Genetic susceptibility to early onset pauciarticular juvenile chronic arthritis: a study of HLA and complement markers in 158 British patients

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SUMMARY To investigate the genetics of susceptibility to early onset pauciarticular juvenile chronic arthritis (JCA), 158 unrelated ethnic British patients with a mean disease onset of 3.2 years, together with controls, were tested for HLA-A, B, C, and DR antigens. Additionally, 117 patients were also investigated for complement Bf and C4 markers. New observations included an increased frequency of the C4B 2 allotype (p corrected(p_c) <0.02) and C4A 4,B 2 phenotype ($p<0.0005$). Findings suggested a unique increase of the haplotype HLA-DRw8, Bf*S, C4A*4, C4B*2, HLA-B39, possibly predisposing to more severe disease. Strong positive associations were confirmed with HLA antigens A2 ($p_c=2.5\times10^{-8}$), DRw8 ($p_c=3.5\times10^{-14}$), DR5 ($p_c<0.02$), DRw52 ($p_c=2.8\times10^{-6}$) and DR5, w8 phenotype ($p_c=3.9\times10^{-6}$), and negative associations with DR7 ($p_c=5.8\times10^{-7}$), DR4 ($p_c<0.002$), and DRw53 ($p_c=0.004$). Antinuclear antibody (ANA) seropositivity correlated with DR5 ($p<0.02$), and in children with chronic iridocyclitis (CIR) Bw62 incidence was raised ($p<0.03$) and B44 reduced ($p<0.03$). HLA-A2 was found in 88% of ANA+, CIR+ patients ($p<0.01$). A significant excess of DR5, w8 heterozygotes was present (relative risk=41.1) and a lack of corresponding homozygotes. Results are inconsistent with a recessive, dominant, or intermediate mode of inheritance of susceptibility, and favour the existence of at least two DR linked 'disease' genes. Moreover, there may be an interaction in heterozygotes of combinatorial factors associated with DR5 and DRw8 in enhancing susceptibility. Possible immunogenetic mechanisms underlying the observed associations with three antigen classes are discussed. Evidence here suggests a role for the HLA-DQ locus in determining susceptibility to this disease.

Key words: major histocompatibility complex, genetic associations, linkage disequilibrium, inheritance, immunogenetics.

Juvenile chronic arthritis (JCA) is a heterogeneous group of disorders^{1 2} which is presently subclassified by the mode of onset into systemic, polyarthritic, or pauciarticular (four or fewer joints). This last type can be further divided into those under 9 years, showing a female predominance, often with chronic iridocyclitis (CIR) and antinuclear antibodies (ANA), those over 9 years, usually HLA-B27 positive boys, and others, which include patients with psoriasis.

The pauciarticular onset form of JCA shows multiple associations with HLA antigens, viz A2, B39, DR5, Dw5, DRw6, DRw8, Dw8, MT1, and MT2 (now DQw1, DRw52 respectively), and B44, B35 in linkage with DR5.³⁻²⁰ Family studies have demonstrated increased haplotype sharing among affected sibling pairs.^{19 21 22} In addition, increased frequencies of the complement haplotypes C4A*3, C4B*1, Bf*S, and C4A*3, C4B*2, Bf*S in early onset cases were reported,²³ while two studies of complement allotypes in pauci-JCA showed no significant deviations in distribution.^{19 24} These studies have shown the involvement of genetic factors in pauci-JCA, but the disease aetiology, pathogenetic

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mechanisms, and mode of inheritance of susceptibility remain undefined.

The reported distribution of antigens associated with pauci-JCA has varied between studies. This might be due to lack of clinical homogeneity, small sample size, racial heterogeneity, and the problems encountered in defining the cross reacting specificities DR5, DRw6, and DRw8, especially when two are present together.

This article describes the genetic aspects of susceptibility to early onset pauciarticular JCA in a clinically well defined group of 158 unrelated patients of British origin. All patients and controls were typed for 67 HLA-A, B, C, and DR antigens. Additionally, 117 patients were also investigated for 38 complement factor B (Bf) and C4 allotypes.

Patients and methods

SUBJECTS

The study comprised 158 patients classified as having pauciarticular onset JCA (EULAR-WHO criteria, 1977), with a mean age of onset of 3.2 years (range 6 months–9 years). All were unrelated, white Caucasians of British extraction. The mean duration of disease was 10.4 years (range 2.5–38 years). The majority of these patients were in ongoing studies relating to (a) ANA and CIR in juvenile chronic arthritis and (b) extending arthritis after a pauciarticular onset. There was therefore a predominance of patients with ANA and also of those in whom disease had extended beyond four joints. One hundred and twenty two patients (77%) were female and 36 (23%) male, all were IgM rheumatoid factor negative, 110 (70%) had ANA present in their serum, and 84 (53%) had developed CIR. Forty one (26%) cases persisted with pauciarticular disease, 47 (30%) had extended to five to nine joints, and 70 (44%) had over nine joints involved. Recorded data were computer based to facilitate analysis.

Controls for the HLA analysis were ethnically matched and comprised 192 random, unrelated, normal individuals, tissue typed either in our laboratory or comparably typed in the Imperial Cancer Research Fund Laboratories, London by Dr Julia Bodmer and staff. Complement allotype distribution in the patients was compared with that of a similar control panel of 200 British subjects typed by Dr K Welsh and colleagues at Guy's Hospital, London.

HLA TYPING

Fifty five HLA-A, B, and C specificities were tested with a standardised panel of 180 established antisera. The DR typing panel consisted of 60 well

validated antisera recognising the antigens DR1 to DRw10, DRw52, and DRw53, obtained from sources both in the UK and abroad, and included 22 sera employed in the 8th International Histocompatibility Workshop. Emphasis was placed on the definition of the related specificities DR5, DRw6, DRw8, and their assignments have been subsequently confirmed on the 9th Workshop typing sets. Typing was carried out by standard, two stage microlymphocytotoxicity visualised for the HLA-A, B, and C antigens by a modified two colour cytofluorochromasia procedure,²⁵ and for the DR antigens by two colour fluorescence on unseparated B cells.²⁶

COMPLEMENT ALLOTYPING

In addition, 117 of the patients together with controls were tested for 34 C4A and C4B allotypes of the classical complement pathway and four Bf allotypes of the alternative pathway.

C4 allotypes were detected by high voltage electrophoresis of desialated plasma, according to the method of Awdeh and Alper,²⁷ using a functional assay to discriminate between C4A and C4B variants. C4 'null' alleles were assigned in single heterozygotes (i.e., in individuals with a 'null' allele at either the A or B locus) on the basis of the ratio of C4A/C4B products as determined by densitometric scanning of the stained typing gels. Although family studies are required for accurate assignment of all null alleles, scanning can give information on the zygosity of the C4 loci and should detect most single heterozygotes.²⁸ This has been confirmed by one of us in extensive family studies (DCB, unpublished observations).

Factor B (Bf) allotypes were determined by the same method, that is, with trometamol (TRIS)-glycine buffer (pH 8.8), except for the following differences: 0.4 g/l calcium lactate was added to the tank buffer and 0.2 g/l was added to the gel buffer (1.4 tank buffer). Samples were not pretreated with neuraminidase for Bf allotyping, nor were any functional tests done: Bf allotype patterns were visualised by staining after immunofixation and washing.

STATISTICAL METHODS

Fisher's exact test (one sided) was used to evaluate comparisons of the frequencies of the genetic markers in patients and controls.²⁹ For the total study analysis the p values were corrected for the number of antigens tested (p_c). Relative risks (RR) were calculated by Woolf's method, with Haldane's correction applied whenever a value was null.³⁰ In addition to the traditional RR, the degree of positive association was estimated for the total study

Table 1 Selected HLA-A, B, and C antigen frequencies (%) in pauci-JCA patients and controls

HLA	Patients (n=158)	Controls (n=192)	Relative risk	Aetiologic fraction (δ)	Fisher's p_c
A2	78.6	46.8	4.2	0.60	2.5×10^{-8}
B27	9.4	6.3	1.5		NS
B35	11.3	16.6	0.6		NS
B39	15.7	4.9	3.6	0.11	3.2×10^{-2}
B44	31.4	30.2	1.1		NS
Bw57	1.3	9.2	0.1		NS
Bw60	26.4	9.2	3.5	0.19	8.8×10^{-4}
Bw62	14.5	9.3	1.7		NS
Cw6	5.1	21.4	0.2		5.4×10^{-4}
C blank	49.7	68.6	0.4		1.7×10^{-2}

NS=not significant at the 5% probability level.

group with the δ value of Bengtsson and Thomson.³¹ This value is identical to the so called 'population attributable risk' or 'aetiologic fraction' (EF) well known in epidemiology.³² For decreased risks the 'preventive fraction' (PF) was used.³² These values give direct measures in genetical terms of the strength of the association between a disease susceptibility or resistance gene and an HLA allele.

Linkage disequilibria (absolute delta or Δ) between loci were computed from the 2×2 table of phenotype frequencies by the formula of Mattiuz *et al*,³³ and the significance assessed by the χ^2 value with Yates's correction and Fisher's exact method. Haplotype frequencies (HF) were obtained from the sum of the product of the gene frequencies and the delta value. The magnitude of the delta value (relative delta or Δ_r) was calculated from the formula defined by Baur and Danilovs.³⁴ Three point linkage disequilibrium was analysed as described by Piazza.³⁵

Results

DISTRIBUTION OF GENETIC MARKERS IN THE TOTAL STUDY GROUP

HLA and complement allotypes

The results for HLA-A, B, and C antigens, which either showed altered frequency compared with controls or were of special interest, are listed in Table 1. There was a highly significant increase of A2, giving an EF value of 0.60. Of those B locus antigens deviating from normal, the raised levels of B39 and Bw60 remained significant after correction. Significantly lowered frequencies of C locus blanks, and particularly Cw6, were seen.

Relative to controls, a marked disturbance in the distribution of DR antigens in the patients was found, as shown in Table 2. The frequencies of DR5 and DRw8 were increased, the latter being associated with a high risk of the disease (RR=12.8). The incidence of DRw6 was also raised but not significantly. Both DR4 and DR7 were much reduced. The low level of DR7 was most significant, with a substantial PF value of 0.28; presence of this antigen carries a fivefold decreased risk of contracting the disease. The two cross reacting groups of DR antigens, DR3, 5, w6, w8 and DR4, 7, w9 were fully included in the 'supertypic' specificities DRw52 and DRw53 respectively, which is reflected in the significant increase of DRw52 and decrease of DRw53.

At the C4 loci the frequency of the C4B 2 marker was increased significantly (Table 3). Among the high risk group of DR5, w8 heterozygotes (vide infra) the incidence of this allotype was found to be even higher (46%). C4A 4 was also raised, though this was not significant after correction. No differences were seen in the distribution of the Bf alleles.

Table 2 HLA-DR antigen frequencies (%) in pauci-JCA patients and controls

HLA	Patients (n=158)	Controls (n=192)	Relative risk	Aetiologic fraction (δ)	Preventive fraction	Fisher's p_c
DR1	14.6	19.8	0.7			NS
DR2	22.2	28.1	0.7			NS
DR3	24.7	27.6	0.9			NS
DR4	9.5	26.6	0.3		0.19	1.9×10^{-3}
DR5	30.1	14.1	2.6	0.18		1.5×10^{-2}
DRw6	33.8	22.0	1.8	0.15		NS
DR7	8.9	33.8	0.2		0.28	5.8×10^{-7}
DRw8	39.2	4.8	12.8	0.36		3.5×10^{-14}
DRw9	5.1	1.0	5.1			NS
DRw10	0.6	1.1	0.5			NS
DRw52	85.4	59.4	4.0	0.64		2.8×10^{-6}
DRw53	22.8	42.7	0.4		0.25	4.0×10^{-3}

Table 3 *Bf and selected C4 complement allotype frequencies (%) in pauci-JCA patients and controls*

Complement	Patients (n=117)	Controls (n=200)	Relative risk	Fisher's p_c
C4				
AQO	30.8	22.7	1.5	NS
A2	6.8	15.0	0.4	NS
A3	75.2	86.0	0.5	NS
A4	17.1	6.8	2.8	NS
A6	2.6	8.7	0.3	NS
BQO	23.9	29.5	0.7	NS
B1	83.8	93.2	0.4	NS
B2	31.6	15.0	2.6*	1.6×10^{-2}
Bf				
F	36.8	42.7	0.8	NS
F1	1.9	1.0	1.9	NS
S	87.4	85.4	1.2	NS
SO-7	00.0	2.5	0.2	NS

*EF(δ)=0.20.*HLA and complement phenotypes*

Eighteen patients carried DR5, w8 (11.4%), 14 DRw6, w8 (8.9%), and 12 DR3, w8 (7.6%). The increased presence of the DRw8, X heterozygote gave a relative risk of 6.8 (Table 4), whereas the DR5, X RR value was 1.5. The DR5, w8 heterozygote showed the most significant increase, conferring a high risk of the disease (RR=24.6). This contrasted with the absence of risk for DR5 or DRw8 homozygotes. It was notable that 58% of the patients carried DR5 or DRw8, or both, compared with 20% in the controls.

Both the HLA-B39, DRw8, and C4A 4, C4B 2 phenotypes were significantly more frequent in patients than controls ($p=1.6 \times 10^{-6}$, 4.1×10^{-4} respectively). Of 36 C4B 2 positive cases, 23 also carried DRw8, with a phenotype frequency of 19.8%. Direct comparison with controls was not possible, but in view of the low control frequency of

Table 5 *Selected haplotype frequencies and linkage disequilibria (Δ) between the HLA and C4 loci in pauci-JCA patients**

Haplotype	Frequency	Δ	χ^2	Fisher's p
HLA-A2, B44	0.099	0.021	0.8	NS
A2, Bw60	0.077	0.015	0.4	NS
A2, Bw62	0.055	0.021	1.8	NS
B39, DRw8	0.068	0.052	27.2	9.7×10^{-8}
C4A*4, B*2	0.091	0.074	48.4	9.4×10^{-13}
A*4, B39	0.027	0.018	4.0	4.4×10^{-2}
B*2, B39	0.063	0.048	18.6	1.3×10^{-6}
A*4, DRw8	0.039	0.020	2.0	NS
B*2, DRw8	0.090	0.054	10.5	1.2×10^{-3}
DRw8, C4B*2, B39	0.065	0.039	6.7	9.8×10^{-3}

*n=158 (HLA); n=117 (C4).

DRw8 this finding is likely to be very significant. The Bf S allotype was present in 92% of the C4B 2 positive patients.

HLA and complement haplotypes

Selected haplotype frequencies and linkage disequilibria in the patients between the HLA loci, and between the HLA and complement loci are presented in Table 5. Established gametic associations in Caucasians,³⁶ which are present in this population, are not included in the table.

HLA antigens B39 and DRw8 were in strong linkage disequilibrium (HF=0.068, $\Delta r=0.82$), which compares with controls (HF=0.005, $\Delta r=0.37$). Similarly, the linkage and haplotype frequency of the complement alleles C4A*4 and C4B*2 were much greater in the patients (HF=0.091, $\Delta r=0.92$) than those found in the controls (HF=0.024, $\Delta r=0.62$). The 'null' allele

Table 4 *Distribution of DR5 and DRw8 phenotypes in pauci-JCA patients and controls*

DR phenotype	Patients (n=158)		Controls (n=192)		Relative risk	Fisher's p
	No	%	No	%		
DR5, 0*	1	0.6	2	1.0	0.6	NS
DR5, X†	28	17.7	24	12.5	1.5	NS
DRw8, 0*	1	0.6	2	1.0	0.6	NS
DRw8, X†	43	27.2	10	5.2	6.8	7.1×10^{-9}
DR5, w8	18	11.4	1	0.5	24.6	3.9×10^{-6}
Total	91	57.6	39	20.3	5.3	4.8×10^{-12}

*No DR antigen detected. Homozygosity confirmed on genotyping.

†DR antigen other than DR5 or DRw8.

Table 8 Linkage disequilibria (Δ) between HLA-DRw8, C4A*4, and C4B*2 in pauci-JCA patients classified by ANA status and by disease course

Patient subgroup	Haplotype					
	DRw8, C4A*4			DRw8, C4B*2		
	Δ	χ^2	Fisher's <i>p</i>	Δ	χ^2	Fisher's <i>p</i>
ANA negative (n=30)	0.009	0.0	NS	0.036	1.0	NS
ANA positive (n=86)	0.026	2.5	NS	0.061	8.5	2.0×10^{-3}
Persistent paucis (n=27)	-0.012	0.0	NS	0.054	0.9	NS
Extended <9 joints (n=31)	-0.012	0.0	NS	0.007	0.0	NS
Extended >9 joints (n=58)	0.049	7.0	7.9×10^{-3}	0.077	11.0	9.0×10^{-4}

DISTRIBUTION OF GENETIC MARKERS IN CLINICAL SUBGROUPS

The pauci-JCA population was classified by disease features and the HLA and complement distribution compared.

Sex, age of onset

Selected HLA antigen frequencies in patients in relation to sex, early age of onset of disease (1–5 years), and later onset (6–9 years) are given in Table 6.

The normal level of DR4 in the later onset subgroup contrasted with a significantly reduced frequency in early onset. The antigen Bw62 was significantly more common in boys when compared with both controls and with girls in either onset group.

Antinuclear antibody, chronic iridocyclitis

Of the 84 patients with CIR, 66 (79%) also carried ANA; of the 74 cases without CIR, 44 (59%) were ANA positive ($p<0.008$).

As shown in Table 7 the DR5 frequency in the ANA positive group was increased compared with that of controls but approached normal frequency in the absence of ANA ($p<0.04$). DRw8 levels were maintained above normal in both subgroups, though were higher in the presence of ANA. Both DR2 and DR4 correlated negatively with ANA, being less frequent in the ANA positive group relative to both normal values and the ANA negative cases (DR2: $p<0.06$, DR4: $p<0.05$). Additionally, HLA-A2 was more prevalent in patients with ANA than in those without ($p<0.02$).

The CIR analysis showed a different pattern of association (Table 7). There were no demonstrable DR differences, but the incidence of B44 was reduced in the presence of CIR, and above normal frequency in its absence ($p<0.03$), whereas Bw62 presented a reciprocal pattern ($p<0.03$). HLA-A2 showed an enhanced association with the presence of CIR ($p<0.05$).

Further partitioning of these data (not shown

here) confirmed a correlation of both DR5 and CIR with ANA positivity, and an exceptionally strong association of HLA-A2 with the presence together of ANA and CIR (frequency=88%, $p<0.01$). Complement allotype distribution did not deviate significantly between any of the subgroups compared.

Of the 18 DR5, w8 heterozygotes, 16 were positive for ANA (RR=3.9, $p<0.05$), and 69 ANA positive patients (63%) carried DR5 or DRw8, or both ($p<0.04$).

The DRw8, C4B 2 phenotype occurred more often in the group with ANA (22%) than in those without ANA (13%) ($p=NS$). Haplotype analysis showed linkage disequilibrium between these two alleles in the ANA positive patients, not evident in the absence of ANA (Table 8), and which was greater than that found in the total study group ($\Delta r=4.6$ v 4.0).

Disease course

Patients were divided between those following a persistent pauciarticular course for at least five years, those extending to five to nine joints involved, and those extending to more than nine joints.

Individual complement and HLA antigen frequencies did not differ significantly between these subgroups, other than that of Bw62 which was increased in patients with persistent pauci-JCA and in those with five to nine joints involved (Table 7).

The DRw8, C4B*2 alleles were found in linkage disequilibrium in the severely extended group but not in those patients more mildly affected (Table 8). This haplotypic association was substantially stronger than that of the total study group ($\Delta r=5.4$ v 4.0).

INHERITANCE OF SUSCEPTIBILITY

Fig. 1 depicts estimates of relative risks of the disease for the DR5 and DRw8 phenotypes, calculated against the absence of DR5 and DRw8 in

patients and controls.³⁷ Also indicated are 95% confidence limits of risks following usual statistical practice. Significant heterogeneity for the risks was shown ($\chi^2=14.0$, $p<0.008$) due primarily to the very high risk for DR5, w8 heterozygotes (RR=41.1, $p=1.3\times 10^{-8}$), and to a much lesser extent for DRw8, X heterozygotes (RR=9.8, $p=1.4\times 10^{-11}$).

The Hardy-Weinberg fit for the DR5 and DRw8 phenotypes is presented in Table 9. Gene frequencies were estimated by the gene counting method of maximum likelihood. There was a lack of Hardy-Weinberg equilibrium in this disease population, with a significant excess of DR5, w8 heterozygotes present and a deficit of the corresponding homozygotes. The observed number of DRw8, X heterozygotes was as expected from the increased incidence of DRw8 in these patients. Similarly, the DRw6 positive phenotypes did not differ from expected numbers.

These are findings inconsistent with the hypothesis of a recessive mode of inheritance of susceptibility to pauci-JCA and point to an additive, 'overdominance' effect of DR5, w8 heterozygotes.

Fig. 1 Relative risk of pauci-JCA for DR5 and DRw8 phenotypes. Relative risks are calculated against the non-DR5, -DRw8 group. Bars indicate 95% confidence intervals of relative risks.

Table 9 Deviation from Hardy-Weinberg equilibrium in pauci-JCA patients

DR phenotype	Number		χ^2
	Observed	Expected	
5, 5	1	3.65	1.92
5, X	28	31.14	0.32
w8, w8	1	6.28	4.44
w8, X	43	40.87	0.11
5, w8	18	9.57	7.43
X, X	67	66.49	0.00
Total	158	158.00	$\chi^2_{(3)} = 14.22$ $p < 0.003$

Discussion

GENETIC ASSOCIATIONS IN EARLY ONSET PAUCI-JCA

The significantly increased incidence of the C4B 2 variant and C4A 4, B 2 phenotype in this study is the first reported association of this disease with complement allotypes. Increases of C4B 2 were found in other pauci-JCA populations but were not significant.^{19 24} Disease associations with C4B 2 have also been reported in Alzheimer's disease and multiple sclerosis.^{38 39} Other recorded C4 associations are with the 'null' alleles C4A*QO and C4B*QO in several autoimmune disorders.⁴⁰ Apart from a weak linkage of C4B*QO and DRw6, there was no evidence here of involvement of the 'null' alleles. This was not surprising in view of the normal or raised serum complement levels found in JCA.⁴¹

We have shown novel observations of disease associations with phenotypic combinations of HLA-B39, -DRw8, C4A 4, C4B 2, and of linkage disequilibria between these alleles, with increased haplotype frequencies. Increased frequencies in patients with early onset JCA were reported of the common, DR5 associated haplotype C4A*3, C4B*1, Bf*S,^{19 23} the uncommon haplotype C4A*3, C4B*2, Bf*S, and in late onset B27 associated cases of the C4A*4, C4B*2, Bf*S haplotype.²³ In the present study C4A*3, C4B*1 phenotype and haplotype frequencies were less than in controls. The non-availability for this study of a control population both HLA and complement typed precluded direct comparisons with the HLA/complement results in the patients. The normally low incidence of DRw8, however, requires a comparatively large control population to evaluate any but strong linkages with this allele. In normal Caucasians included in the 9th Workshop³⁶ DRw8, C4B*2 were not found in linkage disequilibrium and had a relative delta value of $\Delta r=0.08$ and haplotype frequency HF=0.004 compared with $\Delta r=0.40$, HF=0.090 in our pauci-JCA patients. Thus the occurrence of a haplotypic

association between DRw8 and C4B*2, which is supported by preliminary family data, is likely to be unique to these patients. The striking relationship of the B39, DRw8 alleles we report may reflect a functionally significant HLA-DQ α chain variation, as has been suggested for the B15, DR4 associated effect in type 1 diabetics.⁴² These results require verification in further family studies but, taken with the phenotype data, they imply an increased frequency in this pauci-JCA population of a supertype, and possibly an extended haplotype comprising DRw8, Bf*S, C4A*4, C4B*2, B39.

The multiple associations with HLA antigens confirm and extend earlier reports.³⁻²⁰ The increase of A2 was unrelated to increases in other antigens, in agreement with reported family data.¹⁹ Associations with DR5 and DRw8, both singly and in combination, were confirmed, with 58% of patients possessing either or both antigens. The presence of DRw8 confers an almost 13-fold risk of the disease, which, together with the substantial EF (δ) value, signify linkage of this allele to a disease susceptibility gene. In contrast with earlier findings^{4 6 19} the DR5 increase was less marked, possibly reflecting racial differences between studies. Reports of a significant increase in DRw6 incidence^{12 13 15} were not substantiated. The lack of a decrease in this antigen to compensate for increases in other alleles, however, may itself be significant. Recently defined serological subtypes of DR5 (DRw11, DRw12) and DRw6 (DRw13, DRw14)⁴³ may show a stronger association with pauci-JCA. We confirm significant negative correlations with DR4, DR7, and DRw53. The DR4 decrease might be a compensatory one, but the paucity of DR7 positive cases indicates linkage of this allele to a disease resistance gene.

The linkage disequilibrium between the DRw8, C4B*2 alleles found in the total patient group resided primarily and more strongly in the ANA positive subgroup and in those who had extended to over nine joints involved. Confirmation of this finding would indicate that the DRw8, C4B*2 haplotype predisposes to more severe disease. A correlation between ANA seropositivity and DR5 was confirmed,^{4 20} but that reported between ANA and DRw8^{15 17 20} was not evident; the presence of both antigens, however, carried a fourfold increased risk amongst patients of having circulating ANA. Contrary to the results of previous studies,^{4 6 12} the CIR positive group was not associated with any DR antigens, but showed an increase of Bw62 and decrease of B44. The antigen HLA-A2 associated preferentially with CIR, confirming an earlier report,¹⁹ and also with ANA, with a high incidence (88%) seen in the presence of both disease features,

possibly implicating this antigen in disease pathogenesis. Classification by sex and age of onset provided no indication of disease heterogeneity other than an increase of Bw62 in males, and an absence in later onset cases of the abnormally low DR4 frequency shown in the total patient group. The DR4 observation supports an earlier finding,²⁰ but reports of a lack of association in later onset males with HLA-A2, DR5, and DRw8^{9 19} are not substantiated.

A consistent feature of all subgroups of patients divided by disease parameters was the pattern of strong association with the HLA antigens A2, DRw8, and DR7, implying the sharing of a similar background of genetic susceptibility. It may be concluded that the clinical variations encountered are manifestations of the one disease as classified by mode of onset.

INHERITANCE OF SUSCEPTIBILITY TO EARLY ONSET PAUCI-JCA

The lack of fit to Hardy-Weinberg expectations we report for DR5, DRw8 phenotypes is incompatible with a recessive mode of inheritance of susceptibility. A similar view was expressed in the 9th Workshop pauci-JCA report, though some of the data differed from those of the present study.¹⁹ This discrepancy between studies could be attributed to racial or clinical heterogeneity, or the features we describe may be characteristic of the disease in British patients. Investigations of double case, sib pair families have shown significantly increased haplotype sharing in pauci-JCA but no consensus about the mode of inheritance of susceptibility.^{19 21 22}

This study has shown an excess in both the frequency of and risk for DR5, w8 heterozygotes over those for both of the homozygotes. The standard deviations of risks tend to be large, but these observations together are evidence to discount dominant, recessive, and intermediate models of inheritance and introduce the concept in pauci-JCA of at least two different DR linked susceptibility alleles or genes, possibly acting through separate pathogenetic pathways. The affected sib pair data, showing that both haplotypes contribute to susceptibility in most cases, support this hypothesis.^{19 21 22} Moreover, the possible greater risk for the DR5, w8 heterozygote than for the sum of the other heterozygotes, DR5, X and DRw8, X, may indicate that the effect of the DR linked genes is more than just additive. Such a mechanism, involving the DR related HLA-DQ locus, might be the generation of hybrid antigens in particular heterozygotes by gene *trans*-complementation,⁴⁴ which may be implicated in enhanced susceptibility to certain other diseases.^{42 45 46}

Further 'disease' genes in linkage with A2, DR7, and possibly DR4 and DRw6, may contribute to the final susceptibility status of the individual. Also, since the genetic associations and the penetrance of the disease gene(s) are incomplete there are likely to be additional factors predisposing to pauci-JCA.

POSSIBLE MECHANISMS OF SUSCEPTIBILITY TO EARLY ONSET PAUCI-JCA

It is clear from the accumulation of HLA data³⁻²² and the reported abnormalities in the immune system,⁴¹ that there is a strong immunogenetic component of susceptibility to pauci-JCA. Possible mechanisms of susceptibility underlying the associations with three classes of major histocompatibility complex (MHC) gene products observed in this study can be considered.

The singular association, particularly of ANA, CIR positive cases, with the class I antigen HLA-A2 is intriguing. MHC class I molecules function as recognition elements, and possibly receptors, in the host response to a variety of foreign exogenous antigens,⁴⁷ and these mechanisms may underly the known association of certain diseases with these products.⁴⁸ Polymorphism within the serologically defined A2 antigen has been recognised,⁴⁹ and at least four distinct subtypes which are functionally important have been detected.^{50 51} The implication in susceptibility to pauci-JCA of an interaction between an HLA-A2 molecule and a foreign agent, such as a pathogen, becomes an attractive notion.

Strong associations found with the class II antigens DRw8 and DR7, apparent in all clinical subgroups, indicate linkage to major immune-response (Ir) or immune suppression (Is) genes, or both, controlling disease susceptibility and resistance respectively. The DR5 association was weaker and correlated with ANA seropositivity. This DR5 expression in pauci-JCA, and in other autoimmune related diseases,⁵²⁻⁵⁵ may reflect a generalised difference in the immune response to autoantigen effected by a DR linked, non-antigen specific Ir/Is determinant, as proposed for the DR3 associated group of autoimmune related disorders.^{45 56} Further Ir/Is determinants weakly linked to DR2 and DR4 may be present, having a protective role against autoantigen.⁵⁷

Implication of complement in the pathogenesis of this disease is evidenced by its reported activation, particularly via the classical pathway, and possible formation of immune complexes.⁴¹ The contribution of complement system components and MHC encoded gene products in immune defence is known,^{58 59} and their direct involvement in certain DR associated diseases is assumed.⁶⁰ The

concept has been discussed of clusters of linked MHC genes with inter-related functions, where selective interactions favour closer linkage disequilibrium.^{57 61} Our findings give rise to the speculation that susceptibility to early onset pauci-JCA may be mediated through the interaction of a DRw8 related Ir gene and linked complement C4B*2 gene, leading to a defective immune response to unknown antigen(s).

Finally, evidence from this study suggests a role for the HLA-DQ locus in determining susceptibility to pauci-JCA. DNA analysis is revealing substantial genetic variation in the HLA-D region, particularly in the DQ α chain,⁶² which is likely to play an important functional part in resistance to disease, such as through immune response differences.⁴² The reported DNA level polymorphism is correlated with HLA-DR and -DQ types, but in addition, some variation within DR types, such as DR5, has been detected.⁶³ There may be a closer relation between early onset pauciarticular JCA and a DNA defined subtype of DR5 or a putative variant of DRw8, or both, than that shown by serology.

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