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ARTICLE

Genome-wide analysis of insomnia disorder

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Abstract

Insomnia is a worldwide problem with substantial deleterious health effects. Twin studies have shown a heritable basis for various sleep-related traits, including insomnia, but robust genetic risk variants have just recently begun to be identified. We conducted genome-wide association studies (GWAS) of soldiers in the Army Study To Assess Risk and Resilience in Servicemembers (STARRS). GWAS were carried out separately for each ancestral group (EUR, AFR, LAT) using logistic regression for each of the STARRS component studies (including 3,237 cases and 14,414 controls), and then meta-analysis was conducted across studies and ancestral groups. Heritability (SNP-based) for lifetime insomnia disorder was significant ($h^2_g = 0.115$, $p = 1.78 \times 10^{-4}$ in EUR). A meta-analysis including three ancestral groups and three study cohorts revealed a genome-wide significant locus on Chr 7 (q11.22) (top SNP rs186736700, OR = 0.607, $p = 4.88 \times 10^{-9}$) and a genome-wide significant gene-based association ($p = 7.61 \times 10^{-7}$) in EUR for *RFX3* on Chr 9. Polygenic risk for sleeplessness/insomnia severity in UK Biobank was significantly positively associated with likelihood of insomnia disorder in STARRS. Genetic contributions to insomnia disorder in STARRS were significantly positively correlated with major depressive disorder ($r_g =$ 0.44, se = 0.22, $p = 0.047$) and type 2 diabetes ($r_g = 0.43$, se = 0.20, $p = 0.037$), and negatively with morningness chronotype ($r_g = -0.34$, se = 0.17, $p = 0.039$) and subjective well being ($r_g =$ -0.59, se = 0.23, $p = 0.009$) in external datasets. Insomnia associated loci may contribute to the genetic risk underlying a range of health conditions including psychiatric disorders and metabolic disease.

Introduction

Insomnia is highly prevalent, affecting 10–20% of adults in the United States [1] and worldwide [2]. The prevalence of insomnia is even higher (~25–50%) among military veterans, for whom it is frequently associated

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with mental health complications and functional impairment [3–5]. At the population level, insomnia is an important contributor to poor perceived health and disability, and healthcare utilization [1, 6]. Furthermore, chronic insomnia is associated with a multitude of adverse long-term health outcomes, including cardiovascular and metabolic disturbances (such as type 2 diabetes) as well as myriad mental health problems, including post-traumatic stress disorder (PTSD) and suicide [7–11].

Twin studies show that there is a heritable basis to sleep characteristics and insomnia [12–16]. Whereas there have been several recent genome-wide association studies (GWAS) for usual sleep duration [17, 18], including a now-replicated locus at *PAX8* [19], specific genetic risk variants for insomnia are just now being identified at genome-wide significance [20–22]. Given the tremendous variability in causes of insomnia as a symptom, as well as heterogeneity in insomnia as a disorder, it is important to fully specify the phenotype(s) under consideration in insomnia studies. Better understanding of the molecular bases for insomnia will be critical for the development of new treatments [23–25]. These efforts will benefit from careful consideration of the heterogeneity in insomnia at the phenotypic levels in different groups (e.g., causes of insomnia in young versus older cohorts) with the expectation that genetic risk factors may differ across populations.

The purpose of the present study is to use GWAS to elucidate the genetic architecture of insomnia. To achieve this aim we employed survey and genome-wide genetic data from the Army Study To Assess Risk and Resilience in Servicemembers (STARRS) to determine the association between insomnia disorder (approximating *DSM-5* criteria) and specific genetic risk variants. In so doing, we also looked for consistency of our results with those of a large recently published study of sleep disturbance traits in the UK Biobank that included insomnia symptoms [21] and an even more recently published analysis of a largely overlapping UK Biobank sample where a different phenotyping algorithm was used to assign case-control status based on insomnia symptoms [22]. We also determined the extent to which a polygenic risk score for insomnia severity, derived from the largest UK Biobank sample currently available (http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank), was significantly associated with insomnia disorder in STARRS. Lastly, we determined the heritability of insomnia disorder in this generally young and mostly male sample, and explored its genetic relationship to other mental and physical health-related phenotypes by referencing other publicly available GWAS data for those traits [26].

Methods

Subjects

Information in detail about the design and methodology of STARRS can be obtained in our prior report [27]. Each of the participating institutions approved the human subjects and data protection procedures used in the study. As described below, the analyses presented here involved two large components of STARRS.

New Soldier Study (NSS)

New soldiers took part in the NSS at the beginning of their basic training, which took place between April 2011 and November 2012 at one of three Army installations. 39,784 soldiers completed a computerized self-administered questionnaire (SAQ, described below) and, of these, 33,088 (83.2%) gave blood samples for DNA. The first half of the cohort had samples selected to enrich for probable cases of PTSD, suicidality, generalized anxiety disorder, and major depression; and controls with none of these disorders were also selected) (NSS1; $N = 7{,}999$). A subset of samples from the second half of the cohort was subsequently selected for genotyping to include cases of PTSD, suicidal behaviors and controls (NSS2; $N = 2{,}835$).

Pre/Post Deployment Survey (PPDS)

US Army soldiers from three Brigade Combat Teams (BCTs) participated in the PPDS ($N = 7{,}927$ eligible soldiers were genotyped) which began in the first quarter of 2012. The data included in this report were collected at baseline (T0) 4–6 weeks prior to deployment to Afghanistan.

Demographics

The population, sex and age composition of our analyzed samples are shown in Table 1. As expected, the PPDS subjects were older than the new Army recruits in the NSS. Lifetime prevalence estimates (with standard errors) of insomnia disorder are also shown.

Measures

One of the Composite International Diagnostic Interview screening scales (CIDI-SC) [28] was used to determine criteria for major depression. The reliable and valid Brief Insomnia Questionnaire [29] was used to approximate *DSM-5* criteria (which differ immaterially from *DSM-IV* criteria) for Insomnia Disorder. The stem question for insomnia was "Did you ever in your life have insomnia -

Table 1 Study participants (by ancestry) and sex and age distributions in the samples

	NSS1		NSS2		PPDS	
	N	% with Insomnia disorder	*N*	% with Insomnia disorder	*N*	% with Insomnia disorder
Population						
European American	4756	16.51%	1817	19.54%	4900	23.61%
African American	1371	10.07%	406	10.84%	902	16.85%
Latino American	1447	13.41%	498	19.08%	1554	20.4%
Sex (% male)	81.4%		77.8%		92.8%	
Age, yrs (SD)	21.0 (3.3)		20.3 (3.2)		26.0 (5.9)	

that is, problems either getting to sleep, staying asleep, waking too early, or feeling so tired even after a full night's sleep that it interfered with your daytime activities?" Respondents who answered yes were asked "Did you ever in your life have a whole month or longer when you had insomnia at least three nights a week?" Respondents who answered yes were asked 5 questions introduced with "Think of a typical month when your insomnia was worst. During that month, how much did your insomnia interfere with your daytime functioning in the following ways": (1) daytime fatigue, sleepiness, or low motivation, (2) headaches, upset stomach, diarrhea or constipation, (3) moodiness (irritability, nerves, worry, or depression), (4) reduced performance at work or school, and (5) accident proneness. Insomnia Disorder was defined as at least one month of insomnia and reporting of at least "Some of the Time" on one or more of these five symptom items. Controls were defined as failing to meet the definition of Insomnia Disorder.

DNA genotyping, imputation and population stratification adjustment

Detailed information on genotyping, genotype imputation, population assignment and principal component analysis for population stratification adjustment are included in our previous report [30] and in Supplementary Materials. Whole blood samples were shipped to Rutgers University Cell & DNA Repository (RUCDR), where they were frozen for later DNA extraction using standard methods. NSS1 and PPDS samples were genotyped using the Illumina OmniExpress + Exome array with additional custom content (*N* SNP = 967,537). NSS2 samples were genotyped on the Illumina PsychChip (*N* SNP = 571,054; 477,757 SNPs overlap with OmniExpress + Exome array).

Relatedness testing was carried out with PLINK v1.90 [31, 32] and pairs of subjects with π of >0.2 were identified, randomly retaining one member of each relative pair. We used a two-step pre-phasing/imputation approach for genotype imputation, with reference to the 1000 Genomes Project multi-ethnic panel (August 2012 phase 1 integrated release; 2,186 phased haplotypes with 40,318,245 variants). We removed SNPs that were not present in the 1000 Genomes Project reference panel, had non-matching alleles to 1000 Genome Project reference, or had ambiguous, unresolvable alleles (AT/GC SNPs with minor allele frequency (MAF) >0.1). For the Illumina OmniExpress array 664,457 SNPs and for the Illumina PsychChip 360,704 SNPs entered the imputation procedure.

Given the ancestral heterogeneity of the STARRS subjects, samples were assigned into major population groups (European (EUR), African (AFR) or Latino (LAT); see Supplementary Materials; also see ref. [30]). An Asian (ASI) group that was too small for separate analysis was excluded. PCs within each population group were then obtained for population stratification adjustment (see also Supplementary Figures 1–3). For quality control (QC) purposes we kept autosomal SNPs with missing rate <0.05; kept samples with individual-wise missing rate <0.02; and kept SNPs with missing rate <0.02. After QC, we merged our study samples with HapMap3 samples. We kept SNPs with minor allele frequency (MAF) >0.05 and LD pruned at $R^2 > 0.05$. In order to avoid long range LD structure from interfering with the PCA analysis, we excluded SNPs in the MHC region (Chr 6:25–35 Mb) and Chr 8 inversion (Chr 8:7–13 Mb).

Statistical analysis

We used LD score regression (LDSR) [33] and GCTA [34] with imputed data (modified from [35, 36]) to estimate the proportion of variance in insomnia explained by common SNPs (i.e., SNP heritability, h^2_g). We estimated h^2_g of insomnia in EUR with linear mixed models implemented in GCTA software, adjusted for 10 PCs and study.

We used PLINK v1.90 [31, 32] to conduct genome-wide association tests for insomnia disorder on imputed SNP dosage with logistic regression adjusted for age, sex, and the top 10 within-population principal components (PCs). We also conducted sensitivity analyses adjusting for lifetime major depressive disorder, of which insomnia is a common symptom. We filtered out SNPs with MAF < 0.01

or imputation quality score (INFO) <0.6, and performed HWE tests for the top SNPs from the association analysis.

GWAS was conducted in the three studies (NSS1, NSS2 and PPDS) separately within each of the three ancestral groups (EUR, AFR, LAT) and then meta-analyzed within-ancestry group across studies, and then across ancestral groups and studies. We report fixed-effects models as our primary analysis in the Results. Meta-analysis was conducted using an inverse variance-weighted fixed-effects model in PLINK. A p-value $<5 \times 10^{-8}$ was used as the threshold for genome-wide significance whereas results at p-value $<1 \times 10^{-6}$ are reported as genome-wide suggestive.

We used LDSR [33] implemented on LD Hub (http://ldsc.broadinstitute.org) [26] to test the genetic correlation between insomnia disorder and several other traits in European samples using publicly available meta-analytic GWAS for two sleep phenotypes (morningness and sleep duration) [20], two mental disorders frequently comorbid with insomnia (major depression and bipolar disorder) [37, 38], a personality trait frequently associated with insomnia (neuroticism) [39], and subjective well being [40]. We also extended this inquiry to two physical traits that have been frequently epidemiologically associated with insomnia, namely Type 2 diabetes [41] and coronary artery disease [42].

We performed gene-based tests and pathway analysis using software MAGMA [43] and FUMA [44]. The gene-based test can provide association tests for each gene ($N =$ 18,194) by aggregating the SNPs within the gene region and the pathway analysis can identify enriched association signals of insomnia disorder in gene sets aggregated in biological pathways. We used the final meta-analysis in EUR samples and the 1000 Genomes Project European LD reference for this analysis. For the gene-based analysis, we used a combined mean and top SNP association model. We used curated pathways and GO terms obtained from MsigDB with a total of 10,894 pathways. The significance level after Bonferroni correction according to the genes or pathways tested is $0.05/18{,}194 = 2.75 \times 10^{-6}$ for the gene-based tests and $0.05/10{,}894 = 4.59 \times 10^{-6}$ for the pathway analysis. Additional analyses for functional eQTLs were conducted using the Broad GTEx database (https://www.gtexportal.org) [45].

Results

SNP-based heritability of insomnia disorder

We estimated SNP-based heritability (h^2_g) using GCTA [34]. We found significant h^2_g estimates of 0.115 (se 0.033) for insomnia disorder from the EUR meta-analysis across studies ($p = 1.78 \times 10^{-4}$). With LDSR [33], which uses GWAS summary statistics and often provides a lower estimate of heritability than GCTA, we found a significant h^2_g estimate of 0.078 (se 0.026, $p = 0.003$). This latter estimate is similar to the most recently available LDSR-derived heritability estimate from $N = 336{,}965$ EUR individuals for the continuous insomnia symptoms (never/sometimes/usually) variable in UK Biobank ($h^2_g = 0.061$, se $= 0.0037$, $p = 5.15 \times 10^{-62}$; https://nealelab.github.io/UKBB_ldsc/index.html; last updated 20 September 2017). (All estimates reported above are on the observed scale; estimates for insomnia disorder on the liability scale are all higher, given a population prevalence of 0.10 or higher.)

Genome-wide association analyses

Within-ancestry cross-study meta-analyses

EUR ancestry In the meta-analysis of EUR ancestry individuals across the three studies we observed several genome-wide significant SNPs on Chr7 (q11.22) (top SNP rs147549871, odds ratio (OR) = 0.538, $p = 4.90 \times 10^{-9}$) in an intergenic region (Supplementary Figure 4), and several genome-wide suggestive SNPs on Chr 9 (top SNP rs7855172, OR = 0.72, $p = 9.54 \times 10^{-8}$) in *RFX3*. These and other genome-wide suggestive loci are shown in Table 2a.

To test whether the SNPs identified as associated with insomnia were better accounted for by major depression, which frequently has insomnia as a prominent symptom, we conducted the meta-analyses of our largest ancestral group (EUR) across the three studies also adjusting for lifetime major depressive disorder (MDD). These analyses resulted in only very modest attenuation of the GWAS signals associated with insomnia disorder, with multiple SNPs on Chr7 (q11.22) remaining genome-wide significant (top SNP rs147549871, OR = 0.536, $p = 1.91 \times 10^{-8}$).

AFR ancestry In the meta-analysis of AFR ancestry individuals across the three studies we observed a genome-wide significant SNP on Chr 12 (rs7138947, OR = 3.15, $p = 1.92 \times 10^{-8}$) in *NTF3* (Supplementary Figure 5), and a genome-wide suggestive SNP on Chr 3 (rs185334926, OR = 4.38, $p = 4.70 \times 10^{-7}$) in *CACNA1D* (Supplementary Figure 6). These and other genome-wide suggestive loci are shown in Table 2b.

LAT ancestry In the meta-analysis of LAT ancestry individuals across the three studies we observed a genome-wide suggestive SNP on Chr 9 (rs35796756, OR = 2.89, $p = 8.33 \times 10^{-7}$) in the intronic region of *DEC1*. Additional genome-wide suggestive loci are shown in Table 2c.

Summary statistics for all the top SNPs across ancestral groups are shown in Supplementary Tables 3a-5c.

Table 2a NSS1, NSS2 and PPDS GWAS Ancestry-Specific Results for Insomnia Disorder at genome-wide significance $p < 5 \times 10^{-8}$ (in bold) and suggestive results at $p < 10^{-6}$ in Soldiers of European Ancestry

Chr	BP	SNP	A1	A2	MAF	p-value	OR	Gene
4	61,349,132	rs55710816	A	G	0.04809	9.36E−07	0.70	Intergenic
4	150,822,110	rs56676520	A	T	0.07021	9.20E−07	1.50	EST CN411885
4	150,822,180	rs72965338	A	G	0.06822	9.82E−07	0.67	EST CN411885
5	119,118,370	chr5-119118370-D	I5	D	0.3152	2.66E−07	0.82	EST BE896471
7	**67,799,556**	**rs142587679**	**A**	**T**	**0.01476**	**1.03E−08**	**1.82**	
7	**67,799,600**	**rs147549871**	**T**	**G**	**0.01411**	**4.90E−09**	**0.54**	
7	**67,820,045**	**rs186736700**	**A**	**C**	**0.01966**	**1.81E−08**	**0.60**	
7	67,832,096	rs117920677	T	C	0.01728	1.04E−07	1.66	
7	67,915,285	rs60353720	A	C	0.2557	4.80E−07	1.22	
9	3,229,630	rs628884	A	T	0.1172	4.52E−07	1.34	RFX3
9	3,233,835	chr9-3233835-D	I2	D	0.1264	2.54E−07	1.37	RFX3
9	3,234,232	rs577389	C	G	0.1187	1.58E−07	1.38	RFX3
9	3,234,246	rs577434	T	C	0.1188	1.58E−07	1.38	RFX3
9	3,239,284	rs16916157	A	C	0.08846	9.95E−08	1.39	RFX3
9	3,242,236	rs488534	A	G	0.07875	8.64E−07	0.73	RFX3
9	3,242,394	rs653080	C	G	0.0788	8.10E−07	1.37	RFX3
9	3,244,009	chr9-3244009-I	I2	D	0.09904	3.19E−07	1.35	RFX3
9	3,244,165	rs111796799	C	G	0.1014	2.24E−07	0.74	RFX3
9	3,245,080	rs7869158	C	G	0.1054	2.45E−07	0.74	RFX3
9	3,245,127	rs7855172	A	G	0.07337	9.54E−08	0.72	RFX3
9	3,245,605	rs589085	T	G	0.09302	2.08E−07	1.38	RFX3
9	3,245,672	rs528224	A	T	0.1149	2.58E−07	0.74	RFX3
12	45,833,755	rs74081827	A	G	0.03869	6.86E−07	1.66	ANO6
13	67,376,132	chr13-67376132-I	I5	D	0.03019	7.32E−07	1.99	PCDH9

All SNPs in this table were imputed

MAF minor allele frequency

Trans-Ethnic Cross-Study Meta-Analysis

Several SNPs in a region on Chr7 (q11.22) (top SNP rs186736700, OR = 0.607, $p = 4.88 \times 10^{-9}$) were genome-wide significantly associated with insomnia disorder in the pan-ancestral meta-analysis across the 3 studies (Table 3 and Figs. 1 and 2). Polygenic risk scores derived from insomnia disorder in NSS1 + NSS2 were significantly associated with insomnia disorder in PPDS (optimal *p*-value cutoff = 0.05, $R^2 = 0.00028$, $p = 0.0048$, *N* SNPS = 41,566).

Comparison with UK Biobank Sleep Disturbance and Insomnia Symptom GWAS Findings

We did not observe in our EUR data any association between SNPs recently significantly associated with insomnia in UK Biobank GWASs of sleep disturbance traits [21] or insomnia complaints [22]. However, in our trans-ethnic meta-analysis, we observed nominal association of the UK Biobank genome-wide significant or suggestive results for sleep duration [21] including rs1380703 on Chr 2 ($p = 0.032$; where the longer sleep duration effect allele in the UK Biobank Study is associated with greater odds of insomnia in our study), and rs1456031 ($p = 0.036$) and rs10953765 ($p = 0.051$) on Chr 7 (where the shorter sleep duration effect allele in the UK Biobank Study is associated with greater odds of insomnia in our study).

Gene-based and Pathway Analysis

There are 2 significant genes identified via genome-wide gene-association study (GWGAS) with MAGMA after Bonferroni correction for multiple testing. *RFX3* (Regulatory Factor X3; gene ID 5591), on chromosome 9, with a *p*-value $= 7.61 \times 10^{-7}$ obtained by aggregating 738 SNPs in the region. *C1orf100* (chromosome 1 open reading frame 100; gene ID 200159), on chromosome 1, with a *p*-value = 1.8×10^{-6}. We list the top 10 genes with the most significant *p*-values from NSS and PPDS EUR samples in

Table 2b NSS1, NSS2 and PPDS GWAS Ancestry-Specific Results for Insomnia Disorder at genome-wide significance $p < 5 \times 10^{-8}$ (in bold) and suggestive results at $p < 10^{-6}$ in Soldiers of African Ancestry

Chr	BP	SNP	A1	A2	MAF	p-value	OR	Gene
3	53,767,889	rs185334926	T	C	0.01031	4.70E−07	4.38	CACNA1D
4	12,352,522	rs111858442	A	G	0.01012	4.24E−07	0.44	Intergenic
4	80,435,046	rs58777020	T	C	0.01002	6.25E−07	2.84	LINC00989
4	142,518,082	chr4-142518082-I	I2	D	0.01001	9.11E−07	2.88	IL15
4	168,926,494	rs190721506	A	C	0.02951	7.04E−07	0.18	RP11-310I9.1
8	48,770,702	rs7003908	A	C	0.3285	4.63E−07	0.64	PRKDC[a]
12	**5,618,948**	**rs7138947**	**A**	**C**	**0.09706**	**1.92E−08**	**3.15**	**NTF3**
14	40,369,086	rs148431766	T	C	0.04516	1.70E−07	0.48	
16	**7,917,912**	**rs4078004**	**C**	**G**	**0.1178**	**3.77E−08**	**0.35**	**CTD-2535I10.1[b]**
16	**7,955,134**	**rs34670506**	**T**	**C**	**0.1417**	**2.31E−08**	**0.37**	**CTD-2535I10.1[b]**
18	71,552,804	rs76941679	T	G	0.01442	2.57E−07	0.44	g7907.t1[a]
19	52,603,402	rs56701754	T	C	0.03339	4.36E−07	0.59	ZNF616, ZNF841
19	52,604,302	rs73578849	A	G	0.05593	2.93E−07	0.58	ZNF616, ZNF841
19	52,607,777	rs73571238	C	G	0.05213	5.05E−07	0.58	ZNF616, ZNF841

MAF minor allele frequency

[a]Genotyped SNP; all other SNPs in this table were imputed

[b]Predicted gene

Supplementary Table 1. There is no overlap of these genes in our EUR group with those significantly associated with insomnia complaints in the UK Biobank study that also used MAGMA [22]. However, one of our top two most significant ($p = 6.85E{-}05$) genes, *RFX3* (Fig. 3 and Supplementary Table 6), was nominally associated ($p = 0.002$) with UK Biobank insomnia severity [22].

The pathway enrichment analysis did not identify any significant pathway. We listed the top 10 pathways from NSS and PPDS European samples meta-analyses in Supplementary Table 7.

Genetic correlations

LDSR

Insomnia disorder was significantly—and positively—genetically correlated with major depressive disorder ($r_g = 0.44$, se $= 0.22$, $p = 0.047$) and type 2 diabetes ($r_g = 0.43$, se $= 0.20$, $p = 0.037$), but not with bipolar disorder ($r_g = -0.21$, se $= 0.22$, $p = 0.35$), neuroticism ($r_g = 0.280$, se $= 0.32$, $p = 0.38$), sleep duration ($r_g = -0.045$, se $= 0.181$, $p = 0.80$), body mass index ($r_g = 0.051$, se $= 0.125$, $p = 0.68$), or coronary artery disease ($r_g = 0.016$, se $= 0.158$, $p = 0.92$). Insomnia disorder was also significantly negatively genetically correlated with morningness chronotype ($r_g = -0.34$, se $= 0.17$, $p = 0.039$) and subjective well being ($r_g = -0.588$, se $= 0.23$, $p = 0.009$; Table 4). These correlations were uncorrected for multiple tests.

UK Biobank Insomnia sleep disturbance

We calculated genetic correlations in Europeans between our insomnia disorder and UK Biobank sleep disturbance phenotypes [21]. We found that insomnia disorder in our study and insomnia in the UK Biobank study were significantly genetically correlated ($r_g = 0.305$ (se 0.153), $p = 0.046$; Table 4). Neither UK Biobank sleep duration nor excessive daytime sleepiness phenotypes were significantly genetically correlated with insomnia disorder in our study.

We also determined the extent to which the most recently available UK Biobank polygenic risk score (PRS) for insomnia—derived from their continuous sleeplessness/insomnia severity variable, phenotype #1200 in the UK Biobank GWAS Manifest (https://docs.google.com/spreadsheets/d/1b3oGI2lUt57BcuHttWaZotQcI0-mBRPyZihz87Ms_No/edit#gid = 1209628142) -- was associated with insomnia disorder in the STARRS dataset. Results are shown in Supplementary Figure 7 where it can be seen that the UK Biobank PRS for sleepless/insomnia was significantly associated with STARRS insomnia disorder, with the strongest association seen at a p-value threshold of $p = 0.1$ with OR = 1.09 (95% confidence interval 1.04–1.14) involving 75,213 SNPs.

Discussion

Insomnia is associated with substantial deleterious effects on mental and physical health and quality of life [1, 6]. This

Table 2c NSS1, NSS2 and PPDS GWAS Ancestry-Specific Results for Insomnia Disorder with suggestive results at $p < 10^{-6}$ in Soldiers of Latino Ancestry

Chr	BP	SNP	A1	A2	*p*-value	MAF	OR	Gene
2	129,733,260	rs11678417	A	G	8.46E−07	0.09372	1.71	Intergenic
2	129,744,344	rs13013037	A	G	6.74E−07	0.1073	1.68	Intergenic
4	177,070,382	chr4-177070382-I	I2	D	9.23E−07	0.02678	2.85	WDR17
4	177,070,739	rs6838194	A	G	9.10E−07	0.02694	0.35	WDR17
4	177,071,506	rs7356435	A	G	8.75E−07	0.02701	0.35	WDR17
4	177,072,545	rs6826405	T	C	8.30E−07	0.02697	2.86	WDR17
4	177,072,560	rs6826418	T	C	8.29E−07	0.02697	2.86	WDR17
4	177,076,247	rs10012282	T	C	6.71E−07	0.02723	0.35	WDR17
4	185,083,206	rs72699704	A	G	2.36E−07	0.155	1.67	ENPP6
4	185,087,970	rs72699711	T	C	4.94E−07	0.1234	1.69	ENPP6
6	3,915,183	rs9503800	T	C	6.49E−07	0.185	1.54	RP1-140K8.2
6	3,915,406	rs9503802	T	C	9.27E−07	0.1841	0.65	RP1-140K8.2
6	3,915,450	rs9503803	C	G	8.41E−07	0.1841	1.53	RP1-140K8.2
6	3,915,489	rs9503804	A	G	6.17E−07	0.1845	1.54	RP1-140K8.2
6	3,915,755	rs58091956	T	C	5.42E−07	0.1842	1.55	RP1-140K8.2
6	3,915,819	rs9503805	A	G	6.04E−07	0.1842	0.65	RP1-140K8.2
6	3,916,060	rs58048000	A	G	5.45E−07	0.185	1.54	RP1-140K8.2
6	3,916,151	rs60302355	C	G	5.95E−07	0.1841	1.54	RP1-140K8.2
6	3,916,182	rs58239759	A	G	5.44E−07	0.1849	1.54	RP1-140K8.2
6	3,916,207	rs55995704	C	G	5.44E−07	0.1849	1.54	RP1-140K8.2
6	3,916,251	rs11752830	T	C	5.27E−07	0.1849	1.55	RP1-140K8.2
6	3,916,296	rs57213389	A	T	5.37E−07	0.1849	0.65	RP1-140K8.2
6	3,916,713	rs9503806	T	C	5.24E−07	0.1852	0.65	
6	3,916,741	rs9503807	T	C	5.23E−07	0.1852	0.65	
6	3,916,858	rs9503808	A	G	5.21E−07	0.1852	1.55	
6	3,917,007	rs9503809	C	G	5.03E−07	0.1849	0.65	
6	3,917,217	rs9502090	T	C	5.04E−07	0.1852	0.65	
6	3,917,344	rs9502091	C	G	4.10E−07	0.1846	1.55	
6	3,917,519	rs9503813	T	C	4.96E−07	0.1853	1.55	
6	3,917,622	rs9502092	C	G	4.92E−07	0.1853	1.55	
6	3,917,690	rs6915403	C	G	4.91E−07	0.1853	1.55	
6	3,917,918	rs6914617	A	G	4.81E−07	0.1857	0.65	
6	3,918,004	rs6899585	A	G	6.32E−07	0.186	1.54	
6	3,918,537	rs9503815	A	G	5.33E−07	0.1834	1.55	
6	3,918,554	rs145045254	T	C	8.64E−07	0.1766	0.64	
6	3,919,135	rs6905890	A	G	5.34E−07	0.1867	1.54	
6	3,919,215	rs6925458	T	G	2.90E−07	0.1868	0.64	*
6	3,919,380	rs6926967	T	C	1.65E−07	0.2002	0.64	RP1-140K8.5
6	3,919,394	rs6910820	T	C	1.69E−07	0.2003	1.56	
6	3,919,504	rs6927256	A	C	1.68E−07	0.2003	0.64	BX105115
6	3,919,560	rs6906656	A	G	1.45E−07	0.2006	1.57	
6	3,919,714	rs4959896	T	C	1.70E−07	0.2002	0.64	
6	3,920,536	rs9503816	A	G	4.44E−07	0.2015	1.54	
6	3,920,619	rs9503817	T	C	1.63E−07	0.1983	0.64	
9	118,117,399	rs35796756	A	G	8.33E−07	0.01636	2.89	DEC1
16	86,420,361	rs1687657	T	G	2.90E−07	0.06825	2.34	LINC00917

MAF minor allele frequency

*SNP genotyped; all other SNPs were imputed

Table 3 NSS1, NSS2 and PPDS GWAS Trans-Ethnic Meta-analysis Results for Insomnia Disorder at genome-wide significance $p < 5 \times 10^{-8}$ (in bold) and suggestive results $p < 10^{-6}$

CHR	BP	SNP	A1	A2	MAF	*p*-value	OR	Gene
2	5,693,308	chr2-5693308-D	I2	D	0.02341	9.80E−07	0.60	AC107057.1
4	12,352,522	rs111858442	A	G	0.0102	4.24E−07	0.44	—
4	80,435,046	rs58777020	T	C	0.0101	6.25E−07	2.84	LINC00989
4	142,518,082	chr4-142518082-I	I2	D	0.0100	9.11E−07	2.88	
7	**67,799,556**	**rs142587679**	**A**	**T**	**0.01476**	**1.39E−08**	**1.75**	—
7	**67,799,600**	**rs147549871**	**T**	**G**	**0.01411**	**8.76E−09**	**0.56**	
7	**67,820,045**	**rs186736700**	**A**	**C**	**0.01966**	**4.88E−09**	**0.61**	—
7	**67,832,096**	**rs117920677**	**T**	**C**	**0.01728**	**3.57E−08**	**1.65**	—
14	22,325,242	chr14-22325242-I	I2	D	0.02614	2.96E−07	1.48	TRAV8-3
14	48,149,514	rs76049381	C	G	0.1388	3.06E−07	1.23	MDGA2
14	48,159,954	chr14-48159954-D	D	I3	0.1374	3.98E−07	1.23	MDGA2
14	48,161,473	rs8003717	T	C	0.1411	7.38E−07	0.82	MDGA2*

BP: 2009 (GRCh37/hg19) Assembly

MAF minor allele frequency

*SNP genotyped; all other SNPs were imputed

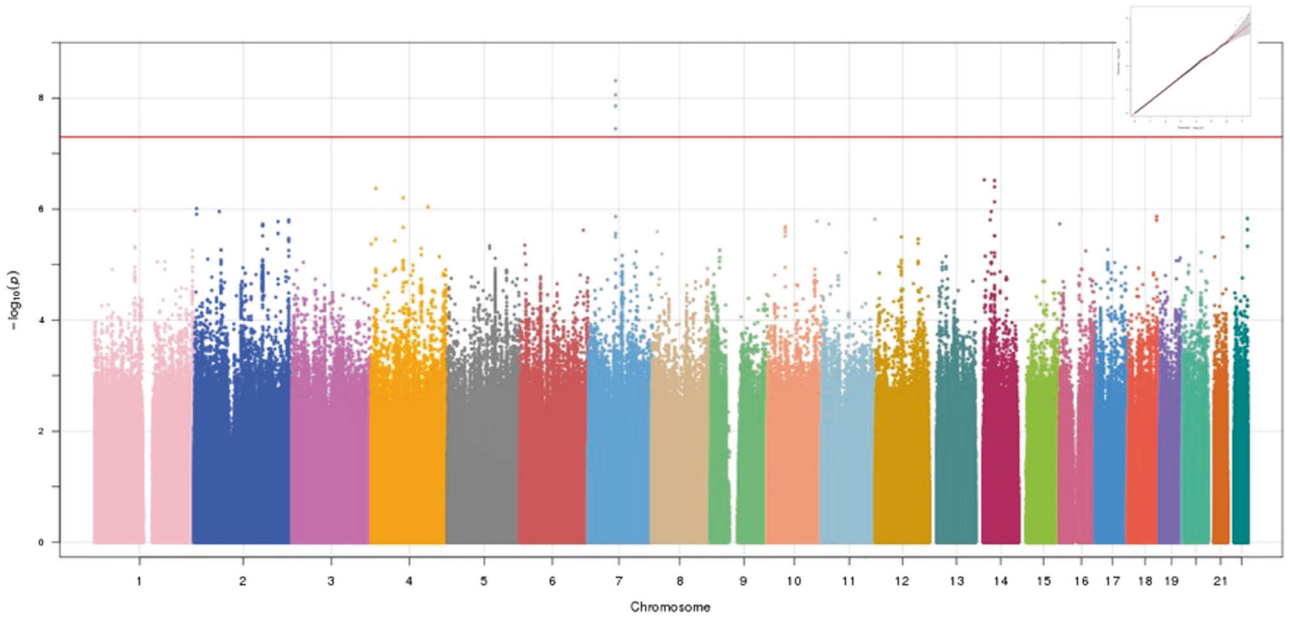

Fig. 1 Manhattan plot (and Q–Q plot, inset) of NSS1, NSS2, and PPDS trans-ethnic meta-analysis genome-wide association study (GWAS)

study is one of the largest GWAS of a phenotype approximating *DSM-5* insomnia disorder conducted to date. As anticipated by twin studies, we confirmed evidence of significant heritability of insomnia disorder using genotype data. Furthermore, consistent with the observation from twin studies that insomnia and major depression share common genetic variance [46], using LDSR we found evidence in our EUR samples of a strong and statistically significant positive genetic correlation between insomnia in our study and major depression based on GWAS summary statistics from the Psychiatric Genomics Consortium [37]. Nonetheless, the genetic association with insomnia is distinct and not explained fully by MDD since covarying for lifetime MDD had only a modest impact on the strength of this association. We also found evidence of a strong and statistically significant positive genetic correlation between insomnia in our study and type 2 diabetes [41]. This latter finding, which was also seen in recent UK Biobank analyses [22], suggests that at least some of the phenotypic association (or "risk") of insomnia and type 2 diabetes is

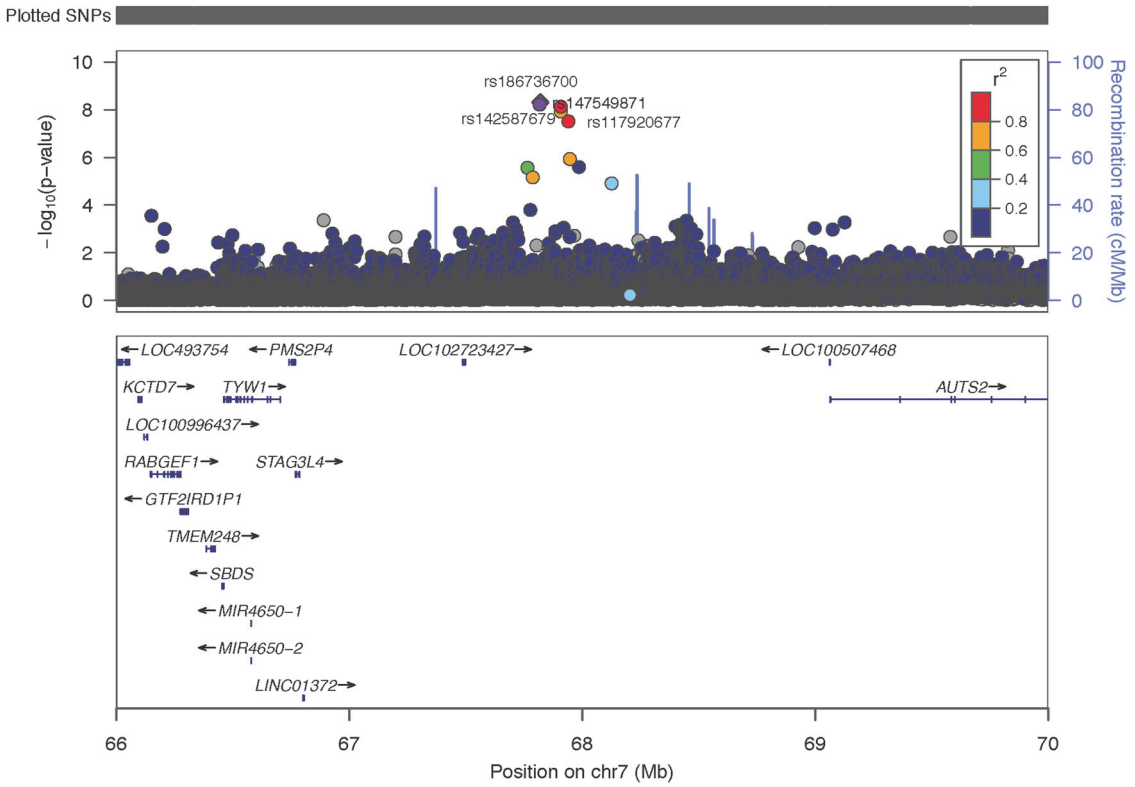

Fig. 2 Locus-zoom plot showing region on Chr 7 containing the genome-wide significant markers in the NSS1, NSS2, and PPDS trans-ethnic meta-analysis

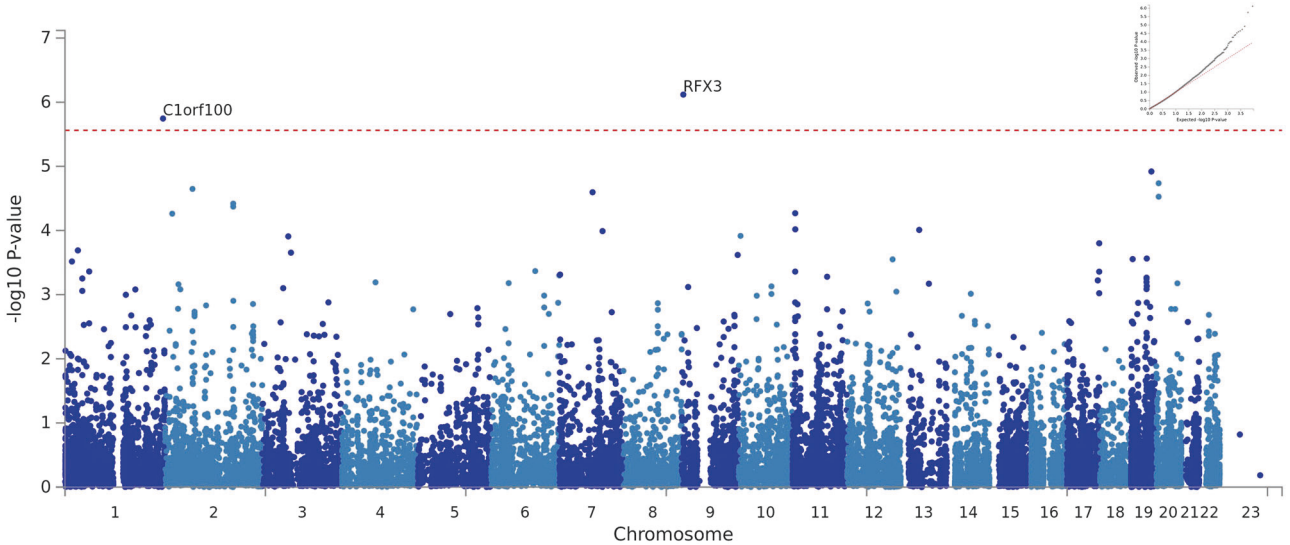

Fig. 3 Manhattan plot (and Q–Q plot, inset) of NSS1, NSS2, and PPDS combined dataset genome-wide gene-association study (GWGAS)

accounted for by shared genetic factors. Interestingly, a recent publication from the CHARGE consortium documented a genetic correlation between sleep duration and type 2 diabetes, although it was longer sleep duration that was associated with increased type 2 diabetes risk [47]. We also found a strong negative genetic correlation between insomnia disorder and subjective well being, a finding that may speak to shared diatheses for the strong relationship between sleep disturbance and reduced quality of life.

Our strongest association with insomnia disorder, with a haploblock on Chr 7 (q11.22) located in an intergenic region, is approximately 1MB away from the nearest gene

Table 4 LDSR determination of genetic correlation in EUR subjects of insomnia disorder with other traits (from external study meta-analyses with PubMed ID [PMID] shown)

Phenotype	PMID	r_g	SE (r_g)	p-value
Major depressive disorder	22472876	0.44	0.22	0.047
Bipolar disorder	21926972	−0.21	0.22	0.351
Neuroticism	24828478	0.28	0.32	0.380
Sleep duration	27494321	−0.05	0.18	0.802
Body mass index	20935630	0.05	0.13	0.683
Coronary artery disease	26343387	0.02	0.16	0.917
Type 2 diabetes	22885922	0.43	0.20	0.037
Chronotype (Morningness)	27494321	−0.34	0.17	0.039
Subjective well being	27089181	−0.59	0.23	0.0091
Insomnia (UK Biobank)	27992416	0.31	0.15	0.046

(*TYW1*). Nearby on Chr 7 (q11.22) is also *AUTS2*, a gene previously associated with alcohol consumption [48], disorders of which are frequently complicated by insomnia [49]. Among the other regions implicated in our study are several potentially interesting candidate genes and/or eQTLs with links to brain development and sleep-related phenotypes. *RFX3*, identified as statistically significant in our GWGAS, is a transcription factor involved in development of brain white matter tracts including corpus callosum [50] and thalamocortical tract [50, 51]. Of potential interest, the thalamocortical tract serves an essential function to coordinate oscillating electrical signals across the brain during sleep [52]. *RFX3* (which, it should be noted, was also a gene nominally associated with insomnia complaints in the UK BIobank GWGAS) [22] was identified in the Broad GTEx database as an eQTL associated with the expression of *CARM1P1*, a nearby gene on chromosome 9. This indicates the associated SNPs may be functional, but could perhaps act on genes other than *RFX3*. *NTF3* is a neurotrophic factor involved in cortical development [53] and synaptogenesis [54]. *DEC1* is a circadian clock modulator previously shown to be induced by sleep deprivation [55, 56]. A genetic homolog of *DEC1*, *DEC2* has been linked to short sleep phenotypes in humans and model organisms [57]. *ANO6* is a membrane bound, non-selective cation channel. The insomnia associated SNP rs74081827 on Chr 12 ($p = 6.86E{-}07$) is located within the 3′-UTR of this gene and is an eQTL associated with *ANO6* expression. Of potential interest, a similar gene on Chr 19, *ANO8* was identified in the gene-based analysis (Table S1), and elsewhere on Chr 12, *ANO2* is immediately adjacent to *NTF3* that harbors an insomnia associated SNP among AFR subjects. *CACNA1D* is an L-type calcium channel gene, previously associated with a variety of other conditions including bipolar disorder [58, 59] (itself almost invariably associated with sleep disturbance) and cardiometabolic disease [60]. A functionally similar L-type calcium channel gene, *CACNA1C* was previously linked with sleep latency [18], and both *CACNA1C* and *CACNA1D* were reported in a genomic pathway analyses to contribute to sleep duration [61]. In addition, the T-type calcium channel gene *CACNA1I*, previously associated with schizophrenia, mediates sleep spindles, a thalamocortical oscillation in stage 2 sleep that also appears to be an endophenotype of schizophrenia [62]. Of note, however, our pathway analyses did not highlight a role for calcium channel genes overall.

Our study differs methodologically in several important ways from the recent UK Biobank Studies of sleep disturbance traits [21] and insomnia complaints, respectively [22]. The former used a one-question phenotype for insomnia symptoms ("Do you have trouble falling asleep at night or do you wake up in the middle of the night" with responses "never/rarely," "sometimes," "usually," and "prefer not to answer") and dichotomized individuals into controls "never/rarely'" versus cases "usually," with the middle category ("sometimes") excluded. It did not specify a time frame (i.e., recent versus remote) or duration, nor did it ascertain the extent to which the insomnia symptoms were associated with disability or distress. Our study more closely approximated lifetime *DSM-5* insomnia disorder criteria (without ruling out other sleep disorders or other causes of insomnia) in requiring a specific number and type of symptoms over a minimal duration. In this regard, our phenotype is more similar to the other recent UK Biobank publication that used insomnia complaints to categorize patients as cases or controls [22], but the questions used to arrive at this determination are quite different from our study, making attempts at true replication impossible. In the general population, women suffer insomnia at higher rates than men and onset is often associated with physical problems of aging. Accordingly, insomnia has higher prevalence in older adults, and especially women. These are two groups that are underrepresented in the STARRS samples that consist primarily of young men, but overrepresented in the UK Biobank sample [63]. Similarly, the UK Biobank subjects tend to be healthier than the general population [63], whereas STARRS insomnia subjects are enriched for risk of psychiatric disorders. As such, in important ways, our results are not directly comparable to these other recent analyses. Whereas the several genetic risk loci shared across studies and the finding of a significant genetic correlation for insomnia across studies likely speak to the common genetic features, the newly reported loci revealed in our study of an earlier onset, younger population may confer risk for distinct features of insomnia.

Our results should be interpreted in light of several additional limitations. First, samples sizes—especially within ancestral groups—are insufficiently powered to detect many loci of modest effect. Second, the insomnia

disorder phenotype is defined on the basis of self-report only. Accordingly, we are unable to distinguish between different types or causes of insomnia. Third, our sample is mostly male. It may be that the genetic factors influencing insomnia vary by sex, but we lacked the power to test this hypothesis. Fourth, the present study of insomnia disorder constitutes analysis of a secondary trait (i.e., it was not the basis for initial ascertainment and selection of subjects for genotyping) and is therefore subject to possible bias or imprecision in the estimated effect sizes [64]. Fifth, the publicly available external GWAS datasets to which we had access consisted solely of individuals of European descent, and therefore the conclusions we have drawn regarding genetic correlations may not generalize to other ancestry groups.

In summary, this set of genome-wide association studies confirms the heritability of insomnia and reveals candidate risk loci. We also find evidence of genetic correlation between insomnia disorder and other psychiatric (e.g., major depressive disorder) and physical (e.g., type 2 diabetes) disorders, suggesting a shared genetic diathesis for these commonly co-occurring phenotypes that recapitulates similar conclusions from prior twin [46] and GWAS [47] studies, respectively. In addition, several of the variants identified rest comfortably among loci and pathways already known to be related to sleep and circadian rhythms. Taken together, these results provide insights into the possible molecular bases for insomnia and related conditions and may inform the development of novel therapeutic targets [23–25].

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Compliance with ethical standards

Conflict of interest Dr. M.B.S. has in the past three years been a consultant for Actelion, Aptinyx, Dart Neuroscience, Healthcare Management Technologies, Janssen, Neurocrine Biosciences, Oxeia Biopharmaceuticals, Pfizer, and Resilience Therapeutics. Dr. M.B.S. owns founders shares and stock options in Resilience Therapeutics and has stock options in Oxeia Biopharmaceticals. Dr. J.W.S. is an unpaid member of the Scientific Advisory Board of PsyBrain, Inc. In the past 3 years, Dr. R.C.K. has been a consultant for Hoffman-La Roche, Inc., Johnson & Johnson Wellness and Prevention, and Sanofi-Aventis Groupe. Dr. R.C.K. has served on advisory boards for Mensante Corporation, Plus One Health Management, Lake Nona Institute, and US Preventive Medicine. Dr. R.C.K. owns 25% share in DataStat, Inc. The remaining authors declare that they have no conflict of interest.

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