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Complex HLA-DR and -DQ Interactions Confer Risk of Narcolepsy-Cataplexy in Three Ethnic Groups

Emmanuel Mignot,[1] Ling Lin,[1] William Rogers,[1] Yutaka Honda[4] Xiaohong Qiu,[1] Xiaoyan Lin,[1] Michele Okun,[1] Hirohiko Hohjoh,[5] Tetsuro Miki,[7] Susan H. Hsu,[8] Mary S. Leffell,[9] F. Carl Grumet,[2] Marcelo Fernandez-Vina,[10] Makoto Honda,[6] and Neil Risch[3]

[1]Center For Narcolepsy, Department of Psychiatry and Behavioral Sciences, [2]Histocompatibility Laboratory, Department of Pathology, and [3]Department of Genetics, Stanford University Medical Center, Palo Alto; [4]Neuropsychiatric Research Institute, Seiwa Hospital, [5]Department of Human Genetics, School of International Health, Graduate School of Medicine, The University of Tokyo, and [6]Tokyo Metropolitan Matsuzawa Hospital, Tokyo; [7]Department of Gerontology, Ehime University School of Medicine, Ehime, Japan; [8]Musser Blood Center, Philadelphia; [9]Institute of Genetic Medicine, School of Medicine, Johns Hopkins University, Baltimore; and [10]National Histocompatibility Laboratory, American Red Cross and Department of Pathology, University of Maryland Medical School, College Park

Human narcolepsy-cataplexy, a sleep disorder associated with a centrally mediated hypocretin (orexin) deficiency, is tightly associated with HLA-DQB1*0602. Few studies have investigated the influence that additional HLA class II alleles have on susceptibility to this disease. In this work, 1,087 control subjects and 420 narcoleptic subjects with cataplexy, from three ethnic groups, were HLA typed, and the effects of HLA-DRB1, -DQA1, and -DQB1 were analyzed. As reported elsewhere, almost all narcoleptic subjects were positive for both HLA-DQA1*0102 and -DQB1*0602. A strong predisposing effect was observed in DQB1*0602 homozygotes, across all ethnic groups. Relative risks for narcolepsy were next calculated for heterozygous DQB1*0602/other HLA class II allelic combinations. Nine HLA class II alleles carried in *trans* with DQB1*0602 were found to influence disease predisposition. Significantly higher relative risks were observed for heterozygote combinations including DQB1*0301, DQA1*06, DRB1*04, DRB1*08, DRB1*11, and DRB1*12. Three alleles—DQB1*0601, DQB1*0501, and DQA1*01 (non-DQA1*0102)—were found to be protective. The genetic contribution of HLA-DQ to narcolepsy susceptibility was also estimated by use of λ statistics. Results indicate that complex HLA-DR and -DQ interactions contribute to the genetic predisposition to human narcolepsy but that additional susceptibility loci are also most likely involved. Together with the recent hypocretin discoveries, these findings are consistent with an immunologically mediated destruction of hypocretin-containing cells in human narcolepsy-cataplexy.

Introduction

Our understanding of the pathophysiology of the sleep disorder narcolepsy (MIM 161400), a disorder associated with HLA-DR and -DQ is rapidly emerging. The gene for an autosomal recessive canine model of the sleep disorder was recently cloned and found to be a receptor for a neuropeptide system, hypocretin/orexin (Lin et al. 1999). This finding was quickly followed by reports that preprohypocretin-knockout mice have symptoms similar to narcolepsy (Chemelli et al. 1999). Finally, human narcolepsy-cataplexy is associated with a loss of hypocretin-containing cells in the perifornical hypothalamus (Peyron et al. 2000; Thannickal et al. 2000) and with resulting undetectable CSF hypocretin-1 levels (Nishino et al. 2000).

In contrast to animal models, human narcolepsy is not a simple genetic disorder (Honda et al. 1983, 1997; Mignot 1998) and does not involve highly penetrant hypocretin-gene mutations (Peyron et al. 2000). Most human cases of narcolepsy are sporadic and carry the HLA haplotype DRB1*1501-DQA1*0102-DQB1*0602 (DR2,DQ1) (Honda et al. 1997; Mignot 1998). Familial cases are the exception rather than the rule, and MZ-twin reports indicate partial (25%–31%) concordance (Mignot 1998), suggesting environmental effects on a specific genetic background.

In spite of the close association that the disease has with HLA, a direct involvement of the immune system in narcolepsy has not yet been demonstrated. All attempts at demonstrating CNS and/or peripheral autoimmunity in narcolepsy have failed (Matsuki et al. 1988; Carlander et al; 1993; Mignot et al. 1995). This has led to the hypothesis that an unknown, nonimmune sleep-control gene mapping in the HLA region is the direct determinant of genetic susceptibility and that the HLA-DR and -DQ

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Address for correspondence and reprints: Dr. E. Mignot, Center For Narcolepsy, Stanford University, Department of Psychiatry and Behavioral Sciences, 1201 Welch Road, P114, Palo Alto, CA 94304-5485. E-mail: mignot@leland.stanford.edu

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associations are secondary (Matsuki et al. 1988; Carlander et al; 1993; Mignot et al. 1995). This hypothesis is now believed to be less likely. In African Americans, narcolepsy is associated with other DRB1-DQB1*0602 haplotypes such as DRB1*1101-DQA1*0102-DQB1*0602 or DRB1*1503-DQA1*0102-DQB1*0602, suggesting that DQA1 and DQB1 play a primary role in susceptibility to the disease (Matsuki et al. 1992; Mignot et al. 1994, 1997*a,* 1997*b;* Pelin et al. 1998). Microsatellite and HLA-haplotype analysis also suggest that HLA-DQB1*0602 has a direct involvement in susceptibility to narcolepsy (Mignot et al. 1997*b*). Finally, extended sequencing in the HLA-DQ region has identified no novel candidate genes (Ellis et al. 1997).

In a recent study, we observed that white American and African American patients homozygous for HLA-DQB1*0602 have twofold-to-fourfold–higher risk compared with that in HLA-DQB1*0602 heterozygotes (Pelin et al. 1998). This observation led us to speculate that HLA alleles other than HLA-DQB1*0602 might have additional effects in influencing the susceptibility to develop narcolepsy. To test this hypothesis, narcoleptic and control subjects from three ethnic groups (African Americans, white Americans, and Japanese) were HLA-DR and -DQ typed, and the effect of HLA alleles other than HLA-DQB1*0602 was analyzed. This study led us to confirm a strong effect of DQB1*0602 homozygosity and to identify other HLA class II alleles as secondary narcolepsy-susceptibility alleles (for sequences of HLA class II alleles, see the IMGT/HLA Sequence Database). We also used the derived relative risks for the various genotypes, to estimate the total recurrence-risk ratio attributable to HLA (Risch 1987).

Subjects and Methods

Subjects

A total of 420 patients and 1,087 control subjects were included in this study. All narcoleptic patients had definite cataplexy, the hallmark of the disorder. Ethnicity was defined as Japanese (both parents of Japanese origin), African Americans (both parents African Americans), or white Americans (both parents white Americans). Japanese narcoleptic patients either were recruited at Seiwa Hospital (by Y.H.; $n = 100$) or were Japanese American patients ($n = 5$) with all four grandparents born in Japan. White American ($n = 238$), Japanese ($n = 5$), and African American ($n = 77$) patients were recruited at Stanford University. Either Y.H. (for all but five Japanese American subjects) or E.M. reviewed all clinical files before the subjects were included in the study. HLA-typed Japanese control subjects ($n = 698$) were kindly provided by Akinori Kimura (Tokyo) and have been described elsewhere (Lin et al. 1997). HLA-typed African American ($n = 243$) and white American ($n = 146$) control subjects were mostly provided by M. S. Leffell (Baltimore) and S. Hsu (Philadelphia), respectively, with a few subjects recruited at Stanford.

HLA Typing

HLA-DRB1 and -DQB1 loci were typed in several laboratories over a period of several years. All narcolepsy samples were HLA-DRB1 typed and HLA-DQB1 typed at Stanford, by either Innotype reverse-dot-blot kits used according to the manufacturer's recommendations (Robbins Scientific, Sunnyvale, CA USA) or a sequence-specific oligonucleotide probe–enzyme-linked immunosorbent assay (SSOP-ELISA) technique (Krishnaswamy et al. 2000). Most control subjects were high-resolution DRB1 typed and DQB1 typed either in Japan or at Stanford, by PCR oligotyping techniques described by Yasunaga et al. (1996). Intermediate- to high-level typing resolution for DRB1 and DQB1 was obtained in all cases. Not all known DRB1 and DQB1 alleles were distinguished by this method, and resolution is reported at the lowest level common to all the techniques used; for example, DQB1*0602 was not distinguished from the rare DQB1*0611 subtype. Japanese control samples were HLA-DQA1 typed in Japan, as described elsewhere (Yasunaga et al. 1996). Intermediate-resolution DQA1 typing was performed on all narcolepsy samples and most control samples, by PCR oligotyping techniques, at the American Red Cross National Histocompatibility Laboratory in Baltimore (Fernandez-Vina et al. 1991).

HLA Class II Haplotype Assignments

Subjects were not phased because of a lack of relatives. Two-locus (DQA1-DQB1) and three-locus (DRB1-DQA1-DQB1) haplotypes were assigned to all subjects on the basis of known associations as described by Fernandez-Vina et al. (1991) and Lin et al. (1997). To do so, (i) it was assumed that the DRB1, DQA1, and DQB1 loci have no blanks, and, on the basis of this assumption, when a single HLA allele was found, the individual was considered to be homozygous for that allele; (ii) in the assignment of haplotypes, priority was given both to combinations known to exist in homozygous B-cell lines or families and, in the analysis of unrelated individuals, to alleles having 100% associations; and (iii) rare associations were accepted when the other complementary haplotypes were well defined (i.e., fitted criteria ii). The frequency of the DRB1-DQA1-DQB1 haplotypes and of the class II alleles of interest was then compared in the control group and the affected group, by χ^2 test.

Analysis of the Effects of HLA Alleles Located in trans *of DQB1*0602*

The goal of this analysis was to determine whether HLA alleles other than DQB1*0602 modulate narcolepsy susceptibility across ethnic groups. This analysis was performed for individual alleles and for two- and three-locus haplotypes. We first compared the relative risks of various DRB1*X-DQA1*0102-DQB1*0602 haplotypes in the three populations. Relative risks were estimated by odds ratios (ORs), with DRB1*15-DQA1*0102-DQB1*0602 homozygotes being used as the referent. This analysis allowed us to expand on the observation that DQB1*0602 is the major susceptibility allele and to confirm that DQB1*0602 homozygosity greatly increases narcolepsy susceptibility across all ethnic groups. For this analysis, we used the Hardy-Weinberg (HW) expected genotype frequencies for the control subjects, rather than the actual observed genotype counts, to obtain greater precision in the OR estimates.

The frequencies of various HLA alleles (DQB1, DQA1, and DRB1) located *trans* to HLA-DQB1*0602 were next compared in narcoleptic subjects versus control subjects. To do this, we calculated ORs for the alleles carried on the non-DQB1*0602 chromosome, separately for the Japanese, white American, and African American samples. The ORs were then summarized into a single estimate across the three ethnicities, by the Mantel-Haenszel (MH) OR estimate, and the significance was determined by an MH χ^2 test.

To assess the heterogeneity of the OR estimates for each allele, we used the MH OR to estimate the number of observations expected to fall within the four cells of each table, given the total number of patients' alleles and of control alleles and the number of alleles of each type (i.e., the 2×2 table margins). This was done for each of the three ethnicities. A χ^2 statistic was then derived for each of these three tables, and the three statistics were summed. The total χ^2 distribution has 2 df (i.e., three independent tables minus one parameter estimate [i.e., OR]). The significance of this homogeneity test was then assessed by comparison of the derived total versus a 2-df χ^2 distribution. This test was performed only when all margin totals for a given table were at least five. In most cases, all three of the populations met this criterion, leading to a homogeneity χ^2 test with 2 df. In a few cases, only two of the three populations met this criterion. In these cases, we performed the goodness-of-fit test on these two populations, with the resulting homogeneity statistics being χ^2 with only 1 df. For those cases in which only one table met the criterion for analysis, no homogeneity test was performed. In total, 26 alleles were subjected to homogeneity testing.

To assess the significance of departure from 1.0, for the estimated MH ORs for each locus, we employed a sequential procedure. At step 1, for a locus with n alleles, we examined the MH χ^2 for each of the n alleles tested. A nominal P value was determined for the maximum χ^2 value. To evaluate the significance of the maximum χ^2, we calculated $P^* = 1 - (1 - P)^n$, which represents the probability that the maximum of n χ^2 statistics has a value as large as or greater than that observed, when the n statistics are assumed to be independent. In this case, the n statistics are modestly negatively correlated, so this derived P value is anticonservative. A more conservative derived P value is the Bonferonni correction, $P_c = nP$, which, in this case, is conservative. However, for small values of P, P^* and P_c are very similar to one another, and we therefore, in general, employed the conservative P_c value.

If an allele was found to be significant (i.e., $P_c < .05$ for the maximum of n alleles), it was removed from the analysis, and the process then was repeated. This procedure was iterated until no more alleles were found to be significant. The importance of doing this stepwise procedure is that, if one allele is strongly associated (positively or negatively) with disease risk, then all other alleles also will appear to deviate from an OR of 1.0 and may be significant as well. This stepwise procedure allowed us to evaluate the effects of alleles after taking into account the background influence of alleles that were more strongly associated.

In a last analysis, the relative risks for various DRB1-DQA1-DQB1 haplotype combinations were calculated. This procedure was performed only for haplotypes carrying the DRB1, DQB1, and DQA1 alleles previously identified, on the basis of the stringent criteria detailed above, as significantly modifying disease susceptibility. The goal of this analysis was to examine the effects of various HLA alleles within the context of each extended haplotype. For this analysis, predisposing haplotypes were ranked by relative risks, compared with reference DRB1*15-DQA1*0102-DQB1*0602 homozygotes in the three ethnic groups.

*Analysis of Patients Who Are Negative for HLA-DQB1*0602*

To assess whether HLA alleles other than DQB1*0602 are associated with narcolepsy in patients without DQB1*0602, we first calculated ORs for the 25 white American non-DQB1*0602 patients (50 chromosomes), comparing them with the same white American controls as had been used previously. For the DQB1*0602 heterozygotes, we evaluated the same alleles for loci DQB1, DQA1, and DRB1 as had been used above, using the same sequential procedure. This led us to estimate ORs for individual HLA class II alleles and to calculate their statistical significance. Second, we tested whether the pattern of ORs for these alleles was similar to that ob-

tained in DQB1*0602 heterozygotes, using the following analysis. For each of the three loci, we calculated the mean and variance of the log ORs across all alleles at that locus, as seen in the DQB1*0602 heterozygotes and in non-DQB1*0602 patients. The log ORs were used because these are more normally distributed than are the untransformed ORs. We then estimated whether the pattern of ORs for all alleles was correlated between the non-DQB1*0602 white Americans and three different DQB1*0602-heterozygote groups: white Americans, all three ethnic groups combined, and Japanese and African Americans combined. For the latter two groups, MH ORs were used. The comparisons with the nonwhite groups were performed to exclude the possibility of a spurious correlation due to differential allele frequencies in white American control subjects and narcoleptic subjects—which could result, for example, from differential recruitments within the United States. Finally, to analyze the three loci together, we also calculated a single correlation coefficient combining the log ORs for all three loci together, as a single group. The significance of the estimated correlation ρ versus the hypothesis H_0: $\rho = 0$ was tested by creating the Fisher Z-transform: $Z = \frac{1}{2}[log(1 + r) - log(1 - r)]$, where r is the estimated correlation, and the standard error of Z is given by $1/\sqrt{n - 3}$, where n is the number of observations (alleles). In this case, because only $n - 1$ of the n ORs at a given locus are independent, we used the formula $1/\sqrt{n - 4}$ for the standard error when there were n alleles.

Estimation of the Genetic Contribution of DQ to Narcolepsy Susceptibility

The main effect observed in this study was that of HLA-DQB1. We thus estimated λ for HLA-DQ in the three ethnic groups, using established DQB1 allele frequencies and MH OR estimates across the three ethnic groups. Alleles included in the calculations were DQB1*0602 and all other significant DQB1 alleles in heterozygote combinations. Calculations were performed as described by Risch (1987).

Results

*HLA-DQB1*0602: The Major Narcolepsy-Susceptibility Allele in Three Ethnic Groups*

HLA-DRB1, -DQA1, and -DQB1 allele frequencies are reported in table 1. As previously reported, DRB1*15, DQA1*0102, and DQB1*0602 frequencies were significantly increased in narcolepsy, across all three ethnic groups. DRB1*11 was also significantly increased in African Americans. DRB1*15 and DQB1*0602 were both similarly associated with narcolepsy in Japanese and white American subjects. In African Americans, DQB1*0602 was more strongly associated with narcolepsy than was HLA-DRB1*15.

*Predisposing Effects of Various HLA–DRB1*X-DQA1*0102-DQB1*0602 Haplotypes*

Relative risks for various DRB1-DQA1-DQB1*0602 haplotypes are reported in table 2. The major goal of this analysis was to compare relative risks for DRB1*11-DQA1*0102-DQB1*0602, a classical African haplotype, with that of DRB1*15-DQA1*0102-DQB1*0602. In this analysis, among African Americans both haplotypes were found to predispose similarly to narcolepsy, but an approximately twofold-higher relative risk was observed for DRB1*11-associated haplotypes in heterozygotes (OR = 2.1; $\chi^2 = 2.96$, $P = .08$) and in homozygotes (not significant). The rare haplotypes DRB1*03-DQA1*0102-DQB1*0602, DRB1*08-DQA1*0102-DQB1*0602, and DRB1*12-DQA1*0102-DQB1*0602 were also found in several narcoleptic patients. As had been reported in previous studies of white Americans and African Americans, DQB1*0602 homozygosity (Pelin et al. 1998) was also found to dramatically increase relative risk, independently of HLA-DRB1. The effect was most striking in Japanese subjects, in whom DRB1*1501-DQA1*0102-DQB1*0602 homozygotes represented 15.2% of the narcolepsy population, compared with 0.4% in the general population, for this haplotype. ORs for DRB1*1501-DQA1*0102-DQB1*0602 heterozygotes versus homozygotes were ~0.2 (table 2), across all three ethnic groups.

*Analysis of HLA-DQB1*0602 Heterozygotes*

ORs calculated for non-DQB1*0602 alleles located *trans* to the predisposing DQB1*0602 haplotype are reported in tables 3–5. At locus DQB1, three alleles were deemed significant (table 3). The allele with the strongest effect was DQB1*0301, with an MH OR of 2.09 ($\chi^2 = 23.86$, $P_c = .000013$). DQB1*0602/DQB1*0301 was found to confer increased risk of development of narcolepsy, across all three ethnic groups. The allele with the next-strongest effect was DQB1*0601, which had an OR of 0.34 ($\chi^2 = 8.65$, $P_c = .04$; this value was obtained after DQB1*0301 was removed from the analysis). Note that DQB1*0601 was found at high frequency primarily in the Japanese population. DQB1*0601/DQB1*0602 heterozygotes were thus found to confer decreased risk of development of narcolepsy. The last allele indicating significance was DQB1*0501, with an OR of 0.51 ($\chi^2 = 7.07$, $P_c = .08$). Although this test showed only borderline significance after correction, at this stage of analysis there were three alleles giving nominal P values <.05, whereas only 11(.05) = .55 would be expected. Thus, it is likely that this allele is also negatively associated with narcolepsy.

Table 1

HLA-DRB1*, -DQA1*, and -DQB1* Allele Frequencies in Three Ethnic Groups

	No. (%) in						
	Japanese		White Americans		African Americans		MH χ^2 for All Three Groups
Allele(s)	Narcoleptic	Control	Narcoleptic	Control	Narcoleptic	Control	
Total	210	1,396	476	292	154	486	
DRB1*:							
01	0 (.00)	100 (7.16)	17 (3.57)	30 (10.27)	2 (1.30)	34 (7.00)	37.21††††
03	0 (.00)	7 (.50)	24 (5.04)	43 (14.73)	11 (7.14)	54 (11.11)	20.56††††
04	32 (15.24)	328 (23.50)	48 (10.08)	28 (9.59)	3 (1.95)	31 (6.38)	6.96††
07	0 (.00)	3 (.21)	39 (8.19)	42 (14.38)	8 (5.19)	58 (11.93)	13.42†††
08	9 (4.29)	182 (13.04)	12 (2.52)	6 (2.05)	9 (5.84)	24 (4.94)	8.62††
09	9 (4.29)	214 (15.33)	0 (.00)	2 (.68)	3 (1.95)	22 (4.53)	22.32††††
10	0 (.00)	5 (.36)	2 (.42)	6 (2.05)	0 (.00)	10 (2.06)	8.55††
11	7 (3.33)	29 (2.08)	32 (6.72)	30 (10.27)	34 (22.08)	58 (11.93)	
12	12 (5.71)	61 (4.37)	5 (1.05)	3 (1.03)	8 (5.19)	21 (4.32)	
13	10 (4.76)	107 (7.66)	33 (6.93)	42 (14.38)	14 (9.09)	80 (16.46)	17.3†††
14	4 (1.90)	91 (6.52)	5 (1.05)	13 (4.45)	1 (.65)	11 (2.26)	16.46†††
15	126 (60.00)	261 (18.70)	256 (53.78)	41 (14.04)	60 (38.96)	75 (15.43)	318.80†††††
16	1 (.48)	8 (.57)	3 (.63)	6 (2.05)	1 (.65)	8 (1.65)	4.69†
DQA1*:							
01	13 (6.19)	462 (33.09)	26 (5.46)	65 (22.26)	8 (5.19)	105 (21.60)	131.65†††††
0102	130 (61.90)	199 (14.26)	280 (58.82)	71 (24.32)	99 (64.29)	129 (26.54)	368.86†††††
02	0 (.00)	3 (.21)	39 (8.19)	42 (14.38)	12 (7.79)	58 (11.93)	9.21††
03	45 (21.43)	576 (41.26)	51 (10.71)	28 (9.59)	6 (3.90)	66 (13.58)	28.85†††††
0401	3 (1.43)	23 (1.65)	9 (1.89)	5 (1.71)	5 (3.25)	39 (8.02)	
05	12 (5.71)	115 (8.24)	71 (14.92)	80 (27.40)	24 (15.58)	88 (18.11)	15.44†††
0601	7 (3.33)	18 (1.29)	0 (.00)	1 (.34)	0 (.00)	1 (.21)	
DQB1*:							
02	0 (.00)	10 (.72)	53 (11.13)	75 (25.68)	23 (14.94)	108 (22.22)	28.83†††††
0301	21 (10.00)	145 (10.39)	71 (14.92)	48 (16.44)	17 (11.04)	85 (17.49)	
0302	11 (5.24)	131 (9.38)	27 (5.67)	16 (5.48)	3 (1.95)	23 (4.73)	
0303	10 (4.76)	220 (15.76)	8 (1.68)	12 (4.11)	0 (.00)	8 (1.65)	24.49††††
0304	0 (.00)	0 (.00)	1 (.21)	0 (.00)	0 (.00)	0 (.00)	
0401	20 (9.52)	187 (13.40)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	
0402	5 (2.38)	42 (3.01)	10 (2.10)	5 (1.71)	4 (2.60)	28 (5.76)	
0501	2 (.95)	105 (7.52)	19 (3.99)	36 (12.33)	7 (4.55)	76 (15.64)	43.80†††††
0502	2 (.95)	27 (1.93)	4 (.84)	6 (2.05)	1 (.65)	12 (2.47)	4.74†
0503	2 (.95)	36 (2.58)	2 (.42)	13 (4.45)	1 (.65)	11 (2.26)	15.00†††
0601	8 (3.81)	297 (21.28)	0 (.00)	4 (1.37)	0 (.00)	4 (.82)	41.92†††††
0602	121 (57.62)	90 (6.45)	255 (53.57)	37 (12.67)	91 (59.09)	89 (18.31)	530.53†††††
0603	0 (.00)	7 (.50)	5 (1.05)	15 (5.14)	2 (1.30)	19 (3.91)	14.18†††
0604	7 (3.33)	91 (6.52)	19 (3.99)	20 (6.85)	3 (1.95)	15 (3.09)	6.77††
0605 / 0609	1 (.48)	8 (.57)	2 (.42)	5 (1.71)	2 (1.30)	8 (1.65)	

† $P < .05$.
†† $P < .01$.
††† $P < .001$.
†††† $P < .0001$.
††††† $P < .000001$.

DQA1 ORs calculated for the three ethnic groups are reported in table 4. The strongest effect, a protective effect, was observed for allele DQA1*01, a broad specificity encompassing all DQA1*01 alleles except DQA1*0102 (e.g., alleles DQA1*0101, DQA1*0103, DQA1*0104, and DQA1*0105). The OR obtained was 0.33 ($\chi^2 = 36.32$, $P_c = .00001$). After allele DQA1*01 was removed from the analysis, DQA1*06, primarily a Japanese allele, was found to be significant, with an OR of 3.23 ($\chi^2 = 8.39$, $P_c = .02$). No other DQA1 alleles were found to be significant.

DRB1 ORs calculated for the three ethnic groups are reported in table 5. The most significant allele was DRB1*04, with an OR of 1.92 ($\chi^2 = 14.49$, $P_c = .007$). After DRB1*04 was removed from the analysis, allele DRB1*12 was found to be significant, with an OR of 2.84 ($\chi^2 = 15.30$, $P_c = .0008$). The next significant allele was DRB1*11, with an OR of 2.00 ($\chi^2 = 11.39$,

Table 2

ORs for Various DRB1*X-DQA1*0102-DQB1*0602 Combinations in Three Ethnic Groups

	Japanese				White Americans				African Americans				All Three Groups	
	No. (%) in				No. (%) in				No. (%) in					
Haplotype 1 / haplotype 2	Narcoleptic (n = 105)	Control (n = 698)	HW Adjusted (n = 698)	OR	Narcoleptic (n = 238)	Control (n = 146)	HW Adjusted (n = 146)	OR	Narcoleptic (n = 77)	Control (n = 243)	HW Adjusted (n = 243)	OR	MH OR	MH χ^2
15-0102-0602 / 15-0102-0602	16 (15.2)	5 (.7)	3 (.4)	1.00	43 (18.1)	3 (2.1)	2 (1.6)	1.00	11 (14.3)	8 (3.3)	4 (1.8)	1.00	1.00	Reference
15-0102-0602 / any haplotype	89 (84.8)	80 (11.5)	84 (12.1)	.20††	169 (71.0)	31 (21.2)	32 (21.1)	.25†	31 (40.3)	47 (19.3)	53 (21.9)	.21††	.22	17.73†††
03-01-0602 / 15-0102-0602									0	1 (.4)	0 (.1)	.00		
03-01-0602 / any haplotype									0	0	1 (.3)	.00		
03-0102-0602 / any haplotype					1 (.4)	0	0	∞	0	1 (.4)	1 (.3)	.00		
08-0102-0602 / 15-0102-0602									1 (1.3)	0 (.0)	0 (.1)	∞		
08-0102-0602 / any haplotype									3 (3.9)	1 (.4)	1 (.3)	1.09		
11-0102-0602 / 11-0102-0602									2 (2.6)	1 (.4)	0 (.1)	∞		
11-0102-0602 / 15-0102-0602									5 (6.5)	0	2 (.9)	.91		
11-0102-0602 / any haplotype									16 (20.8)	14 (5.8)	13 (5.4)	.45		
12-0102-0602 / any haplotype									2 (2.6)	0	0	∞		
13-01-0602 / any haplotype									0	1 (.4)	2 (1.0)	.00		
13-0102-0602 / any haplotype									0	1 (.4)	1 (.3)	.00		
15-02-0602 / any haplotype									0	1 (.4)	1 (.3)	.00		
16-0102-0602 / any haplotype									1 (1.3)	0	0	∞		
Any / any	0	613 (87.8)	611 (87.5)	.00	25 (10.5)	112 (76.7)	111 (76.3)	.01	5 (6.5)	167 (68.7)	162 (66.7)	.01†††††	.01	275.75†††††
Rare 0102-0602 combinations									0	0	1 (.5)	.00		

† $P < .05$.
†† $P < .01$.
††† $P < .001$.
††††† $P < .000001$.

Table 3

ORs, Mantel-Haenszel ORs, and χ^2 for DQB1 Alleles in Three Ethnic Groups

	Japanese			White Americans			African Americans			All Three Groups	
	No.			No.			No.				
DQB1 Allele	Narcoleptic (n = 89)	Control (n = 1,306)	OR	Narcoleptic (n = 170)	Control (n = 255)	OR	Narcoleptic (n = 53)	Control (n = 397)	OR	MH OR	MH χ^2
02	0	10	.00	39	75	.71	17	108	1.26	.85	.82
0301	21	145	2.48†††	55	48	2.06††	17	85	1.73	2.09	23.86††††
0302	11	131	1.26	22	16	2.22†	2	23	.64	1.50	3.30
0303	10	220	.62	8	12	1.00	0	8	.00	.69	1.87
0401	20	187	1.73†	0	0	...	0	0	...	1.73	4.38†
0402	5	42	1.79	8	5	2.47	3	28	.79	1.55	1.97
0501	2	105	.26†	13	36	.50†	6	76	.54	.46	9.76††
0502	2	27	1.09	4	6	1.00	0	12	.00	.77	.30
0503	2	36	.81	1	13	.11†	1	11	.67	.37	4.42†
0601	8	297	.33††	0	4	.00	0	4	.00	.30	11.73†††
0603	0	7	.00	3	15	.29†	2	19	.78	.40	3.94†
0604	7	91	1.14	15	20	1.14	3	15	1.53	1.18	.46
0609	1	8	1.84	2	5	.60	2	8	1.91	1.13	.06

† $P < .05$.
†† $P < .01$.
††† $P < .001$.
†††† $P < .0001$.

P_c = .06). The last significant allele was DRB1*08, with an OR of 2.17 (χ^2 = 7.42, P_c = .046). In this case, all four significant alleles were predisposing.

We also tested whether any alleles demonstrated nominally significant ($P < .05$) heterogeneity among the three ethnicities. Only one allele, DQA1*03, demonstrated such an effect, with ORs of 1.39. 2.89, and 0.42 in Japanese, white Americans, and African Americans, respectively. The χ^2 for this allele was 11.87, which corresponds to P = .005; however, given the fact that 26 such tests were performed, it is not so unlikely that one case with a P value at this level will be observed. Thus, overall, we have little evidence of heterogeneity across populations, for the derived ORs.

In conclusion, a total of nine alleles were found to be significantly associated with either increased or decreased risk of narcolepsy in DQB1*0602 heterozygotes. At this stage, we also recalculated the ORs for all significant alleles at that locus, using, as the reference, all nonsignificant alleles combined. The final ORs for each of the nine alleles in this analysis are given in table 6. Six of the nine alleles are predisposing, and three are protective.

Predisposing Effects of Various Three-Locus Haplotypes When Located trans *to a DQA1*0102-DQB1*0602 Haplotype*

To examine in more detail the pattern of linkage disequilibrium for the identified susceptibility alleles, we next examined relative risks for various DRB1-DQA1-DQB1 haplotype combinations (table 7) carrying these alleles. For this analysis, predisposing haplotypes were ranked by relative risks versus reference DRB1*15-DQA1*0102-DQB1*0602 homozygotes in the three ethnic groups. The analysis displayed in table 7 included only those haplotype combinations with a frequency >1.3% in either narcoleptic subjects or HW-derived control subjects.

In Japanese subjects, the most significant effect was observed in DRB1*15-DQA1*0102-DQB1*0602/DRB1*12-DQA1*06-DQB1*0301. The effect of this Asian-specific haplotype could not be verified in other ethnic groups. Besides effects associated with DQB1*0602 homozygosity, other significant risk increases were observed for DRB1*11-, DRB1*12-, DQB1*0301-, DRB1*08-, and DRB1*04-carrying haplotypes, in agreement with the analysis performed for individual loci. It is noteworthy that several DRB1*04 haplotypes with distinct DQB1 alleles ranked high in susceptibility, suggesting a primary effect of DRB1*04 in some cases. Similar findings were observed for DRB1*08. In contrast, it was more difficult to differentiate DRB1*11/DRB1*12 effects from a potential DQB1*0301 effect; these alleles are almost exclusively associated. The least-susceptible combinations were those including DQB1*0601 and DQB1*0501. The protective effect of DQB1*0601 is remarkable, since this allele is frequently associated with DRB1*15 (table 3). It is noteworthy that the DRB1*08-DQA1*01-DQB1*0601 was also protective, suggesting a primary effect of DQB1*0601.

In white Americans, similar effects were observed,

Table 4

ORs, Mantel-Haenszel ORs, and χ^2 for DQA1 Alleles in Three Ethnic Groups

	Japanese			White Americans			African Americans			All Three Groups	
	No.			No.			No.				
DQB1 Allele	Narcoleptic (n = 80)	Control (n = 1,197)	OR	Narcoleptic (n = 149)	Control (n = 221)	OR	Narcoleptic (n = 46)	Control (n = 354)	OR	MH OR	MH χ^2
01	13	462	.31†††	17	65	.31†††	7	105	.43†	.33	36.32†††††
02	0	3	.00	28	42	.99	9	58	1.24	1.05	.04
03	45	576	1.39	44	28	2.89†††	4	66	.42	1.56	7.53††
04	3	23	1.99	7	5	2.13	4	39	.77	1.35	.82
05	12	115	1.66	53	80	.97	22	88	2.77††	1.40	4.65†
06	7	18	6.28††††	0	1	.00	0	1	.00	4.18	13.56†††

† $P < .05$.
†† $P < .01$.
††† $P < .001$.
†††† $P < .0001$.
††††† $P < .000001$.

with DRB1*08-, DRB1*04-, DRB1*11-, DQA1*0501-, and DQB1*0301-carrying haplotypes increasing susceptibility, whereas the DRB1*01-DQA1*01-DQB1*0501 haplotype was rather protective. Results were more difficult to interpret in African Americans, because of both the smaller sample size and the increased HLA-haplotype diversity. Consistent with the results obtained in other ethnic groups, DRB1*11- and DQB1*0301-carrying haplotypes were generally associated with increased susceptibility, whereas DRB1*01-DQA1*01-DQB1*0501 reduced relative risks. DRB1*13-associated haplotypes were also overrepresented. It is noteworthy that ORs for DRB1*04-DQA1*03-DQB1*0302 did not rank high in this ethnic group.

*Analysis of White American Patients without HLA-DQB1*0602*

ORs for the 25 white American patients were estimated and compared with those in the white American control subjects. Other ethnic groups were not studied, because of the small numbers of non-DQB1*0602 subjects. Results are reported in table 8. Interestingly, DQB1*0301 was marginally increased in this group (OR 1.6; $\chi^2 = 4.38$, $P = .04$), but none of the 25 tested alleles met criteria for significance after adjustment for multiple comparisons. The patterns of ORs reported in table 8 and in tables 3–5 for, respectively, loci DQB1, DQA1, and DRB1, were also strikingly similar; for example, at DQB1, of the four alleles that have ORs >1 in table 8, three have ORs >1 in table 3; similarly, of the four alleles that have ORs <1 in table 8, all four have ORs <1 in table 3. Similarly, extremely concordant patterns were observed for loci DQA1 and DRB1.

The concordance was supported by correlation analysis of the log ORs (table 9). When the white American DQB1*0602 heterozygotes were used for comparison, the correlations for each of the three loci were .57-.82 and .61 for all three loci combined ($P < .01$). Because of the potential for an artifactual correlation due to use of the same control group for calculation of the ORs for the white American DQB1*0602 heterozygotes and non-DQB1*0602 cases, we also calculated the correlation in log ORs, with the MH average of the Japanese and African American ORs as a second comparison; here again the correlations were quite positive, with the corresponding values being .46–.50 and .48, respectively. Thus, the observed correlation in log ORs is unlikely to be an artifact of the comparison with the white American population. Interestingly, the highest correlation was obtained with the MH OR DQB1*0602 heterozygote average for all three ethnic groups, in which the corresponding values were .66–.73 and .67, respectively. This likely reflects the greater precision obtained in the OR estimates when the larger sample of combined ethnicities is used.

Genetic Contribution of HLA-DR and -DQ to Narcolepsy Susceptibility

λ Values (λ_S and λ_O) for HLA-DQ effects were calculated in the three ethnic groups. Only the effects of the major susceptibility alleles—DQB1*0301, DQB1*0501, DQB1*0601, and DQB1*0602—were integrated in the calculation. For this calculation, ORs were assumed to be the same in all three ethnic groups (OR for DQB1*0602/DQB1*0602 = 1.00, OR for DQB1*0602/DQB1*0301 = 0.449, OR for DQB1*0602/DQB1*0501 = 0.134, OR for DQB1*0602/DQB1*0601 = 0.066, and OR for DQB1*0602/DQB1*X = 0.265, with X being any DQB1 allele other than DQB1*0602, DQB1*0301, DQB1*0501, or DQB1*0601, and, for DQB1*Y/DQB1*Y = 0.012, with Y being any DQB1 allele other

Table 5

ORs, Mantel-Haenszel ORs, and χ^2 for DRB1 Alleles in Three Ethnic Groups

	Japanese			White Americans			African Americans			All Three Groups	
	No.			No.			No.				
DQB1 Allele	Narcoleptic (n = 73)	Control (n = 1,122)	OR	Narcoleptic (n = 165)	Control (n = 239)	OR	Narcoleptic (n = 54)	Control (n = 393)	OR	MH OR	MH χ^2
01	2	100	.29	12	30	.55	1	34	.20	.41	8.88††
03	0	7	.00	19	43	.59	7	54	.93	.67	2.71
04	32	328	1.89††	42	28	2.57†††	2	31	.45	1.92	14.49†††
07	0	3	.00	28	42	.96	6	58	.72	.88	.31
08	9	182	.73	8	6	1.98	5	24	1.57	1.08	.08
09	9	214	.60	0	2	.00	2	22	.65	.57	3.02
11	7	29	4.00†††	27	30	1.36	15	58	2.22†	1.87	10.61††
12	12	61	3.42†††	2	3	.97	5	21	1.81	2.41	11.32†††
13	8	107	1.17	25	42	.84	11	80	1.00	.95	.07
14	4	91	.66	2	13	.21†	0	11	.00	.39	5.45†

† $P < .05$.
†† $P < .01$.
††† $P < .001$.

than DQB1*0602). The effect of HLA-DQ was λ_S = 3.54 and λ_O = 3.18 in Japanese, λ_S = 2.70 and λ_O = 2.56 in white Americans, and λ_S = 2.35 and λ_O = 2.23 in African Americans. Most of the effect was due to DQB1*0602, with other alleles contributing little to the λ values. The potential additional effects of DRB1 and DQA1 were not computed, since they would result in only very small changes in λ values once the DQB1 effects were taken into account.

Discussion

In this study, we have demonstrated for the first time that HLA class II alleles other than DQB1*0602 influence susceptibility to narcolepsy-cataplexy. We also have extended what previously had been observed in African Americans and white Americans, by demonstrating that HLA-DQB1*0602 homozygosity increases susceptibility in the Japanese population; in fact, the relative risk in DQB1*0602 homozygotes is extremely high in this population, with 15.2% of patients versus 0.4% of control subjects having this genotype. Homozygosity in this ethnic group increases the risk of development of narcolepsy approximately fivefold, compared with the risk in heterozygotes (OR 0.2; see table 1). In light of the high prevalence (0.16%) reported for narcolepsy in Japanese, as many as 6% of DQB1*0602 homozygotes in Japan could have narcolepsy-cataplexy (in whites, this figure is only 0.6%). This observation is surprising, since previous studies had reported no increased risk of DR2 homozygosity in Japanese subjects (Matsuki et al. 1985; Thomson 1985). Our work now indicates that this effect was not apparent because the various Asian DR2 haplotypes have dramatically different risks for predisposition to narcolepsy. In Japanese, the most frequent DR2 haplotype is the DRB1*1502-DQA1*0103-DQB1*0601, a haplotype that we here found to be protective. Low-resolution DR2 homozygotes in this ethnic group thus included both DRB1*1501-DQA1*0102-DQB1*0602 homozygotes at increased risk and DRB1*1502-DQA1*0103-DQB1*0601, a combination reduced in frequency, with little effect on overall DR2 homozygosity in narcolepsy.

The observation that DQB1*0602 homozygosity increases susceptibility to narcolepsy led us to explore whether other HLA class II alleles also modulate relative risks. We first evaluated the effect that various DR alleles have on a common DQ background, by comparing relative risks for known DRB1*X-DQA1*0102-

Table 6

Mantel-Haenszel Final ORs for Alleles Found to Be Significant, Relative to All Other Alleles Grouped Together

Allele	MH OR for All Three Groups
DQB1:	
0301	1.80
0501	.51
0601	.31
All others	1.00
DQA1:	
01	.34
06[a]	3.23
All others	1.00
DRB1:	
04	2.87
08	2.17
11	2.42
12	3.84
All others	1.00

[a] Found only in Japanese.

Table 7

DR15-DQA1*0102-DQB1*0602 Haplotype Combinations in Three Ethnic Groups

Haplotype 1 / haplotype 2	No. (%)			MH OR	Relative Risk[a]
	Narcoleptic	Control	HW		
	Japanese				
	(n = 105)	(n = 698)	(n = 698)		
15-0102-0602 / 12-0601-0301	7 (6.7)	1 (.1)	1 (.2)	41.5††††	1.094
15-0102-0602 / 15-0102-0602	16 (15.2)	5 (.7)	3 (.4)	43.1††††	1.000
15-0102-0602 / 11-05-0301	7 (6.7)	0	2 (.2)	31.1††††	.788
15-0102-0602 / 08-0401-0402	3 (2.9)	1 (.1)	1 (.1)	20.5††	.563
15-0102-0602 / 12-05-0301	4 (3.8)	5 (.7)	2 (.3)	12.5††	.331
15-0102-0102-0602 / 08-03-0302	3 (2.9)	1 (.1)	2 (.2)	12.0††	.325
15-0102-0602 / 04-03-0401	20 (19.0)	11 (1.6)	12 (1.7)	13.5††††	.301
15-0102-0602 / 04-03-0301	2 (1.9)	0	1 (.2)	9.7	.256
15-0102-0602 / 04-03-0302	8 (7.6)	5 (.7)	6 (.8)	9.6††††	.247
15-0102-0602 / 04-03-0402	2 (1.9)	2 (.3)	2 (.2)	8.0	.208
15-0102-0602 / 14-01-0503	2 (1.9)	1 (.1)	2 (.3)	5.9	.156
Other 0102-0602 combinations	21 (20.0)	33 (4.7)	27 (3.9)	6.2††††	.135
15-0102-0602 / 15-01-0601	5 (4.8)	3 (.4)	10 (1.5)	3.3	.088
15-0102-0602 / 08-01-0601	3 (2.9)	12 (1.7)	9 (1.2)	2.4	.063
15-0102-0602 / 01-01-0501	2 (1.9)	5 (.7)	6 (.9)	2.1	.056
Non–0102-0602 / non–0102-0602	0	613 (87.8)	611 (87.5)	.0††††	.000
	White Americans				
	(n = 238)	(n = 146)	(n = 146)		
15-0102-0602 / 08-04-0402	7 (2.9)	0	0	∞†	∞
15-0102-0602 / 15-0102-0602	43 (18.1)	3 (2.1)	2 (1.6)	13.8††††	1.000
15-0102-0602 / 04-03-0301	18 (7.6)	0	1 (.9)	8.7††	.774
15-0102-0602 / 13-05-0301	5 (2.1)	0	0 (.3)	7.8	.717
15-0102-0602 / 04-03-0302	22 (9.2)	5 (3.4)	2 (1.4)	7.3††	.592
15-0102-0602 / 11-05-0301	26 (10.9)	4 (2.7)	4 (2.5)	4.7††	.386
15-0102-0602 / 01-01-0501	12 (5.0)	2 (1.4)	4 (2.6)	2.0	.170
Other 0102-0602 combinations	80 (33.6)	20 (13.7)	22 (15.1)	2.9†††	.197
Non–0102-0602 / non–0102-0602	25 (10.5)	112 (76.7)	111 (76.0)	.0††††	.012
	African Americans				
	(n = 77)	(n = 243)	(n = 243)		
11-0102-0602 / 11-0102-0602	2 (2.6)	1 (.4)	0 (.1)	21.6	3.001
11-0102-0602 / 13-05-0301	2 (2.6)	1 (.4)	0 (.2)	12.9	1.600
11-0102-0602 / 11-05-0301	3 (3.9)	1 (.4)	1 (.5)	8.2	1.029
15-0102-0602 / 15-0102-0602	11 (14.3)	8 (3.3)	4 (1.8)	9.3††††	1.000
15-0102-0602 / 11-0102-0602	5 (6.5)	0	2 (.9)	8.0†	.923
15-0102-0602 / 13-0102-0501	2 (2.6)	0	1 (.4)	7.2	.844
15-0102-0602 / 08-05-0301	2 (2.6)	0	1 (.5)	5.4	.657
15-0102-0602 / 11-05-0301	5 (6.5)	6 (2.5)	5 (1.9)	3.5	.422
15-0102-0602 / 12-01-0501	2 (2.6)	2 (.8)	3 (1.0)	2.6	.311
15-0102-0602 / 04-03-0302	2 (2.6)	4 (1.6)	3 (1.2)	2.2	.269
Other 0102-0602 combinations	35 (45.5)	52 (21.4)	57 (23.5)	2.7†††	.244
15-0102-0602 / 01-01-0501	1 (1.3)	1 (.4)	5 (1.9)	.7	.087
Non–0102-0602 / non–0102-0602	5 (6.5)	167 (68.7)	161 (66.3)	.0††††	.012

[a] Versus 15-0102-0602 homozygosity.

† $P < .05$.

†† $P < .01$.

††† $P < .001$.

†††† $P < .0001$.

††††† $P < .000001$.

Table 8

ORs for 25 White American Patients without the DQB1*0602 Allele

Allele	No. Narcoleptic	OR
DQB1:		
02	14	.89
0301	14	1.60
0302	5	1.60
0303	0	.00
0402	2	2.01
0501	6	.80
0502	2	1.67
0503	1	.37
0603	2	.64
0604	4	.98
Total	50	
DQA1:		
01	9	.61
02	11	1.41
03	7	1.30
04	2	2.05
05	15	.91
Total	44	
DRB1:		
01	5	.81
03	4	.42
04	6	1.08
07	11	1.39
08	3	2.59
10	1	.83
11	5	.81
12	3	5.23
13	8	.94
14	1	.37
16	3	2.59
Total	50	

DQB1*0602 haplotypes in African Americans (table 2). This analysis indicated that, although all known DRB1*X-DQA1*0102-DQB1*0602 haplotypes predispose to narcolepsy, DRB1 may also influence predisposition. Combinations involving DRB1*11-DQA1*0102-DQB1*0602 haplotypes, for example, generally carried higher relative risks than did those involving the classical DRB1*15-DQA1*0102-DQB1*0602 haplotype (table 1), although these results are only suggestive and not statistically significant.

We next explored the effects that various DQB1, DRB1, and DQA1 alleles located *trans* to the DQB1*0602 haplotype have in heterozygotes. The analysis was performed by first removing, sequentially, the most significant allele for each locus and then rerunning the analysis on residual frequencies until no alleles remained significant. The conservative Bonferonni correction was applied to assess statistical significance. With this procedure, a total of nine alleles were found to be significant (table 6).

Because of linkage disequilibrium between these alleles, we next explored the effect that various DRB1-DQA1-DQB1 haplotypes have when located *trans* to a DQB1*0602 haplotype. This analysis included only haplotypes containing the nine significant alleles. The respective frequencies of each heterozygote combination was ranked by relative risk, with DRB1*15-DQA1*0102-DQB1*0602 homozygotes used as the reference (table 7). The most consistent effect was again the increased risk for DQB1*0301. In all three ethnic groups, all DQB1*0301-associated haplotypes ranked high in relative risks. The DQB1*0301 increase occurred in the context of various HLA haplotypes, such as DRB1*11-DQA1*05-DQB1*0301, DRB1*12-DQA1*06-DQB1*0301, DRB1*12-DQA1*05-DQB1*0301, and DRB1*04-DQA1*03-DQB1*0301, suggesting a primary DQB1*0301 effect. Further analysis made it difficult, however, to conclude that all of the effect is mediated by the DQB1 locus. DRB1*04 and DQB1*08, in particular, may also have strong effects on susceptibility to narcolepsy. Relative risks for DRB1*04-DQA1*03-DQB1*0301 and DRB1*04-DQA1*03-DQB1*0302 were almost identical in white Americans, suggesting an effect due to DRB1*0401 (which is the typical white DRB1*04). Increased predisposition was also observed with the classical Japanese DRB1*04-DQA1*03-DQB1*0401 haplotype, possibly as a result of an association with DRB1*0405 and DRB1*0410. A very robust effect of DRB1*04 was thus observed in white Americans and Japanese but not in African Americans (table 5), a result that may reflect increased DRB1*04-DQB1*03 high-resolution allelic and haplotype diversity in this ethnic group. Similarly, our DRB1 haplotype analysis suggests the possibility that other HLA-DR alleles, such as DRB1*11 or DRB1*12, could have predisposing effects, but, in these cases, it was impossible to identify whether association was primarily with DR or with DQB1*0301. DRB1*11

Table 9

Correlations of Log ORs between White American Patients without DQB1*0602 and DQB1*0602 Heterozygotes

	Correlation of Log ORs for Heterozygous Group (ρ)		
Locus	White Americans	Japanese and African Americans[a]	Three Groups[a]
DQB1	.62	.50	.67[†]
DQA1	.82	.48	.73
DRB1	.57	.46	.66[†]
All three loci	.61[††]	.48[†]	.67[†††]

[a] MH OR estimates were used.
[†] $P < .05$.
[††] $P < .01$.
[†††] $P < .001$.

and DRB1*12 are almost completely associated with DQB1*0301.

Our initial hypothesis was that our previous report of increased DQB1*0602 homozygosity in narcolepsy could be secondary to the protective effects of other frequent DR-DQ haplotypes. This hypothesis was largely disproved by the present analysis; rather, we identified additional predisposing haplotypes. It is noteworthy, however, that two DQB1 alleles, DQB1*0601 and DQB1*0501, were found to be protective against narcolepsy in the presence of DQB1*0602 in *trans* position. The DQB1*0501 effect was observed across all three ethnic groups but was difficult to distinguish from that of the frequently associated DRB1*01 allele. In contrast, the effect of DQB1*0601 was particularly convincing, since it was observed in the context of two frequent DR-DQ Japanese haplotypes, DRB1*08-DQA1*01-DQB1*0601 (presumably DRB1*0803-DQA1*01-DQB1*0601) and DRB1*15-DQA1*01-DQB1*0601 (presumably DRB1*1502-DQA1*0103-DQB1*0601). It is interesting to note that DQB1*0602 and DQB1*0601 are related subtypes but that one is predisposing whereas the other is protective. This suggests that there are narcolepsy-relevant differences in the peptide-binding motifs of these two related DQB1*06 subtypes.

Our analysis indicates complex HLA-DR and -DQ effects in human narcolepsy. A computation of the λ values for the identified HLA-DQ effects indicate a value of only 2–4 in the three ethnic groups, with most of the effects being attributable to DQB1*0602. The effect of HLA-DQ was $\lambda_S = 3.54$ and $\lambda_O = 3.18$ in Japanese, $\lambda_S = 2.70$ and $\lambda_O = 2.56$ in white Americans, and $\lambda_S = 2.35$ and $\lambda_O = 2.23$ in African Americans. These λ values are below the relative risks reported in first-degree relatives, indicating that additional genetic effects are likely to be involved in genetic predisposition to narcolepsy. In whites, several well-designed prevalence studies and family studies indicate a prevalence of ~0.02%–0.04% and a 1%–2% risk in first-degree relatives, indicating a 20–40-fold increased risk (Mignot 1998). In Japanese, the contribution of HLA to overall genetic predisposition might be higher. Family studies have indicated a 1.1% risk in first-degree relatives and a population prevalence of 0.16%, yielding a λ value of only 6.9 (Honda et al. 1983). It is noteworthy, however, that the prevalence value may be significantly overestimated in this ethnic group, leading to artificially low relative risks in relatives. Only one study without polygraphic confirmation has estimated population prevalence in this ethnic group, and additional studies would be needed to confirm this value. Overall, the λ values reported for HLA in this study are unlikely to fully explain increased family clustering in human narcolepsy, even when multiple susceptibility alleles are taken into account. Other experiments aiming to identify additional susceptibility loci are ongoing, with suggestive effects of tumor necrosis factor–α polymorphism (Hohjoh et al. 1999) and genetic linkage to 4q (Nakayama et al. 2000).

The complex pattern of inheritance and the HLA association that are observed in human narcolepsy mirror similar findings reported for other HLA-associated diseases, most of which are autoimmune in nature. The observations of complex homozygosity and compound-heterozygote effects are also consistent with HLA-DR and -DQ being primarily involved in susceptibility to disease. Recent studies have shown that most cases of human narcolepsy are associated with a dramatic loss of hypocretin-containing neurons in the CNS (Peyron et al. 2000; Thannickal et al. 2000). Most of these cases are not due to hypocretin-gene mutations (Peyron et al. 2000), yet hypocretin-1 levels are undetectable in the cerebrospinal fluid (CSF) of most patients (Nishino et al. 2000). The hypocretin system is a unique target, since only a small number (~10,000) of human neurons contain this peptide, and all are discretely localized within the perifornical hypothalamic region (Peyron et al. 2000). These findings, together with the well-established HLA association, suggest that narcolepsy may be an autoimmune disorder involving the hypocretin system.

In spite of this thorough analysis, the largest that has ever been published in the field of narcolepsy research, several points still must to be clarified. A surprising result was the observation that the pattern of HLA class II allele frequencies observed in patients negative for HLA-DQB1*0602 was highly correlated with that observed in DQB1*0602 heterozygotes (table 9). Current data, however, indicate that, although most patients positive for HLA-DQB1*0602 have undetectable CSF hypocretin levels, most patients negative for HLA-DQB1*0602 have measurable, if not normal, levels (E. Mignot, L. Lin, W. Rogers, Y. Honda, X. Qiu, X. Lin, M. Okun, H. Hohjoh, T. Miki, S. H. Hsu, M. S. Leffell, F. C. Grumet, M. Fernandez-Vina, M. Honda, N. Risch, and S. Nishino, unpublished results). The observation that DQB1*0602-positive and -negative patients show some clinical differences (e.g., undetectable CSF hypocretin levels vs. detectable levels) but similar features in their HLA association patterns suggests the presence of a common pathophysiological mechanism. It is noteworthy, however, that, in a previous study, we had reported that DQB1*0602 control subjects, who have normal CSF hypocretin levels (Nishino et al. 2000), have slightly shorter rapid-eye-movement–sleep latency (Mignot et al. 1998). Similarly, we also found in a previous study that HLA-DQB1*0602 homozygotes are present in increased frequency among narcoleptic patients without cataplexy (Pelin et al. 1998) yet that these

subjects also typically do not have undetectable hypocretin CSF levels (E. Mignot, L. Lin, W. Rogers, Y. Honda, X. Qiu, X. Lin, M. Okun, H. Hohjoh, T. Miki, S. H. Hsu, M. S. Leffell, F. C. Grumet, M. Fernandez-Vina, M. Honda, N. Risch, and S. Nishino, unpublished results). It is therefore possible that genetic variation at the level of HLA class II influences sleep patterns and narcolepsy without always resulting in a complete destruction of hypocretin-containing cells and undetectable CSF levels.

Another limitation of the present study was that DRB1 effects were difficult to fully evaluate, because of strong linkage disequilibrium with HLA-DQ, lack of family data, and lower-resolution typing for this locus. Recent studies of other HLA-DQ–associated diseases also now suggest that other HLA-linked polymorphisms frequently contribute additional susceptibility in primarily HLA class II–associated disorders. Additional high-resolution DRB1 studies in trio families, as well as microsatellite studies using markers spanning the HLA complex, are now underway as part of the 13th International Histocompatibility Workshop, in an attempt to address these two issues.

In conclusion, our study demonstrates that the pattern of HLA association in narcolepsy is as complex as that observed in other, better-studied HLA-associated disorders, such as insulin-dependent diabetes mellitus (Friday et al. 1999; Wen et al. 2000) and multiple sclerosis (Allcock et al. 1999; Fogdell-Hahn et al. 2000). Thus, it is likely but not established that narcolepsy has an autoimmune basis, as do some other, better-studied disorders.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

IMGT/HLA Sequence Database, http://www.ebi.ac.uk/imgt/hla/ (for HLA class II sequences)

Online Mendelian Inheritance in Man (OMIN), http://www.ncbi.nlm.nih.gov/Omim/ (for narcolepsy [MIM 161400])

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