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Common variants in *CLOCK* are not associated with measures of sleep duration in people of European ancestry from the Sleep Heart Health Study

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In humans, sleep is primarily regulated by two processes: the circadian process and the homeostatic process of sleep (1; 2). Although much is known about the molecular processes driving the circadian clock, the molecular components of human sleep duration remain elusive. Sleep duration has a genetic component, with heritability estimated at 17–34% (3–11). To date a variant of *PER3* shown to affect diurnal preference reportedly associates with decreased REM and slow-wave sleep (12), a familial mutation in *BHLHE41* (formerly *DEC2)* decreases sleep duration (13), and a region near *MYRIP* may be associated with sleep duration based on a genome-wide association study (5).

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Disclosures

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A recent study by Allebrandt et al. reported a nominal association between two uncorrelated, common *CLOCK* genetic variants and sleep duration in two independent European populations from South Tyrol (n=283) and Estonia (n=1,011) (14). The aim of our study was to replicate these *CLOCK* associations in large samples of European ancestry and to further assess associations using objective data on total sleep time and sleep stage distributions from polysomnography.

Subjects were participants of European ancestry from the Sleep Heart Health Study (SHHS), a prospective study designed to assess the impact of sleep disorders on cardiovascular disease, described in detail elsewhere (7; 15; 16). We used phenotype data from baseline visits of the Atherosclerosis Risk in Communities Study (ARIC) (n=1,812), Framingham Heart Study (FHS) (n=2,221), and Cardiovascular Health Study (CHS) (n=954) parent cohorts. Participants ranged in age from 29–100 years, with mean BMI of 28.82±5.05 kg/m^2 (ARIC), 27.76±5.09 kg/m^2 (FHS) and 27.45±4.58 kg/m^2 (CHS). Daily sleep duration was calculated as the time difference between bedtime and wake time and average sleep duration was calculated as follows: [(Weekday sleep duration * 5) + (Weekend sleep duration * 2)]/7. Individuals with sleep duration less than 3 hours or greater than 14 hours, or shift workers (with bedtime between 4 a.m. and 6 p.m) were excluded from analysis. Mean sleep duration was 7.43±0.98 hours (ARIC), 7.46±1.01 hours (FHS), and 7.7±1.35 hours (CHS). To replicate the findings from Allebrandt et al. (14), we created an age and gender normalized sleep duration variable in SHHS ARIC and SHHS FHS, excluding CHS due to the advanced age of the population. Average sleep duration for each individual was standardized to a "neutral 55 years old" by determining the fitted sleep duration value for a 55 year old and subtracting the residual from the polynomial fit for each specific observation. The mean normalized sleep duration in SHHS ARIC and SHHS FHS was 7.22 and 7.20 hours respectively. Finally, we used sleep duration data from polysomnography conducted in 6,641 participants during a one-night at-home session (17). Percentage of sleep time in each stage was calculated by dividing time scored in a sleep stage divided by the total sleep time.

CARe IBC array genotype data was used to identify *CLOCK* SNPs for replication and to verify self-reported European ancestry (18; 19). To control for relatedness, estimates of pairwise identity-by-descent (IBD) were calculated, and individuals with values >0.125 were pruned from the sample. The *CLOCK* SNP rs11932595 was directly genotyped on the IBC array, and a proxy SNP (rs6843722) was used for *CLOCK* SNP rs12649507 (r-squared=0.965, D′=1 in 1000 Genomes CEU). Both SNPs passed quality control in all three SHHS cohorts (>99% genotyping call rates and Hardy-Weinberg disequilibrium p-value> 0.05). Linear regression analysis was performed in PLINK with normalized sleep duration treated as a continuous variable and raw sleep duration adjusted for age, gender, BMI, and sleep apnea diagnosis (20). BMI was log transformed for analysis. A fixed effects, inverse-variance meta-analysis was performed in METAL (21).

First, we tested both *CLOCK* SNPs for association with normalized sleep duration in our population, but failed to replicate the association (rs11932595, FHS p=0.421, ARIC p=0.649; rs6843722, FHS p=0.712, ARIC p=0.208) (Table 1). In meta-analysis of results from the SHHS ARIC and SHHS FHS studies, we did not observe a significant association between *CLOCK* SNPs and normalized sleep duration. Finally, in a meta-analysis

combining the previous *CLOCK* findings from the Allebrandt et al. study with our current study, again we did not detect a significant association between *CLOCK* variants and normalized sleep duration (rs11932595 p=0.596, rs6843722 p=0.530). In addition, pooled analysis across SHHS cohorts adjusting for cohort did not replicate the association (data not shown).

Second, we performed multiple regression analysis, testing for an association between *CLOCK* variants and raw sleep duration adjusting for age, gender, BMI, sleep apnea diagnosis, and 10 principal components of ancestry. There was no significant relationship between raw sleep duration and variation in *CLOCK (*rs11932595, FHS p=0.1442, ARIC p=0.128, CHS p=0.2621; rs6843722, FHS p=0.202, ARIC p=0.63, CHS p=0.3908) (Table 1). We also performed regression analysis in the component of FHS aged 50 years or younger (N=443). *CLOCK* variants are not significantly associated with raw sleep duration in this subgroup (*P*>0.5; data not shown).

Lastly, we tested for an association between *CLOCK* variants and polysomnographic indices (available for n=4,251). We found no significant association between *CLOCK* SNPs and percent of sleep time spent in stages N1, N2, N3, or REM sleep (data not shown). There was a statistical trend for a nominal association between the *CLOCK* SNP rs11932595 G allele and total polysomnographic sleep time (p=0.067, Beta= −6 minutes, N=509), as well as total time in bed (p=0.055, Beta= −6 minutes, N=569), but this effect was in the opposite direction from that in the discovery cohort in Allebrandt et al. (11).

To date, only a handful of human genetic variants show an association with sleep duration (5; 12; 13; 22) and no associations have been convincingly reproduced. In our study, we find no evidence of an association between *CLOCK* variants and sleep duration in three independent cohorts, despite a sample size three times larger than the previously reported association and >99% power to detect an effect of similar magnitude as previously reported (23). Our analysis does not support the previously reported association of *CLOCK* variants with self-reported sleep duration, nor identifies associations with sleep stage distributions from single night overnight polysomnography.

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Table 1

CLOCK variant association with sleep duration in the Sleep Heart Health Study

		rs11932595						rs12649507*					
		Effect Allele	**MAF**	**N**	**Effect (hours)**	**StdErr (hours)**	**P**	**Effect Allele**	**MAF**	**N**	**Effect (hours)**	**StdErr (hours)**	**P**
Normalized Sleep Duration	SHHS FHS	G	0.415	2017	− 0.025	0.031	0.421	A	0.312	2016	0.012	0.034	0.712
	SHHS ARIC	G	0.396	1596	0.011	0.025	0.649	A	0.309	1597	0.033	0.026	0.208
	Allebrandt Discovery (14)	G	-	275	0.22	-	0.008	A	-	282	− 0.251	-	0.005
	Allebrandt Replication (14)	G	-	1011	− 0.172	0.086	0.047	A	-	1011	− 0.194	0.097	0.045
	META SHHS†	G	0.407	3613	− 0.003	0.019	0.88	A	0.311	3613	0.025	0.021	0.222
	META SHHS + Allebrandt††	G	-	4899	-	-	0.596	A	-	4906	-	-	0.53
Raw Sleep Duration**	SHHS FHS	G	0.415	1876	− 0.051	0.035	0.144	A	0.312	1875	0.048	0.038	0.202
	SHHS ARIC	G	0.396	1577	0.059	0.039	0.128	A	0.309	1578	0.019	0.04	0.63
	SHHS CHS	G	0.404	798	0.073	0.065	0.262	A	0.316	798	−0.06	0.07	0.391
	META SHHS†	G	0.406	4251	0.008	0.024	0.733	A	0.311	4251	0.022	0.026	0.39
PSG total sleep time**	SHHS FHS	G	0.384	509	− 0.109	0.06	0.067	A	0.325	509	0.047	0.068	0.488
PSG total time in bed**	SHHS FHS	G	0.384	569	− 0.099	0.052	0.055	A	0.325	569	0.107	0.058	0.068

*rs6843722 used as a proxy for *CLOCK* SNP rs12649507 in the SHHS FHS, ARIC, and CHS samples (r^2=0.965, D′=1 in 1000 Genomes CEU).

†Indicates inverse variance weighted Meta-analysis.

††Indicates sample size weighted Meta-analysis.

**Analysis adjusted for age, gender, BMI, sleep apnea diagnosis, and 10 principal components of ancestry.