Length polymorphism in the Period 3 gene is associated with sleepiness and maladaptive circadian phase in night-shift workers

CHRISTOPHER L. DRAKE[1], REN BELCHER[1,4], RYAN HOWARD[1,5], THOMAS ROTH[1], ALBERT M. LEVIN[2] and VALENTINA GUMENYUK[1,3]

[1]Sleep Disorders and Research Center, Henry Ford Hospital, Detroit, MI, USA, [2]Department of Public Health Sciences, Henry Ford Hospital, Detroit, MI, USA, [3]Magnetoencephalography Laboratory, Meadowlands Hospital, Secaucus, NJ, USA, [4]Pritzker School of Medicine, University of Chicago, Chicago, IL, USA, and [5]University of Michigan Medical School, Ann Arbor, MI, USA

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Correspondence
Christopher L. Drake, PhD, Sleep Disorders and Research Center, Henry Ford Hospital, 2799 W. Grand Boulevard, CFP-3, Detroit, MI 48202, USA.
Tel.: 313-916-4455;
fax: 313-916-5167;
e-mail: cdrake1@hfhs.org

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SUMMARY

The objective of the current study was to determine if night-shift workers carrying the five-repeat variant of the Period 3 gene show elevated levels of nocturnal sleepiness and earlier circadian phase compared with homozygotes for the four-repeat allele. Twenty-four permanent night-shift workers were randomly selected from a larger study. Participants took part in an observational laboratory protocol including an overnight multiple sleep latency test and half-hourly saliva collection for calculation of dim-light melatonin onset. Period $3^{-/5}$ shift workers had significantly lower multiple sleep latency test during overnight work hours compared with Period $3^{4/4}$ workers (3.52 ± 23.44 min versus 10.39 ± 6.41 min, $P = 0.003$). We observed no significant difference in sleepiness during early morning hours following acute sleep deprivation. Long-allele carriers indicated significantly higher sleepiness on the Epworth Sleepiness Scale administered at 17:00 hours (12.08 ± 2.55 versus 8.00 ± 1.94, $P < 0.001$). We observed a significantly earlier melatonin onset in Period $3^{-/5}$ individuals compared with Period $3^{4/4}$ shift workers (20:44 $\pm$ 6:37 versus 02:46 $\pm$ 4:58, $P = 0.021$). Regression analysis suggests that Period 3 genotype independently predicts sleepiness even after controlling for variations in circadian phase, but we were unable to link Period 3 to circadian phase when controlling for sleepiness. Period $3^{-/5}$ shift workers showed both subjective and objective sleepiness in the pathological range, while their Period $3^{4/4}$ counterparts showed sleepiness within normal limits. Period $3^{-/5}$ night workers also show a mean circadian phase 6 h earlier (i.e. less adapted) than Period $3^{4/4}$ workers. Because Period $3^{-/5}$ workers have maladaptive circadian phase as well as pathological levels of sleepiness, they may be at greater risk for occupational and automotive accidents. We interpret these findings as a call for future research on the role of Period 3 in sleepiness and circadian phase, especially as they relate to night work.

INTRODUCTION

A variable number tandem repeat (VNTR) polymorphism of the Period 3 (PER3) gene has been associated with individual differences in several sleep and circadian functions (Dijk and Archer, 2010; Goel *et al.*, 2009; Groeger *et al.*, 2008; Rupp *et al.*, 2013; Viola *et al.*, 2007). Carriers of the less common five-repeat allele ($PER3^{4/5}$ or $PER3^{5/5}$; hereafter noted $PER3^{-/5}$) make up about 35–40% of the population (Ciarleglio *et al.*, 2008), and $PER3^{5/5}$ individuals are more susceptible to cognitive decrements following sleep deprivation, particularly at the circadian nadir (~05:00 hours; Viola *et al.*, 2007). A recent study comparing PER3 genotypes showed that $PER3^{5/5}$ individuals had higher indicators

of sleepiness, such as impaired sustained attention and slow rolling eye movements, particularly in the early morning hours during total sleep deprivation (Maire *et al.*, 2014). Similarly, under severe sleep restriction to 3 h of allotted time in bed for seven nights, $PER3^{4/5}$ individuals showed greater impairments in daytime neurobehavioural performance compared with $PER3^{4/4}$ individuals (Rupp *et al.*, 2013), but these differences did not manifest until the third day of sleep restriction. In addition, while nocturnal assessments were not performed, deficits were most prominent in the morning hours (~08:00 hours). In contrast, a study of more modest sleep restriction to 4 h per night for five nights did not show an effect of genotype on performance or alertness (i.e. maintenance of wakefulness test; Goel *et al.*, 2009). Finally, a study investigating the cognitive effects of both partial and acute sleep deprivation showed an effect of PER3 genotype on subjective sleepiness and sustained attention with effects most pronounced in the early morning hours (Lo *et al.*, 2012). Taken together, these findings suggest the functional deficits associated with the PER3 genotype may occur following situations that include extensive sleep deprivation and/or involve performing at an adverse circadian phase.

One common situation that involves both restricted sleep for an extensive period of time in conjunction with requirements for maintaining alertness and performance at the circadian nadir is night-shift work (Drake, 2011). Night workers often obtain less sleep than their day work counterparts, and their work schedules inherently require them to maintain alertness throughout the night. In addition, research has shown that the early morning hours (01:00–09:00 hours), which typically include the commute home from night work, are a particularly vulnerable time for shift workers due to the elevated risk of automotive and industrial accidents (Wagstaff and Sigstad Lie, 2011). Given that shift workers ubiquitously experience sleep restriction and exposure to wakefulness at an adverse circadian phase, workers who carry the PER3 five-repeat allele may experience particularly elevated levels of sleepiness during the night-shift and may correspondingly be at greater risk for automotive or occupational accidents.

In addition to the possibility that PER3 genotype may impact nocturnal alertness in night-shift workers, previous studies demonstrating an association of this polymorphism with chronotype (Archer *et al.*, 2003) and circadian phase (Viola *et al.*, 2012) suggest these genotype differences may also influence circadian adjustment to night work. Numerous studies have shown that certain individuals adjust better to night work than others (Drake *et al.*, 2004; Gumenyuk *et al.*, 2012, 2014; Juda *et al.*, 2013; Reinberg *et al.*, 1978). In a review of six studies, Folkard concluded that few night workers experience full adjustment of their circadian rhythms to night work (Folkard, 2008). In particular, late chronotypes ('eveningness/owls') show better adjustment to night work (Ostberg, 1973). Considering the finding that homozygotes for the four-repeat allele are more likely to have a later chronotype (Archer *et al.*, 2003), this suggests the possibility that such individuals may have better circadian adjustment to a night work schedule.

In a recent study, PER3 mediated the efficacy of behavioural adaptation strategies for shift work (Gamble *et al.*, 2011). These authors found significant genotype–strategy interactions, suggesting that $PER3^{-/5}$ nurses adapt better to shift work when they employ certain strategies (i.e. sleep deprivation following the final night-shift in a series), relative to other genotypes. However, circadian phase was not assessed in that study and no objective measures of sleepiness were obtained.

Our study differs from this and other protocols by objectively assessing circadian phase and sleepiness in night-shift workers genotyped for the PER3 VNTR. Our study was designed to test the hypothesis that night-shift workers who are carriers of the five-repeat allele may have elevated levels of nocturnal sleepiness and unadjusted circadian phase consistent with a lack of adaptation to the sleep–wake schedule imposed by night work.

MATERIALS AND METHODS

Night workers were recruited from healthcare and industrial settings to participate in an overnight laboratory study. Participants were required to have worked exclusively on a night-shift for at least 6 months (at least three night-shifts per week, with each shift lasting 8 h or longer, and occurring between 19:00 and 08:00 hours) and must not have worked a rotating shift or 'picked up' daytime shifts during the 6 months preceding the laboratory study. Only individuals who were continuing to be on the night-shift without any anticipated work schedule changes participated. They were required to show low pretest probabilities for obstructive sleep apnea, restless legs syndrome, narcolepsy and psychiatric disorders by providing responses in the normal range on the Berlin questionnaire (0 or 1 positive categories; Netzer *et al.*, 1999), Hamilton Depression Rating Scale ($\leq$7; Hamilton, 1960), as well as in a clinical interview with a sleep medicine physician. Subjects who had been diagnosed with insomnia or excessive sleepiness prior to becoming night-shift workers were excluded. All subjects completed a standardized sleep diary for 2 weeks prior to the laboratory protocol, and were instructed to maintain their usual sleep–wake schedule. Subjects reporting an average time in bed (TIB) <5 h per 24-h period during this screening phase were excluded from participating in the laboratory study. They were required to be free from head injury, hearing problems, alcohol or substance abuse; must have been non-smokers; and must not have consumed more than 300 mg of caffeine, on average, per day. Finally, they were required to be free from all central nervous system (CNS)-acting medication, as well as beta-adrenergic blocking agents and other drugs that are known to affect circadian rhythms or sleep–wake function. Drugs encountered and disqualified included sertraline, tamoxifen, paroxetine, zolpidem, metroprolol, atenolol, bupropion, alprazolam, venlafaxine, methylphenidate,

gabapentin, tramadol, diazepam, triazolam, levocetirizine, buspirone, citalopram, lisdexamfetamine and trazodone. An exception was made for shift workers with insomnia symptoms who had taken melatonin or sleep-promoting agents while working night-time shifts, but had subsequently discontinued medication and had been free of these agents for at least 2 weeks prior to the laboratory study.

Ninety-five night workers initially responded to flyers and online newsletter advertisements. Of these, 35 (36.8%) were disqualified based on an online survey; 13 (13.7%) did not comply with pre-screening requests to keep a sleep diary or failed to keep a screening appointment; five (5.3%) were disqualified at the screening appointment; three (3.2%) were eligible but not enrolled; and one (1.1%) dropped out following enrollment. Thirty-eight participants (40.0%) qualified based on inclusion criteria. Of these 38 participants, 24 (63.2%) were randomly selected to participate in a genetic sub-study, the results of which are reported here. Occupations of participants varied, and included a night inventory stocker, nurses (×3), a night-time pharmacist, various medical/laboratory technologists (×14), security guards (×2), a police officer, a radiographer and a respiratory therapist. Participants were studied in both the summer and winter months. The local Institutional Review Board approved all study procedures, and written informed consent was obtained from subjects prior to participation.

Participating subjects deposited 2 mL of saliva into an Oragene OG-500 collection kit (DNA Genotek; Ontario, Canada). Genomic DNA was extracted and genotyped for on the PER3 VNTR. This VNTR has two alleles: a four-repeat (short) allele; and a five-repeat (long) allele. Preparation and analysis of the samples was performed by the Applied Genomics Technology Center at Wayne State University (Detroit, MI, USA).

The laboratory visit was scheduled to occur immediately following at least two consecutive night-shifts. Participants kept their normal sleep–wake schedules prior to the laboratory visit, including daytime sleep on the day of the study (Table 1). Laboratory procedures were conducted in an isolation facility with low light exposure (<15 lux) for the duration of the protocol. Participants arrived at 16:00 hours and, beginning at 17:00 hours on the day of the laboratory study, saliva samples for melatonin processing were collected at 30-min intervals using a Salivette tube with a cotton insert (Sarstedt Group, Numbrecht, Germany). Prior to 17:00 hours, participants were instructed on the procedure of saliva collection and the amount of saliva required for each sample. Each sample was weighed to ensure that at least 1 mL of saliva was provided. Saliva was then extracted from the cotton insert in a frozen centrifuge, and samples were frozen at −20 °C until being shipped over dry ice to SolidPhase (Portland, ME, USA), where they were radioimmunoassayed. The intra-assay precision was 2.6–20.1% with functional sensitivity of 0.9 pg mL^{-1} and analytical sensitivity of 0.2 pg mL^{-1}. With the exception of the research multiple sleep latency test (MSLT), participants were not permitted to sleep during the protocol, were required to remain out of bed, and were observed by a technician/research assistant throughout. Participants could ambulate throughout the bedroom and a private bathroom and hallway (all <15 lux). Telephone, television and internet usage were permitted during unscheduled times, and three small meals were provided to participants during the course of the protocol. Caffeine, alcohol, milk, bananas and tomatoes were not permitted. Participants were aware of clock time and meals were provided on request. Meals were also supplemented with snacks upon request.

Dim-light melatonin onset (DLMO) is an individual's characteristic point in clock time marking the onset of elevated melatonin levels that normally coincide with the sleep period (Lewy and Sack, 1989; Revell *et al.*, 2005). DLMO was calculated as the time that the amplitude of the fitted LOWESS curve for melatonin concentration rose and remained above a participant's melatonin threshold for at least 1 h. The threshold used was the average of the five lowest concentrations of melatonin during the 24-h phase

Table 1 Demographics and reported habitual sleep by genotype

	4/4	*–/5*	P
% Female	72.7	76.9	0.82
% Asian	18.2	0	0.12
% African–American	54.5	84.6	0.12
Age (years)	36.27 ± 9.83	33.85 ± 8.77	0.53
Total sleep time (h)	5.74 ± 1.02	5.51 ± 1.14	0.61
Bed time (hours)	9:06 ± 0.97	9:54 ± 1.69	0.12
Wake up time (hours)	15:22 ± 1.40	15:59 ± 2.47	0.46
Sleep efficiency (%)	0.91 ± 0.12	0.90 ± 0.09	0.77
Sleep latency (min)	24.48 ± 26.06	16.77 ± 12.04	0.35
Wake after sleep onset (min)	6.46 ± 12.63	11.79 ± 19.52	0.45
Naps during shift (no. per week)	1.2 ± 1.9	2.5 ± 2.6	0.19
n	11	13	

Sleep data were obtained using a standardized sleep diary. Bed times and wake times are calculated for days when subjects worked the night-shift.

assessment, plus 15% of the average of the five highest concentrations. $DLMO_{off}$ was calculated as the time at which the LOWESS curve amplitude fell and remained below the individual's threshold for at least 2 h (Gumenyuk *et al.*, 2012, 2014; Lee *et al.*, 2006).

Objective sleepiness was assessed using a nocturnal and diurnal MSLT. Eight naps were conducted at 2-h intervals from 22:30 to 12:30 hours, following standard research protocol. Thus, participants were awakened following three epochs of stage 1 or one epoch of any other sleep stage. Electrode integrity was checked by physical inspection and electrical biocalibration before each nap. The first four naps (22:30–04:30 hours) coincided with night-shift hours, while the last four (06:30–12:30 hours) evaluated the effects of acute sleep deprivation. Subjective sleepiness was assessed by the Epworth Sleepiness Scale at 17:00 hours, which was well over 1 h after arrival at the sleep laboratory to avoid possible sleep inertia effects. The Stanford Sleepiness Scale (SSS) was used to assess the dynamics of sleepiness throughout the study for each participant, and was administered immediately prior to each of the eight MSLT naps.

To assess the association between the VNTR genotype and sleep phenotypes, a dominant genetic analysis model was used, with individuals heterozygous ($PER3^{4/5}$) and homozygous ($PER3^{5/5}$) for the long allele considered together ($PER3^{-/5}$) for comparison with individuals homozygous for the short allele ($PER3^{4/4}$). This model was selected due to the low frequency of $PER3^{5/5}$ homozygotes in the sample (1 of 13; see Results). Association tests were conducted using independent samples *t*-tests for continuous variables and Pearson chi-square tests for categorical sleep phenotypes. Where appropriate, ordinary least-squares regression models were used to clarify the differential contributions of genotype to sleepiness and circadian phase. *P*-values <0.05 were considered statistically significant. All data analyses were performed using SPSS Version 18.0 for Windows (IBM, Armonk, NY, USA).

RESULTS

Of 24 shift workers in our sample, 13 (54.2%) were homozygous or heterozygous carriers of the long VNTR ($PER3^{-/5}$); 12 of these 13 carriers (92.3%) were heterozygous ($PER3^{4/5}$). The remaining 11 shift workers were homozygous for the short allele ($PER3^{4/4}$). There were no significant differences by carrier status for sex, age, race or reported sleep parameters on work days (Table 1).

$PER3^{-/5}$ shift workers had significantly lower MSLT latency during overnight work hours. Averaged over four naps from 22:30 to 04:30 hours, the mean MSLT score for long-allele carriers was 3.52 ± 3.44 min, compared with 10.39 ± 6.41 min among short-allele homozygotes (P = 0.003). For naps between 06:30 and 12:30 hours (i.e. times corresponding to acute sleep deprivation outside of the circadian nadir), no significant difference (P = 0.249) was observed between carriers of the long-allele (2.17 ± 2.15) and homozygotes for the short-allele (3.16 ± 1.94). MSLT scores for all eight naps are presented in Fig. 1. Sleepiness reports on the SSS administered before each nap did not differ between genotypes during night-shift hours ($PER3^{-/5}$ = 2.95 ± 0.81 and $PER3^{4/4}$ = 2.65 ± 1.12, P = 0.456) or in the morning ($PER3^{-/5}$ = 3.33 ± 1.13 and $PER3^{4/4}$ = 3.58 ± 1.72, P = 0.683). Despite the absence of observed differences on the SSS, long-allele carriers indicated significantly higher sleepiness on the Epworth Sleepiness Scale administered at 17:00 hours: $PER3^{-/5}$ = 12.08 ± 2.55 and $PER3^{4/4}$ = 8.00 ± 1.94 (P < .001).

As shift work constitutes a challenge to the circadian system, we must be careful in interpreting the observed sleepiness differences between genotypes. Other studies refer to the enhanced overnight sleepiness differences in $PER3^{5/5}$ individuals as occurring at the circadian nadir, but it is reasonable to assume that many of the night-shift workers have a circadian phase different from those of typical day workers. Indeed, the mean DLMO time for our sample of shift workers is 23:30 ± 6:34 hours, which is later than that typically reported for the day-working population (Burgess and Fogg, 2008). Testing for genotype differences in circadian phase, we found a significantly earlier DLMO (P = 0.021) in $PER3^{-/5}$ individuals (20:44 ± 6:37 hours) compared with $PER3^{4/4}$ shift workers (02:46 ± 4:58 hours), as well as significantly earlier $DLMO_{off}$ times (P = 0.009) for $PER3^{-/5}$ individuals (07:58 ± 6:19 hours) compared with $PER3^{4/4}$ (14:41 ± 4:51 hours) shift workers. Fig. 2 presents the distribution of DLMO times within the two groups. The duration of melatonin secretion (i.e. time between DLMO and $DLMO_{off}$) was not significantly different between the genotype groups (11:12 ± 1:09 h in $PER3^{-/5}$ individuals versus 11:49 ± 1:09 h in $PER3^{4/4}$, P = 0.224).

To differentiate between the genotype differences in sleepiness and circadian phase, two linear regression models were estimated (Table 2). The first models the relationship between overnight MSLT score and PER3 genotype, adjusting for DLMO. Results from this analysis indicate that

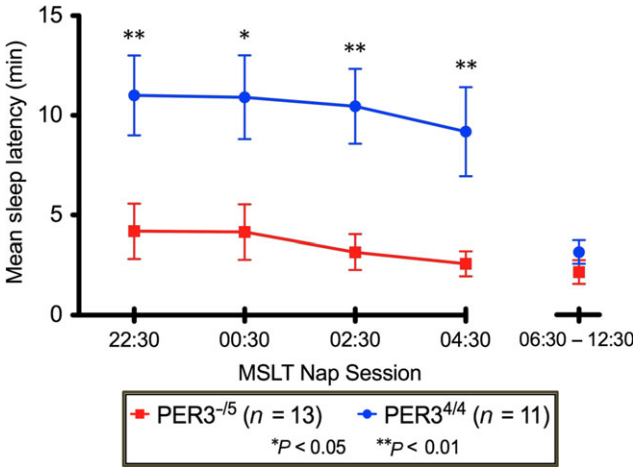

Figure 1. MSLT latencies to sleep by PER3 genotype.

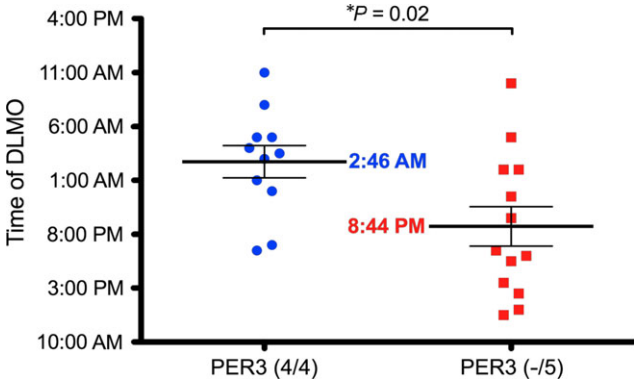

Figure 2. Distribution of dim-light melatonin onset (DLMO) by genotype.

PER3 genotype significantly predicts sleepiness even after controlling for variations in circadian phase. The second models the relationship between circadian phase and genotype, while adjusting for MSLT-assessed nocturnal sleepiness. Results suggest that PER3 is not associated with circadian phase when controlling for the MSLT.

DISCUSSION

The present study provides evidence that shift worker carriers of the five-repeat allele of the PER3 VNTR have significantly higher levels of sleepiness during overnight work hours as well as significantly earlier (i.e. less adapted) circadian phases. These results support the possibility that the PER3 genotype is an important factor in the process of physiological adjustment to the sleep–wake schedule associated with night-shift work, and therefore carries important clinical implications. The PER3 effects on both physiological (MSLT) and self-report (ESS) measures of sleepiness are robust and similar in magnitude (~5 min) to those found with the use of wake-promoting agents to treat patients with shift work disorder (Czeisler *et al.*, 2009). Clinically, the ESS mean for $PER3^{4/4}$ participants fell in the normal range, while the mean for $PER3^{-/5}$ patients was in the pathological range. Although the groups did not differ on the SSS, this may be related to the relative insensitivity of the SSS as a between-groups measure compared with the ESS.

Table 2 Ordinary least-squares regression models

	B	*SE*	β
Model 1. Dependent variable: nocturnal sleepiness (MSLT 22:30–04:30 hours)			
Constant	−0.248	3.304	
PER3 polymorphism	4.769*	1.925	0.482
DLMO	0.170	0.149	0.221
R^2	0.381		
Adjusted R^2	0.322		
Model 2. Dependent variable: circadian phase (DLMO)			
Constant	0.543	1.866	
PER3 polymorphism	0.086	1.087	0.019
Nocturnal MSLT	0.086	0.084	0.243
R^2	0.064		
Adjusted R^2	−0.026		

DLMO, dim-light melatonin onset; MSLT, multiple sleep latency test.
*$P < 0.05$.

Genotype differences in circadian phase were also present. Although these differences did not persist in a linear regression model co-varying for sleepiness, the direction of these differences (with $PER3^{4/4}$ workers being more likely to show delayed phases) suggests that $PER3^{-/5}$ workers are at a severe disadvantage when it comes to shift work adaptation: they suffer from increased sleepiness as well as maladaptive circadian phase. This result is consistent with a study demonstrating more rigid circadian control in $PER3^{5/5}$ homozygotes relative to other PER3 VNTR genotypes (Archer *et al.*, 2008).

A recent model of the effects of the PER3 VNTR on sleep and wakefulness emphasizes the interrelatedness of circadian rhythmicity and sleep homeostasis (Dijk and Archer, 2010). However, we must also consider the possibility that PER3 may not directly influence both sleepiness and circadian phase. While this sample was too small to perform a mediation analysis, existing literature suggests a few possibilities that may account for a possible causal pathway. Our finding of an independent effect of PER3 on sleepiness, but not on circadian phase, suggests that sleepiness may moderate the relationship between genotype and circadian phase. Dijk and Archer have proposed that a reduced build up of homeostatic pressure associated with the PER3 four-repeat genotype may prolong the onset of sleep, potentially leading to differences in circadian phase (Dijk and Archer, 2010). Thus, elevated homeostatic sleep pressure in excessively sleepy $PER3^{-/5}$ shift workers in the present study may have led these individuals to choose an earlier bedtime, which may inhibit a physiological phase delay. On sleep diaries and actigraphy, we did observe later bedtimes on days off in $PER^{4/4}$ workers, although these differences did not reach statistical significance (diary: $PER3^{-/5}$ = 23:17 ± 3:08 hours versus $PER3^{4/4}$ = 24:33 ± 2:48 hours, $P = 0.328$; actigraphy: $PER3^{-/5}$ = 22:25 ± 3:13 hours versus $PER3^{4/4}$ = 24:12 ± 2:25 hours, $P = 0.163$). The present findings are also consistent with a recent study showing that PER3 genotype may interact with sleepiness to affect a worker's chosen sleep strategy (i.e. sleep deprivation) on a shift work schedule and therefore indirectly influence circadian phase (Gamble *et al.*, 2011).

On top of these potential indirect influences of PER3 on circadian phase, there is additional evidence suggesting a direct effect. The five-repeat allele is associated with increased sensitivity to light, resulting in increased suppression of endogenous melatonin (Chellappa *et al.*, 2012). Due to the well-established phase–response curve of

circadian rhythms to light (Minors *et al.*, 1991), early morning light exposure has been found to prevent circadian adjustment (i.e. phase delay) to a simulated shift work schedule (Smith *et al.*, 2009). Thus, PER3$^{-/5}$ individuals (who are putatively more sensitive to light) may experience a larger inhibition to adaptive phase delay from this early morning 'light brake'. Although differences in morning light exposure are associated with differential circadian adjustment in night workers (Gumenyuk *et al.*, 2012), future research is needed to determine if this effect differs between PER3 genotypes.

It is well known that regular night work increases the risk for a variety of cardiovascular (Akerstedt and Knutsson, 1997; Czeisler *et al.*, 2009; Gamble *et al.*, 2011; Leclerc, 2010; Murata *et al.*, 1999; Puttonen *et al.*, 2009) and gastrointestinal (Vener *et al.*, 1989) disorders, as well as breast (Davis *et al.*, 2001; Kamdar *et al.*, 2013; Pijpe *et al.*, 2014; Wang *et al.*, 2013) and prostate (Conlon *et al.*, 2007; Erren *et al.*, 2011) cancers. Thus, in addition to the differences in circadian phase and sleepiness found in the present study, future investigations are needed to determine if night-shift workers with different length polymorphisms in PER3 have differential long-term health outcomes in response to working night-shifts. The differences in circadian phase found in these night workers who have been working the shift for several years (mean time on night-shift in the present sample: 4.34 $\pm$ 3.58 years) suggest this should be a focus of future investigations.

The present results may also have important implications for occupational health. Considering the healthcare industry alone, a litany of evidence indicates that medical and nursing errors increase when health professionals are sleep deprived. Over the past decade, mounting evidence has linked the five-repeat allele of PER3 to accelerated homeostatic dissipation, increased sleepiness at the circadian nadir, and impaired cognitive performance compared with homozygotes for the four-repeat allele. The present results are the first to provide evidence of critical genotype differences affecting objective sleepiness in night workers. A number of ethical questions arise from this finding, including how to handle differential genetic resistance to sleep loss in high-stakes work or educational settings (e.g. graduate medical education; Czeisler 2009). This is an area demanding critical input from sleep scientists, occupational health researchers, policymakers and medical ethicists.

While we believe this study provides compelling evidence that genotypic differences in the PER3 VNTR confer objective and clinically relevant differences in night workers, several limitations should be noted. First, due to sample size, we collapsed homozygotes and heterozygotes for the five-repeat allele. Future research will be needed to determine if homozygous PER3$^{5/5}$ night workers show greater deficits in alertness and circadian adjustment than PER3$^{4/5}$ heterozygotes. Second, circadian phase was only assessed once (after several consecutive night-shifts). Because there may be some variability in circadian phase (especially among shift workers), future studies may benefit from assessing circadian phase after consecutive night-shifts as well as after consecutive days off from work. The lack of differences in the MSLT following the sleep-deprivation phase of the protocol was likely due to a floor effect on the MSLT. Future studies examining sleep deprivation effects related to genotype may benefit from use of an alternative metric, such as the Maintenance of Wakefulness Test, which is less likely to be influenced by floor effects following prolonged sleep deprivation. Additional PER3$^{5/5}$ individuals may have increased the effect sizes observed and should be specifically targeted for recruitment in future trials. Lack of information regarding light history of participants is also a limitation. Finally, our sample was predominately female and African–American, and we were not able to match subjects for age, ethnicity, race or gender.

CONCLUSION

PER3$^{-/5}$ night workers show significantly elevated levels of sleepiness during night-shift hours on both the MSLT and the ESS. In both cases, PER3$^{-/5}$ shift workers showed sleepiness in the pathological range, while their PER3$^{4/4}$ counterparts showed sleepiness within normal limits. PER3$^{-/5}$ night workers also show a mean circadian phase 6 h earlier (i.e. less adapted) than PER3$^{4/4}$ workers. Regression modelling indicates that the five-repeat allele is significantly associated with sleepiness, independent of circadian phase, while the allele does not appear to exert an independent effect on circadian phase net of its effect on sleepiness. We interpret these findings as a call for future research on the role of PER3 in determining differential resilience to the challenges associated with night work, raising a number of practical and ethical questions for our 24-h society.

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AUTHOR CONTRIBUTIONS

CLD, TR and VG designed the research. RH, CLD, VG and RB collected data. RH, CLD, RB and AML analysed data. RB, CLD and AML wrote the manuscript.

CONFLICT OF INTEREST

CLD has served as a consultant or received financial support from Merck, Teva, Purdue, and Jazz. RB reports no conflicts of interest. RH reports no conflicts of interest. TR has served as a consultant for Abbott, Arcadia, AstraZeneca, Aventis, AVER, Bayer, BMS, Cypress, Ferrer, GlaxoSmithKline, Impax, Intec, Jazz, Johnson and

Johnson, Merck, Neurocrine, Novartis, Procter and Gamble, Pfizer, Purdue, Shire, Somaxon, and Transcept. He has received research support from Cephalon, Merck, Transcept, Speakers Bureau, Purdue, and Procter and Gamble. AML reports no conflicts of interest. VG reports no conflicts of interest.

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