HLA and sleep parameter associations in post-H1N1 narcolepsy type 1 patients and first-degree relatives

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Abstract

Study Objectives

To explore HLA (human leukocyte antigen) in post-H1N1 narcolepsy type 1 patients (NT1), first-degree relatives and healthy controls, and assess HLA associations with clinical and sleep parameters in patients and first-degree relatives.

Methods

Ninety post-H1N1 NT1 patients and 202 of their first-degree relatives were HLA-genotyped (next generation sequencing) and phenotyped (semi-structured interviews, Stanford Sleep Questionnaire, polysomnography, multiple sleep latency test). HLA allele distributions were compared between DQB1*06:02-heterozygous individuals (77 patients, 59 parents, 1230 controls). A subsample (74 patients, 114 relatives) was investigated for associations between HLA-loci and continuous sleep variables using logistic regression. Identified candidate HLA-loci were explored for HLA allele associations with hypnagogic hallucinations and sleep paralysis in 90 patients, and patient allele findings were checked for similar associations in 202 relatives.

Results

DQB1*06:02 heterozygous post-H1N1 NT1 patients (84.4% H1N1-vaccinated), showed several significant HLA associations similar to those reported previously in samples of mainly sporadic NT1 i.e. DQB1*03:01, DRB1*04:01, DRB1*04:02, DRB1*04:07, DRB1*11:04, A*25:01, B*35:03 and B*51:01, and novel associations i.e. B*14:02, C*01:02 and C*07:01. Parents HLA alleles did not deviate significantly from controls. The HLA-C locus was associated with sleep parameters in patients and relatives. In patients C*02:02 seems to be associated with protective effects against sleep paralysis and hypnagogic hallucinations.

Conclusion

Our findings of similar risk/protective HLA-alleles in post-H1N1 as in previous studies of mainly sporadic narcolepsy support similar disease mechanisms. We also report novel allelic associations. Associations between HLA-C and sleep parameters were seen independent of NT1 diagnosis, supporting involvement of HLA-C in sleep subphenotypes.

Keywords: narcolepsy, H1N1-vaccination, HLA, hypocretin, hypnagogic hallucinations, sleep paralysis

Statement of Significance

This is the first study to investigate the risk/protective human leukocyte antigen (HLA)-alleles in post-H1N1 (mainly H1N1-vaccinated) narcolepsy type 1 (NT1) patients and first-degree relatives compared to controls. Further, it is the first study to explore associations between HLA and clinical and sleep parameters in post-H1N1 patients and first-degree relatives. A similar risk/protective HLA-allele profile was found in our mainly H1N1-vaccinated NT1 cohort compared to previous studies of mainly sporadic NT1, supporting a similar autoimmune background. Novel associations were detected which might be particularly associated with post-H1N1 NT1. Our results highlight the possible role of HLA-C in sleep subphenotypes and C*02:02 in particularly might have a protective effect for hypnagogic hallucinations and sleep paralysis in patients**.**

Introduction

Narcolepsy type 1 (NT1) is a severe, chronic neurological sleep disorder characterized by excessive daytime sleepiness, unstable regulation of sleep/wakefulness and fragmented nocturnal sleep[1]. Cataplexy (muscle atonia triggered by emotions) is an important clinical feature of NT1, and patients can also experience sleep paralysis and hypnagogic/hypnopompic hallucinations[1-3]. Polysomnography (PSG) and the multiple sleep latency test (MSLT) are part of the diagnostic and phenotype investigations for revealing the increased sleepiness and early occurring rapid eye movement (REM)-sleep[1,3]. Further, lumbar puncture can be performed to detect hypocretin-deficiency (cerebrospinal fluid (CSF) hypocretin-1 <110 pg/ml when a Stanford reference sample[4] is used or ≤1/3 of level in normal population) as NT1 is caused by the loss of specific hypocretin-producing hypothalamic neurons (also called orexins)[5,6]. These hypothalamic neurons have widely distributed connections to other parts of the brain[7-9] and play an important role in regulating sleep, wakefulness and tonus [10,11].

Although the exact mechanisms for the hypothalamic neuron loss are unknown, autoimmunity has been implicated as the most likely cause[3], which is further supported by the >10-fold increase in the incidence of NT1 after the mass vaccinations with Pandemrix® in 2009/2010 in several countries, including Norway[12]. There have been some indications of differences in phenotype for H1N1-vaccinated NT1 from sporadic NT1 (e.g. more prevalent cataplexy near disease onset, shorter time from the debut of excessive daytime sleepiness to cataplexy), however other factors might have influenced this (e.g. disease duration)[13]. Nevertheless, with a still unknown disease mechanism[3] it cannot be ruled out that there could be differences in the etiology of sporadic and H1N1-vaccinated narcolepsy.

The autoimmune hypothesis is supported for both sporadic and H1N1-vaccinated narcolepsy by the strong HLA (human leukocyte antigen)-allele association mainly to a single HLA-allele, HLA-DQB1*06:02[3]. Previous studies have found the HLA-DQB1*06:02 allele strongly associated with both hypocretin-deficient sporadic NT1 patients[14] and H1N1-vaccinated NT1 patients[12,15-17], implicating

CD4+ T cells in the development of NT1[18]. In a study across three ethnic groups [14], sporadic narcolepsy has also been associated with several other HLA class II alleles carried in trans with HLA-DQB1*06:02 which increased the risk of narcolepsy; DQB1*03:01, DQA1*06, DRB1*04, DRB1*08, DRB1*11 and DRB1*12, and in addition detected several protective alleles; DQB1*06:01, DQB1*05:01 and DQA1*01. The predisposing effect of DQB1*03:01 has also been supported in a large sample of mainly sporadic European (3/4 were sporadic patients/included prior to autumn 2009, 1/4 were possibly included after autumn 2009 but H1N1-vaccination/infection was unspecified) and mainly sporadic Chinese narcolepsy patients (included pre- and post-H1N1-pandemy/autumn 2009 but H1N1-vaccination with an unspecified seasonal vaccine was only reported in 14 and H1N1-infection in 27 patients, respectively)[19] and in a Korean study of sporadic narcolepsy[20]. Moreover, the finding of DQB1*05:01 as protective was replicated in another study of presumably mainly sporadic narcolepsy patients (study sample was selected from two previous subsamples: one subsample was sporadic/included prior to autumn 2009, one subsample was published after autumn 2009 but H1N1-vaccination/infection was not specified)[21] which additionally identified three additional alleles as protective; DQB1*06:03, DQB1*06:09, DQB1*02.

Several studies have searched for specific autoantibodies but, with a few exceptions, they have been largely unsuccessful[3]. However, autoreactive hypocretin-specific CD4+ T cells have been detected in narcolepsy type 1 patients [22] and Luo et al.[23] has also shown cross-reactivity between the CD4+ T cells reacting to hypocretin also reacting to a piece of the hemagglutinin flu protein of the pandemic 2009 H1N1 influenza strain. It has alternatively been suggested that the loss of the hypocretin-producing cells might be due to vulnerability towards a more systemic inflammatory challenge rather than a specific immune-mediated attack[24]. However, HLA class I alleles, including A*11:01[19,25], B*51:01[19], B*35:01[25], B*35:03[19], C*04:01[25], have also been shown to increase the risk of narcolepsy, implicating additional cytotoxic responses by CD8+ T cells or natural killer cells in the development of NT1[3]. Furthermore, a recent study[26] has shown that HLA-DQB1*06:02-positive healthy controls have fewer autoreactive CD8+ T cells for narcolepsy-relevant peptides, than NT1

patients and HLA-DQB1*06:02- negative healthy controls, which implies that the autoreactivity of CD8+ T cells, together with HLA-DQB1*06:02 encoded antigen presenting molecules for CD4+ T cells, have a role in the development of NT1.

Increased risk of sporadic narcolepsy in first-degree relatives have been reported in several studies[27-29], and there is 25-31% concordance for narcolepsy in monozygotic twins [27]. In addition to an increased risk of narcolepsy among first-degree relatives, an Asian study[29] described a "narcolepsy spectrum" (defined as MSLT with mean sleep latency ≤ 8 min or sleep-onset REM-sleep (SOREMs)) that could be found in 39.5 % of the first-degree relatives, which suggests the presence of subphenotypes among relatives. However, it must be noted that "narcolepsy spectrum" was also found in 15 % of the healthy control group in the study[29].

It is currently unknown whether a cohort of confirmed post-H1N1 NT1 patients (mainly H1N1-(Pandemrix®)-vaccinated) will have similar HLA-allele distributions as the previous studies of sporadic NT1 and studies of mainly sporadic, but possibly somewhat pre-/post-H1N1 mixed, samples of NT1. Moreover, to our knowledge no previous study has explored the HLA-allele distributions of the first-degree relatives of post-H1N1 NT1-patients, a possibly vulnerable/risk group. This is also the first study, to our knowledge, to explore global HLA associations with clinical and sleep phenotype parameters in both post-H1N1 NT1 patients and their first-degree relatives.

Materials and methods

Participants

Participants were consecutively included from February 2015 to August 2017 at the Norwegian Centre of Expertise for Neurodevelopmental Disorders and Hypersomnias (NevSom). Inclusion criteria for HLA-typing: white European, HLA-DQB1*06:02-positive patients, NT1 diagnosis verified with International Classification of Sleep Disorders (ICSD)-3 criteria[1] with disease onset after the

autumn 2009/the H1N1-vaccinations in 2009/10, and their first-degree relatives. Patients were without narcolepsy medication 14 days prior to the PSG, except for one patient who were without Modiodal for nine days prior to the PSG, two patients were without Venlafaxin for only a week prior to the PSG, and due to comorbidity one patient were on antidepressants during the PSG as well.

Patients medical history and records were examined, hence the disease onset was subsequently corrected for four NT1 patients to prior to the H1N1-vaccination. Pandemrix® was the only H1N1-vaccine used in Norway, and the H1N1-vaccination status for participants was obtained from the official Norwegian Immunisation Registry (SYSVAK). Two patients could not be found in SYSVAK, but reported having been H1N1-vaccinated in their workplace and were included as part of the vaccinated group. Exclusion criteria specifically for the subanalysis involving PSG and MSLT sleep parameters were: insufficient sleep time (<6 hours) on PSG, inability to perform an adequate sleep investigation, PSG apnea–hypopnea index (AHI) ≥ 5, medications (including sedative antihistamines, pain medication and antidepressants) possibly influencing sleep.

Measures of CSF hypocretin-1 were available in 75 of 77 NT1 patients included in the HLA-allele distribution analysis (demographics and clinical data presented in table 1), in 71 of 74 NT1 patients included in the subanalysis of global HLA associations with continuous clinical and sleep phenotype parameters (demographics, clinical data and sleep investigation parameters presented in table 2), and in 87 of 90 NT1 patients included in the analysis of HLA association with dichotomous variables (hypnagogic hallucinations and sleep paralysis) (demographics and clinical data presented in table 3), respectively. Further, as expected we found a high percentage (60%) of HLA-DQB1*06:02-positive first-degree relatives (table 3). Two of the patients (hypocretin-deficient) in the analysis of HLA association with the dichotomous variables did not perform PSG/MSLT. In addition, five of the first-degree relatives (n = 202) that were included in the stratified analysis regarding sleep paralysis, hypnagogic hallucinations and cataplexy-like episodes, did not perform the PSG/MSLT (Epworth Sleepiness scores were 0 in three of these relatives, while the last two reported scores of 8 and 10).

Cataplexy-like episodes in relatives consisted of rare episodes of muscle weakness associated with emotions which usually trigger cataplexy in narcolepsy (laughter, fun, excitement, and surprise). Other kinds of muscle weakness were not considered cataplexy-like. None of these relatives filled the ICSD-3 criteria[1] for narcolepsy.

Written informed consent was obtained from all participants prior to inclusion, and the study was approved by the Norwegian regional committees for medical and health research ethics (REK). 56 patients and 51 first-degree relatives included in the current study have previously been reported on in a diffusion tensor imaging study[30], 41 patients and 41 first-degree relatives have previously been reported on in a functional MRI study [31], 28 of the patients have been reported on in an quality of life study[32] and 70 patients have been reported on in a psychiatric comorbidity study [33].

Narcolepsy diagnosis

The clinical and sleep parameters obtained from patients and first- degree relatives were evaluated by an experienced neurologist, sleep medicine and narcolepsy expert (SK) using the ICSD-3 criteria for diagnoses,[1] including to verify the NT1 diagnoses. The participants (patients and first-degree relatives) underwent semi-structured interviews using a Norwegian translation of the validated Stanford Sleep Questionnaire[34], clinical evaluations and examinations including a neurological examination, actigraphy, PSG, MSLT, HLA-typing and routine blood parameter sampling. Spinal taps in patients were conducted at local hospitals prior to or after inclusion, and analyzed for CSF hypocretin-1 levels at the Hormone Laboratory of Oslo University Hospital using a slight modification to the method of Phoenix Pharmaceutical St. Joseph, MO, USA, as previously reported [35,36].

Polysomnography recordings

Most of the participants were evaluated with 10-14 days of actigraphy (Philips Actiwatch, Respironics Inc., Murrysville, PA, USA) prior to PSG and MSLT. PSG recordings were obtained with the SOMNOmedics system (SOMNOmedics GmbH, Randersacker, Germany) with the electrodes F3-A2, C3-A2, O1-A2, F4-A1, C4-A1 and O2-A1, vertical and horizontal electro-oculography, surface electromyography (EMG) of the submentalis and tibialis anterior muscles, electrocardiography, nasal thermistor, nasal pressure transducer, thoracic respiratory effort and oxygen saturation. Impedance was kept below 10kΩ (preferably 5Ω). The full-night PSG was followed by a 5-nap MSLT, were 2-hour intervals separated the naps (30 minutes). Sleep and event scoring were done according to American Academy of Sleep Medicine (AASM) criteria[1].

HLA genotyping

All Norwegian NT1 patients (N=90) and their first-degree relatives (N=202), excluding monozygotic twin siblings, underwent HLA genotyping using the NGSgo kit from GenDx (GenDx, Utrecht, The Netherlands) for the HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1 and –DPA1 genes. In brief, after amplification and library preparation according to the manufacturer, 2x 150 paired-end sequencing was performed on a MiSeq instrument (Illumina, San Diego, USA) with Miseq Reagent Kit v2 (300-cycles) at the Norwegian Sequencing Centre. HLA-genotypes were then obtained by analysing the sequencing reads with NGSengine (GenDx).

HLA-genotyping data[37] from the Norwegian Bone Marrow Donor Registry for anonymized Norwegian healthy controls was available for analyses. Notably, the HLA-DQB1*06:02 heterozygous healthy controls (N=1230) selected from the Norwegian HLA control dataset [37] for this study, had been genotyped to a lower level of resolution (G-group). Therefore, the patient and first-degree relative data were adjusted to the same typing level for some alleles before the analyses against

controls. Particularly the DQB1*02:01 and DQB1*02:02 were grouped for these analyses, as they belong to the same G-group (see Table S1 for complete list).

Statistical analysis

We compared the HLA-allele distribution between HLA-DQB1*06:02- heterozygous NT1 patients (N=77; 13 patients excluded since they were DQB1*06:02 homozygous) and controls (N=1230) to investigate whether other HLA-alleles were associated with risk or protection for NT1. In addition, we also included 59 HLA-DQB1*06:02- heterozygote parents of NT1 patients, (ICSD-3 diagnosis of Narcolepsy type 1 or 2 was excluded in the parents)[1], in these comparisons.

Subsequently, we conducted a quantitative subanalysis to investigate global HLA associations with the continuous variables (Epworth Sleepiness Scale (ESS) and PSG and MSLT sleep parameters) for 74 of the NT1 patients and 114 of the first-degree relatives with inclusion age as a confounder. Exclusion criteria for this global HLA association analysis: insufficient sleep time on PSG (< 6 hours) or inability to perform an adequate sleep investigation, PSG AHI $\geq$ 5, participants on medications possibly influencing the PSG and MSLT results (including sedative antihistamines, pain medication and antidepressants). For analyses of the dichotomous variables in patients; hypnagogic hallucinations and sleep paralysis, we included all patients (N=90). Notably, cataplexy was not analyzed since 94.4% of the patients had cataplexy. The exclusion criteria for the continuous variables are not relevant for exclusion in the analysis of hypnagogic hallucination and sleep paralysis, hence all 90 patients of the main cohort were included. Finally, we performed stratified analyses for relevant findings in the 202 first-degree relatives regarding sleep paralysis, hypnagogic hallucinations and cataplexy-like episodes. Although antidepressants possibly could diminish occurrence of current sleep paralysis and hypnagogic hallucinations[38,39], we did not exclude participants who were currently on this type of medication as the analysis is regarding a full lifetime occurrence (yes/no) of sleep paralysis and hypnagogic hallucinations.

The analyses program Unphased 3.0.10[40,41] utilizing the expectation-maximization method was used to perform association analyses, quantitative trait analyses and calculate global associations for each locus with a likelihood ratio test. For linkage disequilibrium analyses of the associated HLA-alleles, we utilized the Haploview 2.4 software[42]. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) and uncorrected p-values were calculated using Woolf's formula comprising Haldane's correction. We only tested HLA alleles present at an allele frequency >0.01 in patients, parents or controls. We report both uncorrected p-values and p-values corrected for multiple testing using the Benjamini-Hochberg (BH) procedure[43] (either locus-wise or allele-wise) to control the false discovery rate (FDR) at 5%. The tested loci and alleles are not independent due to high degree of linkage disequilibrium in the HLA complex.

Results

*Additional HLA associations, besides HLA-DQB1*06:02, in post-H1N1 NT1 patients*

Table 1 summarizes the demographic and clinical data for 77 post-H1N1, white European, HLA-DQB1*06:02 heterozygote NT1 patients (84.4% H1N1-vaccinated, two patients were H1N1-vaccinated but with disease onset before the vaccination) who were included in the main analysis of HLA distributions. Global associations with several HLA loci was seen for the patients, i.e. HLA-B (p = 0.004), HLA-C (p =0.005), HLA-DRB1 (p =0.003) and HLA-DQB1 (p = 0.004). All remained significant after correction for multiple testing with the BH procedure (6 loci tested). The individual HLA allele distributions (Table 4) revealed HLA class II alleles associated with significantly increased risk of NT1: DQB1*03:01 (OR = 2.16, p = 0.0007), DRB1*04:01 (OR = 2.24, p = 0.0006), DRB1*04:02 (OR = 7.42, p = 0.005), DRB1*04:07 (OR = 8.04, p = 0.00001), DRB1*11:04 (OR = 7.66, p = 0.001). All remained significant after correction for multiple testing with the BH procedure. Suggestive protective effects were observed with DQB1*06:03 (OR = 0.29, p = 0.04) and DQB1*02:01 (OR= 0.41, p = 0.02). All these alleles have previously been reported to be associated with sporadic and mainly sporadic (but

possibly somewhat pre-/post-H1N1 mixed) NT1 samples[14,19-21]. For the HLA class I alleles, we found significantly increased risk associated with B*35:03 (OR = 7.84, p = 0.00005) and B*51:01 (OR = 2.93, p = 0.002) and a protective association with A*25:01 (OR = 0.06, p =0.006), which have also been previously reported to be associated with sporadic and mainly sporadic NT1 samples [19,25], all remain significant after correcting for multiple testing with the BH procedure. Further increased risk were also associated with B*14:02 (OR = 6.37, p = 0.0006) and C*01:02 (OR = 2.65, p = 0.001) and a protective association with C*07:01 (OR = 0.31, p = 0.005). None of these have been reported previously and all remained signifiant after correction for multiple testing with the BH procedure. Notably, these allelic association are likely independent, as the linkage disequilibrium between the associated alleles was low (r^2<0.24 and r^2<0.08 in cases and controls, respectively). However, DRB1*04:07 and DRB1*11:04 had a D'>0.9 with DQB1*03:01 indicating that these alleles occur together on haplotypes. Among DQB1*03:01 haplotypes, 24% of cases and 6% of controls carry DRB1*04:07 (p=0.003), while 12% of cases and 3% of controls carry DRB1*11:04 (p=0.03). This suggests that these DRB1 alleles influence the risk conferred by DQB1*03:01.

We only observed suggestive associations for the previously reported A*11:01 (OR = 1.77, p= 0.06) and C*04:01 (OR = 1.72, p = 0.05).

*No HLA association in DQB1*06:02 heterozygote parents of NT1 patients*

Table 1 summarizes the demographic and clinical data for the 59 white European DQB1*06:02 heterozygote parents of NT1 patients that were included in the analysis of HLA distributions. There were no global HLA locus associations detected, nor any significant allelic HLA association observed in the parents (data not shown) after correction for multiple testing with the BH procedure.

HLA-C associated with sleep phenotype parameters in NT1 patients and first-degree relatives

We next wanted to investigate whether any of the eight genotyped HLA loci were associated with subphenotypes in NT1 and first-degree relatives. The clinical, demographic information, PSG and MSLT sleep parameters were available for the included 74 post-H1N1 white European NT1 patients and 114 white European, first-degree relatives and are summarized in table 2. Global locus-wise HLA associations was observed with continuous clinical and sleep parameter variables (Table 5), particularly, associations for the HLA-C and -DQB1 loci were significant. HLA-C was associated with the Epworth sleepiness scale (ESS) ($p = 0.002$), sleep latency for N2 ($p = 0.01$), sleep latency for deep sleep ($p = 0.01$) and the periodic limb movement (PLM) index ($p = 0.003$). Interestingly, also the first-degree relatives showed global associations between HLA loci and sleep parameters (Table 6), almost exclusively for HLA-C, including for ESS ($p = 0.002$), sleep latency-PSG ($p = 0.01$), sleep latency-N1 ($p = 0.01$), sleep latency-N2 ($p= 0.01$), sleep latency-deep sleep ($p = 0.003$), percent of N1 during total sleep time ($p = 0.01$) and percent N3 during total sleep time ($p = 0.0003$). Only percent N3 during total sleep time remained significant after correction for multiple testing with the BH procedure.

*A suggestive association between HLA-C*02:02 and clinical phenotypes; hypnagogic hallucinations and sleep paralysis, for NT1 patients.*

We next wanted to investigate the distribution of specific HLA-C alleles among NT1 patients for the dichotomous and clinical phenotypic parameters, hypnagogic hallucinations and sleep paralysis. Demographic and clinical data are summarized in table 3. These parameters are REM-sleep features associated with narcolepsy, and is also found in the normal population to some extent[44], in a previous study of first-degree relatives[29] and in our present cohort of first-degree relatives. In the 90 post-H1N1 NT1 patients (58 females, mean age 20.24 years, 83.3 % had experienced hypnagogic hallucinations and 70 % had experienced sleep paralysis). The allele frequency if HLA-C*02:02 was

significantly less frequent both among NT1 patients with hypnagogic hallucinations (4.00% vs 16.67%; OR = 0.21, p = 0.009) and those with sleep paralyses (3.17% vs 12.96%; OR=0.23, p = 0.01). Notably, none of these remained significant after correction for multiple testing with the BH procedure.

Due to the above suggestive HLA-C association, we stratified the 202 first-degree relatives (table 3) for HLA-C*02:02, but found no significant association with sleep paralysis, hypnagogic hallucination and cataplexy-like episodes.

Discussion

In our present cohort of post-H1N1 NT1 patients, we confirmed findings from previous reports from sporadic NT1 samples[14,20] and mainly sporadic (but possibly somewhat pre-/post-H1N1 mixed) NT1 samples [19,21,25] that not only HLA-DQB1*06:02 but also other HLA alleles are involved in the predisposition to NT1. Importantly, we found similar associations with several of both the HLA Class I and Class II alleles in our post-H1N1 patient cohort, as those previously reported in sporadic narcolepsy[14,20] and studies of mainly sporadic (but possibly somewhat pre-/post-H1N1 mixed) NT1 samples[19,21,25]. In previous large-scale studies[19,21,25] there is limited information provided about the samples, but based on the reported patient inclusion period and/or publication time, to the best of our knowledge, these possibly pre-/post-H1N1 mixed samples consists mainly of sporadic NT1 patients. Our study, although with a smaller sample size, is well-characterized and consists of confirmed post-H1N1 and mainly H1N1-vaccinated patients, which are vaccinated with the proposed narcolepsy-triggering vaccine, Pandemrix®. This supports a similar immunogenetic background for post-H1N1 and sporadic NT1. Taken together, several reports including the present study now show convincing evidence for involvement of both HLA class I and class II-alleles supporting a role for both CD4+ and CD8+ T cells in the presumed autoimmune mechanisms associated with the destruction of the hypocretin-producing cells in NT1.

Specifically, we observed increased disease risk with the alleles DQB1*03:01, DRB1*11:04, B*51:01, B*14:02, B*35:03, C*01:02, and several DRB1*04 subtypes as well as protective associations with A*25:01 and C*07:01 in our post-H1N1, largely H1N1-vaccinated, NT1 cohort. All the alleles withstood correction for multiple testing. However, our cohort has a limited sample size which affects our power. It is though reassuring that many of our findings have also been reported previously in larger NT1 cohorts. A study[14] of 420 sporadic narcolepsy patients with cataplexy also reported increased risk with; DQB1*03:01, DRB1*04 and DRB1*11 among DQB1*06:02-heterozygous individuals. Interestingly, given the higher resolution of our genotyping we saw that the association with DRB1*11 is restricted to DRB1*11:04, while the DRB1*04 association encompass several alleles, i.e. DRB1*04:01, 04:02 and 04:07, but not the frequent 04:04 allele. In particular, the association with DQB1*03:01 has been substantiated in several studies[19,20].

We also observed significant associations for some HLA class I alleles, A*25:01, B*51:01 and B*35:03 associations have been reported previously[19,25]. A*25:01 was reported[25] to show a protective association in patient samples from Switzerland and Poland/Slovakia, and a predisposing association in the patient samples from the Netherlands, France and Germany, although it was not found to be significant for the overall sample including all the countries. We also observed associations with class I alleles not previously reported in narcolepsy associated with significantly increased risk (B*14:02, C*01:02) and protection (C*07:01), hence we speculate that these alleles could potentially be particularly associated with post-H1N1, largely H1N1-vaccinated (Pandemrix®) narcolepsy. Future studies of larger patient samples of post-H1N1 NT1 patients with a high proportion of H1N1-vaccinated patients would be valuable to further replicate our findings.

Both sporadic and the post-H1N1-vaccination (Pandemrix®) related narcolepsy is believed to be caused by an autoimmune destruction of the hypocretin-producing cells[3]. The role of CD4+ T cells have been supported by the strong link between the DQB1*06:02 allele in both sporadic[14] and H1N1-vaccinated narcolepsy patients[12,15-17]. However, as more studies both in studies of largely sporadic narcolepsy (but possibly pre-/post-H1N1 mixed)[19,25] and now our study of post-H1N1, largely H1N1-

vaccinated, narcolepsy patients have found several associations also to HLA class I alleles, this supports the additional role of CD8+ T cells and natural killer cells in the autoimmune mechanisms leading to the development of NT1[3]. This is further supported in a recent study[26] showing that DQB1*06:02-positive healthy controls have fewer CD8+ T cells autoreactive for narcolepsy-relevant peptides, than NT1 patients and DQB1*06:02- negative healthy controls. This implies that autoreactivity in CD8+ T cells together with DQB1*06:02 has a role in the development of NT1. Other autoimmune disorders (e.g diabetes type 1 and multiple sclerosis) have also found independent associations with both class I and class II alleles [45,46]. After the >10 doubling of NT1 cases following the 2009/10 H1N1-vaccinations with Pandemrix®, it has been a matter of attention (and concern) that there is a high prevalence of now H1N1-vaccinated HLA-DQB1*0602-allele carriers in the normal populations and predictably especially in the families of the post-H1N1 NT1 patients. It is still basically a scientific mystery that only 1:16.000 H1N1-vaccinated[3] individuals developed narcolepsy.

Furthermore, we wanted to investigate first-degree relatives of post-H1N1 NT1 patients, due to several studies reporting both increased risk of narcolepsy and appearance of "narcolepsy spectrum" in first-degree relatives of sporadic NT1 patients[27-29]. Narcolepsy spectrum[29] refers to MSLT findings with mean sleep latency ≤ 8 min or SOREMs. Although this could indicate subphenotypes among first-degree relatives, the study also found that 15 % of the healthy control group had "narcolepsy spectrum"[29], which is in concordance with a large-scale community study[44] which found multiple SOREMs in 13.1% of males and 5.6% of females, and the MSLT criteria for narcolepsy were filled by 5.9% of males and 1.1% of females. A previous study[28] of first-degree relatives to sporadic narcolepsy patients using phone interviews found increased risk of what they defined as cataplexy in non-narcoleptic relatives compared to the general population. Therefore, due to the previously shown increased risk of sporadic narcolepsy in first-degree relatives and the possibility of narcoleptic subphenotypes we wanted investigate HLA associations with the phenotype in first-degree relatives of post-H1N1 NT1 patients, which to our knowledge have never previously been done.

Even though we did not find any statistically significant association after correction for multiple testing with HLA-alleles among parents of NT1 patients in our present cohort per se, we observed global associations between HLA-C and several sleep parameters in first-degree relatives including for ESS, sleep latency-PSG, sleep latency-N1, sleep latency-N2, sleep latency-deep sleep, percent of N1 during total sleep time and percent N3 during total sleep time. In the post-H1N1 NT1 patients, sleep parameters were associated with both HLA-DQB1 and HLA-C. For HLA-C specifically, associations with ESS, sleep latency for N2, sleep latency for deep sleep and the periodic limb movement (PLM) index were found. This could indicate that HLA-C play a role in the etiology behind some sleep parameters independent of a narcolepsy diagnosis, and although the immunological functions of the HLA class I molecules are well-known, these molecules have importantly also been implicated in brain development and plasticity [47], and it has been suggested that they could be involved in synpatic pruning [47].

Notably, these results are suggestive as most of them do not remain significant after the correction for multiple testing with the BH procedure and needs to be further explored in larger samples. Further, the sleep parameters are not necessarily independent of each other as for example the different sleep latencies and percentage of different sleep stages of total sleep will be highly dependent on each other.

Another consideration is that there is a wide age range in both the patient (6.5-51.5 years) and first-degree relative (6.8-53.8 years) group, and although the MSLT criteria have been validated for the diagnosis of adult[1] and pediatric NT1[48], there are several sleep parameters that have been previously shown to change with age in a meta-analysis [49] (e.g. sleep latency (increases with age), percent of stage N1 (increases with age) and percent of stage N3 (decreases with age)). Due to this we have controlled for age in our analysis regarding global HLA associations with sleep parameters.

We find that sleep paralysis and hypnagogic hallucinations show a suggestive association with a lower frequency of C*02:02 in the post-H1N1 NT1 patients. Both sleep paralysis and hypnagogic hallucinations have been considered REM-sleep phenomena in narcolepsy[3], and this

similarity of HLA-associations might indicate a possible similar disease mechanisms for these two symptoms in NT1. We did though not find an association with C*02:02 and hypnagogic hallucinations and sleep paralysis in first-degree relatives. Hence, the suggestive C*02:02 finding could be specifically linked to hypnagogic hallucinations and sleep paralysis only when associated with NT1, or we do not have enough power to detect similar associations in first-degree relatives due to lower symptom frequencies. However, we must emphasize that the association between C*02:02 and hypnagogic hallucinations and sleep paralysis in patients is only suggestive and needs to be further explored in future studies with larger sample sizes.

We did not exclude participants who were presently on antidepressants which is a possible limitation regarding the current occurrence of hypnagogic hallucinations, sleep paralysis and cataplexy-like phenomena[38,39]. However in our study the analysis considered only a full lifetime occurrence (yes/no) of these symptoms, and if we were to exclude the current medicated participants we should also have excluded all participants that at some time in their life had used antidepressants and a full lifetime medication history was not available in all. However, we believe this is of less importance as this was as mentioned an analysis based on a lifetime yes/no occurrence and not on frequency. Further supporting this choice the 13 first-degree relatives that currently used antidepressants actually had higher lifetime occurrence (23.1% with sleep paralysis, 30.8% with hypnagogic hallucinations and 7.7% with cataplexy-like phenomena) than the remaining 189 first-degree relatives (not on current antidepressants) where 6.3% had experienced cataplexy-like episodes, 11.7% (data missing for one participant) had experienced hypnagogic hallucinations and 10.6% (data missing for one participant) had experienced sleep paralysis.

In conclusion, we have detected several risk/protective alleles in our post-H1N1 NT1 patients (84.4% H1N1-vaccinated with Pandemrix®) similar to previous findings in studies of sporadic narcolepsy[14,20] and studies of possible pre/post-H1N1 mixed samples (largely sporadic)[19,21,25]. Parents of post-H1N1 NT1 patients, however, did not show a similar allele distribution profile as the patients. Our study further indicates a role for HLA-C regarding several sleep parameters in both first-degree

relatives and NT1 patients. Particularly C*02:02 seems to be interesting regarding a protective effect for both sleep paralysis and hypnagogic hallucinations in NT1 patients. However, most of the associations between HLA-C and sleep parameters are only suggestive as they do not survive correction for multiple testing and needs to be further explored in future studies with larger sample sizes.

Acknowledgments

A special thank you to Janita Vevelstad for being a part of the collection and sleep scoring of PSG/MSLTs. Further, we would like to express our appreciation for Ranveig Østrem's work with the biobanking and DNA extraction, and to Anne Rydland for help with the HLA genotyping.

Disclosure Statement

H.T.J. has received research support from the Norwegian Ministry of Health and Care Services. M.K.V reports no non-financial or financial disclosures. S.E.H.N has received research support from the Norwegian Ministry of Health and Care Services. R.V is supported by a Helse Sør-Øst grant (2017070). L.T.W. reports no non-financial or financial disclosures. P.M.T reports no non-financial or financial disclosures. B.A.L reports no non-financial or financial disclosures. S.K. has been an expert consultant for the Norwegian state and received research support from the Norwegian Ministry of Health and Care Services.

Funding

S. K and B.A.L is partially funded and H.T. J and S.E.H.N is fully funded by research support from the Norwegian Ministry of Health and Care Services. R.V. is supported by a Helse Sør-Øst grant (2017070).

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Table 1. Demographic and clinical data for HLA-allele distribution analysis of *HLA-DQB1*06:02* heterozygote NT1 patients and non-narcoleptic parents

	Narcolepsy type 1 (n = 77)	Parents of NT1 patients (n = 59)
Gender (female), n (%)	51(66.2)	32 (54.2)
Age (years), mean ± SD	19.9± 10.2	47.2± 6.2
Age at disease onset (years), mean ± SD	14.3± 9.8	N/A
Disease duration (years), mean ± SD	5.5 ± 1.7	N/A
H1N1-vaccinated, n (%)	65 (84.4)	31 (52.5)
Cataplexy, n (%)	73 (94.8)	2 (3.4)*
CSF hypocretin-1 ≤ 1/3 of level in normal population, n (%)	75/75 (100) 2 -N/A	N/A
Hypnagogic hallucinations, n (%)	64 (83.1)	3 (5.1)
Sleep paralysis, n (%)	53 (68.8)	3 (5.1)

CSF = cerebrospinal fluid, HLA = human leukocyte antigen, N/A = not available, NT1 = narcolepsy type 1 patients, SD = standard deviation. After inclusion two of the narcolepsy type 1 patients had their disease onset changed to before the H1N1-vaccination. *First-degree relatives with cataplexy-like phenomena (rare episodes of muscle weakness but associated with laughter, fun, excitement and surprise) did not fulfill the International Classification of Sleep Disorders (ICSD)-3 criteria[1] for narcolepsy.

Table 2. Demographic, clinical data, PSG and MSLT measurements for the HLA-associations analysis with clinical data and sleep parameters for NT1 patients and first-degree relatives

	Narcolepsy (n = 74)	First- degree relatives (n = 114)
Gender (female), n (%)	48 (64.9)	68 (59.6)
Age (years), mean ± SD (age range in years)	18.7 ± 8.6* (6.5-51.5)	29.8 ± 14.7* (6.8-53.8)
Age at disease onset (years), mean ± SD	13.3± 8.2	N/A
Disease duration (years), mean ± SD	5.5 ± 1.8	N/A
H1N1-vaccinated, n (%)	62 (83.8)	77 (67.5)
Cataplexy, n (%)	69 (93.2)	9 (7.9)**
*HLA-DQB1*06:02*-positivity, n (%)	74 (100)	70 (61.4)
*HLA-DQB1*06:02* homozygote, n (%)	10 (13.5)	9 (7.9)
CSF hypocretin-1 ≤ 1/3 of level in normal population, n (%)	71/71 (100) 3 -N/A	N/A
Hypnagogic hallucinations, n (%)	63 (85.1)	14/113 (12.4) 1/114- N/A
Sleep paralysis, n (%)	49 (66.2)	12/113 (10.6) 1/114-N/A
Epworth Sleepiness Scale, mean ± SD	17.9± 3.8 3- N/A	5.0 ± 4.0
Total Sleep Time, PSG (TST) (hours) mean ± SD	8.1 ± 0.9	8.0 ± 0.7
Sleep Efficiency, PSG (%) mean ± SD	90.3± 6.8	92.6 ± 5.3
Sleep Latency, PSG (minutes), mean ± SD	6.0± 6.5	14.5 ± 15.1
Sleep Latency N1, PSG (minutes), mean ± SD	6.1± 6.8	14.5 ± 15.1
Sleep Latency N2, PSG (minutes), mean ± SD	19.3 ± 15.7	19.2 ± 16.1
Sleep Latency Deep, PSG (minutes), mean ± SD	35.2± 20.5	32.3 ± 17.8
Sleep Latency REM, PSG (minutes), mean ± SD	29.6± 51.1	94.5 ± 48.2
Sleep Switch Index (divided by TST), PSG, mean ± SD	14.6± 4.3	12.3 ± 3.5

Sleep Switch Index (divided by TIB), PSG, mean ± SD	13.1± 3.6	11.3 ± 3.0
Awakening Index, PSG, mean ± SD	2.5 ± 1.2	1.4 ± 0.9
N1 % of TST, PSG, mean ± SD	9.6± 5.8	6.1 ± 3.7
N2 % of TST, PSG, mean ± SD	38.7± 8.4	47.9 ± 8.4
N3 % of TST, PSG, mean ± SD	25.9 ± 10.3	23.5 ± 11.5
REM % of TST, mean ± SD	25.8 ± 6.7	22.5 ± 4.9
AHI index, PSG, mean ± SD	0.8 ± 1.0	1.2 ± 1.3
PLM index, PSG, mean ± SD	17.8 ± 20.5	7.5 ± 14.3
MSLT sleep latency (minutes), mean ± SD	2.8 ± 2.9	13.4 ± 4.4
MSLT REM-latency (minutes), mean ± SD	1.9 ± 1.5	8.6 ± 5.7***
MSLT SOREMS (yes), n (%)	74 (100)	27 (23.7)
MSLT SOREM, mean ± SD	4.6 ± 0.8	0.3 ± 0.6

After inclusion one narcolepsy type 1 patients had their disease onset changed to before the H1N1-vaccination. AHI = apnea-hypopnea index (Apnea + hypopnea divided by TST (in hours)), Awakening index = number of wake periods during time in bed divided by total sleep time in hours, CSF = cerebrospinal fluid, HLA = human leukocyte antigen, MSLT = multiple sleep latency test, N/A = not available, N1 = N1 sleep stage, N2= N2 sleep stage, N3= N3 sleep stage, NT1 = narcolepsy type 1, PLM = periodic limb movements, PLM index = PLMs divided by TST (in hours), PSG = Polysomnography, REM = rapid eye movement sleep, Sleep latency REM = period of time between sleep onset and the beginning of the first REM epoch, TIB = time in bed, TST = total sleep time, SD = Standard deviation, Sleep efficiency = TST divided by TIB, SOREM = Sleep onset REM. Sleep latency PSG = sleep latency for the whole PSG recording is defined as the period of time between the lights off marker and sleep onset (similar definition for all sleep latencies for stage N1, N2 and deep (N3)). *12 patients and 12 first degree relatives are <12 years and 31 patients and 32 first degree relatives are <16 years. **First-degree relatives with cataplexy-like phenomena (rare episodes of muscle weakness but associated with laughter, fun, excitement and surprise) did not fulfill the International Classification

of Sleep Disorders (ICSD)-3 criteria[1] for narcolepsy. *** MSLT REM-latency only calculated for first-degree relatives with SOREMs.

Table 3. Demographic and clinical data for HLA-allele association analysis for hypnagogic hallucinations, sleep paralysis and cataplexy for NT1 patients and first-degree relatives

	Narcolepsy type 1 (n = 90)	First-degree relatives of NT1 patients (n = 202)
Gender (female), n (%)	58 (64.4)	109 (54.0)
Age (years), mean ± SD	20.2 ± 10.5	35.9 ± 15.2
Age at disease onset (years), mean ± SD	14.6 ± 10.0	N/A
Disease duration (years), mean ± SD	5.6 ± 1.8	N/A
H1N1-vaccinated, n (%)	76 (84.4)	122 (60.4)
Cataplexy, n (%)	85 (94.4)	13 (6.4)*
HLA-DQB1*06:02-positivity, n (%)	90 (100)	121 (60.0)
HLA-DQB1*06:02 homozygote, n (%)	13 (14.4)	17 (8.4)
CSF hypocretin-1 ≤ 1/3 of level in normal population, n (%)	87/87 (100) 3 -N/A	N/A
Hypnagogic hallucinations, n (%)	75 (83.3)	26 (12.9) 1 – N/A
Sleep paralysis, n (%)	63 (70)	23 (11.4) 1 – N/A

CSF = cerebrospinal fluid, HLA = human leukocyte antigen, N/A = not available, NT1 = narcolepsy type 1 patients, SD = standard deviation. After inclusion three of the narcolepsy type 1 patients had their disease onset changed to before the H1N1-vaccination. *First-degree relatives with cataplexy-like phenomena (rare episodes of muscle weakness but associated with laughter, fun, excitement and surprise) did not fulfill the International Classification of Sleep Disorders (ICSD)-3 criteria[1] for narcolepsy.

Table 4. Allele distributions for 77 HLA-DQB1*06:02-heterozygote NT1 patients compared to 1230 HLA-DQB1*06:02-heterozygote controls

	NT1		Controls				
	Risk type	Non-risk type	Risk type	Non-risk type	OR (95% CI)	p	Prev. reported
HLA-A 25:01	0	152	121	2339	0.06 (0.009-0.45)	0.006	[a]
HLA-B 14:02	4	150	11	2449	6.37 (2.22-18.31)	0.0006	
HLA-B 35:03	5	149	11	2449	7.84 (2.90-21.17)	0.00005	*[19]
HLA-B 51:01	10	144	59	2401	2.93 (1.51-5.70)	0.002	*[19]
HLA-C 01:02	13	141	85	2375	2.65 (1.47-4.78)	0.001	
HLA-C 07:01	5	149	264	2196	0.31 (0.13-0.70)	0.005	
HLA-DRB1 04:01	23	129	184	2276	2.24 (1.41-3.55)	0.0006	[b]
HLA-DRB1 04:02	2	150	5	2455	7.42 (1.84-29.94)	0.005	[b]
HLA-DRB1 04:07	6	146	13	2447	8.04 (3.20-20.23)	0.00001	[b]
HLA-DRB1 11:04	3	149	7	2453	7.66 (2.28-25.72)	0.001	[c]
HLA-DQB1 03:01	25	129	205	2255	2.16 (1.39-3.37)	0.0007	*[14,19,20]

All the alleles reported in the table have allele frequencies above 1 % in our sample of NT1 patients or controls. Uncorrected p-values ≤ 0.01 are reported, and all alleles remained significant after correction for multiple testing with the Benjamini-Hochberg procedure, controlling the false discovery rate at 5%. Missing

genotypes have not been included in the total number of alleles. CI = confidence interval, controls = healthy HLA-DQB1*06:02-heterozygote controls, HLA = human leukocyte antigen, Non-risk type = allele not found, NT1 = HLA-DQB1*06:02-heterozygote narcolepsy type 1 patients, OR = odds ratio, risk type = allele found, prev. reported = previously reported in studies of narcolepsy. a) HLA-A*25:01 were found in a previous study by Tafti et al.[25] where it does not reach significance for the overall sample, but it was found to be predisposing in the patient samples from the Netherlands, France, Germany, and protective in the patient samples from Switzerland and Poland/Slovakia. b) Mignot et al.[14] found significantly higher risk for HLA-DQB1*06:02-heterozygote NT1 patients associated with HLA-DRB1*04. c) Mignot et al.[14] found significantly higher risk for HLA-DQB1*06:02-heterozygote NT1 patients associated with HLA-DRB1*11.

Table 5. Global associations between HLA loci and sleep parameters for NT1 patients

	HLA-A	HLA-B	HLA-C	DPA1	DPB1	DQA1	DQB1	DRB1
Disease duration	0.8	1.0	0.4	0.2	0.8	0.2	0.1	0.6
Disease onset	0.5	1.0	0.2	0.2	0.2	0.5	0.6	0.8
ESS	0.9	0.5	0.002*	0.3	0.06	0.4	0.1	0.1
PSG Total sleep time (TST)	1.0	1.0	0.9	1.0	0.8	0.7	0.7	0.9
PSG Sleep efficiency	1.0	1	0.9	0.9	0.5	1.0	1.0	1.0
PSG Sleep latency	0.2	0.6	0.04	0.3	0.4	0.6	0.01*	0.4
PSG latency N1	0.2	0.6	0.04	0.2	0.4	0.6	0.01*	0.4
PSG latency N2	0.2	0.7	0.01*	0.2	0.06	0.9	0.6	1.0
PSG Latency Deep	0.09	0.3	0.01*	0.009*	0.09	1.0	0.07	0.6
PSG REM latency	0.02	0.2	0.03	1.0	0.6	0.1	0.005*	0.3
Sleep stage index (divided by TST)	0.2	1.0	0.6	0.3	0.4	0.1	0.04	0.05
Sleep stage Index (divided by TIB)	0.2	1.0	0.2	0.3	1.0	0.1	0.05	0.1
Awakening index	0.7	0.9	0.3	0.3	0.2	0.09	0.003*	0.003*
PSG REM TST	0.8	0.3	0.06	0.1	0.1	0.08	0.05	1.0
PSG N1 TST	0.4	1.0	1.0	0.2	0.8	0.4	0.2	0.4
PSG N2 TST	0.5	1.0	0.7	0.2	0.03	0.08	0.07	1.0
PSG N3 TST	0.1	1.0	0.3	0.3	0.03	0.6	0.4	0.4
AHI Index	0.4	0.5	0.02	0.3	0.4	0.01*	0.04	0.2
PLM Index	0.2	0.5	0.003*	0.6	0.4	0.05	0.02	0.05
MSLT Sleep latency	0.2	0.2	0.03	0.4	0.4	0.3	0.02	0.8
MSLT REM Latency	0.2	0.6	0.02	0.8	0.8	0.01*	0.08	0.07
SOREMS MSLT	0.9	0.8	0.1	0.4	0.4	0.6	0.7	1.0

Analysis controlled for age. *Global p-values ≤ 0.01. AHI = apnea-hypopnea index (Apnea + hypopnea divided by TST (in hours)), Awakening index = number of wake periods during time in bed divided by total sleep time in hours, HLA = human leukocyte antigen, ESS = Epworth Sleepiness Scale, MSLT = multiple sleep latency test, N1 = N1 sleep stage, N2= N2 sleep stage, N3= N3 sleep stage, NT1 = narcolepsy type 1, PLM = periodic limb movements, PLM index = PLMs divided by TST (in hours), PSG = Polysomnography, REM = rapid eye movement sleep, Sleep latency REM = period of time between sleep onset and the beginning of the first REM epoch, TIB = time in bed, TST = total sleep time, SD = Standard deviation, Sleep efficiency = TST divided by TIB, SOREM = Sleep onset REM. Sleep latency PSG = sleep latency for the whole PSG recording is defined as the period of time between the lights off marker and sleep onset (similar definition for all sleep latencies for stage N1, N2 and deep (N3)).

Table 6. Global associations between HLA loci and sleep parameters for first-degree relatives of NT1 patients

	HLA-A	HLA-B	HLA-C	DPA1	DPB1	DQA1	DQB1	DRB1
ESS	0.5	0.8	0.002*	1.0	0.06	0.02	0.02	0.4
PSG Total sleep time (TST)	0.07	0.8	0.04	0.5	0.7	0.6	0.6	1.0
PSG Sleep efficiency	1.0	0.7	0.8	0.6	1.0	0.9	1.0	1.0
PSG Sleep latency	0.5	0.1	0.01*	1.0	0.7	0.1	0.2	0.6
PSG latency N1	0.5	0.1	0.01*	1.0	0.7	0.1	0.2	0.6
PSG latency N2	0.5	0.1	0.01*	1.0	0.8	0.08	0.2	0.6
PSG latency Deep	0.4	0.06	0.003*	1.0	0.5	0.007*	0.01*	0.6
PSG REM latency	0.4	0.4	0.9	0.5	0.04	0.8	0.3	1.0
Sleep stage index (divided by TST)	0.6	0.6	0.02	1.0	0.3	0.2	0.5	0.7
Sleep Stage Index (divided by TIB)	0.8	0.7	0.2	1.0	0.2	0.3	0.5	0.7
Awakening index	0.3	0.9	0.1	0.8	0.4	0.6	0.3	0.3
PSG REM TST	0.6	0.6	0.3	0.08	0.2	0.3	0.04	0.6
PSG N1 TST	0.6	0.4	0.01*	0.4	0.05	0.06	0.1	0.6
PSG N2 TST	0.3	0.7	0.03	0.02	0.2	0.1	0.09	0.3
PSG N3 TST	0.7	0.4	0.0003*	0.02	0.3	0.1	0.06	0.7
AHI index	0.7	1.0	0.7	0.02	0.3	0.2	0.08	0.5
PLM Index	0.05	0.7	0.08	0.5	0.02	0.04	0.03	0.8
MSLT sleep latency	1.0	0.7	1.0	0.02	0.5	0.03	0.1	0.6
MSLT REM Latency	0.6	0.8	0.08	1.0	0.09	0.7	0.8	1.0
SOREMS MSLT	0.6	0.8	0.7	0.4	0.09	0.3	0.7	1.0

Analysis controlled for age. *Global p-values ≤ 0.01. AHI = apnea-hypopnea index (Apnea + hypopnea divided by TST (in hours)), Awakening index = number of wake periods during time in bed divided by total sleep time in hours, HLA = human leukocyte antigen, ESS = Epworth Sleepiness Scale, MSLT = multiple sleep latency test, N1 = N1 sleep stage, N2= N2 sleep stage, N3= N3 sleep stage, NT1 = narcolepsy type 1, PLM = periodic limb movements, PLM index = PLMs divided by TST (in hours), PSG = Polysomnography, REM = rapid eye movement sleep, Sleep latency REM = period of time between

sleep onset and the beginning of the first REM epoch, TIB = time in bed, TST = total sleep time, SD = Standard deviation, Sleep efficiency = TST divided by TIB, SOREM = Sleep onset REM. Sleep latency PSG = sleep latency for the whole PSG recording is defined as the period of time between the lights off marker and sleep onset (similar definition for all sleep latencies for stage N1, N2 and deep (N3)).