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Narcolepsy

HLA DQB1*0602 is Associated With Cataplexy in 509 Narcoleptic Patients

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Summary: Narcolepsy is a sleep disorder associated with HLA DR15 (DR2) and DQB1*0602. We HLA typed 509 patients enrolled in a clinical trial for the drug modafinil and analyzed the results in relation to cataplexy, a symptom of narcolepsy characterized by muscle weakness triggered by emotions. The patients were either subjects with cataplexy who had a mean sleep latency (SL) of less than 8 minutes and two or more sleep onset rapid eye movement (REM) periods (SOREMPs) during a multiple sleep latency test, or narcoleptic patients without cataplexy but with a mean SL shorter than 5 minutes and two or more SOREMPs. The respective values of DRB1*15 (DR2) and DQB1*0602 as markers for narcolepsy were first compared in different ethnic groups and in patients with and without cataplexy. DQB1*0602 was found to be a more sensitive marker for narcolepsy than DRB1*15 across all ethnic groups. DQB1*0602 frequency was strikingly higher in patients with cataplexy versus patients without cataplexy (76.1% in 421 patients versus 40.9% in 88 patients). Positivity was highest in patients with severe cataplexy (94.8%) and progressively decreased to 54.2% in patients with the mildest cataplexy. A voluntary 50-item questionnaire focusing on cataplexy was also analyzed in 212 of the 509 HLA-typed patients. Subjects with definite cataplexy as observed by an experienced clinician were more frequently HLA DQB1*0602-positive than those with doubtful cataplexy, and the manifestations of cataplexy were clinically more typical in DQB1*0602-positive patients. These results show that the HLA association is as tight as previously reported (85–95%) when cataplexy is clinically typical or severe. We also found that patients with mild, atypical, or no cataplexy have a significantly increased DQB1*0602 frequency (40–60%) in comparison with ethnically matched controls (24%). These results could be explained by increased disease heterogeneity in the noncataplexy group or by a direct effect of the HLA DQB1*0602 genotype on the clinical expression of narcolepsy. **Key Words:** Narcolepsy—REM sleep—Cataplexy—Narcolepsy without cataplexy—HLA—DRB1*15—DR2—DQB1*0602.

Narcolepsy is a disorder characterized by excessive daytime sleepiness (EDS) and symptoms of abnormal rapid eye movement (REM) sleep (cataplexy, sleep paralysis, hypnagogic hallucinations) (1). A restrictive definition of narcolepsy includes documented EDS and cataplexy, a symptom characterized by brief episodes of muscle weakness in response to emotions, most typically laughing or elation. Since the discovery that narcoleptic patients have short REM sleep latencies (2) and that cataplexy, sleep paralysis, and hypnagogic hallucinations are dissociated REM sleep events (3), a less restrictive definition of narcolepsy has generally been adopted (e.g. International Classification of Sleep Disorders). In this classification, patients with either cataplexy or other documented REM sleep abnormalities are considered narcoleptic patients (4).

In 1983, a Japanese group made the observation that narcolepsy is associated with two specific serologically defined HLA class II antigens, DR2 and DQ1 (5). Since then, investigators have studied several hundred Japanese narcoleptic patients, and all subjects have been found to be HLA DR2-positive and DQ1-positive (6). This association was confirmed in Caucasians, with 95–98% of all patients reported DR2-positive and DQ1-positive in Western Europe and North America (7–13). In spite of this high HLA association, however, all attempts to demonstrate that narcolepsy is an autoimmune disorder have failed (14,15).

The report of rare HLA DR2-negative patients in Europe and the United States raised the possibility of

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diagnostic or ethnic effects or disease heterogeneity across countries and cultures. In 1987, Matsuki et al. (16) compared HLA DR2 frequency in narcoleptic patients diagnosed according to various nosological classifications. In all cases, HLA association was lower in patients without cataplexy (6,16). In the opinion of the Japanese group, cataplexy was a prerequisite for the diagnosis of narcolepsy, and the lower association reported in other countries was due to the inclusion of patients with no or clinically doubtful cataplexy (6).

Even when patients without cataplexy were excluded, however, DR2-negative narcoleptic subjects were still observed in North America and western European countries. In one of these reports, four typical cataplectic patients without DR2 but with DQ1 were African-Americans (17), thus suggesting the importance of ethnicity. A study of narcolepsy-cataplexy in African-Americans was therefore initiated, and a low (67%) association with DR2 was confirmed. Strikingly, however, the association was high (95%) with DQB1*0602, a molecularly defined DQ1 subtype tightly associated with DR2 in Caucasians and Asians but not in African-Americans (13,18). Further genetic studies in rare recombinant class II haplotypes and HLA DQ sequencing studies have now established that DQB1*0602 is the actual susceptibility gene for narcolepsy in the HLA class II region (19,20).

In this study, we HLA typed narcoleptic patients diagnosed in 39 sleep centers on the basis of international criteria for narcolepsy in the context of a clinical trial for modafinil as a treatment for EDS. Data were analyzed by ethnic groups and in relation with cataplexy to determine the importance of these factors in determining positivity for these established narcolepsy markers.

METHODS

Patients

Patients were 18–68 years old with a diagnosis of narcolepsy based on the international classification of sleep disorders but meeting more stringent criteria for EDS. The sample included 274 men and 235 women enrolled in two successive clinical trials (studies 301 and 302) conducted in 39 sites in the United States [256 patients in a 18-center study (21) and 253 patients in a 21-center study]. Four hundred twenty Caucasian Americans (White), 71 African-Americans (Black), 14 Hispanic Americans, 4 patients of other ethnic origin, but no Asian-Americans were enrolled. Ethnicity was self-defined.

Patients were included if they had 1) recurrent daytime naps or lapses into sleep occurring almost daily for 3 months, 2) sudden bilateral loss of postural muscle tone in association with intense emotion (cataplexy), and 3) less than 8 minutes' sleep latency (SL) on a four-nap multiple sleep latency test (MSLT). These patients also had to have ≥2 sleep onset REM periods (SOREMPs) during the MSLT (21). Alternatively, patients were included if they had a complaint of excessive somnolence or muscle weakness and associated features (sleep paralysis, hypnagogic hallucinations, automatic behavior, disturbed major sleep episodes), plus an MSLT SL of less than 5 minutes and the combined ≥2 SOREMPs episodes during the MSLT (21).

Patient evaluation

Evaluation included interviews by a sleep specialist, nocturnal polysomnography, maintenance of wakefulness test, MSLT, Epworth sleepiness scale, and a quality of life questionnaire (21). Initial clinical interviews included reports on the presence, absence, and severity of EDS and cataplexy at disease onset and at entry in the study. Severity was rated 1 (mild), 2 (moderate), or 3 (severe) for either time points. Nocturnal polysomnography was used to exclude other causes of EDS, such as obstructive sleep apnea or significant periodic leg movements (21). A voluntary questionnaire focusing on cataplexy was also completed by more than half of the patients. This questionnaire, the Stanford cataplexy questionnaire (50 items), includes the cataplexy section of the longer Stanford Center for Narcolepsy sleep inventory (146 items); this questionnaire is being validated in 1,000 Stanford Sleep Disorders Clinic patients, of whom 6–7% are narcoleptic patients with cataplexy. Information requested included the following: 1) triggering factors, including laughter, anger, excitement, surprise, elation, stress, embarrassment, remembering a happy or an emotional event, making a quick verbal response in a playful or funny context, disciplining children, during sexual intercourse, during or after athletic activities, while being startled or tensed, while playing an exciting game, while having a romantic thought or moment, while telling or hearing a joke, or while moved by something emotional; 2) muscle groups involved (legs, jaw, head and shoulder, arms, speech, or complete attacks); 3) usual duration (from 5 seconds to more than 10 minutes); 4) frequency (from one or more times per day to once per year or less); 5) clinical features associated with cataplexy such as loss of hearing, blurred vision, dreaming or sleeping, loss of bladder/bowel control; 6) age at cataplexy onset; 7) response to anticataplectic medications (i.e. tricyclic antidepressants, selective serotonin reuptake inhibitors, or monoamine oxidase inhibitors); and 8) specific descriptions of three typical cataplectic attacks in real life.

HLA typing

HLA DR and DQ typing was performed in 509 subjects using Innotype reverse-dot blot kits (Robbins Scientific, Sunnyvale, CA). Not all known DRB1 and DQB1 alleles were distinguished using this method. DRB1*15 includes all known subtypes of DRB1*15. This method could not distinguish DQB1*0602 from the rare DQB1*0611 subtype. Intermediate DR and DQ typing resolution is reported for all other subtypes.

Control HLA-typed subjects

We estimated HLA DQB1*0602 and DRB1*15 carrier frequencies using two multiethnic control panels from Dallas (22) and northern California (13). Caucasian DRB1*15 frequencies were 22.8% in 167 controls from Dallas and 20.4% in 49 controls from the Stanford area. DQB1*0602 (DQB1*0602 and DQB1*0611) frequencies were 22.8 and 18.4%, respectively, in these two panels. Mean Caucasian carrier frequencies of 22.2% for DRB1*15 and 21.8% for DQB1*0602 were thus used (n = 216). In African-Americans, DRB1*15 frequencies were 34.8 and 34.9%, whereas DQB1*0602 carrier frequencies were 36.2 and 46.5%, respectively, in the two panels. We thus used mean carrier frequencies of 34.8% for DRB1*15 and 40.2% for DQB1*0602 (n = 112) for this ethnic group. In Hispanic Americans, DRB1*15 and DQB1*0602 frequencies were 22.6 and 20.8%, respectively, using 53 Hispanic Americans from the Dallas area (22).

These values were used to calculate expected control DRB1*15 and DQB1*0602 frequencies in a sample ethnically matched with that of our 509 narcoleptic patients (82.5% Caucasian, 14.0% African-American, and 3.5% Hispanic American). This led us to estimate DRB1*15 and DQB1*0602 frequencies as 24.0 and 24.3%, respectively, in ethnically matched control subjects.

Data analysis

Because of the recruitment procedure, we analyzed data by separating patients with cataplexy (required to have a mean MSLT SL ≤8 minutes) and without cataplexy (required to have a mean MSLT SL ≤5 minutes). We also analyzed frequencies by sex and for studies 301 and 302; but the results were identical, so combined frequency data are reported. We report data only for Caucasian Americans and African-Americans because of sample size limitations in other ethnic groups. Chi-square (or Fisher's exact test when appropriate) and Student's *t* test statistics were used for statistical comparisons.

We considered cataplexy to be present when the investigator reported it at either disease onset and/or inclusion in the clinical trial. Within the cataplexy group, severity scores at disease onset (0 = absent, 1 = mild, 2 = moderate, 3 = severe) and at inclusion (0 = absent, 1 = mild, 2 = moderate, 3 = severe) were added to give an overall score ranging from 0 to 6. We then used the three severity scores (at onset, inclusion, and overall) to evaluate the relation between cataplexy severity and HLA positivity.

Data collected using the Stanford questionnaires were analyzed using two methods. In the first set of evaluations, a single neurologist with more than 20 years of clinical experience in narcolepsy (C.G.) reviewed all questionnaires without knowledge of HLA typing results. The review was designed to separate narcoleptic patients into three groups: no cataplexy, atypical or doubtful cataplexy, or clear-cut cataplexy. Atypical or doubtful cataplexy was defined as either very mild in severity and thus difficult to confirm, or severe enough but clinically atypical (unusual emotional triggers, long duration of attacks, only one or a few attacks ever experienced, atypical descriptions reported in the example section of the questionnaire etc.). Clear-cut cataplexy was defined as brief episodes of weakness in the knees, jaw, face, or neck triggered by laughter, joking, anger, elation, happiness, or excitement with game playing. We used this stratification to compare HLA positivity in the three groups.

In a second set of analyses, we compared cataplexy questionnaire responses in DQB1*0602-positive and DQB1*0602-negative patients who answered at least one question. Questionnaire items were grouped into several categories: type of emotions triggering cataplexy, anatomical localization of muscle weakness, usual duration of cataplexy, frequency of attacks, response to treatment, and age of onset. The types of emotions explored by the questionnaire were grouped as positive (laughter, excitement, remembering a happy moment, making a quick verbal response in a playful or funny context, during sexual intercourse, elation, playing an exciting game, telling or hearing a joke), negative (anger, disciplining children, embarrassment, stress and tension), and not defined (surprise, startle, before and after athletic activities, having a romantic thought or moment, remembering or being moved by an emotional event).

RESULTS

HLA DQB1*0602 is a better marker than DRB1*15 (DR2) across ethnic groups

The respective values of DRB1*15 and DQB1*0602 as markers for narcolepsy were examined

TABLE 1. *HLA DRB1*15 and DQB1*0602 as markers for narcolepsy with and without cataplexy across ethnic groups*

Ethnicity	% Positive DRB1*15 (number of patients)			% Positive DQB1*0602 (number of patients)		
	Cataplexy +	Cataplexy −	All patients	Cataplexy +	Cataplexy −	All patients
Caucasian Americans (n = 420)	73.3 (253/345)	37.3 (28/75)	67.0 (281/420)	73.3[a] (253/345)	37.3 (28/75)	67.0 (281/420)
African-Americans (n = 71)	62.2 (38/61)	50.0 (5/10)	61.0 (43/71)	85.2* (52/61)	80.0 (8/10)	84.5* (60/71)
All patients (n = 509)	70.8 (298/421)	37.5 (33/88)	65.0 (331/509)	76.1[a] (312/421)	40.9 (36/88)	68.0 (348/509)

* $p < 0.005$ using χ^2 statistics versus DRB1*15 as a marker for narcolepsy.
[a] $p < 10^{-8}$ versus cataplexy negative groups.
Plus and minus signs refer to positive and negative, respectively.

across ethnic groups in patients with and without cataplexy (Table 1). As previously reported, DQB1*0602 positivity was identical to DRB1*15 positivity in Caucasians but was significantly higher in African-Americans (Table 1). Relative risks for DRB1*15 and DQB1*0602 were thus similar in Caucasians (7.1 versus 7.3), whereas that of DRB1*15 was lower in African-Americans (2.9 versus 5.5 for DQB1*0602). Relative risks for the overall sample (with and without cataplexy) were 5.9 for DRB1*15 and 6.6 for DQB1*0602.

Table 2 lists the DRB1*15 and/or DQB1*0602-positive haplotypes carried by the narcoleptic patient population. Haplotypes were inferred from established HLA DR and DQ associations, assuming there were no blanks. In both Caucasian Americans and African-Americans, DRB1*15, DQB1*0602 haplotypes were most frequently observed. The second most frequently observed haplotype was DRB1*11, DQB1*0602 in African-American narcoleptic patients (4% haplotype frequency in control African-Americans). Other non-DRB1*15, DQB1*0602-positive haplotypes were observed in the context of DRB1*12, DRB1*08 or DRB1*03. These haplotypes are rare in the general population but have been previously reported in narcoleptic patients (19).

A few haplotypes observed in the narcoleptic population were DRB1*15-positive but DQB1*0602-negative (Table 2). In three cases carrying DRB1*15, DQB1*0502, patients were also DRB1*15, DQB1*0602-positive on their other haplotype, so DQB1*0602 on the other DRB1*15 haplotype is probably involved in disease susceptibility. Three patients carried DRB1*15, DQB1*0601 and were DQB1*0602-negative, a result expected from the frequency of this haplotype in the general population (see Table 5 for details). In one case, an African-American patient with mild cataplexy, the very unusual DRB1*15, DQB1*02 haplotype was inferred; this subject was confirmed to be DQB1*0602-negative, but the presence of DRB1*15 was not verified.

Cataplexy determines HLA DQB1*0602 positivity

HLA DQB1*0602 was much higher in patients with cataplexy (76.1%) than without cataplexy (40.9%), independent of ethnicity (Table 1). We observed this difference even though the noncataplectic patient group had more stringent MSLT criteria for sleepiness than did the cataplectic subjects.

Within the cataplexy group, we added severity scores at disease onset and at inclusion to give an overall score ranging from 1 to 6. Figure 1 plots DQB1*0602 frequency in each subgroup. DQB1*0602 frequency was low in subjects with mild cataplexy (scores 1 and 2), with values ranging from 54.2 to

TABLE 2. *HLA DRB1*15 and/or DQB1*0602 haplotypes in 509 narcoleptic subjects*

Haplotypes	Number of carriers
DRB1*15 − DQB1*0602 (n = 343)	328 (280 Caucasian Americans, 41 African-Americans, 7 others)
DRB1*11 − DQB1*0602 (n = 23)	22 (21 African-Americans, 1 Caucasian American)
DRB1*15 − DQB1*0502 (n = 3)	3 (3 Caucasian Americans)
DRB1*12 − DQB1*0602 (n = 2)	2 (2 African-Americans)
DRB1*15 − DQB1*0601 (n = 2)	2 (1 Caucasian American, 1 African-American)
DRB1*0301 − DQB1*0602 (n = 1)	1 (1 Caucasian American)
DRB1*08 − DQB1*0602 (n = 1)	1 (1 African-American)
DRB1*08 or 13[a] − DQB1*0602 (n = 1)	1 (1 African-American)
DRB1*15 − DQB1*02 (n = 1)	1 (1 African-American
Others (n = 641)	158 (137 Caucasian Americans, 10 African-American, 11 others)

[a] Haplotype could not be determined from known association.
Letter "n" indicates number of specified haplotypes observed in total sample (1018 haplotypes).

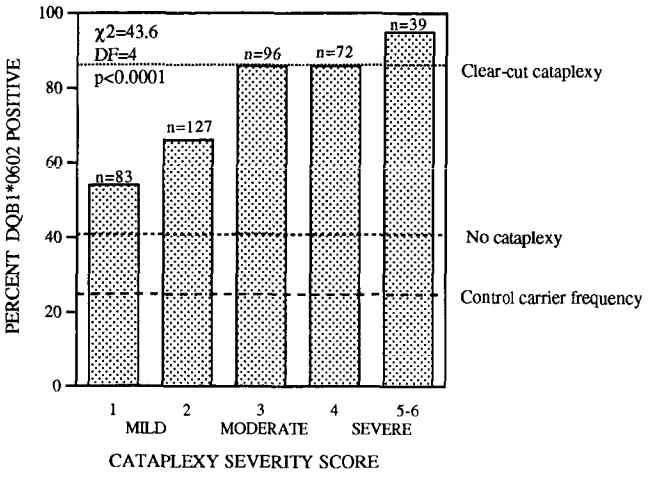

FIG. 1. DQB1*0602 positivity as a function of cataplexy severity. Cataplexy severity scores at disease onset (1 = mild, 2 = moderate, 3 = severe) and at inclusion (1 = mild, 2 = moderate, 3 = severe) were added to give an overall severity score ranging from 1 to 6. Values are percent positivity for each severity category. Letter "n" indicates the number of patients for each severity range. DQB1*0602 frequencies in the noncataplexy group and in an ethnically matched control sample are also indicated for reference.

66.1%. In contrast, patients with the most severe cataplexy (score 5–6) were almost all DQB1*0602-positive (94.8%; Fig. 1). Statistical analysis indicated a highly significant relationship between cataplexy severity and percent DQB1*0602 positivity (Fig. 1). Analysis by cataplexy at severity onset ($\chi^2 = 15.2$, DF = 2, $p < 0.0005$) or at inclusion ($\chi^2 = 18.8$, DF = 2, $p < 0.0001$) yielded identical results, with percent DQB1*0602 positivity ranging from 67% in mild cataplectic patients to 93% in subjects with severe cataplexy (data not shown).

Two hundred twelve narcoleptic patients completed the Stanford cataplexy questionnaire. An independent clinician reviewed the questionnaires and classified patients into noncataplectic patients, doubtful/atypical cataplectic patients, or clear-cut cataplectic patients (see Methods section). These 212 subjects included 181 patients with cataplexy and 31 subjects without cataplexy, as reported by the referring center. In the noncataplexy group (n = 31), the clinician confirmed most patients (77.4%) to be noncataplectic; only seven subjects were found to have doubtful (n = 4) or clear-cut (n = 3) cataplexy, all being DQB1*0602-positive. In the cataplexy group, four of the 181 patients had no cataplexy after review of the questionnaire. In the remaining 177 patients, 152 had clear-cut cataplexy versus 25 doubtful or atypical cataplexy. Table 3 is a compilation of the DQB1*0602 frequencies observed in patients without cataplexy, with doubtful/atypical cataplexy, or with typical clear-cut cataplexy. Subjects with clear-cut cataplexy had significantly higher DQB1*0602 frequency than patients with atypical/doubtful cataplexy (Table 3).

TABLE 3. *Qualitative aspects of cataplexy and DQB1*0602 positivity*

Cataplexy	% DQB1*0602 (number of patients)
No cataplexy	35.70 (10/28)
Atypical/doubtful cataplexy	55.10 (16/29)
Clear-cut cataplexy	83.80 (130/155)

$\chi^2 = 34.2$ (df = 2), $p < 0.0001$, overall; $\chi^2 = 12.3$ (df = 1), $p = 0.005$, atypical/doubtful cataplexy vs. clear-cut cataplexy.

We also compared clinical manifestations of cataplexy in DQB1*0602-positive and DQB1*0602-negative patients using the Stanford questionnaire. Only subjects with cataplexy confirmed by the referring modafinil study center were included. Table 4 contrasts types of triggers, localization of attacks, usual duration, frequency, response to treatment, and age of onset in DQB1*0602-positive and DQB1*0602-negative patients. DQB1*0602-positive subjects more frequently reported positive emotions as triggers for cataplexy. Attacks affecting the head or neck (jaw dropping, sagging, head and shoulder dropping, slurred speech) were also more frequent in DQB1*0602-positive patients. Weakness in the legs and knees was the most frequently observed cataplectic attack, but it did not distinguish the groups. We also found that cataplexy was more frequent in DQB1*0602-positive patients, a finding that parallels our reported increased DQB1*0602 positivity with cataplexy severity. Surprisingly, however, the reported age of onset of cataplexy was lower in DQB1*0602-negative patients.

Genuine HLA DQB1*0602-negative patients with clear-cut and severe cataplexy were observed

Very few DQB1*0602-negative patients with both severe (severity 3 at inclusion) and clear-cut cataplexy (verified by questionnaire) could be documented in the sample. One patient described typical episodes of knee buckling or jaw dropping when telling or hearing a joke, adding the comment "only if it is funny". Examples given included, "when my brother entertains me with funny jokes". Other emotions such as anger, surprise, and game playing were also mentioned. Episodes were always bilateral, occurred several times per week, and had been treated successfully with clomipramine, protriptyline, and now fluoxetine. Another patient documented at Stanford during the trial, described knee buckling, jaw sagging, head dropping, and complete attacks triggered by laughter, anger, and

TABLE 4. *Cataplexy in DQB1*0602-negative and DQB1*0602-positive narcoleptic patients*

Cataplexy		% DQB1*0602-negative	% DQB1*0602-positive	Statistical comparisons
Triggers[a]	Positive emotions	87.8 (31/37)	95.0 (131/137)	$\chi^2 = 5.3$; p = 0.02
	Negative emotions	78.3 (29/37)	75.4 (104/138)	
	Not defined emotions	87.85 (31/37)	83.3 (115/138)	
Localization	Weakness in legs/knees	100.0 (37/37)	97.7 (129/132)	
	Jaw dropping/sagging	41.2 (14/34)	66.2 (86/130)	$\chi^2 = 7.1$; p = 0.008
	Head/shoulders dropping	44.1 (15/34)	76.2 (99/130)	$\chi^2 = 13.1$; p = 0.0003
	Weakness in arms	76.5 (26/34)	79.7 (106/133)	
	Slurred speech	47.2 (17/36)	72.2 (91/126)	$\chi^2 = 7.9$; p = 0.005
	Fall to the ground	40.0 (14/35)	47.4 (63/133)	
Usual duration	5–30 seconds	55.9 (19/34)	70.0 (91/130)	
	30 seconds–2 minutes	23.5 (8/34)	20.0 (26/130)	
	>2 minutes	20.6 (7/34)	10.0 (13/130)	
Frequency	1/day–several/week	38.2 (13/34)	60.0 (75/125)	$\chi^2 = 5.1$; p = 0.02 vs. others
	1/week	20.6 (7/34)	13.6 (17/125)	
	1/month	23.5 (8/34)	17.6 (22/125)	
	1/year or less	17.6 (6/34)	8.0 (11/125)	
Response to treatment	% Improving	50.0 (5/10)	72.3 (34/47)	
Age of onset	Mean ± SEM	20.7 ± 1.8 (n = 37)	25.8 ± 1.2 (n = 129)	Student's $t = 2.1$, $p < 0.05$

[a] Triggers refer to emotions triggering cataplectic episodes. Positive emotions: laughing, telling or hearing a joke, excitement, elation, playing an exciting game, having to make a quick verbal response in a playful or funny context, during sexual intercourse, remembering a happy moment. Negative emotions: anger, embarrassment, stress, tension, when disciplining children. Not defined emotions: surprise, being startled, when having a romantic thought or moment, during or after athletic activities, being moved by something emotional.

many other typical emotions. Typical examples included the following: "I trained myself not to laugh deeply but if and when I do, I end up on the floor"; and "Episodes occur when I get upset because of an unexpected change of events". Mild episodes occurred several times per day.

Noncataplectic patients still exhibit increased DQB1*0602 frequency

Even in patients without cataplexy, the mean DQB1*0602 frequency was still high at 40.9%. This contrasts with a carrier frequency in the general population of 24.3% in an ethnically matched North American sample (Table 1 and Fig. 1).

DQB1*0602-negative patients are younger than DQB1*0602-positive patients independent of cataplexy

Mean age at entrance in the clinical trial was also compiled in DQB1*0602-positive and DQB1*0602-negative patients with and without cataplexy. Mean ± standard error of the mean values were 37.2 ± 1.5 and 40.7 ± 2.2 years in DQB1*0602-negative and DQB1*0602-positive patients, respectively, without cataplexy (t = 1.4, p = 0.16). Corresponding values in the cataplexy-positive groups were 36.8 ± 1.1 (n = 109) and 44.1 ± 0.8 (n = 312) years, a highly significant difference (t = 5.0, $p < 10^{-6}$). DQB1*0602-positive patients without cataplexy were slightly younger than DQB1*0602-positive patients with cataplexy (t = 1.4, p = 0.15).

Non-DQB1*0602 patients do not share any specific HLA haplotype

We analyzed HLA haplotypes in noncataplectic (n = 47) and cataplectic (n = 92) Caucasian DQB1*0602-negative patients. Due to small sample size, we did not report data from other ethnic groups. HLA haplotype frequencies in Caucasian narcoleptic patients were compared to control non-DQB1*0602 American Caucasian frequencies (22). No significant differences were observed (Table 5).

DISCUSSION

This study confirms that HLA DQB1*0602 is a more sensitive marker for narcolepsy than DRB1*15 (DR2) across ethnic groups (13,16,19,23). This was evident in African-Americans, among whom patients without DRB1*15 but with DQB1*0602 were frequently observed, mostly in the context of the African DRB1*11, DQB1*0602 haplotype (19). This study also demonstrates that cataplexy is a critical determinant for HLA DQ association in narcolepsy. Indeed, HLA DQB1*0602 positivity was much higher in patients with cataplexy (76.1%) than without cataplexy (40.9%; Table 1). In both subjects with and without cataplexy, however, patients without HLA

DQB1*0602 were observed, thus limiting the value of this test for diagnosing narcolepsy (24).

Not only was the presence or absence of cataplexy a critical factor in determining HLA genotype positivity, but the quality of the cataplexy reports was equally important. Even within the cataplexy-positive group, DQB1*0602 positivity increased to 94.8% when cataplexy was most severe (Fig. 1) and to 83.8% when the symptom was categorized as clear-cut after independent review of the Stanford cataplexy questionnaire by one of us (Table 3). This last result parallels data recently gathered from 188 narcoleptic patients with cataplexy studied at Stanford University using an identical methodology (23). In this study, DQB1*0602 positivity across ethnic groups was 90.1% when cataplexy was narrowly defined as clear-cut versus 50.0% when patients with atypical or doubtful cataplexy were included (23). A similar picture emerged when the clinical features of cataplexy were compared in DQB1*0602-positive and DQB1*0602-negative cataplectic patients. In this comparison, cataplexy in DQB1*0602-positive patients was more often triggered by typical positive emotions such laughter or related pleasant emotions, known to be the most usual triggers for cataplexy. These data confirm a similar finding observed using an independent sample of DQB1*0602-negative patients with cataplexy gathered at Stanford University (25). Cataplexy in DQB1*0602-positive patients was also shorter in duration, more frequent, and affected different muscles in both groups. Specifically, whereas all patients, whether DQB1*0602-positive or DQB1*0602-negative, reported muscle weakness in the legs or knees buckling with emotions, a much larger proportion of DQB1*0602-positive patients reported rarer but more specific attacks in muscle of the head and jaw (Table 4). These data support the hypothesis that narcolepsy with clear-cut, typical, severe cataplexy is a genetically homogeneous nosological entity.

The DQB1*0602 positivity value observed in patients with doubtful or mild cataplexy (e.g. 50.0–55.1%) is similar to that observed in noncataplectic patients (40.9%). Diagnosing mild or atypical cataplexy may thus not be nosologically useful, and stratifying patients with clear-cut versus possible or no cataplexy might be more relevant for future genetic studies. Even in the noncataplectic group, however, DQB1*0602 frequency is statistically higher than in the corresponding general population (40.9 versus 24.3%). If DQB1*0602-positive narcolepsy is considered a separate etiological entity, one-quarter of these noncataplectic patients must share a similar HLA-associated genetic mechanism to explain the increased DQB1*0602 frequency observed in this sample (versus approximately two-thirds in the cataplexy-positive group). This finding parallels earlier reports obtained using HLA DR2 in Japanese patients with EDS but without cataplexy (6,16,26).

DQB1*0602-positive patients without cataplexy were only slightly younger than DQB1*0602-positive patients with cataplexy (40 versus 44 years of age, respectively) and were thus past the mean age for cataplexy onset in narcolepsy, e.g. 25 years (27–29). In fact, 20 and 11 of these 36 non-DQB1*0602-positive patients without cataplexy were past 40 and 50, respectively, and only four subjects were less than 25 years old. Most of these DQB1*0602-positive patients are thus unlikely to develop cataplexy. The finding of a higher DQB1*0602 frequency in noncataplectic narcoleptic patients thus demonstrates that HLA DQB1*0602 predisposes to narcolepsy both with or without cataplexy.

The finding that cataplexy strongly determines HLA DQB1*0602 positivity could be explained by two mechanisms. One explanation involves disease heterogeneity. In this model, DQB1*0602-associated and DQB1*0602-negative narcolepsy would be distinct disease entities with separate etiological mechanisms. In favor of this hypothesis is the fact that cataplexy, the only pathognomonic symptom of the narcolepsy symptom, is less severe and typical in this population. Another argument in favor of this hypothesis is that DQB1*0602-negative patients are much younger than DQB1*0602-positive subjects, an effect associated with earlier cataplexy onset (Table 4). Non-DQB1*0602 subjects may, in this context, represent a heterogeneous group of patients, some of whom may have another disorder with earlier onset and milder and atypical cataplexy features. Another explanation, possibly complementary to the issue of etiological heterogeneity, could involve a direct effect of DQB1*0602 on the clinical expression of narcolepsy. Specifically, most, if not all, patients diagnosed with narcolepsy could represent a single disease entity, but the presence of HLA DQB1*0602 would increase severity and modify the clinical expression of cataplexy (but not decrease its age of onset). The fact that both HLA DQB1*0602-negative and DQB1*0602-positive patients with narcolepsy-cataplexy are occasionally observed in the context of the same multiplex family (30) may add credence to this hypothesis. Genetic studies aimed at the identification of other, non-HLA narcolepsy susceptibility may provide an answer to this important question.

To explore further the possibility of disease heterogeneity in the sample under investigation, the frequency of haplotypes other than HLA DQB1*0602-bearing haplotypes was examined in DQB1*0602-negative patients. In many other HLA DQ-associated disorders, HLA associations are complex and involve several

TABLE 5. *HLA haplotype frequencies in non-DQB1*0602 narcoleptic and corresponding control Caucasian subjects*

DRB1	DRB3	DRB4	DRB5	DQB1	% Cataplexy − n = 94	% Cataplexy + n = 184	% All patients n = 278	% Controls n = 280
01	—	—	—	0501	16.0 (15)	11.4 (21)	13.0 (36)	14.3 (40)
0301	01	—	—	02	12.8 (12)	9.8 (18)	10.8 (30)	11.1 (31)
0301	02	—	—	02	2.1 (2)	1.7 (3)	1.8 (5)	2.1 (6)
04	—	01	—	03	23.4 (22)	22.3 (41)	22.7 (63)	17.5 (49)
04	—	01	—	02	0.0 (0)	0.5 (1)	0.4 (1)	0.0 (0)
0701	—	01	—	02	7.5 (7)	12.5 (23)	10.8 (30)	12.1 (34)
0701	—	01	—	0303	4.2 (4)	6.5 (12)	5.8 (16)	2.9 (8)
08		—		03	1.1 (1)	1.1 (2)	1.1 (3)	0.7 (2)
08		—		0402	2.1 (2)	3.2 (6)	2.9 (8)	4.3 (12)
0901	—	01	—	02	0.0 (0)	0.0 (0)	0.0 (0)	0.4 (1)
0901	—	01	—	0303	2.1 (2)	1.6 (3)	1.8 (5)	1.1 (3)
1001				0501	2.1 (2)	1.1 (2)	1.4 (4)	0.4 (1)
11	01	—	—	03	0.0 (0)	0.5 (1)	0.4 (1)	0.0 (0)
11	02	—	—	03	4.2 (4)	6.0 (11)	5.4 (15)	10.7 (30)
11	02	—	—	06	0.0 (0)	0.5 (1)	0.4 (1)	0.4 (1)
1201	01	—	—	03	1.1 (1)	1.1 (2)	1.1 (3)	0.0 (0)
1201	02	—	—	03	1.1 (1)	1.6 (3)	1.4 (4)	1.8 (5)
13	01	—	—	03	2.1 (2)	1.6 (3)	1.8 (5)	1.4 (4)
13	02	—	—	03	1.1 (1)	0.5 (1)	0.7 (2)	0.0 (0)
13	03	—	—	05	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (2)
13	01	—	—	06	3.1 (3)	3.8 (7)	4.0 (10)	3.2 (9)
13	01	—	—	06	4.2 (4)	0.5 (1)	1.8 (5)	1.4 (4)
13	03	—	—	06	4.2 (4)	6.5 (12)	5.8 (16)	6.4 (18)
14	02	—	—	0503	3.1 (3)	1.1 (2)	1.8 (5)	3.6 (10)
14	02	—	—	0501	0.0 (0)	0.0 (0)	0.0 (0)	0.4 (1)
15	—	—	01	0601	0.0 (0)	1.1 (2)	0.7 (2)	1.4 (4)
16	—	—	02	0502	2.1 (2)	2.7 (5)	2.5 (7)	1.8 (5)

Plus and minus signs refer to positive and negative, respectively. Letter "n" indicates total number of haplotypes in each group.

susceptibility haplotypes. Typically, patients that do not carry the primary predisposing haplotype display secondary HLA associations, a result supporting the argument for common immune-related mechanisms. In narcolepsy, we could not find evidence for any residual HLA association in non-DQB1*0602 patients with or without cataplexy (Table 5). In fact, a weak association with DRB1*13, DQB1*0603 in non-DQB1*0602 patients with clear-cut cataplexy previously reported by our group (19) could not be confirmed using our sample (Table 5). The absence of any secondary HLA association rather suggests that DQB1*0602-negative subjects mostly represent a patient population with a different etiological mechanism.

In conclusion, HLA typing was performed in 509 narcoleptic patients enrolled in a clinical trial. Results indicate 67% positivity for DQB1*0602, the most closely associated HLA marker for narcolepsy. Further analysis showed that HLA DQB1*0602 is a specific marker (85–95%) for cataplexy in patients with narcolepsy and a more general marker for narcolepsy (40% of patients with EDS, SOREMPs, but no cataplexy). A direct effect of DQB1*0602 on the expression of cataplexy or substantial genetic heterogeneity in this large sample of patients could explain most of the results.

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