Not Only Sleepwalking But NREM Parasomnia Irrespective of the Type Is Associated with HLA DQB1*05:01

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Study Objectives: Despite the high prevalence and clinical relevance of NREM parasomnias, data on supportive genetic markers are scarce, and mainly refer to sleepwalking only.

Methods: We retrospectively analyzed clinical, polysomnographic, and HLA findings of 74 adults (37 men) with NREM parasomnia gathered from four neurological sleep centers. Parasomniac events were classified according to ICSD-2 criteria. HLA DQB1 genotyping was compared to regional-matched reference allele-frequencies.

Results: Fifty-six patients had more than 2 different parasomnia type: 11 sleepwalking, 4 sleep terrors, 3 confusional arousals only. Parasomniac events were documented during video-polysomnography (V-PSG) in 70% (49/70) of subjects (71.4% confusional arousals, 8.2% sleep terrors, 4.1% sleepwalking, 16.3% ≥ 2 NREM parasomnia types). Violent behavior during V-PSG occurred in 8.5% (6/71). NREM parasomnia onset was reported after the age of 30 years in 6.8% (5/74). The HLA DQB1*05:01 allele was present in 41% (29/71) compared to 24.2% in the regional-matched reference allele group (p < 0.05). This haplotype prevalence did not differ within the NREM parasomnia type. Epworth Sleepiness Score was 10 or higher in 28.6% (6/71). Violent behavior during V-PSG occurred in 8.5% (6/71). NREM parasomnia onset was reported after the age of 30 years in 6.8% (5/74). The HLA DQB1*05:01 allele was present in 41% (29/71) compared to 24.2% in the regional-matched reference allele group (p < 0.05). This haplotype prevalence did not differ within the NREM parasomnia type. Epworth Sleepiness Score was 10 or higher in 28.6%.

Conclusions: This is a large polysomnography-based case series of patients with NREM parasomnia. In patients with suspected sleepwalking or sleep terrors, polysomnography is highly useful in detecting arousals from NREM sleep as a marker of NREM parasomnia. We confirmed previous findings by demonstrating a high prevalence of the HLA DQB1*05:01 genotype for different types of NREM parasomnias. Our findings therefore support a common genetic background, and corroborate the importance of video-polysomnography in the work-up of parasomnia.

Keywords: video-polysomnography, genotype, somnambulism, genetics, behavior


INTRODUCTION

NREM parasomnias are characterized by abnormal sleep-related behavior, impaired consciousness during these episodes, and accompanying activation of the autonomic nervous system.1 Although NREM parasomnias are much more prevalent in children, they still affect up to 4% of adults.2–4 Non-bothersome forms seem to be even more frequent in adults, as shown by a recent study that reports a history of non-bothersome sleepwalking in 12% of the healthy adult participants.5

The clinical hallmarks, which allow a distinction between different types of NREM parasomnias, are ambulation in sleepwalking and autonomic symptoms in sleep terrors. There is, however, a notable overlap between the different types.6 Despite the prevalence and clinical relevance of NREM parasomnias, data on supportive genetic markers are scarce. To date, only two studies have been published in patients with sleepwalking.5,7 One of these studies reported the results of a genome-wide investigation of a single family of 22 sleepwalkers. The authors established a significant linkage to chromosome 20q12-q13.12 for this family.7 Genetic covariation has been reported to exist between various parasomnia types.8

Another study investigated the genetic susceptibility to sleepwalking with the regard to HLA system in 60 patients with sleepwalking. The authors demonstrated that the DQB1*05:01 allele is more common in sleepwalkers than in the general population.9 Whether different types of NREM-parasomnias share the same association with the HLA DQB1 allele as shown for sleepwalking has not been investigated.

BRIEF SUMMARY

Current Knowledge/Study Rationale: Despite the prevalence and clinical relevance of NREM parasomnias, data on supportive genetic markers are scarce. Whether different types of NREM-parasomnias share the same association with the HLA DQB1 allele as shown for sleepwalking has not been investigated.

Study Impact: HLA DQB1*05:01 was more frequent in all types of NREM parasomnias. In addition, confusional arousals were frequently observed during V-PSG. Our findings therefore support a common genetic background for all types of parasomnias, and corroborate the importance of video-polysomnography in the work-up of parasomnias.
Since the HLA association was previously described we performed an HLA investigation in our cohort of patients with NREM parasomnia irrespective of the type. As diagnosing NREM parasomnias based on history alone might bear the risk of false positive or negative diagnosis due to the challenging differential diagnostic spectrum, and history-taking alone might underestimate the presence of combined NREM parasomnias, diagnosis of NREM parasomnia in this study was based on both a careful sleep history and video-polysomnography (V-PSG).

METHODS

Subjects

Patients with a diagnosis of NREM parasomnia irrespective of the type (sleepwalking, sleep terror, confusional arousal) were gathered from 4 neurological sleep centers located in Austria and Germany (Innsbruck n = 33, Muenster n = 32, Schwalmstadt-Treysa n = 6, Bremen n = 3). The diagnosis of NREM parasomnia was made according to diagnostic criteria of the International Classification of Sleep Disorders, Second Edition (ICSD-2, 2005). Diagnosis of NREM parasomnia was based on both sleep history and V-PSG. For this investigation eligible patients were identified in the database of the respective centers. Selection criteria were definite diagnosis of NREM parasomnia, presence of at least one night of V-PSG for evaluation, and availability of a blood sample for HLA typing. Clinical data, which were assessed via a structured face-to-face sleep interview (including the interview of the bed partner if available), V-PSG data and HLA genotypes were analyzed. Patients with epilepsy were excluded.

This study was approved by the local ethics committee, at each study site. Participants gave written informed consent prior to study participation.

Video-Polysomnography

Nocturnal V-PSG was performed for 2 consecutive nights (70/74). Second night was used for analysis to exclude first night effects. We abstained from sleep deprivation before V-PSG and forced arousals as has been used in other protocols in order not to influence the semiology of the parasomnian events. Sleep recordings were performed and manually scored according to AASM 2007 standards. V-PSG included electroencephalography (F3, F4, C3, C4, O1, O2, M1, and M2 electrodes), electrooculography, electromyography (mental, both anterior tibialis muscles), and cardiorespiratory recording single channel electrocardiography, nasal airflow (thermositor), nasal pressure cannula, tracheal microphone, thoracic and abdominal respiratory movements (piezo), and transcutaneous oxygen saturation. Throughout the night, patients’ behaviors were recorded by an infrared video camera. Sleepwalking was scored when ambulation during sleep occurred while sleep persisted. Sleep terror was diagnosed when arousal occurred from slow wave sleep with sitting up, frightened facial expressions, accompanied by a loud scream and by signs of significant autonomic nervous system activation such as tachycardia, or increase in respiratory rate. Confusional arousal was diagnosed when recurrent arousal from NREM sleep with minor behavior such as eye opening, sitting up and short lasting signs of apparent disorientation or mental confusion occurred.

HLA-DQB1 Genotyping

Intronic Exon2 enclosing primers (primer sequence on request) were constructed using the reference sequences from the Human genome Browser Version August 2006 (http://genome.ucsc.edu) and the Primer3 program (http://frodo.wi.mit.edu). The Polymerase Chain Reaction (PCR) was performed using standard protocols with HotStar Taq (Qiagen GmbH, Hilden, Germany) and sequencing of Exon2 was performed with the Big Dye Terminator 3.1 chemistry on a 3730 DNA-Analyzer (Applied Biosystems, Darmstadt, Germany). Sequence electropherograms were compared to reference sequences from the IMGT/HLA Database (http://www.ebi.ac.uk/ipd/imgt/hla/) using the SeqMan program (DNASTAR). This method allowed typing of DQB1*06 and 05. To obtain a sufficient sample size and a representative population with a comparable hereditary disposition, allele frequencies were compared to regional matched reference frequencies in the allele frequency database (http://www.allelefrequencies.net/), containing 174 German Caucasians and 200 Austrian Caucasians.

The allele-frequencies of the regional-matched reference group were 24.2% for DQB1*05:01 and 27.6% for DQB1*06:02.

Statistical Analysis

Statistical analysis was performed using IBM-SPSS version 22.0. Normal distribution was tested by applying the Shapiro Wilk test. All data are given as either numbers (percentages) or medians and ranges, as the majority of variables were not normally distributed. Subgroup analysis of PSG data was performed using the Kruskal-Wallis test. A p value below 0.05 was considered to indicate statistical significance.

RESULTS

Demographic and Clinical Findings

Seventy-four patients (37 men, 37 women) with a diagnosis of NREM parasomnia were included in this study. Taking into account clinical history and V-PSG data 56 patients had ≥ 2 NREM parasomnias, 11 sleepwalking, 4 sleep terrors, or 3 confusional arousals only. The median age of patients was 24.5 years (range 13–66 years).

Fifty percent of patients (37/74) had a self-reported disease onset before the age of 10 years, whereas only 6.8% (5/74) reported a disease onset after the age of 30 years. Ninety-seven percent of patients (72/74) had a typical history of NREM parasomnia: 47.2% had a history suggestive of sleepwalking, 36.1% had a combination of different NREM parasomnias, 13.9% of sleep terrors, and 2.7% of confusional arousals. Reported frequency of parasomnial events ranged between one and 150 per month (median 11/month). One patient reported on episodes of confusional arousals at an average of five times per night. Triggering factors for the occurrence of parasomniac symptoms were present in 32.5% of patients (24/74). The most common triggering factor was emotional stress (62.5%).
Violent behavior was reported by 8.5% of patients (6/71). Fifty-six patients were able to give information about dream mentation. Detailed dream mentation related to parasomnias, was reported by 15 patients (26.3%) another 26.3% reported at least fragmentary dream mentation, whereas 46.4% did not recall a dream related to any parasomniac episodes. A positive family history of NREM parasomnia in a first-degree relative was reported by 42.4% of participants (28/66), 7.2% (4/55) reported at least fragmentary dream mentation, whereas 46.4% did not recall a dream related to any parasomniac episodes. A concomitant psychiatric disorder was reported by 9.5% of patients (7/74: 3 major depressive disorder, 1 mixed anxiety depression, 1 post-traumatic stress disorder, 1 borderline personality disorder).

**Video-Polysomnographic Findings**

V-PSG data were available from 70 NREM parasomnia patients (see Table 1). Distribution and relative quantity of sleep stages did not differ between the different parasomnias taking into account that the group sizes for confusional arousals and sleep terrors were small (p > 0.05). The respiratory variables did not show any group differences either: only 2 patients had an apnea-hypopnea index > 5/h. Fifteen patients (20.2%) showed a periodic limb movement during sleep index > 15/h.

Of note, during V-PSG, arousals from NREM sleep with accompanying abnormal behaviors were found in 70.0% (49/70) of patients: in 71.4% (35/70) of patients, V-PSG revealed behavioral confusional arousals, in 8.2% (4/70) sleep terrors, and in 23.1% (16/70) sleepwalking. The finding that at least subtle behavioral abnormalities were observed during V-PSG underlines the importance of V-PSG in the workup of NREM-parasomnia, as it does not only serve to rule out the challenging differential diagnostic spectrum but also to confirm the disorder itself. Indeed, confusional arousals might be even considered the signature of during V-PSG. Similar findings were found for sleep terrors (history: 9, V-PSG: 4) and combined NREM parasomnias (history: 25, V-PSG: 8). In contrast, confusional arousals were more frequently observed in the sleep laboratory setting than expected by medical history (history: 2, V-PSG: 35).

### HLA Genotyping

Patients were assigned to 4 groups according to NREM parasomnia diagnosis (confusional arousals, sleep terrors, sleepwalking, combined NREM parasomnia). Results of DQB1 HLA genotyping, were available for 71 patients and are shown in details in Table 2. The DQB1*05:01 allele was found in 29 (40.8%) patients, among whom 13 were homozygous. The DQB1*06:02 allele was present in 22 (31.9%) of patients. Five patients were heterozygous for both DQB1*05:01 and DQB1*06:02. The results of the additional DQB1 loci investigated in the study as well as allele frequencies of the different NREM parasomnia types are shown in Table 2. Of note the DQB1*05:01 was found in 3/3 of patients with sleep terrors.

### DISCUSSION

This study reports PSG and HLA data from one of the largest case series of patients with different types of NREM parasomnias. HLA DQB1*05:01 was more frequent in all types of NREM parasomnias in our study population than a regional matched reference group. In addition, confusional arousals were frequently observed during V-PSG.

### Importance of Polysomnography in the Work-up of Parasomnia

The finding that at least subtle behavioral abnormalities were observed during a one-night V-PSG underlines the importance of V-PSG in the workup of NREM-parasomnia, as it does not only serve to rule out the challenging differential diagnostic spectrum but also to confirm the disorder itself. Indeed, confusional arousals might be even considered the signature of...
NREM parasomnia itself as half of all patients exhibited confusional arousals during V-PSG, while, not unexpectedly, the incidence of sleep terrors or sleepwalking was rare. We also did not find violent behavior during PSG, although it was reported by 8.5% of patients. This number is lower than reported, but might have relevant therapeutically implications in some cases due to potential injury behaviors. The frequency of simultaneous presence of more than one parasomnia type was shown to be considerably higher when performing a diagnosis based on both history and V-PSG (75.7%) in contrast to medical history alone (36.1%). This finding was likely explained by the fact, that a significant number of patients and bed partners were unaware of probably milder forms of confusional arousals.

Previous studies reported aggravating comorbid sleep disorders as mentioned before. In difference to their findings, we did not find an increased prevalence of sleep disordered breathing (SDB) or periodic limb movements (PLM) in our study population. Another striking finding was that overall sleep macro-architecture was not different compared to healthy normal controls. This is in line with Labelle et al., but contradicts Espa’s findings who reported a decreased sleep efficiency, a decrease of N2 sleep, and an increase of slow wave sleep. Arousal rates during slow wave sleep, cyclic alternating pattern rates, hypersynchronous delta waves, or slow oscillation just before parasomniac episodes have, however, not been specifically investigated in the present study. Therefore, we cannot exclude that incorporating these measures we might have found more signs of minor sleep disruption.

Of note, excessive daytime sleepiness was present in approximately 30% of patients. This finding underlines the reports of other authors that excessive daytime sleepiness is not only an important characteristic of sleepwalking, but NREM parasomnia itself. Therefore, it contradicts the concept that NREM parasomnias affect nocturnal sleep only.

Genetic Susceptibility
In a previous study in patients with sleepwalking the genetic susceptibility with the HLA genotype DQB1*05:01 was 35.0% compared to 13.3% in healthy controls. We extended this finding by choosing patients with a V-PSG supported diagnosis of NREM parasomnia including patients with all clinical types of NREM parasomnia. We found the HLA DQB1*05:01 allele in 41% of all patients irrespective for the parasomnia type. This proportion is similar to the one previously reported in patients with sleepwalking only, and markedly higher than that in the general Caucasian population. Thus, the DQB1*05:01 genotype might be part of one or several genetic subsets which convey genetic susceptibility to all types of NREM parasomnia.

In the present study, the prevalence of the DQB1*05:01 allele did not differ between patients with either: sleepwalking, sleep terrors, or confusional arousals. Although the groups of different parasomnia types are small, the HLA genotyping did not allow a distinction between the different types of NREM parasomnia.

The DQB1*06:02 allele, which can be found in the vast majority of patients with narcolepsy and which explains most of the risk in narcolepsy with cataplexy in Europe, US, and Japan, was found in 30% of our parasomnia patients. Interestingly, in patients with narcolepsy with cataplexy DQB1*05:01 was found to be protective in presence of DQB1*06:02 in transposition in different ethnic groups.

This study provides further evidence that the considerable overlap between the NREM parasomnia types might even challenge the usefulness of their nosological differentiation, not only from the genetic point of view as sleepwalking and sleep terrors also show a considerable overlap by sharing similar clinical features.

A positive history for NREM parasomnia was frequent in 42% of the patients indicated a first-degree relative with a known NREM parasomnia, which is in line with previous reports from the literature. Moreover, 7.2% referred to epilepsy in first-degree relatives.

This increased frequency of epilepsy in first-degree relatives is well in line with a prospective familial aggregation study in nocturnal frontal lobe epilepsy, which demonstrated that arousal disorders are much more frequent not only in patients with nocturnal frontal lobe epilepsy, but also their relatives when compared to controls. In addition, a retrospective chart review of 100 patients with nocturnal frontal lobe epilepsy revealed that 39% of epilepsy patients fulfilled the diagnostic criteria for parasomnias.

Potential Limitations
A potential limitation of this study is that a bed partners’ input or a history of the relatives was not available in all subjects.
Therefore, the reported rate of subtle motor behaviors might be underestimated, and the onset of NREM parasomnias might be reported at a later age. Moreover, we used a regional matched reference allele group to compare HLA allele frequencies of the patient group. This reference group did not undergo polysomnography, and we cannot exclude that a minority of subjects had indeed NREM parasomnia (the estimated prevalence of NREM parasomnia is approximately 4.0% in the general adult population\textsuperscript{2–4}). Given the total sample size of 374 subjects we do not think that this would have a relevant impact on our findings; on the contrary, eliminating the few cases with NREM parasomnias in the reference group would rather strengthen than weakening the present results.

CONCLUSIONS

In summary, this study is a large polysomnography-based case series of patients with different types of NREM parasomnias. We showed that V-PSG is highly useful to confirm suspected NREM parasomnia with confusional arousal being the most frequent manifestation. Furthermore, we replicated and extended previous findings by demonstrating a high prevalence of the HLA DQB1*05:01 genotype in all types of NREM parasomnias. Our findings therefore support a common genetic background for all types of parasomnias, and corroborate the importance of video-polysomnography in the work-up of parasomnias.

ABBREVIATIONS

AASM, American Academy of Sleep Medicine
HLA, human leucocyte antigen
NREM, non-rapid eye movement
CA, confusional arousal
ICSD, International Classification of Sleep Disorders
PLM, periodic limb movements
min, minute
PCR, polymerase chain reaction
SDB, sleep-disordered breathing
ST, sleep terror
SW, sleepwalking
V-PSG, video-polysomnography

REFERENCES