https://doi.org/10.5664/jcsm.11300

JCSM | Journal of Clinical Sleep Medicine

SCIENTIFIC INVESTIGATIONS

Association between human leukocyte antigen class II-DR-DQ and narcolepsy: a case control study

Andrea Bacelar, MD, PhD[1]; Oscar Fernandez, MD, PhD[2]; Eduardo Paradella, MD, PhD[1]; Raimundo Nonato Rodrigues, MD, PhD[3]; Claudia Roberta de Castro Moreno, PhD[4]; Regina Alvarenga, MD, PhD[1]

[1]*Department of Neurology, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil;* [2]*Genetic Lab in Carlos Haya University Hospital, Málaga, Spain;* [3]*Department of Neurology, University of Brasilia, Brasilia, Brazil;* [4]*Department of Health and Society, School of Public Health, University of São Paulo, São Paulo, Brazil*

Study Objectives: Narcolepsy is a neurologic disorder characterized by irresistible sleep attacks. Although its etiology is unknown, it is strongly associated with genetic variances in the human leukocyte antigen (HLA) complex. We investigated the association of HLA class II-DR-DQ alleles in a sample of patients with narcolepsy-cataplexy (narcolepsy type 1 [NT1]) and patients with narcolepsy without cataplexy (narcolepsy type 2) with a control group. Additionally, we compared demographic, clinical, and laboratory characteristics of patients with narcolepsy with or without the *DQB1*06:02* allele.

Methods: This case control study included 21 patients with NT1 (56.8%), 16 patients with narcolepsy type 2 (43.2%), and 100 controls. Sequence-based typing identified HLA-*DRB1* alleles, and HLA-*DQB1* typing was done using polymerase chain reaction (PCR)-sequence-specific oligonucleotide. Allele and haplotype frequencies were calculated by direct counting. Nocturnal polysomnography and Multiple Sleep Latency Test were performed in all participants.

Results: In the NT1 group, only 1 allele had a significantly higher frequency than in the narcolepsy type 2 group: *DQB1*06:02* (61.9% vs 18.8%). Compared to controls, *DQB1*06:02* (61.9% vs 18.0% in controls) and *DRB1*15:01* (47.6% vs 8.0%), had higher frequencies in patients with NT1. Multiple analyses showed that patients with NT1 had an increased chance of being HLA-*DQB1*06:02* positive. HLA-*DRB1*15:01-DQA1*01:02-DQB1*06:02* haplotype is associated with NT1 in our Brazilian patients. Polysomnography was identified in *DQB1*06:02* positive subgroup rapid eye movement sleep latency ≤ 15 minutes, and all patients had 2 or more sleep-onset rapid eye movement periods at Multiple Sleep Latency Test.

Conclusions: This study showed a strong association between HLA-*DQB1*06:02* and the haplotype HLA-*DRB1*15:01-DQA1*01:02-DQB1*06:02* in patients with NT1. Patients with *DQB1*0602* allele showed shorter rapid eye movement sleep latencies at polysomnography. These results reinforce the suggestion of *DQB1* genotyping as relevant to narcolepsy screening.

Keywords: narcolepsy, cataplexy, HLA, polysomnography

Citation: Bacelar A, Fernandez O, Paradella E, Rodrigues RN, de Castro Moreno CR, Alvarenga R. Association between human leukocyte antigen class II-DR-DQ and narcolepsy: a case control study. *J Clin Sleep Med*. 2024;20(12):1945–1953.

BRIEF SUMMARY

Current Knowledge/Study Rationale: Our study is the first in Brazil to investigate the association between human leukocyte antigen class II-DR-DQ alleles in patients with narcolepsy type 1 and narcolepsy type 2 and a control group. We confirm the impact of genetic variants on narcolepsy risk within the Brazilian population.

Study Impact: Narcolepsy has been underdiagnosed, yet it can pose significant dangers, especially for certain populations such as professional drivers. Studies aimed at contributing to the diagnosis and treatment of this disorder, particularly when cataplexy is present, are essential to mitigate the risk of accidents.

INTRODUCTION

Narcolepsy is a neurologic disorder characterized by irresistible sleep attacks, hypnagogic/hypnopompic hallucinations, and sleep paralysis.[1] Although their etiology is unknown, the human leukocyte antigen (HLA) complex types HLA-*DQB1*06:02* and *DQA1*01:02* are those most susceptible to narcolepsy, and the *DRB1*15:01-DQA1*01:02-DQB1*06:02* haplotype (DR2) also have been reported to be associated with narcolepsy.[1–3] In a study conducted in Europe, for instance, a high odds ratio (equal to 251) of HLA-*DQB1*06:02* was found for narcolepsy.[4]

Twenty percent of the general population carry the same HLA-*DRB1*15:01 DQB1*06:02* haplotype, however, more individuals with narcolepsy are homozygous for this haplotype. HLA class II alleles other than *DQB1*06:02* influence susceptibility to narcolepsy-cataplexy.[1,5] In Saudi Arabian patients, the association between HLA-*DQB1*06:02* alleles frequency and narcolepsy type 1 (NT1) was above 80%.[6] The association between narcolepsy and *DQB1*06:02* and *DQA1*01:02* was found in non-*DRB1*15* patients with narcolepsy and cataplexy. Thus, *DQB1*06:02* and *DQA1*01:02* were found to be better markers than DR2 for narcolepsy across all ethnic groups.[7]

Mignot et al reported that *DQB1*0602* frequency was strikingly higher in patients with cataplexy (76.1%), and severe cataplexy (NT1) (94.8%) compared to patients without cataplexy (narcolepsy type 2 [NT2]) (40.9%). These results indicate that

the HLA association with narcolepsy is tightest when clearcut cataplexy is present.[7–9]

Comparing papers with patients with narcolepsy/cataplexy of various ethnic groups, such as Japanese, Korean, Caucasian African-American, and Chinese, we observe in common (1) the requirement of at least 1 copy of *DQA1*01:02-DQB1*06:02* (estimated at more than 98% of 70 cases); (2) a predisposing effect of *DQA1*01:02-DQB1*06:02* homozygosity or the presence of *DQB1*0301* in trans of *DQA1*01:02-DQB1*06:02*. The *DQB1*03:01* predisposing effect was particularly strong in the Chinese population, as well as in Mexican population with narcolepsy. *DQB1*06:02/DQB1*03:01* genotype was present in 15.6% of Mexican cases, conferring a high risk.[10,11]

Few papers have been published about narcolepsy and genetic susceptibility in Latin America. The overall positive rate for *DQB1*0602* was 65.6% and 67% of Mexican and Brazilian patients with narcolepsy/catalepsy, verified in a single publication from each country, respectively.[10,12]

In this sense, the primary aim of our study was to investigate the association of HLA class II-DR-DQ alleles in a sample of patients with NT1 and NT2 with a control group. The second aim was to compare the demographic, clinical, and laboratory characteristics patients with narcolepsy, with or without the *DQB1*06:02* allele.

METHODS

Ethics

The research protocol received approval from the Gaffree and Guinle University Hospital Ethical Committee, Federal University of Rio de Janeiro, Brazil, and all participants provided written informed consent to participate in the study.

Study site and patients-controls

To compare the expression of HLA types in patients with and without narcolepsy, we conducted a case-control study involving 21 patients with narcolepsy-cataplexy (NT1), 16 patients with narcolepsy and without cataplexy (NT2), and 100 controls participants. We considered proportions inequality (allocation ratio 2.7) for the sample size calculation, 2 independent groups (unconditional), an odds ratio of 2.9, a power sample of 80%, and an alpha error of 5%; the total sample size was 122 (G*Power software, Heinrich-Heine-Universität Düsseldorf, Germany). Most patients and controls self-reported being White (58% and 68%, respectively, $P > .31$).

Patients were recruited from the Sleep Disorder Center of Carlos Bacelar Clinic in Rio de Janeiro, Brazil, between July 2004 and July 2014, and the majority were female (64.9%/ mean age = 35 years old, 14–71 years old). They were diagnosed with narcolepsy according to the *International Classification of Sleep Disorders* criteria,[13] exhibiting excessive daytime sleepiness, scoring higher than 10 on the Epworth Sleepiness Scale, and experiencing rapid eye movement (REM)-related sleep events such as sleep paralysis, hypnagogic hallucinations, and/or cataplexy. No other sleep disorders were identified, and all patients had normal neurological examinations. Polysomnography (PSG) was conducted using the BNT-36 electroencephalogram amplifier (EMSA, Rio de Janeiro, Brazil) and scored according to the American Academy of Sleep Medicine rules and criteria.[13] The Multiple Sleep Latency Test (MSLT) was performed, with a mean sleep latency of ≤ 8 minutes and 2 or more sleep-onset REM periods (SOREMPs) considered suggestive of narcolepsy in our sample. Psychostimulant medications and antidepressant drugs, if used, were discontinued for at least 2 weeks before PSG and MSLT recordings.

Patients meeting the following inclusion criteria were enrolled: (1) NT1 or NT2 diagnosis based on clinical data and the results of the nocturnal PSG and the MSLT. Patients were excluded if they: (1) had a total sleep time of less than 6 hours the night before MSLT, (2) had an apnea-hypopnea index > 15 events/h and/or periodic limb movement index > 15 events/h, (3) had irregular sleep-wake cycles or insufficient sleep, or (4) a history of medication/substance use that could cause false-positive MSLT results.

Control participants had no personal or family history of narcolepsy and were selected from the hospitals. The individuals were healthy, with no history of associated diseases confirmed by HLA. Based on clinical history, the study coordinating physician carefully excluded individuals with excessive daytime sleepiness, other sleep disorders, circadian or mental disorders, medication, or substance abuse.

Genotyping

Genomic DNA was isolated from peripheral blood samples collected in ethylenediaminetetraacetic acid tubes, using a QIAamp 96 DNA Blood kit (Qiagen, Hilden, Germany). High-resolution HLA class II (DRB1, DQA1, and DQB1) typing was performed by polymerase chain reaction, followed by sequence-specific oligonucleotide probe hybridization (PCR-SSO Inno-Lipa, Innogenetics, Ghent, Belgium) or by polymerase chain reaction amplification with sequence-specific primers using the One Lambda kit (Canoga Park, California), according to the manufacturer's instructions. *DRB1*15/16* subtypes were identified with sequence-specific primers (Dynal AllSet SSPDRB1*15/16; Dynal Biotech Ltd., Bromborough, United Kingdom).

Statistical analyses

The clinical and HLA data were entered into a database and analyzed with SPSS11.5 for Windows (SPSS Inc., Chicago, Illinois). The allelic and haplotype frequencies in patients and controls were calculated by direct counting, and the significance of the association was determined using either the chi-square or 2-sided Fisher's exact test. Corrected P values were obtained using the Bonferroni method (P_c = probability value × the number of alleles compared). P values ≤ .05 were considered statistically significant. The genotypic frequency refers to the percentage of the sample population that expresses at least 1 allele. To estimate the strength of the association between the narcolepsy and HLA alleles, the odds ratios (ORs) were calculated by Woolf and Haldane's method modified, and 95% confidence intervals (CIs) measured the precision of the estimates.

To assess whether age, sex, ethnicity, cataplexy, sleep paralysis, hypnagogic hallucinations, mean REM sleep latency

(REML), mean sleep latency, and SOREMPs were associated with HLA-*DQB1*06:02*, a univariate logistic model was performed. All variables with $P < .20$ were tested on the multiple model (stepwise forward technique). The model fit was assessed by Akaike information criteria.

RESULTS

Genotypic frequencies

At the HLA-DRB1 locus, 40 different alleles were found, with the most common being: *DRB1*15:01* (35.1%) and *DRB1*03* (27%) in the narcolepsy group and *DRB1*11:01* (27%), *DRB1*07:01* (23%), and *DRB1*03:01* (22%) in the control group.

At the HLA-DQA1 locus, 12 alleles were identified, with *DQA1*05:01/03/05* (narcolepsy: 40.5%; controls: 53%) and *DQA1*01:02* (narcolepsy: 40.5%; controls: 38%) being the most common ones in both groups. *DQA1*03:01* was more common in narcolepsy group (32.4%) and *DQA1*02:01* (22%) in the control group. At the HLA-DQB1 locus, 18 alleles were identified. The most frequent alleles were *DQB1*06:02* (43.2%) in the narcolepsy group and *DQB1*03:01* (40%) in the control group. *DQB1*02:01* (narcolepsy: 21.6%; controls: 24%) and *DQB1*05:01* (narcolepsy: 27%; controls: 20%) alleles were observed in both groups. **Table S1** in the supplemental material shows the genotypic frequencies of the HLA class II alleles in patients with narcolepsy.

Table 1 presents the genotypic frequencies of HLA class II alleles associated with narcolepsy patients compared to controls, which were significant. Four HLA class II alleles showed significantly higher frequencies in the narcolepsy group than in the control group: *DRB1*15:01* (35.1% vs 8%), *DQA1*03:01* (32.4% vs 17%), *DQB1*06:02* (43.2% vs 18%), and *DQB1*04:01* (8.1% vs 0.0%). Three additional alleles showed significantly higher frequencies in the control group: *DRB1*07:01* (5.4% vs 23.0%), *DRB1*11:01* (2.7% vs 27%), and *DQB1*03:01* (16.2% vs 40%). After the Bonferroni correction, only the *DRB1*15:01* ($P_c < .01$) and the DQB1*06:02 ($P_c = .04$) were statistically significant (Figure 1).

Comparison between NT1, NT2, and controls

Table 1 lists the phenotypic frequencies of HLA class II alleles in the control group and patients with narcolepsy with or without cataplexy, which were significant. All alleles found are shown in **Table S2** in the supplemental material. The phenotype frequency of the HLA class II alleles differed between these groups. In the NT1 group, only 1 allele had a significantly higher frequency than in the NT2 group: *DQB1*06:02* (61.9% vs 18.8%). Compared to controls, *DQB1*06:02* (61.9% vs 18.0% in controls) and *DRB1*15:01* (47.6% vs 8.0%), had higher frequencies in the NT1 group (Table 1). These 2 alleles were statistically significant after the Bonferroni correction.

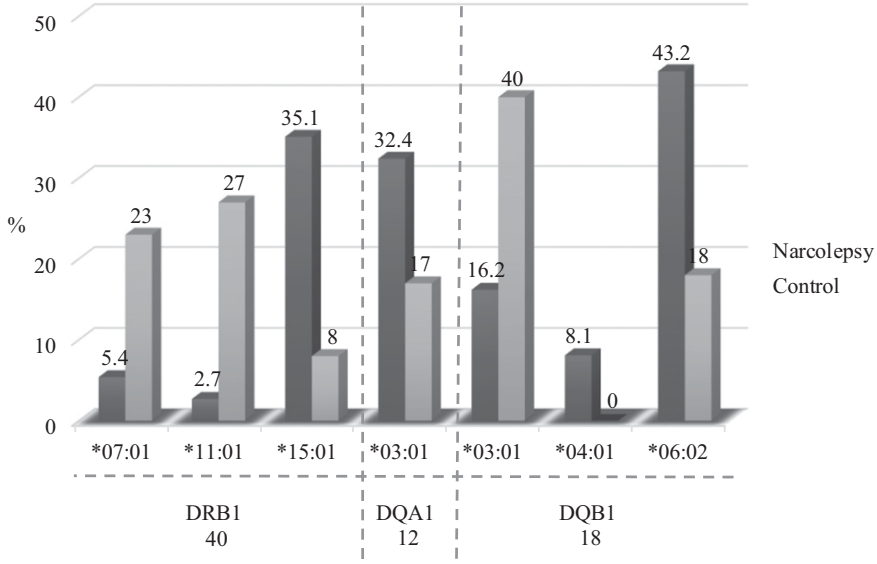
Six DR2 alleles were overrepresented in the NT2 group: *DRB1*01:02* (12.5% in NT2 vs 0% in control), *DRB1*04* (43.8% in NT2 vs 9.5% in NT1), *DRB1*16:02* (18.8% in NT2 vs 1.0% in control), *DQA1*05:02* (12.5% in NT2 vs 0.0% in control), *DQB1*03:04* (12.5% in NT2 vs 1.0% in control), and

Table 1—Phenotype frequencies of HLA class II DRB1, DQA1, and DQB1 alleles in control group, NT1, and NT2.

Alleles		NT1 (n = 21)	NT2 (n = 16)	Control (n = 100)	NT1 vs Control		NT2 vs Control		NT1 vs NT2	
					P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
DRB1 40	*01:02	0 (0.0)	2 (12.5)	0 (0.0)	—	—	.02	1.14 (0.95–1.38)	.18	0.88 (0.73–1.05)
	*04	2 (9.5)	7 (43.8)	14 (14.0)	.74	0.65 (0.14–3.09)	.01	4.78 (1.53–14.91)	.02	0.14 (0.02–0.79)
	*04:03	0 (0.0)	2 (12.5)	1 (1.0)	1.00	0.99 (0.97–1.01)	.05	14.14 (1.20–166.34)	.18	0.88 (0.73–1.05)
	*04:11	0 (0.0)	2 (12.5)	0 (0.0)	—	—	.02	1.14 (0.95–1.38)	.18	0.88 (0.73–1.05)
	*11:01	1 (4.8)	0 (0.0)	27 (27.0)	.04	0.14 (0.02–1.06)	.02	0.73 (0.65–0.82)	1.00	1.05 (0.95–1.16)
	*15:01	10 (47.6)	3 (18.8)	8 (8.0)	< .01	10.46 (3.41–32.06)	.18	2.65 (0.62–11.30)	.07	3.94 (0.86–18.01)
	*16:02	0 (0.0)	3 (18.8)	1 (1.0)	1.00	0.99 (0.97–1.01)	.02	22.85 (2.21–236.19)	.07	0.81 (0.64–1.03)
DQA1 12	*05:02	0 (0.0)	2 (12.5)	0 (0.0)	—	—	.02	1.14 (0.95–1.38)	.18	0.88 (0.73–1.05)
DQB1 18	*03:01	4 (19.0)	2 (12.5)	40 (40.0)	.07	0.35 (0.11–1.13)	.03	0.21 (0.05–0.99)	.68	1.65 (0.26–10.36)
	*03:04	0 (0.0)	2 (12.5)	1 (1.0)	1.00	0.99 (0.97–1.01)	.05	14.1 (1.20–166.34)	.18	0.88 (0.73–1.05)
	*04:01	1 (4.8)	2 (12.5)	0 (0.0)	.17	1.05 (0.95–1.16)	.02	1.14 (0.95–1.38)	.57	0.35 (0.03–4.25)
	*06:02	13 (61.9)	3 (18.8)	18 (18.0)	< .01	7.40 (2.68–20.48)	1.00	1.05 (0.27–4.08)	.01	7.04 (1.52–32.63)

CI = confidence interval, HLA = human leukocyte antigen, NT1 = narcolepsy type 1, NT2 = narcolepsy type 2, OR = odds ratio.

Figure 1—Phenotype frequency of HLA class II DRB1 40, DQA1 12, and DQB1 18 alleles in patients with narcolepsy vs the control group.



Alleles		Narcolepsy vs. Control	
		OR (95% CI)	p
DRB1 40	*07:01	0.19 (0.04-0.86)	0.01
	*11:01	0.08 (0.01-0.58)	<0.01
	*15:01	6.23 (2.32-16.74)	<0.01
DQA1 12	*03:01	2.34 (0.99-5.56)	0.05
DQB1 18	*03:01	0.29 (0.11-0.76)	<0.01
	*04:01	1.09 (0.99-1.20)	0.02
	*06:02	3.47 (1.52-7.93)	<0.01

CI = confidence interval, HLA = human leukocyte antigen, OR = odds ratio.

*DQB1*04:01* (12.5% in NT2 vs 0.0% in control) (**Table 1**). None of them were statistically significant after the Bonferroni correction.

Haplotype comparison between NT1, NT2, and controls

We tested 81 extended 3-locus haplotypes (**Table S3** in the supplemental material). Still, only 2 of them showed a statistically significant difference in the NT1 group vs control group, as shown in **Figure 2**: *DRB1*15:01-DQA1*01:02-DQB1*06:02* (16.7% in the NT1 group vs 4.0% in controls), *DRB1*13:01-DQA1*03:01-DQB1*06:02* (4.8% vs 0.0%). Only 1 haplotype showed significant differences between control and NT1 groups: *DRB1*11:01-DQA1*05:01/3/5-DQB1*03:01* (10.0% in controls vs 0.0% in NT1). No difference was found between the NT1 and NT2 haplotypes.

HLA DR/DQ typing in patients with narcolepsy with and without cataplexy

Almost 62 percent (13/21) of the NT1 group were *DQB1*06:02*-positive and 42.9% (9/21) were positive for both *DRB1*15:01* and *DQB1*06:02*. These rates were much higher than in the NT2 group. Additionally, 81% (13/16) of the NT2 group were *DQB1*06:02*-negative and 75% (12/16) were negative for both *DRB1*15:01* and *DQB1*06:02*, which is much higher than in the NT1 group (**Figure 3**).

Characteristics of patients with narcolepsy and influence of HLA-*DQB1*06:02* on patient characteristics

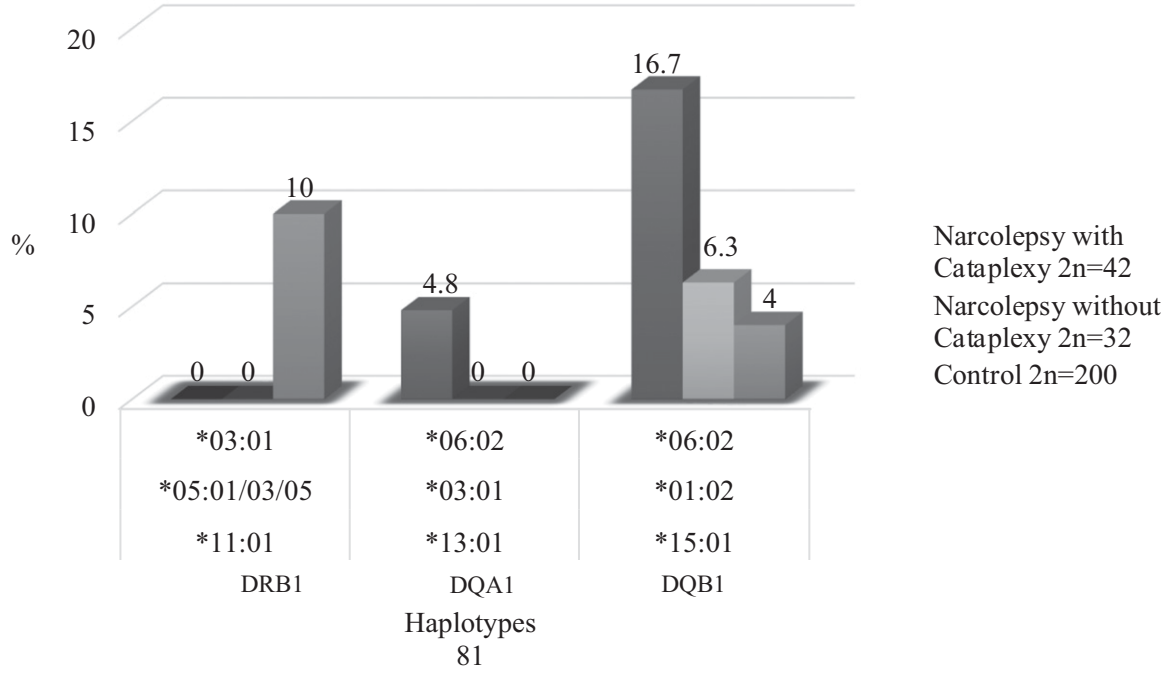
Of the 16 patients with NT2, 2 had SOREMPs during the nocturnal PSG; however, in the MSLT, one had 2 SOREMPs and the other had 3 SOREMPs. This was the criterion used for the diagnosis of narcolepsy. In the initial questionnaire, before recommending the neurophysiological evaluation, we asked patients when their symptoms began. Of the 16 patients with NT2, only 4 had less than 10 years of symptoms, and 2 of these were HLA-*DQB1*06:02* positive, making it relevant to consider the possibility that these 2 patients might develop cataplexy in the future. We did not find differences in the means of mean sleep latency and SOREMPs between NT1 and NT2 (**Table 2**). The average duration of symptoms for patients with NT2 was 13.8 years.

We compared the demographic, clinical, and laboratorial characteristics between narcolepsy patients with or without *DQB1*06:02* allele. The subgroups did not differ in terms of age, sex, and clinical characteristics. Patients who self-reported being White were more frequent in both *DQB1*06:02* positive and negative subgroups. However, the *DQB1*06:02* positive subgroup had a significantly larger difference: 2 non-White to 14 White (**Table 3**).

*DQB1*06:02* analysis showed that 13/16 patients with *DQB1*06:02* are patients with NT1 and that 13/21 patients without the allele are patients with NT2. The REML observed in the PSG was lower in the *DQB1*06:02* positive subgroup and had more SOREMPs in the MSLT in comparison with the *DQB1*06:02* negative subgroup, as shown in **Table 3**.

The univariate model showed that HLA-*DQB1*06:02* was associated with self-reported skin color, cataplexy, REML (in the PSG), mean sleep latency and SOREMPs (in the MSLT). Patients who self-reported being White are 6.36 more likely (95% CI = 1.15–35.23) to be HLA-*DQB1*06:02* positive than patients who self-reported being non-White. Individuals have a higher chance of being HLA-*DQB1*06:02* positive if

Figure 2—Frequency of HLA class II DRB1-DQA1-DQB1 haplotypes in NT1, NT2 vs control group.



Haplotypes			Narcolepsy with Cataplexy vs. Control		Narcolepsy without Cataplexy vs. Control		Narcolepsy with Cataplexy vs. Narcolepsy without Cataplexy	
81								
DRB1	DQA1	DQB1	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
*11:01	*05:01/03/05	*03:01	0.90 (0.86-0.94)	0.03	0.90 (0.86-0.94)	0.09	-	-
*13:01	*03:01	*06:02	1.05 (0.98-1.12)	0.03	-	-	1.05 (0.98-1.12)	0.50
*15:01	*01:02	*06:02	4.80 (1.64-14.08)	0.01	1.60 (0.32-7.90)	0.63	3.00 (0.58-15.55)	0.28

CI = confidence interval, HLA = human leukocyte antigen, NT1 = narcolepsy type 1 (with cataplexy), NT2 = narcolepsy type 2 (without cataplexy), OR = odds ratio.

they have cataplexy (OR = 7.04; 95% CI = 1.52–32.63), mean REML ≤ 15 minutes (OR = 20.00; 95% CI = 2.14–186.87), mean sleep latency ≤ 5 (OR = 0.21; 95% CI = 0.04–1.03) and > 2 SOREMPs (OR = 5.46; 95% CI = 1.26–23.77). The multiple analyses showed that HLA-*DQB1*06:02* was associated with cataplexy, and REML (P = .04 and .02, respectively).

DISCUSSION

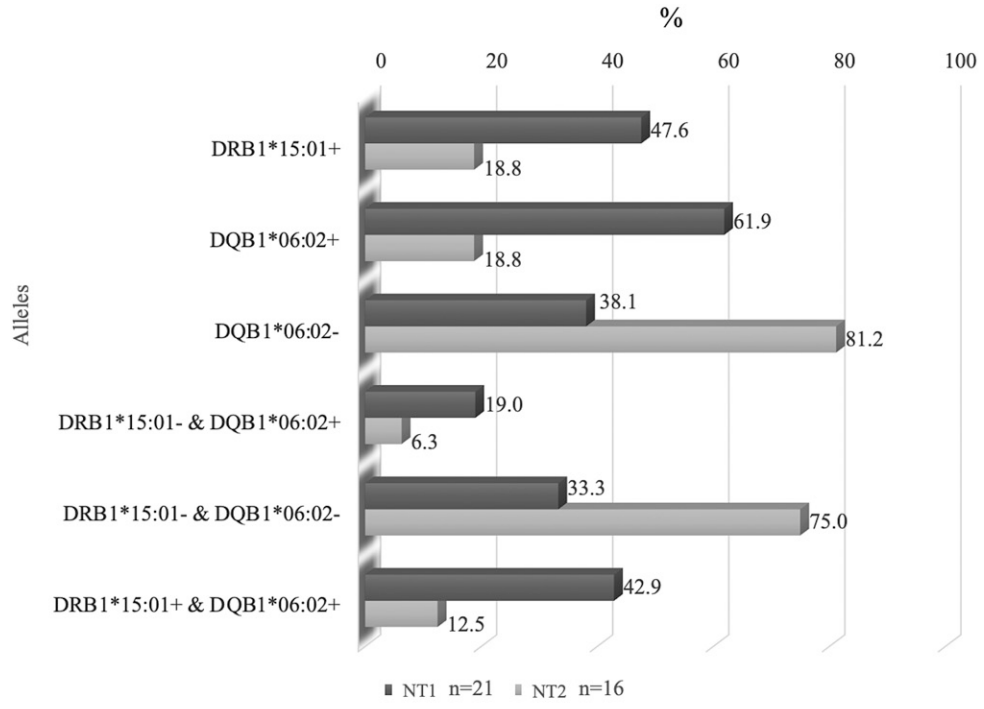
Our study is the first in Brazil to examine the association of HLA class II-DR-DQ alleles in a sample of patients with NT1 and NT2 with a control group. In our group, the alleles *DRB1*15:01* and *DQB1*06:02* were statistically significant in patients with narcolepsy compared to controls. Moreover, *DQB1*06:02* had a significantly higher frequency in the NT1 group than in the NT2 group. Two haplotypes, *DRB1*15:01-DQA1*01:02-DQB1*06:02* and *DRB1*13:01-DQA1*03:01-DQB1*06:02* showed statistically significant differences in patients with NT1 compared to control group. Individuals have a higher chance of being HLA-*DQB1*06:02* positive if they have cataplexy, mean REML ≤ 15 minutes and > 2 SOREMPs.

We reported that *DQB1*06:02* and *DRB1*15:01* showed the strongest association with narcolepsy in our sample. Patients who self-reported being White are 6.36 more likely to be HLA-*DQB1*06:02* positive than patients who self-reported being non-White. Sixteen of 37 DQB1-typed patients were positive for *DQB1*06:02* (allele frequency = 43.2% vs 18% in the controls) and 13/37 were positive for *DRB1*15:01* (allele frequency = 35.1% vs 8% in the controls).

HLA typing confirmed that HLA-*DQB1*06:02* is more commonly found in patients with NT1 than in controls or in patients with NT2. These results indicate that the association of this HLA type with narcolepsy is tighter when clear-cut cataplexy is present.[7]

Mignot et al[8] reported that *DQB1*06:02* frequency was remarkably higher in patients with NT1 (76.1%) compared to NT2 (40.9%), and even higher in patients with severe cataplexy

Figure 3—Positive HLA DR/DQ typing in NT1 and NT2.



Alleles	NT1 vs.NT2	
	OR (95% CI)	p
DRB1*15:01+	3.94 (0.86-18.01)	0.07
DQB1*06:02+	7.04 (1.52-32.63)	0.01
DQB1*06:02-	0.14 (0.03-0.66)	0.01
DRB1*15:01- & DQB1*06:02+	3.53 (0.35-35.16)	0.36
DRB1*15:01- & DQB1*06:02-	0.17 (0.04-0.71)	0.01
DRB1*15:01+ & DQB1*06:02+	5.25 (0.95-29.18)	0.07

CI = confidence interval, HLA = human leukocyte antigen, NT1 = narcolepsy type 1, NT2 = narcolepsy type 2, OR = odds ratio.

(94.8%). Our study showed that this allele is present in 61.9% of patients with NT1 and only 18.8% of patients with NT2. Multiple analysis has shown that patients with NT1 have 7.04 more chance to be HLA-*DQB1*06:02* positive. In addition, 81% of patients NT2 vs 38% of patients NT1 did not have the *DQB1*06:02* allele. It has been demonstrated that in patients with clear-cut cataplexy, approximately 10% are *DQB1*06:02* negative.[9] This may result from the HLA distribution diversity across different ethnic groups, the heterogeneity of narcolepsy, and the strictness of current diagnostic criteria.

When the presence of HLA-DR/-DQ was analyzed, we showed that 42.9% of the patients with NT1 were positive for both *DRB1*15:01* and *DQB1*06:02*, which is much more common than in patients with NT2, despite the absence of statistical significance. HLA-*DRB1*1501*, **15:03* (African American)[9] and *DQB1*06:02* alleles are known to be associated with NT1 in different ethnic groups, and the association is the strongest among Asians, especially in the Japanese.[9,14]

Some authors found that ethnicity influences the frequency of *DRB1*15* in patients with NT1 (67.2% in African patients, 84.5% in Caucasian patients, and 95.8% in Asian patients).[7,9] Our results show that 47.6% (10/21) of patients with NT1 and 18.8% (3/16) of patients with NT2 were *DRB1*15:01* positive. Only 2 of our patients who self-reported being non-White had *DRB1*15:03* allele. A limitation of our study is that we do not have information about the ethnicity of our participants, preventing us from making this association.

Association among narcolepsy and *DQB1*06:02* and *DQA1*0102* was found in non-*DRB1*15* (**1501*) NT1 patients.[15,16] Increased narcolepsy risk was associated with homozygotes for these alleles.[17] Although our sample included 15 patients with *DQA1*01:02* allele, 3 of them homozygotes,

Table 2—Mean sleep latency and SOREMPs of NTI and NT2.

Variables	Groups	Mean (SD)	95% CI	*P* (Mann-Whitney)
Mean sleep latency (min)	NT1 (n = 21)	3.73 (2.22)	2.78–4.68	.89
	NT2 (n = 16)	3.43 (1.94)	2.48–4.38	
SOREMPs (times)	NT1 (n = 21)	2.52 (0.68)	2.23–2.81	.29
	NT2 (n = 16)	2.31 (0.60)	2.02–2.61	

CI = confidence interval, NT1 = narcolepsy type 1, NT2 = narcolepsy type 2, SD = standard deviation, SOREMP = sleep-onset rapid eye movement period.

Table 3—PSG parameters to the presence of DQB1*06:02.

		Narcolepsy with HLA-DQB1*06:02 Positive	Narcolepsy with HLA-DQB1*06:02 Negative	HLA-DQB1*06:02 Positive vs HLA-DQB1*06:02 Negative		
		n = 16	n = 21	*P*	P_c	OR (95% CI)
Demographic Variables						
Age, years, median (range)		29.5 (18–72)	33 (14–53)	.54	NS	(−12.23 to 6.54)
Sex, M:F ratio		05:11	08:13	.67	NS	(−0.26 to 0.40)
Self-reported skin color: non-White:White ratio		02:14	10:11	.02	NS	(−0.65 to −0.05)
Clinical Features						
Cataplexy	With	13 (81.3)	8 (38.1)	.01	NS	7.04 (1.52 to 32.63)
	Without	3 (18.8)	13 (61.9)	.01	NS	0.14 (0.03 to 0.66)
Sleep paralysis		11	16	.72	NS	0.69 (0.16 to 2.96)
Hypnagogic hallucination		13	18	1.00	NS	0.72 (0.13 to 4.17)
Nocturnal PSG						
Sleep latency ≤ 15 minutes		11	17	.46	NS	1.93 (0.42 to 8.81)
REM sleep latency ≤ 15 minutes		8	1	< .01	NS	0.05 (0.01 to 0.47)
Microarousals > 10 events/h		9	12	.96	NS	0.96 (0.26 to 3.58)
MSLT						
Mean sleep latency	≤ 5 minutes	9	18	.07	NS	0.21 (0.05 to 1.03)
	> 5 to ≤ 8 minutes	7	3	.07	NS	4.67 (0.97 to 22.46)
SOREMPs	= 2 times	7	17	.02	NS	0.18 (0.04 to 0.80)
	> 2 times	9	4	.02	NS	5.46 (1.26 to 23.77)

CI = confidence interval, F = female, HLA = human leukocyte antigen. M = male, MSLT = Multiple Sleep Latency Test, NS = non-significant, OR = odds ratio, PSG = polysomnography, REM = rapid eye movement, SOREMP = sleep-onset rapid eye movement period.

no statistical difference was found, compared with the control group (40% vs 38%). It was not possible to demonstrate that this allele alone is necessary for susceptibility to narcolepsy, and *DQB1*06:02* and *DQA1*01:02* alleles together, when present, were also accompanied by *DRB1*15:01* allele. Although different HLA alleles were linked with the narcolepsy risk, there is still no consensus about specific contributions of alleles other than *DRB1*1501* and *DQB1*06:02*.[18,19]

It has been reported that several genes have been implicated in narcolepsy risk, and mainly the aggregate effects of genetic variants on narcolepsy risk, which has been called the genetic risk score .[20] In spite of this, we confirm the association of the HLA-*DRB1*15:01-DQA1*01:02-DQB1*06:02* haplotype with NT1 ($P < .01$; OR = 4.80), already described in Chinese and Japanese patients, where 15% of these patients were typed as *DRB1*15:01-DQA1*01:02-DQB1*06:02* homozygotes.[21,22] In our study, 77% (7/9) of the patients that had the *HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02* haplotype also had cataplexy. Other haplotype investigated here that has shown statistical difference between patients with NT1 and controls, was the *DRB1*13:01-DQA1*03:01-DQB1*06:02* ($P = .03$; OR = 1.05). To our knowledge, it has not been previously described.

Narcolepsy disease characteristics and symptom severity measures varied dose-response depending on HLA-*DQB1*06:02* status,[23] which was also true for sleepiness as measured by nap propensity and the Epworth Sleepiness Scale. Sleep paralysis and hypnagogic hallucinations, despite being part of the narcolepsy pentad, have been considered to have low diagnostic value,[22,24] and they were removed from the *International Classification of Sleep Disorders*, second edition, diagnostic criteria due to insufficient specificity for narcolepsy.[9] We found no significant difference when we compared these symptoms with the *DQB1*06:02* status. Two patients who were homozygous for *DQB1*06:02* had increased clinical and neurophysiological severity of the disease. *DQB1*06:02* homozygotes had a 2- to 4-fold increased risk of developing NT1 compared to the heterozygous.[25]

PSG showed that an increased number of patients had REML ≤ 15 minutes in *DQB1*06:02* positive subgroup ($P < .01$, OR = 0.05, 95% CI = 0.01–0.47), as well as more SOREMPs in the MSLT, compared with the *DQB1*06:02* negative subgroup ($P = .02$, OR = 5.46, 95% CI = 1.26–23.77). This agrees with previous studies.[22,25,26]

Andlauer et al[25] showed that among patients being evaluated for possible narcolepsy short REML (≤ 15 minutes) at PSG had high specificity and positive predictive value and may be enough for the diagnosis without the use of MSLT. Probably for this reason, in 2023, the *International Classification of Sleep Disorders*, third edition, text revision, already classifies as NT1 if REML is < 15 minutes on PSG, obviating the need for MLST. For the diagnosis of NT2, only 1 SOREMP on

MSLT is required if REML < 15 minutes on PSG. In addition, it is extremely important in clinical practice allows the diagnosis of NT2 without cerebrospinal fluid hypocretin-1 level. In our study, we have no data regarding CSF hypocretin-1 concentration as the cerebrospinal fluid punction is an invasive procedure, and difficult to perform among volunteers, which prevents the Ethical Committee to approve it for research goals. This classification suggests reduced hypocretin levels are sufficient for NT1 diagnosis. Although we suggest that differences in sleep parameters may exist between patients depending on *DRB1*1501, DQA1*0102,* and *DQB1*06:02* status, further studies are necessary to confirm the relationship of HLA alleles with clinical and PSG findings in patients with narcolepsy.

CONCLUSIONS

This study revealed a robust association between HLA-*DQB1*0602* and the haplotype HLA-*DRB1*15:01-DQA1*01:02-DQB1*06:02* in patients with NT1. Patients with *DQB1*0602* allele showed shorter REMLs at PSG. Thus, PSG may be considered as an important tool for diagnostic as it has a high specificity and positive predictive value.

In conclusion, we recommend that complete HLA typing be performed with larger samples to characterize the complex HLA-DQ and HLA-DR interactions. In addition, DQB1 genotyping may be relevant to narcolepsy screening.

ABBREVIATIONS

CI, confidence intervals
HLA, human leukocyte antigen
MSLT, Multiple Sleep Latency Test
NT1, narcolepsy type 1
NT2, narcolepsy type 2
OR, odds ratio
PSG, polysomnography
REM, rapid eye movement
REML, rapid eye movement sleep latency
SOREMP, sleep-onset rapid eye movement period

REFERENCES

1. Mignot E, Lin L, Rogers W, et al. Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. *Am J Hum Genet.* 2001;68(3): 686–699.
2. Juji T, Satake M, Honda Y, Doi Y. HLA antigens in Japanese patients with narcolepsy. All the patients were DR2 positive. *Tissue Antigens.* 1984;24(5): 316–319.
3. Lin L, Hungs M, Mignot E. Narcolepsy and the HLA region. *J Neuroimmunol.* 2001;117(1-2):9–20.
4. Tafti M, Hor H, Dauvilliers Y, et al. DQB1 locus alone explains most of the risk and protection in narcolepsy with cataplexy in Europe. *Sleep.* 2014;37(1):19–25.
5. Hor H, Kutalik Z, Dauvilliers Y, et al. Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy. Published correction appears in *Nat Genet*. 2011;43(4):388. *Nat Genet.* 2010;42(9): 786–789.
6. Al Shareef SM, AlAnbay E, AlKhathlan MA, et al. HLA-DQB1*06:02 allele frequency and clinic-polysomnographic features in Saudi Arabian patients with narcolepsy. *Sleep Breath.* 2019;23(1):303–309.
7. Roh EY, Park MH, Park H, et al. Association of HLA-DR and -DQ genes with narcolepsy in Koreans: comparison with two control groups, randomly selected subjects and DRB1*1501-DQB1*0602–positive subjects. *Hum Immunol.* 2006; 67(9):749–755.
8. Mignot E, Hayduk R, Black J, Grumet FC, Guilleminault C. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. *Sleep.* 1997;20(11): 1012–1020.
9. Rogers AE, Meehan J, Guilleminault C, Grumet FC, Mignot E. HLA DR15 (DR2) and DQB1*0602 typing studies in 188 narcoleptic patients with cataplexy. *Neurology.* 1997;48(6):1550–1556.
10. Alaez C, Lin L, Flores-A H, et al. Association of narcolepsy-cataplexy with HLA-DRB1 and DQB1 in Mexican patients: a relationship between HLA and gender is suggested. *BMC Med Genet.* 2008;9:79.
11. Han F, Lin L, Li J, et al. HLA-DQ association and allele competition in Chinese narcolepsy. *Tissue Antigens.* 2012;80(4):328–335.
12. Coelho FM, Pradella-Hallinan M, Pedrazzoli M, et al. Traditional biomarkers in narcolepsy: experience of a Brazilian sleep centre. *Arq Neuropsiquiatr.* 2010; 68(5):712–715.
13. American Academy of Sleep Medicine. *International Classification of Sleep Disorders*. 3rd ed. Darien, IL: American Academy of Sleep Medicine; 2014.
14. Kuwata S, Juji T, Sasaki T, et al. The first international collaborative study on narcolepsy. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991, Proceedings of the Eleventh International Histocompatibility Workshop and Conference Held in Yokohama.* New York, NY: Oxford University Press; 1992: 730–734.
15. Mignot E, Kimura A, Lattermann A, et al. Extensive HLA class II studies in 58 non-DRB1*15 (DR2) narcoleptic patients with cataplexy. *Tissue Antigens.* 1997; 49(4):329–341.
16. Mignot E, Kimura A, Lattermann A, et al. HLA class II studies in non DRB1*1501 patients with narcolepsy cataplexy. In: Charron D, ed. *Genetic Diversity of HLA: Function and Medical Implication.* Paris, France: EDK; 1997:436–441.
17. Weiner Lachmi K, Lin L, Kornum BR, et al. DQB1*06:02 allele-specific expression varies by allelic dosage, not narcolepsy status. *Hum Immunol.* 2012;73(4): 405–410.
18. Planelles D, Puig N, Beneto A, et al. HLA-DQA, -DQB and -DRB allele contribution to narcolepsy susceptibility. *Eur J Immunogenet.* 1997;24(6):409–421.
19. Hohjoh H, Terada N, Honda Y, Juji T, Tokunaga K. Negative association of the HLA-DRB1*1502-DQB1*0601 haplotype with human narcolepsy. *Immunogenetics.* 2001;52(3-4):299–301.
20. Ouyang H, Han F, Zhou Z, Zheng Q, Wang Y, Zhang J. Association between genetic risk scores and risk of narcolepsy: a case-control study. *Ann Transl Med.* 2020;8(4):103.
21. Trachtenberg E, Vinson M, Hayes E, et al. HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1, DPB1) alleles and haplotypes in the Han from Southern China. *Tissue Antigens.* 2007;70(6):455–463.
22. Woo HI, Joo EY, Hong SB, Lee KW, Kang ES. Use of PCR with sequence-specific primers for high-resolution human leukocyte antigen typing of patients with narcolepsy. *Ann Lab Med.* 2012;32(1):57–65.
23. Watson NF, Ton TG, Koepsell TD, Gersuk VH, Longstreth WT Jr. Does narcolepsy symptom severity vary according to HLA-DQB1*0602 allele status? *Sleep.* 2010; 33(1):29–35.
24. Thorpy MJ, Goswami M. Treatment of narcolepsy. In: Thorpy M, ed. *Handbook of Sleep Disorders*. New York, NY: Marcel Dekker; 1990:235–257.
25. Andlauer O, Moore H, Jouhier L, et al. Nocturnal rapid eye movement sleep latency for identifying patients with narcolepsy/hypocretin deficiency. Published correction appears in *JAMA Neurol*. 2013;70(10):1332. *JAMA Neurol.* 2013;70(7): 891–902.
26. Hong SC, Hayduk R, Lim J, Mignot E. Clinical and polysomnographic features in DQB1*0602 positive and negative narcolepsy patients: results from the modafinil clinical trial. *Sleep Med.* 2000;1(1):33–39.

ACKNOWLEDGMENTS

The authors thank Libbs Pharmaceutical Ltd. for providing the HLA genotyping kit and Dr. Oscar Fernandez from the Genetic Lab, Carlos Haya University Hospital, Málaga, Spain, and Dr. Eduardo Paradela from PPGNeuro and Brazilian Institute of Medical Rehabilitation, Brazil, for HLA typing. Furthermore, the authors thank Carlos Bacelar Clinic, Rio de Janeiro, Brazil, for conducting all polysomnography examinations in their Sleep Lab.

SUBMISSION & CORRESPONDENCE INFORMATION

Submitted for publication April 18, 2024
Submitted in final revised form July 16, 2024
Accepted for publication July 17, 2024
Address correspondence to: Andrea Bacelar, MD, PhD, São Francisco Xavier, 150, Rio de Janeiro-RJ, CEP 20550012, Brazil; Tel: +5521 25741650; Email: clinica@bacelar.com.br

DISCLOSURE STATEMENT

All authors have seen and approved the manuscript. All authors report the absence of financial support and no conflicts of interest.