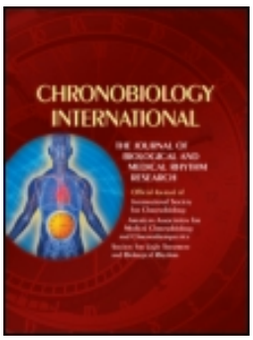

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REPORT

Circadian gene variants influence sleep and the sleep electroencephalogram in humans

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ABSTRACT

The sleep electroencephalogram (EEG) is highly heritable in humans and yet little is known about the genetic basis of inter-individual differences in sleep architecture. The aim of this study was to identify associations between candidate circadian gene variants and the polysomnogram, recorded under highly controlled laboratory conditions during a baseline, overnight, 8 h sleep opportunity. A candidate gene approach was employed to analyze single-nucleotide polymorphisms from five circadian-related genes in a two-phase analysis of 84 healthy young adults (28 F; 23.21 ± 2.97 years) of European ancestry. A common variant in *Period2* (*PER2*) was associated with 20 min less slow-wave sleep (SWS) in carriers of the minor allele than in noncarriers, representing a 22% reduction in SWS duration. Moreover, spectral analysis in a subset of participants (n = 37) showed the same *PER2* polymorphism was associated with reduced EEG power density in the low delta range (0.25–1.0 Hz) during non-REM sleep and lower slow-wave activity (0.75–4.5 Hz) in the early part of the sleep episode. These results indicate the involvement of *PER2* in the homeostatic process of sleep. Additionally, a rare variant in *Melatonin Receptor 1B* was associated with longer REM sleep latency, with minor allele carriers exhibiting an average of 65 min (87%) longer latency from sleep onset to REM sleep, compared to noncarriers. These findings suggest that circadian-related genes can modulate sleep architecture and the sleep EEG, including specific parameters previously implicated in the homeostatic regulation of sleep.

KEYWORDS

Circadian genes; sleep EEG; slow-wave sleep; slow-wave activity

Introduction

Sleep is regulated by two main physiological processes: (1) the circadian timing system (process C), regulating daily rhythms of sleep and wakefulness; and (2) the sleep homeostatic process (process S), which tracks accumulating sleep pressure with time awake and which decreases with time asleep (Borbély, 1982). The prevalence and amplitude of slow waves in the sleep electroencephalogram (EEG), typically quantified by slow-wave activity (SWA; 0.75–4.5 Hz power density) or slow-wave sleep (SWS), serve as markers of homeostatic sleep pressure (Achermann et al., 1993). The EEG is highly heritable, both during wakefulness (h^2 = 76–89%) (Lennox et al., 1945; van Beijsterveldt & van Baal, 2002) and during sleep (h^2 = 50–96%) (Ambrosius et al., 2008; Landolt & Dijk, 2010; Linkowski, 1999). Surprisingly, despite the high heritability of EEG traits, little is known about the genetic basis of these inter-individual differences in the sleep EEG.

Several sleep-associated genetic variants have been identified in human candidate-gene studies but associations have not been consistently

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reproduced; namely in *PER3, DQB1*0602, ADA, ADORA2A, BDNF* and *COMT* (Bachmann et al., 2012; Bodenmann et al., 2012; Bodenmann & Landolt, 2010; Guindalini et al., 2014; Mignot et al., 1999; Retey et al., 2005; Viola et al., 2007). Of these genes, however, only *PER3* is considered a genetic component of the circadian molecular timing system shown to alter the sleep EEG in humans (Viola et al., 2007). Gene variants in *BDNF*, a circadian-related gene, have also been implicated in changes to EEG during sleep (Bollen & Curran, 2005; Guindalini et al., 2014).

In the current study, we used a candidate gene approach to identify associations between single-nucleotide polymorphisms (SNPs) in circadian-related genes and polysomnographic (PSG) measures collected under controlled laboratory conditions, an ideal setting for reducing environmental and behavioral variation to identify genetic variants with a strong effect. Common SNPs in five circadian-related genes were genotyped in our laboratory sample: *Circadian Locomotor Output Cycles Kaput* (*CLOCK*), *Cryptochrome2* (*CRY2*), *basic helix-loop-helix family, member e41* (*BHLHE41*; or *DEC2*), *Period2* (*PER2*) and *Melatonin Receptor1B* (*MTNR1B*). As an initial exploration of the role of circadian genes in sleep physiology, we selected these genes as candidates based on: (1) previously reported associations with sleep phenotypes in the case of *CLOCK, DEC2* and *PER2* (Allebrandt et al., 2010; He et al., 2009; Toh et al., 2001); and (2) association with type 2 diabetes (T2D) risk (Dupuis et al., 2010; Lyssenko et al., 2008; Prokopenko et al., 2008), in the case of *CRY2* and *MTNR1B*. Furthermore, *MTNR1B* gene variants were included in this analysis because of previously documented association with metabolic measures (Dupuis et al., 2010; Lyssenko et al., 2008; Prokopenko et al., 2008) and its role as an output of the clock, not because of a role in the core molecular clock. Because sleep disturbances and short sleep duration lead to increased risk of T2D (Nedeltcheva & Scheer, 2014; Spiegel et al., 2005), we hypothesized that changes in sleep quality and/or duration may provide a mechanistic link between known variants in this gene and T2D risk (Hanlon & Van Cauter, 2011).

Methods

Overview

This genetic study includes data from individuals who previously participated in one of several inpatient physiological protocols in which their sleep EEG was recorded in the Intensive Physiological Monitoring Unit of the Brigham and Women's Hospital. Written informed consents, both for the original in-laboratory studies and for the genetic studies, were obtained from each study participant. The Partners Human Research Committee approved the protocol and study procedures conformed to the Declaration of Helsinki.

Study participants and screening procedures

A total of 101 healthy adults aged 18–35 years (34 F; mean age ± SD 23.10 ± 3.28 years) were enrolled in this study. A PSG recording collected during an 8 h baseline overnight sleep episode (first or third night; see below; Figure 1) and genetic samples obtained from each participant were used for association analyses.

Prior to admission, study participants completed extensive screening procedures to ensure eligibility. Inclusion criteria required that participants were 18–35 years of age and in good health, free from medical or psychological conditions or disorders. While the protocols in which the volunteers participated varied slightly from each other with respect to specific screening procedures, all protocols included a physical examination, laboratory tests of blood and urine samples, a 12-lead electrocardiogram (ECG), and an interview with a clinical psychologist to confirm good medical and psychological health. Exclusion criteria included night work in the prior 3 years and travel across >1 time zone in the prior 3 months. During the 3-week pre-admission screening interval, participants were asked to refrain from taking medications, alcohol, caffeine and nicotine; and they maintained a self-selected, stable, 8 h sleep schedule each night, which was verified with daily sleep diaries, time-stamped telephone calls at bedtime and wake time, and wrist actigraphy for a minimum of 1 week prior to admission.

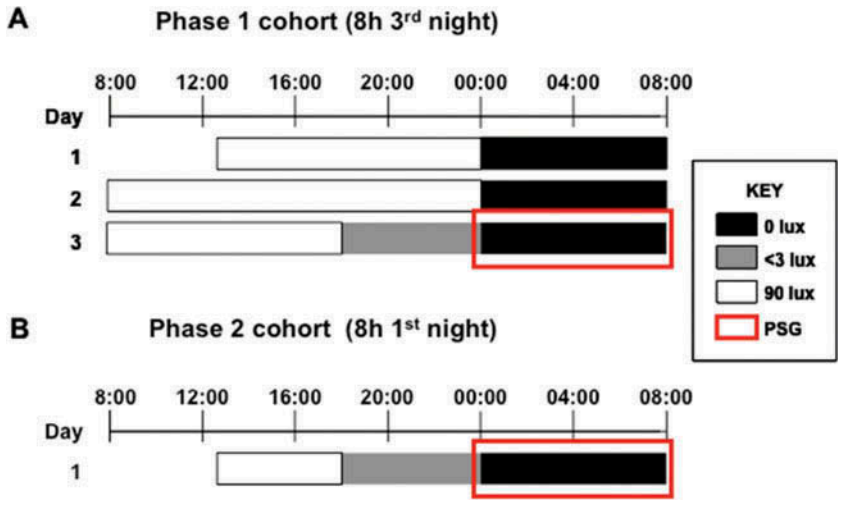

Figure 1. Raster plots of laboratory protocols show PSG recorded during 8 h sleep episodes for a representative participant with a habitual sleep timing of 00:00–08:00. For all study protocols, the ambient room light was dim (gray) for at least 6 h before the sleep episodes (black) during which PSGs were recorded. (a) In the phase 1 cohort, PSG was recorded (red box) during the third baseline night. (b) The first baseline night was recorded in the phase 2 cohort.

Genotyping

DNA was extracted from whole blood using Qiagen Autopure LS (Qiagen, Limburg, The Netherlands). A total of 116 SNPs tagging common variation and rare missense variants from the five candidate genes (*CLOCK, CRY2, BHLHE41, PER2* and *MTNR1B*; ±20 kb) were selected, providing full coverage for each gene (see supplementary table 1 for full list). SNPs with a minor allele frequency (MAF) > 0.05 were selected using a pairwise tagging threshold ($r^2 > 0.8$) in four populations: Northern and Western European (CEU), Yoruba African (YRI), Han Chinese (CHB) and Japanese (JPT) in HapMap release 21 in Tagger on Haploview (Barrett et al., 2005). Additionally, 58 African-American and Hispanic ancestry informative markers were genotyped to test and correct for population stratification. Genotyping was performed using the Sequenom platform (Broad Institute, Cambridge, MA).

Standard quality control criteria included SNP genotyping rate > 90%, MAF > 1% and Hardy–Weinberg equilibrium (HWE) $p > 10^{-6}$ and were assessed using PLINK (Purcell et al., 2007). Also, 11 SNPs were excluded due to low genotyping rate, 20 SNPs were excluded due to low MAF and 3 SNPs were excluded on the basis of departure from HWE; several of the SNPs were excluded by more than one criterion leaving 87 SNPs that passed all quality control filters. For primary association analysis, 84/101 participants that were genetically categorized as being of European ancestry (within 4 SD of the HapMap release 3 CEU panel based on principal components analysis) were selected (see supplementary figure 1).

PSG recordings and sleep measures

PSG recordings were continuously monitored and recorded throughout an 8 h baseline sleep episode using a Vitaport-3 recording system (TEMEC Instruments, B.V. Kerkrade, The Netherlands). Recordings included the EEG (derivations C3, C4, O1 and O2, referenced to contralateral mastoids), right and left electrooculogram, electromyogram and the ECG. Electrode impedances were <10 kΩ prior to the beginning of each recording. EEG signals were filtered (high-pass EEG filter 0.23 Hz; low-pass EEG filter 70.1 Hz; 24 dB/octave, sampling rate 256 Hz). PSG records were scored (blind to genotype) visually in 30 s epochs according to conventional criteria of Rechtschaffen and Kales (1968). Sleep measures included total sleep time (TST); sleep efficiency (TST/time in bed as a %); sleep latency (interval between lights off and first stage of sleep); duration for stage 1, stage 2, and SWS (SWS; stages 3 and 4 combined), REM (rapid eye movement) sleep and

wakefulness; wake after sleep onset (WASO); wake after final awakening (WAFA); and number of awakenings longer than 30 s. Duration of sleep stages and wakefulness was calculated in minutes. For sleep stage 2, SWS and REM (rapid eye movement) sleep, latencies were computed from sleep onset. PSG measures that were not normally distributed were transformed to achieve a normal distribution, including SWS duration and number of awakenings (log transformation) and REM sleep latency (inverse normal transformation).

Spectral analysis of C3/A2 EEG recordings was also completed in the subset of male participants whose PSG was recorded on the third baseline night (n = 37; see below). Signals were first visually inspected, and 4 s epochs containing artifacts arising from body movements, eye blinks or eye movements were removed. The remaining 4 s epochs were subjected to fast Fourier transformation, using a 10%-cosine-tapered window (Vitascore, TEMEC, The Netherlands). A maximum of 10 consecutive, overlapping 4 s epochs were averaged into 30 s epochs and matched with sleep scores. Data were reduced further by averaging power densities into 0.5 Hz bins. For a specific SNP that was associated with SWS, power spectra were compared between genotype groups (risk-allele carriers vs. noncarriers) by ANOVA (SAS 9.2, SAS Institute Inc., Cary, NC, USA).

Genetic analysis and protocol groups

Genetic analysis was initiated in a cohort in whom PSG was recorded during the 8 h sleep episode on the third baseline night (phase 1; n = 59; Figure 1a). Potential associations with significance ($p < 0.001$) were then tested in a separate cohort of different individuals in whom PSG recordings were collected during the 8 h sleep episode on the first baseline night in the laboratory (phase 2; n = 25; Figure 1b). Procedures for the collection of PSG, sleep schedule, room conditions and ambient light levels prior to the sleep episode were consistent between phase 1 and 2 cohorts. Both groups were independent with no overlap of individuals, and the cohorts were selected *a priori*. The phase 2 group in which PSG was collected during the first baseline night was selected to provide a more stringent test of replication for any potential associations that were identified in the phase 1 cohort, assuming greater variability in measures due to first-night effects.

An additive genetic model was used to test for association of SNPs with sleep measures in linear regression analyses. All analysis presented was adjusted for covariates age, sex and ancestry (five principal components). Permutation testing was used to evaluate significance after correction for multiple comparisons. As power for single-variant association testing was low and individual effects may be subtle, an additional gene-based analysis was conducted using sequence kernel association testing (SKAT) to detect combined effects of variants (Ionita-Laza et al., 2013). This analysis provides a more comprehensive view of potential associations between candidate genes and phenotypes by including all variants from one gene, rather than each individual SNP, in the analysis. Furthermore, this allowed for a reduced number of comparisons (the number of genes as opposed to the number of SNPs).

Results

Summary sleep measures for both cohorts are shown in Table 1. The cohorts differed significantly only in REM sleep duration, with a shorter REM sleep duration in the phase 2 cohort, which may have been due to a first-night effect in that cohort (Agnew et al., 1966). Results for SNPs most strongly associated with sleep phenotype measures in the phase 1 cohort, in the phase 2 cohort and from meta-analysis of the entire sample are listed in Table 2. In phase 1, an SNP in the *PER2* gene (rs6753456) showed a significant association with SWS duration; a second *PER2* SNP (rs3739064) was associated with the number of awakenings; and a *MTNR1B* SNP (rs7942988) was associated with REM sleep latency. None of these associations remained significant in the smaller phase 2 cohort in which only first-night sleep data were assessed. Nonetheless, two associations remained significant in the meta-analysis of both phases of analysis: the *PER2* SNP with SWS duration and the *MTNR1B* SNP with REM sleep latency. For the two phenotypes that showed significant association in the meta-analysis, the association with all tested SNPs is shown in supplementary figure 2; and association plots for remaining sleep phenotypes with all SNPs are shown in supplementary figure 3.

Table 1. Summary of sleep measures for phase 1 (n = 59) and phase 2 (n = 25) cohorts.

PSG measure	Phase 1 mean (SD)	Phase 2 mean (SD)	t-Test p
TST (min)	440.98 (21.56)	431.76 (38.79)	0.272
Sleep efficiency (%)	91.85 (4.49)	89.92 (8.07)	0.269
Sleep latency (min)	10.59 (7.02)	12.26 (8.89)	0.410
Stage 1 duration (min)	26.17 (10.80)	21.38 (11.19)	0.077
Stage 2 duration (min)	209.90 (35.16)	219.18 (32.62)	0.250
Stage 2 latency (min)	3.54 (6.86)	4.46 (3.22)	0.407
SWS duration (min)	87.53 (34.74)	92.08 (28.99)	0.539
REM duration (min)	118.08 (23.52)	99.40 (20.96)	**7.31E-04**
REM latency (min)	73.28 (36.75)	80.02 (37.85)	0.456
WASO (min)	22.05 (20.44)	32.88 (34.49)	0.153
Awakenings >30 s	10.46 (6.92)	11.44 (8.43)	0.610
WAFA (min)	3.76 (14.02)	3.40 (7.68)	0.880

Significant p values ($p < 0.05$) are shown in bold.

Table 2. Genetic association results with PSG phenotypes.

Gene	*PER2*	*PER2*	*MTNR1B*
SNP	rs6753456	rs3739064	rs7942988
Minor allele	G	G	T
Frequency (%)	37.5	21.3	3.7
PSG measure	SWS duration (min)	# awakenings/night	REM latency (min)
Phase 1 cohort			
n	59	57	57
β (SE)	−24.37 (7.00)	−9.64 (2.42)	81.19 (17.50)
β (SE)*	−0.29 (0.08)	−0.93 (0.25)	3.90 (1.00)
p*	**9.2E-04**	**6.0E-04**	**3.1E-04**
Phase 2 cohort			
n	25	25	25
β (SE)	−19.38 (10.41)	−2.22 (4.51)	27.27 (27.66)
β (SE)*	−0.19 (0.11)	−0.02 (0.39)	1.47 (1.43)
p*	0.103	0.959	0.32
Meta-analysis			
n	84	82	82
β (SE)	−20.46 (5.58)	−6.30 (2.23)	64.55 (14.46)
β (SE)*	−0.24 (0.06)	−0.54 (0.21)	0.61 (0.17)
p*	**4.4E-04**	**1.3E-02**	**7.4E-04**
p_{adj}*	**4.6E-02**	0.505	**4.6E-02**

Three SNPs (rs6753456, rs3739064 and rs7942988) showed association ($p < 0.001$) with PSG sleep phenotypes in the phase 1 cohort. The β and p value of transformed data are denoted by asterisk. Adjusted p values (p_{adj}) were adjusted for multiple comparisons. Mean (SE) values for the associated phenotypes are listed by genotype for each SNP for phase 1, phase 2 and meta-analysis of both cohorts; note these values are unadjusted, while the betas are adjusted.

Results from association testing of the two sleep-associated SNPs with circadian phenotypes are shown in supplementary table 2.

PER2

A common polymorphism in the *PER2* gene (rs6753456; MAF 38%) was significant in the phase 1 sample for SWS duration with 24 min less SWS in risk allele carriers compared to non-carriers, a difference of 27% ($p = 9.2 \times 10^{-4}$; Table 2). While the magnitude of the effect of this *PER2* SNP was similar in the phase 2 cohort (19 min less of SWS), this association was not statistically significant ($p = 0.103$). Meta-analysis of both phases showed association with 20 min of less SWS duration, equivalent to a 22% reduction compared to noncarriers ($p = 4.43 \times 10^{-4}$). This difference was significant after correction for multiple comparisons ($p_{adj} = 0.046$). Consistent results of a study-wide significant association ($p = 3.39 \times 10^{-4}$) with no heterogeneity of genetic effect between the more controlled phase 1 cohort and the noisier phase 2 cohort ($I^2 = 0\%$, $p_{het} = 0.49$; (Han & Eskin, 2011) were seen using a modified random effect meta-analysis approach, indicating

that the genetic signal was robust to differences between the data sets. Model-estimated mean differences ± SE for SWS duration for the phase 1, phase 2, and meta-analysis groups are shown in Figure 2a. Results from meta-analysis of both phase groups revealed lower SWS in homozygous and heterozygous groups compared to the nonrisk allele carriers (Figure 2a).

EEG spectral analysis was carried out in the 37 male participants from the phase 1 sample in order to eliminate heterogeneity by sex, given that SWS power density differs between men and women (Armitage, 1995; Dijk et al., 1989; Ehlers & Kupfer, 1997) and because there was a very unequal ratio of men to women in the genotype groups. Genotype affected the power spectrum during non-REM sleep in a frequency-specific manner (genotype × EEG frequency bin, $p = 0.049$; Figure 3a). *Post hoc* analysis showed that this effect arose mainly from lower power density in the low delta range (0.25–1.0 Hz; $p < 0.044$, *F*-test) in individuals with one or two copies of the risk allele compared to noncarriers. Moreover, genotype affected the time course of the typical SWA decline across the night that is a measure of the decrease in homeostatic sleep pressure across the night. The biggest effects of genotype were observed in the first half of the night, with lower EEG SWA in the risk allele carriers and a shallower decline of SWA across the sleep episode in the carriers (Figure 3b). EEG power spectra during REM sleep were not affected by genotype (data not shown).

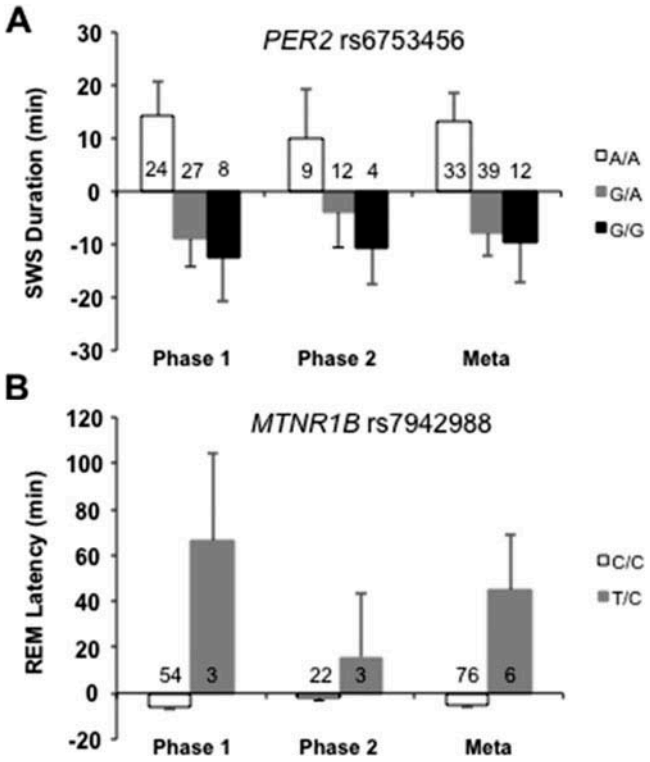

Figure 2. Model-estimated mean differences by genotype for (a) *PER2* SNP rs6753456 and SWS duration and (b) *MTNR1B* SNP rs7942988 and REM sleep latency. Model-estimated means are set to 0 min for each cohort and mean differences were adjusted for covariates. Mean difference, standard error (SE) and corresponding n for each genotype group are shown for homozygous (black bars) and heterozygous (gray bars) carriers for the risk allele and noncarriers (white bars).

Another common *PER2* SNP (rs3739064; MAF 21.3%) showed notable association with the number of awakenings in the phase 1 cohort ($p = 6.0 \times 10^{-4}$; Table 2). Minor allele homozygotes (G/G) had an average of eight fewer awakenings per night than either those with a single copy of the minor allele (G/A) or noncarriers (A/A). This association was not significant in the phase 2 sample and meta-analysis did not show significant association after correction of multiple comparisons ($p = 0.013$, $p_{adj} = 0.505$).

MTNR1B

A SNP in the *MTNR1B* gene (rs7942988; MAF 3.7%) was significantly associated with REM sleep latency from sleep onset ($p = 3.10 \times 10^{-4}$; Table 2) in the phase 1 cohort. Individuals carrying the risk allele showed much longer latencies to REM sleep (by 81 min) than noncarriers, corresponding to a doubling of REM sleep latency (111% increase). This association was not significant in the phase 2 sample alone ($p > 0.1$), but significant ($p = 4.14 \times 10^{-4}$, $p_{adj} = 0.046$; Table 2) in the overall sample, with the latency from sleep onset to REM sleep 65 min (87%) longer in carriers than in noncarriers. In a secondary meta-analysis assuming random effects and testing for heterogeneity, this association demonstrated a consistent effect between the phase 1 and phase 2 cohorts ($p = 6.5 \times 10^{-3}$, $I^2 = 8.7\%$, $p_{het} = 0.30$). The risk allele of rs7942988 (T) is relatively rare with an MAF of 3.7% in our population (6 T/C and 0 T/T individuals). The distribution of REM sleep latency values from both the phase 1 and phase 2 cohorts are plotted in supplementary figure 4 and show that half of the risk allele carriers had REM sleep latencies that were more than twice

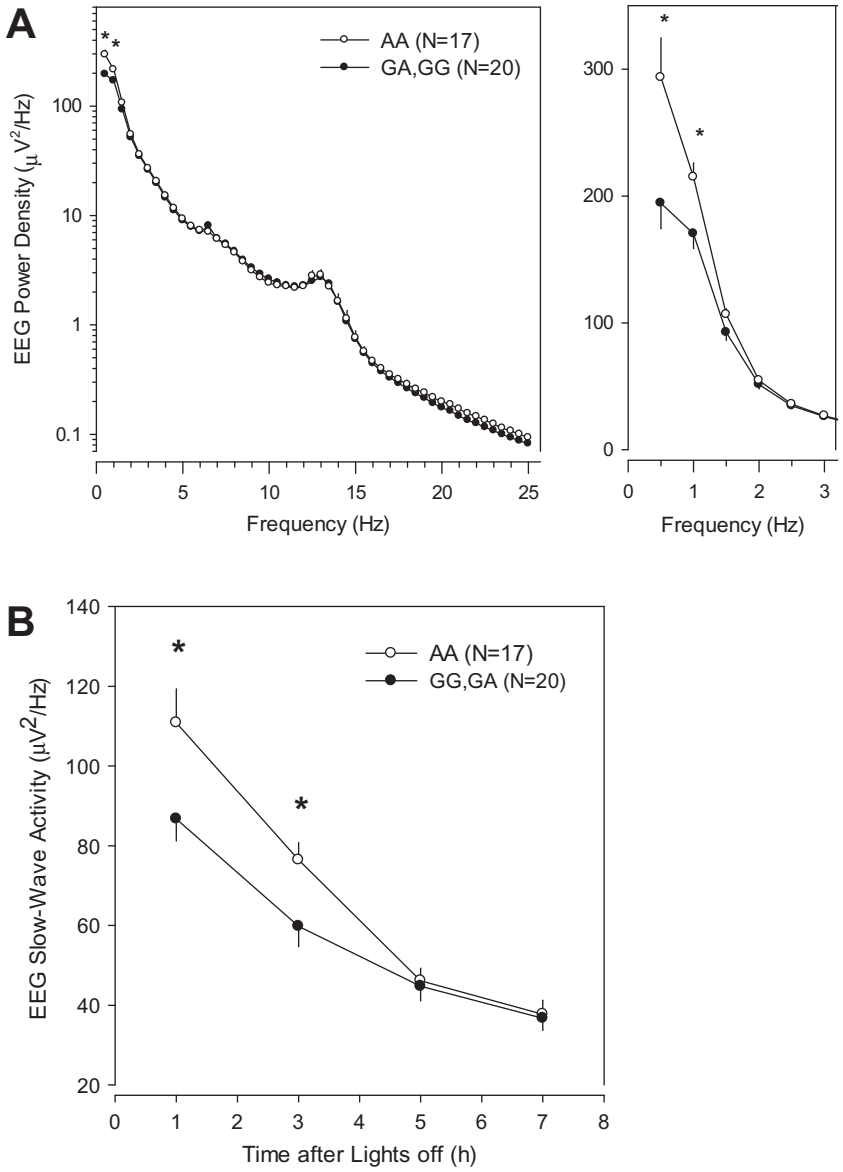

Figure 3. Effect of *PER2* rs6753456 genotype on EEG power density during NREM sleep in the male subset (n = 37) of the phase 1 cohort. (a) Mean power density spectra (*left*: 0.25–25.0 Hz; *right* (*enlarged*): 0.25–3.0 Hz) are shown for risk allele carriers (filled symbols) and noncarriers (open symbols). Risk allele carriers have significantly reduced spectral power density in the low delta-frequency range (0.25–1.0 Hz). (b) Mean slow-wave activity (SWA; 0.75–4.5 Hz) is plotted for consecutive 2 h bins after lights off. Significant differences between genotypes ($p < 0.05$, F-tests) are denoted by the asterisks and error bars show SE.

as long as the group average (>120 min). *Post hoc* analysis found that genotype had a significant effect on the chance of having an REM sleep latency >120 min using both χ^2 analysis (OR = 8.79; CI = 1.48–52.07; p = 0.008) and Fisher's exact test (p = 0.015). Figure 2b displays the model-estimated mean differences for REM sleep latency in the phase 1 and phase 2 cohorts by genotype.

This same *MTNR1B* gene variant rs7942988 that associated with REM sleep latency had a significant association (p = 0.013 with the duration of melatonin secretion, calculated as the difference between the dim light melatonin onset (DLMO) and offset (DLMOff). Because melatonin was collected only in the phase 1 sample, there were only three individuals with this rare allele (T) who had melatonin phase assessments available, but they had a significantly reduced duration of nocturnal melatonin secretion by 1.33 h compared to noncarriers, corresponding to a 13.1% reduction (supplementary table 2). This association

with the melatonin secretion duration remained significant after correcting for multiple testing of the three SNPs (p_{adj}= 0.039). This SNP was also associated with an earlier diurnal preference (p = 0.041), although this association was no longer significant after correction for multiple testing of three SNPs.

Gene-based analysis

Results of SKAT gene-based analysis showed associations for *BHLHE41* (*DEC2*) with the duration of REM sleep (p = 0.005), *PER2* with SWS (p = 0.027) and *MTNR1B* with REM sleep latency (p = 0.028) in phase 1 cohort. Only the *BHLHE41* association remained significantly associated with REM sleep duration after correcting for multiple comparisons (five genes; p_{adj} = 0.01).

Discussion

Associations between single variants in two candidate genes and sleep PSG measures were identified in our phase 1 sample. Meta analyses also showed study-wide significant associations for *PER2* SNP rs6753456 with SWS duration and *MTNR1B* SNP rs7942988 with REM sleep latency that withstood correction for multiple tests. Neither association was observed in the smaller phase 2 sample alone; however, similar effect estimates and concordant direction of the allelic effects were seen in both samples. While statistical power ranged from 83% to 89% (with α = 0.05) in the phase 2 sample to replicate the effect that was observed in the phase 1 sample, lack of replication may be due to "winner's curse" in the initial sample, the small size of the phase 2 sample, and/or less favorable/more noisy experimental conditions during which the PSG was recorded (first night in phase 2 cohort versus third night in phase 1 cohort). As expected for a "first-night" effect in the phase 2 cohort, during which sleep is typically more disturbed due to sleeping in an unfamiliar laboratory environment and PSG recordings for the first time, there was significantly less REM sleep in the phase 1 cohort (Agnew et al., 1966). First-night effects in the phase 2 cohort therefore could have confounded associations with the SNPs. Indeed, we purposefully selected a "noisier" phase 2 cohort to perform a stringent validation of significant findings from the phase 1 cohort, reasoning that replication despite the increased instability of first-night measurements would favor true associations over false positives. Replication in additional large studies with EEG measurements will be important to validate these associations. Our study identified a common variant of *PER2* (rs6753456) that was significantly associated with decreased SWS duration in the combined analysis of both cohorts after correction for multiple comparisons and showed a large effect size (20 min reduction of SWS).

This SNP (rs6753456) is located in the upstream promoter region of *PER2*, a core circadian clock gene, and is in strong linkage disequilibrium (r^2 = 0.8) in Europeans with rs11894491, a regulatory variant annotated with promoter and enhancer histone marks, DNAse hypersensitivity sites and polymerase II binding in multiple cell lines (Ward & Kellis, 2012). However, these SNPs are uncorrelated to another *PER2* SNP in the 5' untranslated region that was reported to be associated with diurnal preference (Carpen et al., 2005). In the current study, *PER2* gene variant rs6753456 was not associated with diurnal preference or any other circadian phenotype, such as timing of sleep, as was seen with a rare *PER2* missense mutation in a family with advanced sleep phase disorder (Toh et al., 2001). A study in mice showed that the effects of an acute 6 h sleep deprivation on increasing *PER2* expression were dependent on the time of day (Curie et al., 2013). In that report, the authors suggest that *PER2* acts as an integrator of both circadian and homeostatic signals. Our findings are consistent with a role of *PER2* in homeostatic sleep regulation. First, the greatest genotype-dependent differences in power density were found in the low delta range of the non-REM sleep EEG, which is particularly sensitive to changes in homeostatic sleep pressure (Aeschbach et al., 1996). Second, the differences in SWA were specifically seen during the early part of sleep, that is, a time when homeostatic sleep pressure and its manifestation in the EEG are typically highest. To further corroborate the potential role of circadian genes in homeostatic sleep regulation, it will be important to subject individuals with different genotypes to challenges of the homeostatic system (e.g. sleep deprivation).

The *MTNR1B* variant rs7942988 showed significant association with REM sleep latency. The low prevalence of this allele in our population may explain the lack of replication. Although only 6/84 individuals were carriers for the risk allele, the effect size was considerable (>1 h longer REM sleep latency) and half of the carriers displayed REM sleep latencies of more than 2 h (120 min), indicating a "skipped" first REM sleep episode. It is remarkable that three of the six minor allele carriers were in the small subgroup of nine individuals who displayed a long REM sleep latency, consistent with skipping the first REM sleep episode (see supplementary figure 4). *Post hoc* analysis to test whether the chance for skipping of the first REM sleep episode was significantly affected by the genotype found a significant association using both χ^2 analysis and Fisher's exact test. Since REM sleep is the sleep state most influenced by circadian phase (Czeisler et al., 1980) and *MTNR1B* is expressed in the central circadian pacemaker, the suprachiasmatic nucleus (SCN) (Pandi-Perumal et al., 2008), the association between an *MTNR1B* gene variant and REM sleep latency may indicate that *MTNR1B* function modulates the SCN control of REM sleep propensity. This would need to be determined in future studies. It is also notable that we observed a significant association with a circadian phenotype (1.33 h shorter duration of melatonin profile) and a nominally significant association with diurnal preference (see supplementary table 2).

Although it is not clear how longer REM sleep latency and shorter duration of melatonin secretion are related, these findings suggest a possible role for *MTNR1B* in the circadian regulation of sleep, specifically REM sleep. Interestingly, and consistent with such a potential relationship, REM sleep latency has been reported to be increased in tetraplegia patients with complete cervical spinal cord transection and abolished nighttime peak in circulating melatonin (Berlowitz et al., 2012; Scheer et al., 2006). More recently, in a separate analysis, we have found that the *MTNR1B* variant, rs10830963, previously associated with increased fasting glucose levels and increased risk of T2D is associated with longer duration of elevated melatonin levels and a delay in circadian phase of melatonin offset in a similar inpatient sample as the one in this analysis (Lane et al., 2015). The variant in the current study, rs7942988, is not itself associated with risk of T2D (http://diagram-consortium.org) or in strong linkage disequilibrium with common or rare *MTNR1B* variants previously associated with T2D (Dupuis et al., 2010; Lyssenko et al., 2008; Prokopenko et al., 2008).

Results of the gene-based analysis showed trends of these associations for the *PER2* and *MTNR1B* variants with SWS duration and REM sleep latency, respectively. In addition, the gene-based analysis showed a significant association in a third gene, *BHLHE41*, with REM sleep duration, which was not identified by the analysis of individual SNPs. A rare variant in this gene (also known as *DEC2*) was associated with shorter sleep duration in humans (*hDEC2-P385R*) and less sleep time in transgenic mice carrying this mutation as compared to controls (He et al., 2009). Furthermore, sleep deprivation studies of both transgenic *hDEC2-P385R* mice and *Dec2* knockout mice showed slower recovery following sleep loss, indicating the importance of this gene in sleep regulation (He et al., 2009). We did not find this rare variant in our entire sample, not surprising given the very low frequency of this allele, and thus were not able to replicate this previously reported association. Taken together, however, our findings support the evidence that *BHLHE41* plays a role in the genetic regulation of sleep.

The small sample size was a limitation of this study, and due to limited power in this sample, we examined relatively common variants. A small sample size in addition to an unequal male/female ratio in the genotype groups for *PER2* SNP rs6753456 was a limitation for the EEG spectral analysis. It will be important to replicate these findings in large samples, especially for the rare *MTNR1B* variant. Given the low number of minor allele carriers for this variant (six) and the finding that three of these showed significantly longer REM sleep latencies, it is theoretically possible that skipping of the first REM sleep episode could have influenced assessment of SWS and SWA, measures of sleep homeostasis. We found no direct association between *MTNR1B* SNP rs7942988 and SWS, however, making it unlikely that carriers were under increased homeostatic sleep pressure. Additionally, we focused our primary analysis on a European subset of the sample in order to examine a homogeneous group. Another limitation was that we tested only a few candidate genes as

opposed to a more comprehensive panel or GWAS, although this reduced our multiple-testing burden. Selection of candidate genes was partially based on previously reported associations with circadian rhythms (Toh et al., 2001), sleep phenotypes (Allebrandt et al., 2010; He et al., 2009) and/or diabetes risk (Dupuis et al., 2010; Lyssenko et al., 2008; Prokopenko et al., 2008); however, our aim was full coverage of each candidate gene by tagged SNPs rather than only previously associated SNPs. Notably, we found associations with SNPs in two of the five genes selected for this study. These positive results are likely due to the use of the phenotypic measures that were collected under exceedingly well-controlled conditions in carefully screened participants and involved the application of consistent and stringent procedures for the collection and analysis of data across protocols. Further studies examining these and other circadian gene variants with sleep EEG collected under different conditions are needed for replication of our results to determine functional pathways and tissue specificity for gene expression and for identification of other genetic associations.

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References

Achermann P, Dijk DJ, Brunner DP, Borbély AA. (1993). A model of human sleep homeostasis based on EEG slow-wave activity: Quantitative comparison of data and simulations. Brain Res Bull. 31:97–113.

Aeschbach D, Cajochen C, Landolt H-P, Borbély AA. (1996). Homeostatic sleep regulation in habitual short sleepers and long sleepers. Am J Physiol. 270:R41–R53.

Agnew HW, Jr., Webb WB, Williams RL. (1966). The first night effect: An EEG study of sleep. Psychophysiol. 2:263–266.

Allebrandt KV, Teder-Laving M, Akyol M, et al. (2010). CLOCK gene variants associate with sleep duration in two independent populations. Biol Psychiatry. 67:1040–1047.

Ambrosius U, Lietzenmaier S, Wehrle R, et al. (2008). Heritability of sleep electroencephalogram. Biol Psychiatry. 64:344–348.

Armitage R. (1995). The distribution of EEG frequencies in REM and NREM sleep stages in healthy young adults. Sleep. 18:334–341.

Bachmann V, Klein C, Bodenmann S, et al. (2012). The BDNF Val66Met polymorphism modulates sleep intensity: EEG frequency- and state-specificity. Sleep. 35:335–344.

Barrett JC, Fry B, Maller J, Daly MJ. (2005). Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 21:263–265.

Berlowitz DJ, Spong J, Gordon I, et al. (2012). Relationships between objective sleep indices and symptoms in a community sample of people with tetraplegia. Arch Phys Med Rehabil. 93:1246–1252.

Bodenmann S, Hohoff C, Freitag C, et al. (2012). Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Br J Pharmacol. 165:1904–1913.

Bodenmann S, Landolt HP. (2010). Effects of modafinil on the sleep EEG depend on Val158Met genotype of COMT. Sleep. 33:1027–1035.

Bollen KA, Curran PJ. (2005). Latent growth curve models: A structural equation perspective. Hoboken, NJ: Wiley.

Borbély AA. (1982). A two process model of sleep regulation. Hum Neurobiol. 1:195–204.

Carpen JD, Archer SN, Skene DJ, et al. (2005). A single-nucleotide polymorphism in the 5'-untranslated region of the hPER2 gene is associated with diurnal preference. J Sleep Res. 14:293–297.

Curie T, Mongrain V, Dorsaz S, et al. (2013). Homeostatic and circadian contribution to EEG and molecular state variables of sleep regulation. Sleep. 36:311–323.

Czeisler CA, Weitzman ED, Moore-Ede MC, et al. (1980). Human sleep: Its duration and organization depend on its circadian phase. Science. 210:1264–1267.

Dijk DJ, Beersma DGM, Bloem G. (1989). Sex differences in the sleep EEG of young adults: Visual scoring and spectral analysis. Sleep. 12:500–507.

Dupuis J, Langenberg C, Prokopenko I, et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 42:105–116.

Ehlers CL, Kupfer DJ. (1997). Slow-wave sleep:Do young adult men and women age differently? J Sleep Res. 6:211–215.

Guindalini C, Mazzotti DR, Castro LS, et al. (2014). Brain-derived neurotrophic factor gene polymorphism predicts interindividual variation in the sleep electroencephalogram. Journal of neuroscience research. 92:1018–1023.

Han B, Eskin E. (2011). Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. Am J Hum Genet. 88:586–598.

Hanlon EC, Van Cauter E. (2011). Quantification of sleep behavior and of its impact on the cross-talk between the brain and peripheral metabolism. Proceedings of the National Academy of Sciences of the United States of America. 108 Suppl 3:15609–15616.

He Y, Jones CR, Fujiki N, et al. (2009). The transcriptional repressor DEC2 regulates sleep length in mammals. Science. 325:866–870.

Ionita-Laza I, Lee S, Makarov V, et al. (2013). Sequence kernel association tests for the combined effect of rare and common variants. Am J Hum Genet. 92:841–853.

Landolt HP, Dijk D. (2010). Genetic basis of sleep in healthy humans. In MH Kryger, Roth T, WC Dement (Eds). Principles and Practice of Sleep Medicine. St. Louis, MO: Elsevier Saunders, pp. 175–183.

Lane JM, Chang AM, Consortium C, et al. (2015). Impact of common variation at diabetes trait loci MTNR1B and CRY2 on sleep, circadian and melatonin physiology. Sleep. 38:A5–6.

Lennox WG, Gibbs EL, Gibbs FA. (1945). The brain-wave pattern, an hereditary trait. The Journal of Heredity. 36:233–243.

Linkowski P. (1999). EEG sleep patterns in twins. J Sleep Res. 8 Suppl 1:11–13.

Lyssenko V, Nagorny CL, Erdos MR, et al. (2008). Common variant in MTNR1B associated with increased risk of type

2 diabetes and impaired early insulin secretion. Nat Genet. 41:82–88.

Mignot E, Young T, Lin L, Finn L. (1999). Nocturnal sleep and daytime sleepiness in normal subjects with HLA-DQB1*0602. Sleep. 22:347–352.

Nedeltcheva AV, Scheer FA. (2014). Metabolic effects of sleep disruption, links to obesity and diabetes. Curr Opin Endocrinol Diabetes Obesity. 21:293–298.

Pandi-Perumal SR, Trakht I, et al. (2008). Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways. Prog Neurobiol. 85:335–353.

Prokopenko I, Langenberg C, Florez JC,et al. (2008). Variants in MTNR1B influence fasting glucose levels. Nat Genet. 41:77–81.

Purcell S, Neale B, Todd-Brown K, et al. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81:559–575.

Rechtschaffen A, Kales A. (1968). A manual of standardized terminology, techniques and scoring system for sleep stages of human Subjects. Washington, D.C.: US Government Printing Office.

Retey JV, Adam M, Honegger E, et al. (2005). A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. PNAS. 102:15676–15681.

Scheer FA, Zeitzer JM, Ayas NT, et al. (2006). Reduced sleep efficiency in cervical spinal cord injury; association with abolished night time melatonin secretion. Spinal Cord. 44:78–81.

Spiegel K, Knutson K, Leproult R, et al. (2005). Sleep loss: A novel risk factor for insulin resistance and Type 2 diabetes. J Appl Physiol. 99:2008–2019.

Toh KL, Jones CR, He Y, et al. (2001). An h Per2 phosphorylation site mutation in familial advanced sleep-phase syndrome. Science. 291:1040–1043.

van Beijsterveldt CE, van Baal GC. (2002). Twin and family studies of the human electroencephalogram: A review and a meta-analysis. Biol Psychol. 61:111–138.

Viola AU, Archer SN, James LM, et al. (2007). PER3 polymorphism predicts sleep structure and waking performance. Curr Biol. 17:1–6.

Ward LD, Kellis M. (2012). HaploReg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40:D930–934.