Genome-Wide Association Study of Sleep Disturbances in Depressive Disorders

Lindsay M. Melhuish Beaupre^{a, d} Vanessa F. Gonçalves^{b, d} Clement C. Zai^{a–d}
Arun K. Tiwari^{b, d} Ricardo S. Harripaul^{a, e} Deanna Herbert^d Natalie Freeman^d
Daniel J. Müller^{a, b, f} James L. Kennedy^{a, b, d}

^aInstitute of Medical Sciences, University of Toronto, Toronto, ON, Canada; ^bDepartment of Psychiatry, University of Toronto, Toronto, ON, Canada; ^cLaboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; ^dMolecular Brain Science Research Department, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada; ^eMolecular Neuropsychiatry and Development Lab, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada; ^fPharmacogenetics Research Clinic, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada

Keywords

Genome-wide association study · Sleep disturbances · Depression · Genetics

Abstract

Sleep disturbance affects about 75% of depressed individuals and is associated with poorer patient outcomes. The genetics in this field is an emerging area of research. Thus far, only core circadian genes have been examined in this context. We expanded on this by performing a genome-wide association study (GWAS) followed by a preplanned hypothesis-driven analysis with 27 genes associated with the biology of sleep. All participants were diagnosed by their referring physician, completed the Beck Depression Inventory (BDI), and the Udvalg for Kliniske Undersøgelser Side Effect Rating Scale at baseline. Our phenotype consisted of replies to 3 questions from these questionnaires. From standard GWAS chip data, imputations were performed. Baseline total BDI scores ($n = 364$) differed significantly between those

with and those without sleep problems. We were unable to find any significant GWAS hits although our top hit was for changes in sleep and an intergenic marker near *SNX18* ($p = 1.06 \times 10^{-6}$). None of the markers in our hypothesis-driven analysis remained significant after applying Bonferroni corrections. Our top finding among these genes was for rs13019460 of Neuronal PAS Domain Protein 2 with changes in sleep ($p = 0.0009$). Overall, both analyses were unable to detect any significant associations in our modest sample though we did find some interesting preliminary associations worth further exploration.

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Depression is a debilitating mood disorder that affects 11–16% of the population throughout their lifetime [1–3]. Most individuals with depression experience substantial impairment, making it the second leading cause of years lived with a disability [2, 4]. One of the core symptoms of depression, as stated by the Diagnostic and Statistical Manual of Mental Disorders (5th ed), is hyper-

somnia and/or insomnia [5]. In fact, about 75% of these individuals experience sleep disturbances [6]. These disturbances are known to have marked effects on the course of the disorder and treatment. For example, irregular sleep physiology is associated with a greater risk of relapse, a greater rate of requiring pharmacological therapy, not responding to psychotherapy and a higher chance of not experiencing remission [7–11]. They have also been found to be a prodromal symptom of depressive disorders [12]. For example, The Munich Vulnerability study found that about a quarter of the high-risk individuals studied (those that have a first degree relative with an affective disorder) experienced changes in their sleep physiology for 10 years leading up to the onset of an affective disorder [13].

Genetics of sleep in depression is an emerging area of research. Thus far, there have been only a limited number of studies done investigating the genetics of sleep disturbances in depression. Of these few studies, all have focused on circadian genes because of their involvement in the regulation of sleep-wake cycles [14]. The only positive findings thus far have been for variants of *TIMELESS* and *CLOCK* [15–17]. For *TIMELESS*, the minor “A” allele of rs7486220 and the “AA” haplotype of rs2291738-rs7486220 have been linked to daytime fatigue in females, whereas the “T” allele of rs1082214 was linked to earlier morning awakenings in males. The findings for *CLOCK* are mixed as 1 study has found an association with rs1801260 and initial insomnia while another was unable to replicate these findings [15, 17].

Genetic studies in MDD research have mainly focused on disease risk. A recent mega-analysis performed by the Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium, combined 9 samples for a total of 18,759 unrelated subjects of European ancestry (9,240 cases and 9,519 controls), but was unable to detect any genome-wide significant associations. A follow-up study done on a large replication sample ($n = 9,238$ MDD subjects and $n = 8,039$ controls) also failed to identify any significant loci [18]. More recently, Hyde et al. [19] identified 15 genetic loci associated with a risk of self-reported major depression in $n = 307,354$ individuals of European ancestry ($n = 65,607$ cases and $n = 231,747$ controls from 23 and Me). The most significant finding was for rs2806933 of *OLFM4*, a gene encoding for olfactomedin-4, that has been previously associated with childhood obesity. The marker rs768705, which is located at a locus containing myocyte enhancer factor 2C and *TMEM161B* (transmembrane protein 161B) was also significant [19, 20]. A second marker at myocyte enhancer factor 2C-

Table 1. Genes included in the hypothesis-driven analysis

CLOCK	TIMELESS	PER1
PER2	PER3	CRY1
CRY2	BMAL1	ARNTL2
NPAS2	RORA	RORB
RARA	HCRT	HCRT1
HCRT2	MCHR1	MCHR2
SIRT1	MTNR1A	MTNR1B
KCNH4	AANAT	USP2
OPN4	ADORA2A	ADA

CLOCK, Clock Circadian Regulator; TIMELESS, Timeless Circadian Regulator; PER1, Period Circadian Regulator 1; PER2, Period Circadian Regulator 2; PER3, Period Circadian Regulator 3; CRY1, Cryptochrome Circadian Regulator 1; CRY2, Cryptochrome Circadian Regulator 2; BMAL1, Aryl Hydrocarbon Receptor Nuclear Translocator Like; ARNTL2, Aryl Hydrocarbon Receptor Nuclear Translocator Like 2; NPAS2, Neuronal PAS Domain Protein 2; RORA, RAR Related Orphan Receptor A; RORB, RAR Related Orphan Receptor B; RARA, Retinoic Acid Receptor Alpha; HCRT, Hypocretin Neuropeptide Precursor; HCRT1, Hypocretin Receptor 1; HCRT2, Hypocretin Receptor 2; MCHR1, Melanin Concentrating Hormone Receptor 1; MCHR2, Melanin Concentrating Hormone Receptor 2; SIRT1, Sirtuin 1; MTNR1A, Melatonin Receptor 1A; MTNR1B, Melatonin Receptor 1B; KCNH4, Potassium Voltage-Gated Channel Subfamily H Member 4; AANAT, Aralkylamine N-Acetyltransferase; USP2, Ubiquitin Specific Peptidase 2; OPN4, Opsin 4; ADORA2A, Adenosine A2a Receptor; ADA, Adenosine Deaminase.

TMEM161B, rs10514299, showed a promising association with risk to MDD in a replication sample [19]. None of these 3 genes has been reported to be associated with sleep measures in depressive disorders.

To our knowledge, this current study is the first to perform a genome-wide association study (GWAS) investigating the genetics of sleep disturbances in depressive disorders. Our hypothesis-free approach investigated the genetic basis of each of 3 items from our questionnaires, namely, changes in sleep, increased sleep, and decreased sleep, in depressed individuals. As a following step, we apply a hypothesis-driven approach to test the role of selected markers in key genes (Table 1) involved in the biology of sleep using the same sample and measures of sleep disturbances from step 1. In this approach, we are able to examine more closely the key markers in the biology of sleep, including the *TIMELESS* gene.

Methods

Subjects

The sample will be a subset of the IMPACT study ($n > 11,000$) [21]. This study was originally designed to examine the association of genetic variants with treatment response and side effects of antidepressants and antipsychotics. Participants were asked to pro-

vide a saliva sample for genetic analyses and fill out a series of questionnaires depending on their diagnoses. All individuals filled out the Beck Depression Inventory (BDI) [22] and the Udalvg for Kliniske Undersogelser (UKU) Side Effect Rating Scale [23]. These individuals were followed prospectively for ~8 weeks and asked to fill out the same questionnaires at a 1-month follow-up and a 2-month follow-up. The diagnoses were made by the referring physician and included: major depressive disorder, depression, depressive episode NOS, depression with psychosis, unipolar and dysthymic disorder. The analyses in this report focused on individuals that had a BDI score >19 at baseline to represent moderate to severe depression [24], and were restricted to European ancestry to reduce population stratification and to allow for the greatest sample size. The ratio of males to females was 110:256. This study was approved by the Centre for Addiction and Mental Health (CAMH) Research Ethics Board. All individuals provided informed consent.

Genotyping

Genomic DNA was extracted at CAMH from saliva collected in Oragene saliva kits using the magnetic bead DNA extraction protocol on the chemagic Magnetic Separation Module I (Perkin Elmer, Waltham, MA, USA) automated DNA extractor as per the manufacturer's standard protocol. A subset ($n = 2,116$) of individuals was genotyped using the Infinium Omni 2.5–8 chip at The Centre for Applied Genomics at The Hospital for Sick Children, Toronto. This chip contains ~2.5 million markers well-suited for genotyping individuals of European ancestry [25].

Quality Control

Genetic quality control (QC) was done on 2,116 individuals who were examined with the Illumina Infinium Omni 2.5–8 chip array using PLINK (version 1.9) [26] and standard protocols described in other GWAS studies [27–29]. The participants in our sample were checked for sex discordance, individual genotype missingness (which was restricted to 2%), heterozygosity rate, relatedness (identity-by-descent), and genetic ancestry to compare with self-reported ancestry. For heterozygosity rate, individuals were removed if they were ± 3 SDs away from the mean to avoid potential confounding of samples or inbreeding. A principal component analysis (PCA) was done to measure self-reported ancestry. To do this, we plotted our sample versus the 1,000 Genomes Phase 3 reference population [30] using R [31]. After individual QC and selecting for European ancestry by the initial PCA analysis, we were left with $n = 1,733$ before the final PCA analysis. Individuals considered to be ancestral outliers were removed from the study if they were ± 6 SDs from the mean [32]. After this final PCA step, we were left with $n = 1,098$ individuals and next we selected those with a clinical depressive disorder leaving 623 people. Finally, we excluded individuals with a BDI score <20, and we were left with 366 individuals.

Genome-wide marker QC was also done to check for significant deviation from Hardy-Weinberg equilibrium ($p < 5 \times 10^{-8}$), minor allele frequency (<1%), and genotype call rates >98% for both markers and individuals. After conducting these QC procedures, we were left with 1,295,909 markers.

Imputations

Whole-genome imputations were conducted using IMPUTE version 2.2 in 5,000,000 Mb segments per chromosome after pre-

phasing was performed with SHAPEIT2 and the 1,000 Genomes reference panel (Phase 3, NCBI build 37) [30, 33, 34]. After performing the imputations, we conducted the same marker QC procedures as described above [27–29]. This time, we excluded markers with a minor allele frequency <0.05 in the European population, <95% genotyping rates, and variants that had an info score threshold of ≥ 0.8 [35]. After applying all QC procedures we were left with 5,932,083 markers.

Sleep Measures

The sleep measures of interest were questions 16 in the BDI (changes in sleep) and in the UKU 1.7 (increased sleep) and 1.8 (decreased sleep). Both the BDI and the UKU were given to participants at baseline, at the 1-month follow-up and 2-month follow-up. Question 16 from the BDI asks about “Changes in Sleeping Pattern” over the course of 2 weeks with 0 = “I have not experienced any change in my sleeping pattern,” 1a = “I sleep somewhat more than usual,” 1b = “I sleep somewhat less than usual,” 2a = “I sleep a lot more than usual,” 2b = “I sleep a lot less than usual,” 3a = “I sleep most of the day,” 3b = “I wake up 1–2 h early and can't get back to sleep.” The question is coded such that individuals that choose 1a or b get a score of 1, those who choose 2a or b receive a score of 2 and those who choose 3a or b get a score of 3. Question 1.7 of the UKU asks regarding an “Increased Duration of Sleep” and 1.8 asks about a “Reduced Duration of Sleep” over the last 3 days. Both questions are provided with a Likert scale from 0 to 3 with 0 = “No side effects,” 1 = “Mild side effects that do not interfere with the patient's performance,” 2 = “Side effects that interfere moderately with the patient's performance” and 3 = “Side effects that interfere markedly with the patient's performance.” All 3 questions were recoded into binary data with 0 representing those who scored a 0 on the question of interest and 1 representing those who scored a 1, 2, or 3 on the question of interest.

Statistics

A binary logistic regression was performed using PLINK (version 1.9) [26] independently for each of the 3 questions of interest for both the GWAS and hypothesis-driven data, using an additive model of inheritance for all single nucleotide polymorphism (SNP) data. Results were considered significant in the GWAS data if they had a p value $< 5.0 \times 10^{-8}$. Nyholt test corrections for linkage disequilibrium (LD) were applied to the hypothesis-driven data [36]. After correcting for 1953 (“sleep”) SNPs in the hypothesis-driven data, a p value of 0.000025 was considered significant. Chi-square analysis was performed to determine whether sex should be analyzed as a covariate. An independent sample t test was conducted to determine if age and baseline BDI score should be analyzed as covariates.

Results

After removing all individuals that did not have a depressive disorder and did not complete the BDI after performing imputations and QC procedures, we were left with $n = 364$ participants. Table 2 for clinical demographics. Note that analyses were limited to those with Euro-

Fig. 1. Manhattan plot for the BDI 16.

Table 2. Clinical demographics

	BDI 16		UKU 1.7		UKU 1.8		Total
	sleep problems		sleep problems		sleep problems		
	absent	present	absent	present	absent	present	
Sample size	36	329	128	223	144	219	366
Gender, male:female	10:26	100:229	43:85	62:161	37:107	71:148	110:256
Current age, mean (SD)	44.67 (15.23)	47.34 (15.49)	47.4 (15.57)	47.23 (15.34)	46.5 (15.21)	47.74 (15.45)	47.01 (15.5)
BDI score, mean (SD)	30.11 (7.5)	33.7 (8.77)	31.78 (8.22)	34.42 (8.86)	31.85 (8.42)	34.42 (8.73)	33.33 (8.71)
Concurrent psychiatric diagnoses, <i>n</i> (%)	24 (66.7)	193 (58.7)	81 (63.3)	136 (61)	87 (60.4)	141 (64.4)	231 (63.1)
Concurrent anxiety diagnoses, <i>n</i> (%)	21 (58)	173 (52.6)	73 (57)	116 (52)	72 (50)	121 (55.2)	195 (53.3)

Some people did not fill out all of the questions, hence why the sample size varies.

BDI, Beck Depression Inventory; UKU, Udvalg for Kliniske Undersogelser Side Effect Rating Scale.

Fig. 2. QQ plot for BDI 16.

pean genetic ancestry. The baseline total BDI score was significantly different between individuals with or without sleep problems for the BDI 16 (changes in sleep; $t = -2.68$, $df = 46.15$, $p = 0.01$), UKU 1.7 (increased sleep; $t = -2.81$, $df = 281.31$, $p = 0.005$), and UKU 1.8 (decreased sleep; $t = -28$, $df = 313.81$, $p = 0.005$). There were no significant correlations between age for either the BDI 16 ($t = -1$, $df = 43.31$, $p = 0.32$), UKU 1.7 ($t = 0.96$, $df = 261.53$, $p = 0.92$), or UKU 1.8 ($t = -0.76$, $df = 309.47$, $p = 0.45$). There was also no significant sex effect found for the BDI 16 ($\chi^2 = 0.106$, $df = 1$, $p = 0.75$), UKU 1.7 ($\chi^2 = 1.3$, $df = 1$, $p = 0.25$), or UKU 1.8 ($\chi^2 = 1.88$, $df = 1$, $p = 0.17$). Thus, we only included baseline BDI score as a covariate in our analyses, although this did not influence the results.

Hypothesis-Free GWAS

From the hypothesis-free approach, none of the SNPs was significantly associated with sleep disturbances with any of the questions of interest (Fig. 1–6). There were some nominally significant associations, for example, the top finding from our analyses for changes in sleep (BDI 16) was for rs2565010, located 44kb 3' of *SNX18* (OR 0.22, $p = 1.06 \times 10^{-6}$) and 2 SNPs in the N-deacetylase and N-sulfotransferase 3 (*NDST3*) coding region, rs7436139 and rs6835510 (OR 0.26, $p = 5.21 \times 10^{-6}$ and OR 0.19, $p = 5.9 \times 10^{-6}$, respectively). Other top hits were intergenic regions (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000505804). For the in-

creased sleep (UKU 1.7), our top findings were for 19 intergenic SNPs (online suppl. Table 2), as well as rs57288534 of *RASGEF1C* (OR 3.31, $p = 8.82 \times 10^{-6}$) and 2 SNPs in ryanodine receptor 2 (*RYR2*), rs10802610 and rs12041884 (OR 0.4, $p = 8.9 \times 10^{-6}$ and OR 0.4, $p = 9.45 \times 10^{-6}$, respectively). Lastly, our top findings for decreased sleep (UKU 1.8) were for rs4076190 of *ANTXR1* (OR 0.46, $p = 2.77 \times 10^{-6}$) and several other intergenic SNPs (online suppl. Table 3).

Hypothesis Driven

None of our SNPs was significantly associated with sleep disturbances on any of the 3 questions of interest after applying Nyholt corrections for multiple testing. Our top findings for changes in sleep were 17 variants in Neuronal PAS Domain Protein 2 (*NPAS2*), with the top hit being rs13019460 (OR 2, $p = 0.0009$). Other nominal findings were 6 SNPs in Period Circadian Regulator 2 (*PER2*; top hit: rs78639475, OR 2, $p = 0.0067$) and rs2234975 of Sirtuin 1 (*SIRT1*; OR 2.4, $p = 0.0068$) with increased sleep and 14 SNPs in hypocretin/orexin receptor 2 (*HCRTR2*) and decreased sleep, with the top hit being rs3134696 (OR 1.7, $p = 0.0062$).

Discussion

First, we performed a GWAS investigating the genetics of sleep disturbances in depressive disorders in the CAMH IMPACT sample. Following this, we tested the association of 5,895 SNPs related to the biology of sleep with 3 measures of sleep disturbances in the same depression sample. We were unable to detect any genome-wide associations in our hypothesis-free analyses, though we did find some nominally significant (p value $< 9.99 \times 10^{-6}$) associations that warrant further investigation. The coding region of the *NDST3*, which is involved in heparin biosynthesis and heparan sulfate metabolism [37, 38], was the top hit in terms of known coding regions. It is expressed both in the fetal and adult brains [39]. Both heparin and haparan sulfate play an important role in many vital cellular processes including cell growth and signaling [40]. Lencz et al. [41] found that rs11098403 of *NDST3* was previously associated with schizophrenia and bipolar disorder in an Ashkenazi Jewish sample. Although neither of the SNPs identified in our study is in LD with the SNP found in Lencz et al. [41], it does provide some support that the gene itself might be implicated in sleep disturbances in depressive disorders. Another promising hit was for 2 variants in the *RYR2*. Ryanodine receptors play a role in the release of calcium from calci-

Fig. 3. Manhattan plot of the UKU 1.7.

um channels in mammalian skeletal and cardiac muscle [42]. Numerous mutations in this gene have been associated with catecholaminergic polymorphic ventricular tachycardias, which may result in sudden cardiac death [43, 44]. More recent research has suggested that ryanodine receptors are regulated by the CLOCK/BMAL1 complex [45]. CLOCK and BMAL1 proteins are found in the suprachiasmatic nucleus (SCN), the master clock of the body. The SCN contains a set of genes responsible for regulating the body's circadian rhythms and sleep-wake cycle [46]. As such, it is plausible that *RYR2* plays a role in the SCN and regulating sleep-wake cycles, although extant research is limited and further work is warranted.

In our secondary, hypothesis-driven analyses, we were unable to detect any significant associations. Our top hit was for a variant (rs13019460) in *NPAS2*, a core circadian gene found in the SCN. *NPAS2* is considered homologous to the *CLOCK* gene. It has been found that in *CLOCK*-deficient mice, *NPAS2* will form a heterodimer with

Fig. 4. QQ plot of the UKU 1.7.

Fig. 5. Manhattan plot for the UKU 1.8.

Fig. 6. QQ plot for the UKU 1.8.

BMAL1, which allows the SCN to maintain circadian rhythmicity [47]. Furthermore, Evans et al. [48] found an association between rs3768984 of *NPAS2* and objectively measured sleep parameters using actigraphy. Soria et al. [49] also implicated another variant of the gene, rs11123857 in depression. Although neither of these SNPs is in LD with our SNP of interest, taken together, the evidence suggests that *NPAS2* could play a role in sleep disturbances among depressed individuals. *PER2*, another core circadian gene has had several variants associated with depression susceptibility versus mental resilience in a sample of $n \sim 430$ individuals, although no variant in the gene was associated with depression in case-control analyses [50, 51]. *PER2*, like *NPAS2* is part of the core clock machinery, as it is part of the positive and negative transcriptional feedback loops in mammals that regulates the 24-h (circadian) cycle in the SCN [52, 53]. As such, it seems plausible that *PER2* plays a role in sleep disturbances in depressed individuals. However,

Utge et al. [16] were unable to find any association with variants in *PER2* and sleep disturbances in depression in either males or females. We also observed nominal promising results for rs2234975, located in the untranslated region of *SIRT1*. Although *SIRT1* is not considered a core circadian gene, it has been shown to bind to the CLOCK-BMAL1 complex and directly regulate *PER2*, suggesting it plays a role in the regulation of circadian rhythms [54]. Therefore, it seems like a promising gene to study in this context. Lastly, we also found nominal associations for several variants in the *HCRT2*. This gene has several lines of evidence supporting its role in the sleep-wake cycle. Lin et al. [55] were the first to discover that disruption of *HCRT2* led to narcolepsy in canines. There is also evidence supporting the involvement of hypocretin/orexin in promoting wakefulness [56]. More recently, a GWAS study investigating sleep traits identified rs3122163, a variant near *HCRT2* to be linked to shorter sleep duration [57]. Although this SNP is not in LD with any of the SNPs found in our study, all of the evidence together suggests that *HCRT2* may play a role in sleep disturbances.

A limitation of this study is that we have a modest sample size, which means that our results could be false-positive results. Second, our study is based on subjective measures, which can be distorted by recall bias or other biases in the participants. Lastly, a confound may lie in the fact that most participants had comorbid psychiatric diagnoses, as in the case in most samples of psychiatric disorders.

Overall, the research into the genetics of sleep disturbances is limited. As such, we sought out to perform a hypothesis-free GWAS study, followed by a secondary hypothesis-driven analysis to explore biologically informed genetic marker associations with sleep disturbances in a subset of the CAMH IMPACT study. We were unable to detect any significant associations in any of our analyses. We provide potentially useful preliminary data and methodological approaches to inform future investigations of this important area.

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Statement of Ethics

The research was conducted ethically in accordance with the CAMH Ethics Board.

Disclosure Statement

J.L.K. is a member of the Scientific Advisory Board of Myriad Genetics (unpaid) and has several patents pertaining to pharmacogenetic testing for psychiatric illnesses. All other authors have no conflicts of interest to declare.

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Author Contributions

L.M.M.B.: performed the imputations, data analysis, interpretation of results, and the write-up of the manuscript. V.F.G.: provided support and guidance on performing the imputations, data analysis, and interpretations of the results. She also helped draft the manuscript. C.C.Z. and A.K.T.: provided guidance on the data analysis, interpretation of results, and the write-up of the manuscript. D.H. was involved in the clinical data collection, analysis of the data, and editing of the manuscript. N.F. was involved in the generation of data from the lab, methods design, and the drafting and editing of the manuscript. D.J.M. helped with the data analysis, interpretation of results, and editing of the manuscript. J.L.K. oversaw and assisted with the data analysis, interpretation of the results, and the drafting of the manuscript.

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