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HLA and prognosis in multiple sclerosis

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Abstract The patients of a multiple sclerosis (MS) incidence cohort with 25 years of longitudinal follow-up were typed for HLA-DR and DQ. This type of cohort provides reliable data for gene frequencies and prognostic studies. The influence of sampling bias, mainly due to mortality during the long follow-up, was accounted for. A positive association between MS and DR15,DQ6 was confirmed, but this haplotype did not influence prognosis. There was no difference in haplotype frequency between relapsing-remitting and primary chronic progressive MS. DR17,DQ2 was significantly overrepresented in the quartile with the most malignant course. The haplotype DR1,DQ5, which was found rather less frequently in MS patients, also tended to be associated with a poorer prognosis.

Key words Multiple sclerosis
HLA antigens · Prognosis
Life-table analysis

Introduction

Multiple sclerosis (MS) is a chronic neurological disease of unknown aetiology. Both environmental [32] and genetic factors appear to play a role in its development. The importance of genetic factors is supported by twin studies. In a virtually population-based study, the concordance rate in monozygotic twins was 25.9%, but only 2.3% in dizygotic twins, and 1.9% in non-twin siblings [8]. An association between MS and the HLA class II allele DR2 (or its split DR15) has been known for several years [15] and several population studies in Caucasians have confirmed this finding. In other populations, different HLA-DR associations have been reported: DR4 in Sardinians [21] and Jordanian Arabs [16] and DR6 in Japanese [22] and Mexican mestizos [12]. These reports have, however, not been confirmed.

It has been suggested that MS is more closely associated with DQ than with DR, more precisely with DQ1 or its split DQ6 [10, 13]. There is, however, a strong linkage disequilibrium between DR and DQ, so that certain DR and DQ alleles always or nearly always occur together as haplotypes. It has been shown that the primary and most important HLA association in MS is with the haplotype DR15,DQ6,Dw2 [23], which is the most common DR2 haplotype in Caucasians. In fact, this association is the only HLA association in MS which is established beyond all doubt. The existence of resistance genes has also been suggested. Several investigators have reported DR1/Dw1 to be less common in MS patients than in controls [1, 11, 14, 20].

While MS usually starts with relapses and remissions (RRMS), about 15% of the patients experience gradual onset of symptoms without remissions, primarily chronic progressive MS (PCPMS) [31]. Different HLA associations have been reported in these two clinical forms of MS [24]. An increase of DR3 or the synonymous haplotype DR17,DQ2 was found in patients with RRMS compared with controls, but not in patients with PCPMS. Because of the immunogenetic difference, it has been suggested that the two types of MS might be different disease entities. Similar suggestions have come from epidemiological [19] and magnetic resonance imaging (MRI) studies [36].

As the prognosis in MS is highly variable, prediction of outcome is conceivably as important as diagnosis. Efforts have been made to correlate MS prognosis to specific HLA types. The results have varied. In some studies,

no such correlation has been found [6, 25, 28, 30]. DR2/Dw2 has been related to a poor prognosis by some investigators [7, 9, 34], but the opposite finding has also been reported [20]. Likewise, DR3 (DR17) has been associated with both a good [7] and a poor prognosis [20].

A population-based incidence cohort is appropriate for the study of gene frequencies. This type of patient material with a long follow-up is particularly well suited to the study of prognostic questions, allowing for the elimination of sampling bias which is almost inevitable in this type of study. The main aim of this study was to investigate if HLA haplotypes, in particular DR2, have any specific relationship to MS prognosis. Secondly, we wanted to test the hypothesis of immunogenetic difference between the two clinical forms of MS. In the present study these issues were investigated in a clinically well-defined incidence cohort which was previously used to study prognostic variables [31].

Patients and methods

The patients all belong to an incidence cohort consisting of 308 patients [31, 35]. The onset of MS was between 1 January 1950 and 31 December 1964; all patients were living in Göteborg at the time of onset. All these patients have been subjected to clinical follow-up for at least 25 years since onset. The clinical course and outcome in the cohort, including analysis of prognostic factors, have been published previously [31].

One hundred and sixteen patients had died before the start of the study. In 66 of these death was mainly caused by MS. Of the 192 surviving patients, 153 had a diagnosis of definite or probable MS according to the criteria of Poser et al. [27]. Blood samples were obtained from 121 of these. In 32 patients with definite or probable MS, no blood sample could be obtained, in most cases because the patient lived far away or refused to donate blood. The majority of these 32 patients had benign MS, and a more favourable outcome than the investigated patients. Twenty-six patients with "possible MS" were also HLA typed, but the results presented here refer only to patients with definite or probable MS.

Two subgroups were defined according to clinical course at onset. Fourteen patients had a progressive course from onset (PCPMS). One hundred and two patients had a relapsing-remitting course from onset (RRMS). This latter group includes patients who later experienced a secondary progressive course. Five patients could not be allocated to either of these groups.

The controls consisted of 30 healthy 85-year-old persons and 110 healthy blood donors.

HLA typing

DNA was isolated from peripheral blood and digested with the restriction enzyme *Taq* 1. Southern blots [33] of digested DNA were hybridized with DQB and DRB probes. These consisted of a 627 bp *Ava*1-*Ava*1 fragment from clone pII-β-1 [18], and a 1323 bp *Bam*H1-*Bam*H1 fragment from clone 45.1 DRβ008 [37]. Restriction fragment patterns were analysed as previously described [3–5]. With this method, it is not possible to discriminate between DR7,DQ9 and DR9,DQ9 [2]. These two haplotypes were combined in the statistics.

Statistical methods

The distribution of haplotypes in patients and controls was compared with the relative predispositional effect method using a chi-square test [26]. With this method, the allele with the greatest predispositional effect is identified first. The procedure is then repeated after excluding the allele identified in the previous round from both patients and controls. Relative risks were calculated according to Woolf [38].

When appropriate, P values were corrected for multiple comparisons by the Bonferoni inequality method with a factor of 24 for the number of DR-DQ haplotypes ($n = 17$) and for the number of DQ alleles ($n = 7$). P values were not corrected when previously published associations were investigated.

The long-term prognosis of the patients with different haplotypes was calculated by life-table analysis. As end-points we used the start of a progressive course and level 6 (assistance required for walking) on the Kurtzke disability status scale (DSS) [17]. For the former end-point, the analysis was restricted to patients with relapsing-remitting course from onset. The end-points were defined within a yearly interval. Significance testing was performed using Mantel's test. No correction of P values was made. Life-table analysis of prognostic factors in this cohort has been described in detail previously [31]. To test for bias in outcome between the HLA-typed patients and the patients not typed, these two groups were compared using the same life-table method.

In order to eliminate the sampling bias, a stratification analysis was made for the haplotypes of major importance (DR1,DQ5 and DR17,DQ2). The patients of the cohort were ranked according to the degree of benign or malignant course, measured as the time to the start of a progressive course (only patients with RRMS were included). Six patients were excluded. They could not be classified into the ranking list as they had died or were lost to follow-up before the 25th year without having a progressive course. None of these six patients were HLA-typed. The ranking list was then divided into four quartiles. The number of patients with the haplotypes DR1,DQ5 and DR17,DQ2 was determined in each quartile. The distribution was analysed with a test for trend in contingency table and Fisher's exact test.

Results

Distribution of haplotypes in MS patients and controls

The distribution of DR-DQ haplotypes in MS patients and controls is shown in Table 1. One patient was found to have an aberrant DR-DQ association (DR1,DQ7). The existence of aberrant associations has been described previously [2]. The haplotype DR15,DQ6 was strongly associated with MS ($P < 0.0001$). It was increased in both clinical types of MS, although less markedly in the PCPMS group.

After subtraction of DR15,DQ6 haplotypes, a negative association was seen with the haplotype DR1,DQ5 ($P = 0.0193$). This negative association has been reported previously, and it can be debated whether correction for multiple comparisons should be made. If correction is made, the association is not significant. DQ5 is in linkage disequilibrium not only with DR1, but also with DR16, DR10 and some DR14. The allele DQ5 was found to have a negative association with MS, which was stronger than for

Table 1 Distribution (%) of HLA-DR-DQ haplotypes in patients with definite or probable multiple sclerosis (*MS*) and controls (*PCPMS* primarily chronic progressive MS, *RRMS* MS with relapses and remissions)

Haplotype	All MS (n = 121)	PCPMS (n = 14)	RRMS (n = 102)	Controls (n = 140)
DR1, DQ5	7	7	7	18
DR15, DQ6	63	43	66	29
DR16, DQ5	0	0	0	1
DR17, DQ2	22	29	21	20
DR4, DQ7	7	7	7	11
DR4, DQ8	25	21	25	24
DR11, DQ7	9	7	10	9
DR12, DQ7	1	0	1	4
DR13, DQ6	21	36	19	35
DR13, DQ7	1	0	1	2
DR14, DQ5	2	0	2	3
DR7, DQ2	7	14	7	11
DR7/DR9, DQ9	6	7	5	4
DR8, DQ4	17	14	18	13
DR8, DQ7	1	0	1	0
DR8, DQ6	1	0	1	0
DR10, DQ5	0	0	0	2
Aberrant	1	0	1	0

the combined DR1,DQ5 haplotype (P = 0.0031), but it was not significant after correction for multiple comparisons (P_{corr} = 0.074). No other significant positive or negative associations were found.

Associations with clinical course at onset

The minor differences between the two clinical forms of MS were not significant. DR17,DQ2, which has been reported to be associated with RRMS, was actually slightly more frequent in PCPMS.

Haplotypes and prognosis

The end-points "start of a progressive course" and "DSS 6" gave similar results. The figures show the results for DSS 6. The prognosis was noted to be almost exactly the same for DR15-positive and DR15-negative patients (Fig. 1). The haplotype that seemed to differ most from the average was DR1,DQ5. Patients with this haplotype had a poorer than average prognosis. Figure 2 shows the outcome for patients having this haplotype compared with DR1-negative patients. However, only eight patients had this haplotype, and the difference is not significant (P = 0.22).

DR3 (DR17,DQ2) has previously been reported to be of prognostic importance and also to be specifically associated with RRMS [24]. Patients with DR17,DQ2 tended

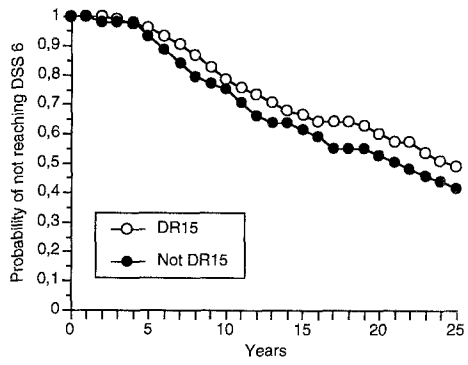

Fig. 1 Life-table analysis with the endpoint DSS 6 for patients positive (n = 76) and negative (n = 45) for DR15,DQ6

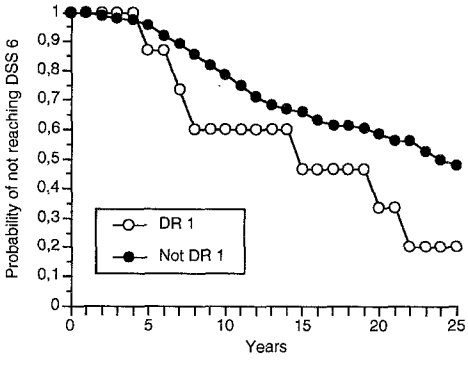

Fig. 2 Life-table analysis with the endpoint DSS 6 for patients positive (n = 8) and negative (n = 113) for DR1,DQ5

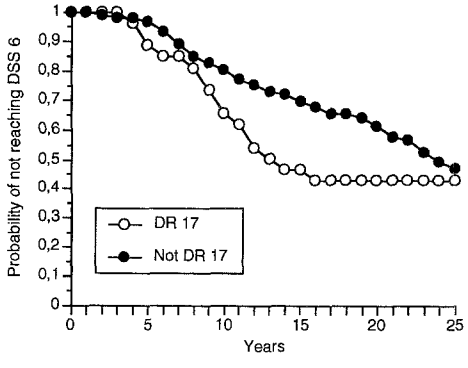

Fig. 3 Life-table analysis with the endpoint DSS 6 for patients positive (n = 27) and negative (n = 94) for DR17,DQ2

to have a poorer prognosis, as shown in Fig. 3. The difference was significant for 15 years of follow-up with the end-point progressive course (P = 0.04) and close to significance for the end-point DSS 6 (P = 0.052). After a longer follow-up, the difference levelled out. There were no other significant differences in prognosis for any of the haplotypes.

Table 2 shows the distribution of the haplotypes DR1,DQ5 and DR17,DQ2 in the four quartiles. Both

Table 2 Distribution of patients positive and negative for DR1,DQ5 and DR17,DQ2 in the four quartiles. Total numbers with percentages in parentheses. Only patients with RRMS are included

Number of quartile	Total number HLA-typed	DR17+	DR17–	DR1+	DR1–
First (benign course)	30	7 (23)	23 (77)	1 (3)	29 (97)
Second	27	0 (0)	27 (100)	1 (4)	26 (96)
Third	26	5 (19)	21 (81)	2 (8)	24 (92)
Fourth (malignant course)	19	9 (47)	10 (53)	3 (16)	16 (84)

tended to be over-represented in the quartile with the most malignant course. The overall trend in the contingency table was close to significance for DR17,DQ2 ($P = 0.06$). The increased proportion of DR17,DQ2 in the quartile with the most malignant course was significant (Fisher's exact test; $P < 0.01$).

For both end-points, the prognosis was significantly better for the HLA-typed patients compared with the patients of the cohort that were not typed ($P < 0.001$).

Discussion

We have confirmed the well-established association between MS and the haplotype DR15,DQ6. In this study, the relative risk was 4.22. In spite of this association, we found no difference in prognosis between patients with and without this haplotype. Neither did we find any significant difference in haplotype frequency between RRMS and PCPMS.

The haplotypes DR17,DQ2 and DR1,DQ5 tended to be associated with a poorer prognosis. These conclusions were based on life-table analysis supplemented by a stratified analysis of proportions of haplotypes in order to eliminate the sampling bias of DNA specimens in the cohort (Figs. 2, 3, Table 2). We found a probable negative association with DR1 and DQ5. The former has been reported in a few studies before [1, 11, 14, 20]. Therefore, the allele DQ5 or the combined haplotype DR1,DQ5 could be resistance factors in MS.

An alternative explanation for the finding of a negative association between DR1,DQ5 and MS diagnosis could be the bias introduced by differing prognoses for patients with different haplotypes. The eight patients positive for DR1,DQ5 had a poorer outcome than the rest (Fig. 2). Because of the small number, the difference was not significant. As the outcome for the HLA-typed patients of this MS cohort was better than for the average of the cohort, a haplotype could be under-represented in this study if it is associated with a poor prognosis. Most studies on MS and HLA have been performed on clinic- or hospital-based patients, and not epidemiological cohorts. Clinic- or hospital-based patients may be expected to have a poorer outcome than epidemiologically based ones [29]. Prognostic differences between haplotypes could explain the different results between studies, as the proportions of benign and malignant cases might differ considerably. This might explain why DR1,DQ5 has been significantly low in a few previous studies, but not in most studies. DR17,DQ2 was associated with a poor prognosis in the present study. Therefore, its frequency could be expected to be reduced in this study, in analogy with the reasoning about DR1. This is not the case and could indicate that the frequency of DR17,DQ2 was in fact increased in the complete cohort. There is, however, no indication in the present study that such an increase should comprise exclusively RRMS, as reported by Olerup and coworkers [23, 24].

Our finding of a lack of association between DR2 and disease outcome has support in at least four previous studies [6, 25, 28, 30]. These include the three largest studies of HLA and MS prognosis, comprising 224 [25], 200 [6] and 160 patients [28] respectively. However, DR2 was associated with a poor prognosis in three studies [7, 9, 34], and a favourable prognosis in one study [20]. Our observation of an association between DR3/DR17 and a malignant course is supported by the study of Madigand et al. [20], but Duquette et al. [7] reported the opposite. To our knowledge, all previous studies of HLA and MS prognosis have been clinic-based, not population-based. Varying methods and cohorts of patient might explain the divergent results. The disease duration is often not stated. In three studies, the duration was 13 years [7, 20] and 17.4 years [9]. In the present study there was a longitudinal follow-up of 25 years. Survival analysis was not used in any previous study.

To investigate the influence of HLA haplotypes on the prognosis of MS patients, two conditions are essential:

1. An unbiased and representative group of patients, preferably and incidence cohort. The present clinical material is an incidence cohort which is probably almost complete [31, 35]. It has been well investigated clinically, the follow-up time (at least 25 years in all patients) is much longer than in any previous study of MS genetics, and a multifactorial prognostic analysis has been performed. It should therefore be well suited to investigate prognostic questions.

2. Complete coverage of DNA samples in the cohort. In the present study, the patients with the most malignant forms of the disease had died long before the blood samples were taken. Therefore, the HLA-typed patients had a more favourable outcome than did the incidence cohort as a whole. This bias implies a risk of underestimating the negative prognostic influence of a haplotype. Using the cohort character of the material to reduce this bias, we ex-

amined the probably unbiased frequencies in each four strata of the incidence cohort, after stratification according to severity. Quartiles were chosen, as four was the highest number that allowed ranking including the group that did not reach the endpoint. This procedure confirmed the trends obtained by the life-table. A malignant subgroup associated with DR17,DQ2 was found, and patients with DR1,DQ5 tended to have a poorer prognosis. However, the number of patients positive for each of the haplotypes was small, except for DR15,DQ6. It was concluded that this haplotype, though associated with an increased risk of MS, has no influence upon prognosis.

While it is desirable to re-evaluate these results in a larger group of patients fulfilling the two essential conditions of freedom from bias as outlined above, these conditions are difficult to achieve.

Conclusions

1. The association of the haplotype DR15,DQ6 with MS was confirmed in this study. However, this haplotype does not influence prognosis.
2. No immunogenetic differences in the HLA frequencies were found between the two major clinical types of MS, relapsing-remitting and primarily chronic progressive MS.
3. The haplotypes DR1,DQ5 and DR17,DQ2 were associated with a poor prognosis in the present study.

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