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## Brief communication

# HLA-DRB1 in eight Finnish monozygotic twin pairs concordant for rheumatoid arthritis

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rheumatoid arthritis.

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This study presents the results of HLA-DRB1 typing of the eight monozygotic twin pairs with both members affected by rheumatoid arthritis (RA), sampled in the nationwide Finnish twin cohort. The shared epitope, associated with RA in case-control studies, was present in all eight twin pairs, being significantly more frequent than among RA patients in a recent Dutch case-control study. Furthermore, 4 out of 8 twin pairs were homozygous for the shared epitope, while in 73 Dutch healthy controls encoding the shared epitope only 13 (18%) were homozygous; this suggests a gene dose effect in RA susceptibility. Combining these results with data from other sources may help to clarify the contribution of HLA alleles in the genetic predisposition to RA.

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While early reports (1, 2) showed an association of the serologically defined HLA-DR4 and -DR1 specificities with rheumatoid arthritis (RA), more recent case-control studies show that this disease is associated with HLA-DRB1 alleles encoding a short structural motif in the third hypervariable region, the “shared epitope” (SE). This shared epitope may be encoded by any of three highly homologous amino acid sequences at positions 67 to 74 of this molecule: LLEQKRAA, LLEQRRAA or LLERRAA (single letter amino acid code: A = alanine, E = glutamic acid, K = lysine, L = leucine, Q = glutamine, R = arginine) (3, 4).

While case control studies in the general population help to define the association between DRB1 alleles and disease susceptibility, data from diverse sources are needed to describe the genetic model for a given disease, e.g. mode of inheritance, the number of genes involved or the relative contribution of HLA to genetic predisposition. Twin studies, assessing the frequency of disease concordance in mono- and dizygotic twins, are very informative in this respect (5–9).

In RA, among the twin studies performed, the Finnish twin cohort study (6, 7) is unique in one aspect: By linking the Finnish twin cohort with the Sickness Insurance Register nearly all relevant

twins are included in the study, resulting in virtually complete ascertainment. In such a study the risk of bias is reduced, e.g. concordant twin pairs are not more likely to enter the study than discordant pairs. Avoiding these biases is important when differentiating between several genetic models. Since in RA these genetic models have to incorporate the effects of HLA-DRB1 alleles on RA risk, we performed HLA-DRB1 typing of the Finnish monozygotic concordant affected twins.

From 4137 monozygotic twin pairs, in total eight monozygotic twin pairs, both individuals fulfilling the 1987 criteria of the American College of Rheumatology for RA, were identified (6, 7). The sampling procedure, zygosity testing and clinical characteristics of these twins have been described elsewhere (6, 7). All 16 twin members were seropositive at some stage, 14 had erosive disease, 12 had been treated with gold and 7 had rheumatoid nodules. Peripheral blood was available from all individuals except patient 1A and 8B.

For HLA-typing DNA was isolated from peripheral blood, amplified in a polymerase chain reaction (PCR) using primers P1 and P2 specific for exon 2 (10), and screened using sequence specific (SSO) probes 1003 and 2810 described in the 11th workshop (11), probes 88–144, 88–148, 88–150, 88–151,

Table 1  
HLA-DRB1 alleles in 8 Finnish RA concordant affected monozygotic twins

No <sup>a</sup>	DR serology	HLA-DRB1	age at onset	sex	RF <sup>b</sup>	nodules	erosive
#1	4	0404-13	46 48	F	++	++	++
#2	1,4	0101-0401	58 60	F	++	+-	++
#3	1,5	0101-11	43 46	F	++	--	--
#4	4	0401-0404	40 61	M	++	+-	++
#5	3/6,4	03-0401	42 43	M	++	--	++
#6	1,7	0101-07	39 40	F	++	++	++
#8	4	0401-0401	36 43	F	++	--	++
#9	1,4	0101-0401	60 63	F	++	+-	++

<sup>a</sup> Numbering as in Aho et al. (6). Pair #7 did not meet the ACR criteria and is left out of this analysis.

<sup>b</sup> Rheumatoid factor positivity in some phase of disease (Latex and/or Waaler-Rose; see ref (7))

88-153, 89-55, 89-61 and 89-62 (10, 12) and probes 3, 4, 5, 6, 10, 11, 20, 21, 22 and 23 (13). Subtyping of the HLA-DR4 associated alleles was performed using primers 336C and 337C, and SSO probes 152B, 153B, 156C, 157C, 315B, 769C, GT86 (14), probe 10 (13) and probe 102G with the sequence 5'-AGAGGAGTCCGTGCGCT-3' (Generously provided by JSS Lanchbury, London). In every screening procedure, SSO probes were end-labelled using T4-kinase (15), hybridized to the dot-blotted PCR product, stringency washed using TMAcI (16), and detected by autoradiography. PCR-amplified DNA of homozygous typing cells from the Tenth International Histocompatibility Workshop was processed together with the samples to ascertain specific hybridization.

Table 1 shows the typing results of the eight monozygotic concordant affected twin pairs. When blood was available from both twins typing results were identical within twin pairs; results are therefore not separately presented. Of the eight twin pairs six were HLA-DR4 positive, while in all at least one HLA-DRB1 allele encoded the shared epitope. Two twin pairs were homozygous for HLA-DR4, while in four pairs both HLA-DRB1 alleles encoded the shared epitope. All individuals with rheumatoid nodules encoded the shared epitope amino acid sequence LLEQRRAA (DRB1\*0404 or DRB1\*0101); no other associations of typing results with clinical features were observed.

To date, no HLA-DRB1 typing data on Finnish RA patients are available; therefore typing results from a recent Dutch case-control study were used for reference. Compared to Dutch RA patients (17) the concordant monozygotic twins showed a significantly higher allele frequency of the shared epitope (12/16 vs 159/334; Odd's Ratio OR=4.72; confidence interval CI=1.70-13.10; Fisher exact P=0.004), while the higher frequencies of HLA-DR4 (OR=2.15; P=0.17), of homozygosity for HLA-DR4 (OR=2.60; P=0.25) and of homozygosity

for SE (OR=3.28; P=0.10) did not reach significance.

If homozygosity and heterozygosity for SE encode identical RA risks, the ratio between SE homozygous and heterozygous individuals should be identical in these monozygotic twin pairs and healthy controls. However, 4 in 8 monozygotic twins were homozygous for SE, while 13 in 73 SE-positive healthy controls were homozygous (OR=4.63; CI=1.02-20.89; P=0.05), thus supporting the concept that homozygosity for SE is associated with a higher RA risk.

Jawaheer et al. (9) reported earlier that concordance in monozygotic twins is rare in the absence of the shared epitope. Our study confirms these findings. Furthermore, it shows an increased frequency of homozygosity for the shared epitope alleles among these twins compared to normal controls, implicating a role for homozygosity in susceptibility to RA, in agreement with the HLA typing results in the UK nationwide RA twin study. This indicates that a genetic model for RA must include the effects of homozygosity. Combining the data presented here with HLA typing data and epidemiological data from other sources may help to clarify the role of HLA alleles in the genetic predisposition to RA.

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