Genetic structure of the population with rheumatoid arthritis in north east England: a genetic approach to define different subtypes

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SUMMARY Clinically and immunologically rheumatoid arthritis (RA) is possibly a heterogeneous disorder. Despite numerous efforts clearer definition of this heterogeneity has been of limited success. Measurements of rheumatoid factor (RF) and antinuclear antibodies (ANA) by conventional methods define subpopulations of patients with RA and in a few recent studies an association of human leucocyte antigens (HLA) undoubtedly indicates the immunogenetic differences in the susceptibility of RA patients with different status of autoantibodies. The studies on a few isolated non-HLA genetic markers in RA are controversial. To understand the role of genetic factors in susceptibility 24 single gene characters other than HLA were investigated in 225 patients with RA classified by humoral status (presence or absence of RF and ANA) into three groups and in 104 healthy control individuals from the north east of England. Locus by locus comparison suggested associations of MN, Lewis, and Bf system with RF positive patients. Although the associations with MN and Lewis blood groups require further investigations, the involvement of the Bf locus is in agreement with the immunological component of the disease suggested by HLA associations and it could be due to the phenomenon of linkage disequilibrium. Measures of genetic distance applied to the subpopulations of patients with RA, divided according to the presence or absence of humoral factors, suggest that RF⁺ANA⁺, RF⁻ANA⁻, and RF⁺ANA⁻ subgroups are distinct genetic diseases, each affecting a different subsection of the population which is genetically distinct. Such genetic heterogeneity may suggest a different pathogenetic mechanism for each of these subpopulations of rheumatoid arthritis.

Key words: blood groups, serum proteins, red cell enzymes, phenotypic variation, genetic heterogeneity.

Present evidence that genetic factors may play an important part in the development of rheumatoid arthritis (RA) comes from twin studies, pedigree analysis, and associations with it of marker variants in genetic polymorphic systems.¹⁻⁷ The initial investigations on monozygotic cotwins showed an incidence of the disease 33 times that expected, and family studies showed a relative risk ranging between 1 and 15.2 for the first degree relatives. These

observations suggested strong genetic susceptibility to the disease.

In the last two decades a few retrospective analyses of the literature cast doubt on the validity of the existing data on familial aggregation of RA, and suggested a weak genetic element in the aetiology of the disease.^{8,9} Meanwhile, research on its association with human leucocyte antigens once again drew attention to the possibility of genetic predisposition coded at or near the HLA-D locus situated on chromosome 6 in man, or a defective immune response gene which may be in linkage disequilibrium with the HLA-D locus.^{4, 10-12}

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One of the fundamental reasons for the inconsistency of these lines of evidence—on the one hand, a strong genetic element suggested by the association with B lymphocyte alloantigens, and on the other, a weaker genetic contribution indicated by the family studies—may be the disease heterogeneity, which was perhaps not taken sufficiently into consideration in the initial investigations. Several observations in man clearly indicate that different genetic factors may be involved in different types of rheumatoid arthritis.¹² A strong association between HLA-DR4 and seropositive (rheumatoid factor; RF⁺) rheumatoid arthritis patients is established in white Europeans, whereas in contrast the frequency of DR4 in seronegative (RF⁻) patients is similar to that of the control.¹³ These HLA results in man, though there is some lack of consistency, are supported by experimental studies in certain mammals,¹⁴ and overall they indicate that seronegative and seropositive patients may be two different genetic subsections of the disease population, with different pathogenetic mechanisms bringing about phenotypically similar diseases.

Another autoantibody frequently encountered in patients with RA is associated with nuclear antigen. The extent of genetic control of antinuclear antibodies (ANA) is not clearly understood, but their association with 'autoimmune' disease suggests that they may play an integral part in the development of the disease.¹⁵ Once again, depending on the presence or absence of ANA, two populations of patients with RA can be distinguished. The interaction of those gene(s) responsible for the production of the autoantibodies may influence the course and severity of the RA.^{16 17}

The aim of the present study, therefore, was to apply available genetic methods to inquire if the patients of different humoral status represent different populations of the same disease. Since the phenotypic expression of the disease will depend on the interactions within the total genotype of the patients the approach here followed was to investigate a large number of single gene markers in systems other than HLA in patient groups of different humoral status, and summarise these data in a single measure of genetic heterogeneity.

Patients and methods

PATIENT AND CONTROLS

A random collection of 225 adult patients fulfilling the criteria of the American Rheumatism Association for the classification of rheumatoid arthritis was included in this study.¹⁸ All the patients were white and from the north east of England. They had been

followed up in outpatient clinics for three to 30 years. Eighty per cent of the total patient population were over 50 years of age and 40% over 60 years. The control group of 104 individuals was matched proportionately for sex and region and was mainly drawn from the electoral register.

EXPERIMENTAL PROCEDURE

Five millilitres of blood was obtained from each patient and control by venepuncture, into ethylenediaminetetra-acetate (EDTA) tubes. The samples were sent to the laboratory within four hours of collection. The plasma sample was separated and stored at -30°C for serum protein examination, and the red cells were analysed for blood groups. After washing the red cells with normal saline, lysates were prepared with equal amounts of water for the examination of red cell enzyme.

BLOOD GROUPS

The red cells were typed using the following antisera: A₁, A₂, B, A+B, M,N, S,s, C,c,C^w, E,e,D, Fy^a, Fy^b, P, K,k, Jk^a, Jk^b, Lu^a, Lu^b, Le^a. The tests were carried out by standard serological methods.¹⁹

SERUM PROTEINS

The complement components (C3 and Bf) were analysed immediately after arrival of the sample by high voltage agarose electrophoresis as described by Alper and Propp.²⁰ Haptoglobin and transferrin were typed by horizontal starch gel electrophoresis by the method of Smithies.²¹ The group specific components (Gc) were subtyped by isoelectric focusing (IEF) on thin layer polyacrylamide gels as described from this laboratory.²²

RED CELL ISOENZYMES

The red cell enzymes adenosine deaminase (ADA), acid phosphatase (AP), phosphoglucomutase (PGM), adenylate kinase (AK), 6-phosphoglucomutase dehydrogenase (6PGD), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), and superoxide dismutase (SOD) were analysed by horizontal starch gel electrophoresis with the specific conditions given by Harris and Hopkinson,²³ and the phosphoglucomutase (PGM) subtypes were analysed by IEF on thin layer polyacrylamide gels.²²

HUMORAL STATUS

The presence of rheumatoid factor was tested by the haemagglutination technique.²⁴ A serum showing a reaction at a dilution of 1:40 was considered positive. Patients were characterised as seronegative

if all repeated tests for RF were negative. The presence of antinuclear antibody (IgG) was tested for by an indirect immunofluorescent technique with rat liver.²⁵

Results

Patients were divided into groups depending upon the presence or absence of the humoral factors (RF and ANA). The composition of the groups and the patients tested in each are given in Table 1. Among the total patient group (RA) five subgroups were distinguished: RF⁺ ANA⁻, RF⁺ANA⁺, RF⁺ ANA⁺, and RF⁻ANA⁻.

The phenotype numbers and frequencies for each system (blood groups, red cell enzymes and serum proteins) in the total number of patients with RA, the subgroups, and the controls are listed in Table 2. Tested by χ^2 , no system shared any deviation of phenotype numbers from the expected. Compared with other surveys of healthy subjects the P blood groups showed slightly aberrant frequencies in the present controls, therefore interpretation of associations if any with this system requires caution. The gene frequencies of the various systems are given in Table 3.

BLOOD GROUPS

The frequencies of the A and B genes were lower in all the five subgroups than in the control group, but the differences were small and not statistically significant. The absence of B blood group from the seronegative group (RF⁻ANA⁻) is probably due to the small sample size. The raised Rh positive frequency observed in other studies²⁶ could not be

Table 1 Subgroups of patients with rheumatoid arthritis (RA) depending on the humoral factors

Subgroup No	Subgroup	Number tested	Abbreviation used
1	Total number of patients with RA	225	RA
2	Rheumatoid factor positive/antinuclear antibody negative	165	RF ⁺ ANA ⁻
3	Rheumatoid factor positive/antinuclear antibody positive	44	RF ⁺ ANA ⁺
4	Rheumatoid factor negative/antinuclear antibody negative	15	RF ⁻ ANA ⁻
5	Rheumatoid factor positive	209	RF ⁺
6	Antinuclear antibodies negative	180	ANA ⁻
7	Control (north east England)	104	Control

One individual (RF⁻ANA⁺) was excluded from the analysis.

confirmed in the present investigation. In the MN system the M gene showed a significantly raised frequency in the RF⁺ and RF⁺ANA⁺ groups ($\chi^2=4.6$ and 3.85 , 1 df; $p<0.05$) but not in the other subsamples. For Kell, Duffy, Kidd, and Lutheran although appreciable differences existed between certain subgroups of patient and the control, in no system were the differences statistically significant. For the Lewis blood group system the Le^{a+} frequencies among the patient groups ranged from 12% to 23% compared with 25% in the controls. The lowest frequency of the Le^{a+} antigen was found in the RF⁺ANA⁺ group, the difference from the controls being significant (Fisher's exact $p=0.05$).

SERUM PROTEINS

For haptoglobin, transferrin, and the C3 system the difference between each patient group and the control was not significant, though the range of frequency among the patient group for Hp*1 was appreciable (35–41%). The suballele frequencies of the Gc system in the control sample were slightly different from those in the north eastern sample reported from this laboratory previously,²⁷ but not significantly so ($\chi^2=6.48$, 4 df; NS). The overall trend was the same in all RA patient groups; there was an increase of some 5% in Gc*2 allele frequency in all the patient groups except the few seronegative patients (RF⁻ANA⁻) where the increase was slightly less. In the Bf system all the patient groups showed an increase of between 5% and 8% in the Bf*S allele frequency. The individuals with the Bf*S allele overall had a statistically significant high relative risk of developing RA of 1.7 ($\chi^2=4.12$, 1 df; $p<0.05$). Although an increased relative risk occurred within each of the patients groups, it was only significant for the RF⁺ and RF⁺ANA⁻ groups (Table 4).

RED CELL ENZYMES

For the red cell enzyme polymorphisms there was a considerable range of gene frequency variation among patient groups, shown for example by the alleles GPT*2 (39–53%); PGM1*1⁺ (60–73%); AP*C (2–7%); EsD*2 (9–17%), and ADA*2 (4–10%). Overall there was no significant association found between RA and the genes of any enzyme system.

MULTIVARIATE ANALYSIS

Consideration allele by allele of the differences between patients with RA and controls, and among the various RA patient groups, is not very informative in the detection of genetic differences among them. It is by no means unusual that minor differences in a number of features which seem to

Table 2 *Observed phenotype numbers and phenotype frequencies for patients with rheumatoid arthritis and controls*

System and phenotypes	Total No RA patients		RF ⁺ ANA ⁻		RF ⁺ ANA ⁺		RF ⁻ ANA ⁻		RF ⁻ ANA ⁺		ANA		Control	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
ABO														
O	115	51.1	84	50.9	23	52.3	8	53.3	107	51.2	92	51.1	45	43.3
A1	66	29.3	48	29.1	12	27.3	5	33.3	60	28.7	53	29.4	34	32.7
A2	22	9.8	17	10.3	3	13.3	2	13.3	20	9.6	19	10.6	8	7.7
B	16	7.1	11	6.7	5	11.4	0	0.0	16	7.7	11	6.1	13	12.5
A1B	3	1.3	3	1.8	0	0.0	0	0.0	3	1.4	3	1.7	2	1.9
A2B	3	1.3	2	1.2	1	2.3	0	0.0	3	1.4	2	1.1	2	1.9
Rhesus (Rh)														
ccDec	4	1.7	2	1.2	1	2.3	1	6.7	3	1.4	3	1.7	2	1.9
CCDEe	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
CcDEe	23	10.2	15	9.0	6	13.6	2	13.3	21	10.1	17	9.4	9	8.7
ccDEe	26	11.5	16	9.6	7	15.9	3	20.0	23	11.0	19	10.6	11	10.6
CCDec	37	16.4	30	18.2	6	13.6	1	6.7	36	17.2	31	17.2	16	15.4
CcDec	88	39.1	70	42.4	13	29.5	5	33.3	83	39.7	75	41.7	47	45.2
Ccddcc	2	0.9	2	1.2	0	0.0	0	0.0	2	1.0	2	1.1	0	0.0
ccDec	4	1.7	3	1.8	1	2.2	0	0.0	4	1.9	3	1.7	2	1.9
ccddcc	38	16.9	27	16.3	8	18.2	3	20.0	34	16.8	30	16.7	15	14.4
CC*Dec	1	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
cC*Dec	2	0.9	0	0.0	2	4.5	0	0.0	2	1.0	0	0.0	0	0.0
C	37	16.4	30	18.2	6	13.6	6	6.7	36	17.2	31	17.2	17	16.3
Cc	113	50.2	87	52.7	19	43.2	7	46.7	106	50.7	94	52.2	56	53.8
c	72	32.0	48	29.1	17	38.6	7	46.7	65	31.1	55	30.6	30	28.8
C*c	1	0.4	—	—	—	—	—	—	—	—	—	—	1	1.0
D	185	82.2	136	82.4	36	81.8	12	80.0	172	82.3	148	82.2	89	85.6
d	40	17.8	29	17.6	8	18.2	3	20.0	37	17.7	32	17.8	15	14.4
E	4	1.8	2	1.2	1	2.3	1	6.7	3	1.4	3	1.7	2	1.9
Ee	49	21.8	31	18.8	13	29.4	5	33.3	44	21.1	36	20.0	21	20.2
e	172	76.4	132	80.0	30	68.2	9	60.0	162	77.5	141	78.3	81	77.9
MMSS	19	8.4	15	9.1	3	6.8	1	6.7	18	8.6	16	8.9	6	5.8
MNSS	4	1.9	3	1.9	1	2.3	0	0.0	4	1.9	3	1.7	2	1.9
NNSS	1	0.4	1	0.6	0	0.0	0	0.0	1	0.5	1	0.5	1	0.6
MMSSs	38	16.9	27	16.4	9	20.5	2	13.3	36	17.2	29	16.1	13	12.5
MNSSs	54	24.0	41	24.8	8	18.2	5	33.3	49	23.4	46	25.6	28	26.9
NNSSs	9	4.0	6	3.6	2	4.5	1	6.7	8	3.8	7	3.9	6	5.8
MMss	22	9.8	16	9.7	5	11.4	0	0.0	21	10.0	16	8.9	9	8.7
MNSSs	51	22.7	36	21.8	13	29.5	2	13.3	49	23.4	38	21.1	21	20.2
NNss	27	12.0	20	12.1	3	6.8	4	26.7	23	11.0	24	13.3	19	18.3
M	79	35.1	58	35.2	17	38.6	3	20.0	75	35.9	61	33.9	28	26.9
MN	109	48.4	80	48.5	22	50.0	7	46.7	102	48.8	87	48.3	51	49.0
N	37	16.4	27	16.4	5	11.4	5	33.3	32	15.3	32	17.8	25	24.0
S	24	10.7	19	11.5	4	9.1	1	6.7	23	11.0	20	11.1	8	7.7
Ss	101	44.9	74	44.8	19	43.2	8	53.3	93	44.5	82	45.6	47	45.2
s	100	44.4	72	43.6	21	47.7	6	40.0	93	44.5	78	43.3	49	47.1
P														
P1	175	79.9	131	80.9	34	81.0	10	71.4	165	80.9	141	80.1	91	87.5
P2	44	20.1	31	19.1	8	19.0	4	28.6	39	19.1	35	19.9	13	12.5
Kell														
Kk	14	6.2	10	6.1	3	6.8	1	6.7	13	6.2	11	6.1	10	9.6
kk	211	93.8	155	93.9	41	93.2	14	93.3	196	93.8	169	83.8	94	90.4
Fy														
Fy (a ⁺ b ⁻)	45	20.0	32	19.4	11	25.0	2	13.3	43	20.6	34	18.9	20	19.2
Fy(a ⁺ b ⁺)	109	48.4	83	50.3	17	38.6	8	53.3	100	47.8	91	50.6	47	45.2
Fy(a ⁻ b ⁺)	71	31.6	50	30.3	16	36.4	5	33.3	66	31.6	55	30.6	37	35.6

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System and phenotypes	Total No RA patients		RF ⁺ ANA ⁻		RF ⁺ ANA ⁺		RF ⁻ ANA ⁻		RF ⁺		ANA ⁻		Control	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Kidd (Jk)														
Jk(a ⁺ b ⁻)	68	30.2	49	29.7	14	31.8	4	26.7	63	30.1	53	29.4	32	30.8
Jk(a ⁺ b ⁺)	106	47.1	76	46.1	21	47.7	9	60.0	97	46.4	85	47.2	44	42.8
Jk(a ⁻ b ⁺)	51	22.7	40	24.2	9	20.5	2	13.3	49	23.4	42	23.3	28	26.9
Lutheran (Lu)														
Lu(a ⁺ b ⁺)	14	6.2	9	5.5	5	11.4	0	0.0	14	6.7	9	5.0	6	5.8
Lu(a ⁻ b ⁺)	211	93.8	156	94.5	39	88.6	15	100.0	195	93.3	171	95.0	98	94.2
Lewis (Le)														
Le(a ⁺)	45	20.2	38	23.2	5	11.7	2	13.3	43	20.8	40	22.3	26	25.0
Le(a ⁻)	96	43.0	73	44.5	18	41.9	4	26.7	91	44.0	77	43.0	68	65.4
Haptoglobin (HP)														
1	30	13.5	20	12.1	6	14.0	3	20.0	26	12.6	23	12.8	14	13.7
1-2	106	47.5	76	46.1	24	55.8	6	40.0	100	48.3	82	45.8	55	53.7
2	87	39.0	68	41.2	13	30.2	6	40.0	81	39.1	74	41.3	33	32.4
Transferrin (TF)														
C	223	99.6	164	99.4	43	100.0	15	100.0	207	99.5	179	99.4	103	99.0
CB	1	0.4	1	0.6	0	0.0	0	0.0	1	0.5	1	0.6	1	1.0
Complement component 3 (C3)														
S	140	62.5	102	6.2	28	63.6	10	66.7	130	62.5	112	62.6	64	61.5
FS	70	31.3	53	32.3	12	27.3	4	26.7	65	31.1	57	31.8	32	30.8
F	10	4.5	6	3.7	3	6.8	1	6.7	9	4.3	7	3.9	4	3.8
SS096	1	0.4	1	0.6	0	0.0	0	0.0	1	0.5	1	0.6	1	1.0
FS096	1	0.4	1	0.6	0	0.0	0	0.0	1	0.5	1	0.6	0	0.0
F107S	2	0.9	1	0.6	1	2.3	0	0.0	2	1.0	1	0.6	2	1.9
SS094	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
Properdin factor B (BF)														
S	165	73.3	123	74.5	31	70.5	10	66.7	154	73.7	133	73.9	63	61.2
FS	50	22.2	36	21.8	10	22.7	4	26.7	46	22.0	40	22.2	29	28.2
F	4	1.8	3	1.8	1	2.3	0	0.0	4	1.9	3	1.7	6	5.8
SS07	4	1.8	2	1.2	2	4.5	0	0.0	4	1.9	2	1.1	2	1.1
F1S	2	1.8	1	0.6	0	0.0	1	6.7	1	0.5	2	1.1	3	2.9
Group specific component (GC)														
1S	62	28.1	44	27.0	14	33.8	4	26.7	58	28.3	48	27.0	29	28.7
1F1S	37	16.7	30	18.4	4	9.5	3	20.0	34	16.6	33	18.5	24	23.8
1F	1	0.5	1	0.6	0	0.0	0	0.0	1	0.5	0	0.6	1	1.0
1S2	76	34.4	56	34.4	15	35.7	5	33.3	71	34.6	61	34.3	26	25.7
1F2	20	9.0	12	7.4	6	13.6	2	13.3	18	8.8	14	7.9	12	11.9
2	25	11.3	20	12.3	3	7.1	1	6.7	23	11.2	21	11.8	9	8.9
Glutamic-pyruvate transminase (GPT)														
1	38	19.7	27	18.8	8	20.5	3	33.3	35	19.1	30	19.6	24	26.1
1-2	106	54.9	80	55.6	21	53.8	5	55.6	101	55.2	85	55.6	51	55.4
2	48	24.9	36	25.0	10	25.6	1	11.1	46	25.1	37	24.2	16	17.4
1-3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.1
2-3	1	0.5	1	0.7	0	0.0	0	0.0	1	0.5	1	0.7	0	0.0
Adenosine deaminase (ADA)														
1	204	90.7	153	92.7	38	86.4	12	80.0	191	91.4	165	91.7	92	88.5
1-2	20	8.9	12	7.3	5	11.4	3	20.0	17	8.1	15	8.3	12	11.5
2	1	0.4	0	0.0	1	2.3	0	0.0	1	0.5	0	0.0	0	0.0
Esterase D (EsD)														
1	178	79.1	134	81.2	32	72.7	11	73.3	166	79.4	145	80.6	79	76.0
1-2	44	19.6	30	18.2	11	25.0	3	20.0	41	19.6	33	18.3	23	22.1
2	3	1.3	1	0.6	1	2.3	1	6.7	2	1.0	2	1.1	2	1.9

System and phenotypes	Total No RA patients		RF ⁺ ANA ⁻		RF ⁺ ANA ⁺		RF ⁻ ANA ⁻		RF ⁺		ANA ⁻		Control	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Phosphoglucomutase 1 (PGM)														
1 ⁺	98	43.6	74	44.8	16	36.4	8	53.3	90	43.1	82	45.6	38	36.5
1 ⁺ 1 ⁻	34	15.1	22	13.3	10	22.7	2	13.3	32	15.3	24	13.3	18	17.3
1 ⁻	2	0.9	0	0.0	2	4.5	0	0.0	2	1.0	0	0.0	1	1.0
2 ⁺	8	3.6	6	3.6	2	4.5	0	0.0	8	3.8	6	3.3	9	8.7
2 ⁺ 2 ⁻	3	1.3	2	1.2	1	2.3	0	0.0	3	1.4	2	1.1	2	1.9
1 ⁺ 2 ⁺	53	23.6	40	24.2	10	22.7	2	13.3	50	25.9	42	23.3	23	22.1
1 ⁻ 2 ⁺	9	4.0	6	3.6	2	4.5	1	6.7	8	3.8	7	3.9	2	1.9
1 ⁻ 2 ⁻	1	0.4	1	0.6	0	0.0	0	0.0	1	0.5	1	0.6	2	1.9
1 ⁺ 2 ⁻	17	7.6	14	8.5	1	2.3	2	13.3	15	7.2	16	8.9	8	7.7
2 ⁻	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
Acid phosphatase (AP)														
A	31	13.8	23	13.9	6	13.6	2	13.3	29	13.9	25	13.9	15	14.4
BA	81	3.6	56	33.9	19	43.2	6	40.0	75	35.9	62	34.4	40	38.5
B	88	39.1	65	39.4	17	38.6	5	33.3	82	39.2	70	38.9	42	40.4
CB	19	8.4	15	9.1	2	4.5	2	13.3	17	8.1	17	9.4	6	5.8
CA	6	2.7	6	3.6	0	0.0	0	0.0	6	2.9	6	3.3	1	1.0
Adenylate kinase (AK)														
1	210	93.3	155	93.9	39	88.6	15	100.0	194	92.8	170	94.4	98	94.2
1-2	15	6.7	10	6.1	5	11.4	0	0.0	15	7.2	10	5.6	6	5.8
Phosphogluconate dehydrogenase (PGD)														
A	216	96.0	157	95.2	43	97.7	15	100.0	200	95.7	172	95.6	101	97.1
CA	9	4.0	8	4.8	1	2.3	0	0.0	9	4.3	8	4.4	3	2.9

LDH, MDH, and SOD were monomorphic in all groups.

Table 3 Gene frequencies in patients with rheumatoid arthritis and controls

System and allele	RA patients (n=225)	RF ⁺ ANA ⁻ (n=165)	RF ⁺ ANA ⁺ (n=44)	RF ⁻ ANA ⁻ (n=15)	RF ⁺ (n=209)	ANA ⁻ (n=180)	Control (n=104)
ABO							
A1	0.1675	0.1687	0.1482	0.1835	0.1644	0.1699	0.1923
A2	0.0696	0.0722	0.0556	0.0862	0.0686	0.0733	0.0622
B	0.0501	0.0496	0.0708	—	0.0540	0.0454	0.0855
O	0.7128	0.7095	0.7254	0.7303	0.7130	0.7131	0.6599
Rhesus (Rh)							
C*De	0.0067	—	0.0227	—	0.0048	—	0.0041
cDe	0.1267	0.1061	0.1705	0.2333	0.1196	0.1167	0.1125
cde	0.4270	0.4255	0.4285	0.4667	0.4261	0.4291	0.4181
C*de	—	—	—	—	—	—	0.0007
CDc	0.4082	0.4327	0.3523	0.3000	0.4155	0.4218	0.4298
Cde	0.0096	0.0127	0.0000	0.0000	0.0104	0.0116	0.0000
CDE	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0077
cDe	0.0219	0.0230	0.0260	—	0.0237	0.0209	0.0270
C*							
C	0.0067	—	0.0227	—	0.0048	—	0.0048
c	0.4178	0.4455	0.3523	0.3000	0.4258	0.4333	0.4375
c	0.5756	0.5545	0.6250	0.7000	0.5694	0.5667	0.5577
D							
d	0.5784	0.5808	0.5736	0.5528	0.5792	0.5784	0.6202
d	0.4216	0.4192	0.4264	0.4472	0.4208	0.4216	0.3798
E							
e	0.1267	0.1061	0.1705	0.2333	0.1196	0.1167	0.1202
e	0.8733	0.8939	0.8295	0.7667	0.8804	0.883	0.8789

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System and allele	RA patients (n=225)	RF ⁺ ANA ⁻ (n=165)	RF ⁺ ANA ⁺ (n=44)	RF ⁻ ANA ⁻ (n=15)	RF ⁺ (n=209)	ANA ⁻ (n=180)	Control (n=104)
Ms	0.2820	0.2905	0.2544	0.2944	0.2826	0.2911	0.2484
Ns	0.0492	0.0489	0.0525	0.0389	0.0499	0.0478	0.0545
Ms	0.3114	0.3034	0.3820	0.1389	0.3202	0.2895	0.2661
Ns	0.3575	0.3572	0.3112	0.5277	0.3472	0.3716	0.4310
M	0.5933	0.5939	0.6364	0.4333	0.6029	0.5806	0.5144
N	0.4067	0.4061	0.3636	0.5667	0.3971	0.4194	0.4856
S	0.3311	0.3394	0.3068	0.3333	0.3325	0.3389	0.3029
s	0.6689	0.6606	0.6932	0.6667	0.6675	0.6611	0.6971
P	(n=219)	(n=165)	(n=42)	(n=14)	(n=204)	(n=180)	(n=104)
1	0.5518	0.5626	0.5636	0.4655	0.5628	0.5541	0.6465
2	0.4482	0.4374	0.4364	0.5345	0.4372	0.4459	0.3535
Kell (K)	(n=225)	(n=165)	(n=44)	(n=15)	(n=209)	(n=180)	(n=104)
K	0.0311	0.0303	0.0341	0.0333	0.0311	0.0306	0.0481
k	0.9689	0.9697	0.9659	0.9667	0.9689	0.9694	0.9519
% Duffy (Fy)							
Fy ^a	0.4422	0.4455	0.4432	0.4000	0.4450	0.4417	0.4183
Fy ^b	0.5578	0.5545	0.5568	0.6000	0.5550	0.5583	0.5817
Kidd (Jk)							
Jk ^a	0.5378	0.5273	0.5568	0.5667	0.5335	0.5306	0.5192
Jk ^b	0.4622	0.4727	0.4432	0.4333	0.4665	0.4694	0.4808
Lutheran (Lu)							
Lu ^a	0.0311	0.0273	0.0568	—	0.0335	0.0250	0.0288
Lu ^b	0.9689	0.9727	0.9432	1.000	0.9664	0.9750	0.9712
Lewis (Le)	(n=223)	(n=164)	(n=43)	(n=15)	(n=207)	(n=179)	(n=104)
Le a+ (%)	20.2	23.2	11.6	40.0	20.8	22.3	25.0
Haptoglobin (HP)							
1	0.3722	0.3537	0.4186	0.4000	0.3671	0.3575	0.4069
2	0.6278	0.6463	0.5814	0.6000	0.6328	0.6425	0.5931
Transferrin (TF)	(n=224)	(n=165)	(n=43)	(n=15)	(n=208)	(n=180)	(n=104)
C	0.9978	0.9970	1.0000	1.0000	0.9976	0.9972	0.9952
D	0.0022	0.0030	—	—	0.0024	0.0028	0.0048
Complement component 3 (C3)	(n=224)	(n=164)	(n=44)	(n=15)	(n=208)	(n=179)	(n=104)
S	0.7880	0.7896	0.7841	0.8000	0.7885	0.7905	0.7885
F	0.2031	0.2012	0.2045	0.2000	0.2019	0.2011	0.1923
SO94	—	—	—	—	—	—	0.0048
SO96	0.0045	0.0061	—	—	0.0048	0.0056	0.0048
F107	0.0045	0.0031	0.0114	—	0.0048	0.0028	0.0096
Properdin factor B (BF)	(n=225)	(n=165)	(n=44)	(n=15)	(n=209)	(n=180)	(n=104)
S	0.8578	0.8636	0.8409	0.8333	0.8589	0.8611	0.7767
F	0.1289	0.1273	0.1364	0.1333	0.1292	0.1278	0.1990
SO7	0.0089	0.0061	0.0227	—	0.0096	0.0055	0.0097
F1	0.0044	0.0030	—	0.0333	0.0024	0.0055	0.0146
Group specific component (GC)	(n=221)	(n=163)	(n=42)	(n=15)	(n=205)	(n=178)	(n=101)
1S	0.5362	0.5337	0.5595	0.5333	0.5390	0.5337	0.5347
1F	0.1335	0.1350	0.1190	0.1667	0.1317	0.1376	0.1881
2	0.3303	0.3313	0.3214	0.3000	0.3293	0.3287	0.2772

System and allele	RA patients	RF ⁺ ANA ⁻	RF ⁺ ANA ⁺	RF ⁻ ANA ⁻	RF ⁺	ANA ⁻	Control
Glutamic-pyruvate transaminase (GPT)	(n=193)	(n=144)	(n=39)	(n=9)	(n=183)	(n=153)	(n=92)
1	0.4715	0.4653	0.4744	0.6111	0.4672	0.4793	0.5435
2	0.5259	0.5313	0.5256	0.3889	0.5301	0.5229	0.4511
3	0.0026	0.0035	0.0000	0.0000	0.0027	0.0033	0.0054
Adenosine deaminase (ADA)	(n=225)	(n=165)	(n=44)	(n=15)	(n=209)	(n=180)	(n=104)
1	0.9511	0.9636	0.9205	0.9000	0.9545	0.9583	0.9423
2	0.0489	0.0364	0.0795	0.1000	0.0455	0.0417	0.0577
Esterase D (EsD)							
1	0.8889	0.9030	0.8523	0.8333	0.8923	0.8972	0.8702
2	0.1111	0.0970	0.1477	0.1667	0.1077	0.1028	0.1298
Phosphoglucomutase 1 (PGM)							
1 ⁺	0.6667	0.6788	0.6023	0.7773	0.6627	0.6833	0.6010
1 ⁻	0.1067	0.0879	0.1818	0.1000	0.1077	0.0889	0.1154
2 ⁺	0.1800	0.1818	0.1932	0.1000	0.1842	0.1750	0.2163
2 ⁻	0.0467	0.0515	0.0227	0.0667	0.0454	0.0528	0.0673
Acid phosphatase (AP)							
A	0.3311	0.3273	0.3523	0.3333	0.3325	0.3278	0.3413
B	0.6133	0.6091	0.6250	0.6000	0.6124	0.6083	0.6250
C	0.556	0.636	0.0227	0.0667	0.0550	0.0639	0.0337
Adenylate kinase (AK)							
1	0.9667	0.9697	0.9432	1.0000	0.9641	0.9722	0.9712
2	0.0333	0.0303	0.0568	—	0.0359	0.0278	0.0288
Phosphogluconate dehydrogenase (PGD)							
C	0.9800	0.9758	0.9886	1.0000	0.9785	0.9778	0.9856
A	0.0200	0.0242	0.0114	—	0.0215	0.0222	0.0144

Table 4 Association of Bf system with rheumatoid arthritis

Group comparison	Bf ⁺ S/pos	Bf ⁺ S/neg	RR	χ^2	p Value
1 RA patient	165	54			
Control	63	35	1.7	4.12	<0.05
2 RF ⁺ ANA ⁻ patient	123	39			
Control	63	35	1.8	4.05	<0.05
3 RF ⁺ ANA ⁺ patient	31	1			
Control	63	35	1.6	1.2	>0.05
4 RF ⁻ ANA ⁻ patient	10	4			
Control	63	35	1.4	0.50	>0.05
5 RF ⁺ patient	154	50			
Control	63	35	1.7	4.08	<0.05
6 ANA ⁻ patient	133	43			
Control	63	35	1.7	3.37	>0.05

RR=relative risk.

show little discrimination when considered one by one can when combined produced an overall meaningful pattern. For example, the faces of two individuals appear to the human eye quite distinct, yet one would find it very difficult to distinguish between them say by the interorbital distance or

upper lip length considered alone. When all characters are combined into an overall picture by the human eye they are seen to be quite different. Such a procedure is paralleled by the statistical techniques of multivariate analysis, and their application is a well established method of assisting the interpretation of genetic characters.

Many measures have been defined of the genetic similarity or dissimilarity among various populations; one group of multivariate measures are genetic distance statistics, another relates to kinship. If patients with different humoral status represent different genetic subsets of the population then such measures may be useful indicators of the genetic heterogeneity of the disease.

Of the groups of multivariate statistics that represent kinship, the index of Harpending and Jenkins²⁸ is particularly useful. The Harpending R matrix is based on the mean standardised deviation of gene frequencies in each group from the total mean (r_{ii}) and on the average covariance of the gene

frequencies in a pair of subgroups relative to the mean (r_{ij}). In this way the frequencies of gene K from the subgroups are transformed into a single value matrix of scaled gene frequency covariance, where the r_{ij} element of R matrix is:

$$r_{ij} = \frac{1}{k} \sum \frac{(p_{ik} - \bar{p}_k)(p_{jk} - \bar{p}_k)}{\bar{p}_k(1 - \bar{p}_k)}$$

where p_{ik} is the frequency of allele k in group i and $\bar{p}_k$ is the mean frequency of allele k over all groups. The elements r_{ij} are obtained as:

$$r_{ii} = \frac{1}{k} \sum \frac{(p_{ik} - \bar{p}_k)^2}{\bar{p}_k(1 - \bar{p}_k)}$$

Table 5 gives the R matrix obtained from the gene frequency data of blood groups, serum proteins, and red cell enzymes. Genetic distinction between the control and the rheumatoid population was shown by the consistently negative values of the control group compared with each of the other subsamples. The patients with both humoral factors present showed negative values against the RF⁺ANA⁻ and RF⁻ANA⁻ subgroups, suggesting the least affinity with these groups. Genetic distance was analysed only on the patient subgroups (RF⁺ANA⁻; RF⁺ANA⁺; RF⁻ANA⁻) and controls.

The genetic distances (d^2) derived from the R matrix are given in Table 6. The largest distance of 0.034 was found between the RF⁺ANA⁺ and RF⁻ANA⁻ subgroups. Other large distances were found between RF⁻ANA⁻ and controls, and RF⁻ANA⁻ and RF⁺ANA⁻ groups (0.026 and 0.025 respectively).

The d^2 matrices were reduced to two dimensional plots by using the first two eigenvectors in a principal component analysis and a dendrogram based on the maximum linkage method (Figs 1 and 2 respectively). The isolated position in these plots of the RF⁻ANA⁻ group is probably partly due to their small sample size, but the genetic distinction of the patients with both humoral factors is beyond doubt.

Table 6 Genetic distance (d^2) between control and RA subgroups

Group		1	2	3	4
1	RF ⁺ ANA ⁻	—			
2	RF ⁻ ANA ⁺	0.0131	—		
3	RF ⁻ ANA ⁻	0.0252	0.0336	—	
4	Control	0.0105	0.0144	0.0256	—

Fig. 1 Eigen vector diagram showing genetic relation of RA subgroups and controls.

Discussion

As an illustration of the usefulness of polymorphism for understanding genetic heterogeneity and disease aetiology this investigation of the association of genetic markers and rheumatoid arthritis is quite encouraging. It is appreciated that in multiple testing some apparently conventionally significant results may arise by chance, and this may be one interpretation of the findings in the MN, Le^a, and Bf

Table 5 R matrix calculated from gene frequency data on control and RA groups for 19 polymorphic systems

Group	No	1 Total RA patients	2 RF ⁺ ANA ⁻	3 RF ⁺ ANA ⁺	4 RF ⁻ ANA ⁻	5 RF ⁺	6 ANA ⁻	7 Controls
Total RA patients	1	0.0000						
RF ⁺ ANA ⁻	2	0.0009	0.0000					
RF ⁺ ANA ⁺	3	0.0001	-0.0018	0.0000				
RF ⁻ ANA ⁻	4	-0.0019	0.0028	-0.0046	0.0000			
RF ⁺	5	0.0007	0.0012	0.0003	-0.0032	0.0000		
ANA ⁻	6	0.0006	0.0015	-0.0020	-0.0012	0.0008		
Controls	7	-0.0010	-0.0009	-0.0002	-0.0024	-0.0008	-0.0011	0.0000

Fig. 2 Dendrogram of various RA subgroups and control constructed from Harpending d^2 matrix.

results. The p values are given, however, because they draw attention to the systems where further investigation may be profitable, but the significance of these individual systems is of little relevance to the general directions shown by the multivariate analysis.

There is marked association with Bf in the total group of patients with RA and patients with the rheumatoid factor. Other immunogenetic studies suggest an association of HLA-DR4 or an unknown immune response gene in linkage disequilibrium with the D locus in seropositive (RF⁺) rheumatoid arthritis patients. The Bf gene is also located within the major histocompatibility region, and the association found with it could well suggest an association with an immune response gene through the phenomena of linkage disequilibrium. Further analysis of extended haplotypes in families with RA may be helpful in predicting the defective immune response and precise genetic susceptibility to rheumatoid arthritis. It will also be useful to include other gene markers, like α_1 antitrypsin, which are thought to be associated with severe seropositive RA.

Another interesting association seems to be with the Lewis blood group, with a decrease of the Le(a) blood groups in patients with RA in whom both RF and ANA are present. How this Lewis association may be related to the aetiology of disease is not clear, but it seems to be worth further investigation since in another rheumatic disorder, primary Sjögren's syndrome, an association with the Lewis antigen has recently been shown.²⁹

Most studies of disease association have been

conducted in terms of variation at a single locus. The present investigation shows that genetic distance approach, which incorporates variation of a large number of loci simultaneously, can be profitably used to examine the genetic heterogeneity of a disease. The present results clearly show that seronegative patients (RF⁻ANA⁻) and patients with both humoral factors (RF⁺ANA⁺) are two distinct entities. These two patient groups represent different subsections of the population, which are genetically distinct. The difference seen in the clinical course of the disease may be the result of interaction of genes controlling the production of these humoral factors with genetic environmental and physiological factors. Certainly in conclusion it seems that this multivariate analysis shows that rheumatoid arthritis is a genetically heterogeneous disease, and the various subtypes of the disease involve genetically distinct individuals.

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