GWAS Meta-Analysis Reveals Shared Genes and Biological Pathways Between Major Depressive Disorder and Insomnia

Yi-Sian Lin 1, Chia-Chun Wang 1, and Cho-Yi Chen 1, 2, *

1 Institute of Biomedical Informatics, National Yang Ming Chiao Tung University, Taipei 11221, Taiwan;
2 Brain Research Center, National Yang Ming Chiao Tung University, Taipei 11221, Taiwan;
* Correspondence: choyichen@nycu.edu.tw

Abstract: Major depressive disorder (MDD) is one of the most prevalent and disabling mental disorders worldwide. Among the symptoms of MDD, sleep disturbance such as insomnia is prominent and the first reason patients may seek professional help. However, the underlying pathophysiology of this comorbidity is still elusive. Recently, genome-wide association studies (GWAS) have begun to unveil the genetic background of several psychiatric disorders, including MDD and insomnia. Identifying the shared genomic risk loci between comorbid psychiatric disorders could be a valuable strategy to understand their comorbidity. This study seeks to identify the shared genes and biological pathways between MDD and insomnia based on their shared genetic variants. First, we performed a meta-analysis based on the GWAS summary statistics of MDD and insomnia obtained from Psychiatric Genomics Consortium and UK Biobank, respectively. Next, we associated shared genetic variants to genes using two gene mapping strategies: (a) positional mapping based on genomic proximity and (b) expression quantitative trait loci (eQTL) mapping based on gene expression linkage across multiple tissues. As a result, a total of 719 shared genes were identified. Over half (51%) of them are protein-coding genes. Functional enrichment analysis shows that the most enriched biological pathways are related to epigenetic modification, sensory perception, and immunologic signatures. We also identified druggable targets using a network approach. Together these results may provide insights into understanding the genetic predisposition and underlying biological pathways of comorbid MDD and insomnia symptoms.

Keywords: GWAS; MDD; Insomnia; eQTL; comorbidity; STRING; gene network; meta-analysis

1. Introduction

Major depressive disorder (MDD) is one of the most prevalent and disabling mental disorders worldwide, with a lifetime prevalence of 15% [1]. Sleep disturbance is the core symptom of MDD that occurs in up to 90% of patients and is the first reason patients seek professional help [2, 3]. Also, according to the DSM-5, the major depressive episode includes “insomnia or hypersomnia nearly every day” [4]. Insomnia is a sleep problem that individuals have difficulty sleeping and is often chronic, negatively affecting their quality of life [5]. On the other hand, studies have shown that people with insomnia are more likely to develop depression and increase suicidal ideation if diagnosed with MDD [6, 7]. Moreover, drugs and behavioral treatments for comorbid MDD and insomnia symptoms can improve both outcomes [8, 9]. Therefore, the relationship between MDD and insomnia may be bi-directional [10, 11].

Although the underlying mechanism of MDD remains elusive, MDD is recognized as a complex disorder contributed by both genetic and environmental factors. The heritability of MDD is 40% to 50% suggested by twin studies [11]. However, the genetic component of insomnia is hard to estimate because it can coexist with other medical and psychiatric conditions. Recent genome-wide association studies (GWAS) have identified genetic variants for depression [12–17] and insomnia disorder [18–23], respectively. GWAS is a powerful approach to test genome-wide genetic variants of population-level to identify
genotype-phenotype associations [24]. Notably, insomnia and MDD are genetically correlated as shown by Lane et al. ($r_g = 0.34$ and 0.24 in two studies) [18, 21], Hammerschlag et al. ($r_g = 0.41$) [19], Stein et al. ($r_g = 0.44$) [20], and Jansen et al. ($r_g = 0.59$) [22, 25]. Therefore, identifying the shared genomic risk loci between MDD and insomnia would be a valuable strategy to associate the underlying pathophysiology of MDD with insomnia.

In this study, we exploit this strategy to explore the shared candidate genes and related biological pathways involved in the pathogenesis of MDD and insomnia. First, we performed a GWAS meta-analysis of MDD and insomnia using the summary statistics from Wray et al. [16] and Lane et al. [21], respectively, to identify shared genetic links and new associated variants between the two psychiatric conditions. Next, to characterize the functional roles of the variants, we conducted positional and expression quantitative trait loci (eQTL) mapping followed by a series of functional enrichment analyses. Finally, to provide potential druggable targets of MDD with insomnia, we prioritized the genes (targets) based on their connectivity degree in the human protein-protein interaction (PPI) network and searched for potential drugs using the drug-gene interaction databases.

2. Materials and Methods

2.1 GWAS data and meta-analysis

GWAS summary statistics of MDD and insomnia were downloaded from Psychiatric Genomics Consortium (PGC) (http://www.med.unc.edu/pgc) and Sleep Disorders Knowledge Portal (http://kp4cd.org/datasets/sleep), respectively. The original GWAS studies can be referred to Wray et al. [16] and Lane et al. [21]. Meta-analysis of MDD and insomnia was performed with an inverse-variance weighted model using METAL [26]. Specifically, SNP ID, weight, alleles, frequency, effect size, standard error, and p-value were provided from both GWAS summary statistics for METAL to execute.

2.2 Identification of candidate SNPs, gene mapping, and functional annotation

FUMA [27] (v1.3.6) was used to identify candidate SNPs. Linkage disequilibrium (LD) blocks from 1000 Genomes Project Phase 3 [28] EUR population were used as a reference panel to compute r2 and MAF. Candidate SNPs were mapped to genes using positional and eQTL mapping approaches separately. Gene window for positional mapping was set at default maximum distance of 10 kb on both sides and was based on ANNOVAR [29] annotation. Cis-eQTL mapping mapped SNPs to genes up to 1 Mb, and two sets of tissue types were used: (1) whole body tissues in GTEx v8 [30] (54 tissue types, including brain regions), and (2) 13 brain-only regions. Only eQTLs with FDR ≤ 0.05 were considered statistically significant. Biotypes of mapped genes were annotated by Ensembl BioMart (Ensembl build v92). Functional enrichment analyses were performed using hypergeometric tests. Pathway and functional gene set information was obtained from MSigDB v7.0 [31].

2.3 MAGMA gene-based tests

The gene-based analysis was performed using MAGMA [32] v1.08 with SNP-wise mean model as part of the FUMA pipeline. Gene annotation window of 10 kb upstream and 10 kb downstream was used. SNPs were mapped to 19,383 genes obtained from Ensembl build v92 GRCh37. Tissue expression (gene-property) analysis was performed to test the genetic associations of highly expressed genes in a specific tissue based on GTEx v8 [30] RNA-Seq data.

2.4 Cell type specificity analysis

MAGMA gene-property analyses were performed to test the relationship between cell-type-specific gene expression profiles and phenotype-gene associations. Mouse cerebellar single-cell RNA-Seq data were obtained from DropViz [33]. Primary cell types and their sub-clusters were used for analysis. Human adult brain single-cell data were obtained from GEO Accession GSE67835 [34].

2.5 Identification of druggable targets
We selected 713 union genes from two mapping strategies and obtained their gene network using the Search Tool for Retrieval of Interacting Genes (STRING) [35]. Genes were ranked by connectivity degree using the Cytoscape [36] plugin cytoHubba [37]. To identify druggable targets from these genes, we characterized the drug-gene interactions in the Drug—Gene Interaction Database (DGIdb v4.1.0) [38]. Approved drugs were used as a preset filter. The known targets for MDD and insomnia were acquired using Open Targets v3.19.0 [39] and then used as input to find their interacted drugs using DGIdb.

3. Results

3.1 Shared genetic variants

We conducted a genome-wide association meta-analysis of MDD and insomnia based on two previous GWAS studies: (1) Wray et al. [16] identified 44 risk variants in 135,458 major depression cases versus 344,901 controls from seven cohorts. (2) Lane et al. [21] identified 57 loci for self-reported insomnia symptoms in 345,022 cases and 108,357 controls from the UK Biobank. Figure 1a shows the workflow of our analyses. In the meta-analysis, we identified 62 lead variants (P < 5 x 10^{-8}) at 54 risk loci, among 7,062 candidate associated SNPs (P < 0.05). The signal (measured by the number of SNPs) was stronger in meta-analysis than in MDD or insomnia study alone (Figure 1b, Supplementary Figure 1, Supplementary Table 1-2). The most significant associated variant was rs113831554 (P = 1.64 x 10^{-22}) lies in the intronic region of MEIS1, a gene associated with restless legs syndrome (RLS) [40]. This SNP was also reported in Lane et al. [21] with the strongest signal. The second strongest signal we identified was rs12658032 (P = 3.77 x 10^{-19}), located in the intron of lincRNA RP11-6N13.1. This locus was not reported by Wray et al. [16] or Lane et al. [21] but was shown to be associated with MDD and attention deficit/hyperactivity disorder (ADHD) [41]. The third significant SNP was rs12552 (P = 5.69 x 10^{-16}) in the 3’UTR of OLFM4, which was also reported in Wray et al. [16] (Supplementary Fig. 2). Furthermore, four other SNPs (rs10156602, rs10865954, rs4577309, and rs12405761) identified by Lane et al. [21] were replicated in our meta-analysis. Notably, a list of variants, including rs201018268, rs558237097, rs575346808, rs8013655, rs1520946, rs529656112, rs75606464, rs35735593, rs71573104, rs6765491, rs17043773, rs62519760, rs12125521, rs12537732, rs12607631, and rs360241, were not previously reported for being associated with any depressive or insomnia traits, suggesting that such variants were new variants shared by of MDD and insomnia.
3.2 Tissue expression and cell type specificity

Tissue enrichment analysis was performed using MAGMA to investigate the tissue expression of variant-associated genes. We found those genes were majorly expressed in the brain but not in the peripheral tissues (Figure 2a). The top enriched brain region was the cerebellum, regardless of hemisphere, followed by the cortex, frontal cortex (FC) BA9, pituitary, and anterior cingulate cortex (ACC) BA24. (Supplementary Table 3) Our results coincide with Wray et al. [16] (MDD enrichment in FC BA9, cortex, and ACC BA24) and Lane et al. [21] (insomnia enrichment in the cerebellum, FC, ACC, and hypothalamus).

Next, we focused on the cell type specificity in the brain regions, including the cerebellum and the frontal cortex (containing Brodmann area BA9 and BA24). It shows that MDD- and insomnia-associated gene expression were enriched in neurons but not glial cells in the cerebellum and the frontal cortex (Figure 2b-d, Supplementary Table 4-6). This result was consistent with Wray et al. [16]. Sub-cell type analysis reveals that such cerebellar neurons were both glutamatergic (Slc17a7) and GABAergic (Gad1Gad2) (Supplementary Fig. 3a). Interestingly, cortical neurons were predominately enriched in glutamatergic (Slc17a7) (Supplementary Fig. 3b).
3.3 Gene mapping and functional enrichment

To better understand how these shared variants contribute to the underlying pathophysiology of MDD and insomnia, we have to associate these variants with genes in the genome. Here we adopted two gene mapping strategies: (a) positional mapping based on genomic proximity and (b) eQTL mapping based on linked gene expression across multiple tissues.

In positional mapping, 507 genes were found proximal to MDD-insomnia shared SNPs (Supplementary Table 7). The largest proportion of the SNPs fell in the intergenic region, followed by genes in the intronic region and then the intronic region of ncRNA (Supplementary Fig. 4). The top significant enriched Reactome gene sets were HDACs deacetylate histones, HATs acetylate histones, and DNA methylation. The most significant enriched GO biological pathways were sensory perception of smell and sensory perception of chemical stimulus (Figure 3a, Supplementary Table 10).

The eQTL mapping identifies variants associated with gene expression across multiple tissues. The following two contexts were considered in the eQTL mapping: (a) eQTLs found in the whole body tissues (54 tissues in GTEx), and (b) eQTLs found in the brain...
regions (prefix with Brain in Supplementary Table 3). For (a) whole body tissues, 444 genes were mapped (Supplementary Table 8). These genes are enriched in Butyrophilin (BTN) family interactions and several immunologic signatures, including macrophage transcriptional response to Interleukin-6, CD4+ T cell pathway, and HMC-1 cell activation (Figure 3b, Supplementary Table 11). For (b) brain-only regions, 148 genes were mapped (Supplementary Table 9). Again, the gene set “Butyrophilin family interactions” was enriched (Figure 3c, Supplementary Table 12). Three BTN genes (BTN3A2, BTN2A2, BTN3A3) show strong associations in both mapping contexts.

Overall, a total of 719 shared genes were identified with at least one of the mapping contexts. Over half (367/719, 51%) of them are protein-coding genes. The remaining half (352/719, 49%) consists of 144 pseudogenes, 66 antisense, 67 lincRNAs, 21 miRNAs, and 54 other biotypes. Of note, shared genes via positional mapping and eQTL mapping are highly overlapped (232 genes in common) (P < 5 x 10^-16, Fisher’s exact test) (Figure 3d), suggesting that a large proportion of shared variants have the potential to influence the expression of proximal genes.
Figure 3. Functional enrichment of shared genes. Bar-dot plots show (a) top 10 significant enriched Reactome gene sets and top 10 enriched GO biological processes of positional mapping genes; (b) enriched Reactome gene sets and top 10 enriched immunologic signatures of eQTL mapping genes (54 tissues); (c) enriched Reactome gene sets of eQTL mapping genes (brain regions). (d) A Venn diagram showing the relationships of mapped genes under different mapping strategies.

3.4 Druggable targets identified by network approach

Genes associated with MDD and insomnia may provide a list of candidates for finding druggable targets. To this end, we prioritized the identified shared genes according to their protein connectivity in the human protein-protein interaction (PPI) network. A
A subnetwork consisting of 275 nodes was created using STRING. We then ranked these 275 nodes by their connectivity degree in the network and searched for their potential drugs based on known drug-gene interactions using DGIdb (Supplementary Table 13). Genes with query scores over 6 and their top 3 interacted drugs were shown in Figure 4. Notably, RHOA was in the gene set of synapse organization in our GO enrichment result. MST1R was related to pathways involved in H3K4me3 and H3K27me3. STAT1, NT5C2, CACNA2D2, PML, and EYA2 were enriched in immune-related pathways (Supplementary Table 10-12).

![Figure 4](image)

**Figure 4.** Druggable targets prioritized in the STRING network. A total of 275 shared genes are ordered by their connectivity degree in the network. The color of nodes from red to yellow represents the degree from high to low. Druggable targets are annotated with their potential drugs (drug listed under the target gene name).

To compare the drugs we found with known targets and drugs for MDD and insomnia, we searched for known drug targets for MDD and insomnia in the Open Target database [39] and DGIdb [38]. Among the 1405 unique drugs for MDD and 684 drugs for insomnia, 630 drugs are in common, suggesting agents relieve shared symptoms in MDD and insomnia (Supplementary Table 14). Among them, 49 drugs approved for both MDD and insomnia are also reported in our proposed drug list (Table 1), showing the potential of our shared gene strategy and network approach in the application of drug discovery and repurposing.

**Table 1.** List of proposed drugs with use approvals for both MDD and insomnia.

| Name of drug | Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 | doi:10.20944/preprints202109.0113.v1 |
4. Discussion

In this study, we conducted a meta-analysis from GWAS summary statistics of MDD and insomnia and identified common variants underlying the pathophysiology of these two psychiatric conditions. The variants were mapped to genes, and functional enrichment analysis was performed to suggest their functions. We also provide a list of druggable targets and potential drugs for future validation, which may benefit future drug development against the comorbidity of depression and insomnia symptoms.

Our meta-analysis was able to capture the significant signal from both MDD and insomnia summary statistics. We identified 62 variants linked to shared genetics of MDD and insomnia symptoms, with signals near MEIS1, RP11-6N13.1, OLFM4, HEXIM1, and TCF4 strongest. Some loci have been reported in previous studies with similar findings. First, a study has reported that MEIS1 shows a pleiotropy effect on RLS and insomnia[19]. A study using bioinformatics and transgenic mice approaches indicated the regulatory role of MEIS1 in neuropeptide substance P expression in the amygdala, suggesting a mechanism underlying anxiety and depression [42]. Second, the RP11-6N13.1 locus has been implicated in early sleep timing [43] and how it contributes to insomnia remains to be determined. Besides our finding of rs126580832, rs40465 near RP11-6N13.1 has been reported to be associated with broad depression [15]. Third, evidence has indicated loci in OLFM4 related to major depression [13, 44] and insomnia [22]. Fourth, we found a variant rs201018268 located in the exonic region of HEXIM1. HEXIM1 is a transcription regulator suggested for its role in cancer [45, 46] and linked to insomnia [21], but its association with MDD still lacks. It is worth further study to investigate whether variation in the exonic region of HEXIM1 affects MDD phenotype. Fifth, our gene-based analysis showed that TCF4 was the only gene that reached genome-wide significance in MDD and insomnia. GWAS studies have identified SNPs in TCF4 susceptible to schizophrenia [47] and corneal endothelial dystrophy [48]. Besides, a rare mutation in TCF4 leads to Pitt-Hopkins syndrome, a rare neurodevelopmental disorder [49, 50]. A recent study shows the contribution of TCF4 in mutual influences between MDD and insomnia [51], which aligns with our result. Although the above loci have been discovered for their associated trait, our study links these risk loci to both MDD and insomnia, suggesting a possible pleiotropy effect on their comorbid phenotypes.

Brain regions and neural network alterations in MDD patients have been found with neuroimaging studies. Specifically, abnormalities in the prefrontal cortex, ACC, amygdala, hippocampus, thalamus, and basal ganglia have been indicated in MDD [52–56]. Moreover, alternations in the inferior frontal gyrus/anterior insula, orbitofrontal cortex,
and suprachiasmatic nuclei were found in patients with MDD and co-occurring insomnia [57–59]. In line with the evidence above, our results show that genes associated with shared variants are highly expressed in the cortex, FC, ACC, and, surprisingly, the cerebellum. The cerebellum has been recognized to be involved in cognitive and affective functions besides functions in motor coordination [60]. Recently, abnormality in cerebellum structure and functions has been reported in depression [61, 62]. Sleep disorders, including insomnia, have also been linked to cerebellar malfunction [63, 64]. However, none of the research focuses on cerebellum changes in MDD with insomnia to our knowledge. Therefore, it could be a novel direction to investigate cerebellum in coexisting MDD and insomnia. We also found enriched gene expression of MDD and insomnia in the pituitary, a neuroendocrine gland in the hypothalamic-pituitary-adrenal (HPA) axis. HPA axis mediates stress response, and its hyperactivity has been implicated in the etiology of MDD, stress-related disorders, and insomnia [65–67]. In summary, to better understand the comorbidity of MDD and insomnia, further transcriptomic experiments are needed to identify differentially expressed genes and region-specific transcripts in the aforementioned brain regions.

Our results also suggest that glutamatergic and GABAergic neurons in the cerebellum and glutamatergic neurons in the frontal cortex may play a role in the comorbidity of MDD and insomnia. Indeed, cortical glutamate and GABA dysregulation were observed in MDD and primary insomnia [68, 69]. Specifically, patients with MDD have decreased glutamatergic metabolites in the medial frontal cortex [70], whereas subjects with primary insomnia or mood disorder had lower GABA levels in the occipital cortex and ACC [71]. GABA is an inhibitory neurotransmitter, and activation of GABA receptors has been targeted for sleep-promoting agents [72]. Current treatment for depression also showed effects on counteracting GABAergic deficit, increasing hippocampal neurogenesis and maturation [73]. One possible explanation of our excitatory glutamate enrichment results in the frontal cortex could be that the associated gene expression in the glutamatergic neuron down-regulates the glutamate synthesis or release in the frontal cortex. However, its effect on the reduction of GABA transmission and their interplay remained to be explored. On the other hand, although previous studies have suggested a role of glutamate and GABA transmission in MDD and insomnia, none of the studies focus on such neurotransmitter levels in the cerebellum. Our result highlights genetic variation linked to cerebellar excitatory and inhibitory neurotransmission in MDD and insomnia, providing brain region and cell type-specific targets for future research to treat such disorders.

Our functional enrichment analysis reveals that genes linked to MDD and insomnia are involved in several biological pathways. Reactome enrichment of HDACs deacetylate histones, HATs acetylate histones, and DNA methylation together suggests epigenetic effects on these disorders. These results imply that both genetic and epigenetic factors contribute to complex psychiatric disorders. Stressful life experiences were correlated with dysregulation of HDAC2 and HDCA5 levels [74, 75], and preclinical studies have suggested HDAC inhibitors as a potential therapeutic agent for MDD [76, 77]. Studies also suggested the roles of HDACs in sleep deprivation and melatonin receptors, which are closely related to insomnia [78, 79]. Our results also show pathway enrichment in sensory perception of the chemical stimulus, such as smell. This is consistent with evidence showing the correlation between smell and taste alterations in older MDD patients [80]. However, no study so far focuses on the roles of smell perception in insomnia. Butyrophilins (BTNs) are regulators of the immune response. Together with our enrichment in several immunologic signatures, our result indicated that MDD with insomnia is associated with a dysfunctional immune system. Accumulating evidence has suggested that MDD is linked to elevated proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and chemokines [81–84]. Studies also indicated that the phase shift of IL-6 and TNF secretion is associated with chronic insomnia [85]. These findings suggested that anti-inflammatory drugs might become promising medications for treating MDD with insomnia. Our result supports the previous finding of immune dysregulation...
in MDD with insomnia and provides a new direction in studying epigenetic or sensory perception-related pathways in this disorder.

Recent pharmacological treatments for MDD with insomnia include antidepressants with sleep-promoting properties, such as Mirtazapine [86]. The action of such antidepressants has long been based on the “monoamine hypothesis”, most of which acts on 5-HT (serotonin), norepinephrine, or histamine receptors [87, 88]. However, antidepressant medications can be non-effective and hardly improve the subjective rating of sleep quality [89]. Although alternative agents can be prescribed for resistant insomnia in depression, for example, benzodiazepine drugs or melatonin, drug dependence and their modest effects have been a concern, respectively [90–92]. This study identified potential druggable targets for MDD with insomnia and their existing drugs for drug repurposing. However, some limitations existed. MDD and insomnia are polygenic disorders; hence, it is difficult to quantify the single gene contribution, and drug design for multi-targets has been challenging. Although we prioritized the targets based on their interaction with other proteins, it does not equate to a higher contribution to the disorders’ pathophysiology. Further studies and technologies are demanded to conquer the above problems to improve drug development for MDD with insomnia.

5. Conclusions

In conclusion, we identified risk loci that link to individuals’ susceptibility to developing MDD with insomnia. Our analyses further revealed tissue and cell-type specific gene expression associated with these two disorders. Functional enrichment analysis suggested pathways in epigenetic, sensory perception, and immune functions in MDD with insomnia. Finally, we provide a list of druggable targets and potential drugs for future medication in treating comorbid MDD and insomnia conditions.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Manhattan plot and Q-Q plot of MDD and insomnia, Figure S2: Regional association plots of the top 5 variants, Figure S3: Second level of cell type specificity analysis, Figure S4: Functional consequences of SNPs on genes in positional mapping, Supplementary Tables S1-S14.

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