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HLA-DQB1*06:02 allele frequency and clinic-polysomnographic features in Saudi Arabian patients with narcolepsy

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Abstract

Background Narcolepsy is an uncommon neurological disorder characterised by irresistible spells of sleep associated with abnormal rapid eye movement (REM) sleep. The association between narcolepsy and human leukocyte antigen HLA-DQB1*06:02 has been established elsewhere but remains to be investigated among Saudi Arabian patients with narcolepsy.

Methods A total of 29 Saudi patients with type I or type 2 narcolepsy comprising of 23 (79%) males and 6 (21%) females with a mean age of 17.2 ± 9.6 years were included in this study. Type 1 or type 2 narcolepsy was diagnosed by full polysomnography followed by a multiple sleep latency test in accordance with International Classifications of Sleep Disorders-3 criteria. HLA typing for DQB1 alleles was performed by polymerase chain reaction and hybridization with sequence-specific oligonucleotide probes. Differences in clinical and sleep parameters were compared by univariable analyses. HLA-DQB1*06:02 frequency was systematically compared with the published literature.

Results Type 1 narcolepsy was diagnosed in 19/29 (65.5%) patients, whereas 10/29 (34.5%) patients had type 2 narcolepsy. DQB1*06:02 was present in 25/29 (86.2%) patients; 15/19 (78.9%) narcolepsy type 1 patients and 10/10 (100%) narcolepsy type 2 patients harboured the DQB1*06:02 allele. REM latency was significantly lower in DQB1*06:02-positive patients compared to DQB1*06:02-negative patients (17.6 ± 32.3 min vs. 106.0 ± 86.0 min; $p = 0.025$). Epworth Sleepiness Scale scores were significantly higher among type 1 than type 2 narcolepsy patients (19.7 ± 3.2 vs 15.3 ± 3.6; $p = 0.02$).

Conclusions DQB1*06:02 allele frequencies among Saudi patients with narcolepsy were consistent with previously published data.

Keywords Sleep · Hypersomnia · Haplotype · Sleep studies · Autoimmune disease, DQB1*06:02

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Introduction

Narcolepsy is an uncommon neurological disorder characterised by an irrepressible need to sleep and pathological rapid eye movement (REM) sleep [1, 2]. This disorder is frequently under-recognised by physicians [3] most likely due to the fact that about a quarter of patients with narcolepsy show the classical clinical tetrad of compulsive sleep attacks, cataplexy, hypnagogic hallucinations and sleep paralysis [2, 4]. Narcolepsy is a multifactorial disorder with both genetic and environmental components; there is 20–30% concordance for narcolepsy between monozygotic twins, and 1–2% of first-degree relatives of affected individuals also develop the disease [5]. Hypocretin-1 (orexin-A) is usually reduced or undetectable in the cerebrospinal fluid of narcoleptic patients, and the hypothalami of narcoleptic individuals show a severe deficiency of hypocretin-producing neurons [6, 7]. Over 30 years

ago, narcolepsy with cataplexy was shown to have a strong association with HLA (DR2) [8]. Subsequent studies in the 1990s revealed familial and trans-ethnic associations with the HLA-DQB1*06:02 haplotype [5, 9, 10]. In addition, circulating anti-Tribbles-2-specific antibodies thought to target hypocretin-secreting neurons have also been detected among the affected individuals [11–14]. Although the mechanisms involved in the causation of narcolepsy remain elusive, the presence of auto-antibodies, HLA associations and familial predisposition points to an autoimmune pathogenesis.

Despite the substantial body of literature on narcolepsy, little is known about this disorder among Arab patients. The prevalence of narcolepsy in Saudi Arabia is approximately 40/100,000 [4], in line with the reported prevalence of 25–50/100,000 in other countries and ethnic groups [2]. We previously reported that the clinical presentation of narcolepsy in Saudi Arabia is no different from the clinical presentation elsewhere with a greater prevalence among males than females, narcolepsy without cataplexy in about a quarter of patients, and greater disturbances in quality of sleep among individuals with cataplexy [4]. Nevertheless, the frequency of HLA-DQB1*06:02 positivity in Arab patients with narcolepsy is yet to be reported. This study was performed to assess the prevalence of HLA-DQB1*06:02 haplotype among patients with narcolepsy and its association with other demographic and sleep study variables. Furthermore, we performed a comprehensive review of the literature on human subjects with narcolepsy in which DQB1*06:02 genotyping was performed to compare our genotyping data and assess for any possible ethnic differences.

Patients and methods

Twenty-nine patients with narcolepsy were included in the study. Data were collected from patients attending two tertiary sleep centres: The University Sleep Disorders Centre (USDC) at King Saud University and the Paediatrics Sleep Disorders Centre (PSDC) at Prince Sultan Military Medical City in Riyadh, Saudi Arabia. The USDC accepts referrals from all age groups from across Saudi Arabia, while the PSDC accepts sleep disorder cases from the paediatric population from all military health care services in Saudi Arabia.

Diagnosis of narcolepsy was based on the third version of the International Classifications of Sleep Disorders Diagnostic (ICSD-3) [15]. Each patient was rechecked and verified by a sleep medicine specialist after full polysomnography followed by a multiple sleep latency test (MSLT) in accordance with the ICSD-3 [15]. Briefly, in addition to a subjective complaint of sleepiness, narcolepsy type 1 was diagnosed by a mean latency of < 8 min on MSLT with evidence of sleep-onset rapid eye movement periods (SOREMPs) and clear cataplexy [defined as "more than one episode of generally brief (< 2 min), usually bilaterally symmetrical, sudden loss of muscle tone with retained consciousness"]. Narcolepsy type 2 included similar MSLT requirements of a mean latency < 8 min and two SOREMPs (or one SOREMP on PSG and one or more on MSLT) but without cataplexy.

All patients (or their guardians) provided written informed consent for inclusion in the study. This study was approved by the Institutional Review Board of King Saud University.

Investigations

All participants underwent full history and examination by a sleep specialist, nocturnal polysomnography and MSLT. Clinical interviews included reports on the presence or absence of sleepiness, cataplexy, hypnagogic and hypnopomic hallucinations, sleep paralysis, nightmares, and sleep interruptions. The Epworth Sleepiness Scale (ESS) was used to assess daytime sleepiness. The ESS is a validated questionnaire consisting of eight items that assess the likelihood of falling asleep during a variety of daily living situations [16].

Sleep studies

Level 1 attended nocturnal sleep studies were performed at the University Sleep Disorders Centre (USDC) at King Saud University and the Pediatrics Sleep Disorders Centre (PSDC) at the Prince Sultan Military Medical City in Riyadh, Saudi Arabia, as described previously [17, 18]. Alice 5 and 6 Laboratory Diagnostic Equipment (Respironics, Inc., Murrysville, PA) was used for acquisition of standard polysomnography data with four leads for electroencephalography (F4/M1, F3-M2, C4-M1, C3-M2 and O1-M2, O2-M1), electrooculography (EOG), chin electromyography (EMG), electrocardiography (EKG), oxygen saturation, chest and abdominal wall movements, airflow (thermistor and nasal pressure), and sleep position. An MSLT with five nap tests was performed according to standard recommendations to determine sleep latency and sleep-onset REM periods (SOREMPs) [19, 20]. A certified polysomnography technologist scored the data manually in a blinded fashion.

HLA-DQ typing by reverse sequence-specific oligonucleotide probe

HLA typing for DQB1 alleles was performed for all participants by PCR and hybridization with sequence-specific oligonucleotide probes (SSOPs). DNA extraction and purification were performed using the QIAmp DNA Mini and QIAmp Blood Mini Kit (Qiagen, Hilden, Germany). HLA-DQ typing by SSOP was performed using the Luminex™/One Lambda LABType™ RSSO according to the manufacturer's instructions (Luminex, Austin, TX). The method involves use of Luminex™ microspheres, incorporated with infrared and red

dyes to assign each microsphere a unique spectral address allowing 100 different bead classifications. Each bead classification is bound with a particular oligonucleotide probe; PCR was performed to amplify the DNA samples using biotinylated primers. After amplification, the PCR products were hybridised to a mixture of microspheres carrying a predetermined set of oligonucleotide probes. PCR products were allowed to detect complimentary sequences and bind with the specific probe (bead). Individual sample reactions were washed to remove unbound PCR products. Samples were stained with R-phycoerythrin (PE)-conjugated streptavidin, which binds to the biotinylated primer, and finally, each sample was processed in Luminex™ flow analyser. The fluorescent intensity of PE on each microsphere classification was translated into a positive or negative reaction. HLA assignment was based on the reaction pattern and analysed using current HLA sequence data. Analysis was performed using One Lambda LABType Visual Software (Thermo Fisher Scientific, Waltham, MA). All samples testing positive for DQB1*06 alleles were further resolved using sequence-specific primers (SSP) by One Lambda SSP High-resolution Procedure for detection of DQB1*06:02 allele.

Literature search

The PubMed database was searched until March 2018 for all articles on HLA typing in narcolepsy using the search terms: ("HLA"[Journal] OR "hla"[All Fields]) AND ("narcolepsy"[MeSH Terms] OR "narcolepsy"[All Fields]) AND DQB1 [All Fields]. Inclusion criteria were studies in human subjects with narcolepsy (with or without cataplexy) in which DQB1*06:02 genotyping was performed. A total of 298 articles were retrieved. Where more than one study represented the same (or subset of a) cohort, the largest population was compared. After application of the inclusion criteria, 13 studies were selected for comparison [21–33].

Data analysis

Data were analysed using SPSS Statistics v23 (IBM Statistics, Chicago, IL) computer software. Measures are reported as the mean ± standard deviation (SD) or percentages as appropriate. Data were compared using the Mann-Whitney test or chi-squared test for continuous or categorical variables, respectively. A p value either equal to or less than 0.05 was considered statistically significant.

Results

The patient demographics and sleep characteristics are shown in Table 1. The mean age of the study population was 17.2 ± 9.6 years, and the majority (23/29, 79.3%) of patients were males. The mean age of onset was 12.9 ± 7.8 years with a mean age at the time of diagnosis of 17.4 ± 9.9 years. Out of the 29 patients, 10 (34.5%) were being treated with modafinil. The average ESS score was 19.9 ± 4.0, denoting "excessively sleepy" status in the majority of cases. Table 2 shows data for the polysomnographic characteristics of the study group.

Out of the total number of patients, 19 (65.5%) had narcolepsy type 1, and narcolepsy type 2 was present in 10/29 (34.5%). DQB1*06:02 was present in 25/29 (86.2%) patients, and 15/19 (78.9%) narcolepsy type 1 patients harboured DQB1*06:02 vs. 10/10 (100%) narcolepsy type 2 patients; the difference, however, was not statistically significant (chi-squared test $p = 0.12$).

Differences in all clinical and sleep parameters were compared between (i) DQB1*06:02-positive patients and DQB1*06:02-negative patients; and (ii) type 1 and type 2 patients by univariate analyses. The most striking difference was a significantly lower REM latency during PSG in the DQB1*06:02-positive patients compared to DQB1*06:02-negative patients (17.6 ± 32.3 min vs. 106.0 ± 86.0 min; $p = 0.03$; Table 2). Furthermore, sleep paralysis was not present in 3/4 DQB1*06:02-negative patients compared to 4/25 DQB1*06:02-positive patients ($p = 0.01$; Table 1). All DQB1*06:02-negative patients were on medication. With respect to differences between type 1 and type 2 patients, ESS scores (taken at baseline prior to any medication) were significantly higher in type 1 compared to type 2 patients (19.7 ± 3.2 vs 15.3 ± 3.6; $p = 0.02$).

Thirteen studies documented DQB1*06:02 allele frequency in patients with narcolepsy [21–33], ten of which had data on both type 1 and type 2 cataplexy and one study grouped the study population based on ethnicity (Table 3). The average DQB1*06:02 frequency was 74.9% in all narcolepsy patients (range 56.3–100%), 83.8% in type 1 narcolepsy patients (range 56.3–100%) and 41.3% in type 2 narcolepsy patients (range 20.0–83.3%).

Discussion

Here, we present, for the first time, the HLA-DQB1*06:02 allele frequency among Saudi Arabian patients with narcolepsy. Our data show that, overall, DQB1*06:02 allele frequencies were consistent with previously published data and type 1 (cataplexy-positive) patients, being positive in 86.2% and 78.9% of patients, respectively, compared to averages of 75% and 82.8% across all studies examined representing a wide range of ethnicities (Table 3). All type 2 patients were DQB1*06:02 positive in the current study. This is much higher than generally reported and the average of 44.2% DQB1*06:02-positivity calculated for the published studies examined here (see Table 3). We interpret this result with caution given the low number of patients in the type 2 group

Table 1 Demographics, genotype and clinical characteristics of the study cohort

		All patients		DQB1*06:02-positive ($n = 25$)		DQB1*06:02-negative ($n = 4$)		p value
		n/average	%/SD	n/average	%/SD	n/average	%/SD	
Age (years)		17.2	9.6	16.1	9.5	24.5	7.1	0.06
Gender	Male	23	79.3	19	76.0	4	100	0.27
	Female	6	20.7	6	24.0	0	0	
Narcolepsy type	1	19	65.5	15	60.0	4	100	0.12
	2	10	34.5	10	40.0	0	0	
HLA	DQB1*06:02	25	86.2	25	100	0	0	*< 0.0001*
	Other	4	13.8	0	0	4	100	
Age of onset		12.9	7.8	12.0	7.4	18.7	9.6	0.14
Age of diagnosis		17.4	9.9	16.2	9.8	24.8	7.8	0.06
Epworth sleepiness scale ($n = 17$)		17.9	4.0	17.5	4.5	19.0	1.2	0.55
Sleep paralysis	No	7	24.1	4	16.0	3	75.0	*0.01*
	Yes	22	75.9	21	84.0	1	25.0	
Hypnagogic hallucinations	No	11	37.9	10	40.0	1	25.0	0.57
	Yes	18	62.1	15	60.0	3	75.0	
Hypnopomic hallucinations	No	24	82.8	20	80.0	4	100	0.33
	Yes	5	17.2	5	20.0	0	0	
Nightmares	No	18	62.1	14	56.0	4	100	0.27
	Yes	9	31.0	9	36.0	0	0	
	Missing	2	6.8	2	8.0	0	0	
Sleep interruption	No	11	37.9	10	40.0	1	25.0	0.75
	Yes	19	58.6	14	56.0	3	75.0	
	Missing	1	3.4	1	4.0	0	0	
Naps refreshing	No	6	20.7	6	24.0	0	0	0.27
	Yes	23	79.3	19	76.0	4	100	
H1N1 vaccinated	No	23	79.3	19	76.0	4	100	0.71
	Yes	3	10.3	3	12.0	0	0	
	Missing	3	10.3	3	12.0	0	0	
On medication	No	19	65.5	19	76.0	0	0	*0.003*
	Yes	10	34.5	6	24.0	4	100	

Italic indicates a significant difference

($n = 10$), but a genuine difference in Saudi narcolepsy patients with a higher prevalence of DQB1*06:02-positivity cannot be definitively excluded as has been reported in Chinese patients with narcolepsy [34]. Moreover, type 2 group in this study may have high possibility of hypocretin-1 deficiency and may develop cataplexy in the future (as they are still young) [35].

Our results therefore suggest that, consistent with the published data, HLA-DQB1*06:02 is strongly correlated with narcolepsy with cataplexy in Saudi patients and that DQB1*06:02 negativity is present in a minority of patients with definite cataplexy, as reported previously [9]. Although the DQB1*06:02 haplotype and hypocretin deficiency are relatively homogenous in narcolepsy patients, differences do exist in terms of disease prevalence, the predisposition or protective effects of other HLA class 2 alleles, and clinical features across ethnic groups [5]. For instance, in Japanese and Caucasian patients, the disease is associated with DRB1*15:01-DQA1*01:02 DQB1*06:02 haplotypes, while the DQB1*06:02 haplotype is associated with DRB1*11:01 and DRB1*15:03 in African-Americans [5]. While our data support a role for DQB1*06:02 in increasing risk for narcolepsy in Arab patients, unfortunately, we did not conduct comprehensive haplotyping in order to further stratify our population. Future studies will involve contiguous genomic sequencing across the region to establish other susceptibility loci.

In terms of clinical or polysomnographic differences between DQB1*06:02-positive and DQB1*06:02-negative patients, REM latency was lower in the DQB1*06:02-negative compared to DQB1*06:02-positive group. Furthermore, sleep paralysis was more common in the DQB1*06:02-positive

Table 2 Polysomnography and MSLT characteristics of the study cohort

	All patients		DQB1*06:02-positive ($n = 25$)		DQB1*06:02-negative ($n = 4$)		p value
	n/average	%/SD	n/average	%/SD	n/average	%/SD	
Sleep latency (min)	4.3	4.5	4.4	4.7	3.5	3.1	0.98
Sleep efficiency	77.4	12.8	76.4	13.3	83.5	7.0	0.34
REM latency (min)	93.9	86.1	106.1	86.0	17.6	32.2	*0.03*
Stage 1%	13.3	7.7	13.8	8.0	10.4	5.0	0.37
Stage 2%	48.9	11.9	47.2	10.7	59.9	14.9	0.11
Deep sleep %	17.4	9.3	18.6	9.4	9.7	3.4	0.08
REM (%)	20.6	7.6	20.7	6.7	19.9	13.1	0.93
Arousal index	16.9	20.0	13.7	14.6	36.5	37.8	0.11
Apnoea Hypopnea Index	9.2	12.9	7.7	10.9	18.9	21.3	0.11
Sleep onset times (min)	3.1	2.8	3.3	2.9	1.9	2.1	0.30
SOREMP (average)	3.29	0.98	3.17	0.96	4.0	0.82	0.09

Italic indicates a significant difference

group. These findings are consistent with Hong et al. [36], who reported the same findings of different REM latency and sleep paralysis between haplotypes, and is consistent with the hypothesis that DQB1*06:02 might affect the narcolepsy phenotype; of note, normal subjects with the DQB1*06:02 haplotype have been shown to have shorter nocturnal sleep latency [37]. Furthermore, DQB1*06:02 allele status has been shown to exert a linear effect on narcolepsy severity [38], further favouring a direct effect for the allele on disease modification. Alternatively, DQB1*06:02 may merely mark an etiologically homogeneous group of patients, and indeed DQB1*06:02-negative narcolepsy has been shown to have more clinical variability [9].

Given the dominant effect of DQB1*06:02-positivity either in terms of clinical phenotype or patient subdivision, it was perhaps unsurprising that few differences were apparent between the type 1 and type 2 narcolepsy groups; all cataplexy-negative type 2 patients were DQB1*06:02

Table 3 Comparison of DQB1*06:02 allele frequencies in different ethnic groups

Study	Country/ethnicity	Overall DQB1*06:02 frequency	DQB1*06:02 frequency, type 1 (cataplexy-positive)	DQB1*06:02 frequency, type 2 (cataplexy-negative)
This study	Saudi Arabia	25/29 (86.2%)	15/19 (78.9%)	10/10 (100%)
Rogers et al. 1997 [31]	USA, mixed ethnicity	162/185 (87.6%)	154/171 (90.1%)	8/14 (57.1%)
Mignot et al. 1997 [9], Pelin et al. 1998 [30], Hong et al. 2000 [36]	USA/19.5% African-American	113/127 (90.0%)	103/115 (89.6%)	10/12 (83.3%)
	USA/80.5% Caucasian American	373/524 (71.2%)	345/449 (76.8%)	28/75 (37.3%)
Hong et al. 2002 [27]	Korea	18/20 (90.0%)	18/20 (90.0%)	NA
Dolenc-Grosel et al. 2002 [24]	Slovenia	11/11 (100%)	11/11 (100%)	NA
Hong et al. 2007 [28] and Jeong et al. 2007 [40]	Korea	83/101 (82.2%)	73/79 (92.4%)	10/22 (45.5%)
Wing et al. 2008 [33]	Southern Han Chinese	33/47 (70.2%)	30/37 (81%)	3/10 (30%)
Alaez et al. 2008 [21]	Mexico	21/32 (65.6%)	21/32 (65.6%)	NA
Coelho et al. 2009 and 2010 [23, 41]	Brazil	35/54 (64.8%)	29/43 (67.4%)	6/11 (54.5%)
Chen et al. 2013 [22]	Han Chinese	57/74 (77.0%)	50/52 (96.2%)	7/22 (31.2%)
Han et al. 2014 [26]	Multinational (USA, Europe, China, and South East Asia)	485/666 (72.8%)	456/522 (87.4%)	29/144 (20.0%)
Miyagawa et al. 2015 [29]	Japan	748/1328 (56.3%)	748/1328 (56.3%)	NA
Vrana et al. 2016 [32]	Czech Republic	150/198 (75.8%)	129/137 (94.2%)	21/61 (34.4%)
Geremew et al. 2017 [25]	Iran	20/44 (45.5%)	15/19 (78.9%)	5/25 (20.0%)
Average (published studies)		74.9%	83.3%	41.3%

positive. Nevertheless, ESS scores were on average higher in patients with cataplexy, consistent with Harsh et al. [39], who reported a number of differences between cataplexy-positive and cataplexy-negative patients including ESS scores, which were also higher in narcolepsy patients with cataplexy. It may be that type 1 narcolepsy represents a more severe form of the disorder, but there is some controversy regarding the appropriateness of the narcolepsy diagnosis in patients without cataplexy, and misdiagnosis in the type 2 group may be more likely in spite of rigid adherence to ICSD-3 criteria. Furthermore, as noted above, the high rate of DQB1*06:02-positivity in the type 2 group may have masked differences in symptoms between the clinically defined groups.

This study has some limitations in addition to the already mentioned narrow scope of genotyping and relatively small numbers of patients. The cohort originated from only two tertiary referral centres, and therefore, there may be selection bias. Furthermore, we were unable to assay for CSF hypocretin-1 (orexin-A), which is usually reduced or undetectable in the cerebrospinal fluid of narcolepsy type 1 patients, with values below 110 pg/ml considered diagnostic [6, 7]; this may have helped with diagnostic classification.

Regardless of these limitations, this is the first report of HLA typing in an Arab narcolepsy population, and it supports the use of haplotyping in the clinical management of narcolepsy patients in Saudi Arabia. Future studies will focus on further examining the genotypes of Arab narcolepsy patients.

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