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Genetic basis for clinical expression in multiple sclerosis

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Summary

Multiple sclerosis is a clinically heterogeneous demyelinating disease and an important cause of acquired neurological disability. An underlying complex genetic susceptibility plays an important role in multiple sclerosis aetiology; however, the role of genetic factors in determining clinical features of multiple sclerosis is unknown. We studied 184 stringently ascertained Caucasian multiple sclerosis families with multiple affected cases. A detailed evaluation of patient histories identified clinical variables including age of onset, initial clinical manifestations and disease severity. The concordance within families for continuous and categorical clinical variables was investigated using an intraclass correlation or Cohen's kappa coefficient, respectively. Genetic analyses included model-dependent, model-independent and association methodology. Linear and logistic regression models were used to evaluate the effect of human leucocyte antigen (HLA)-DR2 (DRB1*1501, DQB1*0602) on clinical outcome, taking account of correlation within families. Significant concordance for early clinical manifestations within families was observed for individuals with exclusive optic neuritis and/or spinal cord involvement as first and second multiple sclerosis attacks ($P < 10^{-6}$). Linkage (LOD = 3.80, $\theta = 0.20$) and association ($P = 0.0002$) to HLA-DR were present in the dataset; however, linkage was restricted to families in which the DR2 haplotype was present in at least one nuclear member. No evidence for linkage to HLA-DR in DR2-negative families was observed. When families were stratified by concordance of early clinical manifestations, a significant DR2 association was present in all subgroups. Concordance for early manifestations of multiple sclerosis was present in this familial dataset, but was not associated with HLA-DR2. The association of DR2 in families with different clinical presentations suggests that a common basis exists for susceptibility in multiple sclerosis. However, non-HLA genes or other epigenetic factors must modulate disease expression. Locus heterogeneity at the HLA region suggests a distinct immunopathogenesis in DR2 negative patients.

Keywords: human; autoimmunity; multiple sclerosis; *HLA-DR*; EAE

Abbreviations: EDSS = Expanded Disability Status Scale; HLA = human leucocyte antigen; ON = optic neuritis; PDT = pedigree disequilibrium test; SC = spinal cord

Introduction

Multiple sclerosis is an autoimmune demyelinating disease of the central nervous system that affects >1 000 000 people in the western world and is second only to trauma as a cause of neurological disability in young adults (Noseworthy *et al*., 2000; Hauser and Goodkin, 2001). Multiple sclerosis shares several characteristics with other autoimmune disorders such as: polygenic inheritance; consisting of a human leucocyte antigen (HLA)-associated gene and other largely undefined susceptibility genes; and evidence that an environmental exposure of some type is involved. From a clinical perspective, symptoms of multiple sclerosis are extremely variable, and the course may be relapsing–remitting or progressive, severe or mild, and involve the neuraxis in a widespread fashion or predominantly affect the spinal cord and optic nerve. This latter variant, termed 'opticospinal multiple sclerosis', is common in Asians but relatively uncommon in

Caucasians (Kira *et al*., 1996). Recent studies of the pathology of multiple sclerosis lesions, including detection of autoantibodies deposited *in situ*, have suggested that multiple sclerosis is not a unitary disorder, but may represent an overlapping spectrum of related disorders (Genain *et al*., 1999; Raine *et al*., 1999; Lucchinetti *et al*., 2000). Essentially, nothing is known about the underlying cause or significance of disease variability in multiple sclerosis.

Family studies represent a powerful resource to identify multiple sclerosis susceptibility genes and to dissect inherited contributions to the clinical expressivity of multiple sclerosis (Barcellos *et al*., 2000). The HLA-DR2 (DRB1*1501, DQB1*0602) haplotype on chromosome 6p21 has consistently demonstrated both association and linkage with multiple sclerosis in case-control and family studies (Allen *et al*., 1994; Hillert *et al*., 1994; Barcellos *et al*., 1997; Haines *et al*., 1998); however, the role of a gene within this region or other regions in determining clinical features or subtypes of multiple sclerosis is unknown. We studied a large stringently ascertained familial dataset with numerous multiple sclerosis cases. In this paper we report concordance within families for early clinical manifestations of multiple sclerosis and also extend genetic evidence indicating locus heterogeneity within the MHC (major histocompatibility complex) region defined by the presence or absence of HLA-DR2. The clinical variables examined in this study, including those found to segregate in some families, were not influenced by HLA-DR2, suggesting that non-HLA genes, or epigenetic factors such as environmental influences, determine the varied clinical manifestations of multiple sclerosis.

Subjects and methods

Families

Rigorous clinical criteria were employed to identify and collect 184 US families each containing multiple cases of multiple sclerosis. Diagnostic criteria have been previously described (Goodkin *et al*., 1991) and the annual follow-up of all families continues. In keeping with the variety of family structures seen in the general population, ascertainment was not restricted to a single family type. All affected members were interviewed, and parental and ancestral information was recorded by countries of origin. All known ancestors were Caucasian and European in origin. In order to limit possible confounding effects of disease heterogeneity and misdiagnosis, families in which a primary progressive course was present in two or more members were excluded; 94.5% of patients had relapsing–remitting multiple sclerosis at onset. Of the 184 multiple sclerosis families collected, 26 were large pedigrees (with four to seven affected individuals, total number affected = 114) and 158 families primarily comprised affected sib pairs (total number of sib pairs = 239, total number affected = 359). Age of onset was defined as the first episode of neurological dysfunction suggestive of demyelinating disease (Doolittle *et al*., 1990). To determine the age of onset, medical records were reviewed and the patient was asked to recall his or her initial neurological symptom, including visual blurring due to optic neuritis or diplopia, focal weakness, sensory symptoms, incoordination, vertigo, tic douloureux, Lhermitte's symptom or paroxysmal symptoms. Isolated mild sensory symptoms of uncertain significance, fatigue or bladder symptoms alone were not considered as initial symptoms. In most patients with visual blurring, a distinction between optic neuritis and brainstem diplopia could be made with confidence, but in uncertain cases no designation was made. More difficult was the identification of cases with a spinal cord onset; this designation was restricted to cases in which (i) bilateral sensory and or motor signs appeared acutely with evidence of a sensory level; or (ii) the site of the attack was confirmed by MRI scanning of the spinal cord. Each patient was categorized as having optic neuritis (ON) only, spinal cord (SC) involvement only, both ON and SC (without other sites of involvement) or other initial symptoms (e.g. unilateral sensory or motor symptoms, brainstem dysfunction, incoordination, etc). The second multiple sclerosis attack was similarly recorded. Disability was assessed at entry with the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). Mild multiple sclerosis was defined as an EDSS score of <3 after 10 years of symptoms. In general, patients in this group can walk normally or have mild gait disability only. Severe multiple sclerosis was defined as wheelchair dependency (EDSS $\geqslant 7$) within 10 years of onset. Complete clinical data was available for over 90% of patients. The collection of subjects and all experiments were performed under the approval the Committee of Human Research at the University of California at San Francisco. All study participants provided informed consent.

HLA typing

High molecular weight DNA was isolated using a standard desalting procedure. HLA typing used a non-radioactive PCR-SSOP (polymerase chain reaction-based sequence specific oligonucleotide probe) reverse line-blot assay (Dynal, Oslo, Norway). Complete HLA genotyping results were available for all families. Generation of genotypes was performed blind to pedigree structure and to the clinical status of the family members. Data were formatted in Microsoft Excel and transferred to pedigree files drawn with Cyrillic 2.1 software, where Mendelian inheritance was automatically checked and confirmed. Allele information was then exported back into Excel and saved in ASCII files for direct downloading into the LAPIS data management system and storage in the PEDIGENE® database (Haynes *et al*., 1995).

Statistical analysis

The concordance of family members with respect to continuous clinical variables (age of onset and years to EDSS $\geqslant 7$) was investigated using an intraclass correlation

coefficient as previously described (Zar, 1974; Barcellos *et al*., 2000). The concordance of common (>10%) categorical clinical phenotypes was assessed and tested using Cohen's kappa coefficient (Fleiss, 1981; Robertson *et al*., 1996), which is analogous to the intraclass correlation coefficient obtained from ANOVA (analysis of variance) models for quantitative measurements, and can accommodate families with more than two affected members. Similarly, if concordance for a particular phenotype within families is the same as between families, kappa = 0; when perfect concordance is present within families, kappa = 1. For analyses of concordance, families in which clinical information was not present for all members for a particular phenotype were excluded.

Patients were stratified by HLA-DR2 phenotype (presence/absence of DR2) to test for effects of HLA-DR2 on clinical variables. Age of disease onset and years to EDSS $\geqslant$7, both continuous outcomes, were analysed in the multiplex families using linear mixed models (models with both fixed and random effects) as implemented in Proc Mixed in SAS Version 6.12 (SAS Institute, Cary, NC, USA) (Laird and Ware, 1982; Jennrich and Schlucter, 1986). These models are fitted by restricted maximum likelihood and use random effects to take into account any correlation between members of the same family, which would violate the independence assumption of ordinary linear regression models. Binary clinical outcomes such as site of first and second multiple sclerosis attack (ON, SC involvement or both compared with all others) and disease severity (mild and severe forms) were analysed using logistic models estimated by generalized estimating equations (Liang and Zeger, 1986; Zeger and Liang, 1992), which also take into account any correlation between family members, as implemented in Proc Genmod in SAS. Similarly, the effects of age of onset and early symptoms on disease severity and years to EDSS $\geqslant$7 were examined using the methods described above. Correction for multiple comparisons was considered too conservative and was not generally applied. However, when multiple categorizations of patients were used to look for effects on clinical phenotypes, as in the case of first symptoms (n = 4 categories) on disease severity (n = 2 categories) and DR2 status on clinical phenotypes (n = 8 categories), a significance criterion of P = 0.00625 (0.05/8) was used for each analysis.

Linkage analysis of the HLA-DR locus included model-dependent and model-independent methods. Since the mode of multiple sclerosis inheritance is not known with certainty, both an autosomal dominant model assuming a multiple sclerosis disease allele frequency of 0.05, and an autosomal recessive model with a multiple sclerosis disease allele frequency of 0.20 were used for LOD score analysis. Both of these models used phenotypic information only on affected individuals, thus eliminating the phenotypic information on all individuals not clinically definite for multiple sclerosis. Calculations utilized FASTLINK (Schaffer *et al*., 1994). The two-point LOD scores for DR were examined for evidence of locus heterogeneity using HOMOG (Ott, 1999). Sib-pair analysis (assuming a locus specific λ_s = 3) was completed using the sib-phase option of ASPEX computer package (Hinds, 1998). Family-based association studies of HLA-DR alleles used the pedigree disequilibrium test (PDT) (Martin *et al*., 2000). The PDT is a powerful analytical method that utilizes genetic data from related nuclear families and discordant sibships within extended pedigrees. Families were stratified for linkage and association analyses by DR2 status. DR2 positive families were defined as those in which the DR2 haplotype was present in a nuclear family member (affected or unaffected); DR2 negative refers to families in which DR2 was not present in any nuclear family member (affected or unaffected). Families were also stratified by concordance of early clinical manifestations for association testing of DR alleles. Only families in which clinical data were available for all affected members were used in analyses.

Results

Clinical features

The multiple sclerosis data set, summarized in Table 1, consisted of 184 families, 341 affected females and 132 affected males (sex ratio 2.6 : 1). The mean age of onset was 30.0 ($\pm$8.8) years with a range of 12–56 years, and the mean disease duration was 17.0 ($\pm$10.7) years; 335 (70.8%) patients had a disease duration of at least 10 years. Male patients had a marginally significant later age of onset than females, 31.3 ($\pm$9.5) versus 29.4 ($\pm$8.5) years, respectively (P = 0.05); 22.1% of males (n = 29) had an age of onset $\geqslant$40 years, compared with 13.1% (n = 44) in females (P = 0.05). The majority of patients had an EDSS score between 3 and 5.5 (35.2%) at time of examination, and the mean number of years to EDSS $\geqslant$7 was 16.3 ($\pm$8.5) years. No differences in EDSS categories or mean years to EDSS $\geqslant$7 were observed between male and female patients.

Initial multiple sclerosis symptoms consisted of ON in 90 patients (21.2%), SC in 114 (26.9%) and ON and/or SC in 205 (48.3%). In this group, restricted ON/SC involvement was maintained with the second clinical attack in 113 patients (26.9%). Mild multiple sclerosis was present in 37 (11.5%) individuals and severe multiple sclerosis in 19 (5.9%) patients. A total of 322 (69.4%) patients were positive for HLA-DR2, 62 (13.4%) were homozygous for DR2. Similar distributions for these clinical parameters were also observed when index (unrelated) cases (n = 184) were considered alone (data not shown). Gender differences were not present for any of the categorical clinical variables examined in this study. Furthermore, differences in clinical expression were not observed in individuals from larger pedigrees (four to seven affected) when compared with those from smaller pedigrees (two or three affected, data not shown).

A severe disease course was positively associated in individuals with a SC onset [OR (odds ratio) = 4.37, 95% CI (confidence interval) 1.60, 11.96, P = 0.004], and negatively

Table 1 *Clinical summary of 184 multiplex Caucasian multiple sclerosis families*

Clinical/demographic information		All multiple sclerosis patients	Female only	Male only
Total no. of individuals with MS (%)		473	341 (72.1)	132 (27.9)
Mean age of onset (years) (SD)		30.0 (±8.8)	29.4 (±8.5)	31.3 (±9.5)*
Age of onset [*n* (%)]	⩽20	72 (15.4)	52 (15.5)	20 (15.3)
	21–39	322 (69.0)	240 (71.4)	82 (62.6)
	⩾40	73 (15.6)	44 (13.1)	29 (22.1)**
Mean disease duration† (years) (SD)		17.0 (±10.7)	17.2 (±10.8)	16.6 (±10.7)
EDSS categories (*n*) (%)	<3	71 (15.8)	53 (16.4)	18 (14.4)
	3–<6	158 (35.2)	114 (35.2)	44 (35.2)
	6	71 (15.8)	52 (16.0)	19 (15.2)
	6.5	47 (10.5)	35 (10.8)	12 (9.6)
	⩾7	102 (22.7)	70 (21.6)	32 (25.6)
Mean no. of years to EDSS ⩾7 (SD)		16.3 (±8.5)	15.7 (±8.7)	17.7 (±8.3)
First attack (*n*) (%)	ON only	90 (21.2)	67 (21.9)	23 (19.5)
	SC only	114 (26.9)	87 (28.4)	27 (22.9)
	ON/SC‡	205 (48.3)	154 (50.3)	51 (43.2)
First two attacks§ (*n*) (%)	ON/SC only	113 (26.9)	87 (28.5)	26 (22.6)
Disease course¶ (*n*) (%)	Mild MS	37 (11.5)	28 (12.1)	9 (10.0)
	Other	284 (88.5)	203 (87.9)	81 (90.0)
	Severe MS	19 (5.9)	14 (6.0)	5 (5.6)
	Other	305 (94.1)	220 (94.0)	85 (94.4)
HLA-DR2 (*n*) (%)	Positive	322 (69.4)	237 (71.0)	85 (65.4)
	Negative	142 (30.6)	97 (29.0)	45 (34.6)

MS = multiple sclerosis; ON = optic neuritis; SC = spinal cord. *$P = 0.05$; **$P = 0.05$. †Mean disease duration determined at age of examination. ‡Refers to patients with optic neuritis, spinal cord or involvement of both areas in first attack of symptoms. §Refers to patients with optic neuritis, spinal cord or involvement of both areas in first and second attacks. ¶Mild multiple sclerosis defined as EDSS <3 after 10 years following onset of first symptoms; severe multiple sclerosis defined as EDSS ⩾7 in 10 years or less following onset of first symptoms.

Table 2 *Concordance of initial clinical manifestations in multiple sclerosis families*

Clinical expression†	Intrafamilial correlation for clinical phenotype (kappa)	*P* value*
First attack		
ON	0.14	0.016
SC	0.06	0.33
ON and/or SC	0.19	0.0014
First two attacks		
ON and/or SC	0.31	$<10^{-6}$

Only families in which clinical data were available for each member were used for analysis. *P value refers to significance of kappa coefficient for correlation within family for clinical phenotype. †Refers to clinical expression of phenotype: ON versus others, SC versus others and ON and/or SC versus others. Families in which all affected had first attack: ON = 6.2%, SC = 4.5%, ON and/or SC = 24.8%; first two attacks: ON and/or SC = 10.5%.

associated with ON (OR = 0.10, 95% CI 0.06, 0.17, $P = 0.004$). Conversely, a mild course was positively associated with ON and negatively associated with SC (OR = 0.38, 95% CI 0.10, 0.92, $P = 0.04$), though this was not significant after correction for multiple testing. Furthermore, a modest but significant inverse relationship was present between age of onset and years to EDSS ⩾7 ($\beta = -0.24$, 95% CI –0.45, –0.03, $P = 0.03$). These epidemiological characteristics (i.e. ON has a favourable course and motor onset an unfavourable course) have been previously reported in sporadic multiple sclerosis patients (Weinshenker and Ebers, 1987), further emphasizing the similarity, from a clinical perspective, of the multiple sclerosis phenotype between multiple affected member families and single affected member families (see also Multiple Sclerosis Genetic Group, 1998). There was no association between initial ON/SC symptoms, considered together, and disease severity (data not shown).

Concordance of early clinical manifestations within families

As shown in Table 2, there was significant concordance within families for a pattern of exclusive ON and/or SC manifestations with the first and second attacks of multiple sclerosis, respectively (kappa = 0.19, $P = 0.0014$, and kappa = 0.31, $P < 10^{-6}$). A weak but significant correlation for age of onset within families was also observed ($r = 0.14$, $P < 0.05$), but was not present for years to EDSS of ⩾7 ($r = 0.20$, $P = 0.29$). There was no evidence of concordance in families for mild or severe disease course (data not shown).

Linkage and association studies of HLA-DR in multiple sclerosis families

To take full advantage of the power of this dataset, a multi-analytical strategy was applied, including parametric LOD

Table 3 *Linkage and association results for HLA-DR*

	Max LOD score		Sib-pair analysis		PDT
	AD†	AR	MLS	% sharing	*P* value
All families*	3.80	2.91	2.00	57.4	0.0002
DR2 positive families	4.62	3.95	2.37	58.8	0.0002
DR2 negative families	–0.03	–0.04	0.00	50.0	0.87

*Total number of multiple sclerosis families (n = 184); DR2 positive (n = 150) and DR2 negative (n = 34). DR2 positive families were defined as those in which DR2 was present in any nuclear family member (affected or unaffected); DR2 negative refers to families in which DR2 was not present in any nuclear family member (affected or unaffected). †Refers to autosomal dominant (AD) and recessive (AR) model specification in two point linkage analyses; see Subjects and methods.

Table 4 *HLA-DR association in families stratified by concordance for early ON/SC manifestations*

Multiple sclerosis families‡	PDT* *P* value	HLA-DR2† *P* value
First attack		
Families concordant for ON/SC (n = 36)	0.0042	0.0023
All other families (n = 116)	0.03	0.0005
First two attacks		
Families concordant for ON/SC (n = 16)	0.10	0.008
All other families (n = 132)	0.007	0.0001

*Global *P* value for PDT analysis of HLA-DR alleles; †*P* value for PDT analysis of HLA-DR2 only. ‡Only families in which complete clinical data were available for all members were used in analyses.

scores, sib-pair and PDT analyses (Table 3). Linkage and association to HLA-DR (LOD = 3.80, $\theta = 0.20$ and $P = 0.0002$, respectively), and a strong association with the specific DR2 haplotype ($P = 1.2 \times 10^{-6}$) were observed in the overall dataset, as previously reported in a subset of this population (Haines *et al*., 1998). As shown in the table, essentially all of the linkage information and evidence for association is derived from families in which DR2 was present in at least one nuclear member. No genetic effect of the HLA-DR locus could be discerned in the DR2-negative family set. In fact, our results exclude linkage for at least 20 cM around the DR locus (LOD score of $\leqslant -2$) in the DR2-negative families for both autosomal dominant and recessive models. There was also no evidence that a subset of the DR2-negative families were linked, as the admixture test was not significant (Ott, 1999). These data provide strong evidence that heterogeneity at the HLA locus exists in multiple sclerosis.

The familial dataset was then stratified by concordance in families for early clinical manifestations to look for HLA-DR associations (Table 4). Thirty-six families were concordant for ON/SC involvement in the initial multiple sclerosis attack, and 16 families were concordant for ON/SC involvement in both first and second attacks, whereas 116 and 132 families were not concordant for ON/SC involvement in initial or secondary attacks, respectively. Significant HLA-DR2 associations were present in all family subsets.

HLA-DR2 and clinical expression in patients

The effect of HLA-DR2 on age of onset, early clinical manifestations, mild and severe disease course was also examined in patients. Significant associations were not observed (data not shown). Interestingly, progression in DR2 positive patients to EDSS $\geqslant 7$ was on average 3.9 years sooner than in DR2 negative patients, but this result did not reach statistical significance ($P = 0.08$).

Discussion

These data demonstrate that early clinical manifestations of multiple sclerosis, specifically symptoms that are confined to the optic nerve and/or spinal cord, aggregate within families. Thus, familial factors influence multiple sclerosis expression. The occurrence of a distinct form of demyelinating disease, characterized by restricted involvement of the optic nerves and spinal cord, was first proposed more than a century ago (Erb, 1880; Devic, 1894). This syndrome, generally classified as Devic's disease or neuromyelitis optica, may be acute and non-recurrent or evolve into a chronic, usually relapsing–remitting, demyelinating disorder with predominantly optic nerve and spinal cord involvement (Wingerchuk *et al*., 1999). This latter disorder, also known as opticospinal or 'Asian-type' multiple sclerosis (Kira *et al*., 1996), is relatively common in Japanese, and may also occur in other non-

Caucasian populations that have a low population prevalence for multiple sclerosis, including African-Americans and individuals residing in the Indian sub-continent (Bansil *et al*., 1996). In contrast, this variant is uncommon in Caucasians (Wingerchuk *et al*., 1999). Prior descriptions of Asian-type multiple sclerosis have been limited to retrospective case series of sporadic cases that are subject to ascertainment bias and, although diagnostic criteria for this syndrome have been recently proposed, they have not been validated (Wingerchuk *et al*., 1999). Current data, derived from a population of rigorously ascertained Caucasian multiple sclerosis-prone families, support the concept that opticospinal clinical manifestations constitute a discrete phenotype with an underlying genetic basis.

Genetic analysis of the MHC region revealed that strong evidence for overall linkage to *HLA-DR* in the multiple sclerosis families was present (LOD score of 3.80), and the results obtained using the PDT statistic were highly significant for both DR ($P = 0.0002$), and specifically for the DR2 allele ($P = 1.2 \times 10^{-6}$). These results highlight the power of family-based association studies to detect disease genes in complex traits such as multiple sclerosis. The frequency of DR2 in familial multiple sclerosis index cases was higher (69.4%) than has been generally reported in sporadic multiple sclerosis (Allen *et al*., 1994; Barcellos *et al*., 1997), and the DR2 frequency tended to increase further as the number of affected individuals per family increased. Although this may be simply the result of the high frequency of DR2 in the founders of the pedigrees, these data argue that genetic loading for multiple sclerosis susceptibility genes is increased in multiply affected families, and provide further evidence that the high recurrence rate has an underlying genetic basis.

These results also extend the current evidence for locus heterogeneity at *HLA-DR* in multiple sclerosis. The linkage and association data indicate that essentially all of the genetic information was derived from DR2-positive families. Furthermore, no evidence for association of other DR alleles in the DR2-negative families was observed, suggesting a fundamentally different disease mechanism in the DR2-negative families without discernible genetic influence of other DR alleles or HLA genes. The genetic association of HLA with multiple sclerosis is likely to be due to the class II genes themselves (DR, DQ or both), related to the known function of these molecules in the normal immune response (antigen binding and presentation and T-cell repertoire determination; for a review, see Oksenberg and Hauser, 1998). Currently available disease-modifying agents for multiple sclerosis consist of interferon-β (Avonex®, Betaseron®) and glatiramer acetate (Copaxone®), the so-called ABC drugs. These immunomodulatory drugs have been shown to decrease clinical relapses, reduce brain MRI activity and possibly slow progression of disability. Glatiramer acetate is thought to act as a molecular mimic of a region of myelin basic protein that is immunodominant in HLA-DR2 positive individuals (Fridkis-Hareli *et al*., 1994). Interferon-β is likely to function by interfering with antigen processing and antigen-mediated triggering of encephalitogenic T cells (Yong *et al*., 1998); each drug is only partially effective. The pharmacogenomic implications of HLA locus heterogeneity in multiple sclerosis are substantial and could potentially explain individual differences in treatment response, a possibility that should be easily testable.

Limited data in Japanese multiple sclerosis patients indicated that HLA-DR2 was associated with disseminated, but not with restricted opticospinal, variants of multiple sclerosis (Kira *et al*., 1996). In contrast, we found that DR2 was significantly associated with ON/SC as well as with other presenting manifestations. Optic neuritis in Caucasians has long been known to have a DR2 association that is as strong, or nearly as strong, as that for multiple sclerosis (Hauser *et al*., 2000). A later age of onset and a more severe disease course, both associated with opticospinal multiple sclerosis (Kira *et al*., 1996; Wingerchuk *et al*., 1999; Yamasaki *et al*., 1999), were also not present in the current series. This is probably due to the large numbers of pure optic neuritis onset cases in our series known to have a favourable prognosis. Most of the current cases with opticospinal onset developed disseminated manifestations of multiple sclerosis at a later time in the disease course (data not shown) and are thus not phenotypically pure.

These results emphasize the power of family-based studies to identify biologically meaningful clinical variants of complex disease traits and to investigate genotype–phenotype correlations. Earlier studies have reported possible intrafamilial concordance for disease course (Robertson *et al*., 1996), disease severity (Brassat *et al*., 1999) and age of onset (Doolittle *et al*., 1990; Bulman *et al*., 1991). Patients in the current series comprised almost exclusively relapsing–remitting or secondary progressive multiple sclerosis, thus concordance for primary progressive multiple sclerosis, recently reported in a sample of UK multiple sclerosis families (Robertson *et al*., 1996), could not be confirmed. In sporadic multiple sclerosis patients, HLA-DR2 has been variously reported to be associated with an earlier age of onset, female gender, severe, relapsing–remitting and mild multiple sclerosis courses (Engell *et al*., 1982; Madigand *et al*., 1982; Duquette *et al*., 1985, 1992; Masterman *et al*., 2000; Weatherby *et al*., 2001) or to have no influence on disease course (Poser *et al*., 1981; Runmarker *et al*., 1994; Weinshenker *et al*., 1998; McDonnell *et al*., 1999; Celius *et al*., 2000). Many of these positive findings could represent type I errors. In the current family-based study, intrafamilial concordance or HLA-DR2 effects on age of onset, first symptoms, a mild course or evolution to a severe course were not observed. Interestingly, correlation within families for years to EDSS $\geqslant 7$ was 0.20 (similar to that reported by Brassat *et al*., 1999), and HLA-DR2 positive individuals progressed on average 3.9 years faster to EDSS $\geqslant 7$ than HLA-DR2 negative patients; neither of these trends were statistically significant. Several studies examining the influ-

ence of other non-HLA genes on disease course and severity in multiple sclerosis have been recently reported, but have been negative or await confirmation (Weinshenker *et al*., 1997; Fukazawa *et al*., 1999; Schrijver *et al*., 1999; Barcellos *et al*., 2000).

In conclusion, concordance for early manifestations of multiple sclerosis was present in this large familial multiple sclerosis dataset. Despite the clear evidence of locus heterogeneity defined by HLA-DR2, we found no influence of HLA-DR2 on any of the clinical multiple sclerosis outcomes examined. The association of DR2 in families with diverse clinical presentations suggests that there exists a common genetic basis to various clinical phenotypes of multiple sclerosis. In the multiple sclerosis disease model EAE (experimental allergic encephalomyelitis), it appears that MHC genes primarily influence penetrance, whereas other loci modulate specific phenotypes such as location in brain or spinal cord, demyelination and severity of inflammation (Butterfield *et al*., 2000). Epigenetic factors, such as the selection of different disease-inducing antigens, also influence the location and severity of EAE lesions (Sobel, 2000). It is likely that a similar interplay of genetic and epigenetic factors operates in human multiple sclerosis. In addition to the HLA region at 6p21, a second susceptibility locus for multiple sclerosis exists at 19q13 (Pericak-Vance *et al*., 2001) and several other suggestive loci have been proposed as well (Oksenberg *et al*., 2001). As the genomic map of multiple sclerosis loci is increasingly refined, multiple sclerosis families concordant for opticospinal manifestations will represent a valuable stratifying element for gene identification and genotypic–phenotypic correlations.

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