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DQB1*0301 and DQB1*0601 Modulate Narcolepsy Susceptibility in Koreans

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ABSTRACT: The association of narcolepsy with HLA-DQB1*0602 is established in Japanese, African-Americans, European, and North American Caucasians. We examined DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1 in 163 patients with centrally mediated daytime sleepiness (100 with narcolepsy) and 211 Korean controls. In this population, the DQB1*0602 association was always evident in the context of the DRB1*1501-DQA1*0102-DQB1*0602 haplotype. The DQB1*0602 association was highest in cases with hypocretin deficiency (100% vs 13% in controls), most of which had narcolepsy-cataplexy (81%). A weaker DQB1*0602 (45%) association was present in cases without cataplexy. No human leukocyte antigen (HLA) association was present in idiopathic hypersomnia or in cases with normal cerebrospinal fluid (CSF) hypocretin-1. As in other populations, DQB1*0602 homozygosity increased risk in cases with cataplexy and/or hypocretin deficiency (odds ratio = 2.0 vs heterozygotes). Non-DQB1*0602 allelic effects were also observed but could not be interpreted in the context of DQB1*0602 overabundance and linkage disequilibrium. We therefore next analyzed compound heterozygote effects in 77 subjects with either hypocretin deficiency or cataplexy and one copy of DRB1*1501-DQA1*0102-DQB1*0602, a sample constructed to maximize etiologic homogeneity. In this analysis, we found additional predisposing effects of DQB1*0301 and protective effects for DQA1*0103-DQB1*0601. Unexpectedly, the predisposing effects of DQB1*0301 were present in the context of various DQA1-bearing haplotypes. A predisposing effect of DQA1*0303 was also suggested. These results indicate a remarkable consistency in the complex HLA association present in narcolepsy across multiple ethnic groups. *Human Immunology 68, 59–68 (2007).* © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: Narcolepsy; cataplexy; hypocretin; orexin; HLA; DQB1*0602; DQB1*0301; DQB1*0601

ABBREVIATIONS
CSF: cerebrospinal fluid; HLA: human leukocyte antigen

INTRODUCTION

The sleep disorder narcolepsy is characterized by excessive daytime sleepiness, cataplexy (sudden episodes of loss of muscle tone triggered by emotions), and rapid transitions into rapid eye movement sleep [1, 2]. Recent studies have shown that the human disorder is caused by the destruction of most of approximately 70,000 hypothalamic neurons that secrete a wake-promoting neuropeptide called hypocretin (orexin) [3, 4]. Measuring hypocretin-1 in the cerebrospinal fluid (CSF) of patients is now an accepted diagnostic test, with low levels indicating narcolepsy [2, 5, 6]. Based on the observations of the human leukocyte antigen (HLA) association in the disorder, an autoimmune-mediated destruction of hypocretin-containing cells has been proposed as the cause of most human cases. In spite intensive research, however, an involvement of the immune system in the pathophysiology of the disorder remains unproven [7, 8].

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In 1984, Juji *et al.* first reported a strong association between HLA-DR2, DQ1, and narcolepsy in Japanese patients (100% vs 30%) [9]. The DR2 association was rapidly confirmed in Caucasians [10, 11], whereas it was found to be more variable in African-Americans [12]. Further studies in Caucasians and Japanese have shown that the association is with the DRB1*1501, DQA1*0102, DQB1*0602 haplotype [13]. More recently, studies in other ethnic groups have shown a primary effect of DQB1*0602 rather than DRB1*1501. In African-Americans, narcolepsy is associated with DQB1*0602 haplotypes bearing distinct DRB1 alleles, most commonly DRB1*1503, DRB1*1501, DRB1*1101, and DRB1*0806 [14, 15].

Interestingly, the association with DQB1*0602 is extremely strong only in subjects with typical cataplexy [16, 17], most of which also have low CSF hypocretin [13]. The DQB1*0602 association is decreased or absent in subjects without cataplexy. Additionally, less than 1% of subjects with low hypocretin levels and cataplexy are DQB1*0602 negative [13]. These findings suggest disease heterogeneity or partial hypocretin deficiency that is not reflected by decreased CSF levels in HLA-DQB1*0602–negative subjects and patients without cataplexy [13].

Effects more complex than a simple dominant effect of DQB1*0602 on narcolepsy susceptibility can also be detected and are still under investigation. A twofold to fourfold increased risk in DQB1*0602 homozygous versus heterozygotes has been demonstrated in Japanese, Caucasians, and African-Americans in case-control samples [18, 19]. Additional susceptibility effects have also been detected in selected DQB1*0301/X heterozygotes. Most notably, DQB1*0602/DQB1*0301 heterozygotes are at increased risk, whereas decreased susceptibility is observed in DQB1*0602/DQB1*0601 and DQB1*0602/DQB1*0501 heterozygotes [19]. These findings have been replicated using a conditional transmission disequilibrium test trio family study in the context of the 13th International Histocompatibility Workshop [20]. A possible additional effect of DRB1*0407 on DRB1*0407-DQB1*0301 was also observed for the first time in this later study [20].

Much has been learned in the HLA field through transethnic association studies [19–25]. In this study, we extended our HLA analysis to a large sample of Korean narcolepsy patients. Well-characterized patients with classical narcolepsy-cataplexy, without cataplexy, and with other related disorders of excessive daytime sleepiness were studied [26]. The study was also extended to include DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1 typing. The results suggest DQB1*0301 effects independent of DQA1, and they reveal a remarkably consistent transethnic pattern of association in narcolepsy-cataplexy.

SUBJECTS AND METHODS

Patients

One hundred sixty-three consecutive patients (2000–2004) with unexplained sleepiness and 211 controls recruited at St. Vincent's Hospital, Korea were included and gave informed consent for the study. The gold standard for diagnosis was *International Classification of Sleep Disorders,* 2nd edition criteria [2]. Polysomnography (87%), a multiple sleep latency test (96%), and CSF hypocretin-1 measurements (53%) were conducted in patients to further confirm the diagnosis. Patients were separated into diagnostic groupings based on the *International Classification of Sleep Disorders,* 2nd edition. [2]. This categorization resulted in the following groupings: (1) narcolepsy with cataplexy (presence of cataplexy triggered by strong emotions, most typically joking and laughing), (2) narcolepsy without cataplexy (no or atypical cataplexy but multiple rapid eye movement sleep transitions during the multiple sleep latency test), and (3) idiopathic hypersomnia cases (unexplained sleepiness without multiple transitions into rapid eye movement sleep during the multiple sleep latency test). Detailed clinical evaluation for these patients has been published elsewhere [26]. We also constructed a sample of 83 likely hypocretin-deficient patients, as described in Figure 1. The sample included all subjects with documented low-CSF hypocretin-1 (n = 55, all DQB1*0602 positive) and subjects with typical cataplexy and DQB1*0602 (n = 28). Based on previous observations and DQB1*0602 frequency in the Korean population, this sample is likely to contain only subjects with hypocretin deficiency.

HLA-DR and DQ Typing

All samples were HLA-DRB and DQB1-typed at Stanford University using sequence specific amplification followed by allele sequencing using an Applied Biosystem capillary sequencer (Applied Biosystems, Foster City, CA USA). High-level typing resolution for DRB1 and DQB1 was obtained in all cases. DQA1 typing was performed at the Fred Hutchinson Cancer Research Center, Seattle, using a reverse probe format sequence specific oligonucleotide probes (SSOP) typing system Innogenetics line probe assay (INNO-LIPA) HLA-DQA1, Innogenetics, Belgium). The INNO-LIPA reagents employ multiplex amplification of exons 1, 2, and 3. Thirty-five probes to polymorphisms distinguishing all of the known HLA-DQA1 alleles were bound on nylon membrane strips. Hybridization and detection were performed with the Auto-LiPA 48 machine and analysis was

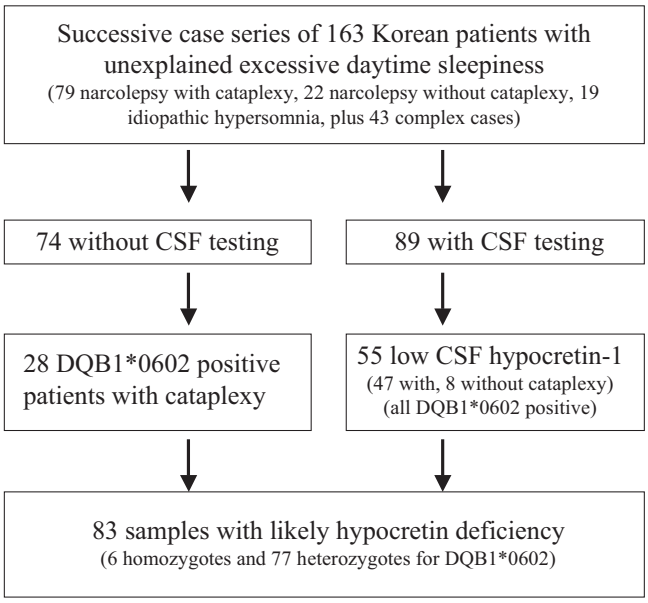

FIGURE 1 Construction of the sample under study. One hundred sixty-three consecutive patients with a complaint of excessive daytime sleepiness not obviously explained by sleep apnea, insufficient sleep, or circadian rhythm disorder were initially studied. These patients are clinically described in Hong *et al.* and included 79 patients with cataplexy, 22 patients without cataplexy, and 19 subjects with idiopathic hypersomnia [26]. Diagnosis was made according to the *International Classification of Sleep Disorders,*2nd edition [2], thus patients without cataplexy may have atypical or cataplexylike symptoms, whereas patients with cataplexy must have typical cataplexy triggered by usual emotions. Of these 163 patients, 89 were tested for CSF hypocretin-1, and 55 showed hypocretin deficiency. These patients were all included in the final sample for human leukocyte antigen (HLA) analysis. Of 74 subjects who were not tested for CSF hypocretin-1, 30 had cataplexy and 28 of those were HLA-DQB1*0602 positive. Because over 98% of HLA-positive Korean patients with cataplexy are known to be hypocretin deficient, these 28 subjects were added to the 55 subjects with low CSF hypocretin-1 to form a sample of 83 subjects with likely hypocretin deficiency.

accomplished with the LiPA software program (Innogenetics, Ghent, Belgium).

HLA Class II Haplotype Assignments

Two (DQA1-DQB1) and three (DRB1-DQA1-DQB1) locus haplotypes were assigned to all subjects on the basis of known Korean associations according to the following [27]: (1) It was assumed that the DRB1, DQA1, and DQB1 loci have no blanks. Based on this assumption, when a single HLA allele was present, the individual was considered homozygous for that allele. (2) In the assignment of haplotypes, priority was given to combinations known to exist in homozygous B-cell lines or families and to alleles having 100% associations in the analysis of unrelated individuals. (3) Rare associations were accepted when the other complementary haplotypes were well defined (*i.e.,* fitted criteria).

Statistical Analysis

Simple comparisons of case and control carrier and allele frequency were first performed across various groups of patients (Table 1). As several studies have shown increased DQB1*0602 homozygotes, we next evaluated this possibility in the Korean population (Table 2). Finally, we studied DQB1*0602 heterozygotes. In this analysis, we selected 77 heterozygote patients from 83 subjects with low CSF hypocretin, or most likely to be hypocretin deficient (DQB1*0602 positive and with typical cataplexy, Figure 1). Allele counts in narcolepsy versus non-DQB1*0602 haplotypes in controls were compared, as previously performed [19]. Fisher exact tests were used for statistical comparison. Odds ratios were reported to estimate the magnitude of the effects whenever the comparisons were significant ($p \leq 0.05$).

RESULTS

HLA Frequencies in Korean Narcolepsy Versus Controls

Table 1 reports on carrier frequencies in various diagnostic groups. We found a very tight HLA-DRB1*1501 and DQB1*0602 association (90% frequency vs 13% in controls) in narcolepsy with typical cataplexy and a reduced association (40%) in narcolepsy without cataplexy. Patients with hypocretin deficiency had a complete DQB1*0602 association (100%). DRB5 positivity was also increased (Table 1). In all narcolepsy cases, DRB1*1501 and DQB1*0602 were present in the context of a predicted DRB1*1501-DRB5 positive-DQA1*0102-DQB1*0602 haplotype (n = 78 haplotypes in 79 patients with cataplexy and 11 haplotypes in 22 patients without cataplexy). In one control, DRB1*1501 was found in the context of a DRB1*1501, DQA1*0505, DQB1*0301, a rare haplotype we previously found in Japanese controls.

A number of other alleles were decreased in the narcolepsy-cataplexy group versus controls, most notably DRB1*0803, DRB1*1302, DRB1*1502, DQA1*0101, DQA1*0103, DQA1*0301, DQB1*0302, DQB1*0501, and DQB1*0601. No differences in DRB3 and DRB4 positivity were noted. Importantly, however, odds ratios for most alleles were passively decreased as the result of increased DRB1*1501, DQA1*0102, DQB1*0602 allele counts, magnifying the significance of any decrease; the effect of these alleles was therefore next studied in heterozygotes (see below).

In a small sample size of idiopathic hypersomnia cases, DQB1*0301 was decreased significantly, a result of unknown significance. Exploratory analysis was also performed in 33 cases with normal CSF hypocretin-1 and in

TABLE 1 HLA allele carrier frequencies in four diagnostic groups

	Control		Narcolepsy with typical cataplexy				Narcolepsy without cataplexy				Idiopathic hypersomnia			
DRB1	$n = 211$	%	$n = 79$	%	p Value[a]	OR[b]	$n = 22$	%	p Value[a]	OR[b]	$n = 19$	%	p Value[a]	OR[b]
0101	22	10.4	1	1.3	0.02	0.11	2	9.1			3	15.8		
0301	10	4.7	2	2.5			0	0.0			0	0.0		
0401	3	1.4	3	3.8			1	4.5			0	0.0		
0402	0	0.0	1	1.3			0	0.0			0	0.0		
0403	10	4.7	1	1.3			3	13.6			0	0.0		
0404	6	2.8	3	3.8			0	0.0			0	0.0		
0405	31	14.7	12	15.2			4	18.2			4	21.1		
0406	23	10.9	1	1.3	0.02	0.10	1	4.5			1	5.3		
0407	1	0.5	0	0.0			0	0.0			1	5.3		
0410	5	2.4	2	2.5			0	0.0			0	0.0		
0701	30	14.2	6	7.6			3	13.6			3	15.8		
0802	5	2.4	1	1.3			1	4.5			1	5.3		
0803	33	15.6	3	3.8	0.01	0.20	4	18.2			0	0.0		
0901	34	16.1	7	8.9			5	22.7			3	15.8		
1001	8	3.8	2	2.5			0	0.0			1	5.3		
1101	22	10.4	7	8.9			3	13.6			0	0.0		
1108	1	0.5	0	0.0			0	0.0			0	0.0		
1201	18	8.5	6	7.6			0	0.0			2	10.5		
1202	16	7.6	5	6.3			1	4.5			0	0.0		
1301	2	0.9	0	0.0			0	0.0			0	0.0		
1302	39	18.5	3	3.8	0.003	0.17	1	4.5			3	15.8		
1401	14	6.6	4	5.1			2	9.1			4	21.1		
1402	1	0.5	1	1.3			0	0.0			0	0.0		
1403	3	1.4	1	1.3			0	0.0			1	5.3		
1405	19	9.0	6	7.6			1	4.5			0	0.0		
1406	3	1.4	0	0.0			0	0.0			0	0.0		
1407	1	0.5	0	0.0			0	0.0			1	5.3		
1501	26	12.3	73	92.4	<0.0001	86.57	10	45.5	0.0001	5.93	3	15.8		
1502	20	9.5	0	0.0	0.01	0.00	1	4.5			3	15.8		
1602	6	2.8	2	2.5			0	0.0			1	5.3		
DRB3	120	56.9	34	43.0			7	31.8			8	42.1		
DRB4	121	57.3	33	41.8			13	59.1			11	57.9		
DRB5	51	24.2	72	91.1	<0.0001	32.27	9	40.9			7	36.8		
DQA1														
0101	25	11.8	1	1.3	0.01	0.096	1	4.5			3	15.8		
0102	69	32.7	73	92.4	<0.0001	24.7	12	54.5			7	36.8		
0103	50	23.7	1	1.3	<0.0001	0.04	5	22.7			3	15.8		
0104	34	16.1	10	12.7			2	9.1			5	26.3		
0105	8	3.8	2	2.5			0	0.0			1	5.3		
0201	29	13.7	6	7.6			3	13.6			3	15.8		
0301	37	17.5	4	5.1	0.01	0.25	4	18.2			4	21.1		
0302	37	17.5	11	13.9			5	22.7			3	15.8		
0303	40	19.0	19	24.1			5	22.7			4	21.1		
0401	6	2.8	1	1.3			1	4.5			1	5.3		
0501	10	4.7	2	2.5			0	0.0			0	0.0		
0503	12	5.7	3	3.8			0	0.0			1	5.3		
0505	31	14.7	8	10.1			3	13.6			0	0.0		
0601	17	8.1	7	8.9			2	9.1			0	0.0		
DQB1														
02	40	19.0	7	8.9			3	13.6			2	10.5		
0301	60	28.4	22	27.8			5	22.7			1	5.3	0.05	0.14
0302	37	17.5	4	5.1	0.01	0.25	4	18.2			5	26.3		
0303	39	18.5	11	13.9			5	22.7			4	21.1		
0304	2	0.9	0	0.0			0	0.0			0	0.0		
0401	29	13.7	12	15.2			4	18.2			4	21.1		
0402	16	7.6	5	6.3			1	4.5			0	0.0		
0501	34	16.1	3	3.8	0.009	0.21	2	9.1			4	21.1		
0502	16	7.6	4	5.1			1	4.5			4	21.1		

TABLE 1 (Continued)

	Control		Narcolepsy with typical cataplexy				Narcolepsy without cataplexy				Idiopathic hypersomnia			
DRB1	$n = 211$	%	$n = 79$	%	p Value[a]	OR[b]	$n = 22$	%	p Value[a]	OR[b]	$n = 19$	%	p Value[a]	OR[b]
0503	24	11.4	8	10.1			2	9.1			2	10.5		
0601	48	22.7	1	1.3	<0.0001	0.04	5	22.7			3	15.8		
0602	25	11.8	73	92.4	<0.0001	90.52	10	45.5	0.0007	5.15	3	15.8		
0603	2	0.9	0	0.0			0	0.0			0	0.0		
0604	25	11.8	2	2.5	0.03	0.19	0	0.0			3	15.8		
0609	15	7.1	1	1.3			1	4.5			0	0.0		

Abbreviations: OR = odds ratio.

[a] p value reported using the Fisher exact test.

[b] Odds ratio, reported when $p \leq 0.05$.

six non-DQB1*0602 positive narcolepsy-cataplexy cases (data not shown). In these cases, we did not find significant deviations from expected allele frequencies. The results generally suggested a strong HLA association only in cases with cataplexy and/or hypocretin deficiency. We also did not find increased DQB1*0301 in cases with cataplexy but without DQB1*0602, but sample size was extremely small (six subjects).

Increased Susceptibility in DRB1*1501, DQA1*0102, DQB1*0602 Homozygotes

We next assessed the effect of DQB1*0602 homozygosity in narcolepsy with cataplexy (Table 2); previous studies have shown twofold to fourfold increased risk in DQB1*0602 homozygotes in African-Americans, Caucasians, and Japanese subjects [18, 19]. As in other ethnic groups [19], a twofold to threefold increased risk was found in both cases with typical cataplexy, without cataplexy, and in the 83 cases with likely hypocretin deficiency. Of note, however, the increased risk (approximately twofold to threefold) was lower than previously reported in Japanese (threefold to fourfold) and did not reach statistical significance due to the small sample size.

Heterozygote Analysis Indicates Susceptibility Effects for DQA1*0303, DQB1*0301 and Protective Effects for DRB1*1502/ DRB1*0803-DQA1*0103, DQB1*0601

We next analyzed heterozygote effects in 77 subjects likely to be hypocretin deficient (see Figure 1, from a

TABLE 2 DQB1*0602 homozygote and heterozygote subjects in narcolepsy versus controls

	DQB1*0602 homozygotes among all										
	DQB1*0602 homozygotes			DQB1*0602 heterozygotes			Non-DQB1*0602			DQB1*0602 homozygotes among all DQB1*0602 carriers	
	n	%	RR[b]	n	%	RR[b]	n	%	RR[b]	%	RR[c]
Narcolepsy with typical cataplexy, $n = 79$	5	6.3	192.0	68	86.0	86.0	6	7.6	1	6.8	2.2
Narcolepsy without cataplexy, $n = 22$	1	4.5	19.0	9	40.9	5.7	12	54.5	1	10.0	3.3
Idiopathic hypersomnia, $n = 19$	0	0.0	0.0	3	15.8	1..4	16	84.2	1	0.0	0
Sample with likely hypocretin deficiency, $n = 83$	6	7.2	inf	77	92.8	inf	0	0.0	1	7.2	2.4
Control, observed, $n = 211$	2	1.0		22	10.4		187	88.6		8.3	
Hardy-Weinberg expected values[a]	1	0.4		24	11.6		186	88.1		3.4	

Abbreviations: RR = relative risk; infin = infinite.

[a] Estimated using a DQB1 * 0602 allele frequency of 0.062.

[b] Relative risk versus non-0602 subjects.

[c] Relative risk of homozygoytes versus heterozygotes, calculated as described in Pelin et al. [18].

TABLE 3 DQB1*0602/X heterozygote patient combinations with significant deviations from controls

	Patient allele counts (n = 77)		Control allele counts (n = 395)		Fisher exact[a]	
	Counts	%	Counts	%	p Value	OR
DRB1*0405	12	15.6	32	8.10	0.02	2.09
0602-DQB1						
0602-0301	22	28.6	61	15.4	0.004	2.19
0602-0401	12	15.6	29	7.3	0.01	2.33
0602-0501	2	2.6	34	8.6	0.04	0.28
0602-0601	1	1.3	48	12.2	0.001	0.10
0102-DQA1						
0102-0101	1	1.3	25	6.3	0.05	0.19
0102-0103	1	1.3	50	12.7	0.001	0.09
0102-0303	18	23.4	42	10.6	0.002	2.56
0102-0601	7	9.1	17	4.3	0.05	2.22
0102-0602/DQA1-DQB1						
0102-0602/0101-0501	1	1.3	25	6.3	0.05	0.19
0102-0602/0103-0601	1	1.3	48	12.2	0.001	0.10
0102-0602/0303-0401	12	15.6	29	7.3	0.01	2.33
0102-0602/0505-0301	10	13.0	30	7.6	0.05	1.82
0102-0602/0601-0301	7	9.1	17	4.3	0.05	2.22
1501-0102-0602/DRB1-DQA1-DQB1						
1501-0102-0602/0803-0103-0601	1	1.3	32	8.1	0.02	0.15
1501-0102-0602/1502-0103-0601	0	0.0	16	4.1	0.05	0.00

Abbreviations: OR = odds ratio.

[a] Only combination deviating from control values using a Fisher's exact test, with $p \leq 0.05$ reported.

total of 83 after removing six homozygotes). In these subjects, we compared allele and haplotype counts for all alleles carried in trans of the DRB1*1501, DQA1*0102, DQB1*0602 haplotype (*i.e.,* alleles on the other chromosome) in the 77 heterozygotes in comparison with non-DQB1*0602 allele and haplotype counts in controls. Table 3 reports on combinations reaching statistical significance. The most significant deviations were observed for DQB1*0301 (susceptibility allele), DQB1*0601 (protective allele), DQA1*0103 (protective allele), and DQA1*0303 (susceptibility allele). We also noted that DQA1*0102 homozygosity did not have significant effect when homozygote beyond that occurring in the context of the DQB1*0602 bearing haplotypes (data not shown).

The DQB1*0601 and DQA1*0103 protective effects were dependent of each other, and in this population, almost exclusively present in the context of the DRB1*0803 and DRB1*1502 haplotype, suggesting a primary effect of DQ rather than DR (see numbers in Table 3). In contrast, the DQB1*0301 were present in the context of multiple haplotypes, most notably DQA1*0601, DQB1*0301, and DQA1*0505, DQB1*0301, two combinations that were significantly increased statistically in heterozygotes (Table 3). Further analysis was performed in extended DRB1, DQA1, DQB1*0301 haplotype, and although sample size was small for many haplotypes, no statistical evidence for heterogeneity was present (Table 4).

A similar analysis was performed in DQA1*0303-bearing haplotypes (Table 5). Similar to DQB1*0301, this allele was present in the context of multiple haplotypes all contributing to the effect, with no statistical evidence for heterogeneity (Table 5). In most cases, however, the effect was evident in association with the DRB1*0405, DQB1*0303, DQB1*0401, but the heterozygote combination with this haplotype alone was borderline significant (not shown in Table 3).

DISCUSSION

The present study confirms the extremely tight association of DQB1*0602 in narcolepsy with cataplexy in Korea, especially when associated with hypocretin deficiency (100%). Our survey of narcolepsy and idiopathic hypersomnia patients in this ethnic group also allowed us to explore the possibility of an HLA association in idiopathic hypersomnia and narcolepsy without cataplexy, two conditions clinically close to narcolepsy-cataplexy (Table 1). Unlike in narcolepsy-cataplexy, the DQB1*0602 association decreased significantly in cases without cataplexy, suggesting etiologic heterogeneity when cataplexy was not present. In idiopathic hypersomnia, a condition characterized by daytime sleepiness but

TABLE 4 Distribution of DQB1*0301 haplotypes in patients versus controls

	Patient counts (n = 77)		Control counts (n = 395)		Fisher exact	
	Counts	%	Counts	%	p Value	OR[a]
All 0602-0301	22/77	28.6	61/395	15.44	0.004	2.19
0102-0602/DQA1-0301	n = 22		n = 61			
0102-0602/0302-0301	1	4.5	0	0.0	0.265	Infin
0102-0602/0303-0301	2	9.1	2	3.3	0.230	2.95
0102-0602/0503-0301	2	9.1	12	19.7	0.152	0.41
0102-0602/0505-0301	10	45.5	30	49.2	0.188	0.86
0102-0602/0601-0301	7	31.8	17	27.9	0.200	1.21
1501-0102-0602/DRB1-DQA1-0301	n = 22		n = 61			
1501-0102-0602/0401-0303-0301	2	9.1	2	3.3	0.230	2.95
1501-0102-0602/0803-0601-0301	2	9.1	1	1.6	0.153	6.00
1501-0102-0602/0901-0601-0301	0	0.0	1	1.6	0.735	0.00
1501-0102-0602/1101-0503-0301	0	0.0	1	1.6	0.735	0.00
1501-0102-0602/1101-0505-0301	8	36.4	20	32.8	0.196	1.17
1501-0102-0602/1108-0505-0301	0	0.0	1	1.6	0.735	0.00
1501-0102-0602/1201-0302-0301	1	4.5	0	0.0	0.265	Infin
1501-0102-0602/1201-0503-0301	1	4.5	3	4.9	0.431	0.92
1501-0102-0602/1201-0505-0301	2	9.1	9	14.8	0.248	0.58
1501-0102-0602/1202-0601-0301	5	22.7	15	24.6	0.227	0.90
1501-0102-0602/1402-0503-0301	0	0.0	1	1.6	0.735	0.00
1501-0102-0602/1403-0503-0301	1	4.5	3	4.9	0.431	0.92
1501-0102-0602/1406-0503-0301	0	0.0	3	4.9	0.392	0.00
1501-0102-0602/1501-0505-0301	0	0.0	1	1.6	0.735	0.00

Abbreviations: OR = odds ratio; infin = infinite.

[a] Odds ratio calculated against controls.

no rapid eye movement sleep abnormalities, we found a negative association with DQB1*0301, but sample size was small (n = 19 patients). This finding will need replication and may be the result of multiple testing.

Our study was also a relatively complex pattern of HLA-allele association in Korean narcoleptic patients. As in Caucasians and Japanese, the primary association was present with DRB1*1501, DQA1*0102, DQB*0602 [15, 19, 20]; other studies in African-Americans have shown a primary role of DQA1*0102 and DQB1*0602 [14, 15]. As both alleles are in almost complete linkage disequilibrium in all populations, the independent role of DQB1*0602 without DQA1*0102 cannot be investigated. Nonetheless, it is suspected the products en-

TABLE 5 Distribution of DQA1*0303 haplotypes in patients versus controls

	Patients		Controls		Fisher exact	
	Counts	%	Counts	%	p Value	OR[a]
All 0102-0303	18/77	23.4	42/395	10.6	0.002	2.56
0102-0602/0303-DQB1	n = 18		n = 42			
0102-0602/0303-0301	2	11.1	2	4.8	0.270	2.50
0102-0602/0303-0401	12	66.7	29	69.0	0.230	0.90
0102-0602/0303-0402	4	22.2	11	26.2	0.246	0.81
1501-0102-0602/DRB1-0303-DQB1	n = 18		n = 42			
1501-0102-0602/0401-0303-0301	2	11.1	2	4.8	0.270	2.50
1501-0102-0602/0404-0303-0402	2	11.1	5	11.9	0.336	0.93
1501-0102-0602/0405-0303-0401	11	61.1	29	69.0	0.194	0.70
1501-0102-0602/0405-0303-0402	1	5.6	3	7.1	0.424	0.76
1501-0102-0602/0410-0303-0402	2	11.1	3	7.1	0.322	1.63

Abbreviations: OR = odds ratio.

[a] Odds ratio calculated against controls.

coded by these two alleles act as antigen-binding heterodimers in mediating narcolepsy susceptibility. In this study, we extended our class II typing study in this population, adding the typing of DQA1 and characterizing haplotypes located in trans of DRB1*1501, DQA1*0102, DQB1*0602.

Surprisingly, the pattern of DQB1 association in trans of DQB1*0602 was remarkably similar to previous studies in African-Americans, Caucasians, and Japanese subjects, indicating a strong predisposing effect of DQB1*0301, a strong protective effect of DQB1*0601, and a weaker protective effect of DQB1*0501 [19, 28]. We also observed for the first time a predisposing effect of DQA1*0303, and unlike for DQB1*0602, did not find any independent effect of DQA1*0102 homozygosity.

Unlike previous studies [19, 28], we conducted high-resolution DRB1 and DQA1 typing, allowing the study of these additional loci. This led to several interesting findings. The relatively higher frequency of the DRB1*0803, DQA1*0103, DQB1*0601 in this population relative to DRB1*1502, DQA1*0103, DQB1*0601 in the Japanese, allowed us to conclude that the primary protective effect of the DRB1*1502, DQA1*0103, DQB1*0601 haplotype reported by us and others [19, 28] was conferred by DQA1*0103, DQB1*0601 rather than DRB1*1502. Interestingly, DRB1*13, DQA1*0103, DQB1*0603 haplotypes confer moderate protection in Caucasians and African-Americans [19]. Part of the protective effect for the DQA1*0103, DQB1*0601 haplotype may thus be mediated through DQA1*0103. Interestingly, *in vitro* studies have shown that structurally similar DQα and DQβ chains, for example, members of the same families (*i.e.,* DQ1), can form stable transheterodimers, whereas structurally diverse members do not (*i.e.,* DQα1*0102 and DQ β1*0302) [29–31]. In this context, both DQα1*0103 and DQβ1*0601 may be able to pair with transencoded DQβ1*0602 and DQα1*0102, respectively, thereby reducing availability for the major narcolepsy susceptibility heterodimer, DQα1*0102/DQβ1*0602.

Similarly, DQA1*01, DQB1*0501 has also been shown to be protective in narcolepsy in African-Americans, Caucasians, and Japanese [19]. As for DQA1*0103, DQB1*0601, such protection could be mediated by the formation of compatible transdimers, such as DQα1*0101/DQβ1*0602 and DQα1*0102/DQβ1*0501. Transeffects on disease susceptibility have been reported in other HLA-DQ associated diseases, such as celiac disease [23, 25] and type 1 diabetes [22, 32]. In these diseases, it is hypothesized that specific residues within the DQα1/DQβ1 binding pockets can present specific antigens, leading to the abnormal immune response. An alignment of various DQß1*06 suggests the importance of a combined number of residues, including Y25, Q34, R41 in DQα1*0102, and F9, G13, M14, L26, Y30, Y37, A38, D57, G70, A86, F87 for DQβ1*0602 [29]. All of these residues but αR41 and ßM14 are in pockets 1, 3, 6, and 9, known to be important for peptide binding [33], with revised renumbering for DQA1 residues. Unlike these other diseases, however, no direct evidence has implicated the immune system in narcolepsy nor have putative antigens been discovered.

Another surprising result was the observation that the DQB1*0301 predisposing effect was observed in the context of multiple DQA1-bearing haplotypes (Tables 3 and 4), most notably those with DQA1*0505 and those with DQA1*0601. This result was suspected from prior data in Japanese and Caucasian samples [19], but was not proven due to the absence of DQA1 typing in these previous studies. Four possibilities could explain this result. In the first, narcolepsy susceptibility for the DQ7 heterodimer is primarily conferred by DQB1*0301 encoded residues independent of DQA1 encoded residues. This may be possible if the primary antigen-binding ability for the various heterodimers is only directed by polymorphisms on the DQβ1 molecule, for example, in binding pocket 7 [33]. A second may involve DQB1 effects on narcolepsy that do not involve DQB1 pairing with DQA1 and peptide binding. A third possibility may an unusual transpairing effect between DQA1*0102 and DQB1*0301 encoded chains; however, this hypothesis is rather unlikely considering the lack of pairing of DQA1*01 and DQB1*0302 encoded proteins [30, 31]. Finally, it could be that DQB1*0301 is a linkage marker for a close by polymorphism located immediately centromeric to DQB1, which is genuinely involved in disease susceptibility. This last possibility is also rather unlikely, considering available sequence data and the phylogenic distance separating the DQA1*0505, DQB1*0301 and DQA1*0601, DQB1*0301.

A similar complex effect was also present with DQA1*0303, as susceptibility in trans of DQA1*0102, DQB1*0602 was observed in the context of multiple DQA1, DQB1 haplotypes. No evidence for heterogeneity for the effects was observed (Table 5). In this case, however, the association will need further confirmation in other populations, because it was observed for the first time.

In summary, our study extends on previous work and indicates a remarkable consistency of the HLA association pattern across multiple ethnic groups and cultures. This is unlike other diseases, such as type I diabetes [22] or multiple sclerosis [21, 24], and may suggest a more etiologically narrow disease entity in terms of HLA interacting factors. In contrast to these other diseases,

however, whereas a simple mechanism—an autoimmune destruction of hypocretin producing cells—has been proposed as the primary etiology, all attempts at demonstrating such mechanism have failed [7, 8]. The role of HLA and the immune system in the pathophysiology of narcolepsy is thus still unknown. It is interesting to speculate that the great specificity of the HLA association may suggest that the pathogenic disease-causing antigens in narcolepsy patients are very limited and relatively restricted to specific class II peptide-presenting molecules. Alternatively, it may be that DQB1 plays an independent role to immune modulation in the brain, which is involved in narcolepsy. The fact that some of the DQB1 association in narcolepsy may be independent of DQA1, unlike in the prototypical DQA1*05, DQB1*02 associated celiac disease [23, 25], may raise the possibility of a novel function for DQB1.

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