Multiple Sclerosis in French Canadians: Evidence for HLA Class II Susceptibility and Resistance Genes

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ABSTRACT: HLA class II DRB1, DQB1 and DQA1 gene probes were used to study DNA from unrelated French Canadian multiple sclerosis (MS) patients and controls by restriction fragment length polymorphism (RFLP) analysis. An MS-associated and linked series of allele-specific RFLPs or allogenotypes was identified among this relatively homogeneous ethnic group; the allogenotypes include DRw15, DQw6 and a DQA1 allogenotype termed DQα1b. An additional allogenotype which cross-hybridizes with DQA1 and is termed DQA2 upper (DQA2U), was shown not only to be part of the MS-associated extended haplotype, but also to be independently associated with MS in DRw15-negative patients. Conversely a second DQA2 allogenotype, termed DQA2 lower (DQA2L) and a DQB1 allogenotype (DQw7) linked to DQA2L showed negative correlations with MS. It seems likely that the relationship of the HLA class II gene region to MS is complex and that MS susceptibility may reflect interaction between disease susceptibility and resistance genes.

RÉSUMÉ: La sclérose en plaques chez les canadiens-français: indications qu'il existe des gènes HLA de classe II de susceptibilité et de résistance Nous avons utilisé des sondes géniques HLA de classe II DRBI, DQB1 et DQA1 pour étudier des polymorphismes de restriction (RFLP) de l'ADN de patients canadiens-français non apparentés, atteints de sclérose en plaques (SEP), et de contrôles. Des séries de RFLP liés, spécifiques d'un allèle ou allogénotypes, et associés à la SEP, ont été identifiés parmi ce groupe ethnique relativement homogène: les allogénotypes incluent DRw15, DQw6 et un allogénotype DQA1 nommé DQα1b. On a démontré qu'un allogénotype additionnel nommé DQA2 supérieur (DQA2S), qui a une réaction d'hybridation croissée avec DQA1, est non seulement une partie de l'haplotype étendu associé à la SEP, mais qu'il est associé indépendamment à la SEP chez les parients DRw15 négatifs. Réciproquement, un second allogénotype DQA2, appelé DQA2 inférieur (DQA2I), et un allogénotype DQB1 (DQw7) lié à DQA2I démontraient une corrélation négative avec la SEP. Il semble probable que la relation de la région génique HLA de classe II à la SEP est complexe et que la susceptibilité à la SEP peut témoigner d'une interaction entre les gènes de susceptibilité et de résistance à la maladie.

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Multiple sclerosis (MS) is a demyelinating disease in which genetic factors play a role in disease susceptibility.[1] In most but not all Caucasian populations, study of MS patients has documented an association between MS and various HLA alleles, particularly the HLA class II alleles DRw15 and DQw6;[2,3] these alleles were previously termed DR2 and DQw1 respectively.[4] Class II alleles have been identified most frequently by serology, mixed lymphocyte culture and cellular typing but recent studies have used gene probes to DR and DQ.[5-10] These probes can unequivocally identify restriction fragment length polymorphisms (RFLPs) that are specific for DR and DQ alleles and these allele-specific RFLPs are termed allogenotypes.[11-13] Recently we performed DR and DQ allogenotypic analysis of a group of unrelated Canadian Caucasian MS patients and identified a series of closely linked alleles (allogenotypes) which is present in more than 50% of MS patients and includes DRw15, DQw6 and a DQα allele termed DQαlb.[7] A possible caveat with this study was that both controls and patients were of diverse ethnic origins and patient-control differences in allelic frequencies could have reflected these differences in ethnicity. To overcome this potential difficulty, we have now used sequential HLA-DRB1, -DQB1 and -DQA1 gene probes together with RFLP analysis to study the chromosomes of French Canadian MS patients and controls because together the French Canadian population forms a relatively homogeneous ethnic group.[14,15] Not only did we confirm the association of DRw15, DQw6 and DQαlb with MS but also identified additional class II alleles that may independently confer susceptibility or resistance to MS.

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Materials and Methods

Patients and Controls

Sixty French Canadian MS patients followed at the Montreal Neurological Hospital MS clinic were selected at random and these formed the DR-unmatched patient group. All patients had definite MS by standard criteria.[16] Ninety percent of these patients were classifiable into one of two clinical subgroups: relapsing-remitting MS (n = 37) and progressive MS (n = 17). DR-unmatched controls consisted of 58 adult French Canadians who were not age matched to the MS patients but had no clinical evidence of MS or other "autoimmune disease". Both patients and control groups consisted of unrelated individuals and 25% of patients gave a family history of MS. Fifty-three percent of patients and 53% of controls had their geographic origins in Montreal and the remainder of patients and all but one control were French Canadians from the province of Quebec; one control was a French Canadian from Nova Scotia. All patients and controls were DR-typed by RFLP analysis using the DRB1 probe in Taq 1 digests; the accuracy of DR-RFLP typing vs DR serotyping has been established previously by ourselves[6,7] and others.[11]

Individuals among the DR-unmatched patient and control groups shown to be DRw15-positive (DRw15$^+$) by RFLP analysis were selected to form the DRw15$^+$ patient and control groups. Additionally 10 DRw15$^+$ individuals previously serotyped as potential organ donors were included in the DRw15$^+$ control group.

DRw15-negative (DRw15$^-$) individuals among the total DR-unmatched patient and control groups formed the DRw15$^-$ patient and control groups.

Data analysis

The χ^2 (chi-square) test was used to analyze differences between patient and control chromosomes in 2×2 tables. Since multiple comparisons were made of class II allele-specific RFLPs (allogenotypes) a correction for multiple comparisons was included as follows: the α level ($\alpha = 0.05$) was adjusted by performing each χ^2 at an $\alpha' = \alpha$ divided by the number of tests, ie for Table 1, $0.05 \div 5 = 0.001$ and for Tables 2 and 3, $0.05 \div 2 = 0.025$.

DNA isolation, restriction enzyme digestion and Southern blotting

DNA was isolated by phenol-chloroform extraction, digested to completion with Taq 1, electrophoresed in a 0.8% agarose gel, depurinated, denatured, neutralized and transferred to a nylon membrane (Hybond N, Amersham) as described previously.[6,7]

DNA probes, hybridization and autoradiography

Southern blots of Taq 1-digested DNA were hybridized sequentially with a 517 bp DRB1 cDNA probe, then a 627 bp DQB1 probe and finally with a 900 bp DQA1 probe as previously described in detail;[6,7,17] for nomenclature of the DR-DQ genes see reference 4. The probes were labelled with ^{32}PdCTP, and hybridization, washing of membranes and autoradiography (3-8 days) were described before.[6,7]

Results

Class II alleles (allogenotypes) of DR-unmatched patient (n = 120) and control (n = 108) chromosomes (Table 1)

Genomic DNA samples from all patients and controls were Taq 1-digested and the resulting Southern blots were probed sequentially with radiolabelled DRB1, DQB1 and DQA1 probes so as to assign DR and DQ allelic (allogenotypic) specificities. A significantly higher percentage of patient than control chromosomes were DRw15$^+$ ($\chi^2 = 11.4$; $p < 0.001$) and DQw6$^+$ ($\chi^2 = 10.9$; $p < 0.001$); the RFLPs characteristic of these alleles in Taq 1 digests have been described previously by Bidwell and ourselves.[6,7,11,17] In Taq 1 digests, the DQA1 probe hybridizes not only with DQA1 alleles but also cross-hybridizes with two fragments of 2.2 and 2.0 kb (Figure 1) which represent DQA2 alleles and are termed DQA2 upper (DQA2U) and DQA2 lower (DQA2L) respectively.[6,7,11,17] A higher percentage of patient than control chromosomes were positive for the 6.5 kb DQA1 allogenotype which is termed DQα1b (Figure 1, lanes 1-3) ($\chi^2 = 7.4$; $p < 0.008$) and for the DQA2U allogenotype (Figure 1, lanes 2,3) ($\chi^2 = 13.2$; $p < 0.001$). Conversely a higher percentage of control than patient chromosomes were positive for the DQA2L allogenotype (Figure 1, lanes 1,2,4) ($\chi^2 = 13.2$; $p < 0.001$).

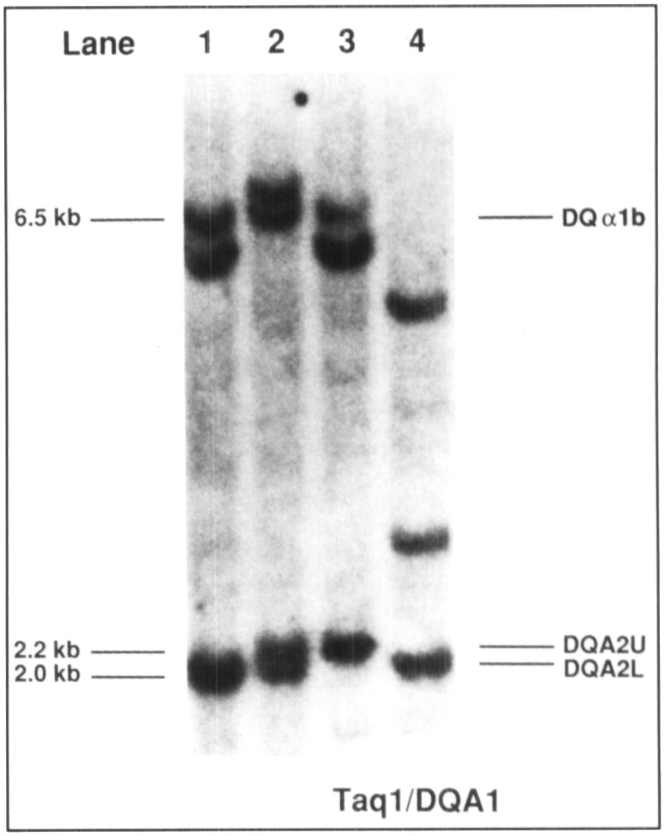

Figure 1 — Allogenotypes detected with the DQA1 probe. The molecular weights of the DQα1b hybridizing band and the cross-hybridizing DQA2 allogenotypes are shown.

Extended HLA class II haplotypes among DRw15$^+$ individuals

The normal associations between DRB1 alleles (allogenotypes) and DQB1 and DQA1 alleles in Caucasians have been well defined in more than 1,100 controls studied by Bidwell[11,17] and these associations have been confirmed by ourselves[6,7] with identical DR and DQ probes in Taq 1 digests. In French Canadian MS patients analysis of the linkage patterns of

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DRw15 allogenotypes with DQB1 and DQA1 alleles reveals that all DRw15 alleles (n = 34) are linked to DQw6 and DQαlb allogenotypes. In French Canadian controls 100% of DRw15 alleles (n = 21; see Materials and Methods) are similarly linked to DQw6 and DQαlb. Thus it is clear in French Canadians that these three alleles are in strong linkage disequilibrium and together form an extended class II haplotype which is associated with MS.

DQA2U and DQA2L alleles in DRw15-negative (DRw15⁻) patients and controls

An association between DQA2U and DRw15 has been previously reported among British Caucasoids.[18] However, analysis of French Canadian chromosomes reveals that 14% of control (n = 21) and 15% of patient (n = 34) chromosomes show a definite DRw15-DQA2L association and 83% and 86% respectively show DRw15-DQA2U associations. Therefore, association of DQA2U with MS (see Table 1) is likely due mainly to linkage disequilibrium with the DRw15-linked extended haplotype but the possibility arises that some of the association of DQA2U with MS is independent of linkage to DRw15. To investigate the latter possibility the chromosomes of DRw15⁻ patients and controls were further analyzed (Table 2); whereas a higher percentage of patient than control chromosomes were DQA2U⁺ ($\chi^2 = 6.35$; $p < 0.02$), a higher percentage of control than patient chromosomes were DQA2L⁺ ($\chi^2 = 7.53$; $p < .008$). Thus, DQA2U and DQA2L are candidates for disease susceptibility and resistance genes respectively.

Table 1. Frequency of HLA class II alleles in normal control and MS chromosomes unmatched for DR-type

	Allogenotypes (%)				
	DRw15	DQw6	DQαlb	DQA2U	DQA2L
Patient chromosomes (n = 120)	28*	37*	35**	54*	46*
Normal chromosomes (n = 108)	10	17	18	30	70

* $p < 0.001$
** $p < 0.008$

Table 2. Frequency of HLA class II alleles in normal control and MS chromosomes in DRw15-negative individuals

	Allogenotypes (%)	
	DQA2U	DQA2L
Patient chromosomes (n = 64)	44*	53**
Control chromosomes (n = 90)	25	74

* $p < 0.02$
** $p < 0.008$

Linkage of DQB1 alleles to DQA2L and associations of these DQB1 alleles with MS

Previously linkages of DQA2U and DQA2L with various other class II alleles have been established in 2 × 2 contingency tables.[18] Similar analysis of French Canadian controls revealed no linkage between DQA2U and any DR or DQ allele except DRw15 (see above). However, there was strong linkage between DQA2L and both DQw7 and DQw5; 92% of DQw7 alleles and 87% of DQw5 alleles were linked to DQA2L by 2 × 2 analyses (for details of this type of analysis see Awad et al).[18] We therefore investigated whether these particular DQB1 alleles also show a negative correlation with MS. Significantly more control than patient chromosomes were DQw7⁺ among DR-unmatched ($\chi^2 = 13.6$; $p < 0.001$) and DRw15⁻ individuals ($\chi^2 = 5.7$; $p < 0.02$) (Table 3).

Table 3. DQw7 and DQw5 allelic frequencies in normal control and MS chromosomes unmatched for DR-type and in DRw15-negative individuals

	Alleles (%)	
	DQw7	DQw5
DR-unmatched individuals		
Patient chromosomes (n = 120)	7*	13
Normal chromosomes (n = 108)	24	21
DRw15-negative individuals		
Patient chromosomes (n = 64)	9**	17
Normal chromosomes (n = 90)	25	23

* $p < 0.001$
** $p < 0.02$

Discussion

In a previous study of Canadian Caucasoids we documented an association between MS and the HLA class II alleles DRw15, DQw6 and DQαlb.[7] However, both controls and patients were of diverse ethnic origins. The possibility arises therefore that the observed class II associations with MS reflected differences in patient-control ethnicity and in particular that more Northern Europeans were present among patients than controls; 43% of normal Scandinavians are DRw15⁺[2] which approximates the percentage of Canadian MS patients who are DRw15⁺[7]. Conversely, several class II alleles were less frequent among controls of French Canadian than non-French Canadian origin yet French Canadians represented a higher percentage of the patient than the control group (unpublished data). To overcome this confusion in allelic frequencies created by ethnic heterogeneity, we have now studied a group of DR-unmatched French Canadian patients and controls who together form a relatively homogeneous ethnic group. The present French Canadian population is descended from approximately 8,000 immigrants from France; since 1763 and until the last 20 years, there has been minimal French immigration into this population and minimal "cross-breeding" with Canadians of other ethnic origins.[14,15] The HLA class II alleles, DRw15, DQw6 and DQα1b were significantly increased among French Canadian MS patient chromosomes compared with normal chromosomes. Moreover 100% of DRw15⁺ control and patient chromosomes were also DQw6⁺ and DQαlb⁺. In other words, these three alleles are in strong linkage disequilibrium and together form an extended class II haplotype which is associated with MS. This contrasts with the majority of previous Caucasian studies which have concentrated either on DRw15 or on DQw6 (DQw1)[2,3,5,8-10] and the present correlation of an extended class II haplotype with disease may reflect the requirement for both DRw15 and DQw6 products in determining MS susceptibility. A further possibility is that

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DRw15 and DQw6 are simply markers of a disease susceptibility locus, namely DQαlb, which interacts with the DQβ allele which encodes the DQw6 serotype. We cannot distinguish between these possibilities or an alternative possibility that two or more of these alleles have an hierarchical and additive effect in susceptibility to MS as postulated in other "autoimmune disease".[20]

The role of HLA genes in susceptibility to MS was complicated by our finding that the DQA2U allele was more frequent in patient than control chromosomes not only in DR-unmatched but also in DRw15$^-$ individuals. This indicates not only that DQA2U is part of the MS-associated DRw15$^-$ linked extended haplotype, but also that association of DQA2U with MS is partly independent of the DRw15-extended haplotype. One possible interpretation of this latter finding is that DQA2U *per se* is not important in susceptibility to MS but rather is in linkage disequilibrium with other class II alleles that are themselves disease susceptibility genes. A precedent for this was established in a study of class II gene linkage in British Caucasoids which showed strong DQA2U linkage not only with DRw15 but also with DR3, w13 and a subset of DR4.[18] However, among our French Canadian controls similar linkage was not observed except with DRw15. An alternative possibility is that disease susceptibility genes are actually encoded in the DQA2 gene region as has been previously suggested in diabetes mellitus, celiac disease and dermatitis herpetiformis[21-23] where there is also a positive disease correlation with DQA2U. Since no DQA2 gene products have been identified,[11] a further and more likely possibility is that there are as yet undescribed genes linked to DQA2U which play a role in MS susceptibility. An important caveat to this interpretation derives from the composition of the patient and control groups. Similar numbers of patients (53%) and controls (53%) are from Montreal but the remainder are French Canadians from outside the Montreal region. A possibility which cannot be completely excluded is that geographic differences in HLA frequencies could explain observed patient-control HLA differences. However, our comparison of allelic frequencies have failed to detect such HLA differences between controls from Montreal and those from outside (unpublished data).

Previously we demonstrated a negative association between MS and DQA2L.[6,7] It was felt important to confirm this finding both in DR-unmatched (Table 1) and in DRw15$^-$ patient and control groups (Table 2). This was because the presence of DQA2U-DRw15 linkage means that a decrease in DQA2L among MS patients could simply reflect the increased number of DRw15$^+$ chromosomes in patients compared to controls. However this was shown not to be the case as there was a negative correlation between MS and DQA2L in chromosomes from DRw15$^-$ patients. Previously we postulated the presence of a disease resistance gene in the DQA2L gene region.[6] However, we now find that DQA2L is in strong linkage disequilibrium with DQw7 which also shows a negative association with MS. It seems likely therefore that the negative association of DQA2L with MS is due to strong linkage disequilibrium of DQA2L with DQw7 and the latter becomes a candidate for a new disease resistance gene in MS.

The present findings of both positive and negative associations of HLA class II genes with MS indicate that susceptibility to MS likely reflects interaction between disease susceptibility and resistance genes. Since susceptibility to MS is likely polygenic,[24] the interactive role of susceptibility and resistance genes both within and outside the HLA region including T cell receptor genes[25,26] merits further investigation.

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