

# A study of the association of HLA DR, DQ, and complement C4 alleles with systemic lupus erythematosus in Iceland

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## Abstract

**Objective**—To perform an exploratory analysis of the relative contribution of single MHC genes to the pathogenesis of systemic lupus erythematosus (SLE) in a homogenous white population.

**Methods**—MHC class II alleles and C4 allotypes were determined in 64 SLE patients and in ethnically matched controls. HLA-DR and DQ typing was performed by polymerase chain reaction amplification with sequence specific primers. C4 allotypes were determined by agarose gel electrophoresis.

**Results**—The frequency of C4A\*Q0 was significantly higher in patients than in controls (46.9% v 25.3%,  $p=0.002$ ). HLA-DRB1, DQA1, and DQB1 alleles in the whole group of SLE patients were not significantly different from those of controls. On the other hand increase in DRB1\*03 was observed in the group of patients with C4A\*Q0, as compared with patients with other C4A allotypes ( $p=0.047$ ). There was no significant correlation between severe and mild disease, as judged by the SLEDAI, and HLADR, DQ alleles and comparing the patients with C4A\*Q0 with those with other C4A allotypes there was no significant difference regarding clinical manifestations.

**Conclusion**—The results are consistent with the argument that C4A deficiency contributes independently to susceptibility and the pathogenesis of SLE. C4A\*Q0 in SLE patients in Iceland shows weaker linkage disequilibrium with DR3 genes than reported in most other white populations and emphasises the role of ethnicity.

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A number of studies have shown associations of certain serological HLA specificities with systemic lupus erythematosus (SLE); B8, DR2, and DR3 have shown the strongest associations.<sup>1</sup> The nature of this relation is, however, obscure as the strength of these associations varies from study to study and associations can vary in ethnically different populations.<sup>2</sup>

The two components of complement component four, C4A and C4B are encoded by separate two closely linked polymorphic loci. Thirty five different alleles have been identified, including non-expressed, or “null”

alleles (“\*Q0”), 13 alleles for C4A and 22 for C4B.<sup>3</sup> C4A null has been associated with SLE in several ethnically diverse populations.<sup>1</sup> There are however a few studies that do not find such an increased frequency of C4A\*Q0 in SLE.<sup>4,5</sup>

The molecular basis for C4A deficiency differs from one racial group to another and even within the same ethnic group. A large deletion, involving most of the C4A gene and part of the 21 OHA gene has been reported to account for up to two thirds of C4A null in a white population with SLE by virtue of HLA-B8, DR3.<sup>1</sup> However, recent studies indicate that gene deletion may not be the basis for C4A\*Q0 in other ethnic groups with diverse HLA types.<sup>3</sup> Non-deleted but non-functional C4A alleles have been described in Asian<sup>6</sup> and Mexican patients as well as in some white and black populations.<sup>7</sup>

Therefore the genetic basis for C4A\*Q0 in SLE seems to be heterogenous, and linkage disequilibrium makes it difficult to assess the relative contribution of HLA and C4A\*Q0 to disease susceptibility. The need to pay attention to ethnicity in both patients and controls in genetic studies of white populations in SLE has recently been emphasised.<sup>4</sup>

## Methods

### PATIENTS AND CONTROLS

The Icelandic population (260 000) is white, with ethnic origin most likely from the Nordic countries and Ireland. The patient group consisted of 57 women and seven men (table 1). They all fulfilled four or more ARA criteria for the classification of SLE. Disease severity was judged by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).<sup>8</sup> Included in this group of 64 patients there are 16 patients from seven multicase families where the intra-familial relationship is of the first or second degree. Patients and controls were from the Icelandic population.

Table 1 Clinical characteristics of the 64 patients

Total number of patients	64
Women (%)	57 (89.1)
Men (%)	7 (10.9)
Mean age and range at first symptoms	29.4 (9–70)
Mean number of ARA criteria	6.3 (4–10)
Major organ involvement (%)	28 (43.7)
Renal involvement (%)	13 (20.3)
ANA (%)	64 (100)
Anti-dsDNA antibodies (%)	51 (79.6)

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## HLA TYPING

HLA-DR and DQ typing was performed on genomic DNA extracted from peripheral blood leucocytes by polymerase chain reaction amplification with sequence specific primers (PCR-SSP).<sup>9</sup>

## PHENOTYPING OF C4

C4 allotypes were determined by high voltage agarose electrophoresis on carboxypeptidase and neuraminidase treated samples followed by immunofixation.<sup>10</sup>

An attempt was made to select serum samples for C4 electrophoresis from periods of low disease activity.

## STATISTICAL METHODS

The  $\chi^2$  test with Yates's correction was used for statistical analysis. The level of significance was selected as 0.05. Ninety five per cent confidence intervals are given for differences in proportions.

## Results

The frequencies of HLA-DRB1, DQA1, and DQB1 alleles were not significantly different in the whole group of patients compared with controls (table 2). On the other hand the frequency of C4A\*Q0 was significantly higher than in controls ( $p=0.002$ ). Four patients were homozygous for C4A\*Q0. C4B\*Q0 was not significantly different in patients compared with controls.

DRB1\*03 was higher in the group of patients with C4A\*Q0 compared with those with other C4A allotypes ( $p=0.047$ ) (table 3).

As relationship between the 16 patients in the seven families might bias the results, the following was done to assess if this had any effect: (1) all 16 patients from the seven families were excluded and alternatively, (2) at random only one patient from each of the seven families was included. This did not change the results: C4A\*Q0 was found in 50% and in 49% of SLE patients respectively compared with 25.3% in controls.

There was no significant correlation between severe and mild disease, as judged by the highest SLEDAI during the disease course, and

HLA DR, DQ alleles. Comparing all of the patients with C4A\*Q0 with those with other C4A allotypes there was no significant difference regarding the mean age of onset or the severity of disease including renal involvement.

## Discussion

The findings in this study are different from most previous studies in white populations including studies on SLE patients from Scandinavia<sup>4 11</sup> in which B8, DR2, DR3 have shown the strongest associations. On the other hand the increased frequency of C4A\*Q0 in our patient group is consistent with many other reports in ethnically diverse populations.<sup>1 5 11</sup>

Only 23.4% of the whole group of the Icelandic SLE patients, 36.7% of those with C4A\*Q0 and only 15.7% of the controls had DR3. This is different from other white populations and suggests different linkage disequilibrium in the Icelandic population.

This study along with the studies by Schur *et al* from USA and by Goldstein *et al* from Canada<sup>4</sup> emphasise ethnic difference regarding MHC markers in SLE even within white populations. As a possible explanation for the difference in the frequencies of HLA alleles in our study and some other studies in Scandinavians the question arises if there is a selection bias regarding the severity of disease. However, in this study no significant difference was found between mild and severe disease, as judged by the SLEDAI, regarding HLA-DR, DQ and C4A alleles, but the power of these calculations is small because of the small numbers in these subgroups.

The findings in this study are consistent with the hypothesis that C4A deficiency contributes to susceptibility of SLE. There are several strong arguments suggesting that in SLE the disease predisposition may primarily result from a deficiency of the C4A protein. The findings of increased frequency of C4A\*Q0 in different ethnic groups irrespective of HLA associations, the molecular heterogeneity of C4A\*Q0 in SLE, and the finding of markedly increased relative risk when C4A\*Q0 occurs in the homozygous form are consistent with that theory.<sup>12</sup>

Additional support for this theory is the association of SLE with deficiencies of other complement components; C1q, C1r and C1s and C3; the structural genes for these components show no linkage to the MHC.<sup>13</sup>

Recent studies of immune complex handling and success with replacement therapy in a patient with C2 deficiency and SLE have further elucidated the importance of intact function of the classic complement pathway in the clearance of immune complexes and supported the theory of a causal relation between complement deficiency and SLE.<sup>14 15</sup> It seems reasonable to speculate that deficiency of the C4 protein would predispose to immune complex disease in a similar manner. Whether the same is true for partial deficiency (heterozygote) has yet to be demonstrated.

This study was done on an ethnically homogenous and well defined group of patients and controls. There are not many previous

Table 2 Distribution in the group of 64 Icelandic patients of those DR, DQ, and C4A alleles most frequently reported to be associated with SLE in a white population

	SLE n (%)	Control n (%)	p Value
DRB1*03	15/64 (23.4)	37/236 (15.7)	NS (0.3)
DQA1*0501	16/64 (25.0)	47/170 (27.6)	NS
DQB1*0201	13/64 (20.3)	33/180 (18.3)	NS
DRB1*15	26/64 (40.6)	95/236 (40.3)	NS
DQA1*0102	37/64 (57.8)	91/170 (53.5)	NS
DQB1*0602	26/64 (40.6)	70/180 (38.9)	NS
C4A*Q0	30/64 (46.9)	49/194 (25.3)	0.002 (Difference 22.1% with 95% CI 8.6, 35.6%)

Table 3 Frequency of DRB1\*03, DQA1\*0501, and DQB1\*0201 in patients with C4A\*Q0 compared with other C4A allotypes

	C4A*Q0 n (%)	Other C4A allotypes n (%)	p Value
DRB1*03	11/30 (36.7)	4/34 (11.8)	0.04 (Difference 24.9% with 95% CI 4.1, 45.0%)
DQA1*0501	10/30 (33.3)	6/34 (17.6)	NS (0.25)
DQB1*0201	10/30 (33.3)	6/34 (17.6)	NS (0.25)

studies of SLE patients in which careful ethnic matching of controls has been attempted. We therefore consider this study an important contribution to genetic studies on SLE.

The results of our study support the theory that deficiency of complement proteins, in this instance C4A\*Q0, may contribute to the pathogenesis of SLE. However, many studies indicate that the inheritance of SLE may be polygenic and there may be additional unidentified genes that determine susceptibility to SLE. Our results strongly support the importance of ethnic background and indicate that the relative importance of different genes may vary in different populations.

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