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SCIENTIFIC INVESTIGATIONS

Genetic risk for subjective reports of insomnia associates only weakly with polygraphic measures of insomnia in 2,770 adults

Jonathan Foldager, MS[1,2,3]; Paul E. Peppard, PhD[4]; Erika W. Hagen, PhD, MS[4]; Katie L. Stone, PhD[5]; Daniel S. Evans, PhD, MPH[5]; Gregory J. Tranah, PhD, MS[5]; Helge Sørensen, PhD[2]; Poul Jennum, MD, DMSc[6]; Emmanuel Mignot, MD, PhD[3]; Logan D. Schneider, MD[3,7,8]

[1]Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kgs. Lyngby, Denmark; [2]Department of Health Technology, Technical University of Denmark, Kgs. Lyngby, Denmark; [3]Stanford Center for Sleep Sciences and Medicine, Stanford University, Palo Alto, California; [4]Department of Population Health Sciences, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, Wisconsin; [5]Research Institute, California Pacific Medical Center, San Francisco, California; [6]Danish Center for Sleep Medicine, Department of Clinical Neurophysiology, Rigshospitalet, Glostrup, Denmark; [7]Stanford/VA Alzheimer's Research Center, Palo Alto, California; [8]Sierra Pacific Mental Illness Research Education and Clinical Centers, VA Palo Alto Health Care System, Palo Alto, California

Study Objectives: Subjective insomnia complaints and objective sleep changes are mostly studied outside of clinical trial studies. In this study, we tested whether 240 genetic variants associated with subjectively reported insomnia were also associated with objective insomnia parameters extracted from polysomnographic recordings in three studies.

Methods: The study sample (total n = 2,770) was composed of the Wisconsin Sleep Cohort (n = 1,091) and the Osteoporotic Fractures in Men (n = 1,026) study, two population-based studies, and the Stanford Sleep Cohort, a sleep center patient-based sample (n = 653). Seven objective polysomnographic features related to insomnia defined outcome variables, with each variant allele serving as predictor. Meta-regression was performed, accounting for common confounders as well as variance differences between studies. Additionally, a normalized genetic risk score was generated for each subject to serve as a predictor variable in separate linear mixed models assessing objective insomnia features.

Results: After correction for multiple testing, single-nucleotide polymorphisms associated with subjective insomnia were not significantly associated with 6 of 7 objective sleep measures. Only periodic limb movement index was significantly associated with *rs113851554* (*MEIS1*), as found in previous studies. The normalized genetic risk score was only weakly associated with arousal index and duration of wake after sleep onset.

Conclusions: Our findings suggest that subjective insomnia does not have a strong genetic signature mapping onto objective (polysomnographic) sleep variables.

Keywords: insomnia, polysomnography, single-nucleotide polymorphism

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Distinctions between subjective and objective insomnia and combinations of the two are rarely addressed. Large-scale studies of the genetic architecture of insomnia have predominantly relied upon subjective reports, as a matter of practicality, but it is unclear whether these findings are associated with objective polysomnographic metrics.

Study Impact: Few of the genetic associations with subjective insomnia were confirmed when attempting to replicate previous findings with objective insomnia measures obtained from polysomnography. Our results suggest that future studies should account for both objective and subjective insomnia-related findings.

INTRODUCTION

Insomnia, defined as unrefreshing sleep, difficulty falling asleep, difficulty staying asleep, or early morning awakening resulting in daytime fatigue, is the second most prevalent mental disorder.[1] Acute insomnia is the most common type of insomnia,[2] and treatment is not always required, whereas treatment of chronic insomnia is recommended.[3] The *International Classification of Sleep Disorders*, third edition[4] did away with differentiation between the previously defined 8 subtypes of insomnia, which were primarily based on "humanly observable" differences, distinguishing now only between early, middle, and late insomnia. It is notable, however, that none of the updated or prior categorizations relies upon sleep changes quantified by objective methods such as polysomnography (PSG). More recently, subtypes of insomnia have been defined based on life history and personality traits.[5]

Quantitative parameters of insomnia have been used in the description of the disorder, but, as mentioned above, subjective patient reports remain key to diagnosis.[6] PSG quantitative features, however, are more commonly used in the context of US Food and Drug Administration–approved pharmacological studies in addition to subjective assessments.[7] As a consequence, the subtypes of insomnia are merely based on what patients report and additional objective evidence is only recommended for diagnostic management and to identify possible confounders.[4] The complexity of the condition can therefore simply be revealed by the fact that some individuals experience objectively short sleep times but have no sleep complaints or daytime dysfunction, while others reporting to be

sleep-deprived turn out to have normal sleep when assessed objectively.[8] This leads to the question of whether insomnia is a symptom of sleep dissatisfaction rather than a disease of insufficient sleep.[9] Moreover, insomnia is highly likely to be observed with a variety of other psychiatric comorbidities such as depression and anxiety,[10] making controlled experimentation challenging.

The current definition of insomnia and understanding of its relation to objective sleep changes raises the question as to whether sleep experts are not yet able to consistently and objectively define insomnia, or whether insomnia is a complaint that relates more to mental perception and other comorbidities rather than objective changes. Nevertheless, recent advances within genetics open up possibilities to correlate subjective sleep reports/perceptions, objective sleep macroarchitecture, and individual genetic risk profiles. An example of this comes from Jansen et al,[11] who recently studied a population of over 1.3 million individuals, revealing > 200 genetic risk loci related to subjectively reported insomnia. Of interest was the fact that loci found in this study overlapped mostly with self-reported short sleep duration, restless legs syndrome (RLS), and with psychiatric traits such as anxiety and depression. Because of the aforementioned dissociation between subjective and objective sleep, it is uncertain whether these genetic associations would remain when assessed in relation to objective measurements of sleep quality and quantity, such as changes in the macroarchitecture of the PSG.

In this study we aimed to examine the association between objective, quantitative parameters extracted from PSG recordings and previously identified genetic risk loci implicated in subjective insomnia complaints—specifically, 240 independent single-nucleotide polymorphisms (SNPs) representing newly discovered as well as previously reported findings, as documented by Jansen et al.[11] While replication attempts have been made with actigraphic data,[12] which are only partially reflective of PSG (overestimating sleep and underestimating wake[13]), to our knowledge this is the first study of this size in which both polysomnographic and genetic data were able to explore this question.

METHODS

This study was approved by the Stanford Institutional Review Board and the institutional review boards of the respective study sites. All participants gave written informed consent for usage of their data. Consistent with other sleep-based analyses of these cohorts, this study includes the first visit from Wisconsin Sleep Cohort[14] (WSC, n = 1,091), the second sleep visit from Osteoporotic Fractures in Men[15,16] (MrOS, n = 1,026), and the first visit from the Stanford Sleep Cohort[17] (SSC, n = 653). Only one visit from each cohort was used to simplify modeling. We included the second visit for MrOS, as this included questionnaires on insomnia, depression, and anxiety. The study procedures for the WSC and MrOS cohorts have been described elsewhere, and only relevant aspects will be detailed, in brief, alongside the details of the SSC.

Assessment of psychiatric disorders

Anxiety and depression were assessed differently between the three cohorts. In the clinically based SSC, diagnoses were based on chart review of doctor's notes and patient reports, ie, if either the patient reported symptoms of or the doctor's notes documented anxiety or depression. Based on the time at which these cohorts were established, common *International Classification of Diseases, Ninth Revision* (ICD-9) codes used in the specification of anxiety or depressive disorders were also used. The WSC accounted for individuals who had been clinically diagnosed with anxiety and depression or treated for these conditions. Finally, for MrOS, the Goldberg Anxiety Scale[18] and the Geriatric Depression Scale[19] were used to derive estimates of prevalent anxiety (≥ 5) and depressive symptoms (≥ 5), respectively. Additionally, we accounted for whether individuals were on antidepressant/anxiolytic medications at the time of the visit, as an additional means for determining whether an individual had a diagnosis of depression or anxiety. Data were taken from the visit associated with the sleep study used for analysis.

Assessment of sleep disorders

Sleep disorder assessments related to the relevant PSG data were gathered using methods similar to those used in defining anxiety and depression. Chart reviews focusing on relevant ICD-9 codes (indicative of insomnia or RLS) as well as free-text physicians' notes were used for determining the presence/absence of insomnia and/or RLS in the SSC. The determination of insomnia in the WSC was predicated on survey responses indicating at least one of four following insomnia complaints at least five times per month: (1) difficulty falling asleep, (2) waking repeatedly, (3) waking too early in the morning, and (4) waking at night and inability to go back to sleep. Additionally, participants in the WSC were identified as having RLS symptoms based on their responses to questions regarding how often, when sitting or lying down, the individual experienced (a) a repeated urge to move the legs, (b) strange and uncomfortable feelings in the legs, and (c) the duration of several leg jumps or jerks. If replying with anything other than "never" for all 3 prompts, 2 subsequent questions were asked: (1) Do these feelings get better when you start walking? and (2) Do these feelings disrupt your sleep? Similar to our work and other groups' prior studies in this cohort,[20] individuals reporting "never" or "less than once a month" for prompts a–c were classified as not having RLS symptoms, while all other responses were classified as RLS symptoms, and any individual with incomplete responses was classified as missing data. In the MrOS cohort, insomnia symptoms were defined as having insomnia severity index ≥ 7, whereas RLS symptoms were defined by a 2-step question sequence: "Has a doctor or health care provider ever told you that you have a sleep disorder other than sleep apnea?," which was followed by a 5-item list (including "restless legs") if the primary question was answered affirmatively.[21] Finally, we also included whether the patient had RLS symptoms. Daytime sleepiness was assessed in all 3 cohorts, using the standard Epworth Sleepiness Scale.[22]

Polysomnographic phenotypes

PSG was collected and staged/scored according to clinically accepted standards.[23–25] A set of standard, easily interpretable features from PSG was extracted: total sleep time, sleep onset

latency, sleep efficiency, wake after sleep onset, arousal index, periodic limb movement index (PLMI), and apnea-hypopnea index, as well as time and percentage in each sleep stage. All features were computed from manual annotations in the time period from lights off to lights on, except for arousal index in SSC and WSC, for which a validated, automatic, deep-learning tool developed by Brink-Kjaer et al[26] was used due to the lack of manual annotations.

Genetic models

Genotyping was performed in each cohort using commercially available genome-wide arrays: Illumina HumanOmni1_Quad_v1-0 H in the MrOS cohort[27], Affymetrix 6.0 and Affymetrix 500K DualGeneChip+ in the WSC, and Affymetrix 6.0 in the SSC. All raw genotypes were brought to the same build—b37/hg19—using University of California Santa Cruz's liftOver resource (http://genome.ucsc.edu/).[28] Subsequently, all raw genotypes were subjected to conventional quality control (QC) methods including filtering for genotyping call rate > 90%, minor allele frequency > 5%, and nondivergence from Hardy–Weinberg equilibrium ($P > 10^{-6}$) in controls. In each cohort-specific QC, multiple steps of genotyping missingness were used to remove inadequately genotyped samples. Prior to minor-allele frequency thresholding, individuals were removed if there was > 10% missingness in the genotype. Following minor-allele frequency thresholding a stricter removal of missing genotypes was applied, such that samples with > 3% genotype missingness were removed. Additionally, samples were assessed for cryptic relatedness and removed if $\hat{\pi} > 0.5$. Using the Wellcome Trust's SHAPEIT and IMPUTE2 software pipeline, all genotypes were subsequently phased and imputed to the 1000 Genomes Phase 1 reference panel. Postimputation QC was, again, performed, filtering for a minor allele frequency > 5% and only markers that were of high quality (ie, info score > 0.9) in all of the imputed data were retained. After imputation and QC the allele dosages of any of the remaining 240 SNPs of interest were extracted using PLINK v1.90 for usage in SNP-based meta-regressions, whereas only preimputed, QCed, raw genotypes (ie, actual allele calls rather than imputed alleles) were used in the construction of the normalized genetic risk score (detailed below).

Statistical models

We assumed an additive relationship between each dose of a given allele and the phenotype of interest and thus performed a study-level meta-regression with linear mixed models, in order to adjust for common confounders while also accounting for study-specific factors that may have influenced the variance.[29] As such, in addition to modeling the phenotypes from fixed effects (SNP dosage, age, body mass index, and sex), a number of random effects are introduced to account for systematic bias introduced by intercohort variations, specifically study site (in the case of MrOS), cohort (WSC vs SSC vs MrOS), and the first 5 dimensions from cohort-specific multidimensional scaling analyses (dimensions nested within cohort variables) in order to account for population stratification. One set of beta coefficients is fitted for each SNP and the corresponding *P* value is obtained to explore if the SNP significantly associated with the phenotype, after accounting for multiple comparisons using the Bonferroni method.[30]

Since our QC of the genetic data resulted in 4% missing data on average, with a handful of individuals having > 20% missing allele information, using only genotyped (rather than probabilistically imputed) SNPs we calculated a normalized genetic risk score (nGRS) as the fraction of an individual's SNP dosage multiplied by the insomnia-positive effect from Jansen et al[11] and divided by the maximum possible risk (ie, from 2 doses of all alleles), as in the following equation:

$$\text{nGRS} = \frac{\sum_i \beta_i \chi_i}{\sum_{i'} 2\beta_{i'}}.$$

Here, β_i is the i'th SNP effect in the positive direction and x_i and n are the allele dosage for the n'th individual on the i'th SNP. This nGRS allows us to compare individuals with differing amounts of missing data. Study-level meta-regression was, again, performed for all of the outcome variables of interest with the nGRS along with age, sex, and body mass index as fixed effects, as well as random effects for study site (for MrOS), cohort, and, in order to account for population stratification per cohort, the first 5 dimensions of multidimensional scaling coefficients nested within the cohort variable.

In recognition of the multiplicity of tests run in genetic analyses, we performed a priori power and sample size calculations to estimate our expected likelihood of externally verifying the insomnia–SNP associations, under the assumption of a 100% base rate of positivity within the 240 previously associated SNPs. For the binary outcome of insomnia, the University of Michigan CaTS power calculator[31] was used with the following assumptions: 1,013 cases, 1,572 controls, 33% population prevalence,[32] a multiplicative model, and a significance level of $\alpha = 0.05/240 = 2*10^{-4}$, which suggested sufficient power to detect multiple SNPs over the range of expected allele frequencies (0.05–0.95) and genotype relative risks (1.00–1.91) reported by Jansen et al[11] (see **Figure S1** in the supplemental material). For the continuous phenotypes, the *genpwr* package in R v4.0.3 (R Project for Statistical Computing, Vienna, Austria)[33] was used to determine, for an additive genetic model, what beta coefficient effect sizes would be needed to achieve 80% power at a per-SNP significance threshold of $\alpha = 0.05$, given the population sample size, as well as the mean and standard deviation of the sleep feature. Based on this, very reasonable effect sizes were estimated to be sufficient for each objective sleep feature across the range of effect allele frequencies (see **Figure S2**). Thus, we believe that, with our sample sizes, there is sufficient power to detect meaningful findings and, therefore, negative findings can be relied upon in their confirmation of the null hypothesis, ie, no significant association between the genetic factor (SNP or nGRS) and the outcome phenotype.

RESULTS

A summary table of all variables is provided in **Table 1**. Comparisons of relevant differences between individuals with insomnia

Table 1—Comparison of variables between individuals who reported subjective symptoms consistent with insomnia vs those who did not.

	Insomnia μ ± SEM or n (%)	No Insomnia μ ± SEM or n (%)	OR (95% CI)	B	Partial η^2	*P*
	n = 1,013 (39%)	**n = 1,572 (61%)**				
Age	65.73 ± 0.4 (n = 1,013)	62.98 ± 0.49 (n = 1,568)	1.01 (1.00, 1.02)	.39	.00	1.13E-01
BMI	30.37 ± 0.22 (n = 977)	27.71 ± 0.15 (n = 1,482)	1.01 (1.00, 1.03)	.18	.00	1.24E-01
Sex (female)	666 (66%) (n = 1,013)	365 (23%) (n = 1,572)	0.76 (0.58, 1.01)	−.25	.00	5.56E-02
ESS	**8.88 ± 0.13 (n = 1,011)**	**8.08 ± 0.12 (n = 1,571)**	**1.07 (1.04, 1.10)**	**.62**	**.01**	**1.15E-07**
SOL	19.54 ± 0.74 (n = 835)	12.67 ± 0.5 (n = 1,375)	1.01 (1.00, 1.01)	.32	.00	4.68E-03
TST	354.66 ± 2.21 (n = 1,013)	357.25 ± 1.9 (n = 1,571)	1.00 (1.00, 1.00)	−.31	.00	4.82E-03
ArI	23.74 ± 0.37 (n = 1,006)	21.38 ± 0.27 (n = 1,562)	1.01 (1.00, 1.02)	.24	.00	1.82E-02
N1%	0.11 ± 0 (n = 912)	0.13 ± 0 (n = 1,533)	0.94 (0.27, 3.26)	−.01	.00	9.23E-01
N1	38.19 ± 0.79 (n = 912)	45.1 ± 1.03 (n = 1,533)	1.00 (0.99, 1.00)	−.18	.00	2.21E-01
N2%	0.65 ± 0 (n = 912)	0.63 ± 0 (n = 1,533)	1.46 (0.74, 2.87)	.13	.00	2.77E-01
N2	231.1 ± 1.91 (n = 912)	226.13 ± 2.05 (n = 1,533)	1.00 (1.00, 1.00)	−.23	.00	8.96E-02
N3%	0.07 ± 0 (n = 912)	0.09 ± 0 (n = 1,533)	0.73 (0.18, 2.98)	−.06	.00	6.64E-01
N3	24.33 ± 0.91 (n = 912)	31.94 ± 1 (n = 1,533)	1.00 (0.99, 1.00)	−.14	.00	2.99E-01
REM%	0.17 ± 0 (n = 912)	0.18 ± 0 (n = 1,533)	0.24 (0.05, 1.13)	−.21	.00	7.04E-02
REM	60.97 ± 0.94 (n = 912)	64.92 ± 0.77 (n = 1,533)	0.99 (0.99, 1.00)	−.31	.00	7.06E-03
WASO	92.11 ± 1.94 (n = 1,013)	104.03 ± 1.68 (n = 1,571)	1.00 (1.00, 1.00)	.35	.00	2.59E-03
SE	**0.77 ± 0 (n = 1,013)**	**0.88 ± 0.07 (n = 1,572)**	**0.23 (0.10, 0.53)**	**−7.10**	**.01**	**5.36E-04**
AHI	9.07 ± 0.38 (n = 1,013)	15.27 ± 0.41 (n = 1,571)	1.00 (0.99, 1.01)	−.01	.00	9.46E-01
PLMI	12.15 ± 0.6 (n = 1,013)	8.7 ± 0.47 (n = 1,563)	1.00 (1.00, 1.01)	.14	.00	1.61E-01
RLS	**664 (66%) (n = 1,011)**	**199 (13%) (n = 1,484)**	**2.30 (1.80, 2.94)**	**.70**	**.02**	**3.72E-11**
Antidepressant	794 (82%) (n = 973)	180 (12%) (n = 1,552)	1.41 (1.04,1.92)	.25	.00	2.78E-02
Depression	**557 (62%) (n = 905)**	**280 (18%) (n = 1,533)**	**2.51 (1.99, 3.16)**	**.84**	**.03**	**8.39E-15**
Anxiety	**821 (84%) (n = 979)**	**32 (2%) (n = 1,557)**	**5.31 (3.40, 8.29)**	**.91**	**.02**	**1.95E-13**

Using a dichotomous outcome variable for insomnia, we perform logistic regression with each predictor variable, adjusting for relevant confounders. Marked in bold are the Bonferroni-adjusted significant ($\alpha = 0.05/23 = 2.17*10^{-3}$) coefficients from the adjusted linear mixed model. All sleep-stage variables are excluded in further analysis to maximize level of significance. B represents the standardized beta coefficient. Sample sizes differ between metrics based on data availability. AHI = apnea-hypopnea index, ArI = arousal index, BMI = body mass index, CI = confidence interval, ESS = Epworth Sleepiness Scale, N1 (%) = N1 sleep time (percent), N2 (%) = N2 sleep time (percent), N3 (%) = N3 sleep time (percent), OR = odds ratio, PLMI = periodic limb movement index, REM (%) = rapid eye movement sleep time (percent), RLS = restless legs syndrome, SE = sleep efficiency, SOL = sleep onset latency, TST = total sleep time, WASO = wake after sleep onset.

and those without were assessed using a linear mixed model with a binomial response variable for insomnia. In order to account for confounding, cohort and site-specific random effects were incorporated into the modeling of fixed effects for age, sex, and body mass index, whereas all other variable comparisons were adjusted for age, sex, and body mass index, again with cohort and site contributing random effects to the models.

From these comparisons, the only statistically significant differences between individuals with insomnia and those without were increased levels of self-reported daytime sleepiness (Epworth Sleepiness Scale of 8.88 ± 0.13 vs 8.08 ± 0.12, $P = 1.15*10^{-7}$) and lower sleep efficiency (77% ± 0% vs 88% ± 7%, $P = 5.36*10^{-4}$). Similarly, individuals with reports of RLS-like (odds ratio 2.30, $P = 3.72*10^{-11}$), depressive (odds ratio 2.51, $P = 8.39*10^{-15}$), or anxiety symptoms (odds ratio 5.21, $P = 1.95*10^{-13}$) were substantially more likely to meet criteria for insomnia.

While not statistically significant, some of the objective sleep features demonstrated expected differences between individuals with insomnia and those without, despite a lack of statistical significance (namely the percent of total sleep time spent in N2, N3, or rapid eye movement), whereas total sleep time, percent of total sleep time in N1 sleep, sleep onset latency, and wake after sleep onset lacked clinically meaningful differences between the groups, in addition to the lack of statistical significance. As no sleep stage variables were significantly different between individuals with insomnia and those without, they were not included in subsequent analyses, due to the multiplicative increase in the number of comparisons. Finally, while not satisfying the adjusted significance threshold, women were noted to have 32% higher odds of having insomnia when compared to males, which is quite close to what prior studies report.[34] This analysis is replicated for each individual cohort with similar results, as can be seen in **Table S3**, **Table S4**, and **Table S5**. It should, however, be noted that prevalence of sleep and mental disorders obtained in WSC and MrOS are quite similar to each other, not surprisingly considering their similar demographics and ascertainment methods, while these are lower in the SSC, these being based on presence of clinical diagnosis.

Genetic associations with objective insomnia measures

In **Table 2**, the 5 most significantly associated (ie, lowest *P* value) SNPs derived from adjusted study-level meta-regression models assessing each of the 240 SNPs for each outcome measure independently.

No association was found between any of the previously reported SNPs and the presence of insomnia, even in light of a priori power estimates that indicated a reasonable likelihood of replicating at least a handful of SNPs over a range of allele frequencies and genotype relative risks. Given a maximum effect size of about 25% for the nonsignificant SNP associations with insomnia (see **Tables S6**), it is surprising that none of these existing hits was replicated. Also, despite reasonable power estimates regarding the ability to verify the previously reported SNPs (see **Figure S2**), only one of the 240 SNPs had an association, namely rs113851554 (*MEIS1*) and PLMI, which has been previously shown by Moore et al[35] and Winkelman et al[36] in two of these cohorts. The full list of associated SNPs for both meta-level regression and individual cohorts can be seen in **Table S7**, **Table S8**, **Table S9**, and **Table S10**.

Relationships between the nGRS and each phenotype demonstrated a similar paucity of associations with all of the outcome phenotypes of interest (**Table 3**). While all of the associations were in the expected direction, the only objective outcome phenotypes that were significantly associated with the nGRS were the arousal index (with an increase of 0.34 events/h for every 1% increase in nGRS, $P = 1.20*10^{-3}$) and wake after sleep onset (with an increase in 1.72 minutes for every 1% increase in nGRS, $P = 2.67*10^{-3}$). Given that it is somewhat hard to conceptualize what the normalized score effect size is, the "Max Δ" was provided in the table to express the greatest difference in phenotypic variables over the maximum range of normalized genetic risk scores: 0.411–0.567 (see **Figure S3** and **Figure S4**), where an nGRS score of 0 indicates no risk alleles and a score of 1 indicates a double dose of the risk allele at all 240 loci. Notably, the odds of insomnia increased by only 7% when considering the individual with the maximum number of risk alleles compared to the individual with the minimum number of risk alleles. Cohort-by-cohort analyses of nGRS are provided in **Table S11**.

DISCUSSION

In an effort to reconcile the clinically apparent discrepancies between subjective reports of insomnia and objective PSG features that reflect insomnia,[8] we sought to expand upon recent advances in genetic associations with subjective insomnia afforded by large cohort sizes,[11] by exploring the reported genetic associations of subjective insomnia with objectively defined PSG signs of insomnia. Despite the hypothesis that at least some of the SNPs associated with subjectively reported insomnia would replicate in this more targeted analysis (correcting for a smaller number of SNPs and/or associating with stronger, objective phenotypes), we were unable to confirm strong associations of any of the SNPs with either clinically defined insomnia or most of the objective polysomnographic features classically considered to be consistent with insomnia (eg, prolonged sleep onset latencies). Nonetheless, we were able to confirm prior findings associating the *MEIS1* polymorphism, rs113851554, with periodic limb movements of sleep[35,36] through SNP-wise regression. Additionally, the nGRS–objective phenotype associations were all in the correct direction, with 2 objective measures of insomnia—arousal index and wake after sleep onset—reaching statistical significance. Together these findings suggest that the methods chosen are robust.

Among the 3 cohorts in this study, a number of aspects differed between those with insomnia and those without (**Table 1**). Expectedly, the prevalence of RLS symptoms, depression, and anxiety was higher in the insomnia group. Additionally, it was interesting to note that few other traditional measures of insomnia, aside from lower sleep efficiency, were found to have clinically or statistically meaningful associations with the presence of insomnia. Comparatively, the higher Epworth Sleepiness

Table 2—Each phenotype with its corresponding top 5 most significant SNPs.

SNP	CHR	A1	A2	R^2*	Estimate	*P*	SNP	CHR	A1	A2	R^2*	Estimate	*P*
Insomnia (Yes/No)					**OR**		**Arousal Index (events/h)**					**β**	
rs7615602	3	C	G	.01	1.24	7.38E-03	rs61765555	1	T	C	.13	1.33	7.89E-04
rs17005118	4	A	G	.01	1.26	7.49E-03	rs28552587	8	G	A	.12	−1.07	1.08E-03
rs17223714	5	G	A	.01	0.78	1.11E-02	rs11119409	1	C	T	.12	.87	7.19E-03
rs34104813	4	T	C	.01	1.2	2.36E-02	rs4592425	11	G	T	.12	−.96	7.41E-03
rs1580173	3	G	A	.01	0.84	2.55E-02	rs9964420	18	A	C	.12	0.89	1.27E-02
Periodic Limb Movement Index (events/h)					**β**		**Total Sleep Time (min)**					**β**	
rs113851554	**2**	**T**	**G**	**.05**	**8.68**	**1.99E-09**	rs4260410	3	T	C	.11	7.17	1.78E-03
rs35110063	3	G	A	.04	1.84	1.72E-03	rs623025	1	T	C	.11	−6.48	5.80E-03
rs12187443	5	C	T	.04	−1.62	7.12E-03	rs7306710	12	C	T	.1	3.87	7.65E-03
rs3902952	16	T	C	.03	1.86	8.41E-03	rs4744240	9	T	C	.11	5.94	7.80E-03
rs62068188	17	C	T	.03	2.36	8.66E-03	rs5877	1	C	T	.1	−5.68	8.68E-03
Sleep Efficiency (%)					**β**		**Wake after Sleep Onset (min)**					**β**	
rs16967103	15	C	T	0	0.09	7.52E-03	rs871994	8	A	C	.15	4.66	8.34E-03
rs34592089	4	A	G	0	−0.18	8.17E-03	rs34104813	4	T	C	.15	4.34	2.11E-02
rs6967168	7	G	T	0	−0.09	9.64E-03	rs28552587	8	G	A	.15	−4.04	2.40E-02
rs2216427	3	G	C	0	0.08	1.38E-02	rs1861412	2	A	G	.15	3.99	2.43E-02
rs4792858	17	C	A	0	−0.07	1.59E-02	rs2806933	13	A	C	.14	4.14	2.53E-02
Sleep Onset Latency (min)					**β**								
rs9964420	18	A	C	.01	−1.85	2.85E-03							
rs16990210	4	C	T	.01	−2.44	3.17E-03							
rs9527083	13	G	A	.01	1.66	4.56E-03							
rs4547518	2	T	C	.01	−1.51	1.15E-02							
rs12724444	1	A	G	.01	−2.93	1.47E-02							

Only rs113851554 (marked in bold) when predicting periodic limb movement index is below the Bonferroni-adjusted threshold ($2.97*10^{-5}$, after accounting for 7*240 tests). All analyses were adjusted for age, sex, body mass index, and site/cohort. *R^2 marginal measures the explanatory value of the fixed effects in the model. Abbreviations: A1 - first (usually minor) allele at the position, A2 - second allele at the position, CHR - chromosome, OR - odds ratio, SNP - single nucleotide polymorphism (indicated by rsID).

Table 3—Effect sizes and *P* values of normalized genetic risk score (nGRS) as a predictor for each phenotype.

Phenotype	Estimate	Max Δ	$R^2_{marginal}$	*P*
Insomnia (odds)	1.00*	1.07	.01	8.65E-01
PLMI (events/h)	0.31	4.84	.03	9.04E-02
SE (%)	−.02	−.28	.00	1.15E-01
SOL (min)	.20	3.17	.01	2.59E-01
ArI (events/h)	**.34**	**5.31**	**.12**	**1.20E-03**
TST (min)	−1.15	−17.91	.11	9.04E-02
WASO (min)	**1.72**	**26.90**	**.15**	**2.67E-03**

One percentage point increase in nGRS leads to the expected change in response variable. Max Δ represents the maximum phenotypic difference from individuals with the highest nGRS (0.567; ie, the most risk alleles) and the lowest nGRS (0.411; ie, the least risk alleles). The Bonferroni-adjusted threshold for statistical significance was α = .05/7 = 0.007, with statistically significant associations indicated in bold. *Estimate is the odds ratio. ArI = arousal index, PLMI = periodic limb movement index, SE = sleep efficiency, SOL = sleep onset latency, TST = total sleep time, WASO = wake after sleep onset.

Scale score was an unexpected finding, possibly suggesting that the characterization of insomnia across these cohorts may still not reflect the "tired but wired" phenotype that classically defines a primary insomnia.

The replication of prior findings of association between the *MEIS1* polymorphism, rs113851554, and periodic limb movements demonstrates that even with small sample sizes (from a genetic association study standpoint) phenotypic refinement

and focused genetic exploration can improve statistical power, even crossing the threshold of genome-wide significance ($5*10^{-8}$). Although it has been reported before,[37] and has been integrated into some models of sleep-state instability in insomnia,[38] PLMs are not commonly evaluated in insomnia patients. However, the role that PLMs play in insomnia is not quite clear. While increased PLMI is primarily mentioned in the context of sleep fragmentation and daytime sleepiness—a controversial disorder called periodic leg movement disorder—the clinical import of PLMs outside of RLS remains quite nebulous. In trying to explore the RLS-independent effects of PLMs, Leary et al[20] found significant interactions between daytime sleepiness measures in individuals with PLMs with and without RLS symptoms, wherein individuals with elevated PLMs but no symptoms of RLS were more alert, albeit at the trend level of statistical significance. Also, in a recent study by El Gewely et al,[39] carefully phenotyped individuals with insomnia without RLS had fewer PLMs than individuals with chronic insomnia symptoms with RLS: 13/h (normal for the age of the population studied) vs 31/h, respectively. While there was no exploration of PLMs in an insomnia-free cohort in this study, this may suggest that RLS may constitute a subtype of insomnia that develops in susceptible individuals with PLMs. This overlap may be highlighted by the shared genetic association of *MEIS1*[12,40] with both insomnia and RLS and suggests the need for further explorations of the role of PLMs, with or without symptoms of RLS, in individuals with insomnia.

Despite most polysomnographic phenotypes lacking strong associations with the presence of insomnia—as defined by these cohorts—and lacking replicable SNP-wise genetic associations in all instances but the aforementioned PLMI–*MEIS1* case, the nGRS was significantly associated with two objective insomnia features. While all nGRS associations were in the expected direction—with higher normalized genetic risk scores being noted alongside more severely disturbed sleep features—individuals in the cohort with the greatest genetic risk (ie, highest nGRS of 0.567) relative to those with the least risk (ie, lowest nGRS of 0.411) had 5.31 more arousals per hour and 26.90 more minutes of wake after sleep onset. It is notable that the 2 features that are most likely to suggest disruptions of the sleep period—arousals and waking bouts—were the only objective features to find significant associations with SNPs derived from a genetic analysis of subjective insomnia symptoms. In general, these findings further support the notion that these analyses were, in fact, looking at features that should associate with genetic factors that contribute to symptomatic insomnia but may also highlight that any given SNP or combination of SNPs truly contributes only modestly to the complex clinical phenotype of insomnia. The lack of strong association of our nGRS with the objective phenotypes of interest is in line with the relatively low polygenic association initially reported by Jansen et al (maximum $R^2 = 2.6\%$).[11]

The inability to replicate more of the previously discovered SNPs independently or through a normalized genetic risk score highlights a number of possibilities. First, the concept of a "winner's curse" has been demonstrated throughout the genetic literature, with many initial findings not being able to be replicated in subsequent studies and initially strong effect sizes diminishing in subsequent, larger cohort studies.[41] Another possibility is that exploring the genetic architecture of subjectively reported phenotypes, such as insomnia, is even more challenging than the study of complex traits. This may make reproducibility of findings in genetic studies even more difficult, as the personal experience of insomnia may lack a common genetic architecture, particularly with so many patterns of sleep disturbance contributing to the experience of insomnia that is able to be captured in large population-based studies.

Limitations

There were a number of limitations to this study. Most evident was the heterogeneity between cohorts, with regard to diagnostic labels and PSG (see **Table S1**, **Table S2**, and **Table S12**). Nonetheless, the more rigorous definitions of the objectively quantifiable PSG variables used should result in improvements over the vague, unverified reports of "insomnia" in the large, pooled cohorts from which we derived the SNPs of interest.[11] Moreover, even with heterogeneity in PSG between cohorts and over time, we variously employed machine-learning feature-extraction methods, in order to reduce the influence of such systematic bias. Finally, in order to account for these and other systematic differences between cohorts, we employed study-level meta-regressions, adjusting our models with random effects accounting for cohort-specific factors. Additionally, the variation in genotyping platforms resulted in differences in genotyping rates at our SNPs of interest, so we employed a normalization procedure that assumed a maximum potential polygenic risk for each individual given the effect sizes from Jansen et al.[11] The nGRS provided us the possibility to compare across various missing patterns in the data, while also magnifying the expected genetic associations in this complex, polygenic disease. While the populations studied are not entirely comparable to the original cohorts of Jansen et al. (specifically with our cohorts being older), the fact that the genetic architecture of subjective insomnia was not strongly associated with most measures of objective sleep from the PSG despite a high prevalence of subjective insomnia in these cohorts should raise concerns about the highly variable nature of the subjective experience of insomnia, which may highlight the influence of age rather than sleep itself. A final factor that could have resulted in an inability to replicate the findings of Jansen et al[11] is the fact that sleep in a sleep laboratory is not completely reflective of naturalistic sleep, with certain individuals with self-reported insomnia even having a paradoxically improved sleep on the night of a PSG.[42]

CONCLUSIONS

Insomnia is a complex, heterogeneous disorder that has no consistent objective sleep characteristics. In this project, the association of subjective-insomnia-associated genetic factors and objective polysomnographic features were explored. The genetic results showed that only rs11385155 (*MEIS1*) significantly associated with PLMI, confirming findings from others. Comparatively, despite ample power and stronger, objective phenotypes derived from quantitative analysis of the PSG, none of the other objective standards of insomnia—sleep efficiency, sleep onset latency, wake after sleep onset, total sleep time, or sleep stage proportions—associated independently with the 240 SNPs identified

in the large, multicohort genome-wide association study exploring subjective insomnia symptoms. While we were unable to replicate the association between these risk alleles (even in an aggregated genetic risk score) and a subjectively defined insomnia, the two objective polysomnographic features most reflective of sleep disturbances—arousals and wake after sleep onset—were found to significantly and meaningfully associate with a normalized polygenic risk score, suggesting that standard definitions objectively quantifying insomnia may not actually relate to the underlying genetic architecture of the experience of insomnia. In summary, the lack of correspondence of subjective insomnia SNP associations with objective, quantitative PSG features suggests that subjective insomnia and objective insomnia may have different genetic underpinnings, warranting study designs that account for this subjective–objective disconnect. As such, we strongly encourage a revision of the diagnosis of insomnia and suggest that distinctions should be made between subjective and objective insomnia. We claim that these two regimes are crucial in future studies as they should be treated as two different conditions. Furthermore, we suggest a subcategorization of each regime into whether both are present or if it is one or the other. In order to meaningfully analyze insomnia patients, the evidence of this study along with previous studies suggests that future studies should control for both objective and subjective insomnia-related findings. This may be especially important to consider in the context of clinical trials, which traditionally mandate a combination of objective and subjective criteria for inclusion and measurement of therapeutic responses.

ABBREVIATIONS

MrOS, Study of Osteoporotic Fractures in Men
nGRS, normalized genetic risk score
PLM(D/I), periodic limb movement (disorder/index)
PSG, polysomnogram/polysomnography
QC, quality control
RLS, restless legs syndrome
SNP, single-nucleotide polymorphism
SSC, Stanford Sleep Cohort
WSC, Wisconsin Sleep Cohort

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Address correspondence to: Logan Schneider, MD, Stanford/VA Alzheimer's Research Center, 3801 Miranda Ave, Building 4, 1st Floor, C-141, Palo Alto, CA 94304; Email: logands@gmail.com

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