid arthritis depending on their combination in genotypes.

Conclusions: When assessing the influence of HLA genes on the susceptibility to rheumatoid arthritis, not only should the HLA-DR or -DQ alleles or haplotypes be unravelled but also the genotype. The effect of HLA class II region genes is more complicated than any of the existing hypotheses can explain.

See end of article for authors' affiliations

Correspondence to:
Dr S Laivoranta-Nyman,
Medical Research
Laboratory, Turku
University, Tykistökatu 6A,
FIN-20520 Turku, Finland;
susanna.laivoranta-
nyman@utu.fi

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Rheumatoid arthritis is a polygenic autoimmune disease. The most important genes that influence the susceptibility to the disease are located within the human major histocompatibility complex (MHC) or the HLA region on the short arm of chromosome 6 (6p21). The first findings of an increase in HLA-Dw4 and -DR4 specificity among patients with rheumatoid arthritis were made in the 1970s by Stastny.^{1,2} Thereafter our knowledge of the associations of the different DRB1 alleles in various ethnic groups with rheumatoid arthritis susceptibility has increased gradually.³ In 1987 Gregersen with coworkers formulated the shared epitope (SE) hypothesis, based on the finding that HLA-DRB1 alleles associating with the disease (DRB1*0101, *0401, *0404, *0405, and *0408) share a common amino acid sequence (Q⁷⁰(K/R)RAA⁷⁴) in their third hypervariable region (HVR3).⁴ The hypothesis was strengthened later on by the finding that the rare allele DRB1*1402 with the same QRRAA motif was associated with susceptibility to rheumatoid arthritis in native North Americans.⁵ DRB1*1001 (with RRRAA in the HVR3) has also been included in shared epitope alleles to explain the association of DRB1*1001 with rheumatoid arthritis in some other populations.^{6,7} Later on, several studies on various ethnic groups have proved the association between the shared epitope alleles and rheumatoid arthritis, although there is a discrepancy over whether the association is more pronounced with the severity of the disease than with the susceptibility.^{8–14}

On the other hand, in addition to susceptibility genes there are also HLA class II alleles or haplotypes that appear to provide protection against rheumatoid arthritis.^{15–19} In 1999,

Zanelli and his coworkers formulated a so called rheumatoid arthritis protection (RAP) model.²⁰ According to this, the haplotypes HLA-DQA1*01-DQB1*0501 (DQ5) (linked to DRB1*0101, *0102, *0103, and *1001) and DQA1*03-DQB1*03 (DQ3) (linked to DRB1*0901 or any DRB1*04 allele) predispose to rheumatoid arthritis, while the DRB1 alleles carrying the motif D⁷⁰ERAA⁷⁴ in their HVR3 (*0103, *0402, *1102, *1103, *1301, and *1302) protect against the disease.²¹ Afterwards, studies both supporting and contradicting this model have been published.^{22–24}

Alternative ways to interpret the effect of non-susceptibility or shared epitope negative HLA-DRB1 alleles have also been presented. Revron and coworkers classified the shared epitope negative alleles according to the electric charge in the P4 pocket of the DRB1 molecule.¹⁸ They found that DRB1 alleles having either neutral or negative electric charge in the P4 pocket (DRB1*0103, *0402, *07, *08, *11 (except *1107), *12, and *13) protect against rheumatoid arthritis, while the alleles having a positive electric charge in the P4 pocket (DRB1*03, *0403, *0406, *0407, *0901, *1107, *14, *15, and *16) are neutral with respect to predisposition to rheumatoid arthritis. A similar concept was proposed by de Vries *et al*, who noted that isoleucine (I) at position 67 and aspartic acid (D) at position 70 were shared by alleles protective against rheumatoid arthritis.¹⁹ In that study they emphasised the

Abbreviations: AFBAC, affected family based artificial controls; HLA, human leucocyte A; HVR, hypervariable region; MHC, major histocompatibility complex; RAP, rheumatoid arthritis protection; RF, rheumatoid factor

HLA associated risk encoded by amino acid positions 67–74 of the HLA-DRB1 molecule, and included both the effect of the shared epitopes and the protective DRB1 alleles.

Our aim in the present study was to clarify the contribution of HLA-DR-DQ haplotypes to rheumatoid arthritis susceptibility and to analyse how the various models for HLA associated risk for this disease fit with affected Finnish patients. The genotypic effect of both inherited haplotypes was also analysed to detect additional or synergistic risk effects as well as the mode of possible protection.

METHODS

All patients gave informed consent before inclusion and all local ethics committees of participating centres approved this research.

The study included 322 patients with rheumatoid arthritis from three separate Finnish studies:

- 97 patients were probands of multiplex rheumatoid arthritis families (some of whom were also included in two earlier studies^{25–26});
- 65 patients were from the HLA haplotype work by Tuokko *et al*^{27–28};
- 160 patients were from the so-called FIN-RACo trial.

All patients fulfilled the rheumatoid arthritis criteria of the American College of Rheumatology (formerly the American Rheumatism Association).²⁹ In all, 217 patients (67%) were female and 249 (77%) were rheumatoid factor (RF) positive. The RF status was not known for five patients. Erosions were found in the hand or foot joints in 215 of the patients (67%). Information about the presence of erosions was missing in six cases.

As controls we used untransmitted haplotypes found in a series of 622 Finnish families with a diabetic child.³⁰ Affected family based artificial controls (AFBAC) were formed by combining untransmitted haplotypes found only in healthy parents. This type of control series has been shown to be representative of the background population^{31–32}; it can thus be regarded as a suitable control group, although in this case the special advantage offered by familial selection was not obtained. The representativeness of the AFBAC group for the Finnish population is also shown by the fact that no significant differences in haplotype frequency of AFBAC groups from diabetic and multiple sclerosis families were seen.^{30–33}

For all the study subjects, DNA was extracted from anticoagulated blood samples using a salting out method.³⁴ HLA-DRB1 alleles were determined by sequence specific polymerase chain reaction (PCR) amplification.³⁵ For DRB1*04 subtyping we used either a sequence specific PCR and dot-blot hybridisation³⁵ or a high resolution, time resolved fluorometer based technique.³⁶ HLA-DQB1 typing was undertaken using either the so called HLA-DQB1 “full house” typing capable of detecting DQB1*02, *0301, *0302, *0303, *04, *0501, *0502, *0503, *0601, *0602, *0603, and *0604 alleles,³³ or as a smaller screening procedure, capable of detecting *02, *0301, *0302, *0602, *0603, and *0604 alleles.³⁷ The principle of both HLA-DQB1 typing methods was the same as that described earlier by Sjöroos *et al*.³⁶ HLA-DQB1 typing was complemented by detection of HLA-DQA1 when it provided further information about the definition of the haplotypes. HLA-DQA1 typing was done using time resolved fluorescence hybridisation, as described earlier.³⁸ HLA-DQB1 full house or DQB1 screening and DRB1 typing were done in all patients except the nine in whom the HLA-DRB1 typing was not done. In those cases, as well as in the AFBACs, the HLA-DR-DQ haplotypes were deduced from the DQB1 full

house typing and if necessary the results were also complemented by DR4 subtyping and DQA1 typing.

The great majority of the DQB1, DQA1, and DRB1 combinations observed in both patients and controls (AFBAC) were consistent with standard northern European haplotypes^{39–40} and the results among patients did not differ depending on the initial typing techniques used. We excluded two patients of the 67 from Tuokko’s study because the HLA DR-DQ haplotype was unconventional. For the same reason we excluded 11 patients from the FIN-RACo group, where DNA samples were available from 176 patients; one patient whose information was insufficient and four already included in the family study were also excluded from this group. Homozygosity for a given allele was assumed when only one allele could be found in all the HLA typings made. Within the control material the number of homozygotes did not differ from that expected based on the Hardy–Weinberg equilibrium.

Statistics

The frequencies of HLA haplotypes and genotypes were compared using a χ^2 test with continuity correction. Fisher’s exact test was used when appropriate. Relative risk (RR) was calculated as the odds ratio (OR) with 95% confidence intervals in 2×2 tables; Haldane’s correction was used if one of the numbers in the 2×2 table was zero.⁴¹

RESULTS

Susceptibility haplotypes determined from HLA-DR-DQ haplotypes

The frequencies of the DR-DQ haplotypes in the 322 patients with rheumatoid arthritis (644 haplotypes) and 622 AFBACs (1244 haplotypes) are shown in table 1. The susceptibility haplotype group included all shared epitope haplotypes. The highest risk was associated with the haplotype DRB1*0401-(DQA1*03)-DQB1*0301 (OR = 7.56 (95% confidence interval, 4.02 to 14.42); $p < 10^{-6}$). Interestingly, the same HLA-DRB1 specificity *0401 in the haplotype DRB1*0401-(DQA1*03)-DQB1*0302 was less associated with susceptibility (OR = 2.93 (2.13 to 4.02); $p < 10^{-6}$) than in the haplotype associated with DQB1*0301 (DRB1*0401-(DQA1*03)-DQB1*0301 *v* DRB1*0401-(DQA1*03)-DQB1*0302, OR = 2.71 (1.34 to 5.52); $p = 0.0039$). Further, the two other shared epitope related haplotypes—DRB1*0408-(DQA1*03)-DQB1*0301 and DRB1*0404-(DQA1*03)-DQB1*0302—also showed increased susceptibility for rheumatoid arthritis: OR = 5.59 (2.07 to 15.93); $p = 0.00013$; and OR = 2.00 (1.25 to 3.18); $p = 0.0028$, respectively. Thus we grouped these four haplotypes as “strong susceptibility” haplotypes (S) (table 1). The most frequent susceptibility haplotype both in patients (162 of 644; 25.2%) and in controls (225 of 1244; 18.1%) was (DRB1*01/10)-(DQA1*01)-DQB1*0501. This carried only a slightly increased risk for rheumatoid arthritis (OR = 1.52 (1.20 to 1.93); $p = 0.00039$) and was therefore classified as a “weak susceptibility” haplotype (s).

Protective haplotypes

In comparison with controls, seven haplotypes were significantly decreased among the patients with rheumatoid arthritis and were thus designated protective haplotypes. The most protective was the HLA-(DRB1*16)-(DQA1*01)-DQB1*0502 (OR = 0.17 (0.02 to 1.30); $p = 0.048$). However, the frequency of the haplotype in both the patients and the AFBACs was very small (1/644 (0.2%) and 11/1244 (0.9%), respectively). Additionally, three other haplotypes—(DRB1*07)-DQA1*0201-DQB1*0303, (DRB1*1301)-(DQA1*01)-DQB1*0603, and (DRB1*1302)-(DQA1*01)-DQB1*0604—were associated with low odds ratios to rheumatoid arthritis and are grouped as “strongly protective” (P). The

Table 1 Frequencies of all defined HLA-DR-DQ haplotypes in 322 patients with rheumatoid arthritis and 622 affected family based artificial controls, and their classification into haplotype risk groups

Haplotype risk group	DRB1-DQA1-DQB1 haplotype	Patients (n = 644 haplotypes)		Controls (AFBAC) (n = 1244 haplotypes)		OR	95% CI	p Value*
		N	%	N	%			
Susceptibility haplotypes								
S	0401-(03)-0301	51	7.9	14	1.1	7.56	4.02 to 14.42	<10 ⁻⁶
S	0408-(03)-0301	17	2.6	6	0.5	5.59	2.07 to 15.93	0.00013
S	0401-(03)-0302	109	16.9	81	6.5	2.93	2.13 to 4.02	<10 ⁻⁶
S	0404-(03)-0302	41	6.4	41	3.3	2.00	1.25 to 3.18	0.0028
s	(01/10)-(01)-0501	162	25.2	225	18.1	1.52	1.20 to 1.93	0.00039
Neutral haplotypes								
N	0407-(03)-0301	2	0.3	3	0.2	1.29	0.10 to 9.45	
N	(03)-05-02	37	5.7	95	7.6	0.74	0.49 to 1.11	
N	(07)-0201-02	19	3.0	51	4.1	0.71	0.40 to 1.25	
N	(09)-03-0303	18	2.8	52	4.2	0.66	0.37 to 1.17	
Protective haplotypes								
P	(15)-(01)-0602	65	10.1	200	16.1	0.59	0.43 to 0.80	0.00050
p	(08)-(04)-04	39	6.1	125	10.0	0.58	0.39 to 0.85	0.0046
p	(11/12)-05-0301	35	5.4	114	9.2	0.57	0.38 to 0.86	0.0058
P	(1302)-(01)-0604	9	1.4	42	3.4	0.41	0.18 to 0.87	0.018
P	(1301)-(01)-0603	21	3.3	109	8.8	0.35	0.21 to 0.58	0.000012
P	(07)-0201-0303	4	0.6	31	2.5	0.24	0.09 to 0.73	0.0020†
P	(16)-(01)-0502	1	0.2	11	0.9	0.17	0.02 to 1.30	0.048†
Other haplotypes								
O	0403-(03)-0302	7	1.1	7	0.6	1.94	0.61 to 6.17	
O	(09)-03-02	0	0	1	0.1	0.64		
O	(14)-(01)-0503	6	0.9	26	2.1	0.44	0.16 to 1.13	
O	0403-(03)-0304	0	0	2	0.2	0.39		
O	x-03-0301	1	0.2	8	0.6	0.24	0.02 to 2.21	

*Only statistically significant p values are shown.

†By Fisher's exact test.

AFBAC, affected family based artificial controls; CI, confidence interval; N, neutral; O, other; OR, odds ratio; P, strongly protective; p, weakly protective; S, Strong susceptibility; s, weak susceptibility.

most frequent protective haplotype both in rheumatoid patients and AFBACs was HLA-(DRB1*15)-(DQA1*01)-DQB1*0602. Its protective effect against rheumatoid arthritis was significant (OR = 0.59 (0.43 to 0.80); $p = 0.00050$), although weaker than the strongly protective haplotypes. This and two other protective haplotypes—(DRB1*08)-(DQA1*04)-DQB1*04 and (DRB1*11/12)-DQA1*05-DQB1*0301 (with ORs of 0.58 and 0.57, respectively)—were grouped as “weakly protective” (p) (table 1).

Neutral and other haplotypes

Four haplotypes—(DRB1*07)-DQA1*0201-DQB1*02, DRB1*0407-(DQA1*03)-DQB1*0301, (DRB1*03)-DQA1*05-DQB1*02, and (DRB1*09)-DQA1*03-DQB1*0303—were grouped as “neutral haplotypes” (N), as no significant differences in their frequencies were found in rheumatoid patients and AFBACs. Five further haplotypes were associated either with high or low odds ratios, but differences in the frequencies of these relatively rare haplotypes were not

Table 2 HLA-DRB1-DQA1-DQB1 and risk group genotypes among which either an increase or a decrease of the odds ratio was found to be statistically significant

DRB1-DQA1-DQB1 genotype	Risk group genotype	Patients (n = 322)		Controls (AFBAC) (n = 622)		OR	95% CI	p Value
		n	%	n	%			
0401-(03)-0301, 0401-(03)-0302	SS	15	4.7	1	0.2	30.34	4.20 to 121.91	8.7 × 10 ^{-7*}
0401-(03)-0301, 0401-(03)-0301	SS	6	1.9	0	0	25.57†	3.13 to 208.73†	0.0015*
0401-(03)-0301, (14)-(01)-0503	SO	3	0.9	0	0	13.64†	1.52 to 122.53†	0.039*
0401-(03)-0301, (1/10)-(01)-0501	Ss	10	3.1	2	0.3	9.94	2.04 to 38.64	6.2 × 10 ^{-4*}
0408-(03)-0301, (1/10)-(01)-0501	Ss	8	2.5	2	0.3	7.90	1.55 to 34.60	0.0038*
0408-(03)-0301, (11/12)-05-0301	Sp	4	1.2	1	0.2	7.81	0.83 to 83.87	0.049*
0401-(03)-0302, (1/10)-(01)-0501	Ss	39	12.1	13	2.1	6.46	3.27 to 12.95	<10 ⁻⁶
0401-(03)-0302, 0401-(03)-0302	SS	7	2.2	3	0.5	4.59	1.06 to 19.37	0.022*
0401-(03)-0302, 0404-(03)-0302	SS	7	2.2	3	0.5	4.59	1.06 to 19.37	0.022*
(1/10)-(01)-0501, (1301)-(01)-0603	sP	6	1.9	30	4.8	0.37	0.14 to 0.96	0.038
(15)-(01)-0602, (1301)-(01)-0603	pP	2	0.6	16	2.6	0.24	0.05 to 1.08	0.027*
(08)-(04)-04, (1301)-(01)-0603	pP	1	0.3	10	1.6	0.19	0.02 to 1.44	0.067*
(07)-0201-0303, (15)-(01)-0602	Pp	0	0	6	1.0	0.15†	0.02 to 1.20†	0.082*
(15)-(01)-0602, (1302)-(01)-0604	pP	0	0	7	1.1	0.13†	0.02 to 1.02†	0.054*
(03)-05-02, (1301)-(01)-0603	NP	0	0	8	1.3	0.11†	0.01 to 0.89†	0.035*
(03)-05-02, (1302)-(01)-0604	NP	0	0	8	1.3	0.11†	0.01 to 0.89†	0.035*

*By Fisher's exact test.

†Haldane's correction is used when odds ratios and 95% confidence intervals are calculated.

AFBAC, affected family based artificial controls; CI, confidence interval; OR, odds ratio.

Table 3 Risk group genotypes in 322 rheumatoid arthritis patients and 622 affected family based artificial controls

Risk group genotype	Patients (n = 322)		Controls (AFBAC) (n = 622)		OR	95% CI	p Value*
	n	%	n	%			
S/S	38	11.80	7	1.13	11.76	4.96 to 29.21	<10 ⁻⁶
S/s	64	19.88	26	4.18	5.69	3.44 to 9.45	<10 ⁻⁶
N/S	19	5.90	18	2.89	2.10	1.04 to 4.26	0.038
s/s	17	5.28	17	2.73	1.98	0.95 to 4.14	
S/P	13	4.04	16	2.57	1.59	0.71 to 3.55	
S/p	41	12.73	62	9.97	1.32	0.85 to 2.05	
N/s	22	6.83	39	6.27	1.10	0.62 to 1.94	
s/p	28	8.70	76	12.22	0.68	0.42 to 1.10	
N/p	22	6.83	67	10.77	0.61	0.36 to 1.03	
s/P	12	3.73	46	7.40	0.48	0.24 to 0.96	0.037
p/p	20	6.21	79	12.70	0.46	0.26 to 0.78	0.0029
N/N	4	1.24	21	3.38	0.36	0.11 to 1.12	0.037†
p/O	2	0.62	16	25.72	0.24	0.05 to 1.08	0.027
N/P	3	0.93	29	4.66	0.19	0.06 to 0.67	0.0012†
P/p	6	1.86	60	9.65	0.18	0.07 to 0.43	0.000016
P/P	0	0	18	2.89	0.05	0.01 to 0.38	0.00050

*Only statistically significant p values are shown.

†By Fisher's exact test.

AFBAC, affected family based artificial controls; CI, confidence interval; OR, odds ratio.

significant and they were thus grouped as "other haplotypes" (O) (table 1).

Genotypes grouped according to the HLA-DR-DQ haplotypes

The total number of various genotypes found in this study was 136. Those with either significant susceptibility or protective odds ratios against rheumatoid arthritis are listed in table 2. We found nine susceptibility and seven protective genotypes. The respective odds ratios in the susceptibility and protective groups varied from 4.59 to 30.34 and from 0.11 to 0.37, respectively (table 2). Although there was a wide overlap within the confidence intervals of the odds ratio values, the genotypes with the strongest association with rheumatoid arthritis were those combining two strong susceptibility haplotypes. Only one genotype combining a protective haplotype ((DRB1*11/12)-DQA1*05-DQB1*0301) with the risk associated haplotype DRB1*0408-(DQA1*03)-DQB1*0301 was seen among susceptibility genotypes, although the number of patients was small and the statistical significance borderline.

Genotypes grouped according to the haplotype risk groups

Because of the small numbers of several individual genotypes we also regrouped the genotypes according to the haplotype risk groups. In table 3 both the significant and non-significant susceptibility and protective genotypes are shown, arranged according to their odds ratios.

As expected, the individuals homozygous for the strong susceptibility haplotypes (S/S) proved to carry the highest risk for rheumatoid arthritis (OR = 11.76 (4.96 to 29.21); $p < 10^{-6}$), while those homozygous for strongly protective haplotypes (P/P) had the lowest risk for rheumatoid arthritis (OR = 0.05 (0.01 to 0.38); $p = 0.00050$). No dominant protection could be found by either strongly or weakly protective haplotypes in combination with a strong susceptibility haplotype (S/P, S/p): OR = 1.59 (0.71 to 3.55); NS; and OR = 1.32 (0.85 to 2.05); NS, respectively. On the other hand, both strongly and weakly protective haplotypes were found to be protective when in combination with a weak susceptibility haplotype (s/P, s/p). Nevertheless, only the associated with the strongly protective haplotype reached statistical significance (s/P: OR = 0.48 (0.24 to 0.96); $p = 0.037$; table 3). The difference in the risk of developing rheumatoid arthritis among patients with the disease was most prominent when

two strong susceptibility haplotypes (S/S) and two strongly protective haplotypes were compared (S/S v P/P: OR = 87.28 (11.92 to 639.40); $p < 10^{-8}$; not shown).

Comparison of current results with established models

Table 4 summarises the characterised haplotypes and shows whether they include the shared epitope, the proposed rheumatoid arthritis predisposing DQ antigen (DQ^{RA}), and the DERAA motif. The electrical charge in the P4 pocket of the DRB1 molecules as well as the HVR3 67–74 amino acid sequences are also presented in table 4.

All protective haplotypes in the present study share either isoleucine (I) at position 67 or aspartic acid (D) at position 70 (table 4), fitting the reshaped shared epitope hypothesis.¹⁹ There was only one haplotype with both isoleucine at position 67 and aspartic acid at position 70 which did not show a protective effect—namely, the haplotype (DRB1*07)-DQA1*0201-DQB1*02. This was found to be neutral.

In accordance with the RAP model, the present results also show that all our HLA-DR-DQ haplotypes including DRB1 alleles with the DERAA motif in HVR3 could be classified as either strongly or weakly protective (P/p).²⁰ However, we also found four other haplotypes—(DRB1*15)-(DQA1*01)-DQB1*0602, (DRB1*08)-(DQA1*04)-DQB1*04, (DRB1*07)-DQA1*0201-DQB1*0303, and (DRB1*16)-(DQA1*01)-DQB1*0502—which showed significant protection. Further, the protective effect of these haplotypes also remained detectable in their combined genotypes without haplotypes positive for the DERAA motif: OR = 0.39 (0.20 to 0.75); $p = 0.0034$ (table 5).

On the other hand, as proposed by Revirón *et al.*,¹⁸ the P4 pocket in the HVR3 of DRB1 molecule of all the protective haplotypes found, with the exceptions of (DRB1*15)-(DQA1*01)-DQB1*0602 and (DRB1*16)-(DQA1*01)-DQB1*0502, were either negatively or neutrally charged. Only the (DRB1*07)-DQA1*0201-DQB1*02 haplotype with neutral electrical charge in the P4 pocket of the DRB1 molecule appeared to be neutral rather than protective against rheumatoid arthritis. On the other hand, all DRB1 alleles seen in this study that have neutral or negative charge in their pocket 4 had aspartic acid (D) at position 70 in the HVR3 (table 4).

Table 6 shows frequencies of the genotypes organised according to the previously proposed models for the HLA associated risk for rheumatoid arthritis. The first three genotypes are grouped according to the genotype risk ratings

Table 4 Presence of the shared epitope, proposed rheumatoid arthritis predisposing DQ antigen (DQ^{RA}), DERA motif, and HLA-DR-DQ haplotype risk groups seen in the present study, with the amino acid sequences in the HVR3 and the electrical charge in P4 pocket of DRB1 molecule of those included

DRB1-DQA1-DQB1 haplotype	SE	DQ ^{RA} /DERAA	Haplotype risk group	Electrical charge in P4 pocket	HVR3 amino acid positions				
					67	70	71	73	74
0401-(03)-0301	+	DQ3	S	++	L	Q	K	A	A
0408-(03)-0301	+	DQ3	S	++	L	Q	R	A	A
0401-(03)-0302	+	DQ3	S	++	L	Q	K	A	A
0404-(03)-0302	+	DQ3	S	++	L	Q	R	A	A
(01/10)-(01)-0501	+	DQ5	s	+/++	L	Q/R	R	A	A
0407-(03)-0301	-	DQ3	N	+	L	Q	R	A	E
(03)-05-02	-	-	N	++	L	Q	K	G	R
(07)-0201-02	-	-	N	n	I	D	R	G	Q
(09)-03-0303	-	DQ3	N	+	F	R	R	A	E
(15)-(01)-0602	-	-	p	+	I	Q	A	A	A
(08)-(04)-04	-	-	p	n	F	D	R	A	L
(1101)-05-0301	-	-	p	n	F	D	R	A	A
(1102)-05-0301	-	DERAA	p	-	I	D	E	A	A
(1103)-05-0301	-	DERAA	p	-	F	D	E	A	A
(12)-05-0301	-	-	p	n	I	D	R	A	A
(1302)-(01)-0604	-	DERAA	P	-	I	D	E	A	A
(1301)-(01)-0603	-	DERAA	P	-	I	D	E	A	A
(07)-0201-0303	-	-	P	n	I	D	R	G	Q
(1601)-(01)-0502	-	-	P	+	F	D	R	A	A
0403-(03)-0302	-	DQ3	O	+	L	Q	R	A	E
(09)-03-02	-	-	O	+	F	R	R	A	E
(14)-(01)-0503	-	-	O	+	L	R	R	A	E
0403-(03)-0304	-	-	O		L	Q	R	A	E

n, neutral; N, neutral haplotype; O, other haplotype; P, strongly protective haplotype; p, weakly protective haplotype; S, strong susceptibility haplotype; s, weak susceptibility haplotype; SE, shared epitope.

found, as presented in table 3. In all, 121 patients with rheumatoid arthritis (37.6%) and 51 AFBACs (8.2%) were homozygous for the susceptibility haplotypes and were classified as risk genotypes. On the other hand, 47 patients with rheumatoid arthritis (14.6%) and 269 AFBACs (43.3%) were grouped to genotypes protecting significantly against rheumatoid arthritis. Interestingly, almost exactly the same frequencies of rheumatoid patients and AFBACs were homozygous for either the shared epitope positive or negative haplotypes, although the selections of the patients in these two groupings were not exactly identical.

We saw that a strongly protective haplotype appears to be dominant when combined with a weak but not a strong risk haplotype (table 3). Similarly, in contrast to DQ3+/DERAA+ the genotype DQ5+/DERAA+ shows protection against rheumatoid arthritis (table 6). Finally, the rheumatoid patients homozygous for the haplotype not containing either the predisposing DQ^{RA} or the protective DERA motif (DQ^{RA}-/-) showed a statistically significant decrease in the risk of rheumatoid arthritis (OR = 0.48 (0.31 to 0.74); $p = 0.00056$), again suggesting the existence of other protective genetic elements than the DERA motif.

DISCUSSION

Our study was aimed at clarifying the effects of HLA-DR-DQ haplotypes and their genotypic combinations on the

susceptibility to rheumatoid arthritis and at evaluating the feasibility of published models for interpreting the association between HLA-DR, HLA-DQ, and rheumatoid arthritis in Finnish patients with the disease. The limitation of the study was the deduction of HLA-DR-DQ haplotypes in controls and in nine patients. Unconventional haplotypes were found in 13 patients (13/336, $\approx 3.9\%$) and, as earlier mentioned, those were left out of the study. The amount of these aberrant or rare haplotypes is similar to those observed in the earlier studies.^{39, 40} HLA haplotypes were divided into different groups according to the haplotype specific disease risk they conferred, and their effects in various genotypes were further evaluated.

Our results confirm that the protective DR-DQ haplotypes include DRB1 molecules with HVR3 amino acid similarities, although the classification using these specific positions (67, 70, 71, 73, and 74) in HVR3 is not sufficient to explain the haplotypic effects we detected. The reshaped shared epitope hypothesis suggested by de Vries and coworkers emphasises the protective effect of isoleucine (I) at position 67 and aspartic acid (D) at position 70. A protective effect of alleles encoding aspartic acid (D) at position 70 has also been described by del Rincón *et al* and by Matthey *et al*.^{15, 16}

Our results also confirm that amino acid changes in the HVR3 of DRB1 molecule—both at position 67 (leucine (L) to isoleucine (I)) and at position 70 (glutamine (Q) to aspartic

Table 5 The risk effect on rheumatoid arthritis susceptibility according to the different protective genotypes

Genotype	Patients (n = 322)		Controls (AFBAC) (n = 622)		OR	95% CI	p Value
	n	%	n	%			
DERAA +/+	4	1.2	24	3.9	0.31	0.10 to 0.96	0.016*
P _{tot} -DERAA/P _{tot} -DERAA	13	4.0	60	9.6	0.39	0.20 to 0.75	0.0034
DERAA +/P _{tot} -DERAA	9	2.8	73	11.7	0.22	0.10 to 0.45	0.0000066

*By Fisher's exact test.

AFBAC, affected family based artificial controls; P_{tot}-DERAA, the sum of the both strongly and weakly protective haplotypes (p+P) except those positive for the DERA motif.

Table 6 The genotypes classified according to different models in 322 patients with rheumatoid arthritis patients and 622 affected family based artificial controls

Genotype	Patients (n = 322)		Controls (AFBAC) (n = 622)		OR	95% CI	p Value*
	n	%	n	%			
Susceptibility	121	37.6	51	8.2	6.74	4.61 to 9.87	<10 ⁻⁶
Neutral	143	44.4	277	44.5	0.99	0.75 to 1.32	
Protective	47	14.6	269	43.3	0.22	0.16 to 0.32	<10 ⁻⁶
SE+/SE+	119	37.0	50	8.0	6.71	4.57 to 9.85	<10 ⁻⁶
SE+/SE-	142	44.1	267	42.9	1.05	0.79 to 1.39	
SE-/SE-	61	18.9	305	49.0	0.24	0.17 to 0.34	<10 ⁻⁶
DQ3+/+	47	14.6	15	2.4	6.92	3.68 to 13.17	<10 ⁻⁶
DQ3+/DQ5+	71	22.0	35	5.6	4.74	3.02 to 7.47	<10 ⁻⁶
DQ5+/+	17	5.3	17	2.7	1.98	0.95 to 4.14	
DQ3+/DERAA+	29	9.0	51	8.2	1.11	0.67 to 1.83	
DQ5+/DERAA+	15	4.7	55	8.8	0.50	0.27 to 0.94	0.028
DERAA+/DERAA+	4	1.2	24	3.9	0.31	0.10 to 0.96	0.016†
DQ ^{RA} +/+	135	41.9	67	10.8	5.98	4.21 to 8.50	<10 ⁻⁶
DQ ^{RA} +/DERAA+	44	13.7	106	17.0	0.77	0.52 to 1.15	
DQ ^{RA} -/-	32	9.9	117	18.8	0.48	0.31 to 0.74	0.00056

The susceptibility genotype includes S/S+S/s+N/S genotypes (table 3); the neutral genotype includes s/s+S/P+S/p+N/s/s/p+N/p genotypes (table 3); the protective genotype includes s/P+p/p+N/N+p/O+N/P+p/P genotypes (table 3).

*Only statistically significant p values are shown.

†By Fisher's exact test.

AFBAC, affected family based artificial controls; DQ^{RA}-/-, patients homozygous of the haplotypes not included DQ^{RA}+ or DERAA+ alleles.

acid (D)), either alone or in combination—appear to protect against rheumatoid arthritis. Only the (DRB1*07)-DQA1*0201-DQB1*02 haplotype was not found to be protective but neutral, although it includes both isoleucine at position 67 and aspartic acid at position 70 in its HVR3. The different disease risks associated with the two DRB1*0401 positive haplotypes—DRB1*0401-(DQA1*03)-DQB1*0301 (DQ7) and DRB1*0401-(DQA1*03)-DQB1*0302 (DQ8) (odds ratios of 7.56 and 2.93, respectively)—also point to the importance of the whole DR-DQ haplotype and not only the DR allele when assessing the risk for rheumatoid arthritis. The various effects of the haplotypes on susceptibility to rheumatoid arthritis may be influenced by DQB1 alleles, but other alleles in the HLA region could also be important.^{27–42–44}

In the RAP model, all the DRB1 alleles carrying the HVR3 motif D⁷⁰ERAA⁷⁴ (*0103, *0402, *1102, *1103, *1301, and *1302) were considered protective.²⁰ All these alleles share aspartic acid at position 70 and, in addition, all except allele *1103 also share isoleucine (I) at position 67 in the HVR3 of the DRB1 molecule. Thus the difference between the RAP and the reshaped shared epitope hypothesis, which is partly supported by our study, is a more strict definition of the protective alleles; however, it leaves out several clearly protective alleles or haplotypes which we detected (table 5). When susceptibility alleles according to the RAP model are considered, the difference from the reshaped shared epitope hypothesis is not remarkable either, although the underlying theory is different. In practice the result is almost the same, because all shared epitope positive DRB1 alleles are found in haplotypic combinations with the proposed rheumatoid arthritis-predisposing DQB1 antigen (DQ^{RA}) specificities. There were three DQ3 positive DQ^{RA} haplotypes—DRB1*0407-(DQA1*03)-DQB1*0301, (DRB1*09)-DQA1*03-DQB1*0303, and DRB1*0403-(DQA1*03)-DQB1*0302—which were not positive for shared epitope. None of these was found to be associated with an increased risk for rheumatoid arthritis, supporting the importance of DR alleles and the shared epitope. In our population all these haplotypes are relatively rare and do thus not much affect the size of risk groups.

Though we are not able to explain all our findings by the reshaped shared epitope hypothesis,¹⁹ it fits better to the present results than either the rheumatoid arthritis

protection model or “the electric charge in P4 pocket of the DRB1 allele” model proposed by Revirion *et al.*^{18–20} Most probably the compatibility of all the models varies markedly in different populations depending on the frequencies of deviating haplotypes.

In line with several earlier studies, our present results emphasise the importance of genotypes for disease risks.^{9–12–16} Furthermore, our results are in line with the so called gene dose effect hypothesis, according to which the risk for rheumatoid arthritis increases in line with the number of inherited predisposing genes.⁴⁵ The gene dose effect was clearly seen whether considering the issue with respect to the susceptibility haplotypes (either weak or strong) or the protective haplotypes (either weak or strong) (table 3). One should also emphasise that shared epitope haplotypes can clearly be divided into those associated with high (DR4 positive) and low (DR1 positive) rheumatoid arthritis risk.

Conclusions

Our results confirm the importance of looking not only at the HLA-DR or HLA-DQ alleles or haplotypes, but also at the genotypes when evaluating their influence on the susceptibility to rheumatoid arthritis. Although our results fit the reshaped shared epitope hypothesis, we suggest that the influence of the HLA class II region genes on the susceptibility to rheumatoid arthritis is so complicated that none of the existing hypotheses can explain it.

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MEMBERS OF THE FIN-RACO TRIAL GROUP

J Ahonen, H Blåfield, S Forsberg, C Friman, M Hakala, M Hakola, P Hannonen, K Ilva, P Järvinen, H Julkunen, H Kautiainen, K Koota, M Korpela, L Laasonen, M Leirisalo-Repo, R Luukkainen, T Möttönen, M Nissilä, L Paimela, I Pälvimäki, H Piirainen, K Puolakka, K Vuori, U Yli-Kerttula.

Authors' affiliations

S Laivoranta-Nyman, J Ilonen, R Hermann, Turku Immunology Centre and Department of Virology, University of Turku, Turku, Finland
T Möttönen, A Toivanen, Department of Medicine, Turku University Central Hospital
J Tuokko, Department of Medical Microbiology, University of Turku
R Luukkainen, Department of Rheumatology, Satalinna Hospital, Satalinna, Finland
M Hakala, Department of Internal Medicine, University of Oulu, Oulu, Finland
P Hannonen, Department of Medicine, Jyväskylä Central Hospital, Jyväskylä, Finland
M Korpela, U Yli-Kerttula, Department of Medicine, Tampere University Hospital, Tampere, Finland

*Members of the group are listed at the end of the article

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