Genotyping Sleep Disorders Patients

Daniel F. Kripke1  
Farhad F. Shadan1
Arthur Dawson1
John W. Cronin1
Shazia M. Jamil1
Alexandra P. Grizas2
James A. Kozlo1
Lawrence E. Kline1

1Scripps Clinic Sleep Center, La Jolla, California, 2Yale University School of Public Health New Haven, Connecticut, 3The Scripps Research Institute La Jolla, California, USA

Objective  The genetic susceptibility factors underlying sleep disorders might help us predict prognoses and responses to treatment. Several candidate polymorphisms for sleep disorders have been proposed, but there has as yet inadequate replication or validation that the candidates may be useful in the clinical setting.

Methods  To assess the validity of several candidate associations, we obtained saliva deoxyribonucleic acid (DNA) samples and clinical information from 360 consenting research participants who were undergoing clinical polysomnograms. Ten single nucleotide polymorphisms (SNPs) were genotyped. These were thought to be related to depression, circadian sleep disorders, sleep apnea, restless legs syndrome (RLS), excessive sleepiness, or to slow waves in sleep.

Results  With multivariate generalized linear models, the association of \textit{TEF} rs738499 with depressive symptoms was confirmed. Equivocal statistical evidence of association of rs1801260 (the C3111T SNP in the \textit{CLOCK} gene) with morningness/eveningness and an association of \textit{Apolipoprotein E} (\textit{APOE}) rs429358 with the Epworth Sleepiness Scale (ESS) were obtained, but these associations were not strong enough to be of clinical value by themselves. Predicted association of SNPs with sleep apnea, RLS, and slow wave sleep were not confirmed.

Conclusion  The SNPs tested would not, by themselves, be of use for clinical genotyping in a sleep clinic.

Psychiatry Investig 2010;7:36-42

Key Words  Sleep, Genotype, Sleep apnea, Depression, \textit{TEF}, \textit{APOE}.

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

In a sleep clinic, the causes of our patients’ distress can be complex and obscure. We might better understand prognosis and likely responses to treatment interventions when the etiologies can be identified. For example, a patient with excessive sleepiness may have both sleep apnea and depressive symptoms, both of which may have genetic components. A patient complaining of insomnia may have genetically-mediated delayed sleep phase disorder and restless legs combined with a cognitive-behavioral disturbance. Since reported sleep duration appears to be as much as 50% heritable, both short sleep complaints and long sleep complaints may arise in large part from genetic susceptibilities. Recognizing genetic susceptibilities for depression, delayed sleep phase, restless legs or short sleep, for example, might help confirm diagnostic formulations and anticipate the responses to treatment options.

With the rapid pace of genomic discoveries, many research groups and not a few corporations are exploring the idea that genotyping individuals may help guide their health care. With the long-term goal of applying genotyping to our care of sleep clinic patients, we have begun to genotype patient volunteers on a research basis when they are undergoing clinical polysomnograms. We hoped to validate the association of candidate sleep disorder susceptibility factors with various symptom phenotypes, and to assess the extent to which genotypes might become helpful in identifying the etiologies of our patients’ complaints.

To date, only a limited number of genetic polymorphisms have been suggested as causes of the diverse sleep disorders. The alleles of 4 single nucleotide polymorphisms (SNPs)
possibly related to circadian rhythms and affective disorders, 3 SNPs possibly related to sleep apnea, 2 SNPs possibly related to restless legs syndrome (RLS) or periodic limb movements in sleep (PLMS), and 1 SNP possibly related to slow wave sleep were identified (Table 1). These 10 SNPs were selected from the larger number with previously-reported sleep associations based on ease of genotyping and the predicted likelihood of association in our Clinic sample.

Our prospective hypotheses were as follows. Based on a finding among sib pairs with a proband with early-onset recurrent unipolar major depression, it was prospectively predicted that the common T allele of rs738499 of TEF (a gene participating in circadian system feedback loops) would be associated with the QIDS-SR measure of depressed mood. Likewise, based on prior evidence that rs2314339 (a SNP in NR1D1 or Rev-erb-alpha, a component of circadian system feedback loops) was associated with bipolar disorder, it was predicted that rs2314339 would be associated with the QIDS-SR, even though rs2314339 had not been significantly associated with unipolar major depression in the prior study. Because of the evident functional role of circadian SNPs, it was also prospectively predicted that the BALM morningness-eveningness scale would be associated with rs738499, rs2314339, the Phe876Leu amino-acid changing SNP in Rev-erb-alpha (a gene previously reported to be related to morningness-eveningness and to insomnia).7 Further, based on previous reports, it was prospectively predicted that sleep apnea might be associated with rs405509 and rs429358 [SNPs related to the Apolipoprotein E 4 (APOE 4) genotype] and rs1800629, a -308 tumor necrotic factor α (TNF α) promoter polymorphism which influences TNFα expression.8-13 Also, rs5751876 in ADORA2A, the A2A adenosine receptor, is associated with higher electroencephalography (EEG) amplitudes especially below 5 Hz regardless of sleep or wake, so we predicted rs5751876 would be associated with the slow wave sleep percentage or with total sleep time (TST).14 We further predicted that MEIS1 rs12469063 and MAP2K5 rs 1026732 would be associated with restless legs frequency, the 4 essential questions for diagnosis of RLS, and the polysomnographic periodic limb movement index.15-17 Several secondary hypotheses were also examined.

### Table 1. Gene targets with observed genotype frequencies

| Gene | TEF | NR1D1 | TNFα | CLOCK | PER2 |
|------|-----|-------|------|-------|------|
| SNP  | rs738499 | rs2314339 | rs1800629 | rs1801260 | Phe876Leu* |
| Genotypes | GG : TT | GG : TT | AA : GG | CC : TT | AA : AG |
| N     | 21 : 145 : 190 | 252 : 93 : 14 | 4 : 98 : 257 | 22 : 140 : 196 | 345 : 14 |
| SNP  | rs405509 | rs429358 | rs5751876 | rs12469063 | rs1026732 |
| Genotypes | GG : GT : TT | GG : TT | CC : CT : TT | AA : AG : GG | GG : GA : AA |
| N     | 99 : 181 : 79 | 3 : 98 : 258 | 125 : 165 : 69 | 212 : 125 : 22 | 124 : 165 : 40 |

Each assayed single nucleotide polymorphism (SNP) is identified by an “rs” number, which links to detailed documentation available, e.g., through the SNP data base provided by NCBI Entrez at http://www.ncbi.nlm.nih.gov/sites/entrez or the UCSC Genome Browser available at http://www.genome.ucsc.edu/cgi-bin/hgGateway.

### Methods

#### Consent and questionnaire

When a clinical research staff member was available and the clinical context permitted, patients arriving at our sleep laboratory for clinical recordings or who had recently undergone sleep recordings were invited to participate in our genetics research program. First, the study was explained. Then a signed consent approved by the Scripps Institutional Review Board was obtained. If it had not already been completed as part of our clinical patient intake procedure, a comprehensive sleep questionnaire was completed (form can be requested from the authors). The questionnaire asked about habitual bedtimes, falling-asleep times, wake-up times, and uptimes both on weekdays and weekends. It included dozens of multiple-choice questions about the frequency and severity of various sleep symptoms. Embedded in the questionnaire were the Bbaln morningness-eveningness scale (a simplified-language abbreviation of the Horne-Östberg morningness-eveningness scale18 with which it correlates *r*=0.96), the QIDS-SR (a modern self-report depression rating scale which has been validated against the Hamilton Depression Rating Scale and against DSM-IV major depression diagnoses),19,20 and the Epworth Sleepiness Scale (ESS) (a widely used rating of daytime sleepiness),21 along with the four questions essential for a diagnosis of RLS, often associated with PLMS.22-24

#### Polysomnogram

Participants underwent standard clinical polysomnograms in our sleep laboratory using Compumedics Profusion PSG 2.10.0.131 software on a Compumedics E Series platform. Recordings were scored according to the current criteria of the American Academy of Sleep Medicine. In 59% of the recordings, the patient was awakened after sleep apnea was detected, and nasal continuous positive airway pressure (CPAP) treatment was titrated during the latter part of the recording.
In these split night studies, scoring of the polysomnograms was divided into two portions. Phenotypes were computed from the first portion of split recordings unmasked by CPAP, except in 10 recordings where there was so little sleep in the first portion that use of the CPAP portion appeared more representative.

**DNA collection**

The participants were asked to provide about 2 cc of saliva in an Oragene deoxyribonucleic acid (DNA) Self-collection Kit (DNA Genotek, Inc., Ottawa, Ontario, Canada), used according to the manufacturer’s directions. DNA was stored with preservative in the Oragene kits at room temperature until it could be extracted and purified according to the manufacturer’s protocol, producing an average of 80-120 mcg. of purified DNA. DNA was then frozen in split samples for further study.

**Phenotype determination**

In accordance with the consent granted, polysomnographic data, clinical diagnoses from multiple visits, and clinical referrals were compiled with coded identifiers in research data bases. For this study, the following 10 quantitative phenotypic traits were derived from the data bases: 1) ESS score for daytime sleepiness; 2) BALM score for room morningness-eveningness circadian trait; 3) QIDS-SR score for current depression; 4) number of 4 essential RLS complaints (restless legs essential features) answered affirmative; 5) answer on a 6-point scale of frequency of “a feeling of restless legs”, 6) polysomnographic slow wave sleep percentage, i.e., stage 3%+stage 4%, 8) polysomnographic periodic leg movement index, 9) polysomnographic apnea-hypopnea index (AHI), 10) a global score summarizing the extent of clinician documentation of an obstructive sleep apnea diagnosis. This apnea diagnostic score was compiled from arbitrary weights for the number of visits and interval of months over which an apnea diagnosis was recorded clinically; likewise the number of visits, interval, and stated confidence in which a separate research diagnosis of obstructive apnea was recorded: whether a referral for CPAP or mandibular advancement device treatment was recorded; the polysomnographic AHI (if 15 or more) and the index of oximetry desaturations of ≥4% (if 15 or more per hour). Each of these phenotypic traits reflecting a manifestation of sleep disorders could then be examined for its association with particular genotypes.

**Assays**

DNA was genotyped for the first 360 participants at the DNA Core Laboratory of the Molecular and Experimental Medicine division of the Scripps Research Institute, using allele-specific oligonucleotide hybridization. A single subject was dropped for inadequate DNA quantity. Technical difficulties assaying certain SNPs and expense limited this report to 10 SNPs from a much larger number which might have been of interest (Table 1).

**Statistics**

The quantitative phenotypes were tested as dependent variables in multivariate generalized linear models, using as independent predictors the participant’s age (continuous), gender (binary), race (self-reported and recoded as white or non-white), and the genotypes. Additive, dominant, and recessive models were tested in these analyses when applicable. For the dominant and recessive models, each SNP was modeled as a dichotomous variable with one genotype (either dominant or recessive) chosen as the reference group, and the other two genotypes combined into one category. For the additive model, the number of dominant alleles was used as the variable. No adjustments for multiple comparisons were conducted in the analyses, since the primary analyses tested independent prospective hypotheses. Analyses were implemented in Systat V.12 (Systat Software, Inc., Chicago, IL, USA).

**Results**

The 359 participants ranged from age 21 to 92 years, with a mean age of 57.4 (±standard deviation 13.9) years. Participants were 33% women and 67% men. The sample was 90% White by self-report. The global obstructive apnea scores ranged from 3 to 94 with a mean of 25. Apnea global scores indicated some clinical consideration of an apnea diagnosis for every patient, though in many cases, a clinician might have coded an apnea diagnosis based on clinical supposition which later was not confirmed by polysomnography. The number of times clinical and research diagnoses of apnea were recorded, treatment referrals, AHIs, and oxygen desaturation indices (ODIs) (4% ODIs) contributed 48%, 15%, 10%, 17%, and 10% respectively to the global apnea scores. In 10% of participants, sleep apnea (any kind) was not a first diagnosis, e.g., primary diagnoses of insomnia, delayed sleep phase disorder, rapid eye movement behavior disorder, and narcolepsy. Polysomnographically, 22% had an AHI of less than 5 (suggesting no clinically significant sleep apnea), and only 4% had no AHI of greater than 15 (a commonly-used threshold indicating when CPAP treatment is indicated.) Parenthetically, 43% had ODI<5. In only 10 of the recordings was it necessary to utilize the CPAP titration portion of a split PSG for analysis because there was insufficient sleep in the first part. Of these 10 recordings during CPAP, 7 had an AHI<5, but presumably the CPAP suppressed the AHI as compared to what would be observed without CPAP. Partly because of the split night procedures, the mean TST for the recording segments utilized was only 253 minutes.

Examining the association of the QIDS-SR depression score with TEF rs738499 as well as age, gender, and race as
independent control variables, the overall regression yielded $R^2=0.06$, with all independent variables being significant (Table 2). The adjusted mean QIDS-SR was 6.5±standard error (SE) 0.9 for the GG rs738499 genotype, 6.9±SE 0.4 for the GT genotype, and 7.9±SE 0.3 for the TT genotype. Controlling for age, gender, and race, the rs738499 T allele being present or absent was significantly associated with the QIDS-SR. Topping for age, gender, and race, the rs738499 T allele being present or absent was significantly associated with the QIDS-SR. Controlling for age, gender, and race, the rs738499 T allele being present or absent was significantly associated with the QIDS-SR.

Since this association had been prospectively predicted as the highest priority hypothesis, this association was considered significant. The NR1D1 SNP genotype was not significantly related to the QIDS-SR.

Although with CLOCK rs1801260, the BALM score averaged 33.8±SE 1.9 in the CC genotypes, compared to 36.8±SE 0.8 in the TC genotypes and 37.4±SE 0.7 in the TT genotypes, the probability of association was only $p=0.071$, two-tailed. This result might be considered equivocally significant by one-tailed criteria, considering that the association and its direction (less morningness with the C allele) was predicted in advance based on prior work. The NR1D1, TEF, and PER2 SNP genotypes were not significantly associated with the BALM score.

The predictions that TNFα rs1800629 and the APOE SNPs rs405509 and rs429358 would be associated with the AHI or with the global apnea score were confirmed. However, in rs429358, the more common T allele was weakly associated with the ESS (Table 3), an association which had not been prospectively predicted. The other two SNPs, rs405509 and rs1800629, were not associated with the ESS.

MEIS1 rs12469063 and MAP2K5 rs1026732 were not significantly associated with the four diagnostic questions for RLS syndrome, with the reported frequency of “restless legs,” or with the polysomnographic periodic leg movement index. ADORA2A rs5751876 was not significantly associated with polysomnographic TST nor with the slow wave sleep percentage.

In retrospective Post Hoc computations (which do not meet Bonferroni significance criteria for multiple testing), the T allele of TEF rs738499 was associated with the percentage of slow wave sleep (sleep stages 3 and 4), controlling for covariates of gender, age, and race ($p<0.05$). Similarly, EEG TST was associated with the C allele of CLOCK rs1801260 ($p=0.04$) and the A allele of TNFα rs1800629 ($p<0.025$). Selecting on those roughly 50% of participants with AHI<15, to minimize confounding with apnea, the PLMI was greater among those with the C allele of CLOCK rs1801260 ($p<0.05$), the number of the 4 RLS questions answered affirmatively was associated both with the A allele of MEIS1 rs12469063 ($p<0.01$, but not the predicted direction of association) and with the T allele of TEF rs738499 ($p<0.025$), and the T allele of NR1D1 rs2314339 was positively associated with the reported frequency of a feeling of restless legs. No such associations were noted in the group with AHI≥15. Moreover, no such associations were noted when groups answering the four RLS questions were dichotomized into those answer yes to all 4 or <4.

### Discussion

The genomic revolution is a work in progress, providing remarkable developments in methodology, exciting new discoveries, and many disappointments and frustrations. For example, the whole genome association chips have led to numerous new discoveries but also to an appreciation that tens of thousands of subjects may be needed to locate the relevant

---

**Table 2. Association of QIDS-SR with the TEF rs738799 genotype, age, gender, and race**

| Source   | SS     | df | Mean squares | F-ratio | p-value |
|----------|--------|----|--------------|---------|---------|
| TEF rs738799 | 107.140 | 2  | 53.570       | 3.709   | 0.025   |
| Age      | 70.652 | 1  | 70.652       | 4.892   | 0.028   |
| Gender   | 61.431 | 1  | 61.431       | 4.253   | 0.040   |
| Race     | 69.475 | 1  | 69.475       | 4.810   | 0.029   |
| Error    | 5,040.868 | 349 | 14.444     |         |         |

**Table 3. Association of the ESS with APOE rs429358 genotype, age, gender, and race**

| Source   | SS     | df | Mean squares | F-ratio | p-value |
|----------|--------|----|--------------|---------|---------|
| APOE rs429358 | 159.552 | 2  | 79.776       | 3.332   | 0.037   |
| Age      | 0.493  | 1  | 0.493        | 0.021   | 0.886   |
| Gender   | 79.127 | 1  | 79.127       | 3.305   | 0.070   |
| Race     | 230.436 | 1  | 230.436      | 9.624   | 0.002   |
| Error    | 8,428.494 | 352 | 23.945      |         |         |

ESS: Epworth Sleepiness Scale
polymorphisms for complex disorders such as hypertension and diabetes, in which dozens or hundreds of variants may make small contributions to the phenotype. Few of the associations demonstrated by this new technology explain more than a small fraction of each disease phenotype, perhaps because many rare variants rather than a few common variants produce the genetic susceptibility. Distinguished observers have argued that there is little value for individual patients in current genotyping methods for most major disorders. 

Research in a patient sample undergoing clinical polysomnograms had the strength of relevance to patients for whom diagnostic determination and treatment choice was a high priority. Another advantage was clinical support for the diagnostic assessment. Serious limitations of this approach included lack of consistent diagnostic criteria, since the national standards changed during the study, diagnostic uncertainty or multiple diagnoses in many instances, and the variability in the portion of the polysomnogram which could be utilized if split-night CPAP titration procedures were begun. Also, even without CPAP titrations, there is considerable night-to-night variability in polysomnographic measures such as the periodic leg movement index. With a larger patient sample, we might have been able to demonstrate more significant associations of genotypes with sleep phenotypes, but the lower the attributable risk of an association and the larger the sample needed to detect association with a genotype, the less the clinical value of genotype association will be. In the prospectively predicted results, none of the associations were strong enough to be considered statistically conclusive without further replication. Of the post-hoc analyses, none of the nominal findings would have been significant after correction for multiple testing, so they should merely be considered hypothetical relationships needing further replication.

Because depression is commonly comorbid with sleep apnea, insomnia, restless legs, and narcolepsy, depression is very commonly seen among Sleep Clinic patients. A large component of depression is hereditary, so depression might be one of the sleep-disturbing disorders most identifiable by genetic means. Unfortunately, the genetic basis of depression has proved surprisingly elusive, as indicated by the disappointing results of the GAIN whole genome association study, which used over 3,500 subjects. Our result replicating an association of TEF rs738799 with a quantitative trait of depression was therefore of considerable scientific interest, though the level of statistical confidence even combining this and the earlier study was not so great as to make further replication unnecessary. It is possible that rs738799 is not the functional SNP associated with depression, in which case, a search for the functional SNP or a group of functionally significant polymorphisms might yield an association of greater magnitude. By itself, rs738799 might not display sufficient association with depression to make its identification valuable for predicting the usefulness of antidepressant treatment.

Remarkable new findings with RLS and PLMS may promise that genotyping could identify a larger proportion of the risk for these disorders than most whole genome association results for other disorders. To our surprise, we were not able to confirm that genotyping identified a useful proportion of the risk for restless legs or PLMS, but we were only able to genotype two of the several SNPs for which the strongest associations had been reported, and MEISI rs12469063 was technically difficult to genotype in our assay. Part of the explanation for our failure to demonstrate association may be the limited positive predictive value of the four criterion questions which we used to identify restless legs. Another problem may be the interference of sleep apnea with recognizing RLS, since there was a nominally significant association of the RLS questions with MEISI rs12469063 among those participants with lower AHI scores. The retrospective evidence for nominal association with PLMS or restless legs symptoms with three circadian SNPs (CLOCK rs1801260, TEF rs738499, and NRI1 rs2314339), consistent with the strong circadian modulation of restless legs symptoms, might be explained by regulation of dopamine by MAOA, which is under control of the circadian network. Circadian system genetic feedback loops regulate the expression and activity of the CLOCK-ARNTL heterodimer, which binds to an e-box promoter site on the MAOA gene. The greater the expression of MAOA, the faster the degradation of dopamine, thus regulating dopaminergic mediation of restless legs symptoms.

There is as yet little knowledge of the genetic factors in sleep apnea. We could not confirm reports that TNFα and APOE are substantial risk factors for sleep apnea, as some reports have suggested, but our results support the recent meta-analysis which concluded that APOE is not associated with sleep apnea. The SNP rs405509 does not fully define the APOE4 haplotype, but it is very highly associated with Alzheimer’s Disease. As we expand our sample, we will have more participants with no indication of sleep apnea, which may make the discrimination of sleep apnea syndrome stronger. However, since half of the participants had an AHI<15, the sample may have been well-balanced for detecting polymorphisms associated with apnea in need of treatment. We found statistically equivocal evidence that an APOE variant is associated with sleepiness, but the possible association noted was too weak to be of any current practical value. Likewise, we have as yet little knowledge of the genetic factors determining sleep duration, despite its considerable heritability.

In ADORA2, rs5751876 had been reported to influence EEG slow wave amplitude, but it had not been reported to influence either TST or the slow wave sleep percentage. We tested the novel hypotheses that a polymorphism which increases EEG slow waves should increase the percentage of slow wave sleep scored and perhaps the TST, but could not confirm...
these hypotheses. The nominal associations of TEF rs738499 with slow wave sleep percentage and of CLOCK rs1801260 and TNFα rs1800629 with TST should not be regarded as conclusive findings unless they can be replicated. Patel et al. found that TNFα concentrations were higher in the blood of those reporting longer habitual sleep, but TNFα was associated with shorter objective PSG durations.

Examination of four SNPs of the circadian system provided an equivocal confirmation of the influence of rs1801260 in CLOCK, which did not quite satisfy the strictest significance criteria. This previously-reported association has been controversial, but the current results tend to support a weak association. Linkage disequilibrium extends throughout the large CLOCK gene, so this SNP may be linked to the true functional SNP only in certain populations. Elsewhere, we have reported SNPs with a stronger association with the BALM scale. We believe that as knowledge of circadian polymorphisms grows, it may be possible to better recognize the genetic circadian component of sleep disorders.

This study replicated some reported associations of SNP polymorphisms with sleep phenotypes, found other reported associations not to be replicable, and contributed preliminary suggestions of some new associations. These results add to the body of knowledge of genetic polymorphisms which influence sleep. Significance of associations of some of these 10 SNPs with phenotypes might be more conclusively demonstrated with larger samples, e.g., the association of the CLOCK SNP rs1801260 with the BALM, but if more subjects are needed, such associations are likely to explain too little phenotypic variance to be of clinical value by themselves. Our sample was larger than that of most of the papers which originally needed, such associations are likely to explain too little phenotypic variance to be of clinical value by themselves. Our sample was larger than that of most of the papers which originally reported the associations examined. Because so many associations with phenotypes in genetics have proven nonreplicable, failures to replicate associations are informative, as a minimum for highlighting the modesty of effect sizes.

The discovery of the genetic factors in sleep disorders is still in its infancy. New reports of polymorphisms associated with sleep disorders are appearing. For example, our assays were performed before the very recent report relating polymorphisms in a T cell receptor to narcolepsy, and there are many other interesting SNPs we have not yet examined. As the knowledge base expands, many more polymorphisms worth testing will emerge, and more extensive genotyping will become progressively more economically practical as the technology develops. In conditions with complex genetic susceptibility factors, it may become possible to identify a useful amount of genetic susceptibility by assaying much larger numbers of markers. The advancement of genetic knowledge to assist sleep disorders treatment depends on the foresighted collection of still larger DNA samples of several homogeneous racial groups, along with reliable data bases of phenotypes. We remain hopeful that future discoveries will make genotyping useful for the sleep clinician.

Acknowledgments

J. Steven Poceta, MD, Diem Vo, Susan Abel, RN, and Richard Lov- ing, RN, DNeS recruited participants for this research. Pauline Lee, Ph.D. and Jessica Nichols genotyped the Sleep Clinic participants in the Core DNA Laboratory, which was supported by the Stein Endowment Fund at the MEM division of the Scripps Research Institute. The research was supported by Scripps Clinic Academic Funds.

REFERENCES

1. Partinen M, Purkonen PTS, Kaprio J, Koskenvuo M. Hereditary and environmental determination of human sleep length. In: Karger S, ed.-itor. Sleep 1982: 6th European Congress on Sleep Research, Zurich 1982. Basel. 1983, p.206-208.
2. de Castro JM. The influence of heredity on self-reported sleep pat- terns in free-living humans. Physiol Behav 2002;76:479-486.
3. Klei L, Reitz P, Miller M, Wood J, Maendel S, Gross D, et al. Herita-

www.psychiatryinvestigation.org 41
31. Mosko SS, Dickel MJ, Ashurst J. Night-to-night variability in sleep
26. Beutler E, Gelbart T. Large-scale screening for HFE mutations
29. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic varia
28. Kraft P, Hunter DJ. Genetic risk prediction--are we there yet? N Engl
21. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt
20. Hening WA, Allen RP, Washburn M, Lesage SR, Earley CJ. The four
19. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt
18. Levinson DF. The genetics of depression: a review. Biol Psychiatry
14. Possible role for the presynaptic protein piccolo. Mol Psychiatry 2009;
12. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. The 3111T/C
10. Patel SR, Zhu X, Storfer-Isser A, Mehra R, Jenny NS, Tracy R, et al. Sleep duration and biomarkers of inflammation. Sleep 2009;32:200-
8. Thakre TP, Mantani MR, Kulkarni H. Lack of association of the APOE epsilon 4 allele with the risk of obstructive sleep apnea: meta-
7. Hampp G, Ripperger JA, Houben T, Schmutz I, Blex C, Perreaux-Lenz S, et al. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. Curr Biol 2008;18:678-683.
6. Desautels A, Turecki G, Montplaisir J, Brisebois K, Sequeira A, Adam B, et al. Evidence for a genetic association between monoamine oxidase A and restless legs syndrome. Neurology 2002;59:215-219.
5. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. The 3111T/C polymorphism of hClock is associated with evening preference and de-
4. Pedrazzoli M, Louzada FM, Pereira DS, Benedito-Silva AA, Lopez AR, Martynghak BJ, et al. Clock polymorphisms and circadian rhythmicity in a sample of the Brazilian population. Chronobiol Int 2007;24:1-8.
3. Winkelmann J, Schombarb M, Lichtner P, Röpke S, Xiong L, Jalilzadeh S, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 2007;39:1000-1006.
2. Hallmayer J, Faraco J, Lin L, Hesselson S, Winkelmann J, Kawashima M, et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat Genet 2009;41:708-711.
1. Allen RP, Burchell BJ, MacDonald B, Hening WA, Earley CJ. Validation of the self-completed Cambridge-Hopkins questionnaire (CHRLSq) for ascertainment of restless legs syndrome (RLS) in a population survey. Sleep Med 2009;10:1097-1100.

al. MEIS1 intronic risk haplotype associated with restless legs syndrome affects its mRNA and protein expression levels. Hum Mol Genet 2009;18:1065-1074.
18. Brown FM. Psychometric equivalence of an improved Basic Language Morningness (BALM) scale using industrial population within comparisons. Ergonomics 1993;36:191-197.
19. Rush AJ, Trivedi MH, Ibrahim HM, Carmedy TJ, Arnow B, Klein DN, et al. The 16-item Quick Inventory of Depressive Symptomatology (QIDS), clinical rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. Biol Psychiatry 2003;54:573-583.
20. Rush AJ, Bernstein IH, Trivedi MH, Carmedy TJ, Wisniewski S, Mundt JC, et al. An evaluation of the quick inventory of depressive symptomatology and the hamilton rating scale for depression: a sequenced treatment alternatives to relieve depression trial report. Biol Psychiatry 2006;59:493-501.
21. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-545.
22. Kushida CA. Clinical presentation, diagnosis, and quality of life issues in restless legs syndrome. Am J Med 2007;120:S4-S12.
23. Hening WA, Allen RP, Washburn M, Lesage SR, Earley CJ. The four diagnostic criteria for Restless Legs Syndrome are unable to exclude confounding conditions ("mimics"). Sleep Med 2009;10:976-981.
24. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J. Restless Legs Syndrome Diagnosis and Epidemiology workshop at the National Institutes of Health: International Restless Legs Syndrome Study Group. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4:101-119.
25. Beutler E, Gelbart T. Large-scale screening for HFE mutations: methodology and cost. Genet Test 2000;4:131-142.
26. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med 2009;360:1759-1768.
27. Goldstein DB. Common genetic variation and human traits. N Engl J Med 2009;360:1696-1698.
28. Kraft P, Hunter DJ. Genetic risk prediction--are we there yet? N Engl J Med 2009;360:1701-1703.
29. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. Nat Rev Genet 2009;10:241-251.
30. Mosko SS, Dickel MJ, Ashurst J. Night-to-night variability in sleep apnea and sleep-related periodic leg movements in the elderly. Sleep 1988;11:340-348.
31. Levinson DF. The genetics of depression: a review. Biol Psychiatry 2006;60:84-92.
32. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, et al. Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry 2009;14:359-375.
33. Winkelmann J, Schombarb M, Lichtner P, Röpke S, Xiong L, Jalilzadeh S, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 2007;39:1000-1006.
34. Hallmayer J, Faraco J, Lin L, Hesselson S, Winkelmann J, Kawashima M, et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat Genet 2009;41:708-711.
35. Allen RP, Burchell BJ, MacDonald B, Hening WA, Earley CJ. Validation of the self-completed Cambridge-Hopkins questionnaire (CHRLSq) for ascertainment of restless legs syndrome (RLS) in a population survey. Sleep Med 2009;10:1097-1100.
36. Desautels A, Turecki G, Montplaisir J, Brisebois K, Sequeira A, Adam B, et al. Evidence for a genetic association between monoamine oxidase A and restless legs syndrome. Neurology 2002;59:215-219.
37. Hampp G, Ripperger JA, Houben T, Schmutz I, Blex C, Perreaux-Lenz S, et al. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. Curr Biol 2008;18:678-683.
38. Thakre TP, Mantani MR, Kulkarni H. Lack of association of the APOE epsilon 4 allele with the risk of obstructive sleep apnea: meta-analysis and meta-regression. Sleep 2009;32:1507-1511.
39. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer’s disease. Nat Genet 2009;41:1088-1093.
40. Patel SR, Zhu X, Storfer-Isser A, Mehra R, Jenny NS, Tracy R, et al. Sleep duration and biomarkers of inflammation. Sleep 2009;32:200-
41. Robilliard DL, Archer SN, Arendt J, Lockley SW, Hack LM, English J, et al. The 3111 Clock gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. J Sleep Res 2002;11:305-312.
42. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. Am J Med Genet B Neuropsychiatr Genet 2005;133B:101-104.
43. Pedrazzoli M, Louzada FM, Pereira DS, Benedito-Silva AA, Lopez AR, Martynghak BJ, et al. Clock polymorphisms and circadian rhythmicity phenotypes in a sample of the Brazilian population. Chronobiol Int 2007;24:1-8.
44. Friedman L, Zeitzer JM, Kushida C, Zdanavova I, Noda A, Lee T, et al. Scheduled bright light for treatment of insomnia in older adults. J Am Geriatr Soc 2009;57:441-452.
45. Johansson C, Willeit M, Smedh C, Ekholm J, Paunio T, Kieseppa T, et al. Circadian-clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. Neuropsychopharmacology 2003;28:734-739.
46. Ioannidis JP. Why most published research findings are false. PLoS Med 2005;2:e124.
47. Hirshhorn JN, Lehmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002;4:45-61.