differences between populations, possibly owing to a selective pressure of *S. mansoni* infection, may motivate further studies.

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Multiple Sclerosis in Sardinia Is Associated and in Linkage Disequilibrium with HLA-DR3 and -DR4 Alleles

To the Editor:

The preponderance of genetic factors in attempts to account for susceptibility to multiple sclerosis (MS), a common inflammatory and demyelinating disease of young adults, has recently been demonstrated (Ebers et al. 1995). The inheritance of MS appears to be complex and is believed to involve several genes (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996). Methodological approaches to the study of genes conferring susceptibility to MS include association studies, which measure the frequency of a specific allele in affected and healthy populations, and linkage studies, which trace the inheritance of a gene from parents and correlate these genes to disease susceptibility. The genetic approach to MS has preferentially been performed on genes involved in immune mechanisms—in particular, on HLA genes (Haegert and Marrosu 1994). Association studies have established a link between the disease and the HLA class II DRB1*1501-DQA1*0102-DQB1*0602 haplotype (the serologically defined DR2,DQw1 allele [Bodmer et al. 1995]) in Caucasian MS patients (Hillert and Olerup 1993). Nevertheless,

the association determined by case-control studies can be prone to artifacts due to population stratification.

In Sardinia, an Italian island, a high incidence and prevalence of MS has been reported (Rosati 1994); a recent study showed an incidence of 5.7/100,000/year during 1987–91 and a prevalence of 102.6/100,000 (Rosati et al. 1996). As demonstrated by genetics from population studies (Cavalli-Sforza et al. 1988), the phylogeny of the Sardinian population is the result of a Caucasoid root, but it is genetically distinct from that of other Europeans. The concomitance of both the particular genetic structure of Sardinians and the high incidence of MS on the island offers a unique opportunity for evaluation of whether the reported association (Hillert and Olerup 1993) is due to population stratification. In this sense, genetic studies of MS in Sardinians may be regarded as a touchstone for findings related to other genetically different populations. The present study was performed in order to test the association of HLA-DR genes to MS, by performing a large case control–association study in a population with little genetic heterogeneity. Then, we tested for linkage to the same HLA alleles in an independent data set from the same population, using the transmission-disequilibrium test (TDT), in order to distinguish between linkage disequilibrium (the presence of both linkage and association between marker and disease) and associations that arise from population structure in absence of linkage.

DNA of patients and controls was extracted from peripheral blood by use of standard methods. Both amplification of the polymorphic second exon of DRB1 gene and dot-blot analysis of amplified DNA with sequence-specific oligonucleotide probes (SSO) were performed by use of a procedure described elsewhere (Bell et al. 1989). All subjects had been born in and were living in Sardinia and were of Sardinian descent. The majority of patients and controls came from southern Sardinia, whereas a minority came from the northern or central part of the island. All patients had clinically definite MS, according to the criteria of Poser et al. (1983). Informed consent was obtained from all subjects.

For the association study, we HLA-DRB1–typed 240 unrelated MS patients (UMS)—82 males and 158 females, mean age 35.9 years (range 14–62 years). The course of the disease in UMS patients was relapsing-remitting in 123, secondary chronic progressive in 96, and primary chronic progressive in 21. As controls, we typed 80 healthy persons—26 males and 54 females, mean age 41.2 years (range 27–63 years, who came from the hospital staff and who denied personal or familial history of either MS or autoimmune diseases. Moreover, we typed both healthy parents in each of 90 families of UMS patients, considering the parenteral alleles not transmitted by parents to the affected child to be a "control" population, according to the affected family–based controls (AFBAC) method (Thomson 1995). Statistical evaluation was performed by use of either the χ^2 test with Yate's correction or Fisher's exact test, as appropriate. Significance level was set at .05. Moreover, we have corrected P values ($P_{corrected}$) by multiplying by 5—that is, the number of DRB1 alleles showing gene frequency >5% in healthy Sardinians. Since the allelic frequency in healthy controls was similar to that obtained with AFBAC method, we pooled the data in order to obtain an unbiased estimate of frequency in the overall population. Only UMS-patient DRB1 allele frequencies significantly deviating from those in controls are shown in table 1. The allelic frequency of the DRB1*0301 allele is 31.4% in UMS patients and 20.6% in controls ($P_{corrected}$ = .003). Considering all subjects who were DRB1*040.. positive, we found a gene frequency of .21 in UMS patients and .14 in controls ($P_{corrected}$ = .05). When DRB1*040..-positive subjects were split into various subtypes, we found that the gene frequency of the DRB1*0405 allele was .14 in UMS patients and .07 in controls ($P_{corrected}$ = .01). The present study, performed with a group of MS patients totally different from those studied elsewhere (Marrosu et al. 1988), confirms the previously reported MS-DR4 association in Sardinians (Marrosu et al. 1988) and shows a novel DRB1 association—namely, DRB1*0301. DR3 and DR4 alleles are also implicated in insulin-dependent diabetes mellitus (IDDM) (Svejgaard and Ryder 1989), a high-incidence disease among Sardinians (Green et al. 1992).

Linkage disequilibrium has been studied by typing 52 MS siblings (MSS) (26 pairs)—21 males and 31 females, mean age 38.3 years (range 26–61 years), and 35 parents and 66 healthy sibs of MSS (HS), all of whom were found, on the basis of recent interview and neurological examination, to be clinically unaffected. MSS patients were not used in the initial case-control study. Among the MSS patients, the course of the disease was relapsing-remitting in 35, secondary chronic progressive in 15, and primary progressive in 2.

The transmission-disequilibrium test (TDT) (Spiel-

Table 1

HLA-DRB1 Allelic Frequency in 240 Unrelated UMS Patients and 170 Healthy Controls

	No. (%) in		
	UMS Patients (n = 480)	Controls (n = 340)	$P_{corrected}$
DRB1*0301	151 (31.4)	70 (20.6)	.003
DRB1*040.. (all)	101 (21.0)	48 (14.1)	.05
DRB1*0405	67 (13.9)	26 (7.0)	.01

Table 2

TDT for DRB1*0301 and DRB1*040.. Alleles in MSS Pairs and HS

Group and Allele	i[a]	$h - i - j$[b]	j[c]	h[d]	χ^2_{td}[e]	P[f]
MSS pairs:						
DRB1*040..	8	5	2	15	4.8	.05
DRB1*0301	12	6	3	18	9	.005
HS:						
DRB1*040..	3	4	1	8	1	NS
DRB1*0301	4	7	4	15	0	NS

[a] Number of parents who transmit the DRB1*0301 allele or the DRB1*040.. allele to both children.

[b] Number of parents who transmit the DRB1*0301 allele or the DRB1*040.. allele to one child and the alternate allele to the other child.

[c] Number of parents who transmit the alternate allele to both children.

[d] Total number of heterozygous parents.

[e] $2(i - j)2/h$.

[f] NS = not significant.

man et al. 1993) has been used to test for linkage of the HLA-DR alleles associated with MS in the population-based study. To ensure that any differences found in the TDT were not due to an artifact of meiotic distortion, we have performed the TDT in HS (Spielman et al. 1993). The significance level in TDT has been set at .05, by use of the χ^2_{td} test (Spielman et al. 1993). Considering both the DRB1*0301 and the DRB1*040.. alleles associated with MS in UMS patients, we sought evidence for linkage disequilibrium, using the TDT for the DRB1*0301 and DRB1*040.. alleles. The DRB1*0301 and DRB1*040.. alleles were not present in 2 of 26 families studied, whereas one or both alleles were present in 24 of 26 families. The TDT has been calculated for 18 DRB1*0301-positive parents and 15 DRB1*040..-positive parents. Linkage disequilibrium with both the DRB1*0301 allele ($\chi^2_{td} = 9$, $P < .005$ [table 2]) and the DRB1*040.. allele ($\chi^2_{td} = 4.8$, $P < .05$ [table 2]) has been found in MSS. There has been no evidence of segregation distortion in unaffected offspring from 15 DRB1*0301-transmitting/-nontransmitting and 8 DRB1*040..-transmitting/-nontransmitting parents (table 2); nevertheless, because of the small number of unaffected subjects, the power to detect statistical differences in this group is low. Our results show evidence of linkage disequilibrium between both the DRB1*040.. allele and the DRB1*0301 allele and MS in patients. Sardinians appear to be a unique population carrying a genetic predisposition to MS, a predisposition that is in linkage disequilibrium with two different DRB1 alleles.

Using identity-by-descent analysis in affected sibling pairs, previous studies have reported either no linkage (Ebers et al. 1982) or only a suggestion of linkage (Hauser et al. 1989; Voskuhl et al. 1996) between MS and the DR2,DQw1 allele. In this study, haplotype-sharing values in affected sib pairs do not significantly diverge from "random"-sharing values (data not shown), but it is well known that the power to detect linkage by use of this method may require hundreds of sib pairs; on the contrary, in terms of detection of linkage, the TDT is much more sensitive and effective than the haplotype-sharing test, even with a relatively small number of sibling pairs, when association with a marker has already been established in a large population-based study (Spielman et al. 1993), as in the present report.

We also analyzed the transmission of DRB1*0301 and DRB1*040.. alleles from healthy heterozygous parents to affected offspring. The transmitting/nontransmitting ratio for DRB1*0301 mothers was 11/1, whereas that for fathers was 12/10 ($P = .03$; Fisher's exact test); the same ratio for the DRB1*040.. was 11/5 for mothers and 6/4 for fathers (probability not significant; data not shown). With regard to parental inheritance, MS susceptibility has been reported to be transmitted more frequently by mothers than by fathers (Sadovnick et al. 1991). In the sib pairs that we studied, the disease segregates with DRB1*0301 maternal alleles, whereas there is no significant difference in the segregation of the DRB1*040.. allele, although there are more transmitting mothers than nontransmitting mothers. These data may suggest an "imprinting" mechanism in the transmission of the DRB1*0301 allele, but caution is necessary because of the limited number of pairs studied. Voskuhl et al. (1996) reported, in a small number of families, no difference in the parental tranmission frequency of the DR2,DQw1 allele. A possibility that cannot be excluded is that the different parent-of-origin alleles involved in MS susceptibility in Caucasians and Sardinians may be inherited in a different way.

Recent results from a genome screen for genes implicated in MS susceptibility have shown heterogeneous results regarding the contribution made by all regions of interest, in various data sets (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996). However, in two (Ebers et al. 1996; Sawcer et al. 1996) of three studies, a weak effect of major-histocompatibilty-complex (MHC) genes was evident. The failure, in all studies (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996), to obtain homogeneous data regarding the MHC region may be due to the ethnically diverse origin of the patients. On the basis of such different results (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996), it can be problematic to establish a strategy for future genetic studies. Data presented in this study, obtained from a highly homogeneous population, seem to exclude the hypothesis that the association

between MS and HLA genes may be due to population stratification and, in this sense, contribute to sustaining the importance of the MHC region in MS susceptibility. However, the presence of different HLA class II allele associations, such as DRB1*1501 (DR2) in Caucasians (Hillert and Olerup 1993) and DRB1*040.. (DR4) and DRB1*0301 (DR3), demonstrated both elsewhere (Marrosu et al. 1988) and in this report, suggests the possibility that any of these alleles may be the locus primarily involved in susceptibility to MS. The absence of polymorphic sequences shared by DRB1*1501, DRB1*0301, and DRB1*040.. alleles (Marsh and Bodmer 1995) reinforces this opinion. However, we cannot exclude the possibility that susceptibility to MS is indeed carried by different DRB alleles, which interact, in a complex way, with other genes located outside or within chromosome 6.

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