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Sleep, Diurnal Preference, Health, and Psychological Well-being: A Prospective Single-Allelic-Variation Study

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Individual differences in sleep and diurnal preference associate with physical and mental health characteristics, but few genetic determinants of these differences have been identified. A variable number tandem repeat (VNTR) polymorphism in the *PERIOD3 (PER3)* gene (rs57875989) has been reported to associate with diurnal preference, i.e., preferred timing of waking and sleep. Here, the authors investigate in a prospective single-candidate genetic variant study whether allelic variation for this polymorphism associates also with reported actual sleep timing and sleep duration, as well as psychological and health measures. Six hundred and seventy-five subjects, aged 20 to 35 yrs, completed questionnaires to assess sleep and psychological and health characteristics and were genotyped for the *PER3* VNTR. Homozygosity for the longer allele ($PER3^{5/5}$) of the VNTR was associated with increased morning preference, earlier wake time and bedtime, and reduced daytime sleepiness. Separate analyses of work and rest days demonstrated that the increase in time in bed during rest days was greatest in $PER3^{5/5}$ homozygotes. *PER3* genotype modified the effects of sleep timing and duration on fluid intelligence and body mass index. Genotype was not associated with physical or psychological characteristics as assessed by the SF-36 Health Questionnaire, the General Health Questionnaire, the Big Five Inventory, the Behavioral Inhibition System–Behavioral Activation System scales, and the Positive and Negative Affect Scale, even though these measures varied significantly with diurnal preference as assessed by the Morningness-Eveningness Questionnaire. Whereas diurnal preference also predicts mental health and psychological characteristics, as well as sleep timing, the *PER3* VNTR specifically affects measures of sleep timing and may also modify the effects of sleep on health outcome measures. (Author correspondence: a.lazar@surrey.ac.uk)

Keywords: Body mass index, Clock genes, Diurnal preference, Genetic polymorphism, Intelligence, *Per3*, Sleepiness

INTRODUCTION

There is a growing interest in the contribution of the circadian timing system to individual differences in sleep. This is in part because sleep complaints and disturbances of the circadian timing system are common in psychiatric disorders (Emens et al., 2009; Monteleone & Maj, 2008). Furthermore, epidemiological studies have identified associations between individual differences in sleep timing and duration and health outcome measures, including mortality (Gallicchio & Kalesan, 2009; Hublin et al., 2007; Paudel et al., 2010), cardiovascular disease (Ferrie et al., 2007; Schwartz et al., 1999), diabetes (Yaggi et al., 2006), body mass index (BMI) and obesity (Baron et al., 2011; van den Berg et al., 2008), and depression (Baglioni et al., 2011; Franzen & Buysse, 2008; Gaspar-Barba et al., 2009; Kitamura et al., 2010). Identifying factors contributing to individual differences in sleep and how these factors associate with physiological and psychological profiles may aid our understanding of the role of sleep and the circadian system in mental health.

Individual differences in sleep characteristics have most frequently been investigated by single questions about sleep duration, or by assessing diurnal preference or chronotype using questionnaires, such as the Morningness-Eveningness Questionnaire (MEQ) (Horne & Östberg, 1976) or the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al., 2003). These approaches have led to identification of a number of polymorphisms that are associated with diurnal preference, sleep timing, or sleep duration (Dijk & Franken, 2005; von Schantz, 2008). Within the general population, sleep timing has been linked to variants of the gene *NPSR1* and sleepiness

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to *PDE4D* (Gottlieb et al., 2007). Sleep duration has been associated with variants of the circadian gene *CLOCK* (Allebrandt et al., 2010). Variation in the MEQ score has been associated with variants in a noncoding region in the circadian gene *CLOCK* (Katzenberg et al., 1998; Mishima et al., 2005), although this association has not been replicated in all studies (Pedrazzoli et al., 2007; Robilliard et al., 2002). Extreme diurnal preference has been linked to a single-nucleotide polymorphism in the 5'-untranslated region of *hPER2* (Carpen et al., 2005) and a silent polymorphism in *PER1*(Carpen et al., 2006; Chang et al., 2011).

We have focused on the gene *PERIOD3* (*PER3*). In humans, this paralogue is one of the most robustly rhythmically expressed clock genes, as assessed in leukocytes (Archer et al., 2008) or hair follicles (Akashi et al., 2010). Several polymorphisms and haplotypes of *PER3* have been investigated (Archer et al., 2010; Ebisawa et al., 2001). A primate-specific (Jenkins et al., 2005) variable number tandem repeat (VNTR) polymorphism (RS57875989) in the coding region of *PER3* associates with diurnal preference and delayed sleep phase disorder (Archer et al., 2003; Pereira et al., 2005). This association has been reported to be significant in young subjects (18–29 yrs of age) and attenuates with aging (Jones et al., 2007).

Laboratory studies have shown that this polymorphism affects sleep structure at baseline in young (Viola et al., 2007) and older people (Viola et al., 2011), and in response to partial sleep deprivation (Goel et al., 2009). The *PER3* VNTR has also been reported to modulate the decline of executive function during a night without sleep as measured behaviorally (Groeger et al., 2008; Viola et al., 2007) and by functional magnetic resonance imaging (fMRI) (Vandewalle et al., 2009). This polymorphism was found to co-vary with differences in adaptation to shiftwork in nurses (Gamble et al., 2011), to associate with the age of onset of the first episode in bipolar disorder (Benedetti et al., 2008), and with the postpartum onset of bipolar disorder (Dallaspezia et al., 2011). Most of these studies were not prospective single-candidate polymorphism studies and replication studies have been rare. Discrepancies between studies and lack of replication may be related to different study populations, or small effect sizes (e.g., Barclay et al., 2011; Osland et al., 2011). The latter is likely to lead to nonsignificant results, in particular, in cases in which the replication studies use a smaller sample size than previous studies (e.g., Osland et al., 2011). Additional limitations include the quality of the phenotyping. In our study, we aimed to overcome these limitations by using a new and large sample and phenotyping based on questionnaires used in the original studies (i.e., the MEQ) as well as other questionnaires that are meant to assess the same phenotypic complex (e.g., MCTQ).

Very limited data are available regarding the effects of this polymorphism on actual sleep timing and duration. In most past studies, only single questionnaires have been used and comprehensive assessment of sleep, health, and psychological profiles were not available. In this study, subjects were genotyped only for the *PER3* VNTR, and we characterized its effects on preferred and actual sleep-wake timing, sleep duration, sleep quality, and daytime sleepiness, as measured by multiple validated questionnaires. To better understand the interaction between biological factors and social constraints, we analyzed the effect of genotype on sleep-wake timing and sleep duration separately for workdays and rest days, an aspect of sleep-wake regulation that has not yet been sufficiently explored in epidemiological studies, although emphasized within recent chronobiological studies (Roenneberg et al., 2003; Wittmann et al., 2006). Since health and psychological characteristics have been associated with diurnal preference (Adan et al., 2010; Baron et al., 2011; Emens et al., 2009; Gaspar-Barba et al., 2009; Hasler et al., 2010; Hogben et al., 2007; Killgore & Killgore, 2007; Kitamura et al., 2010; Monteleone & Maj, 2008; Prat & Adan, 2010; Roberts & Kyllonen, 1999; Selvi et al., 2010; Tonetti et al., 2009; Urbán et al., 2011) and sleep duration (Ferrie et al., 2007; Gallicchio & Kalesan, 2009; Geiger et al., 2010; Hublin et al., 2007; van den Berg et al., 2008; Yaggi et al., 2006), we also investigated the effect of the *PER3* VNTR polymorphism on self-reported multiple health measures, individual traits of personality, general motivation systems, and general affective orientation, using questionnaires and scales widely used in psychological and psychiatric research. We also investigated whether the genotype modulates the association between sleep timing and duration and health and psychological variables, such as BMI (Baron et al., 2011) and intelligence (Killgore & Killgore, 2007; Roberts & Kyllonen 1999), which have been reported to associate with sleep timing and duration. To put any effects of the polymorphism into perspective, we also analyzed the effect of diurnal preference as assessed by the MEQ on these variables in our sample.

MATERIALS AND METHODS

Participants

The study was reviewed favorably by the University of Surrey Ethics Committee and by the Institutional Review Board of the Air Force Research Laboratory and conducted in accordance with the principles of the Declaration of Helsinki and standards of the journal (Portaluppi et al., 2010). Participants were recruited using posters, advertisements in local newspapers, by radio, and through Web sites. No exclusion criteria related to ethnicity or country of origin were applied. Seven hundred and four healthy nonsmoking participants aged between 20 and 35 yrs participated in the screening phase of three laboratory-based sleep research projects. Eligibility criteria for screening were that the subjects did no shiftwork during the last year and had a BMI ranging between 18 and 30. Recruitment and screening

took place between November 2008 and March 2010. Participants provided written informed consent before providing a buccal swab for genotyping and completing any further study-related procedures.

Genotyping

Genotyping of the *PER3* VNTR was performed according standard procedure, as described previously (Vandewalle et al., 2009) using the following polymerase chain reaction (PCR) primers:

Forward: 5' GTGTCTTTTCATGTGCCCTTACT 3',
Reverse: 5' TACCCCAATATACCTGACAAAAA 3'

Six hundred seventy-five participants (262 female) out of 704 were genotyped successfully (Table 1).

Characterization of the Phenotype

Diurnal preference, habitual sleep-wake timing, quality of sleep, and daytime sleepiness, as well as psychological and self-reported health characteristics were assessed by multiple questionnaires and scales (see Supplement 1 for details).

Demographic data, such as age, sex, BMI, shiftwork, ethnicity, number of workdays and rest days, etc., were assessed by a medical questionnaire (MQ) and the British Sleep Survey (BSS) (Groeger et al., 2004).

Diurnal preference was assessed using the total score of the MEQ (Horne & Östberg, 1976) and the single question from the MCTQ referring to self-assessed chronotype (Roenneberg et al., 2003).

Sleep-wake timing and duration were assessed using multiple questionnaires that differed from each other with respect to the time period to which they refer (Pittsburgh Sleep Quality Index [PSQI]—last month [Buysse et al., 1989], British Sleep Survey [BSS]—last week [Groeger et al., 2004], Karolinska Sleep Diary [KSD]—last day [Åkerstedt et al., 1994], MCTQ—usual workdays and rest days, and MEQ—preferred). From these questionnaires, we directly extracted self-reported bedtime and wakeup time (WT), sleep latency, sleep duration, and duration of naps. From these self-reported measures, we derived some further sleep timing parameters: sleep onset time (SOT), midpoint of sleep (MS), and time in bed (TIB). SOT was calculated by adding sleep latency (time between intent to go to sleep and actual sleep onset) to bedtime. MS indicated the midpoint of the time period spent in bed between SOT and WT. TIB indicated the time period between bedtime and WT. For all variables extracted from the MCTQ, we also computed the difference and the average between workdays and rest days. The average was weighted by number of workdays and rest days reported by the participants (Allebrandt et al., 2010), e.g., $BTA = (BTW \times WDn + BTR \times RDn)/7$, where BTA = average bedtime, BTW = bedtime on workdays, WDn = number of workdays, BTR = bedtime on rest days, and RDn = number of rest days. MS during rest days was also corrected for the sleep debt accumulated during rest days according to the following formula: $MSRcorr = MSR - .5 \times (TIBR - [TIBW \times WDn + TIBR \times RDn]/7)$, where MSRcorr = corrected MS during rest days, MSR = MS on rest days, TIBW = TIB on workdays, WDn = number of workdays, TIBR = TIB on rest days, and RDn = number of rest days (Roenneberg et al., 2004).

Quality of sleep was assessed by the PSQI and the Insomnia Severity Index (ISI) (Bastien et al., 2001).

Daytime sleepiness was assessed by the Epworth Sleepiness Scale (ESS) (Johns, 1991) and the Karolinska Sleepiness Scale (KSS) (Gillberg et al., 1994).

Nonverbal fluid intelligence was assessed using the short version of the Raven's Advanced Progressive Matrices (APM), with a maximum score of 12 (Raven, 1978).

Verbal abilities were assessed by the Verbal Reasoning Task (VRT) (Baddeley, 1968).

Health profile was assessed across several domains using Version 2 of the 36-Item Short-Form Health Survey (SF-36v2) (Ware & Sherbourne, 1992) and General Health Questionnaire (GHQ) (Goldberg, 1978).

Eating behavior was assessed using the English version of the Dutch Eating Behavior Questionnaire (DEBQ) (Vanstrien et al., 1986).

Personality was explored by the means of the Big Five Inventory (BIF) (Goldberg, 1990; McCrae & Costa, 1987).

Individual traits of general motivation systems were assessed by the Behavioral Inhibition System–Behavioral Activation System (BIS-BAS) scales (Carver & White, 1994).

TABLE 1. Demographics

	Males		Females		Total		Age (years)		BMI (kg/m^2)	
	N	%	*N*	%	*N*	%	Mean	SD	Mean	SD
Total	413	61.2	262	38.8	675	100	26.1	4.0	23.7	2.8
Ethnicity										
European	284	42.1	184	27.3	468	69.3	25.1	4.1	23.3	2.8
African	51	7.6	42	6.2	93	13.8	26.8	4.2	23.8	3.1
Indian	34	5.0	8	1.2	42	6.2	26.7	4.7	23.8	2.9
Oriental	20	3.0	11	1.6	31	4.6	26.0	3.0	22.1	3.1
Other	11	1.6	11	1.6	22	3.3	26.0	4.5	24.3	2.2
No answer	13	1.9	6	.9	19	2.8	26.1	4.1	24.0	3.0

N = number of participants in a category; SD = standard deviation.

General affective orientation was measured by the Positive and Negative Affect Scale (PANAS) (Watson et al., 1988).

Statistical Analyses

All statistical analyses were conducted using SAS (Version 9.1; SAS, Cary, NC, USA).

For the assessment of the effect of genotype on categorical variables, we used chi-square tests. The effect of genotype on continuous variables was assessed by one-way parametric (analysis of variance [ANOVA]) or nonparametric (Kruskal-Wallis) tests, depending on whether or not the distribution was normal. This was followed by *t* test or Mann-Whitney *U* test for quantifying the contrasts between the genotypes and/or other categorical predictors.

For repeated-measures analyses, we used mixed models that allowed the assessment of fixed effects (e.g., genotype) controlled for random effects represented by the individuals. If non-Gaussian distribution emerged, we applied transformations (e.g., log transformation) for mixed-model analyses in such a way that variables fitted normal distribution (e.g., BMI and sleep-wake timing). For normality assessment, residuals were visually inspected in each analysis, and normality cutoffs applied at 2 standard errors of the skewness and kurtosis parameters of the distribution (Tabachnick & Fidell, 2007).

No correction for multiplicity was applied to measures related to diurnal preference and sleep-wake timing because this is a prospective study investigating one genetic polymorphism based on well-established *a priori* assumptions. For some of the variables, we designed robust repeated-measures statistical models in which variables were assessed simultaneously, thereby considerably reducing the number of comparisons. For the analyses related to psychological profiles and health outcomes, no correction for multiplicity was applied, because many of the studied variables were not independent from one other, and these analyses were considered exploratory. To assess to what extent findings could have been related to the heterogeneity of our sample, with respect to ethnicity, for some variables effects of *PER3* VNTR genotype were also assessed by excluding all ethnicities but Europeans.

RESULTS

The prevalence of the three genotypes was independent of sex and age (Tables 2 and 3). The prevalence of the genotypes varied significantly across self-reported ethnicity categories (Table 2). The relative frequency of the $PER3^{5/5}$ genotype was highest in the African (18%) and lowest in Oriental (3%) ethnic categories. $PER3^{4/4}$ was most frequent in the Oriental (74%) and rarest in the African ethnicity group (34%).

Genotype Effect on Diurnal Preference

The distribution of the *PER3* VNTR genotypes across the three MEQ categories of diurnal preference was not uniform (Figure 1A). The relative frequency of the $PER3^{5/5}$ genotype was above the overall sample average (11.3%) in the morning preference group (15.4%) and considerably below it in the evening preference group (7.5%). When treated as continuous variables, both the composite score from the MEQ and the self-assessment question from the MCTQ differed significantly across genotypes (Table 3), with no significant effect of sex (Figure 1B, D). The $PER3^{5/5}$ group exhibited higher morning preference compared to both of the other genotypes as measured by the MEQ, whereas the heterozygous group exhibited higher evening preference as compared to both homozygous genotypes as measured by the MCTQ (Table 3, Figure 1B, D). Morningness

TABLE 2. Distribution of genotypes across sex and ethnicity groups

	$PER3^{4/4}$			$PER3^{4/5}$			$PER3^{5/5}$			Total				
	N	%[a]	%[b]	N	%[a]	%[b]	N	%[a]	%[b]	N	%[a]	%[b]	χ^2	p
Sex														
Male	183	27.1	44.3	184	27.3	44.6	46	6.8	11.1	413	61.2	100	.437	ns
Female	122	18.1	46.6	110	16.3	41.9	30	4.4	11.5	262	38.8	100		
Ethnicity														
European	215	31.9	44.9	201	29.8	42.9	52	7.7	11.1	468	69.3	100	19.88	.011*
African	32	4.7	34.4	44	6.5	47.3	17	2.5	18.3	93	13.8	100		
Indian	18	2.7	42.9	21	3.1	50.0	3	.4	7.1	42	6.2	100		
Oriental	23	3.4	74.2	7	1.0	22.6	1	.2	3.2	31	4.6	100		
Other	17	2.5	41.5	21	3.1	51.2	3	.4	7.3	41	6.1	100		
Total	305	45.2	n/a	294	43.6	n/a	76	11.3	n/a	675	100	n/a		

N = number of participants in a category; %[a] = relative frequency of the sexes and reported ethnicities within a genotype group; %[b] = relative frequency of the genotypes within a sex or an ethnic category. Due to the low number of *N*, "Other" ethnicity category includes the "No answer" category as well. There was a significant difference in the distribution of genotypes across ethnic groups ($\chi^2 = 19.88$, $p = .011$). The frequency of the long ($PER3^5$) and short ($PER3^4$) alleles can be calculated from the frequency of the genotypes according to the following formula: [Frequency allele (%) = frequency homozygous genotype (%) + .5 × frequency heterozygous genotype (%)].

TABLE 3. Genotype and diurnal preference effects on studied variables

			Genotype effects												
			PER3$^{4/4}$			PER3$^{4/5}$			PER3$^{5/5}$			*Main effect*		*Contrasts* PER3		
Areas	Questionnaires	Studied variables (effect of diurnal preference)	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	χ^2/F	*p*	*4/4-5/5*	*4/5-5/5*	*4/4-4/5*
Demographics	Medical Questionnaire (MQ)	Age (yrs)****	305	25.51	4.13	294	25.51	4.13	76	25.84	4.38		ns			
		BMI (kg/m^2)$_{med}$	299	22.76	3.73	290	23.38	3.56	72	23.24	4.20		ns			
Diurnal preference	Morningness-Eveningness Questionnaire (MEQ)	Total score$^{N/A}$	300	51.06	7.89	289	49.88	8.08	74	53.31	7.90	$F = 5.75$	.003	*	***	
	Munich ChronoType Questionnaire (MCTQ)	Self-reported morningness****	301	2.94	1.53	291	3.22	1.58	75	2.76	1.51	$\chi^2 = 7.46$	.024		*	*
Sleep-wake timing	MCTQ—workdays *(In general)*	Bedtime^a****	303	23:16	0:59	290	23:24	1:00	75	23:06	0:54		ns			
		Sleep latency^b*	304	17.4	12.7	289	16.3	11.1	75	14.7	8.2		ns			
		Sleep onset time^a****	303	23:33	1:01	289	23:40	1:00	75	23:21	0:55		ns			
		Midpoint of sleep^a****	301	03:41	0:58	287	03:45	0:58	75	03.21	0:49	$\chi^2 = 10.23$	.006	**	***	
		Wakeup time^a****	301	07:48	1:12	287	07:52	1:11	75	07:21	1:03	$\chi^2 = 11.18$	.004	***	***	
		Time in bed^c	301	8:33	1:05	287	8:28	1:04	75	8:14	1:05		ns			
		Nap duration^b*	108	38.1	18.9	97	43.5	28.7	26	31.4	17.2		ns			
	MCTQ—rest days *(In general)*	Bedtime^a****	301	00:09	1:05	286	00:23	1:13	75	23:58	1:06	$F = 5.03$	.007	**		*
		Sleep latency^b	296	15.2	10.8	284	14	11	74	13.6	9.2		ns			
		Sleep onset time^a****	296	00:24	1:06	283	00:37	1:13	74	00:12	1:06	$F = 4.87$	.008	**		*
		Midpoint of sleep^a****	296	04:36	1:06	283	04:48	1:07	74	04:28	1:09	$F = 3.69$	.026	*		*
		Midpoint of sleep$^a_{corr}$****	286	04:34	1:05	274	04:46	1:10	71	04:21	1:05	$F = 4.66$	.01	*		*
		Wakeup time^a****	305	08:48	1:23	291	08:59	1:23	76	08:45	1:30		ns			
		Time in bed^c***	301	8:40	1:14	286	8:37	1:22	75	8:46	1:16		ns			
		Nap duration^b	101	41.8	22.7	91	47	35	24	44.2	34.3		ns			
	MCTQ—change from workdays to rest days [rest days – workdays] *(In general)*	Bedtime^c****	299	00:52	0:48	284	01:00	0:57	74	00:51	.54		ns			
		Sleep latency^b	295	-2.1	8.9	281	-2.4	7.6	73	-1.1	5.3		ns			
		Sleep onset time^c****	294	00:51	0:47	280	00:58	0:56	73	00:51	0:53		ns			
		Midpoint of sleep^c****	292	0:56	0:49	278	1:03	0:53	73	1:08	1:01		ns			
		Midpoint of sleep$^c_{corr}$****	286	00:53	0:45	274	01:00	0:53	71	01:00	0:56		ns			
		Wakeup time^c****	301	00:59	1:13	286	01:06	1:13	75	01:24	1:25		ns			
		Time in bed^c*	297	0:07	1:15	281	0:07	1:16	74	0:33	1:13	$F = 3.93$	.02	*	*	
		Nap duration^b	84	3.5	15.9	74	.3	23.1	20	10	23.2		ns			
	MQ *(In general)*	Self-reported sleep duration^c*	296	7:50	0:45	289	7:58	0:50	73	7:47	0:53		ns			
	Pittsburgh Sleep Quality Index (PSQI) *(Last month)*	Bedtime^a****	305	23:30	1:02	294	23:38	1:08	76	23:12	0:56	$\chi^2 = 9.57$	.008	**	***	
		Sleep latency^b	305	16.8	11.2	294	16.5	10.9	76	15.8	9.8		ns			
		Sleep onset time^a****	305	23:47	1:03	294	23:55	1:09	76	23:28	0:58	$\chi^2 = 10.36$	.006	**	***	
		Midpoint of sleep^a****	305	03:58	1:05	294	04:07	1:11	76	03:36	0:57	$F = 6.72$	.001	*	****	
		Wakeup time^a****	305	08:09	1:19	294	08:20	1:25	76	07:44	1:13	$F = 5.95$	.003	*	****	
		Time in bed^c*	305	8:39	1:00	294	8:41	1:02	76	8:32	1:06		ns			
		Self-reported sleep duration^c*	305	07:50	0:52	294	07:53	0:58	76	7:47	1:04		ns			
	British Sleep Survey (BSS) *(Last week)*	Bedtime^a****	304	23:34	1.05	293	23:41	1:07	76	23:24	1:07					
		Wakeup time^a****	303	08:15	1:20	292	08:23	1:24	76	07:48	1:12	$\chi^2 = 9.9$	.007	*	***	
		Time in bed^c***	302	8:41	1.09	292	8:42	1.03	76	8:24	1:09		ns			
		Self-reported sleep duration^c**	305	07:57	0:50	293	08:04	1:00	75	7:54	1:00		ns			
	Karolinska Sleep Diary (KSD) *(Last day)*	Bedtime^c****	304	23:40	1:16	288	23:52	1:15	74	23:31	1:15	$\chi^2 = 6.38$	.041		*	
		Sleep latency^b	299	22.6	26.1	285	19.2	28.7	74	13.7	22.8		ns			
		Sleep onset time^a****	299	00:02	1:14	285	00:10	1:16	74	23:44	1:16	$\chi^2 = 8.38$	.015	*	***	
		Midpoint of sleep^a****	297	04:04	1:03	280	04:09	1:03	73	03:48	1:01	$F = 6.26$	.034		*	
		Wakeup time^a****	302	08:05	1:15	283	08:09	1:12	74	07:52	1:09		ns			
		Time in bed^c	302	8:24	1:21	283	8:18	1:18	73	8.21	1:24		ns			
	MEQ *(Preferred)*	Bedtime^a****	304	23:54	1:09	294	00:01	1:10	75	23:38	1:13	$F = 3.21$	.041		*	
		Wakeup time^a****	304	08:48	1:14	294	08:58	1:12	75	08.34	1:22	$F = 3.39$	.034		*	
		Time in bed^c*	304	8:53	1:10	294	8:57	1:14	75	8:56	1:22		ns			
Sleepiness	Epworth Sleepiness Scale (ESS) *(Recent times)*	Usual daytime sleepiness*	302	4.91	3.18	292	5.04	3.19	76	5.08	3.21		ns			
	Karolinska Sleepiness Scale (KSS) *(Actual)*	Actual daytime sleepiness****	305	3.75	2.00	292	3.67	2.00	75	3.15	2.00	$\chi^2 = 8.26$	.016	*	***	

Continued

TABLE 3. *Continued*

Areas	Questionnaires	Studied variables (effect of diurnal preference)	Genotype effects: PER3$^{4/4}$ N	Mean	SD	PER3$^{4/5}$ N	Mean	SD	PER3$^{5/5}$ N	Mean	SD	*Main effect* χ^2/F	p	*Contrasts* PER3 4/4-5/5	4/5-5/5	4/4-4/5
Sleep quality	PSQI	General sleep quality****	280	3.16	1.83	273	3.23	1.79	71	3.04	1.92		ns			
	KSD	Previous night sleep quality***	304	3.50	1.39	284	3.46	1.42	74	3.35	1.45		ns			
	Insomnia Severity Index (ISI) *(Last 2 wks)**	Insomnia****	294	3.40	3.45	280	3.40	2.96	73	2.99	3.16		ns			
Health	MQ	Weekly alcohol intake****	299	4.52	4.12	286	4.28	4.10	73	3.58	3.61		ns			
		Daily caffeine intake*	222	1.26	.97	212	1.32	1.01	53	1.46	1.23		ns			
	General Health Questionnaire (GHQ)* *(Last 4 wks)*	Total score****	301	8.55	3.81	288	8.06	3.37	76	7.97	2.98		ns			
	Short Form-36 Health Questionnaire (SF36)	Physical Health	301	57.68	4.05	284	57.69	3.81	75	57.99	3.44		ns			
	(Last 4 wks)	Mental Health****	301	52.23	6.49	284	52.42	6.35	75	52.39	6.57		ns			
	Ducth Eating Behaviour Questionnaire (DEBQ)	Restrained eating*	301	2.24	.85	289	2.19	.75	73	2.17	.79		ns			
	(In general)	Emotional eating*	300	1.89	.68	291	1.97	.76	74	1.92	.76		ns			
		External eating****	301	2.99	.63	291	3.11	.67	74	2.93	.63	$F = 3.78$	.023		*	
Psychology	Ravens Advanced Progressive Matrices (APM)	Nonverbal intelligence*	304	6.31	2.38	291	6.45	2.61	76	5.63	2.83		ns			
	Verbal Reasoning Test (VRT)	Verbal reasoning ability*	299	24.98	12.12	287	26.17	14.00	76	23.78	12.33		ns			
	Big Five Inventory (BIF)	Openness	296	38.42	5.25	287	38.68	5.07	76	37.84	5.26		ns			
	(In general)	Conscientiousness****	295	34.40	5.53	284	33.81	5.92	76	34.79	4.33		ns			
		Extraversion*	298	29.02	5.20	291	28.92	4.90	75	28.83	4.52		ns			
		Agreeableness****	299	36.12	4.65	287	35.75	4.90	76	37.01	5.08		ns			
		Neuroticism****	300	18.32	5.51	288	18.05	5.50	75	16.91	4.93		ns			
	Behavior Inhibition System–Behavior	BIS***	299	16.19	3.45	288	16.68	3.75	72	17.07	3.64		ns			
	Approach System (BIS-BAS)	BAS reward*	295	7.72	2.03	289	7.71	1.82	73	7.71	2.00		ns			
	(In general)	BAS drive	297	8.40	2.10	289	8.30	2.21	75	8.31	2.17		ns			
		BAS fun seeking	297	6.99	2.08	290	6.90	1.99	75	7.35	2.06		ns			
	Positive Affect-Negative Affect	Positive affect****	296	37.24	6.65	290	37.62	5.81	76	38.43	5.08		ns			
	Scales (PANAS)	Negative affect****	298	15.69	5.07	286	15.85	4.99	71	16.01	5.69		ns			
	(Last few wks)															

Number of observations, mean values, standard deviations (SD), and one-way analyses main effects and contrasts are indicated for each variable within each questionnaire. The time period to which each questionnaire refers is also indicated in brackets. For parametric analyses (ANOVA) F values and for nonparametric analyses (Kruskal Wallis) χ^2 values are indicated. Main effects are indicated only if $p < .05$. Asterisks indicate level of significance levels (*$p < .05$, **$p < .01$, ***$p < .005$, ****$p < .001$). Contrasts are not indicated unless main effects are indicated. Contrasts: (*PER3*$^{4/4}$ vs. *PER3*$^{5/5}$), (*PER3*$^{4/5}$ vs. *PER3*$^{5/5}$), (*PER3*$^{4/4}$ vs. *PER3*$^{4/5}$); superscripts following variable names indicate unit of measure ([a]clock time, [b]minutes, [c]hours:minutes); asterisks following the variable names in the column Studied variables indicate significance levels related to the effect of diurnal preference on studied variables. Diurnal preference was used as a categorical variable comprising three groups. Each group represented approximately one third of the studied populations classified along cutoffs (evening: <48; intermediate [3]48 and £54 morning >54). Subscript "med" indicates that median and interquartile range are presented. Subscript "corr" indicates that the midpoint of sleep was corrected for sleep debt (see Materials and Methods).

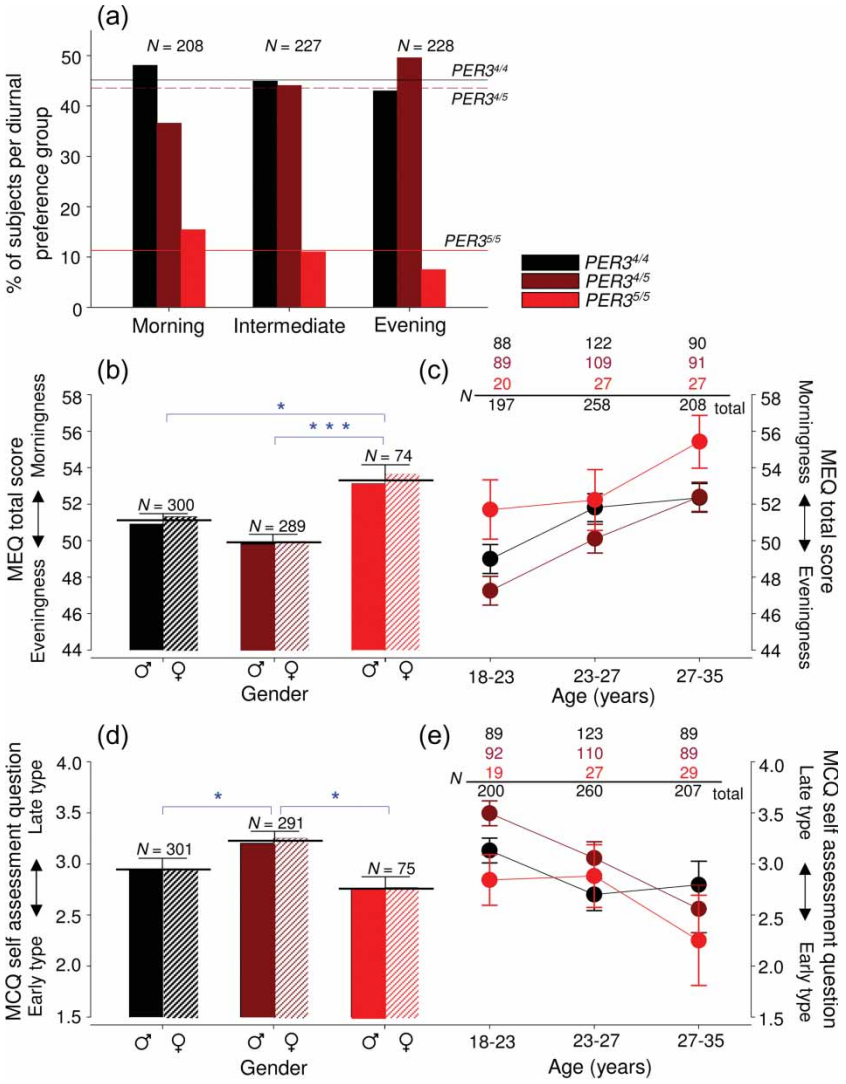

FIGURE 1. The effect of the *PER3* VNTR polymorphism on diurnal preference. Black = $PER3^{4/4}$, dark red = $PER3^{4/5}$, red = $PER3^{5/5}$. Blue horizontal lines indicate significant contrasts between genotypes (*$p < .05$, **$p < .01$, ***$p < .005$). (A) Distribution of the PER3 VNTR genotypes across three categories of diurnal preference as assessed by the Morningness-Eveningness Questionnaire (MEQ). Each category represented approximately one third of the studied populations classified along cut-offs of the total MEQ score (evening: <48; intermediate >= 48 and <= 54; morning >54). Vertical bars indicate relative frequency of the three studied genotypes within each diurnal preference group. Horizontal reference lines indicate prevalence of the studied genotypes in the entire group of subjects. The distribution of the genotypes across the three categories of diurnal preference was not uniform ($\chi^2 = 11.01$, $p = .026$, $N = 663$). (B and C) Effect of genotype, sex, and age on diurnal preference as measured by the MEQ. The total score of the 19 items ranges from 16 to 86; higher values indicate morning preference. (B) Least square means and standard errors by genotype and sex groups. The MEQ score differed across the genotypes ($F_{2,659} = 5.73$, $p = .003$) and was similar in both sexes without an interaction between the two categorical predictors. It was higher in the $PER3^{5/5}$, i.e., higher morning preference, as compared to the $PER3^{4/4}$ ($p = .033$) and $PER3^{4/5}$ ($p = .001$) groups. (C) Least square means and standard errors by genotype and age groups. Each of the three age groups represent approximately one third of the studied populations classified along cutoffs (youngest: <23, intermediate: ≥23 and ≤27, oldest: >27 yrs of age). Morning preference differed between the genotypes ($F_{2,658} = 5.45$, $p = .005$) and increased with age ($F_{2,658} = 15.28$, $p < .001$). No interaction was observed. $PER3^{5/5}$ homozygotes displayed stronger morning type as compared to the $PER3^{4/4}$ ($p = .04$ and the $PER3^{5/5}$ ($p = .002$) groups. Each age group significantly differed from the other two groups: youngest vs. oldest ($p < .001$), youngest vs. intermediate ($p < .001$), intermediate vs. oldest ($p = .028$). (D and E) Effect of genotype, sex, and age on diurnal preference as measured by the self-assessment question from the Munich ChronoType Questionnaire (MCTQ). The self-assessment question ascertains chronotype on a scale ranging from 0 (extreme early type) to 6 (extreme late type). (D) Least square means and standard errors by genotype and sex groups. There was a main effect of genotype ($F_{2,663} = 3.78$, $p = .023$) independent of sex, and there was no interaction between the two categorical predictors. $PER3^{4/5}$ heterozygotes were delayed compared to both homozygote groups, the $PER3^{5/5}$ ($p = .022$) and $PER3^{4/4}$ ($p = .03$) groups. $PER3^{5/5}$ homozygotes were the earliest genotype. (E) Least square means and standard errors by genotype and age groups. Self-assessed diurnal preference differed between the genotypes ($F_{2,662} = 3.49$, $p = .031$) and lateness decreased with age ($F_{2,662} = 5.52$, $p < .004$), with no interaction between the categorical predictors. $PER3^{4/5}$ heterozygotes were delayed compared to $PER3^{5/5}$ ($p = .034$) and the $PER3^{4/4}$ ($p = .03$) homozygotes. The oldest group was advanced compared to the youngest ($p = .001$) and intermediate ($p = .034$) age groups.

increased with age, even within this narrow age range of 18–35 yrs, with no significant interaction between genotype and age groups (Figure 1C, D). After controlling for further factors shown to be associated with morningness, such as conscientiousness, positive or negative emotional status, and intelligence, the genotype effect

on diurnal preference as measured by the MEQ persisted ($F_{2,619} = 4.43$, $p = .012$), and it remained marginally significant for the MCTQ ($p = .06$). When the analysis was limited to Europeans, the effect of genotype on diurnal preference as measured by the MEQ persisted ($F_{2,458} = 6.61$, $p = .002$), and a similar trend was observed for the MCTQ, although it was no longer significant ($p = .055$). When we controlled our analyses for the combined effect of age, sex, and ethnicity as well, the main effect of genotype remained significant for both MEQ ($F_{2,636} = 5.97$, $p = .003$) and the self-assessment question from the MCTQ ($F_{2,642} = 4.12$, $p = .017$).

Genotype Effect on Sleep-Wake Timing and Sleep Duration

The *PER3* VNTR had a significant effect on numerous sleep-wake timing parameters as assessed by individual questionnaires (Table 3) or as estimated by combining questionnaires (MCTQ, BSS, PSQI, KSD, MEQ) in a mixed model (Figure 2). In the latter analysis, bedtime, MS, WT (Figure 2), and SOT ($F_{2,675} = 6.81$, $p = .001$) differed between the genotypes, independent of questionnaire, which also had an effect on all variables ($p < .001$). On average, $PER3^{5/5}$ homozygotes went to bed 15 min earlier than the $PER3^{4/4}$ and 25 min earlier than the $PER3^{4/5}$ individuals. The heterozygous group's bedtime was on average 10 min later than that of $PER3^{4/4}$s. $PER3^{5/5}$ homozygotes woke up 22 min earlier than the heterozygotes, and the MS was 17 min earlier as compared to the $PER3^{4/4}$ individuals and 26 min earlier compared to the heterozygotes (Figure 2). The genotype effect on SOT was similar to the effect on bedtime ($F_{2,675} = 6.81$, $p = .001$). $PER3^{5/5}$ individuals reported to fall asleep on average 17 min earlier compared to the $PER3^{4/4}$ ones ($p = .02$) and 28 min earlier compared to the heterozygous ones ($p < .001$). Heterozygotes had the latest SOT, which was 11 min later compared to the $PER3^{4/4}$s ($p = .047$). Diurnal preference also had an effect of sleep-wake timing and sleep duration. Across all questionnaires, the morning preference group had earlier sleep-wake timing ($p < .001$) and shorter sleep duration (except workdays) compared to the evening preference group (Table 3, Studied Variables (Effect of Diurnal Preference)). When we controlled our analyses for the combined effect of age, sex, and ethnicity, the main effect of genotype remained significant for all studied variables, such as bedtime ($F_{2,648} = 5.20$, $p = .006$), SOT ($F_{2,650} = 5.92$, $p = .003$), MS ($F_{2,652} = 5.84$, $p = .003$), and WT ($F_{2,649} = 4.21$, $p = .015$).

Genotype Effect on Sleep Duration During Workdays and Rest Days

Because sleep-wake timing and sleep duration are, in part, determined by work schedules, we analyzed the effect of genotype and work versus rest day on these parameters. Not surprisingly, bedtime ($F_{1,661} = 730$, $p < .001$), MS ($F_{1,653} = 865$, $p < .001$), and WT ($F_{1,666} = 398$, $p < .001$) were all later on rest days than workdays, and were all affected by genotype on workdays and/or rest days (Table 3). Both the raw and corrected estimates of MS were affected by genotype during both workdays and rest days (Table 3), such that this marker was earliest in the $PER3^{5/5}$. With regard to WT, genotype effects were most pronounced during workdays, with the $PER3^{5/5}$ waking up earlier as compared to both other genotypes. By contrast, for bedtime, genotype effects were most pronounced during rest days, with $PER3^{5/5}$ homozygotes going to bed earlier than $PER3^{4/4}$ homozygotes (Table 3). Analysis of the change from workdays to rest days revealed that these changes in timing had an effect on

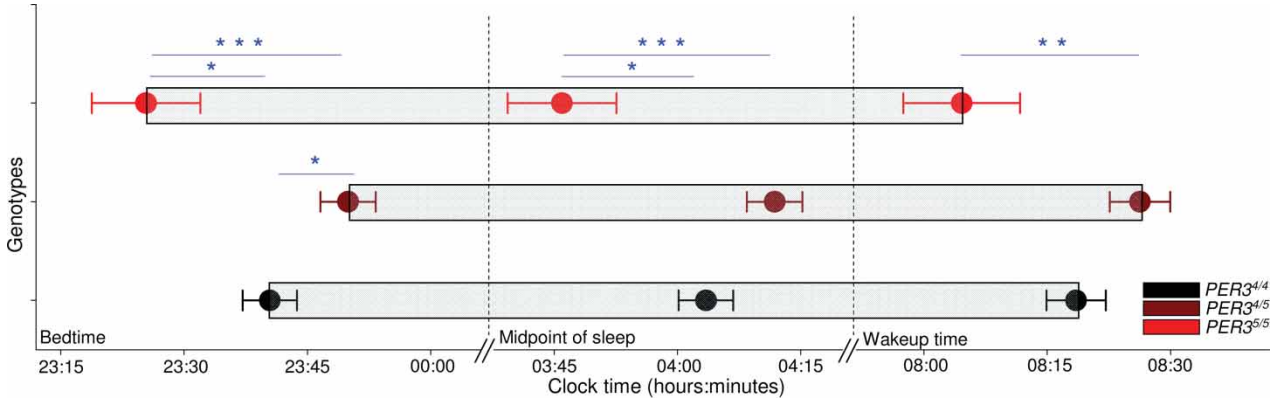

FIGURE 2. Effect of genotype on bedtime, midpoint of sleep, and wakeup time as derived from the Munich ChronoType Questionnaire (MCTQ), British Sleep Survey (BSS), Pittsburgh Sleep Quality Index (PSQI), Karolinska Sleep Diary (KSD), and Morningness-Eveningness Questionnaire (MEQ). All sleep-wake timing variables extracted from the MCTQ were averaged between workdays and rest days (see Materials and Methods). Least square means and standard errors by genotype are indicated. Genotypes are indicated in different colors (black = $PER3^{4/4}$, dark red = $PER3^{4/5}$, red = $PER3^{5/5}$). Horizontal bars indicate time in bed. Blue horizontal lines indicate significant contrasts between genotypes (*$p < .05$, **$p < .01$, ***$p < .005$, ****$p < .001$). Bedtime, ($F_{2,673} = 6.01$, $p = .003$), MS ($F_{2,677} = 6.32$, $p = .002$), and wakeup time ($F_{2,673} = 4.01$, $p = .019$) differed between the genotypes. $PER3^{5/5}$ homozygotes went to bed earlier as compared to the $PER3^{4/4}$ homozygotes ($p = .042$) and heterozygotes ($p = .001$). Heterozygotes also went to bed later as compared to the $PER3^{4/4}$ group ($p = .043$). $PER3^{5/5}$ homozygotes woke up earlier compared to heterozygotes ($p = .006$) and had an earlier MS as compared to the $PER3^{4/4}$ homozygotes ($p = .018$) and heterozygotes ($p < .001$).

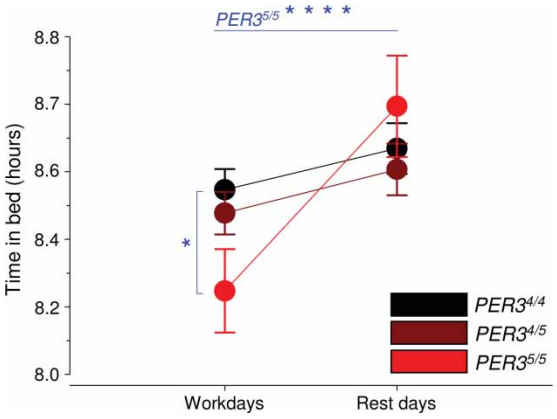

FIGURE 3. Genotype effect on time in bed (TIB) during workdays and rest days as measured by the Munich ChronoType Questionnaire (MCTQ). Least square means and standard errors by genotype and by work versus rest days for TIB. Genotypes are indicated in different colors (black = *PER3*$^{4/4}$, dark red = *PER3*$^{4/5}$, red = *PER3*$^{5/5}$). Blue horizontal/vertical lines indicate significant contrasts between/within genotypes (*$p < .05$, **$p < .01$, ***$p < .005$, ****$p < .001$). There was a significant interaction between genotype and work versus rest days ($F_{2,654} = 3.70$, $p = .025$). During workdays, the *PER3*$^{5/5}$ homozygotes spent on average 18 min less time in bed than the *PER3*$^{4/4}$ homozygotes ($p = .031$). TIB during rest days was 33 min longer in *PER3*$^{5/5}$ homozygotes ($p < .001$), and only 7 min longer in both of the other genotypes as compared to workdays.

TIB, such that the *PER3*$^{5/5}$ group showed a larger "weekend" increase in TIB compared to both the *PER3*$^{4/4}$ and *PER3*$^{4/5}$ groups (Table 3). This was confirmed in a mixed model, in which we included work versus rest day in addition to genotype (Figure 3). The *PER3*$^{5/5}$ group spent on average 18 min less time in bed (TIB) during workdays compared to the *PER3*$^{4/4}$ group ($p = .031$). TIB during rest days increased by 33 min in the *PER3*$^{5/5}$ genotype ($p < .001$) and only by 7 min in both other genotypes (Table 3, Figure 3). When we controlled our analyses for the combined effect of age, sex, and ethnicity, the interaction remained significant for TIB ($F_{2,642} = 3.51$, $p = .031$).

Genotype Effect on Daytime Sleepiness, Sleep Quality, and Insomnia

Daytime sleepiness, as measured by the KSS, was lowest in *PER3*$^{5/5}$ homozygotes (Table 3). The effect of genotype on the likelihood of falling asleep during the daytime (ESS) was not significant. No significant effects of genotype on other sleep quality or insomnia measures were observed (Table 3). Individuals with morning preference reported lower actual ($p < .001$) and general ($p < .007$) daytime sleepiness, better actual ($p < .001$) and general sleep quality ($p < .001$), and lower insomnia score ($p < .001$) as compared to individuals with evening preference (Table 3).

Genotype Effect on Health and Psychological Profile

External eating behavior, as derived from the DEBQ, differed between the genotypes, and this effect persisted after controlling for BMI ($F_{2,661} = 4.53$, $p = .011$) (not shown). The *PER3*$^{4/5}$ individuals were more associated with external eating than the *PER3*$^{5/5}$ individuals (Table 3).

No significant genotype effects were found for other health measures (SF-36, GHQ) or measures of affect (BIS-BAS, PANAS), personality (BIF), intelligence (APM), and verbal reasoning (VRT). However, we found an effect of diurnal preference on many of these measures. Individuals with morning preference reported better general ($p < .001$) and mental health ($p < .001$), higher conscientiousness ($p < .001$), agreeableness ($p < .001$), behavioral inhibition ($p < .017$), restrained eating ($p < .044$), and positive affect ($p < .001$) and lower alcohol consumption ($p < .001$), verbal reasoning ($p < .001$), intelligence ($p < .038$), neuroticism ($p < .001$), external eating ($p < .001$), and negative affect ($p < .001$) as compared to the evening preference group (Table 3, Studied Variables (Effects of Diurnal Preference)).

Effect of Genotype on Associations Between Sleep-Wake Timing During Workdays and Rest Days and BMI

BMI differed marginally between the genotypes ($p = .057$), such that BMI was highest in *PER3*$^{5/5}$ (Table 3). Delayed sleep timing is associated with higher BMI (Baron et al., 2011), and there is increasing evidence that the *PER3* genotype is related to metabolism and obesity/diabetes (Ando et al., 2009; Dallmann & Weaver, 2010). We, therefore, investigated whether the *PER3* VNTR modulates the association of BMI with sleep-wake timing as measured by MS. This analysis revealed a significant interaction between genotype and MS groups for both workdays and rest days (Figure 4A, B). *PER3*$^{5/5}$ homozygotes reporting delayed sleep-wake timing during workdays showed a strikingly higher BMI as compared to both other genotypes within the same MS category (Figure 4A). In the early and intermediate groups, there was no difference. Also during rest days, a more delayed sleep-wake timing was associated with increasing BMI in the *PER3*$^{5/5}$ and decreasing BMI in the other two genotypes (Figure 4B).

Effect of Genotype on Associations Between Time in Bed During Workdays and Rest Days and Fluid Intelligence

Our overall analyses yielded a marginal nonsignificant effect of genotype on both intelligence ($p < .068$) and sleep duration ($p < .053$), as measured by the MQ (Table 3). Because sleep duration has been repeatedly reported to associate with intelligence (Geiger et al., 2010; Gruber et al., 2010), we investigated whether genotype had an effect on the association of sleep duration during either workdays or rest days with fluid intelligence. In this analysis, TIB values for both workdays and rest days were included as a categorical predictor (Figure 5). There was a main effect of genotype ($F_{2,652} = 6.84$, $p = .001$) and an interaction between the two factors, such that those *PER3*$^{5/5}$ homozygotes who reported sleeping >9 h during workdays performed strikingly poorer on the APM as compared to the other

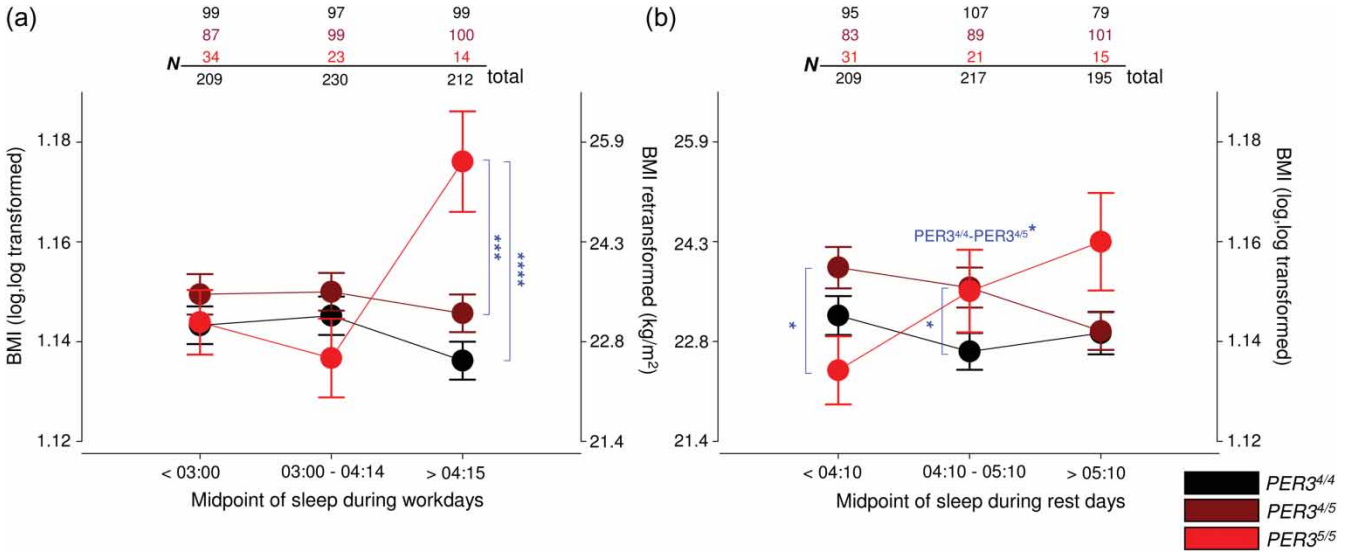

FIGURE 4. The effect of the *PER3* VNTR on association between sleep-wake timing and reported BMI. Sleep-wake timing was estimated by the midpoint of sleep (MS). MS measured on rest day was corrected for sleep debt (see Materials and Methods). MS was included in the model as a categorical predictor. Each of the three MS categories represents approximately one third of the studied populations classified along different cutoffs for workdays (early: <03:00 h, intermediate: 03:00–04:15 h, late: >03:15 h) and rest days (early: <04:10 h, intermediate: 04:10–05:10 h, late: >05:10 h). Genotypes are indicated in colors (black = $PER3^{4/4}$, dark red = $PER3^{4/5}$, red = $PER3^{5/5}$). Blue horizontal and vertical lines indicate significant contrasts between genotypes (*$p < .05$, ***$p < .005$, ****$p < .001$). (A) Least square means and standard errors by genotype and MS groups during workdays. BMI was log-transformed to attain normality and statistics were performed on transformed data. Both transformed (external) and retransformed (internal) y scales are indicated. The association of MS with BMI was different across the genotypes ($F_{4,643} = 3.32$, $p = .01$). $PER3^{5/5}$ homozygotes grouped as late types had a strikingly higher BMI compared to $PER3^{4/4}$ homozygotes ($p < .001$) and heterozygotes ($p = .005$) within the same MS group. (B) Least square means and standard errors by genotype and MS groups during rest days corrected for sleep debt. The effect of MS grouping on rest days was different in the three genotypes ($F_{4,612} = 2.74$, $p = .028$). The BMI was higher in heterozygotes compared to the $PER3^{5/5}$ homozygotes in the early MS group ($p = .01$) and compared to the $PER3^{4/4}$ homozygotes in the intermediate MS group ($p = .018$). The tendency was just the opposite in the late group.

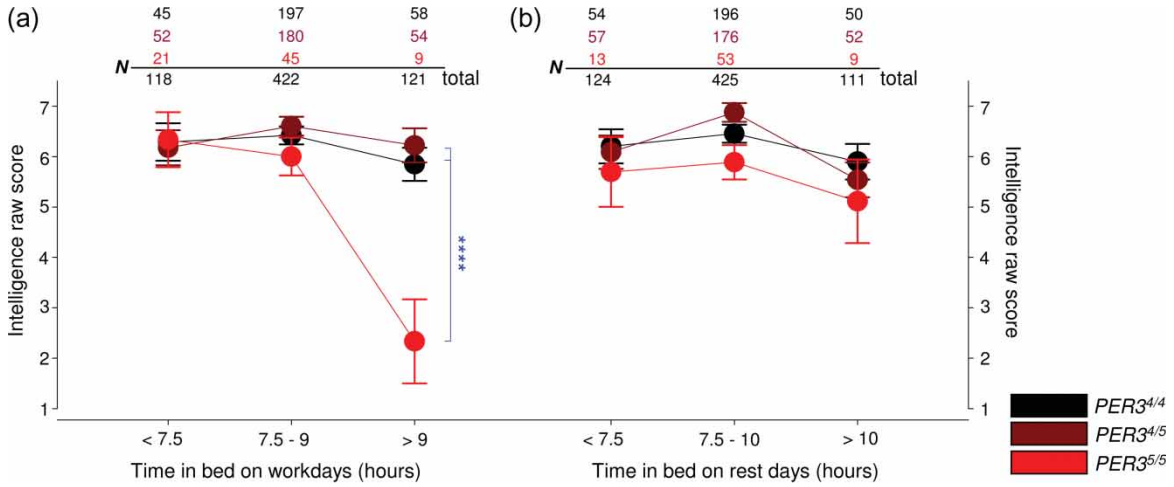

FIGURE 5. The effect of the *PER3* VNTR on association between fluid intelligence and time in bed (TIB) on workdays. Fluid intelligence was measured by the Advanced Progressive Matrices (APM) test. TIB was included as a categorical predictor in the model where the groups were created to show a balanced distribution of the participants in the extreme categories. TIB categories for workdays were: Short sleepers: <7.5 h, intermediate sleepers: 7.5–9 h, long sleepers: >9 h. TIB categories for rest days were: Short sleepers: <7.5 h, intermediate sleepers: 7.5–10 h, long sleepers: >10 h. Genotypes are indicated in colors (black = $PER3^{4/4}$, dark red = $PER3^{4/5}$, red = $PER3^{5/5}$). Blue horizontal and vertical lines indicate significant contrasts between genotypes (****$p < .001$). (A) Least square means and standard errors by genotype and TIB groups during workdays. We found an interaction between genotype and TIB categories during workdays ($F_{4,652} = 3.62$, $p = .006$). The low intelligence score observed in the long sleeper group was due to the $PER3^{5/5}$ individuals, who performed strikingly poorly compared to both of the other genotypes ($p > .001$) from this TIB category. (B) Least square means and standard errors by genotype and TIB groups during rest days. No significant interaction was observed.

genotypes (Figure 5A). Sleep duration during rest days had an effect on intelligence, but neither genotype effect nor interaction was observed (Figure 5B).

DISCUSSION

This is a study in which, based on predictions derived from previous publications, associations between a single gene polymorphism and complex phenotypic characteristics were assessed in a large sample of young, healthy individuals. The prevalence of the three genotypes was similar to a previous report on a UK-based population mixture (Archer et al., 2003). As in some (Archer et al., 2003; Jones et al., 2007; Pereira et al., 2005), but not all (Barclay et al., 2011; Osland et al., 2011), previous studies, the *PER3* VNTR predicted diurnal preference, such that the individuals homozygous for the long allele were more morning type. In contrast to previous studies (Archer et al., 2003; Jones et al., 2007; Pereira et al., 2005), we did not base our analyses only on the extremes of diurnal preference but included all observations. In addition, we found this association using two instruments to ascertain chronotype, i.e., the MEQ and MCTQ, an indication that these associations are robust and not scale specific. Factors that may mask the association between the VNTR and diurnal preference were minimized in our sample. Thus, our current sample consisted of healthy nonsmoking participants who did not report shiftwork, and all fell within a narrow age range. This latter aspect may be important because the association between the VNTR and diurnal preference has been reported to vary with age (Jones et al., 2007). We observed an increase in morningness with age even in this small sample, but did not observe any interaction between genotype and age or a significant effect of sex on diurnal preference, which is at odds with previous studies (Adan & Natale, 2002; Natale et al., 2009). This may be related to the overrepresentation of men in this sample who had expressed interest in participating in a laboratory study. The observed association between *PER3* VNTR genotype and diurnal preference persisted after controlling for other factors known to be connected with diurnal preference, such as conscientiousness (Hogben et al., 2007; Tonetti et al., 2009), affective status (Gaspar-Barba et al., 2009; Kitamura et al., 2010), and intelligence (Killgore & Killgore, 2007; Roberts & Kyllonen, 1999), and was also observed when the analysis was limited to subjects of European descent.

A new aspect of this study was that in addition to self-reported diurnal preference, we assessed the association between genotype and actual reported sleep-wake timing, sleep duration, subjective sleepiness, and sleep quality, as measured by multiple questionnaires and scales. Our results indicate a strong and specific association of the *PER3* VNTR with actual sleep-wake timing and the change in sleep timing and duration from workdays to rest days. The data suggest that the earlier WT in $PER3^{5/5}$ homozygotes during workdays leads to an accumulation of a sleep debt, which is dissipated during the rest days by a considerably longer time spent in bed. Even during rest days, the timing of MS, which is considered the most reliable marker of chronotype (Roenneberg et al., 2004), was earlier in $PER3^{5/5}$ homozygotes. Why $PER3^{5/5}$ individuals wake up earlier and sleep shorter during work days is not clear from the present analyses. One possible explanation is that despite an earlier circadian phase, $PER3^{5/5}$ homozygotes do not go to sleep early because of social pressure, but wake up early in the morning.

For those parameters related to diurnal preference and sleep duration for which the effect of genotype was significant, the heterozygous phenotype was not located intermediately between the two homozygous groups but located close to the $PER3^{4/4}$ genotype. In some cases (e.g., bedtime), the phenotype of $PER3^{4/5}$ was even more extreme than in $PER3^{4/4}$. These findings suggest that the effect of this polymorphism may be overdominant or provide a heterozygous advantage (Gemmell & Slate, 2006). We found alertness, as measured by the KSS, to be significantly different between the genotypes, the $PER^{5/5}$ group exhibiting the highest vigilance as compared to the other two genotypes. Given that alertness on average was assessed around noon, this may very well reflect an increased alertness during the first part of the day as a result of the higher morning preference of the $PER3^{5/5}$ group.

Diurnal preference has been reported to be associated with conscientiousness (Hogben et al., 2007; Tonetti et al., 2009), positive and negative emotional status (Gaspar-Barba et al., 2009; Hasler et al., 2010; Kitamura et al., 2010), and intelligence (Killgore & Killgore, 2007; Roberts & Kyllonen, 1999). We confirmed these associations in our data set and found that diurnal preference is a genotype-independent and strong predictor of most of the studied psychological and health outcome measures. Thus, despite the absence of corrections for multiplicity, we did not find effects of the *PER3* VNTR on the SF-36, General Health Questionnaire, or Big Five Personality questionnaires, or the Behavioral Inhibition System–Behavioral Activation System scales and Positive and Negative Affect Scale. In other words, the polymorphism primarily associates with the sleep timing aspects of diurnal preference and does not affect other psychological and health aspects of diurnal preference. Thus, even though this polymorphism associates with diurnal preference, it clearly does not "re-create" the entire complex phenotype defined by the MEQ. In addition, our data show that diurnal preference is an independent and strong predictor of mental health, but not physical health as measured by the SF-36.

One significant non–sleep-related association with genotype was observed for external eating as assessed by the Dutch Eating Behavior Questionnaire. Whether this observation is linked with the interaction between genotype and the effect of sleep-wake timing characteristics on BMI remains to be established. Late sleep

timing has previously been linked to greater BMI (Baron et al., 2011). In our sample, this association was only observed for the *PER3*$^{5/5}$ homozygotes. We also observed that genotype had an effect on fluid intelligence, such that fluid intelligence scores for *PER3*$^{5/5}$ homozygotes who reported a long sleep duration during workdays were strikingly less intelligent than carriers of the other two genotypes. Longer sleep duration has previously been associated with lower intelligence in children (Geiger et al., 2010). No published studies show such an association in adults. However, in adults there are additional reports of an association between intelligence and diurnal preference. Evening types are reported to be more intelligent (Killgore & Killgore, 2007; Roberts & Kyllonen 1999), and they have also been shown to sleep less (Kitamura et al., 2010). Whether or not the observed interaction with genotype is also mediated by socioeconomic factors remains to be established.

Some limitations of the study should be considered. The study population included more males than females. This was due to a differential response during the laboratory study recruitment phase, which generally attracts more men. Although sex was not significantly different between the genotypes, we controlled for the effect of sex in some of our analyses and the effects of genotype persisted (e.g., diurnal preference). However, there are some limitations in the way these results can be generalized to females. Another limitation is that the study population included several ethnicities across which the distribution of the genotypes differed. The *PER3* polymorphism has variable frequency across different ethnic groups (Ciarleglio et al., 2008; Nadkarni et al., 2005). Although we recognize that a multi-ethnic sample may be considered problematic, we believe that the mixture of ethnicities sampled here is a good representation of the general public and useful for the identification of genetic associations that are meaningful in a real-life context. As has been pointed out previously (Cardon & Palmer, 2003), population stratification may not necessarily be inappropriate for this type of association study. Nevertheless, we have also restricted many of our analyses to subjects of white European heritage only and find that many genotype associations persist. In addition, when we correct for sex, age, and ethnicity, all the main genotype effects remained significant. In most cases in which the associations lose their statistical significance, nonsignificant tendency persists. For example, in the case of the self-assessment question from the MCTQ, the p value changes from $p = .024$ to $p = .055$. Further analyses with other ethnic groups were not possible due to small sample sizes. Future studies should also investigate the phenotypic effects of the single-nucleotide polymorphisms (SNPs) identified in the promoter region of the *PER3* gene (Archer et al., 2010).

The restricted age range of the participants in this sample is a strong point and a limitation as well. It is a strong point because it helps to eliminate many confounding factors that are associated with age, but it is a limitation because it prevents extrapolating our conclusions to the older population. We opted for a narrow age range, above all because we have previously reported that the association between diurnal preference and the *PER3* VNTR changes with aging (Jones et al., 2007). A limitation of many studies is that no distinction is made between rest and workdays and that no information on the use of alarm clocks is present. In analyses aiming at clarifying genetic determinants of a particular phenotype, such as sleep-wake timing, social activities and the use of alarm clocks may represent a confounding factor. However, the assessment of the contribution of genetics to any phenotype always takes place in the context of an environment, and all we can do is assess the contribution of a gene to the genetics-environment interaction. Social activities and alarm clocks are part of the "workday" environment; the other environment is "rest days," and the current data provide evidence for a contribution of this allelic variation to sleep-wake phenomenology in both environments. Although, in our current study, we analyzed work and rest days separately, we did not perform any additional analysis on the use of alarm clocks during either work or rest days.

Data on the use of alarm clocks during rest days were not available and sleep on rest days may, therefore, in theory still be influenced by this and other social factors. This could be a confounding factor for the assessment of chronotype based on the midpoint of sleep during rest days and corrected for the accumulated sleep debt during workdays. Such an analysis could have yielded additional findings, in particular, because we previously reported that *PER3*$^{5/5}$ homozygotes were less dependent on an alarm clock for waking up (Ellis et al., 2009). Despite these potential confounding factors, we observe associations with genotype and large differences between work and rest days. Further, due to the large number of variables, the present study could have benefited from data-reduction methods, such as factor analyses. However, these analyses were far beyond the purpose of the present paper, and they were not necessary for the conclusions drawn.

The present study confirms previous results with respect to the effect of *PER3* VNTR on diurnal preference and reports new effects of the polymorphism on sleep-wake timing, sleep duration, plus subjective sleep quality and sleepiness. The genotype effects appear specific to sleep-wake timing whereas Diurnal Preference as derived from the MEQ also affects many psychological and health measures. In addition, the data suggest that this polymorphism interacts with the effects of sleep timing and sleep duration on BMI and intelligence. This may be a first step towards understanding the factors that mediate and modulate the effects of sleep characteristics in relevant health outcome measures.

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SUPPLEMENT. SHORT DESCRIPTION OF THE QUESTIONNAIRES USED IN THE STUDY:

Medical Questionnaire (MQ). The MQ is a 25-item questionnaire administered according to the Standard Operating Procedures of the Surrey Clinical Research Centre. It includes questions about general demographics (age, sex, weight, and height), health-related information (past and current medical conditions, medications), habitual sleep duration and shiftwork.

British Sleep Survey (BSS). The BSS was developed to provide representative data on British adults. Respondents are asked what their average sleep in bed was per day in the preceding week, what their average non-bed sleep was per day, and which (if any) days during the preceding week differed from this average, and what the estimates are for each of those days. Similar questions are asked with regard to times of retiring and arising. There are also questions related to demographics, including ethnicity (Groeger et al., 2004).

Morningness Eveningness Questionnaires (MEQ). The MEQ represents a widely used tool for assessing preferred timing of wake activities and sleep across 19 questions and provides a quantitative measure (total score) of diurnal preference that ranges from 16 to 86. Higher values indicate increased morning preference (Horne & Östberg, 1976).

Munich ChronoType Questionnaire (MCTQ). The MCTQ asks about sleep habits in the present circumstances and addresses sleep-wake timing separately for workdays and rest days. The self-assessment question assesses chronotype on a scale ranging from 0 (extreme early type) to 6 (extreme late type) (Roenneberg et al., 2003). For all variables extracted from the MCTQ, we also computed the difference between workdays and rest days (rest days – workdays) and the average between workdays and rest days.

Pittsburgh Sleep Quality Index (PSQI). The PSQI is a 24-item questionnaire that generates seven component scores (sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction). The addition of these seven components yields a global score of subjective sleep quality ranging from 0 to 21, where a higher score is indicative of poorer subjective sleep quality. Here, we only report the global score. The questions refer to the last month (Buysse et al., 1989).

Karolinska Sleep Diary (KSD). The KSD is a 10-item questionnaire assessing the last night sleep-wake timing and sleep quality. Sleep quality is assessed on a scale ranging from 1 (best ever) to 9 (worst ever) (Åkerstedt et al., 1994).

Insomnia Severity Index (ISI). The ISI is a 7-item questionnaire assessing dissatisfaction with sleep during the last 2 wks, with a maximum of 28 points. A higher score indicates more severe insomnia (Bastien et al., 2001).

Epworth Sleepiness Scale (ESS). The EES evaluates the chance of dozing-off under typical day-to-day situations in recent times, with a score ranging from 0 (minimal sleepiness) to 24 (maximum sleepiness) (Johns, 1991).

Karolinska Sleepiness Scale (KSS). The KSS assesses sleepiness during the previous 5 min on a 1 (extremely alert) to 9 (extremely sleepy) scale (Gillberg et al., 1994).

Raven's Advanced Progressive Matrices (APM). It provides a nonverbal assessment of general fluid intelligence for performances above average. The test has 12 items and the score ranges from 0 to 12. A higher score indicates better performance. The time limit for the completion of the task was 10 min (Raven, 1978).

Verbal Reasoning Task (VRT). In the VRT participants must decide whether the order in which letters are shown matches the order specified in an accompanying sentence (e.g., X-F: F is not before X). The test has 64 items, and the score ranges from 0 to 64. A higher score indicates better performance. The time limit for the completion of the task was 3 min (Baddeley, 1968).

SF-36 Health Survey (SF-36v2). The SF-36 contains eight subscales assessing physical functioning, role physical, role emotion, vitality, mental health, social functioning, body pain, and general health in

general and during the last 4 wks. For each subscale, scores are summed and transformed to generate a score ranging from a 0 to 100, with higher scores indicating better health-related quality of life. Finally, all dimensions were grouped into two summary or global dimensions: the physical health component and the mental health component. The computation of the aggregate scores consists of several steps. In the first step, a standardized (*Z*-score) is computed by subtracting the mean 0–100 general US population for each SF-36 scale and dividing the difference by the corresponding scale standard deviation. After the *Z*-score has been computed for each SF-36V2 scale, in the second step the aggregate score is computed by multiplying each SF-36 scale *Z*-score by its respective mental factor score coefficient and summing the eight products. In the third step, each component score was transformed to the norm-based (50, 10) scoring by multiplying each aggregate component scale score by 10 and adding the resulting product to 50. A higher score indicates better health based on the findings of the SF-36v2 Health Survey (1992–2002 by Health Assessment Lab, Medical Outcomes Trust, Hanover, Germany, and QualityMetric Incorporated, Lincoln, NE, USA) (Ware & Sherbourne, 1992).

General Health Questionnaire (GHQ). The GHQ is a measure of current mental health consisting of 12 items assessing whether the participant has experienced a specific symptom or behavior during the last 4 wks. Each item is rated on a 4-point scale. The total score ranges between 0 and 36 (i.e., the lower the score the healthier the person) (Goldberg, 1978).

Dutch Eating Behavior Questionnaire (DEBQ). The DEBQ is a widely used scale consisting of 33 items measuring three different psychologically based eating behaviors: (i) emotional eating (food intake as a response to an emotional factor); (ii) external eating (eating in response to food-related stimuli, regardless of the internal states of hunger and satiety); and (iii) restrained eating (effort to refrain from eating). A higher score indicates stronger presence of a certain eating behavior (Vanstrien et al., 1986).

Big Five Inventory (BIF). This instrument is based on the five-factor model of personality and measures openness, extraversion, agreeableness, conscientiousness, and neuroticism. Openness refers to propensity to novelty, tolerance of different values, and interest toward different habits and lifestyles. Extraversion refers to aspects such as activity, assertiveness, and self-confidence. Agreeableness refers to concern and sensitiveness toward others and their needs. Conscientiousness refers to self-regulation in proactive and inhibitory mode. Neuroticism refers to the inability to cope adequately with one's own anxiety and emotionality and to control irritation and anger. A higher score indicates the stronger presence of a certain personality dimension (Goldberg, 1990; McCrae & Costa, 1987).

Behavior Inhibition System–Behavior Activation System (BIS-BAS). The questionnaire is based on the theory that a behavioral approach/activation system (BAS) regulates appetitive motives, which drive approach to something desired. A behavioral inhibition system (BIS) is believed to govern aversive motives, which drive removal from something unpleasant. The BIS-BAS, which consists of 24 items, measures individual differences in the sensitivity of these systems. The characterization is provided along four factors: BAS Drive, BAS Fun Seeking, BAS Reward Responsiveness, and BIS. The BIS comprises 7 items about anticipation of punishment. The BAS Reward Responsiveness (RR) has 5 items about anticipation or occurrence of reward. The BAS Drive has 4 items about pursuit of desired goals. The BAS fun seeking has 4 items about desire for new rewards and impulsive approach to potential rewards. Higher values indicate stronger presence of a certain motive (Carver, 1994).

Positive Affect Negative Affect Scale (PANAS). The PANAS is a reliable 20-item self-report measure of dispositional positive and negative affect experienced during the last few weeks. The positive affect (PA) score reflects positive engagement with the environment, whereas the negative affect (NA) score reflects negative engagement with the environment. The lower ends of each dimension suggest the absence of those affective activations (Watson et al., 1988).