

Circadian Clock Gene Polymorphisms and Sleep–Wake Disturbance in Alzheimer Disease

*Jerome A. Yesavage, M.D., Art Noda, M.S., Beatriz Hernandez, B.A., Leah Friedman, Ph.D., Jauhtai J. Cheng, M.D., Ph.D., Jared R. Tinklenberg, M.D., Joachim Hallmayer, M.D., Ruth O'Hara, Ph.D., Renaud David, M.D., Philippe Robert, M.D., Elizabeth Landsverk, M.D., Jamie M. Zeitzer, Ph.D., the Alzheimer's Disease Neuroimaging Initiative**

Objectives: *One of the hypothesized causes of the breakdown in sleep–wake consolidation often occurring in individuals with Alzheimer disease (AD) is the 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, National Institute on Aging 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 data set. Measurements:* *Determination of sleep–wake state was obtained by wrist actigraphy data for 7 days in Cohort 1 and by the Neuropsychiatric Inventory questionnaire for Cohort 2. Both cohorts were genotyped by using an Illumina Beadstation (Illumina, San Diego, CA), and 122 circadian-related single-nucleotide polymorphisms (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 time 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.*

Received March 25, 2010; revised August 25, 2010; accepted August 28, 2010. From the Department of Veterans Affairs Health Care System (JAY, JJC, JRT, LF, RO, JMZ) and Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine (JAY, AN, BH, LF, JRT, JH, RO, JMZ), Palo Alto, California; Memory Center (CMRR), CIU-S, Université de Nice, France (RD, PR); and ElderConsult Medical Associates, Burlingame, California (EL).

Send correspondence and reprint requests to Dr. Jerome Yesavage, Palo Alto VA Health Care System (151Y), 3801 Miranda Avenue, Palo Alto, CA 94304. e-mail: yesavage@stanford.edu.

*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 coinvestigators from a broad range of academic institutions and private corporations (see www.adni-info.org).

© 2011 American Association for Geriatric Psychiatry

DOI: 10.1097/JGP.0b013e31820d92b2

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.* (Am J Geriatr Psychiatry 2011; 19:635–643)

Key Words: Alzheimer disease, circadian rhythm, sleep–wake disturbances

Nocturnal wakefulness and daytime napping often characterize the sleep–wake disturbance frequently associated with Alzheimer disease (AD). Previously, we longitudinally followed sleep–wake disturbances in AD participants and found significant nocturnal wake time after sleep onset (WASO).¹ We also found that sleep–wake deterioration in individuals with AD behaved as a “trait”; that is, 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 the degeneration of the circadian clock.³ A limited number of genes likely underlie the core circadian oscillation found in neurons of the suprachiasmatic nucleus, 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*, *csnk1e*, *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) by 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 the National Institute of Neurological and Communicative Disorders and Stroke–AD and Related Disorders Association criteria⁷ based on relevant neurologic, medical, neuroimaging, and neuropsychological assessments. Participants were excluded if they had active major medical conditions that would have precluded the collection of actigraphy data. The 124 participants in this cohort were from two sources: 1) 81 participants in an ongoing longitudinal study of AD at the Stanford/Veterans Affairs, National Institute on Aging (NIA) Alzheimer’s Disease Core Center, and 2) 43 French participants from the Memory Disorders Clinic at the University of Nice School of Medicine that collaborated with our group on AD sleep studies. For this analysis, only white participants were selected from both sources, and participants in Cohort 1, as a whole, were 46% men. At the time of actigraphy, the average age of the 124 participants in Cohort 1 was 75.2 years (SD: 8.1; range: 45–88 years) and their mean Mini-Mental State Exam score⁸ was 19.8 (SD: 4.8; range = 4–29).

Cohort 2. Data were also obtained from a second cohort of 176 white participants in the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI) with a diagnosis of probable AD by the National Institute of Neurological and Communicative Disorders and Stroke–AD and Related Disorders Association criteria.

ADNI was launched in 2003 by the NIA, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a \$60-million, 5-year public–private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging, positron emission tomography, other biologic markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment and early 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 ADNI is Michael W. Weiner, M.D., VA Medical Center and University of California, San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and participants have been recruited from more than 50 sites across the United States and Canada. The initial goal of ADNI was to recruit 800 adults, age 55–90 years, to participate in the research.

The ADNI participants were studied by using the same Illumina gene chip technology as that used for Cohort 1, described previously. They did not receive actigraphy but received the Neuropsychiatric Inventory questionnaire (NPI-Q),⁹ which included 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 the selected 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 white participants were selected from the ADNI database; the cohort was 55% men, and the average age was 75.6 years (SD: 7.6; range: 55–91 years). The mean Mini-Mental State Exam⁸ score for the 176 participants in Cohort 2 at the time the NPI data were collected was 23.3 (SD: 2.0; range: 18 to 27).

The institutional review boards for human subjects research at all sites 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). Participants were asked to wear an actigraph 24 hours a day on their nondominant wrists for seven 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, WASO, were obtained by using the computer scoring program supplied by the manufacturer (ACTION software version 1.3, Ambulatory Monitoring Systems, Inc, Ardsley, NY). The ACTION software scores the actigraph recordings following the entry of participants' evening bedtimes 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 article. 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: 5–414). The staff members in Nice, France, were trained for the use of actigraphy at Stanford, and they used the same device. Furthermore, all data collected in Nice were double checked by US 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. 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% were "moderate" and 2% were "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 administered to only the US-based ADNI cohort).

TABLE 1. Association Tests Between 122 Candidate Gene SNPs and NPI Severity Items^a

NPI Item	Item	SNP	Correlation/ Trend, <i>r</i>	Full-Scan Permutation, <i>p</i>
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 talks to people who are not there?	rs10832005	0.23	0.247
Item C severity	Is {P} stubborn and resistive to receive 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	0.493
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

^aScoring was 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]).

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) by using the manufacturer-provided procedures. We utilized the Human 610 Quad gene chip (Illumina, Inc., Hayward, CA) 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 four or five units, has been linked with multiple sleep phenotypic parameters.⁶ This VNTR polymorphism could not be determined by using Illumina technology, so a separate procedure was performed in which the target *hPer3* gene fragment was amplified by using sense (5'-CAAAATTTTATGACACTACCAGAATGGCTGAC-3') and reverse (5'-AACCTTGTACTIONTCCACATCAGTGCCTGG-3') primers. The polymerase chain reaction (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% dimethyl sulfoxide. 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 polymorphism 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*) by 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 by using Illumina 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, Golden Helix, Bozeman, MT) software, SNPs were filtered to exclude failed SNPs according to one or more of the following criteria: call rate less than 0.95; minor allele frequency less than 5%; Fisher's exact test for Hardy-Weinberg equilibrium (HWE), $p < 0.001$. This filtering reduced the number of candidate gene SNPs to 122. Finally, we used the SVS software (Symantec, Software Virtualization Solution, Mountain View, CA) 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.

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 resampling 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 white ($r^2 = 0.117$) 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 data set 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 greater than 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

Circadian Clock Gene Polymorphisms and AD Sleep-Wake Disturbance

TABLE 2. Association Tests Between 122 Candidate Gene SNPs and WASO

	SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend, p^a	Correlation/Trend, r	Full-Scan Permutation, p
1	rs7295750	12	<i>cry1</i>	Flanking_5UT	0.006	0.286	0.269
2	rs11113179	12	<i>cry1</i>	Intron	0.008	-0.279	0.323
3	rs1921120	12	<i>cry1</i>	Flanking_5UT	0.017	0.249	0.596
4	rs10778537	12	<i>cry1</i>	Flanking_5UT	0.035	-0.220	0.855
5	rs10127838	1	<i>per3</i>	Intron	0.036	-0.220	0.861
6	rs228729	1	<i>per3</i>	Intron	0.042	-0.212	0.902
7	rs10778536	12	<i>cry1</i>	Flanking_5UT	0.051	0.204	0.941
8	rs6486099	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.051	0.203	0.942
9	rs10861704	12	<i>cry1</i>	Flanking_5UT	0.065	-0.192	0.976
10	rs10861709	12	<i>cry1</i>	Flanking_5UT	0.065	-0.192	0.976
11	rs4262808	12	<i>cry1</i>	Flanking_5UT	0.065	-0.192	0.976
12	rs135737	22	<i>csnk1e</i>	Flanking_3UT	0.067	-0.191	0.977
13	rs3817444	4	<i>clock</i>	Intron	0.067	0.191	0.979
14	rs12315175	12	<i>cry1</i>	Flanking_5UT	0.067	0.191	0.979
15	rs2585408	17	<i>per1</i>	Flanking_3UT	0.074	-0.186	0.986
16	rs6811520	4	<i>clock</i>	Intron	0.080	0.184	0.990
17	rs7950226	11	<i>arntl = bmal-1</i>	Intron	0.082	0.181	0.990
18	rs135757	22	<i>csnk1e</i>	Intron	0.084	0.181	0.991
19	rs11931061	4	<i>clock</i>	Intron	0.093	0.175	0.995
20	rs11113204	12	<i>cry1</i>	Flanking_5UT	0.094	0.175	0.995
21	rs875994	1	<i>per3</i>	Intron	0.120	0.162	0.999
22	rs11932595	4	<i>clock</i>	Intron	0.124	-0.161	1.000
23	rs11605518	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.124	-0.162	1.000
24	rs10831990	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.131	-0.158	1.000
25	rs1481871	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.147	-0.151	1.000
26	rs3792603	4	<i>clock</i>	Intron	0.151	-0.150	1.000
27	rs10741613	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.151	-0.150	1.000
28	rs3860194	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.162	0.146	1.000
29	rs2412648	4	<i>clock</i>	Intron	0.167	0.144	1.000
30	rs10500773	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.177	0.142	1.000
31	rs1384015	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.183	0.139	1.000
32	rs10462023	2	<i>per2</i>	Intron	0.185	-0.138	1.000
33	rs2304674	2	<i>per2</i>	Intron	0.186	0.138	1.000
34	rs10778528	12	<i>cry1</i>	Intron	0.188	-0.137	1.000
35	rs6001093	22	<i>csnk1e</i>	Intron	0.199	-0.134	1.000
36	rs1921141	12	<i>cry1</i>	Flanking_5UT	0.212	0.130	1.000
37	rs10462018	1	<i>per3</i>	Intron	0.229	-0.125	1.000
38	rs10838527	11	<i>cry2</i>	3UTR	0.230	0.125	1.000
39	rs11038695	11	<i>cry2</i>	Intron	0.230	0.125	1.000
40	rs7126303	11	<i>arntl = bmal-1</i>	Intron	0.234	0.124	1.000
41	rs2304673	2	<i>per2</i>	Intron	0.237	0.123	1.000
42	rs228654	1	<i>per3</i>	Intron	0.247	0.121	1.000
43	rs10832020	11	<i>arntl = bmal-1</i>	Intron	0.249	0.120	1.000
44	rs7306232	12	<i>cry1</i>	Flanking_5UT	0.277	0.113	1.000
45	rs2403661	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.287	-0.112	1.000
46	rs10462021	1	<i>per3</i>	Coding	0.289	0.111	1.000
47	rs10864316	1	<i>per3</i>	Intron	0.289	0.111	1.000
48	rs6486121	11	<i>arntl = bmal-1</i>	Intron	0.308	0.106	1.000
49	rs900145	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.340	-0.100	1.000
50	rs16924750	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.341	0.099	1.000
51	rs2197040	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.341	0.099	1.000
52	rs4757138	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.375	-0.092	1.000
53	rs2374661	12	<i>cry1</i>	Intron	0.389	-0.090	1.000
54	rs969485	11	<i>arntl = bmal-1</i>	Intron	0.389	-0.090	1.000
55	rs11607529	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.398	0.088	1.000
56	rs228642	1	<i>per3</i>	Intron	0.411	-0.087	1.000
57	rs4757122	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.413	0.085	1.000
58	rs11022783	11	<i>arntl = bmal-1</i>	Intron	0.425	-0.083	1.000
59	rs8192440	12	<i>cry1</i>	Coding	0.432	-0.082	1.000
60	rs7111898	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.436	0.081	1.000
61	rs10832000	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.455	0.078	1.000
62	rs9312661	4	<i>clock</i>	Intron	0.462	0.077	1.000

(Continued)

TABLE 2. (Continued)

SNP	Chromosome	Circadian Candidate Gene	Location	Correlation/Trend, p^a	Correlation/Trend, r	Full-Scan Permutation, p	
63	rs2090602	11	<i>cry2</i>	Flanking_5UT	0.464	-0.076	1.000
64	rs707467	1	<i>per3</i>	Intron	0.480	0.074	1.000
65	rs10462020	1	<i>per3</i>	Coding	0.480	0.074	1.000
66	rs2290034	11	<i>arntl = bmal-1</i>	Intron	0.483	0.073	1.000
67	rs7942486	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.492	0.072	1.000
68	rs7975663	12	<i>cry1</i>	Flanking_5UT	0.526	0.066	1.000
69	rs7297614	12	<i>cry1</i>	Flanking_5UT	0.526	-0.066	1.000
70	rs7289981	22	<i>csnk1e</i>	Flanking_5UT	0.537	0.064	1.000
71	rs2278749	11	<i>arntl = bmal-1</i>	Intron	0.539	0.065	1.000
72	rs2374671	12	<i>cry1</i>	Flanking_5UT	0.543	-0.063	1.000
73	rs6431590	2	<i>per2</i>	Intron	0.566	0.060	1.000
74	rs4663868	2	<i>per2</i>	Intron	0.579	0.058	1.000
75	rs12582821	12	<i>cry1</i>	Flanking_5UT	0.583	-0.057	1.000
76	rs6976817	1	<i>per3</i>	Intron	0.595	-0.055	1.000
77	rs10766077	11	<i>arntl = bmal-1</i>	Intron	0.596	0.055	1.000
78	rs4964521	12	<i>cry1</i>	Flanking_5UT	0.618	0.052	1.000
79	rs11022780	11	<i>arntl = bmal-1</i>	Intron	0.621	-0.052	1.000
80	rs4757145	11	<i>arntl = bmal-1</i>	Intron	0.669	-0.045	1.000
81	rs7108752	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.673	0.044	1.000
82	rs7112005	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.673	0.044	1.000
83	rs3816358	11	<i>arntl = bmal-1</i>	Intron	0.679	-0.043	1.000
84	rs11022778	11	<i>arntl = bmal-1</i>	Intron	0.689	0.042	1.000
85	rs6798	11	<i>cry2</i>	3'UTR	0.693	-0.041	1.000
86	rs1997644	22	<i>csnk1e</i>	Flanking_5UT	0.693	-0.041	1.000
87	rs2518023	17	<i>per1</i>	Flanking_5UT	0.711	0.039	1.000
88	rs12808129	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.713	0.038	1.000
89	rs11022693	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.722	-0.037	1.000
90	rs7941871	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.734	0.035	1.000
91	rs2075984	22	<i>csnk1e</i>	Intron	0.743	-0.034	1.000
92	rs7924734	11	<i>arntl = bmal-1</i>	Intron	0.759	0.032	1.000
93	rs10832008	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.759	0.032	1.000
94	rs10832005	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.764	-0.031	1.000
95	rs11022742	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.765	0.031	1.000
96	rs998089	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.765	0.031	1.000
97	rs7949336	11	<i>arntl = bmal-1</i>	Intron	0.767	-0.031	1.000
98	rs7121775	11	<i>cry2</i>	Flanking_5UT	0.774	-0.030	1.000
99	rs16912392	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.777	0.030	1.000
100	rs748923	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.792	0.027	1.000
101	rs3824872	11	<i>cry2</i>	Flanking_3'UT	0.799	0.027	1.000
102	rs3789327	11	<i>arntl = bmal-1</i>	Intron	0.823	-0.023	1.000
103	rs2253820	17	<i>per1</i>	Coding	0.826	0.023	1.000
104	rs4756763	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.832	-0.022	1.000
105	rs11022713	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.849	0.020	1.000
106	rs934945	2	<i>per2</i>	Coding	0.866	-0.018	1.000
107	rs2170436	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.874	0.017	1.000
108	rs10437896	12	<i>cry1</i>	Flanking_5UT	0.876	-0.016	1.000
109	rs11022775	11	<i>arntl = bmal-1</i>	Intron	0.933	0.009	1.000
110	rs10832027	11	<i>arntl = bmal-1</i>	Intron	0.940	0.008	1.000
111	rs1534891	22	<i>csnk1e</i>	Intron	0.948	-0.007	1.000
112	rs7926443	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.957	0.006	1.000
113	rs228682	1	<i>per3</i>	Intron	0.970	0.004	1.000
114	rs4757143	11	<i>arntl = bmal-1</i>	Intron	0.972	0.004	1.000
115	rs5757037	22	<i>csnk1e</i>	Flanking_5UT	0.978	-0.003	1.000
116	rs4757144	11	<i>arntl = bmal-1</i>	Intron	0.978	-0.003	1.000
117	rs3816360	11	<i>arntl = bmal-1</i>	Intron	0.984	0.002	1.000
118	rs10507216	12	<i>cry1</i>	Flanking_5UT	0.988	0.002	1.000
119	rs10861688	12	<i>cry1</i>	Intron	0.988	0.002	1.000
120	rs1481872	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.993	0.001	1.000
121	rs7925536	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.993	0.001	1.000
122	rs7104311	11	<i>arntl = bmal-1</i>	Flanking_5UT	0.999	0.000	1.000

^aDegrees of freedom for the Correlation/Trend test is the degrees of freedom for the χ^2 statistic, which equals one.

TABLE 3. The VNTR Polymorphism Association Tabulated With One *per3* SNP

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

may exist with some SNPs on *cry1* or *per3*; however, none of these correlations at the level of less than 0.30 were significant after the full-scan permutation correction. Such smaller relationships may still be of theoretical interest to those examining basic physiologic relations of genetic measures and sleep–wake phenomena, even if they may not be clinically relevant.

Exploratory analyses were also performed by using the ADNI data set and scores on other NPI items. In these analyses, no SNPs were significantly associated with any of the other NPI measures. We feel that 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 data sets to determine whether they are replicable.

In summary, we examined the 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 on other physiologic mechanisms or interactions among a number of genetic markers.

The authors thank their clinical research staff: Terry Miller, M.D., Helen Davies, R.N.M.S., and Aimee Stepp, for clinical testing and assessments. Special appreciation is expressed to Deryl Wicks, who organized the longitudinal actigraphy follow-up and quantification, and to Chun-Ping (Phoebe) Liao, who performed the Illumina genotyping.

The Foundation for the National Institutes of Health (www.fnih.org) coordinates the private sector participation of the \$60 million ADNI public–private partnership that was initiated by the NIA and supported by the National Institutes of Health. To date, more than \$27 millions have been provided to the Foundation for NIH by Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson & Johnson, Eli Lilly and Co., Merck & Co., Inc., Novartis AG, Pfizer Inc., F. Hoffmann-La Roche, Schering-Plough, Synarc Inc., and Wyeth, as well as non-profit partners, the Alzheimer’s Association and the Institute for the Study of Aging. The Department of Veterans Affairs, War Related Illness and Injury Study Center, Palo Alto, California, provided the support for the purchase and maintenance of the Illumina technology used in this research.

This research was supported by National Institute of Health grants MH40041 (National Institute of Mental Health) and AG17824 (NIA); the Sierra-Pacific Mental Illness Research, Education, and Clinical Center; the Medical Research Service of the Department of Veterans Affairs; and an Alzheimer disease plan grant (Conseil Général des Alpes-Maritimes, Nice, France). ADNI research was supported by The Foundation for NIH (AG024904), which coordinates the private sector participation of the ADNI public–private partnership, as well as nonprofit partners, the Alzheimer’s Association and the Institute for the Study of Aging.

References

1. Yesavage JA, Friedman LF, Kraemer HC, et al: A follow-up study of actigraphic measures in home-residing Alzheimer’s disease patients. *J Geriatr Psychiatry Neurol* 1998; 11:7–10
2. Yesavage JA, Taylor JL, Kraemer H, et al: Sleep/wake disturbance in Alzheimer’s disease: How much is due to an inherent trait? *Int Psychogeriatr* 2002; 14:73–81
3. Swaab DF, Lucassen PJ, Salehi A, et al: Reduced neuronal activity and reactivation in Alzheimer’s disease. *Prog Brain Res* 1998; 17:343–377
4. King DP, Zhao Y, Sangoram AM, et al: Positional cloning of the mouse Clock gene. *Cell* 1997; 89:641–653
5. Katzenberg D, Young T, Finn L, et al: A CLOCK polymorphism associated with human diurnal preference. *Sleep* 1998; 21: 569–576
6. Dijk DJ, Archer SN: PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev* 2010; 14: 151–160
7. McKhann G, Drachman D, Folstein M, et al: Clinical diagnosis of Alzheimer’s disease: a report of the NINCDS-ADRDA workgroup under the auspices of the Department of Health and Human Services Task Force on Alzheimer’s disease. *Neurology* 1984; 34:939–944

8. Folstein MF, Folstein SE, McHugh PR: "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12:189-198
9. Kaufer DI, Cummings JL, Ketchel P, et al: Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. *J Neuropsychiatry Clin Neurosci* 2000; 12:233-239
10. Murphy GM, Jr., Taylor J, Kraemer HC, et al: No association between apolipoprotein E epsilon 4 allele and rate of decline in Alzheimer's disease. *Am J Psychiatry* 1997; 154:603-608
11. Westfall PH, Young SS: *Resampling-Based Multiple Testing: Examples and Methods for p Value Adjustment*. Wiley Interscience, New York, 1993
12. Ebisawa T, Uchiyama M, Kajimura N, et al: Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. *EMBO Rep* 2001; 2:342-346
13. Cohen J: *Statistical Power Analysis for the Behavioral Sciences* 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988. (See also "A Power Primer" under the Wikipedia entry for "Jacob Cohen")