PAPER

HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis

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Background: The association between multiple sclerosis and class II alleles of the major histocompatibiliy complex, in particular the DRB1*1501-DQB1*0602 haplotype, is well established but their role in determining specific features of this clinically heterogeneous disease is unknown as few studies involving large sample sizes have been performed.
Method: 729 patients with multiple sclerosis were typed for the HLA DR15 phenotype. All patients underwent clinical assessment and a detailed evaluation of their clinical records was undertaken.
Results: The presence of DR15 was associated with younger age at diagnosis and female sex but there was no association with disease course (relapsing-remitting or secondary progressive *v* primary progressive type), disease outcome, specific clinical features (opticospinal *v* disseminated form), diagnostic certainty (clinically and laboratory supported definite *v* clinically probable multiple sclerosis), and paraclinical investigations including the presence of oligoclonal bands in the CSF or characteristic abnormalities on MRI imaging of the central nervous system.
Conclusion: Even though DR15 carriers are more likely to be female and prone to an earlier disease onset, the results indicate that there is no association with other specific clinical outcomes or laboratory indices examined here. This suggests that DR15 exerts a susceptibility rather than disease modifying effect in multiple sclerosis.

The disease process in multiple sclerosis is triggered by environmental factors acting in genetically susceptible people. Pedigree analysis indicates that susceptibility is complex and involves several genes. These may act independently or through epistatic interactions.[1 2] To date, the only well established genetic aspect of multiple sclerosis is the association with alleles and haplotypes of the major histocompatibility complex, in particular with DRB1*1501-DQA1*0102-DQB1* 0602 (DR15) in northern Europeans,[3–7] and with DRB1*0301-DQA1*0501-DQB1*0201 (DR3) and to a lesser extent DRB1*0405-DQA1*0501-DQB1*0301 (DR4) in southern Europeans.[7–9] The candidature of all other regions of interest encoding susceptibility genes remains to be confirmed. In addition to the effect on susceptibility, genetic factors may also shape the course and outcome of multiple sclerosis. Family studies have suggested a correlation for disability in siblings, but this effect might also derive from environmental factors.[10] The influence of genes on clinical characteristics is not established and the proposed associations require further investigation.[2 11] Furthermore, the highly variable clinical course seen in multiple sclerosis has led many investigators to debate whether clinically distinct forms of the disease reflect heterogeneity in the pathogenesis.[12–15]

In retrospect, it is clear that most studies investigating the role of major histocompatibility complex alleles in determining specific clinical or paraclinical features in multiple sclerosis have been limited by small sample size. Studies employing large numbers have generally failed to show any significant effect of HLA on disease course or outcome among Europeans although DR15 (2) is associated with female sex and younger age at onset.[16 17] We typed a large number of clinically well characterised patients with multiple sclerosis, stratified for demographic and clinical features, to resolve the issue of whether DR15, the HLA phenotype most commonly associated with multiple sclerosis, identifies particular clinical groups defined by variations in the clinical course or outcome.

MATERIAL AND METHODS

Patients

The 729 patients that comprised this study were index cases from trio families (an affected person and both parents) recruited from throughout the United Kingdom as part of an ongoing genetic analysis of the disease. The patients were white, meeting the Poser criteria[18] for either clinically definite (89%), laboratory supported definite (6%), or clinically probable (5%) multiple sclerosis. Mean age was 38 years and mean Kurtzke expanded disability status score (EDSS)[19] 4.5 (range 0–9.5). The sex ratio was consistent with previous population based series (three female:one male). Each person gave written consent to take part in genetic analysis. Ethical approval was obtained from the Oxford and Anglia multicentre research ethics committee as well as local research ethics committees.

Clinical classification, grading of disability, and investigations

Because primary progressive multiple sclerosis has distinct clinical, paraclinical, and prognostic features from relapsing-remitting and secondary progressive multiple sclerosis,[14] probands were considered in primary progressive or bout onset groups. Primary progressive multiple sclerosis was characterised by historical evidence for a progressive clinical course from onset with no remissions. The bout onset group included patients with relapsing-remitting and secondary progressive disease courses at the time of the last clinical assessment. In addition, probands whose clinical phenotype exclusively affected only the optic nerve and spinal cord were considered in an opticospinal category, although all other patients who showed clinical involvement at multiple sites

Abbreviations: EDSS, expanded disability status score; PCR, polymerase chain reaction

Table 1 Odds ratios or regression coefficients (95%CI) for the probability that DR15 is associated with specific clinical subgroups

	Odds ratio/++regression coefficient (95% CI)	p Value
Sex	0.637 (0.453 to 0.897)	0.010*
Disease course (primary progressive *v* bout onset)	0.993 (0.593 to 1.663)	0.978
Opticospinal *v* diffuse form	1.508 (0.844 to 2.698)	0.116
History of other autoimmune diseases	0.684 (0.354 to 1.322)	0.259
Family history of multiple sclerosis	1.337 (0.911 to 1.961)	0.138
Disease progression	++–0.003 (–0.05 to 0.05)	0.919
Age at diagnosis	++–1.877 (–3.03 to –0.725)	0.001*

*Significant.

were classified as having diffuse multiple sclerosis. There were 69 probands with primary progressive multiple sclerosis and 660 with bout onset disease. The opticospinal phenotype was present in 61 patients and 668 had the diffuse form of multiple sclerosis. Thirty eight patients had a history of other autoimmune diseases and 152 had a family history of multiple sclerosis.

Disability was graded according to the progression index, defined as the ratio of the EDSS and duration of disease. As disability is variable in the early stages of multiple sclerosis, with no linear relation between duration and EDSS score, patients with a history of less than 5 years were excluded from the analysis. Following Weinshenker *et al*,[20 21] who applied the progression index to patients with a longer disease duration (>5 years) and found a roughly normal distribution of ranked severity scores, we used the square root of the progression index for analysis to obtain a normal distribution of progression indices in our sample.

Analysis of CSF for the presence of unpaired oligoclonal bands or raised IgG index had previously been performed in 413/729 patients. Magnetic resonance imaging of the brain or the spinal cord was available in 512/729 probands and considered abnormal if the reporting radiologist judged the images to be typical of multiple sclerosis. Visual evoked potentials had been assessed in 520/729 persons and considered to be indicative of demyelination if delayed with a well preserved wave form. Only unequivocally normal results or those consistent with multiple sclerosis on any paraclinical test were included in the analysis.

HLA typing

The presence or absence of the DR 15 alleles was tested by amplifying genomic DNA using the polymerase chain reaction (PCR) and sequence specific primers *(*5′-CCG CGC CTG CTC CAG GAT -3′ and 5′-TCC TGT GGC AGC CTA AGA G-3′*)*. A positive control for the PCR was provided by primers amplifying the human growth hormone locus**.** The PCR assay was performed in a final volume of 13 μl, containing 30 ng genomic DNA, 20 mM ammonium sulphate, 75 mM Tris HCL (pH 9.0), 0.01% Tween, 2 mM magnesium chloride, 200 μM each of dATP, dCTP, dGTP, dTTP, and 0.125 units Taq. Cycle conditions were 94°C for 2 minutes, followed by 10 cycles of 94°C for 20 seconds and 65°C for 60 seconds, and then 20 cycles of 94°C for 20 seconds, 61°C for 50 seconds, and 72°C for 30 seconds. Amplified products were visualised under ultraviolet light after running in a 2% agarose gel containing TBE buffer and 0.5 mg/μl ethidium bromide for 30 minutes at 100 V. Gel interpretation assigned the presence or absence of DR 15 alleles according to the 1998 nomenclature report.[22] Each patient was typed twice and typing was repeated if discordant results were obtained.

Statistical analysis

Patients were categorised as positive or negative for DR 15. DR 15 homozygotes and heterozygotes were not distinguished. Odds ratios and 95% confidence intervals (95% CIs) were calculated using logistic regression analysis to estimate the relation between HLA status and sex, family history of multiple sclerosis, history of other autoimmune disease, presence of laboratory abnormalities (imaging, CSF, and evoked potentials), disease course (bout onset *v* primary progression), specific clinical features (opticospinal *v* diffuse form), and diagnostic certainty (clinically or laboratory supported definite *v* clinically probable multiple sclerosis). Linear regression analysis was performed to examine the effect of HLA DR15 on age at diagnosis and the transformed progression index as a measure of disease outcome.

RESULTS

DR15, age at diagnosis, and diagnostic subgroups

A total of 729 probands was included in the analysis, of whom 466 (59%) carried one or two DR 15 alleles. The mean age at diagnosis was 30.3 and 32.2 years in the DR15 positive and negative groups, respectively (p=0.001). Comparison of DR15 phenotype frequencies showed no statistically significant difference between the definite and probable diagnostic groups (p=0.636). The DR15 phenotype was significantly more common in women (66%) than men (55%: p=0.01).

DR15 and clinical subgroups

Table 1 shows odds ratios or regression coefficients and 95% confidence intervals for the probability that DR15 is associated with specific clinical subgroups. There was no significant influence of the DR15 phenotype on diseases course (bout onset *v* primary progressive) or opticospinal compared with diffuse forms of the disease. In addition, there was no association between DR15 and other autoimmune diseases or a family history of multiple sclerosis.

Table 2 DR15 phenotype frequencies for patients grouped according to the results of paraclinical investigations

	Normal result (DR15 positive)	Result consistent with multiple sclerosis (DR15 positive)	Test not performed/ result equivocal (DR15 positive)
MRI	23 (10)	408 (250)	298 (197)
CSF	42 (220)	240 (155)	447 (289)
VER	121 (74)	250 (152)	358 (240)

DR 15 and disease outcome

Considering all patients with disease duration of more than 5 years, there was no significant association between disability measured as the square root of the progression index and the presence of the DR15 phenotype (p=0.919).

DR15 and laboratory markers of multiple sclerosis

Table 2 shows DR15 phenotype frequencies for patients grouped according to the results of CSF, evoked potential and MRI studies. There was no association between any of these paraclinical investigations and the heterozygote or homozygote presence of HLA DR 15.

DISCUSSION

Our study involved a large cohort of patients with multiple sclerosis and was designed to resolve the issue of whether DR15, the HLA phenotype which is most commonly associated with multiple sclerosis, identifies particular clinical groups defined by variations in the clinical course or outcome. The DR15 phenotype was associated with a younger age at diagnosis and female sex but not with disease course, type or outcome.

Differences in the clinical, radiological and pathological features have led to the suggestion that primary progressive and bout onset multiple sclerosis represent different disease entities. Primary progressive multiple sclerosis often manifests a spinal phenotype and has a male preponderance, older age at onset, more severe disability, less inflammation but prominent axonal pathology, and fewer cerebral lesions compared to bout onset disease.[14] Provisional evidence has been provided for an increased frequency of DR15 and DR3 in relapsing-remitting multiple sclerosis, and an association with DR4 in the primary progressive form of the disease.[21 23–25] Other investigators have reported associations of relapsing-remitting disease with DR2 and progressive multiple sclerosis with DR3.[26] However, most surveys investigating HLA class 2 alleles and disease course have failed to confirm specifically different associations with any one of these phenotypes.[16 17 27 28] It remains possible that the application of new criteria for assigning patients with primary progressive multiple sclerosis could usefully be used to reinterpret these analyses [29]. The frequencies of DR3 or DR4 between clinical subgroups were not investigated in our study.

Specifically different HLA associations are seen in Japanese patients with multiple sclerosis compared with Europeans. Only the "western" phenotype is associated with DR15 [30] whereas the more prevalent opticospinal phenotype is associated with the DPB1*0501 allele[15] suggesting that (at least in Oriental patients) genetic factors may determine an anatomically restricted form of multiple sclerosis. Although the observations are based on sample sizes, these differences provisionally provide further evidence for genotype-phenotype heterogeneity in multiple sclerosis.

DR2 and DR3 have each been associated with a favourable prognosis.[24 26] But this finding is also unconfirmed and other investigators report that the DR15 phenotype carries a worse prognosis [31–33] whereas, as in the present study, most surveys have found no association between HLA and disease outcome.[21 34] However, many of these studies depended on a small sample size and did not use consistent classifications for patient ascertainment. Significantly, the largest published survey (involving 948 persons) provided no evidence for an effect of HLA on prognosis.[16] Evaluation of disease severity is made difficult in multiple sclerosis by the unpredictable clinical course. Subgroups can only be defined after long term follow up and this limits the available sample size for cross sectional surveys. Methods such as measurement of median time to reach a given point on the EDSS scale and progression indices may increase precision although, to date, the use of novel but incompletely validated methods for assessing prognosis is the likely explanation for differing results in studies assessing HLA associations with the course and clinical features of multiple sclerosis.

Even though paraclinical tests such as examination of CSF and MRI demonstrate abnormalities suggestive of multiple sclerosis in more than 90% of patients with clinically definite disease, these are normal in a proportion of affected people. The identification of additional lesions on MRI in the presence of DR15 is associated with a relative high conversion rate for multiple sclerosis in patients with clinically isolated demyelinating syndromes.[35] Importantly, these risk factors are markers both of independent and interactive effects—the DR15 phenotype, the presence of CSF abnormalities, and MRI abnormalities having additive influences on susceptibility.[16 36] Our failure to confirm the association between DR15 and paraclinical abnormalities may reflect the low number of tested persons lacking these laboratory markers of multiple sclerosis. The alternative explanation is that DR15 acts only as a susceptibility factor and does not influence disease mechanisms that shape the clinical course and outcome of multiple sclerosis. A primary effect on susceptibility rather than the clinical course is consistent with the increased strength of the DR15 association depending on diagnostic verification using the Poser criteria—the probable category necessarily including more persons in whom the diagnosis of multiple sclerosis proves incorrect compared with those classified as having clinically definite disease.[16] We included few patients with probable multiple sclerosis and therefore did not reproduce the specific DR15 association with clinically definite diagnostic categories. It seems likely that the use of new criteria, which only assign persons into groups with or without multiple sclerosis based on clinical and paraclinical evidence,[37] will eliminate this somewhat artificial aspect of the relation between HLA and disease in future studies.

The main finding of our survey, in agreement with other recent studies,[16 17] is that DR15 is more common in females with multiple sclerosis and predisposes to a younger age at onset. The association with DR15 endorses the hypothesis that the increased overall risk of the disease in females is linked to alterations in immune function. Why the clinical onset occurs at a younger age remains unexplained but this might relate to DR15 linked differences in the regulation of autoreactive immune cells, which precipitate inflammatory disease activity at an earlier age. It has previously been suggested that T cells from DR15 positive patients produce increased quantities of TNFα, which plays a part in inflammation and demyelination.[38 39]

Within the limitations of the analyses performed, our study does not add to the evidence for genotype-phenotype heterogeneity in multiple sclerosis. The stratification of clinical and paraclinical subgroups indicates that DR15 does not discriminate particular clinical and prognostic subgroups. This result is consistent with the interpretation that the HLA system mainly influences those aspects of the pathogenesis determining susceptibility rather than the course of the disease.

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