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Circadian Clock Gene Polymorphisms and Sleep/Wake Disturbance in Alzheimer's Disease

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Abstract

Objectives—One of the hypothesized causes of the breakdown in sleep/wake consolidation often occurring in individuals with Alzheimer's disease (AD) is dysfunction of the circadian clock. The goal of this study is to report indices of sleep/wake function collected from individuals with AD in relation to relevant polymorphisms in circadian clock-related genes.

Design—One week of *ad libitum* ambulatory sleep data collection.

Setting—At-home collection of sleep data and in-laboratory questionnaire.

Participants—Two cohorts of AD participants. *Cohort 1* (n=124): individuals with probable AD recruited from the Stanford/Veterans Affairs NIA Alzheimer's Disease Core Center (n=81) and the Memory Disorders Clinic at the University of Nice School of Medicine (n=43). *Cohort 2* (n=176): individuals with probable AD derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) data set.

Measurements—Determination of sleep/wake state was obtained by wrist actigraphy data for seven days in Cohort 1 and by the Neuropsychiatric Inventory (NPI-Q) for Cohort 2. Both cohorts were genotyped using an Illumina Beadstation and 122 circadian-related SNPs were examined. In Cohort 1, an additional polymorphism (variable number tandem repeat in *per3*) was also determined.

Results—Adjusting for multiple tests, none of the candidate gene SNPs were significantly associated with the amount of wake after sleep onset (WASO), a marker of sleep consolidation. Although the study was powered sufficiently to identify moderate-sized correlations, we found no relationships likely to be of clinical relevance.

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*Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI provided data but did not participate in analysis or writing of this report. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations (see www.adni-info.org.)

Conclusions—It is unlikely that a relationship with a *clinically* meaningful correlation exists between the circadian rhythm-associated SNPs and WASO in individuals with AD.

Keywords

Alzheimer's Disease; Sleep/wake Disturbances; Circadian Rhythm

OBJECTIVE

Nocturnal wakefulness and daytime napping often characterize the sleep/wake disturbance frequently associated with Alzheimer's disease (AD). Previously, we longitudinally followed sleep/wake disturbances in AD participants and found significant nocturnal wake after initial sleep onset (WASO) (1). We also found that sleep/wake deterioration in individuals with AD behaved as a "trait", i.e., decline was consistently manifested in some individuals while others never manifested such decline over their illness course (2). This led us to search for genetic variations to explain such "traits". One hypothesized cause of this breakdown in sleep/wake consolidation is degeneration of the circadian clock (3). A limited number of genes likely underlie the core circadian oscillation found in neurons of the suprachiasmatic nucleus (SCN), the location of the circadian clock in mammals (4). Variation within the small number of circadian genes could result in several relevant phenotypes becoming more prominent as compensatory mechanisms are lost during the course of AD. The potential impact of human circadian genetic variation is suggested by recent studies showing that variation in the human *CLOCK* and *per3* genes may be associated with differences in sleep/wake function (5,6). *CLOCK* and *per3* proteins interact with other gene products in the basic circadian system (*per1*, *per2*, *bmal-1*, *csnkle*, *cry1* and *cry2*).

Using actigraphy it is feasible to collect sleep/wake phenotype data in large numbers of AD participants and to analyze their genotype data on candidate gene single nucleotide polymorphisms (SNPs) using gene chip technology. This study reports actigraphy sleep/wake data collected from AD participants in relation to their circadian candidate gene polymorphisms.

METHODS

Participants

The analyses in this report were performed on two cohorts of AD participants.

Cohort 1—Inclusion/exclusion criteria required a diagnosis of probable AD by NINCDS-ADRDA criteria (7) based on relevant neurological, medical, neuroimaging, and neuropsychological assessments. Participants were excluded if they had active major medical conditions that would have precluded collection of actigraphy data. The 124 participants in this cohort are from two sources: 1) 81 participants in an ongoing longitudinal study of AD at the Stanford/Veterans Affairs NIA Alzheimer's Disease Core Center (ADCC), and 2) 43 French participants from the Memory Disorders Clinic at the University of Nice School of Medicine that is collaborating with our group on AD sleep studies. For this analysis, only Caucasians were selected from both sources and participants in Cohort 1, as a whole, were 46% male. At the time of actigraphy, the average age of the 124 participants in Cohort 1 was 75.2 years (SD = 8.1; Range = 45 to 88) and their mean MMSE (8) was 19.8 (SD = 4.8; Range = 4 to 29).

Cohort 2—Data were also obtained from a second cohort of 176 Caucasian participants in the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI) with a diagnosis of probable AD by NINCDS-ADRDA criteria.

The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a \$60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer’s disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of the ADNI is Michael W. Weiner, M.D., VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and participants have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research.

The ADNI participants were studied using the same Illumina gene chip technology as that used for Cohort 1, described above. They did not receive actigraphy but received the Neuropsychiatric Inventory (NPI-Q) (9), which includes sleep and other behavioral ratings. The major reason for including the ADNI/NPI cohort was to provide additional data regarding the possibility that facets of behavior being described by the NPI-Q might be in any way affected by SNPs in the same manner as the sleep data from Cohort 1. Thus since both cohorts were measured on the same SNPs, we felt it worthwhile to provide all available data on behavioral correlates of these SNPs in one analysis.

Only Caucasians were selected from the ADNI database, the cohort was 55% male and the average age was 75.6 years (SD = 7.6; Range = 55 to 91). The mean MMSE (8) for the 176 Cohort 2 participants at the time the NPI data were collected was 23.3 (SD = 2.0; Range = 18 to 27).

The Institutional Review Board for Human Subjects Research at each site in the current analysis approved their respective research protocols. Written informed consent was obtained from each participant or legally authorized representative.

Measures

Actigraphy—Rest/activity data were collected for Cohort 1 by means of a wrist-worn, watch-size ambulatory motion-detecting device, the actigraph (Ambulatory Monitoring Systems, Inc., Ardsley, NY 10502). Participants were asked to wear an actigraph 24 hours a day on their non-dominant wrists for 7 consecutive days and were instructed to remove the device only for bathing or swimming. The actigraph was set to record motion in 30-second epochs.

Measures of nighttime sleep/wake behavior, in particular wake after sleep onset (WASO), were obtained using the computer scoring program supplied by the manufacturer (ACTION software version 1.3). The ACTION software scores the actigraph recordings following entry of participants’ evening bed times and final morning out-of-bed times derived from daily sleep logs completed by the caregiver. The scores used in the data analyses were averages of the consecutive days of actigraphy; night-to-night variability was not examined

in this paper. Not all data were usable due to occasional technical failures of the device, and the amount of actigraph data collected varied across participants depending on their compliance, but 84% of the participants had at least 4 or more days of data per recording session. The mean WASO for the 124 participants in Cohort 1 was 85 minutes (SD = 64; range = 5 to 414). The staff in Nice, France were trained in the use of actigraphy at Stanford and used the same device. Further, all data collected in Nice were double-checked by U.S. staff and both groups used the same diagnostic criteria for AD. An advantage of the actigraph as an outcome measure is that it is not language dependent.

Neuropsychiatric Inventory (NPI-Q)—The NPI-Q is a brief, informant-completed questionnaire assessing the participant’s neuropsychiatric symptoms and the caregiver’s level of associated distress. The NPI-Q is designed to assess dementia patients’ behaviors (delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/indifference, disinhibition, irritability/lability, motor disturbance, nighttime behaviors and appetite/eating) regarding their severity and distress to the caregiver during the past month. The item relating to sleep/wake disturbance in nighttime behaviors on the NPI-Q was analyzed for the 176 AD participants in Cohort 2: “Does the patient awaken you during the night, rise too early in the morning, or take excessive naps during the day? (Item K)” On this item, 74% were scored as ‘not a problem’, 17% were ‘mild’, 7% ‘moderate’ and 2% ‘severe’ (see Table 1, which shows the association tests between gene SNPs and NPI severity items). None of the measures used required translation (the NPI-Q reported here was only administered to the U.S.-based ADNI cohort).

Genotyping Procedures

Genomic DNA extraction from frozen EDTA-containing whole blood or saliva samples was conducted as previously described (10). Genotyping was performed by an Illumina Beadstation (Illumina, San Diego CA) using the manufacturer-provided procedures. We utilized the Human 610 Quad gene chip that genotypes approximately 658,000 SNPs and has the same coverage as that used in ADNI. SNPs on all relevant circadian candidate genes were genotyped for both cohorts.

Additional Genotyping Procedures

The variable number tandem repeat (VNTR) polymorphism in *per3*, where a 54-nucleotide coding region motif is repeated in 4 or 5 units, has been linked with multiple sleep phenotypic parameters (6). This VNTR polymorphism could not be determined using Illumina technology so a separate procedure was performed in which the target hPer3 gene fragment was amplified using sense primer (5'-CAAATTTTATGACTACTACCAGAATGGCTGAC-3') and reverse (5'-AACCTTGACTTCCACATCAGTGCCTGG-3') primers. The PCR reaction was carried out in a final volume of 15 µL consisting of 30 ng of genomic DNA, 50 ng each of sense and antisense primers, 7.5 µL of Taq PCR Master mix (Qiagen #201445) and 10% DMSO. The PCR conditions included an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 1 minute and extension at 72°C for 1 minute and 45 seconds, with a final extension of 8 minutes at 72°C. The 4-repeat polymorphism with a size of 581 bp and the 5-repeat with a size of 635 bp were detected by Western blot.

Analytic Procedures

The analytic approach proceeded along the following steps. We first identified the SNPs available for analysis from the eight circadian candidate genes (*CLOCK*, *per1*, *per2*, *per3*, *bmal-1*, *csnk1e*, *cry1* and *cry2*) using a database linking SNPs on the Illumina Human 610

Quad gene chip with parent genes. There were 136 SNPs from the candidate circadian genes on the Illumina chip. We then performed quality control using Illumina's BeadStudio Software (Version 3.1, San Diego CA) and visually examined each SNP to validate the called genotype clusters. We discovered one SNP with problematic genotyping because one signal channel was not performing adequately (rs1441351) and excluded this SNP from further analysis, leaving 135 SNPs. Next, using Golden Helix SNP and Variation Suite (SVS Version 7.2.2, Bozeman MT) software, SNPs were filtered to exclude failed SNPs according to one or more of the following criteria: call rate < 0.95; minor allele frequency < 5%; Fisher's Exact Test for HWE $P < 0.001$. This filtering reduced the number of candidate gene SNPs to 122. Finally, we used the SVS software genotype association test with the following options: 1) Additive model testing, 2) Correlation/Trend test, 3) Drop missing values, 4) Full scan permutation with 10,000 permutations (a correction for multiple testing), and 5) Correcting batch effects/stratification with Principal Components Analysis (PCA).

RESULTS

Table 2 shows the association testing between the 122 candidate gene SNPs and the relevant sleep/wake phenotype variable, WASO. None of the candidate gene SNPs or the VNTR polymorphism was significantly associated with WASO score after full scan permutation. The full scan permutation is a permutation test over the distribution of minima for the p-values calculated in the full genome-wide scan for association provided by the Golden Helix Software. "Full scan", as defined by Golden Helix, means that the software determines the minimal p-value over the entire set of p-values for the study, randomizing the phenotype with each re-sampling iteration (11).

The VNTR polymorphism appeared to be associated with one of the PER3 SNPs in our sample (rs228729, see Table 3). This is a different SNP from that previously found (rs2640909) to be associated with the PER3 VNTR in a Japanese sample (12). These two SNPs are not well associated in Caucasian ($r^2=0.117$) or Japanese individuals ($r^2=0.014$) (SNP Annotation and Proxy Search, www.broadinstitute.org/mpg/snap/ldsearch.php, based on HapMap release 22).

A similar analysis was also negative using the same SNPs in the 176 cases from the ADNI dataset and their NPI measure of nighttime behavioral disturbance. Parenthetically, we found that there were no full-scan permutation-corrected significant correlations between the selected SNPs and any of the NPI severity measures (see Table 1, which shows the association tests between gene SNPs and NPI severity items).

CONCLUSIONS

The analysis conducted with 124 participants and 122 SNPs has over 80% power to detect a two-tailed correlation of 0.38 for any one of the 122 SNPs with WASO scores. Thus we conclude that it is unlikely that a clinically relevant relationship (one with a "medium" effect size) (13) exists between these circadian rhythm-associated SNPs and WASO, our primary measure of nocturnal sleep disturbance, in individuals with AD, although this is based on a possible 20% false negative rate. It is possible that there may be an association between these SNPs and sleep pathologies commonly found in this population (e.g., sleep disordered breathing and restless legs syndrome), though this remains for future study. These results do not rule out the possibility that smaller correlations exist; however, correlations smaller than 0.38 would explain less than 14% of the variance of the clinical phenomena, hence may not be clinically relevant. The data in Table 2 suggest the possibility that relationships of a smaller magnitude might exist with some SNPs on *cry1* or *per3*; however, none of these correlations at the < 0.30 level were significant after the full scan permutation correction.

Such smaller relationships may still be of theoretical interest to those examining basic physiological relations of genetic measures and sleep/wake phenomena, even if they may not be clinically relevant. Exploratory analyses were also performed using the ADNI dataset and scores on other NPI items. In these analyses no SNPs were significantly associated with any of the other NPI measures. We feel it would be premature to draw any conclusions from these results other than to suggest the need for further examination of these relationships in other datasets to determine if they are replicable.

In summary, we examined genetic sources of variability in two independent samples of AD patients with different but complementary measures of sleep/wake disturbances. We did not find relationships that are likely to be of clinical relevance, even though the study was powered sufficiently to identify correlations of a moderate size. We conclude that sources of variation of neuropsychiatric symptoms in AD do not lie in simple relationships to specific SNPs associated with circadian rhythms but possibly depend upon other physiological mechanisms or interactions among a number of genetic markers.

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Table 1

Table of Association Tests Between 122 Candidate Gene SNPs and NPI Severity Items*

NPI Item	Item	SNP	Correlation/Trend r	Full Scan Permutation p-value
Item A Severity	Does {P} believe that others are stealing from him/her or planning to harm him/her in some way?	rs228654	-0.15	0.976
Item B Severity	Does {P} act as if he/she hear voices? Does he/she talk to people who are not there?	rs10832005	0.23	0.247
Item C Severity	Is {P} stubborn and resistive to help from others?	rs10741613	-0.16	0.910
Item D Severity	Does {P} act as if he/she is sad or in low spirits? Does he/she cry?	rs228654	0.21	0.325
Item E Severity	Does {P} become upset when separated from you? Does he/she have any other signs of nervousness such as shortness of breath, sighing being unable to relax or feeling excessively tense?	rs11932595	0.23	0.213
Item F Severity	Does {P} appear to feel too good or act excessively happy?	rs228729	-0.24	0.156
Item G Severity	Does {P} seem less interested in his/her usual activities and in the activities and plans of others?	rs10778528	0.22	0.252
Item H Severity	Does {P} seem to act impulsively? For example does {P} talk to strangers as if he/she knows them or does {P} say things that may hurt people's feelings?	rs10778528	-0.17	0.861
Item I Severity	Is {P} impatient or cranky? Does he/she have difficulty coping with delays or waiting for planned activities?	rs2075984	-0.20	0.876
Item J Severity	Does {P} engage in repetitive activities such as pacing around the house handling buttons, wrapping strings, or doing other things repeatedly?	rs135757	0.06	1.000
Item K Severity	Does {P} awaken you during the night rise too early in the morning or take excessive naps during the day?	rs10766077	-0.20	0.4933
Item L Severity	Does {P} awaken you during the night rise too early in the morning or take excessive naps during the day?	rs10832005	0.16	0.912

* Scoring as follows: [0=This behavior was not noted to be a problem; 1=Mild (noticeable, but not a significant change); 2=Moderate (significant but not a dramatic change); 3=Severe (very marked or prominent, a dramatic change)].

Table 2

Table of Association Tests Between 122 Candidate Gene SNPs and WASO

SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend p-value*	Correlation/Trend r	Full Scan Permutation p-value
1	12	CRY1	flanking_5UT	0.006	0.286	0.269
2	12	CRY1	intron	0.008	-0.279	0.323
3	12	CRY1	flanking_5UT	0.017	0.249	0.596
4	12	CRY1	flanking_5UT	0.035	-0.220	0.855
5	1	PER3	intron	0.036	-0.220	0.861
6	1	PER3	intron	0.042	-0.212	0.902
7	12	CRY1	flanking_5UT	0.051	0.204	0.941
8	11	ARNTL=BMAL-1	flanking_5UT	0.051	0.203	0.942
9	12	CRY1	flanking_5UT	0.065	-0.192	0.976
10	12	CRY1	flanking_5UT	0.065	-0.192	0.976
11	12	CRY1	flanking_5UT	0.065	-0.192	0.976
12	22	CSNK1E	flanking_3UT	0.067	-0.191	0.977
13	4	CLOCK	intron	0.067	0.191	0.979
14	12	CRY1	flanking_5UT	0.067	0.191	0.979
15	17	PER1	flanking_3UT	0.074	-0.186	0.986
16	4	CLOCK	intron	0.080	0.184	0.990
17	11	ARNTL=BMAL-1	intron	0.082	0.181	0.990
18	22	CSNK1E	intron	0.084	0.181	0.991
19	4	CLOCK	intron	0.093	0.175	0.995
20	12	CRY1	flanking_5UT	0.094	0.175	0.995
21	1	PER3	intron	0.120	0.162	0.999
22	4	CLOCK	intron	0.124	-0.161	1.000
23	11	ARNTL=BMAL-1	flanking_5UT	0.124	-0.162	1.000
24	11	ARNTL=BMAL-1	flanking_5UT	0.131	-0.158	1.000
25	11	ARNTL=BMAL-1	flanking_5UT	0.147	-0.151	1.000
26	4	CLOCK	intron	0.151	-0.150	1.000
27	11	ARNTL=BMAL-1	flanking_5UT	0.151	-0.150	1.000
28	11	ARNTL=BMAL-1	flanking_5UT	0.162	0.146	1.000
29	4	CLOCK	intron	0.167	0.144	1.000

SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend p-value*	Correlation/Trend r	Full Scan Permutation p-value	
30	rs10500773	11	ARNTL=BMAL-1	flanking_5UT	0.177	0.142	1.000
31	rs1384015	11	ARNTL=BMAL-1	flanking_5UT	0.183	0.139	1.000
32	rs10462023	2	PER2	intron	0.185	-0.138	1.000
33	rs2304674	2	PER2	intron	0.186	0.138	1.000
34	rs10778528	12	CRY1	intron	0.188	-0.137	1.000
35	rs6001093	22	CSNK1E	intron	0.199	-0.134	1.000
36	rs1921141	12	CRY1	flanking_5UT	0.212	0.130	1.000
37	rs10462018	1	PER3	intron	0.229	-0.125	1.000
38	rs10838527	11	CRY2	3UTR	0.230	0.125	1.000
39	rs11038695	11	CRY2	intron	0.230	0.125	1.000
40	rs7126303	11	ARNTL=BMAL-1	intron	0.234	0.124	1.000
41	rs2304673	2	PER2	intron	0.237	0.123	1.000
42	rs228654	1	PER3	intron	0.247	0.121	1.000
43	rs10832020	11	ARNTL=BMAL-1	intron	0.249	0.120	1.000
44	rs7306232	12	CRY1	flanking_5UT	0.277	0.113	1.000
45	rs2403661	11	ARNTL=BMAL-1	flanking_5UT	0.287	-0.112	1.000
46	rs10462021	1	PER3	coding	0.289	0.111	1.000
47	rs10864316	1	PER3	intron	0.289	0.111	1.000
48	rs6486121	11	ARNTL=BMAL-1	intron	0.308	0.106	1.000
49	rs900145	11	ARNTL=BMAL-1	flanking_5UT	0.340	-0.100	1.000
50	rs16924750	11	ARNTL=BMAL-1	flanking_5UT	0.341	0.099	1.000
51	rs2197040	11	ARNTL=BMAL-1	flanking_5UT	0.341	0.099	1.000
52	rs4757138	11	ARNTL=BMAL-1	flanking_5UT	0.375	-0.092	1.000
53	rs2374661	12	CRY1	intron	0.389	-0.090	1.000
54	rs969485	11	ARNTL=BMAL-1	intron	0.389	-0.090	1.000
55	rs11607529	11	ARNTL=BMAL-1	flanking_5UT	0.398	0.088	1.000
56	rs228642	1	PER3	intron	0.411	-0.087	1.000
57	rs4757122	11	ARNTL=BMAL-1	flanking_5UT	0.413	0.085	1.000
58	rs11022783	11	ARNTL=BMAL-1	intron	0.425	-0.083	1.000
59	rs8192440	12	CRY1	coding	0.432	-0.082	1.000
60	rs7111898	11	ARNTL=BMAL-1	flanking_5UT	0.436	0.081	1.000

SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend p-value*	Correlation/Trend r	Full Scan Permutation p-value	
61	rs10832000	11	ARNTL=BMAL-1	flanking_5UT	0.455	0.078	1.000
62	rs9312661	4	CLOCK	intron	0.462	0.077	1.000
63	rs2090602	11	CRY2	flanking_5UT	0.464	-0.076	1.000
64	rs707467	1	PER3	intron	0.480	0.074	1.000
65	rs10462020	1	PER3	coding	0.480	0.074	1.000
66	rs2290034	11	ARNTL=BMAL-1	intron	0.483	0.073	1.000
67	rs7942486	11	ARNTL=BMAL-1	flanking_5UT	0.492	0.072	1.000
68	rs7975663	12	CRY1	flanking_5UT	0.526	0.066	1.000
69	rs7297614	12	CRY1	flanking_5UT	0.526	-0.066	1.000
70	rs7289981	22	CSNK1E	flanking_5UT	0.537	0.064	1.000
71	rs2278749	11	ARNTL=BMAL-1	intron	0.539	0.065	1.000
72	rs2374671	12	CRY1	flanking_5UT	0.543	-0.063	1.000
73	rs6431590	2	PER2	intron	0.566	0.060	1.000
74	rs4663868	2	PER2	intron	0.579	0.058	1.000
75	rs12582821	12	CRY1	flanking_5UT	0.583	-0.057	1.000
76	rs697686	1	PER3	intron	0.595	-0.055	1.000
77	rs10766077	11	ARNTL=BMAL-1	intron	0.596	0.055	1.000
78	rs4964521	12	CRY1	flanking_5UT	0.618	0.052	1.000
79	rs11022780	11	ARNTL=BMAL-1	intron	0.621	-0.052	1.000
80	rs4757145	11	ARNTL=BMAL-1	intron	0.669	-0.045	1.000
81	rs7108752	11	ARNTL=BMAL-1	flanking_5UT	0.673	0.044	1.000
82	rs7112005	11	ARNTL=BMAL-1	flanking_5UT	0.673	0.044	1.000
83	rs3816358	11	ARNTL=BMAL-1	intron	0.679	-0.043	1.000
84	rs11022778	11	ARNTL=BMAL-1	intron	0.689	0.042	1.000
85	rs6798	11	CRY2	3UTR	0.693	-0.041	1.000
86	rs1997644	22	CSNK1E	flanking_5UT	0.693	-0.041	1.000
87	rs2518023	17	PER1	flanking_5UT	0.711	0.039	1.000
88	rs12808129	11	ARNTL=BMAL-1	flanking_5UT	0.713	0.038	1.000
89	rs11022693	11	ARNTL=BMAL-1	flanking_5UT	0.722	-0.037	1.000
90	rs7941871	11	ARNTL=BMAL-1	flanking_5UT	0.734	0.035	1.000
91	rs2075984	22	CSNK1E	intron	0.743	-0.034	1.000

SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend p-value*	Correlation/Trend r	Full Scan Permutation p-value	
92	rs7924734	11	ARNTL=BMAL-1	intron	0.759	0.032	1.000
93	rs10832008	11	ARNTL=BMAL-1	flanking_5UT	0.759	0.032	1.000
94	rs10832005	11	ARNTL=BMAL-1	flanking_5UT	0.764	-0.031	1.000
95	rs11022742	11	ARNTL=BMAL-1	flanking_5UT	0.765	0.031	1.000
96	rs998089	11	ARNTL=BMAL-1	flanking_5UT	0.765	0.031	1.000
97	rs7949336	11	ARNTL=BMAL-1	intron	0.767	-0.031	1.000
98	rs7121775	11	CRY2	flanking_5UT	0.774	-0.030	1.000
99	rs16912392	11	ARNTL=BMAL-1	flanking_5UT	0.777	0.030	1.000
100	rs748923	11	ARNTL=BMAL-1	flanking_5UT	0.792	0.027	1.000
101	rs3824872	11	CRY2	flanking_3UT	0.799	0.027	1.000
102	rs3789327	11	ARNTL=BMAL-1	intron	0.823	-0.023	1.000
103	rs2253820	17	PER1	coding	0.826	0.023	1.000
104	rs4756763	11	ARNTL=BMAL-1	flanking_5UT	0.832	-0.022	1.000
105	rs11022713	11	ARNTL=BMAL-1	flanking_5UT	0.849	0.020	1.000
106	rs934945	2	PER2	coding	0.866	-0.018	1.000
107	rs2170436	11	ARNTL=BMAL-1	flanking_5UT	0.874	0.017	1.000
108	rs10437896	12	CRY1	flanking_5UT	0.876	-0.016	1.000
109	rs11022775	11	ARNTL=BMAL-1	intron	0.933	0.009	1.000
110	rs10832027	11	ARNTL=BMAL-1	intron	0.940	0.008	1.000
111	rs1534891	22	CSNK1E	intron	0.948	-0.007	1.000
112	rs7926443	11	ARNTL=BMAL-1	flanking_5UT	0.957	0.006	1.000
113	rs228682	1	PER3	intron	0.970	0.004	1.000
114	rs4757143	11	ARNTL=BMAL-1	intron	0.972	0.004	1.000
115	rs5757037	22	CSNK1E	flanking_5UT	0.978	-0.003	1.000
116	rs4757144	11	ARNTL=BMAL-1	intron	0.978	-0.003	1.000
117	rs3816360	11	ARNTL=BMAL-1	intron	0.984	0.002	1.000
118	rs10507216	12	CRY1	flanking_5UT	0.988	0.002	1.000
119	rs10861688	12	CRY1	intron	0.988	0.002	1.000
120	rs1481872	11	ARNTL=BMAL-1	flanking_5UT	0.993	0.001	1.000
121	rs7925536	11	ARNTL=BMAL-1	flanking_5UT	0.993	0.001	1.000
122	rs7104311	11	ARNTL=BMAL-1	flanking_5UT	0.999	0.000	1.000

* Degrees of Freedom for the Correlation/Trend test is the degrees of freedom for the Chi-Squared statistic, which equals one.

Table 3

The VNTR Polymorphism Association Tabulated with one PER3 SNP

		rs228729			
		AA	AG	GG	Total
VNTR	44	0	1	47	48
	45	0	42	0	42
	55	18	1	1	20
<i>Total</i>		18	44	48	110