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Genetic variants of complement component 3 (C3) in DR4 positive and DR4 negative rheumatoid arthritis

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SUMMARY C3 allotypes were defined in 86 Caucasoid patients with rheumatoid arthritis living in the north west of England and in 80 local, healthy controls. C3 allotype and phenotype frequencies were similar in RA (whether DR4 positive or negative) and control groups.

Key words: genetic marker, immunogenetics, C3 polymorphism, HLA.

There is now a well documented association between rheumatoid arthritis (RA) and HLA-DR4,[1 2] and more recently an association between RA and the Gm variant, G1m(x) (coded for by genes on the 14th chromosome) has been reported.[3 4] Previous studies in this laboratory found an increase in the complement component, properdin factor B (Bf) variant BfS, in both DR4 positive and DR4 negative RA[5]; the BfS bearing haplotype B15, DR4, BfS was found to be between four and five times more frequent in DR4 positive RA as compared with DR4 positive haplotypes from non-RA families.[6] However, it is unlikely that genetic predisposition to RA can be completely accounted for by genes linked to HLA and Gm, thus the above findings suggest that the complement genes themselves or genes linked to the complement genes may also influence susceptibility to RA.

Two previous studies have suggested an association between genetic variants of C3 (coded for by genes on chromosome 19) and RA.[7 8] In this study we have re-examined this association by comparing C3 allotype and phenotype frequencies in rheumatoid and control populations. As the previously described associations between RA and both G1m(x) and BfS were shown to be dependent on DR status we have also looked for evidence of an interaction between C3 and DR4.

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Materials and methods

C3 phenotypes were determined in 86 unrelated Caucasoid patients with classical or definite RA attending the rheumatology unit at Hope Hospital and in 80 normal healthy controls with no known history of RA who were members of hospital staff.

C3 typing was carried out by prolonged agarose gel electrophoresis of ethylenediaminetetra-acetic acid (EDTA) plasma or serum in barbital buffer pH

Table 1 *C3 phenotype and allotype frequencies in RA patients and controls*

	RA (n=86)		*Controls (n=80)*	
	No	*%*	*No*	*%*
Phenotype				
S	53	61	45	56
F	4	5	5	6
SF	28	33	28	35
S1S	0	0	1	1·5
S1F	1	1	0	0
SF1	0	0	1	1·5
Total	86	100	80	100
Allotype				
S	81	94	74	93
F	33	38	33	41
S1	1	0·01	1	0·01
F1	0	0	1	0·01

All differences in frequencies are non-significant (Fisher's exact test).

Table 2 *C3 phenotype and allotype frequencies in DR4 positive and DR4 negative RA*

	DR4 positive RA (n=48)		*DR4 negative RA (n=33)*	
	No	*%*	*No*	*%*
Phenotype				
S	28	58	21	64
F	0	0	3	9
SF	19	40	9	27
S1F	1	2	0	0
Total	48	100	33	100
Allotype				
S	47	98	30	91
F	20	42	12	36
S1	1	0·02	0	0

All differences in frequencies are non-significant (Fisher's exact test).

Table 3 *C3 phenotype and allotype frequencies in rheumatoid factor positive and negative RA patients*

	RF positive RA (n=72)		*RF negative RA (n=14)*	
	No	*%*	*No*	*%*
Phenotype				
S	45	63	8	57
F	3	4	1	7
SF	23	32	5	36
S1F	1	1	0	0
Total	72	100	14	100
Allotype				
S	68	94	13	93
F	26	36	6	43
S1	1	0·01	0	0

All differences in frequencies are non-significant (Fisher's exact test).

8·6.[9] Variants S, F, S1, and F1 were distinguished.

Eighty one (94%) of the patients were also typed for HLA-DR antigens as previously reported,[10] and all patients were tested for rheumatoid factor (RF) by the sheep cell agglutination test (SCAT) with RAHA kit (Fujizoki Inc., Tokyo). A past or present titre of $\geq$1/32 was considered positive.

Statistical analyses were carried out with Fisher's exact test or χ^2 test.

Results

The observed phenotype frequencies for both RA patients and controls were not significantly different from those expected assuming Hardy-Weinberg equilibrium (χ^2_q=4·8, p=0·8; and χ^2_q=8·5, p=0·5 respectively).

Table 1 shows allotype and phenotype frequencies in RA patients and controls. The phenotype and allotype frequencies were the same in both groups. Division of patients into DR4 positive and DR4 negative did not show any differences (Table 2), and there were no significant differences between rheumatoid factor positive and negative patients (Table 3).

Discussion

We have extended our study of immunogenetic markers in patients from the north west of England with RA by searching for an association with genetic variants of complement component C3. C3 allotype and phenotype frequencies were similar in RA and control groups, hence it was not possible to show any independent effect of C3 genes on susceptibility to RA. The C3 allotype and phenotype frequencies were also similar in DR4 positive and DR4 negative RA.

The gene frequencies for our control group are in keeping with previous reports of C3 polymorphism in European populations.[11 12] However, our results conflict with the two previous reports of C3 polymorphism in RA,[7 8] in which increases in the C3*F gene were found.

In the first study Farhud and coworkers found an increase in the C3*F gene frequency in 97 seropositive patients with chronic polyarthritis but not in seronegative patients.[7] In our patients there were no significant differences between RF positive and RF negative RA patients. In the second previous report Bronnestam also found an increase in the C3*F gene frequency in 180 patients with probable, classical, or definite RA as defined by the Rome criteria.[8]

In both these previous studies the gene frequencies reported for the RA group still lie within the accepted range of frequencies for normal European populations.[11 12]

In conclusion, we have found no evidence that genes linked to C3 predispose to seropositive or seronegative RA, either independently or by interaction with HLA-DR4.

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