Nocturnal Sleep and Daytime Sleepiness in Normal Subjects with HLA-DQB1*0602

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Summary: Narcolepsy, a neurological disorder characterized by excessive daytime sleepiness and abnormal REM sleep, is known to be tightly associated with the Human Leukocyte Antigen (HLA) DQ allele DQB1*0602. In this study, we have explored the possibility that normal subjects carrying this HLA allele (25% of the general population) could display subclinical REM sleep abnormalities and increased daytime sleepiness. Data from 525 middle-aged adults enrolled in the Wisconsin Sleep Cohort study were used for this analysis. Nocturnal polysomnography, sleep latency during the multiple sleep latency test (MSLT), and questionnaire items pertaining to excessive daytime sleepiness were compared between DQB1*0602 positive (n=132) and negative (n=393) participants. Results indicate shorter REM latency whether or not the latency was adjusted for wake after sleep onset (p=0.003 and p=0.02 respectively), increased sleep efficiency (p=0.06) and decreased percent time spent in stage I sleep (p=0.02) during nocturnal polysomnography in DQB1*0602 subjects. Data gathered using the Multiple Sleep Latency Test or the Epworth and Stanford sleepiness scales did not differentiate between DQB1*0602 positive and negative subjects. These results support the hypothesis that polymorphisms at the level of HLA DQ modulates sleep tendencies in humans.
Key words: Sleep; HLA DQ; REM sleep; DQB1*0602; Multiple Sleep Latency Test; epidemiology

ALTHOUGH THE EXACT pathophysiological mechanism underlying narcolepsy, a sleep disorder characterized by excessive daytime sleepiness and abnormal REM sleep, remains elusive, one of the susceptibility factor, HLA-DQB1*0602, has now been identified. Subjects homozygous or heterozygous for this HLA-DQB1 allele are at much greater risk of developing narcolepsy than subjects without this antigen.[1,2] DNA sequencing studies,[3] family studies[4] and transethnic association studies[1,2,5] have all shown that DQB1*0602, rather than a yet unidentified gene or the previously identified HLA-DR2 subtype, is the actual susceptibility factor for narcolepsy. HLA-DQB1*0602 is present in 90%-100% of narcoleptic patients with definite cataplexy versus 25% in control Caucasian Americans, 38% in control African Americans and 12% in Japanese control subjects.[1,2]

The observation that most narcoleptic patients share specific HLA alleles (e.g., HLA-DQB1*0602 and the associated HLA-DR2 subtype) suggests that the disorder may be autoimmune in nature.[6-8] Most of the studies conducted to date however rather argue against this possibility. Narcolepsy is not associated with peripheral immune abnormalities[6-10] and pathological examination of the central nervous system has not revealed any obvious inflammatory lesions in patients with narcolepsy.[11-13] In a canine model of narcolepsy where the disorder is transmitted as an autosomal recessive trait, reciprocal bone marrow transplantation was also shown not to have transmitted the disorder.[14]

Other experiments however favor an autoimmune basis for narcolepsy. Like many autoimmune disorders, predisposition to human narcolepsy involves specific HLA alleles, non HLA genes, and environmental factors.[4,15] Recent experiments have also shown that the onset narcolepsy-cataplexy is associated with diffuse increased microglial HLA Class II expression in the white matter[13] and neuronal

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degeneration in the amygala and the Basal Forebrain area in the canine model.[16,17] These abnormalities may indicate immune mediated neuronal destruction leading to abnormal sleep in narcolepsy.

Whether or not narcolepsy is an autoimmune disorder, a large portion of the normal population carry the exact same HLA susceptibility alleles as narcoleptic patients. These alleles have been sequenced in a large number of patients and shown to be identical to control sequence.[5,18,19] In this study, we hypothetized that normal subjects with DQB1*0602 could have subclinical abnormalities indicative of narcolepsy. This hypothesis is substantiated by data gathered in control subjects carrying HLA susceptibility alleles for other HLA associated disorders, such as HLA-B27 associated spondyloarthropathies,[20,21] HLA-DQ2 associated celiac disease,[22-24] HLA-DR3/DR4 associated insulin-dependent type II diabetes mellitus[25-28] and HLA-DR3/DR5 associated autoimmune thyroiditis.[29] In these disorders, a substantial portion of healthy subjects with the corresponding susceptibility antigens have been found to display subclinical abnormalities (e.g., radiological sacroiliac joint abnormalities[20,21] or increased mucosal gluten sensitivity[22-24] in HLA-B27 or HLA-DQ2 normal subjects respectively).

In 1986, a report by Schulz et al. found a reduced REM latency in 19 DR2-positive normal volunteers,[30] a result that they were not able to replicate in a second sample of 20 subjects with and without DR2.[31] In this study, we attempted to replicate this finding using DQB1*0602 rather than DR2 typing as a narcolepsy marker and a much larger sample drawn from the Wisconsin Sleep Cohort.[32] REM sleep latency, mean sleep latency on the Multiple Sleep Latency Test (MSLT) and questionnaire items pertaining to daytime sleepiness were compared in subjects with and without HLA-DQB1*0602. A preliminary analysis using a slightly smaller sample size was recently published in The Lancet.[33]

MATERIAL AND METHODS

Subjects

A population-based random sample of 577 middle-aged adults enrolled in the Wisconsin Sleep Cohort Study was used.[32] These subjects are being studied as part of a longitudinal study on the natural history of sleep disorders. Fifty-two subjects taking psychotropic compounds known to modify sleep architecture (e.g., antidepressant, neuroleptic, anxiolytic, hypnotic, and/or antiepileptic medications) were excluded, thus leaving 525 subjects (95% Caucasians, 57% male) for this analysis.

Data Collection

Nocturnal polysomnography, a Multiple Sleep Latency Test (MSLT),[34] structured interviews on health, medical history, and sleep habits and questionnaire evaluations of daytime sleepiness using the Epworth sleepiness scale and the Stanford sleepiness scale were administered in all subjects.[32] Nocturnal polysomnography included EEG, EOG, EMG, electrocardiography, and monitoring of potential abnormal breathing events (nasal and oral airflow, tracheal sounds, oxymetry, thoracic, and abdominal effort.[32] Bedtime and waketime were at each subject's discretion. Participants height and weight were measured as part of the overnight protocol to calculate their body mass index (BMI, kg/m2). Ethnic heritage data were also collected and pooled into 6 broad geographically based heritage groups (Germany, Great Britain, Scandinavia, Central Europe, South Europe, other).

Measures of subjective and objective daytime sleepiness were also collected. The Stanford sleepiness scale[35] was collected both the evening before and the morning after the nocturnal polysomnography. As part of a mailed questionnaire, participants were asked to complete the Epworth sleepiness scale,[36] to rate on a 5 point likert scale the frequency of excessive daytime sleepiness and to report on their perceived sleep latency on a typical night. Finally, a 4 nap MSLT[34] is conducted 7-14 days following nocturnal polysomnography. The naps are given at 2=hour intervals beginning at 9 or 9:30 am and each nap is interrupted after sleep onset as described in Carskadon et al. for experimental MSLTs.[34] The 4 sleep latencies are then averaged for one measure of sleep latency. Participants are asked to keep a diary of the sleep and wake times for the week prior to their MSLT.

DQB1*0602 Typing

The presence or absence of DQB1*0602 and not full HLA-DQB1 typing was determined. To do so, genomic DNA was extracted for all subjects and amplified using the sequence-specific primers 611F (5'-CCCGCAGAG-GATTTCGTGTT-3') and 611R (5'-AACTCCGCC-CGGGTCCC-3').[37] These primers amplify DQB1*0602 and the rare DQB1*610, DQB1*613, DQB1*614 alleles as a 218 bp PCR product. Primers DRBEX3F(5'-TGC-CAAGTGGAGCACCCAA-3') and DRBEX4R(5'-GCATCTTGCTCTGTGCAGAT-3'), amplifying the third intron of DRB1 genes, were also included in the mix as internal positive control. Thirty-five cycles at 95°C for 30 sec, 63°C for 30 sec, 72°C for 1 min were used. Oligotyping was then performed as previously described[38] in all PCR products to confirm DQB1*0602 positivity and to exclude for the presence of other rare DQB1 subtypes.

Statistical Analysis

Linear regression modeling was used to assess differences between DQB1*0602 positive and negative subjects on polysomnographic parameters, questionnaire items, and

Epworth and Stanford sleepiness scales. All models were adjusted for the potential confounding factors of age, sex, body mass index (BMI), apnea hypopnea index (AHI), Leg Movements (PLM) index, and ethnic heritage. The SAS statistical package was used for all analysis, and p-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

HLA-DQB1*0602 Frequency and Demographic Characteristics

DQB1*0602 was present in 132 of the 525 adults tested (25%). This carrier frequency was compatible with established DQB1*0602 carrier frequency in a mostly Caucasian sample.[1,2] Age, sex, BMI, AHI, PLM index and ethnic heritage did not differ in DQB1*0602 positive and negative subjects (Table 1).

Nocturnal Polysomnography in HLA-DQB1*0602 Positive and Negative Volunteers

As previously reported in a smaller sample,[29] REM latency was shorter in DQB1*0602 positive subjects. This difference was statistically significant whether or not REM latency was corrected for wake after sleep onset before the occurrence of REM sleep (Table 2). A tendency towards increased % REM sleep and decreased nocturnal sleep latency was also observed (Table 2). Other differences were suggestive of increased sleep continuity (i.e.,increased sleep efficiency, decreased % stage I and % wake after sleep onset).

REM Latency, HLA-DQB1*0602, and Other Potential Confounding Factors

Unadjusted REM latencies in all DQB1*0602 positive and negative studies are reported in Figure.1. The effect of DQB1*0602 positivity on REM latency was modest and accounted for an estimated 1.3% of the total variance in this parameter (versus 13% for the overall model). The respective effect on REM latency of DQB1 versus other potentially important factors such as AHI, age, BMI, PLM index, and amount of sleep self reported before polysomnography is reported in Table 3. The DQB1*0602 status was associated with a difference in REM latency that was larger than all other effects. The only other very significant effect was the number of minutes of nocturnal sleep self reported the night before polysomnography, with 60 minutes of additional sleep resulting in an 11 minute longer REM latency.

To examine for the effect of ethnic heritage, REM latency values and DQB1*0602 frequencies were compared by ethnic heritage (Table 4). DQB1*0602 frequency was found not to differ significantly between ethnic heritage and in all cases REM latency was shorter in DQB1*0602 positive subjects (Table 4).

Table 1. Demographic characteristics of the sample

DQB1*0602	positive (n=132)	negative (n=393)
Age (years)	49±8	51±8
Sex (% male)	55%	58%
Body Mass Index (kg/cm2)	31±7	30±7
AHI (events/hr)	1.6; 0-121	1.5; 0-81
Leg movements (events/hr)	22.9; 0-115	21.9; 0-119
% Caucasian	97%	94%
% German	41%	38%

AHI: Apnea, Hypopnea Index. Data are means±SEM, medians with range or percent when applicable.

Table 2. Sleep variables in DQB1*0601 positive and negative volunteers

DQB1*0602	positive (n=132)	negative (n=393)	p value
Time in bed (min)	437±4	442±3	0.33
Total sleep time (min)	378±5	375±3	0.59
Sleep efficiency (%)	86.5±0.8	84.8±0.5	0.06
Sleep onset latency (min)	8.6±1.2	10.0±0.7	0.24
REM sleep latency (min)	106±5	123±3	0.003
Corrected REM sleep latency (min)†	93±4	104±3	0.02
Percent wake after sleep onset	14.3±1.2	16.6±0.7	0.07
Percent stage 1	8.1±0.5	9.4±0.3	0.02
Percent stage 2	56.9±0.9	56.6±0.6	0.75
Percent stage 3	12.1±0.6	12.1±0.4	0.93
Percent stage 4	4.0±0.5	4.0±0.3	0.92
Percent REM sleep	18.8±0.1	17.7±0.4	0.09

Data are mean ±SEM. Adjusted by age, sex, BMI, AHI, ethnicity and amount of sleep before polysomnography.

†: Corrected for wake after sleep onset before REM sleep in 121 DQB1*0602 positive and 365 DQB1*0602 negative subjects.

Table 3. Effect of DQB1, age, sex and other variables on REM latency

Independent variable	Increment in REM latency (beta coefficient)	p value
DQB1	-17.0±5.7	0.003
AHI (5/hr)	2.3±1.2	0.06
Age (10 years)	-6.1±3.2	0.06
Sex (F)	5.0±5.2	0.33
BMI (5 kg/cm2)	4.3±2.1	0.04
Min sleep (60 min)	10.8±2.2	0.001
Leg movements (10/hr)	2.2±1.2	0.06

Data are mean ±SEM. Adjusted for ethnic heritage.

Figure 1. REM latency distribution in DQB1*0602 positive and negative volunteers. REM latency was calculated from sleep onset defined as 3 consecutive epochs of stage 1 or more sleep.

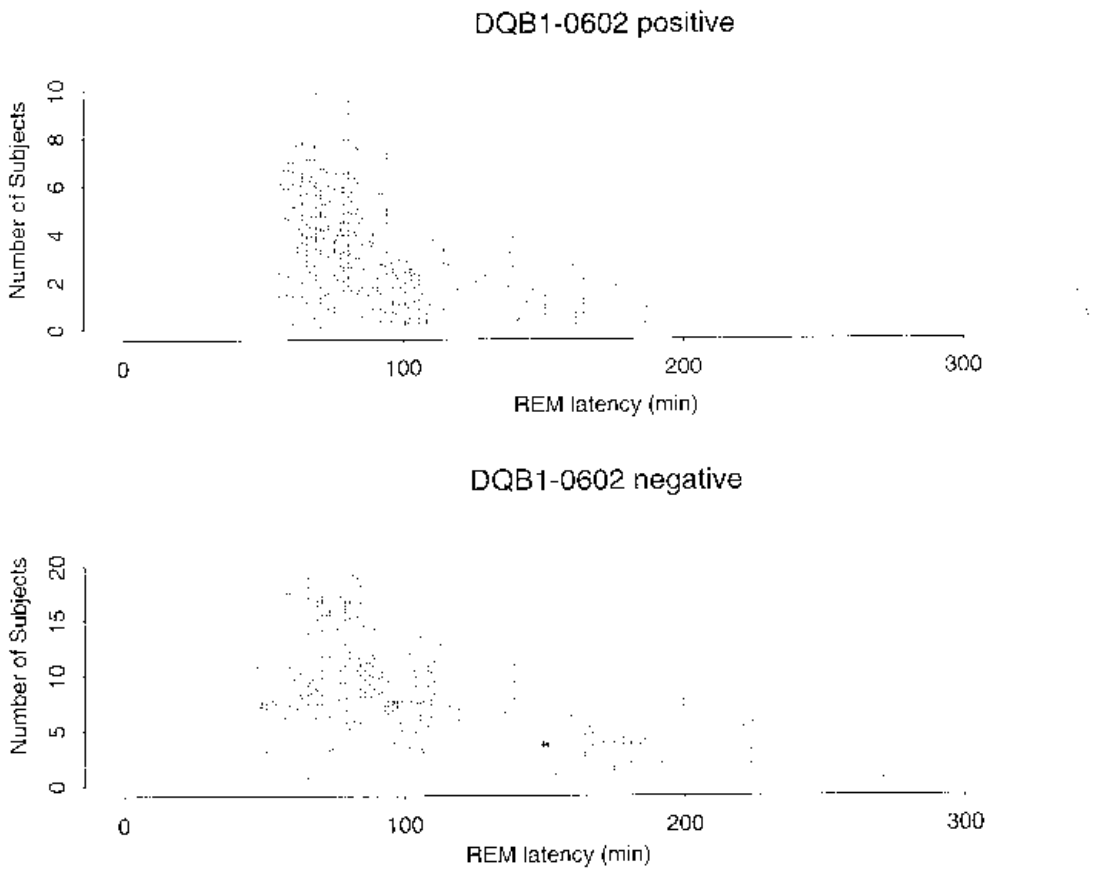

Table 4. REM latency by DQB1 positive and negative stratified by ethnic heritage

Ethnic Heritage	DQB1*0602 frequency	REM latency positive	REM latency negative
American	0.39 (31)	85±16	109±13
Central Europe	0.26 (38)	108±18	136±11
Germany	0.26 (197)	102±8	111±5
Great Britain	0.22 (91)	114±12	127±7
Scandinavia	0.28 (87)	73±39*	155±12
Other	0.21 (56)	97±16	110±8

Data are means±SEM. Adjusted for age, sex, BMI, AHI, PLMS and amount of sleep before polysomnography.

*:p=0.06

Table 5. MSLT and sleepiness by DQB1*0602 status

DQB1*0602	positive (n=132)	negative (n=393)
MSLT sleep latency	9.5±0.6 (89)	9.7±0.4 (266)
Self reported sleep latency	13.6±1.6 (132)	15.9±1.0 (393)
Epworth sleepiness scale	8.1±0.4 (120)	8.7±0.3 (375)
Stanford Sleepiness Scale (am)	2.9±0.1 (132)	2.8±0.1 (393)
Stanford Sleepiness Scale (pm)	3.7±0.1 (132)	3.5±0.1 (393)
Daytime sleepiness†	1.1; 0.6-1.9 (128)	n.a. (373)

Data are means±SEM or odd ratio with range. Number of observation in each group is listed under parenthesis. Adjusted by age, sex, BMI, AHI, PLMS, ethnic heritage and amount of sleep before polysomnography.

†: Odd ratio for daily or almost daily daytime sleepiness in DQB1*0602 positive versus negative.

MSLT results and evaluation of daytime sleepiness

The effect of DQB1*0602 positivity on various parameters indicative of daytime sleepiness is reported in Table 5. Approximately 20% of the sample in both the DQB1*0602 positive and negative group had a MSLT mean sleep latency less or equal to 5 minutes (19.46 vs 21.59 % in DQB1*0602 negative and positive respectively). Results were similar when patients with an AHI greater or equal to 10 were excluded from the analysis (data not shown). None of the parameters distinguished between the two groups.

DISCUSSION

These results extend our preliminary observation using a larger sample size. In this second analysis, all subjects taking psychotropic medications were excluded and results are reported for both nocturnal polysomnography and daytime

assessment of sleepiness. We also report on REM latency and DQB1*0602 across various ethnic heritage to control for possible population stratification effects (Table 4). Data indicates a shorter mean REM latency and changes in nocturnal sleep indicative of greater sleep continuity but no measurable excessive daytime sleepiness in subjects carrying the narcolepsy susceptibility allele DQB1*0602.

The effect observed in this population is unlikely to be due to the presence of a large number of undiagnosed subjects with clinical narcolepsy in the HLA DQB1*0602 positive group. First, narcolepsy-cataplexy as defined by international classifications only affects 0.05% of the general population[4] so less than one patient would be expected in a random sample of 525 individuals. Second, the presence of DQB1*0602 in the cohort was associated not only with reduced REM sleep latency (as observed in narcolepsy) but also with increased sleep efficiency, decreased wake after sleep onset, and decreased stage I sleep (Table 2) and no clinically significant daytime sleepiness (Table 5). In narcoleptic patients, decreased REM latency, increased daytime sleepiness, decreased sleep efficiency, increased percentage stage I, and increased wake after sleep onset are observed.[39-41] A comparison of the distribution of REM sleep latencies in both groups also did not substantiate the hypothesis of undiagnosed narcoleptic patients in the sample. In a recent analysis of nocturnal sleep onset in 530 untreated narcoleptic patients, 45% of the narcoleptic patients studied had a nocturnal REM latency shorter than 15 minutes (sleep onset REM periods) and less than 10% of the subjects had latencies longer than 100 minutes, thus indicating both sleep onset REM periods and generally shorter REM latencies occur in narcoleptic subjects.[41] In our control sample, none of the 132 DQB1*0602 positive subjects had a REM latency shorter than 15 minutes (Fig.1). The difference in REM latency observed was mostly due to a overrepresentation of REM latencies longer than 100 minutes in DQB1*0602 negative subjects (Fig.1). It is thus more likely that the presence of specific HLA DQ alleles influences REM latency and other sleep parameters in a large portion of the general population.

The effect of DQB1*0602 on REM latency was modest if one consider the variability of this parameter in the general population (Fig.1). In comparison with other factors such as age, sex, BMI, AHI, and previous sleep history, the effect of DQB1*0602 was substantial (17 minutes) and highly significant (Table 2). As reported by others, age moderately reduced REM latency[42] while sex had no significant influence (Table 3). Increased BMI, PLM index, or AHI also slightly prolonged REM latency, probably as a result of increased sleep disruption. Most strikingly, the effect of DQB1 was as large and as significant as that of previous sleep history. In this comparison, one hour of extra sleep the night before polysomnography prolonged REM latency for only 11 minutes (Table 3). The effect of DQB1*0602 on REM latency was of smaller magnitude as that frequently reported in other established psychiatric pathologies such as major depression.[43,44]

The mechanism by which polymorphism at the level of HLA-DQ could influence sleep patterns is unknown. Subclinical findings have been reported for other HLA associated disorders known to be autoimmune in nature.[20-29] Reduced REM latency in DQB1*0602 control subjects might thus represent the extension in the general population of an autoimmune-mediated disease spectrum. For narcolepsy, however, there is no direct evidence for an autoimmune mediation of the disorder[6-8] Animal and human data suggest a connection between sleep and immunity but most of the findings are consistent with an involvement of the immune system in slow wave sleep rather than REM sleep regulation.[45-47] Multiple mechanisms might therefore be involved and a better understanding of narcolepsy pathophysiology will likely be needed first to interpret our observation.

Further studies are also warranted replication and extension of this finding. Both sib pair and population-based designed studies in larger samples would ideally be needed. Recent studies in narcolepsy and other HLA associated disorders have also shown complex HLA-DQ allele dosage effects and protective effects.[2,48] It would also therefore be worthwhile to evaluate the effect of other HLA DQ subtypes on REM latency in parallel with similar studies in narcoleptic patients.

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