

Report

Mapping Multiple Sclerosis Susceptibility to the *HLA-DR* Locus in African Americans

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An underlying complex genetic susceptibility exists in multiple sclerosis (MS), and an association with the *HLA-DRB1*1501-DQB1*0602* haplotype has been repeatedly demonstrated in high-risk (northern European) populations. It is unknown whether the effect is explained by the *HLA-DRB1* or the *HLA-DQB1* gene within the susceptibility haplotype, which are in strong linkage disequilibrium (LD). African populations are characterized by greater haplotypic diversity and distinct patterns of LD compared with northern Europeans. To better localize the HLA gene responsible for MS susceptibility, case-control and family-based association studies were performed for *DRB1* and *DQB1* loci in a large and well-characterized African American data set. A selective association with *HLA-DRB1*15* was revealed, indicating a primary role for the *DRB1* locus in MS independent of *DQB1*0602*. This finding is unlikely to be solely explained by admixture, since a substantial proportion of the susceptibility chromosomes from African American patients with MS displayed haplotypes consistent with an African origin.

Evidence of disease risk heritability in multiple sclerosis (MS [MIM 126200]) is supported by familial aggregation of cases (Ebers et al. 1995; Robertson et al. 1996; Sadovnick et al. 1996, 2000) and by the high prevalence in some ethnic populations (particularly those of northern European origin) compared with others (African and some Asian groups) irrespective of geographic location (Compston 1998). Modeling of the available data predicts that the MS-prone genotype results from multiple independent or interacting polymorphic genes, each exerting a small—or, at most, a moderate—effect on the overall risk. The *HLA-DR2* haplotype (*DRB1*1501-DQB1*0602*) within the major histocompatibility complex on chromosome 6p21 has consistently demon-

strated both linkage and association in family and case-control studies (Multiple Sclerosis Genetics Group 1998). However, fine-mapping studies have not settled whether the effect is explained by the *DRB1* gene itself (MIM 142857); by another closely spaced gene within the class II *HLA* region, such as *DQB1* (MIM 604305); or by some other nearby gene in strong disequilibrium with the *HLA-DR* locus. Analyses have been made more complex by extensive linkage disequilibrium (LD) occurring across the region and by the presence of >240 genes within this superlocus, many of which have roles in immune function and are thus plausible disease candidates. Results suggesting that genes of interest for MS exist within the class III (de Jong et al. 2002; Palacio et al. 2002) and/or telomeric to the class I regions (Shinar et al. 1998; Fogdell-Hahn et al. 2000; Marrosu et al. 2001; Rubio et al. 2002) have been reported as well. Because patterns of LD differ between populations, the most direct and practical approach to resolving this complex genetic obstacle will be to scrutinize and compare a large number of MS haplotypes in well-characterized

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data sets from distinct populations. However, studies of *HLA* and MS in low-risk ethnic groups have generally utilized small cohorts, and the results have been inconclusive or conflicting (Serjeantson et al. 1992; Kelly et al. 1995; Caballero et al. 1999; Kwon et al. 1999; Quellenec et al. 2003).

Compared with northern Europeans and white Americans, African Americans are at a lower risk for MS (Kurtzke et al. 1979), but genetic studies of *HLA* and other loci in this population can be extremely valuable for disease gene identification, because of the presence of distinctive patterns of disequilibrium shaped by the population history of the group (Just et al. 1997; Zachary et al. 1997). Some combinations in *cis* of *DRB1* and *DQB1* alleles are unique to African Americans, and, more specifically, the *DRB1**1501 and *DQB1**0602 alleles do not display the high degree of LD and haplotype fixity in African Americans that is characteristic of Europeans.

To localize the *HLA* gene responsible for MS susceptibility, we performed case-control and family-based association studies for *DRB1* and *DQB1* loci in a large African American cohort consisting of 1,003 genotyped individuals. The data set comprised 336 unrelated patients with MS (female:male ratio 4.6:1; mean age at onset 32.7 ± 9.4 years; mean disease duration 9.1 ± 7.1 years), available nuclear family members ($n = 357$), and unrelated control individuals ($n = 310$, primarily patient spouses). Families included 33 complete trios (both parents and the affected individual), 162 families with one parent, and 100 discordant sib pairs (one affected and one unaffected individual). All study participants were self-reported African Americans, but European ancestry in patients and controls was documented on the basis of genotyping results of 186 SNPs. Based on the use of two parent populations, West Africans and Europeans, these SNPs have a mean 54% allele frequency differences between the parental populations and are spaced at least 10 cM from each other across the genome (Parra et al. 1998). Global estimation of European ancestry in 50 patients and 50 controls indicated 19.1% admixture in both groups (data not shown). Institutional review board approval and informed consent were obtained from all study participants. Medical records of all patients were reviewed by one of the authors (S.L.H. or B.A.C.C.) and, in all cases, diagnosis was confirmed using standard criteria (Poser et al. 1983). A total of 92% of patients had relapsing-remitting MS at onset.

Global testing for class II *HLA-DRB1* and *-DQB1* in the African American patient-control data set revealed differences in allele distributions ($P = 4.0 \times 10^{-4}$ and $P = .02$, respectively). Whereas the *DQB1* effect was restricted to the *DQB1**0602 allele only (odds ratio [OR] 1.4; $P = .01$), disease association at the *DRB1* locus was found for both *DRB1**15 and *DRB1**0301

alleles (OR 1.7, $P = 6.0 \times 10^{-4}$ for *DRB1**15; and OR 1.7, $P = .01$ for *DRB1**0301; see table 1). As observed in white MS data sets, there was an increased risk, albeit not as pronounced, for the *DRB1**1501 allele (OR 1.7; $P = .03$) and the *DRB1**1501-*DQB1**0602 haplotype (OR 1.6; $P = .049$) in African Americans (table 1). In addition, associations were observed for both the *DRB1**1503 allele (OR 1.6; $P = .015$) and the common African haplotype *DRB1**1503-*DQB1**0602 (OR 1.5; $P = .02$). Two-locus *DRB1-DQB1* haplotypes were deduced on the basis of known associations (Fernandez-Vina et al. 1991; Lin et al. 1997). As an additional measure of validation, *DR-DQ* haplotype frequencies in patients and controls were also estimated from genotype data via the method of maximum likelihood, through the use of the expectation-maximization algorithm (Arlequin version 2.0 [Arlequin's Home on the Web]) under the assumption of Hardy-Weinberg equilibrium (Excoffier and Slatkin 1995; Long et al. 1995), and were confirmed, whenever possible, by available family data.

It is important that the *DRB1**1501 and *1503 disease associations were independent of *DQB1**0602, as evidenced by the fact that chromosomes carrying the *0602 allele with other *DRB1* alleles were present at identical frequencies in patients and controls (4.0%; $P = .77$). Conversely, *DRB1**15 (including *1501 and *1503) chromosomes carrying other *DQB1* alleles (non-*0602 and denoted by "X") were significantly increased in patients with MS (OR 3.7; $P = .01$; see table 1). Patients and controls were also grouped, by genotype, according to whether they carried at least one *DRB1**15 and/or one *DQB1**0602 allele, to rule out the possibility of *trans* allelic effects (table 2). In these analyses, for example, the effect in an individual of *DRB1**15 on one chromosome in *trans* with *DQB1**0602 on the other chromosome is eliminated (see the *DRB1**15⁻/*DQB1**0602⁺ genotypic category), and an independent role for *DQB1**0602 unmasked by *DRB1**15 can be assessed. As shown in table 2, our results do not indicate the involvement of *DQB1**0602 in MS susceptibility. An increased risk in individuals carrying the *DRB1**15⁻/*DQB1**0602⁺ genotype was not observed (OR 1.0; $P = .99$). Furthermore, the analyses of *DRB1**15⁺/*DQB1**0602⁻ and *DRB1**15⁻/*DQB1**0602⁺ haplotypes did not reveal additional susceptibility alleles acting in an asymmetric fashion. A diverse array of *DRB1* alleles, including *DRB1**03, *08, *11, *13, *14 and *16, were present in similar proportions on *DRB1**15⁻/*DQB1**0602⁺ haplotypes in patients and controls (data not shown). *DRB1**15⁺/*DQB1**0602⁻ haplotypes also displayed marked heterogeneity and included *DQB1**02, *03, *0501, *0502, *601, *0603, *0609, *0615, and *0617 alleles distributed equally in both groups. Nonetheless, much larger data sets will be required to exclude the possibility of minor

Table 1**HLA-DR2- and HLA-DR3-Related Alleles and Haplotypes in African American Patients with MS and Controls**

ALLELE OR HAPLOTYPE	NO. (%) OF		OR	95% CI	P VALUE
	Patients (2N = 672)	Controls (2N = 620)			
Allele ^a :					
<i>DRB1*0301</i>	72 (10.7)	42 (6.8)	1.7	1.1–2.5	.01
<i>DRB1*15^b</i>	149 (22.2)	91 (14.7)	1.7	1.2–2.2	.0005
<i>DRB1*1501</i>	49 (7.3)	27 (4.4)	1.7	1.1–2.8	.03
<i>DRB1*1503</i>	100 (14.9)	64 (10.3)	1.5	1.1–2.1	.014
<i>DQB1*0602</i>	158 (23.5)	111 (17.9)	1.4	1.1–1.9	.01
<i>DRB1-DQB1</i> haplotype ^c :					
<i>0301-0200^d</i>	72 (10.7)	41 (6.6)	1.7	1.1–2.5	.009
<i>1501-0602</i>	41 (6.1)	25 (4.0)	1.7	1.0–2.8	.049
<i>1503-0602</i>	90 (13.4)	61 (9.8)	1.5	1.1–2.1	.02
<i>15-0602</i>	131 (19.5)	86 (13.9)	1.5	1.1–2.1	.004
<i>X-0602</i>	27 (4.0)	25 (4.0)	1.1	.6–1.9	.74
<i>15-X</i>	18 (2.7)	5 (.8)	3.7	1.3–9.9	.007
<i>1501-X</i>	8 (1.2)	2 (.3)	4.1	.9–19.2	.06
<i>1503-X</i>	10 (1.5)	3 (.5)	3.4	.9–12.4	.05

NOTE.—Molecular typing of *HLA-DRB1* and *-DQB1* loci was performed using a nonradioactive PCR-based sequence-specific oligonucleotide probe reverse line-blot assay (Dynal). *DRB1*15/DRB1*03/DQB1*06* subtyping was performed for all individuals, through use of a similar approach, after allele-specific amplification of the *DR2* allele (Bugawan et al. 2002). For other *DRB1* and *DQB1* alleles not distinguished by this method, the resolution is reported at the lowest level common to any particular allele.

^a Reference group: all other *DRB1* or *DQB1* alleles. *P* values (χ^2 or Fisher's exact test, when appropriate), ORs, and CIs were derived using PROC FREQ (SAS version 8.2).

^b Includes *DRB1*1501* and **1503* alleles only. *DRB1*1502* was also present in patients and controls but was very rare (.1% and .8%, respectively).

^c The reference group for *DRB1*0301-DQB1*0200* haplotype comparison was all other haplotypes. To distinguish the *HLA-DR2* related haplotype effect, all non-*DRB1*15* and non-*DQB1*0602* haplotypes were used as the reference group for *DRB1*15-* and *DQB1*0602*-related haplotypes.

^d Primarily *DRB1*0301-DQB1*0201* haplotypes. Does not include *DRB1*03021-DQB1*0402* haplotypes.

heterogeneous multiallelic *DRB1* and *DQB1* interactions influencing disease susceptibility in MS.

All *DRB1*0301*-associated chromosomes in African American patients and controls carried *DQB1*02* alleles; the vast majority were the common autoimmune disease-associated haplotype *DRB1*0301-DQB1*0201*. The effect for the *DRB1*0301* allele considered alone or in combination with *DQB1*02* was identical (OR 1.7; *P* = .01), and no evidence for association in patients with non-*DRB1*0301* haplotypes bearing *DQB1*02* alleles (or *X-DQB1*02*) was observed (OR 0.8; *P* = .14; data not shown). These results further support an independent role for *DRB1*0301* in MS, rather than variation at the *DQB1* locus. The patient data set was also compared with another large published African American control group (*N* = 242 individuals) (Just et al. 1997), with similar results (data not shown). Significant associations for *DRB1*15* (OR 1.4; 95% CI 1.1–1.9; *P* = .02) and *DRB1*0301* (OR 1.6; 95% CI 1.1–2.5; *P* = .03) and no independent association with

*DQB1*0602* (OR 1.0; 95% CI 0.8–1.3; *P* = .94) or *X-*0602* haplotypes (OR 0.6; 95% CI 0.3–1.0; *P* = .04) were observed. Further, the frequencies of predominantly African *DRB1*03021-DQB1*0402* and *DRB1*1304-DQB1*0301* and of northern European *DRB1*0800-DQB1*0402* haplotypes in patient and control groups were statistically indistinguishable (6.6%–5.2%, *P* = .57 for *DRB1*03021-DQB1*0402*; 0.5%–1.2%, *P* = .28 for *DRB1*1304-DQB1*0301*; and 0.5%–0.9%, *P* = .64 for *DRB1*0800-DQB1*0402*). These observations, together with the SNP genotyping results mentioned above, indicate that the patients with MS and the controls used in this study were adequately matched.

To further address the possibility that the case-control results might be influenced by population stratification, family-based analyses of the *HLA-DRB1* and *DQB1* loci were performed in a subgroup of patients for whom informative family controls were available (table 3). Evidence for excess transmission of the *DRB1*1501* allele

Table 2

***DRB1*15* and *DQB1*0602* Genotypes in African American Patients with MS and Controls**

GENOTYPE	No. (%) OF				95% CI	P Value
	Patients	Controls	HW ^b	OR ^a		
<i>DRB1*0602*15⁺/DQB1*0602⁺</i>	123 (36.6)	76 (24.5)	86 (27.7)	1.6	1.1–2.2	.01
<i>DRB1*15⁺/DQB1*0602⁻</i>	11 (3.3)	4 (1.3)	4 (1.3)	3.0	.9–9.6	.05
<i>DRB1*15⁻/DQB1*0602⁺</i>	23 (6.8)	24 (7.7)	25 (8.1)	1.0	.5–1.9	.99

NOTE.—Patients and controls were grouped according to whether they carried at least one *DRB1*15* and/or one *DQB1*0602* allele. For example, the genotypic category *DRB1*15⁺/DQB1*0602⁺* contains individuals carrying at least one *DRB1*15* and one *DQB1*0602* allele, regardless of chromosomal phase, and would include the following combination of genotypes: *DRB1*15-DQB1*0602/DRB1*15-DQB1*0602*, *DRB1*15-DQB1*0602/DRB1*15-X* (where “X” = non-*DQB1*0602* alleles), *DRB1*15-DQB1*0602/X-DQB1*0602* (where “X” = non-*DRB1*15* alleles), *DRB1*15-DQB1*0602/X* (where “X” = all other non-*DRB1*15*–non-*DQB1*0602* haplotypes), and *DRB1*15/X-DQB1*0602* (where “X” = non-*DQB1*0602* alleles). Similarly, the *DRB1*15⁺/DQB1*0602⁻* category comprised individuals with *DRB1*15-X/X* genotypes (where “X” = non-*DQB1*0602* alleles and “X” = all other non-*DRB1*15*–non-*DQB1*0602* haplotypes, respectively) and *DRB1*15-X/DRB1*15-X* genotypes (where “X” = non-*DQB1*0602* alleles). For these analyses, the Hardy-Weinberg expected genotype frequencies for the control subjects were used, rather than the actual observed genotype counts, to obtain greater precision in the OR estimates.

^a The reference group for all OR estimates comprised X/X (*DRB1*15⁻/DQB1*0602⁻*) individuals.

^b Number and percent expected under Hardy-Weinberg conditions.

to affected individuals was observed when the pedigree disequilibrium test (PDT) was used, for all families ($P = .04$) and for trios only ($P = .025$). TRANSMIT (available from David Clayton’s Web site) also yielded significant results for *DRB1*1501* ($P = .0006$). Associations with other *DRB1* or *DQB1* alleles were not observed. Although only a few *DRB1*1501*, *DQB1*X* haplotypes were identified in the family data set, TRANSMIT results for this haplotypic classification were also supportive of an independent *DRB1*1501* association, but not for *DQB1*0602* (data not shown). The failure to detect evidence for statistically significant excess transmission of *DRB1*1503* and *DRB1*0301* alleles was likely due to the small number of informative trios available for analysis.

In summary, we report a selective association with *DRB1*15*, indicating a primary role for the *DRB1* locus in MS independent of *DQB1*. An approach similar to that reported here was previously used to identify the primary class II region gene associated with the sleep disorder narcolepsy, another *HLA-DR2*-related disorder (Mignot et al. 2001). In striking contrast to MS, the specific HLA association in African American narcoleptic patients was with *DQB1*0602* rather than with *DRB1*1501*. A primary role for *DRB1*15* in susceptibility to MS is consistent with a pathogenesis model that involves a T cell–mediated autoimmune response against the 85-99 peptide of myelin basic protein (MBP) (Allegrretta et al. 1990; Pette et al. 1990; Oksenberg et al. 1993; Krogsgaard et al. 2000). The crystal structure of *DRβ*1501* differs from other non-*DR2*-related *DRβ* molecules, in that aromatic residues in the ligand are preferred in the large hydrophobic P4 pocket of the peptide-binding

domain (Smith et al. 1998). For MBP, this pocket is primarily occupied by the aromatic side chain Phe92, acting as an important primary anchor and accounting for its high-affinity binding to the *HLA-DRα*0101/DRβ*1501* heterodimer. Although structural features of *DRB1*1503* have not been described, the two *DRB1*15* alleles differ only at position 30 (Tyr in **1501*, His in **1503*) (fig. 1A). Recent immunologic studies have demonstrated the

Table 3

African American MS Family-Based Association Test Results for *HLA-DRB1* and *HLA-DQB1*

ALLELE	TRANSMISSION		
	PDT (All) ^a	PDT (Trios Only) ^a	TRANSMIT ^b
<i>DRB1</i> global	.33	.73	.057
<i>DRB1*1501</i>	.04	.025	.0006
<i>DRB1*1503</i>	.70	.67	.56
<i>DRB1*0301</i>	.30	.56	.39
<i>DQB1</i> global	.47	.84	.34
<i>DQB1*0602</i>	.61	.53	.40

NOTE.—Two complementary approaches were used to look for evidence of excess transmission of *DRB1* and *DQB1* alleles in African American families. The PDT (Martin et al. 2000, 2001; Pedigree Disequilibrium Test Analysis Program Web site) can utilize both discordant sib pairs and nuclear families from extended pedigrees. TRANSMIT (Clayton and Jones 1999) is restricted to parent-offspring triads but can consider transmission of alleles or haplotypes even in the presence of phase uncertainty and missing parental genotypes.

^a PDT analysis utilized 33 trios and 100 discordant sib pairs.

^b TRANSMIT analysis utilized 162 families.

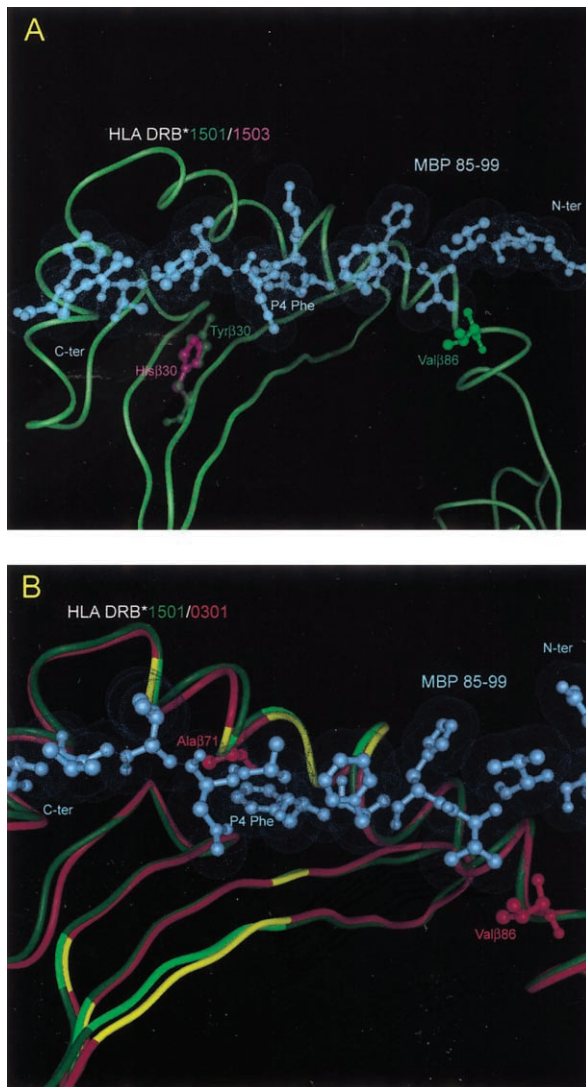


Figure 1 Molecular structures of MS-prone HLA-DR β 1 alleles. **A**, HLA-DR β 1*1501 and -DR β 1*1503 allelic structure are superimposed using MINRMS, a program for finding minimal root mean squared distance alignments between two proteins as a function of the number of matching residue pairs within a heuristically limited search space. The resulting structures were displayed as rods through use of Chimera (UCSF Chimera Home Page), an interactive molecular graphics program (Chiang et al. 2003). The structure of DR β *1503 was developed from the DR β *1501 crystal structure (Protein Data Bank [PDB] ID 1BX2) by mutating the residue at position 30 from Tyr to His, using the software Swiss PDB-viewer. The distinctive residues at position 30—Tyr for *1501 and His for *1503—are shown in green and magenta, respectively. Polymorphic residue Val β 86 (critical for the hydrophobic pocket characteristic of DR2 and DR3 alleles) is shown in green. MBP peptide 85-99 and its electronic cloud are shown in gray. **B**, HLA-DR β 1*1501 (green) is superimposed on DR β 1*0301 (PDB ID 1A6A; red) in a top view, and their differences are highlighted in dark green and yellow, respectively. Most of the allele-specific residues are in close proximity to the peptide and thus likely affect the MBP binding affinity. Residue DR β 1*0301Val 86 is shown in red. Residue labels are colored according to that of their respective molecule. For clarity, the DR α chain was omitted in these representations.

capability of DRB1*1503 to present the immunodominant autoantigen MBP 85-99 peptide to a DRB1*1501 restricted MBP-specific T cell line (Quelvenec et al. 2003) and suggest that *1501 and *1503 molecules may act similarly in MS development.

The polymorphic residue at DR β position 71 is also critically important in creating the necessary space for Phe92 of MBP, and Ala at this position has been observed only for DR15 alleles (DRB1*1501–DRB1*1506) and for DRB1*1309 but not for DRB1*0301. HLA-DRB1*1501, DRB1*1503, and DRB1*0301 alleles, on the other hand, all share a critical Val residue at position 86, where the HLA-DR β chain is also polymorphic, and can encode either Val or Gly. DR β Val86 at the base of the P1 pocket results in a smaller pocket than that observed for DR1 and DR4 (Gly), for example, influencing not only binding and presentation of a number of self antigens, including MBP, but also DR $\alpha\beta$ dimer stability (Verreck et al. 1993; Wucherpfennig et al. 1994; Smith et al. 1998). The Val86/Val86 genotype has been implicated in association studies in Swedish and Australian populations with MS (Allen et al. 1994; Teutsch et al. 1999). The HLA-DR3 (DRB1*0301-DQB1*0201) association with MS described here in African Americans has been demonstrated elsewhere in Sardinian patients (Marrosu et al. 1998) and has been implied in other MS studies of northern European populations as well (Allen et al. 1994). The high-risk HLA-DR2 (DRB1*1501-DQB1*0602) association can also be detected in Sardinian data sets (Marrosu et al. 2001), along with other DRB1-DQB1 haplotypes, emphasizing the complex multiallelic nature of class II involvement in MS. Current understanding of the molecular structure of DRB1*15 alleles, illustrated in the simulated structure comparison shown in figure 1A, also proposes that both *1501 and *1503 have similar functional roles in MS susceptibility. The molecular structures of DRB1*1501/*1503 and DRB1*0301 are significantly divergent in the proximity of the bound peptide, suggesting that they present either a different MBP epitope or a different antigen (fig. 1B).

The 7.3% frequency of the DRB1*1501 allele observed in this African American cohort with MS is low compared with the expected frequency of ~50% in white American populations with MS. On the other hand, it is substantially higher than published frequencies in most control African populations. Genetic admixture is one possible source of DRB1*1501 alleles in African American patients with MS, and some degree of admixture probably accounts for some of the observed DRB1*1501-DQB1*0602 haplotypes in African American patients. However, admixture cannot fully account for this observation, since 16% (8/49) of DRB1*1501 and 12% (18/149) of DRB1*15- (*1501- or *1503-) bearing chromosomes in African Americans were not as-

sociated with *DQB1*0602* alleles in *cis*. The haplotypic features of these *DRB1*1501*-positive chromosomes indicate an older African origin, predating the divergence of human ethnic groups, rather than genetic admixture with people of European descent. Positive selection for *DRB1*1501* appears to have occurred in Europeans but not in Africans, and, although the factors that drove this selection (presumably some infectious pathogen) are unknown, one possible consequence was a heightened susceptibility to MS, a disorder almost nonexistent in Africa (Elian and Dean 1987; Modi et al. 2001). Since *DRB1*1501* and *DRB1*1503* are both present in native Africa, the low prevalence of MS in Africa is likely due to additional protective genetic and/or environmental factors (Todd 1991; Zinkernagel 2001).

The current data underscore the power of ethnically defined cohorts to identify disease genes by association for complex diseases. Here, we have provided strong evidence for the direct involvement of the *HLA-DRB1* gene in MS, as well as the presence of allelic heterogeneity. Taken together, our results indicate that variation at the *DQB1* locus does not play a primary role in disease susceptibility, though a modulating influence on clinical outcome cannot be excluded and warrants further investigation. It is likely that *HLA-DRB1* constitutes the centromeric boundary of the class II *DR-DQ* association in MS, but this will need to be confirmed by additional centromeric genotyping, including analysis of *HLA-DP*. In addition to the potential of this approach for the localization of candidate genes, SNP markers whose frequency differs between Africans and whites/Asians could also be used in a whole-genome strategy to search for new susceptibility or modifier loci (Lautenberg et al. 2000). As the MS genomic map in Northern Europeans is increasingly refined, well-characterized African American MS data sets should prove to be an extraordinarily valuable resource.

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Electronic-Database Information

The URLs for data presented herein are as follows:

- Arlequin's Home on the Web, <http://lgb.unige.ch/arlequin/> (for Arlequin version 2.00)
 David Clayton's Web site, <http://www-gene.cimr.cam.ac.uk/clayton/software/> (for the TRANSMIT program)
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for MS, *DRB1*, and *DQB1*)
 Pedigree Disequilibrium Test Analysis Program, Center for Human Genetics, Duke University Medical Center, <http://wwwchg.mc.duke.edu/software/pdt.html>
 UCSF Chimera Home Page, <http://www.cgl.ucsf.edu/chimera/>

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