EXTENDED REPORTS

Immunogenetic differences between patients with familial and non-familial rheumatoid arthritis

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Abstract

Objective—To search for possible immunogenetic differences between the patients with familial and non-familial rheumatoid arthritis (RA).

Methods—The study compared 129 familial RA patients with 217 non-familial patients for the frequencies of HLA-DR antigens including DR4 subtypes, DR4-DQB1*0301 and DR4-DQB1*0302 haplotypes and HLA-B27 antigen as well as the age of disease onset and existence of rheumatoid factor or joint erosions.

Results—Two major differences between familial and non-familial groups were found: firstly, familial RA patients had increased frequency of HLA-DR4 as compared with the non-familial RA group (68.2 v 54.8%; $p = 0.019$). Secondly, the mean age at onset of RA was significantly lower in the familial than in the sporadic RA patients (42.0 v 46.5 years; $p = 0.0020$) and the difference still remained when the DR4 positive and negative subgroups were compared separately.

Conclusion—These results confirm the more prominent association with HLA-DR4 in familial than in the non-familial cases and suggest that accumulation of HLA risk genes may, at least partly, explain the familial occurrence of the disease. Other susceptibility genes may also be concentrated in multiplex case families as suggested by an earlier age at the onset of RA in both HLA-DR4 positive and negative familial patients.

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Both environmental and genetic factors have a role in the aetiopathogenesis of rheumatoid arthritis (RA). Familial clustering of RA is a phenomenon clearly demonstrated in previous studies.^{1 2} It has been estimated that perhaps 2% to 3% of probands with RA in the population have first degree relatives with RA^{3 4} and a positive family history is the best established risk factor for RA.⁵ The estimated population prevalence of 1%–2% rises to 3%–8% in the first degree relatives of RA sufferers.⁶ Comparisons of the prevalence of RA between patients' relatives and their spouses has shown much

higher prevalence among the relatives.⁷ Although the evidence for an effect of the home environment in adult life is unconvincing, the possibility that the environment in childhood may predispose to arthritis in later life must be considered.¹ What such predisposing environmental factors would be, is at present not known. The concordance rate among monozygotic twins indicates the maximum level of genetic contribution in any disease. The respective rates for monozygotic and dizygotic twins with RA have been reported to vary from 12% to 32% and from 3.5% to 9%.^{1 4 8} Those patients who had developed their disease early in the life had a higher prevalence of RA among their relatives than those who developed their arthritis later in life.⁷

The inheritance of the tendency to develop RA is not a simple Mendelian dominant or recessive pattern but one that seems to be dependent on the simultaneous presence of, at the very least, two and probably three to four genes.⁹ Family based haplotype sharing as well as population based association studies have clearly indicated the presence of an "RA susceptibility" allele in the HLA region.^{6 10-18} Nevertheless it has been estimated that the HLA region only accounts for one third of the genetic contribution to this susceptibility.^{12 19} Some studies suggest that the HLA associated susceptibility is related to the severity and progression of the disease²⁰⁻²⁴ but some others find HLA association also in population based studies including early and mild cases.^{25 26}

Comparison of familial and non-familial RA has shown no major clinical or immunological differences between these two groups justifying the use of multiplex case families to study the pathogenesis of RA.²⁷⁻²⁹ However, already in 1983 Khan and collaborators raised the possibility of an increased frequency of DR4 and homozygosity for DR4 in probands with familial RA as compared with patients with non-familial disease.^{30 31} The increased frequency of HLA-DR4 in familial compared with non-familial RA patients has been found by Sanders and coworkers²⁹ but some other family studies did not find significant differences between the familial and the non-familial RA groups.^{28 32} If immunogenetic factors have an important role in the aetiology of RA, you

Table 1 Clinical and demographic details in familial and non-familial RA patient groups

	Familial (n=129) n (%)	Non-familial (n=217) n (%)	p
Male (%)	35 (27.1)	78 (35.9)	NS
RF positive (%)	111 (86.0)	170 (78.3)	NS
Erosions found (%)	110 (85.3)	61 (93.8)*	NS
Age at disease onset ≤30 (%)	24 (18.6)	15 (6.9)	0.0020
Disease duration (y), mean (SD)	15.6 (10.9)	14.3 (9.8)*	NS

*In the smaller non-familial RA group (66 patients).

would expect them to be pronounced in multi-case families.⁶

To define further the issue we compared in this study familial and non-familial RA groups for HLA-DR antigens including DR4 subtypes, DR4 associated DQ alleles and HLA-B27 antigen, ages at the onset of disease and existence of rheumatoid factor (RF) or bone erosions. In Finland the population is for historical reasons very homogenous and a family material is better suited for genetical studies than in countries with ethnically mixed population.

Methods

FAMILIAL RA PATIENTS

We collected 61 Finnish families in which at least two family members (first degree relatives to each other) have RA. The index cases have been collected from six different rheumatological units (Turku, Paimio, Satalinna, Tampere, Jyväskylä and Oulu). The RA patients who had at least one first degree relative with RA were asked to participate in the study by a questionnaire. The 61 families consisted of 40 affected sibpairs, 15 parent-child pairs and six sibtrios. There was also one child of an index case affected in one sibpair and one mother of another index case affected in another sibpair. Altogether 129 RA patients including both index cases and affected family members were included in this study as familial RA patients.

NON-FAMILIAL RA PATIENTS

Two hundred and seventeen RA patients who were ensured not to have first degree relatives with the disease were combined from two previous Finnish RA studies, namely 151 patients from the so called FIN-RACo trial and 66 patients from another study of RA genetics (Tuokko *et al*, manuscript in preparation). There was one patient whose age at RA onset was not known and six patients of whom the knowledge of bone erosions was missing.

In all patients of both familial and non-familial RA groups the diagnosis of RA was made according to the criteria of the American College of Rheumatology (formerly the American Rheumatism Association).³³

Clinical documentation of patients with RA included sex, age at disease onset (meaning the age when the diagnosis of RA was made), disease duration, serum rheumatoid factor (RF) positivity and the presence of joint erosions. The disease duration in years was known in each patient in the familial and in the smaller non-familial group but in the FIN-RACo trial group we only knew that all the patients had suffered from RA from three to five years. In addition to that, we also knew that the patients

in the FIN-RACo trial have had symptoms of RA on average eight months before the diagnosis of RA. The patients were classified as seropositive if there had been one positive result of a RF test at any time during the disease course. The test results for RF positivity as well as radiographs for erosions were re-evaluated from the case records.

HEALTHY CONTROLS

Altogether 60 healthy controls were included; 50 controls were healthy Finnish blood donors and 10 control samples were constructed with the method described by Falk and Rubinstein from 20 parental HLA haplotypes untransmitted to the RA affected child in a family study.³⁴

HLA TYPING

DNA was extracted from anticoagulated blood samples using a salting out method.³⁵ The HLA-DR alleles were determined by a sequence specific polymerase chain reaction (PCR) amplification.³⁶ The low resolution genomic typing used could identify HLA-antigens from DR1 to DR18 and also DR52 and DR53. When a single HLA-DRB1 allele could be amplified, the alleles were assumed to be homozygous.

The presence of HLA-DQB1 alleles *0301 or *0302, or both, was defined by a method based on hybridisation of PCR product with a panel of lanthanide labelled oligonucleotide probes³⁷ and the HLA-DR4 subtypes and HLA-B27 were defined by using similar method designed for these purposes.^{38, 39}

STATISTICAL METHODS

Statistical analyses were performed by using the χ^2 test with continuity correction when differences in proportions of HLA frequencies, sex, RF or bone erosion status between the familial and non-familial groups were compared. Fisher's exact test was used when appropriate. The differences in the age at onset of disease were compared by the two tailed *t* test.

ETHICAL ISSUES

The study was accepted by the ethical committees of participating hospitals and the samples were collected after informed consents.

Results

Table 1 gives the clinical and demographic data of familial compared with non-familial patients. There were no statistically significant differences in the proportions of men or patients with RF between the familial and non-familial RA groups. Joint erosions were also

Table 2 Mean (SD) age of disease onset (in years) in familial and non-familial RA groups

	Familial	Non-familial	p
All	42.0 (14.3)	46.5 (10.9)	0.0020
Men	47.5 (12.6)	46.6 (10.9)	NS
Women	39.9 (14.4)	46.5 (10.9)	<0.001
RF positive patients	41.2 (13.2)	46.7 (11.3)	<0.001
RF negative patients	46.8 (19.4)	46.0 (9.5)	NS
DR4 positive	41.5 (14.7)	45.2 (11.3)	0.044
DR4 negative	43.0 (13.3)	48.1 (10.2)	0.014

Table 3 Frequencies of HLA-DR antigens in familial and non-familial RA patients and in healthy controls

HLA-DR antigen	Familial RA (n=129) n (%)	F/N-F p	Non-familial RA (n=217) n (%)	N-F/C p	Healthy controls (n=60) n (%)	F/C p
1	49 (38.0)		87 (40.1)		27 (45.0)	
15(2)	18 (14.0)		46 (21.2)		9 (15.0)	
16(2)	0		2 (0.9)		0	
17(3)	9 (7.0)	0.060	31 (14.3)	0.14	14 (23.3)	<0.01
18(3)	0		0		0	
4	88 (68.2)	0.019	119 (54.8)	<0.0001	13 (21.7)	<0.0001
11(5)	5 (3.9)		13 (6.0)		7 (11.7)	
12(5)	4 (3.1)		10 (4.6)		5 (8.3)	
13(6)	12 (9.3)		22 (10.1)	<0.01	15 (25.0)	<0.01
14(6)	5 (3.9)		5 (2.3)		2 (3.3)	
7	10 (7.8)		14 (6.5)		8 (13.3)	
8	10 (7.8)		29 (13.4)		10 (16.7)	
9	9 (7.0)		5 (2.3)		5 (8.3)	
10	1 (0.8)		3 (1.4)		1 (1.7)	
5*	9 (7.0)		23 (10.6)	0.085	12 (20.0)	0.016
6*	17 (13.1)		27 (12.4)	<0.01	17 (28.3)	0.020

F/N-F = familial *v* non-familial RA patients. N-F/C = non-familial RA patients *v* healthy controls. F/C = familial RA patients *v* healthy controls. *The frequencies of both subtypes of this allele have been summarised. If p values are not mentioned, the differences do not reach statistical significance.

found in equal proportions in both familial and non-familial groups when only the smaller non-familial RA group was considered. The 151 non-familial RA patients from the FIN-RACo trial were left out from this comparison because of their significantly shorter disease duration as compared with the others. The mean age at onset was lower in the familial as compared with the non-familial group (42.0 compared with 46.5 years; $p = 0.0020$). We also found a significantly increased proportion of patients who had been diagnosed with RA at early age (≤ 30 years) in the familial compared with non-familial group (18.6 *v* 6.9%; $p = 0.0020$) (table 2).

Both familial and non-familial RA patients were found to have an increased frequency of HLA-DR4 and decreased frequency of

Table 4 Frequencies of the subtypes of HLA-DR4 antigen in the familial and non-familial RA patients positive for DR4

DRB1*alleles (DR4 subtypes)	Familial RA (n=88) n (%)	Non-familial RA (n=119) n (%)	p
0401	64 (72.7)	81 (68.1)	NS
0402	0	0	
0403	1 (1.1)	5 (4.2)	NS
0404	18 (20.5)	29 (24.4)	NS
0405	0	0	
0407	0	2 (1.7)	NS
0408	9 (10.2)	9 (7.6)	NS
0410	0	1 (0.8)	NS
0413	0	1 (0.8)	NS

Table 5 Frequencies of the HLA-DR4-DQB1*0301 and DR4-DQB1*0302 haplotypes in familial and non-familial RA patients positive for DR4

DR4-DQB1*0301/*0302	Familial RA (n=88) n (%)	Non-familial RA (n=119) n (%)	p
4.301	33 (37.5)	35 (29.4)	NS
4.302	64 (72.7)	83 (69.7)	NS
Others	1 (1.1)	6 (0.5)	

Table 6 Shared epitopes in the familial and non-familial RA patients

Shared epitope	Familial RA (n=129) n (%)	Non-familial RA (n=217) n (%)
+/+	62 (48.1)	72 (33.2)
+/-	43 (33.3)	97 (44.7)
-/-	24 (18.6)	48 (22.1)

$\chi^2=7.651$ (df=2), $p=0.022$. +/+ patients homozygous for shared epitope. +/- patients heterozygous for shared epitope. -/- patients negative for shared epitope.

DR13(6) antigen as compared with the control group (table 3). DR17(3) and DR5 broad specificities were significantly decreased in familial but not in non-familial patients as compared with controls. HLA-DR4 was found more frequently in the familial than in the non-familial group (68.2 *v* 54.8%; $p = 0.019$) (table 3). The group of probands (n=61) was very similar (HLA-DR4 positive patients: 42 (68.9%) *v* 119 (54.8%)) to the original familial patient group although the statistical significances in the comparisons decreased ($p=0.070$) because of the smaller frequencies. HLA-DRB1*0401 allele was the most common DR4-subtype in both groups, and no significant difference in the distribution of the subtypes was found between familial and non-familial cases (table 4). We also compared the presence of HLA-DR4-DQB1*0301 and DR4-DQB1*0302 haplotypes in HLA-DR4 positive patients between these two groups but did not find any statistically significant differences (table 5). The shared epitope (SE) common to DRB1*0401/*0404/*0405/*0408/DR1 and DR10 alleles was found in 105 (81.4%) familial and 169 (77.9%) non-familial patients. That difference did not reach the statistical significance although the homozygosity for SE was clearly more common in the familial than non-familial group (48.1 *v* 33.2%; $p = 0.0084$) (table 6).

HLA-B27 antigen was found in 25 of 129 (19.4%) familial and 31 of 215 (14.4%) non-familial RA patients. The total number of the non-familial patients in that comparison was 215 (not 217) because in two non-familial patients the HLA-B27 analysis was not successful. The difference between the familial and non-familial group was not statistically significant.

Discussion

This investigation was aimed at searching for evidence of possible immunogenetic heterogeneity between familial and non-familial RA patients. We found statistically significant increase in the HLA-DR4 frequency in the familial as compared with the non-familial RA group. This study is the largest one so far and the results are in accordance to the previous

findings of Sanders *et al.*²⁹ Most studies that did not find differences between familial and non-familial RA patients are based on small patient numbers.^{28 32 40}

Few previous studies have compared familial and non-familial RA for the HLA-DR4 subtype frequencies. DRB1*0401/*0404 or *0408 heterozygosity (Dw4/Dw14 in terminology based on the use of homozygous typing cells) has been suggested to be a high risk gene combination for susceptibility and severity of RA in white populations.^{21 22 24 41} In this study this risk allele combination was not overexpressed in the familial RA patients as compared with non-familial patients. There were only four (3.1%) patients in the familial and eight (3.7%) patients in the non-familial group with that allele combination. HLA-DRB1*0401 and *0404 are the two common subtypes in our population⁴² and these known RA associated HLA-DR4 subtypes were found in equal frequencies in both our familial and non-familial RA groups, the subtype *0401 was more frequent than *0404 in both study groups. McDonagh and coworkers found a relatively high prevalence of DRB1*0408 (16.7%) in their familial RA patients (no non-familial patients) and suggested the possibility that the DRB1*0408 allele may have a more important role in familial RA.⁴³ In our study HLA-DRB1*0408 was seen in 10.2% of our familial DR4 positive RA patients while the corresponding figure for non-familial group was 7.6%. In addition to the HLA-DR4 subtypes *0401, *0404 and *0408 the subtype *0405 belongs to those so called RA risk alleles. It, however, is very rare in the healthy Finnish population⁴² and we could not find any DRB1*0405 positive persons either in the familial or the non-familial group. The increased frequency of DR4 in familial RA patients was also reflected in the higher amount of so called SE in them. However, other alleles with this epitope, DR1 and DR10, were not more common among the familial than non-familial patients.

All DRB1*0408 positive patients had also DQB1*0301 allele and 10 of 18 (55.6%) of them were positive for B27. We have earlier described this HLA-B27 positive haplotype in reactive arthritis⁴² and RA⁴⁴ where we suggested that it might explain the increased frequency of B27 reported in Finnish RA patients. In this study the frequency of B27 among non-familial patients was similar to that reported for Finnish controls^{45 46} and we could not confirm its higher frequency in RA patients; in familial cases it was only marginally increased (19.4 v 14.4%; NS). Rantapää-Dahlqvist and coworkers reported a remarkably high incidence of HLA-B27 in RA patients with positive family history but not in those with negative family history (42.6 v 18.0%). One explanation for the different results might be that Rantapää-Dahlqvist *et al* accepted into the familial group RA patients with family history of polyarthritis, which might include also ankylosing spondylitis or reactive arthritis in addition to RA in the relatives of familial RA patients.⁴⁷

The frequency of HLA-DR17(3) was decreased in our familial RA group similar to two earlier studies by McDermott *et al* and Ollier *et al* although in those studies the comparison was made only between familial RA patients and healthy controls and no non-familial RA patients were included.^{14 48} The negative associations are difficult to interpret because of the fact that when one allele (HLA-DR4 in RA) is highly increased in frequency, the frequencies of the remaining alleles in the series are consequently reduced.

We also compared the ages at onset of RA between familial and non-familial groups and found that the mean age at onset was significantly lower in the familial as compared with the non-familial group. Almost exactly the same ages at onset were reported by Sanders and coworkers.²⁹ Overall, HLA-DR4 antigen has been found to be associated with an earlier age of onset of RA.^{41 49} This could also explain our findings of the difference between the mean age at onset of the disease in familial compared with non-familial group. However, the difference was found both in the DR4 positive and negative subgroups indicating the existence of other factors besides HLA-DR4. In the bigger non-familial group (151 patients) the symptoms of RA have been seen in average only eight months before the diagnosis of the disease. So it seems that the difference in the mean age at disease onset of RA between our two groups cannot be explained only by the increased awareness of the symptoms of RA in the familial group. For a person an early onset of RA implies a longer period of life with the disease, and subsequently more time to develop more extensive joint damage. In subgroup analysis the difference in the mean age of disease onset between the familial and non-familial group could not be confirmed in men or RF negative patients (table 2).

In conclusion, the increased frequency of HLA-DR4 antigen found in familial RA patients, at least partly, explains the familial aggregation of the disease and the finding of the earlier age at onset in that group also supports the more prominent genetic background in these patients. No differences were observed in clinical parameters measured between familial and non-familial patients. Consequently, similar pathogenetic mechanisms seem to be responsible for the development of the disease in both familial and non-familial patients.

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