PER3 Polymorphism Predicts Sleep Structure and Waking Performance


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Summary

Circadian rhythmicity and sleep homeostasis interact to regulate sleep-wake cycles [1–4], but the genetic basis of individual differences in sleep-wake regulation remains largely unknown [5]. PERIOD genes are thought to contribute to individual differences in sleep timing by affecting circadian rhythmicity [6], but not sleep homeostasis [7, 8]. We quantified the contribution of a variable-number tandem-repeat polymorphism in the coding region of the circadian clock gene PERIOD3 (PER3) [9, 10] to sleep-wake regulation in a prospective study, in which 24 healthy participants were selected only on the basis of their PER3 genotype. Homozygosity for the longer allele (PER344) had a considerable effect on sleep structure, including several markers of sleep homeostasis: slow-wave sleep (SWS) and electroencephalogram (EEG) slow-wave activity in non-rapid eye movement (non-REM) sleep and theta and alpha activity during wakefulness and REM sleep were all increased in PER344 compared to PER355 individuals. In addition, the decrement of cognitive performance in response to sleep loss was significantly greater in the PER355 individuals. By contrast, the circadian rhythms of melatonin, cortisol, and peripheral PER3 mRNA expression were not affected. The data show that this polymorphism in PER3 predicts individual differences in the sleep-loss-induced decrement in performance and that this differential susceptibility may be mediated by its effects on sleep homeostasis.

Results and Discussion

The coding region of the PERIOD3 (PER3) gene contains a variable-number tandem-repeat (VNTR) polymorphism (insertion of nucleotides 3031–3084) in which a motif encoding 18 amino acids is repeated either four (PER34) or five times (PER35) [9]. These repeat units contain a cluster of putative phosphorylation motifs [10]. Retrospective studies have shown that this polymorphism is associated with diurnal preference and delayed-sleep-phase syndrome (DSPS) [10–12]. Here we present the results of a prospective study in which we investigated the functional consequences of this polymorphism for sleep and circadian physiology as well as waking performance. Subjects were selected only on the basis of their genotype. Four hundred and four men and women were genotyped in order to select matched pairs of individuals who were homozygous for the PER34 and PER35 alleles. These individuals then participated in a field study in which we characterized the timing of their sleep-wake cycle. This field study was followed by an intensive physiological monitoring study, in which circadian rhythms and homeostatic aspects of sleep regulation and performance were quantified under baseline conditions and subsequently during sleep loss. Ten PER355 (4 women, 6 men; age ± standard error of the mean = 25.2 ± 1.1 yr) and 14 PER344 (6 women, 8 men; 24.8 ± 1.0 yr) healthy participants, closely matched for age, sex, and ethnicity and without sleep complaints, completed both the field and laboratory studies (see Table S1 in the Supplemental Data available online). Assessment of sleep timing by sleep diary and actigraphy during the field study revealed no significant difference in bed time (PER344, 01:03 ± 0.32; PER355, 01:03 ± 0:21; p > 0.05) or wake time (PER344, 07:57 ± 0:28; PER355, 08:41 ± 0:21; p > 0.05) (Figure 1A). No major difference was observed in reported sleep duration (PER344, 06:55 ± 0:19; PER355, 07:38 ± 0:15; p > 0.05) or actigraphically assessed sleep duration (PER344, 06:51 ± 0:14; PER355, 07:19 ± 0:12; p > 0.05). For assessment of the endogenous phase and amplitude of circadian rhythms in the absence of the confounding effects of light-dark and behavioral cycles, participants underwent a constant-routine protocol, in which they were kept awake in dim light (<5 lux) for approximately 40 hr in a semirecumbent posture [13, 14]. During this period, motor-activity levels did not differ between the genotypes and were near stable (Figure 1A), indicating that the constant-routine protocol was successful in eliminating the 24 hr activity cycle, which masks endogenous circadian rhythms. PER3 mRNA levels in total RNA extracted from peripheral blood mononuclear cells exhibited a robust endogenous circadian rhythm with a peak value occurring at 06:42 ± 0:08 in PER355 and 06:26 ± 0:28 in PER344 (p > 0.05) (Figure 1B). This circadian rhythm is very similar to a previously reported profile [15]. The amplitude and mean levels of PER3 mRNA were also not significantly different between the genotypes. The circadian rhythms of plasma cortisol (Figure 1C) and melatonin (Figure 1D), two hormonal rhythms driven by the circadian pacemaker in the suprachiasmatic nuclei of the hypothalamus, were also remarkably similar in the two genotypes. The average peak value of cortisol occurred at 09:48 ± 1:02 in PER355 and 09:00 ± 0:27 in PER344. No difference was observed in cortisol amplitude. For the melatonin profile, the onset (PER355, 23:27 ± 0:28; PER344, 23:23 ± 0:22)
spent more time in slow-wave sleep (SWS) \(\text{PER3}^{g/s}\), 22.7% ± 1.6% of total sleep time; \(\text{PER3}^{d/d}\), 15.7% ± 1.6% of total sleep time; \(p = 0.006\), a well-established marker of the homeostatic oscillator [16]. SWS is characterized by low-frequency, high-amplitude oscillations in the EEG. Detailed analysis of the spectral composition of the EEG during non-REM sleep (stages 1, 2, 3, and 4), REM sleep, and wakefulness revealed that the effects of the \(\text{PER3}\) genotype on SWS were not caused by a general effect on the amplitude of the EEG. Instead, we found that the effects of the \(\text{PER3}\) genotype on the EEG were specific to frequency and vigilance state. Whereas during non-REM sleep (Figures 2A and 2B) the largest differences were observed in the slow-wave range, during REM sleep (Figures 2C and 2D) and wakefulness (Figures 2E and 2F) the genotype-dependent differences were located primarily in the theta and alpha range. It is well established that slow-wave activity (SWA) during non-REM sleep [17] and EEG activity in the theta and alpha frequency range during wakefulness [18] and REM sleep [17, 19] track homeostatic sleep pressure and sleepiness. Therefore, the data imply that the \(\text{PER3}\) polymorphism affects sleep homeostasis in all three vigilance states.

This interpretation is strengthened by an analysis of the effects of the \(\text{PER3}\) polymorphism on the time course of EEG activity during sleep. Baseline sleep of \(\text{PER3}^{g/s}\) participants was characterized by higher initial values of SWA followed by a steeper decline (Figure 3A). Alpha activity in REM sleep remained higher in \(\text{PER3}^{g/s}\) throughout the sleep episode (Figure 3D). When the sleep homeostatic system was challenged by sleep deprivation, genotype-dependent differences in non-REM and REM sleep persisted. During recovery sleep, the initial values of SWA during non-REM sleep (Figure 3B) and alpha activity in REM sleep throughout the recovery episode (Figure 3E) were higher in \(\text{PER3}^{g/s}\) (\(p < 0.05\)). As indexed by the duration of SWS and REM, the response to sleep loss also differed between the genotypes. In both genotypes, sleep deprivation led to an increase in SWS (Figure 3C), but the associated inhibition of REM sleep in the initial part of recovery sleep was stronger in \(\text{PER3}^{g/s}\) (Figure 3F).

As assessed in the constant-routine protocol, the increase in theta activity in the EEG during sustained wakefulness differed noticeably between the genotypes. In \(\text{PER3}^{g/s}\) individuals, it increased substantially with time awake. By contrast, in the \(\text{PER3}^{d/d}\) subjects, no noticeable change was observed over time (Figure 4A). We also monitored the occurrence of slow rolling eye movements (SEMs), an EEG-independent marker of inattention and drowsiness [14]. Over the course of the 40 hr of wakefulness, SEMs increased more rapidly in \(\text{PER3}^{g/s}\) than in \(\text{PER3}^{d/d}\) homozygotes. The largest differences between the genotypes were observed in the morning hours of the second day of sleep deprivation (Figure 4B). The genotype-dependent difference in the response to sleep deprivation during the biological night of these two markers of sleepiness and inattention raises the possibility of a genetically determined variability in susceptibility to the negative effects of sleep deprivation on performance. We assessed various domains of waking performance (including working memory, attention, and psychomotor

![Figure 1](current-biology-image-url). Endogenous Circadian Rhythms of Hormones and \(\text{PER3}\) mRNA Levels Do Not Differ between \(\text{PER3}^{g/s}\) and \(\text{PER3}^{d/d}\) Homozygotes

(A) Shows the activity during the constant routine. Time courses of (B) \(\text{PER3}\) mRNA levels in total RNA extracted from blood mononuclear cells, (C) plasma cortisol concentration, and (D) plasma melatonin concentration are shown. All data are averaged across ten \(\text{PER3}^{g/s}\) (open symbols) and 14 \(\text{PER3}^{d/d}\) (filled symbols) participants. Error bars represent the standard error of the mean, and time represents local time.

23:45 ± 0:21), offset \(\text{PER3}^{g/s}\), 08:44 ± 0:24; \(\text{PER3}^{d/d}\), 09:18 ± 0:29), midpoint \(\text{PER3}^{g/s}\), 04:06 ± 0:26; \(\text{PER3}^{d/d}\), 04:31 ± 0:24), and amplitude \(\text{PER3}^{g/s}\), 71.37 ± 9.51 pg/ml; \(\text{PER3}^{d/d}\), 72.78 ± 10.82 pg/ml) did not differ between the genotypes (\(p > 0.05\) in all cases).

Despite the absence of significant differences in the timing and amplitude of these markers of central and peripheral circadian oscillators, the two genotypes differed strikingly with respect to sleep propensity, electroencephalogram (EEG) during wakefulness and sleep, and waking performance. Whereas during baseline, no differences were observed in rapid eye movement (REM) sleep, stage 1 or 2 sleep, or total sleep time (see Table S2), \(\text{PER3}^{g/s}\) subjects fell asleep more readily than \(\text{PER3}^{d/d}\) subjects (sleep latency: \(\text{PER3}^{g/s}\), 8.6 ± 1.3 min; \(\text{PER3}^{d/d}\), 18.1 ± 2.6 min; \(p < 0.005\), indicating a greater sleep propensity. Accordingly, these participants also
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During the extended period of wakefulness in a performance test battery consisting of verbal and spatial 1-, 2-, and 3-back tests; a sustained-attention-to-response task; a paced-visual-serial-addition task; a self-paced digit-symbol-substitution test; simple-reaction-time and serial-reaction-time tests; and a motor-tracking task. The performance data were summarized by computation of a composite performance score. Whereas during the first biological day of the constant-routine protocol performance was near stable in both genotypes, the patterns dissociated when wakefulness was extended into the biological night (Figure 4C). Most strikingly, PER3<sup>5/5</sup> homozygotes performed very poorly during the hours after the melatonin midpoint. The decrement in waking performance in the PER3<sup>4/4</sup> homozygotes was far less. These major differences in performance between the two genotypes occurred during the late-night and early-morning hours, a time known from both laboratory and field studies as the nadir of the circadian timing system and during which performance is poorest and sleep propensity at its peak [2]. This is also the time at which many sleepiness-related accidents occur [20] and the greatest impairment is seen in shift-work sleep disorders [21].

The PER3 5-repeat allele, which is the less frequent one in most ethnic groups [22], has been associated with extreme morning preference [10, 11], while the 4-repeat allele has been linked with DSPS in our previous study [10]. The primate-specific expansion of the polymorphic region [23], its association with variation in diurnal preference and DSPS [9–11], its potential impact on phosphorylation of the PER3 protein [10], and its expression in hypothalamic and other brain areas implicated in sleep regulation [24, 25] led us to consider it as a candidate for mediating some of the marked individual differences in sleep-wake regulation. These individual differences include the preferred timing of sleep-wake cycles, the structure of sleep, EEG patterns during sleep and wakefulness, and their response to sleep loss and circadian-phase misalignment [20, 26–29].

Our results indicate that the PER3 polymorphism may contribute to the marked individual differences in performance decrement during sleep loss [29]. The phenotypes that we found to be predicted by homozygosity for the PER3 alleles are also reminiscent of some of the classical phenotypes in human sleep research—morning and evening types [30, 31], and short and long sleepers [32]. Sleep in both morning types and PER3<sup>5/5</sup> individuals is characterized by high initial values of SWA, in particular when compared to evening types who sleep at a similar circadian phase [30]. This is in accordance with the previously reported association between this PER3 polymorphism and diurnal preference in subjects selected from a larger population sample [10]. Thus, this PER3 polymorphism appears to contribute to the differences in sleep structure, but not the differences in circadian timing [33], between morning and evening types.
The sleep of habitual short sleepers is also characterized by high initial values of SWA and high levels of theta activity in the EEG during wakefulness, very similar to those reported here for PER35/5 individuals. The effect of the PER3 polymorphism on SWA is of a comparable magnitude to the effects of genetic variation in an already well-known key component of the homeostatic regulation of sleep, the adenosine 2A receptor and adenosine deaminase system [34].

In summary, this prospective study, in which subjects were selected by genotype alone, demonstrates that the PER3 VNTR polymorphism affects key markers of sleep homeostasis, including sleep latency, SWS, theta activity in the waking EEG, and the decrement in waking performance in response to sleep loss. However, no significant effect on objective measures of sleep-timing and circadian-rhythm parameters was observed. Within the framework of the homeostatic and circadian regulation of sleep and performance, these data imply that the PER3 VNTR polymorphism affects the homeostatic aspect of sleep regulation. This is the first time that such an effect has been demonstrated in humans and contrasts the limited animal data available for Per1 and Per2 [7, 8]. However, in animal studies, other clock genes, such as Clock, Cry1 and Cry2, NPAS2, DBP, and BMAL1 have been shown to influence aspects of the sleep and waking EEG as well as the response to sleep loss [35–39], although effects on performance have not previously been reported for any of these genes. The independence of circadian and homeostatic aspects of sleep regulation has been a mainstay of our current models of sleep regulation [1, 40]. Nevertheless, our data collected from humans, together with data from mice, imply that this concept does not extend to the molecular level, and that some genes previously described as clock genes perform noncircadian roles.

Conclusions

The effects of the PER3 polymorphism on SWS, SWA, and the decrements of waking performance during the biological night, as observed in this study, are significant and substantial. This implies that this polymorphism may be an important marker for individual differences in sleep and susceptibility to sleep loss and circadian-phase misalignment, which are major causes of health problems and accidents in our society.

Supplemental Data

Supplemental Data include Experimental Procedures and two tables and are available with this article online at: http://www.current-biology.com/cgi/content/full/17/7/613/DC1/.

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Figure 4. Deterioration of Waking Performance and Increase of Theta EEG Activity and Slow Eye Movements during Sleep Deprivation Is Greater in PER3<sup>4/4</sup> Than PER3<sup>5/5</sup> Participants

Time course of (A) central EEG theta (5–8 Hz) activity during wakefulness, (B) incidence of slow eye movement (SEMs) (percentage of 30 s epochs containing at least one SEM), and (C) waking performance (composite performance score) are plotted relative to the timing of the plasma melatonin rhythm (D) in ten PER3<sup>4/4</sup> (open symbols) and 14 PER3<sup>5/5</sup> (filled symbols) homozygotes. EEG theta activity, SEMs, and waking performance data were averaged per 2 hr intervals, relative to the midpoint of the melatonin rhythm. (* indicates a significant difference between genotypes, p < 0.05; upper abscissa indicates approximate wake duration.) Error bars represent the standard error of the mean.

References


