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Research Paper

Human Gene Expression Profile Analysis of Insomnia and Pre-insomnia Disorders: A Cellular Study

Vahid Mansouri[1], Babak Arjmand[2, 3], Nastaran Asri[4], Zahra Razzaghi[5], Mostafa Rezaei-Tavirani[1*], Farideh Razi[6], Fatemeh Bandarian[7], Reza M Robati[8], Mitra Rezaei[9, 10]

1. Proteomics Research Center, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
3. Iranian Cancer Control Center (MACSA), Tehran, Iran.
4. Celiac Disease and Gluten-Related Disorders Research Center, School of Medicine, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
6. Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
7. Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
8. Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
9. Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
10. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Introduction: Sleep is a vital process for restoring brain function and is recognized as a fundamental aspect of both physical and mental health. This study aims to assess the molecular mechanisms of insomnia disorder and identify the key dysregulated genes associated with it.

Methods: To study molecular mechanisms of insomnia, GSE208668 was selected from the Gene Expression Omnibus (GEO) database. Total RNA from peripheral blood mononuclear cells of 17 individuals with insomnia disorder was analyzed and compared to 25 controls using the GEO2R program. The gene expression profiles were assessed using box plots, uniform manifold approximation and projection (UMAP) plots, expression density diagrams, and Venn diagrams. The significantly differentially expressed genes (DEGs) were evaluated through a directed protein-protein interaction (PPI) network using the CluePedia plugin of Cytoscape software, taking into account co-expression interactions. The central nodes were identified as the most influential and regulated genes.

Results: Pre-evaluation analysis revealed that insomnia exhibits heterogeneity and can be divided into two groups. The gene expression profiles of the first group were similar to those of the insomnia group. In contrast, the second group of controls was distinguished from the insomnia group by genes such as *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1*, which were identified as key actor genes. Additionally, *IL10*, *IL6*, *TP53*, *PTGS2*, *ESR1*, *PTEN*, *JUN*, *CREB1*, *CDKN1A*, *CDKN2A*, *CXCR4*, and *GATA3* were identified as important regulatory genes.

Conclusion: It can be concluded that many individuals may be potentially involved in insomnia disorder as pre-insomnia. The findings demonstrate that pre-insomnia and insomnia share very similar molecular mechanisms. The critical genes *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1*, along with pathways related to apoptosis, inflammation, immunological response, and changes in sleep quality, are emphasized as particularly relevant to insomnia disorder.

*** Corresponding Author:**
Mostafa Rezaei-Tavirani, Professor.
Address: *Proteomics Research Center, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
E-mail: *tavirany@yahoo.com*

Highlights

- People with insomnia and pre-insomnia share similar gene profiles with no significant DEGs.
- Key genes in insomnia include *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1*.
- Key regulatory genes include *IL10*, *IL6*, *TP53*, *PTGS2*, *ESR1*, *PTEN*, *JUN*, *CREB1*.
- Shared mechanisms include apoptosis, inflammation, and changes in sleep quality.

Plain Language Summary

Sleep is essential for physical and mental health, but many people struggle with insomnia. This study aimed to examine how genes might play a role in insomnia and pre-insomnia. We used blood samples from 17 people with insomnia and 25 without insomnia, analyzing their gene activity with special computer tools. We discovered that some people with pre-insomnia had gene patterns similar to those with insomnia, suggesting they might be in a pre-insomnia stage. The genes *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1*, were Key actors in insomnia, linked to processes including inflammation and cellular changes. Other genes, such as *IL6* and *IL10*, also seemed to control these effects. The results of this study help understand the gene connections and detect insomnia early, even before symptoms fully appear. It also highlights how inflammation, a common bodily response, may be a significant factor in sleep disorders.

Introduction

Sleep is a fundamental process for restoring brain function and is recognized as a basic dimension of physical and mental health (Baglioni et al., 2016). On the other hand, sleep disturbances, such as insomnia, are associated with an increased risk of dementia (Shi et al., 2018), a higher likelihood of dying from cardiovascular diseases (Sofi et al., 2014), and an elevated risk of mental disorders (Hertenstein et al., 2019). The association between insomnia and depression has been acknowledged, with key indicators including high levels of negative emotion and low levels of positive emotion dysregulation (Tomaso et al., 2021). Franzen et al. revealed that 90% of individuals with major depression report disturbances in normal sleep (Franzen & Buysse, 2008). However, recent meta-analytic data concluded no consistent evidence for an intervention effect between sleep disturbances and improvement in depressive symptoms (Mitter et al., 2022). Circadian systems and sleep are key modulators of immune system function, and experimental sleep deprivation leads to increased expression of interleukin *(IL)-6* and tumor necrosis factor (*TNF*) from nighttime to daytime (Vgontzas et al., 2002). Sleep loss may activate nuclear factor-kappa B, a key regulatory pathway in the inflammatory response, increasing levels of *IL-6* and *TNF* (Irwin et al., 2008). Interventional studies have revealed an association between insomnia and inflammation (Carroll et al., 2015). Evidence suggests that inflammation caused by insomnia may be related to hypothalamic-pituitary-adrenal axis activation and glucocorticoid resistance (Irwin, 2019). Genome-wide association studies of insomnia have identified MEIS1 as having a strong association signal, suggesting that MEIS1 may play a role in insomnia and restless leg syndrome (Hammerschlag et al., 2017). However, the inflammation associated with insomnia and depression has not been thoroughly analyzed and remains largely hypothetical (Palagini et al., 2022).

Today, with the assistance of bioinformatics knowledge and powerful data analysis software, gene network analysis and the interpretation of gene interactions are possible (Hammerschlag et al., 2017). Since bioinformatics tools are suitable for detecting the molecular mechanisms of diseases (Arjmand et al., 2024), this study aims to investigate the molecular mechanisms of insomnia and identify the key genes that are dysregulated in relation to it. The findings may be important for managing individuals with insomnia or related disorders.

Materials and Methods

Data collection

To study the molecular mechanisms of insomnia, data were collected from the Gene Expression Omnibus (GEO) database (GSE208668). Total RNA was obtained

from the peripheral blood mononuclear cells of 17 individuals with insomnia disorder and compared to 25 controls for analysis. The data are linked to the published document by Piber et al., titled “sleep disturbance and activation of cellular and transcriptional mechanisms of inflammation in older adults” (Piber et al., 2022). There is evidence that patient cells are a suitable source for studying the molecular mechanisms of the studied disorder or disease (Liu et al., 2023; Zaman et al., 2021).

Pre-evaluation of data

Data were evaluated using the GEO2R program (National Center for Biotechnology Information, NCBI) to identify potential comparisons between samples through box plots, uniform manifold approximation and projection (UMAP) plots, expression density diagrams, and Venn diagrams (Piber et al., 2022). The gene expression profiles that did not match statistically were normalized using the “force normalization” option of the GEO2R program. UMAP plot analysis indicated that normalization had no unfavorable consequence. Significantly differentially expressed genes (DEGs) were selected based on adjusted P<0.05 and a fold change >2. The data were cleaned, and individuals without a characterization were excluded from further analysis.

Protein-protein interaction (PPI) network analysis

The selected significant DEGs were included in a directed PPI network using the CluePedia plugin of Cytoscape software, considering co-expression interactions. The main connected components of the network that were eligible for network topology analysis were assessed using the “Network Analyzer” application of Cytoscape software in “direct” mode. The main connected components were laid out based on outdegree and indegree centrality parameters to identify the critical actor and controlled genes, respectively. The central nodes and their centrality parameters were visualized to highlight the crucial genes.

Statistical analysis

The significant DEGs were selected based on (adjusted P<0.05 and fold change >2. Data analysis was conducted using the GEO2R program (NCBI, version 1.0) to evaluate gene expression profiles from the GEO database (GSE208668). Directed PPI network analysis was performed using the CluePedia plugin (version 1.5.7) of Cytoscape software (version 3.9.1) to evaluate significant DEGs, incorporating co-expression interactions. Central nodes, identified as the most influential and regulated genes, were determined through network topology analysis using the "Network Analyzer" application within Cytoscape, with results visualized to highlight key genes.

Results

Figure 1 presents the visualization of insomnia, consisting of no insomnia (I-NI) samples, using box plots, UMAP plots, and expression density diagrams. As depicted in Figure 1, the NI samples are not uniform and do not match individuals with insomnia. The UMAP plot and density diagram correspond to the two sets of samples in the NI group. Based on the box plot in Figure 1, the gene expression profiles of the NI group were divided into two groups: No Insomnia 1 (NI1) and no insomnia 2 (NI2). NI1 includes GSM6360952, GSM6360955, GSM6360957-60, GSM6360962, and GSM6360967-75, while, GSM6360951, GSM6360953-4, GSM6360956, GSM63609561, and GSM6360963-6 are grouped as NI2. The box plot, UMAP scheme, expression density diagram, and Venn diagram of the I-NI1-NI2 analysis indicate that the two groups of NI gene expression profiles are completely separated from the insomnia group (Figure 2). The samples were normalized and compared, and the results of the I-NI1-NI2 reanalysis are shown in Figure 3. As depicted in Figure 3, the three groups are distinguished by their gene expression profiles (see UMAP plot). Since the intensity diagrams of the samples have a similar pattern, the gene expression profiles of the studied groups are comparable. The UMAP plot demonstrated exactly two distinct groups of no-insomnia samples.

To identify the differentiation among the NI1, NI2, and insomnia groups, the gene expression profiles of each group were compared with those of the others. The results of this analysis are depicted in Figure 4. As shown in Figure 4, the three groups are completely separated from each other based on the significant DEGs. As depicted in Figures 2-4 (the Venn diagrams), many DEGs are dysregulated significantly. The central part of the main connected component of the NI1-NI2 PPI network is shown in Figure 5. According to Figure 5, the genes *CCND1*, *STAT3*, *SOX9*, *NOTCH1*, *IL1B*, *HMOX1*, *EGR1*, *SPI1*, *YY1*, *SP1*, *PTGS2*, and *PTEN* are identified as the main actors that differentiate the two groups from the NI group. These genes are arranged in descending order based on their outdegree values, with CCND1 and PTEN being the strongest and weakest actors, respectively. Next, *IL6*, *PTGS2*, *PTEN*, *ESR1*, *JUN*, *CREB1*, *CDKN1A*, and *CDKN2A* were highlighted as the critical controlled DEGs, with *IL6* and *CDKN2A* identi-

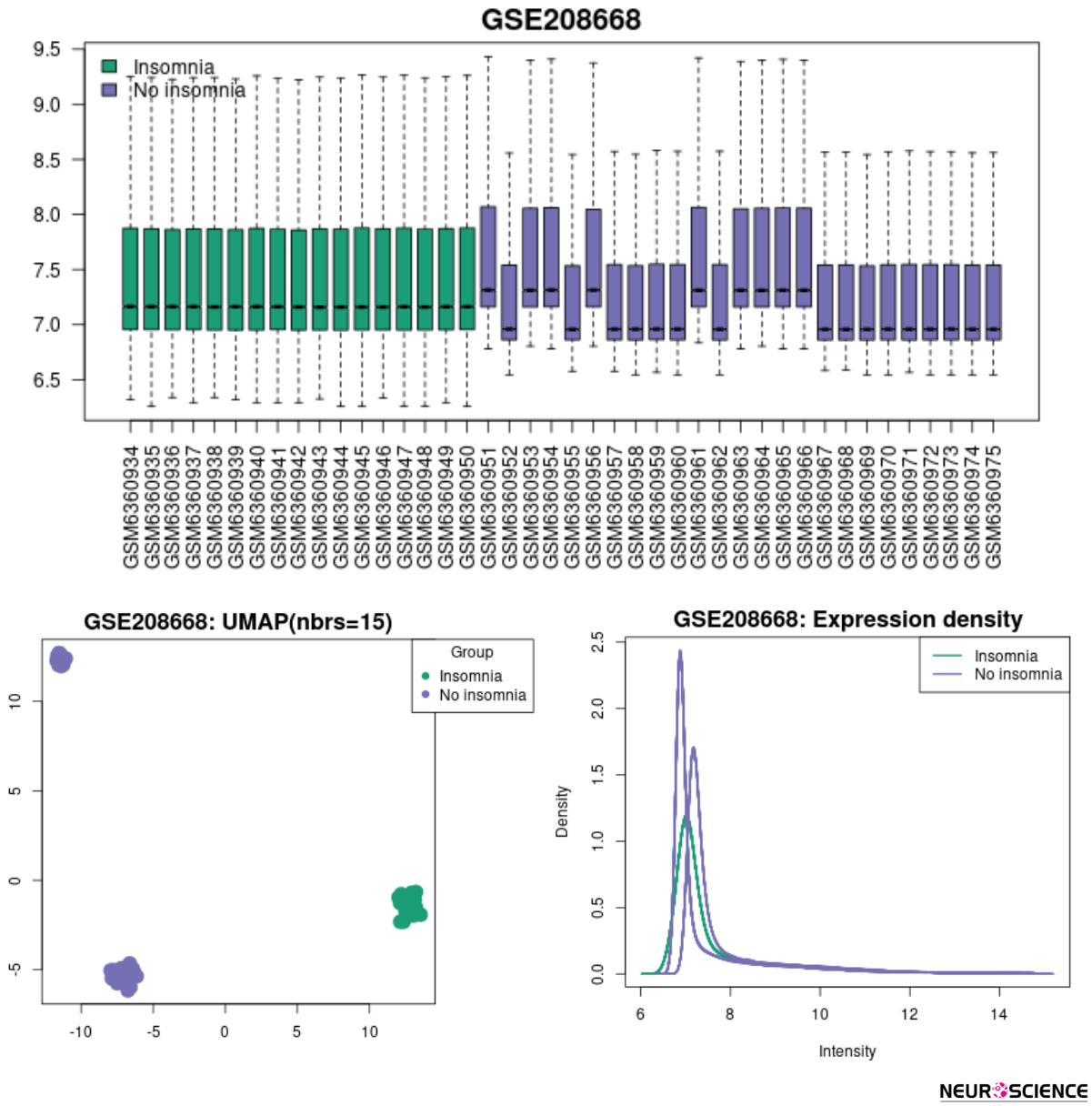

Figure 1. Box plot, UMAP plot, and density diagram of the–NI gene expression profile analysis "NBRS 15" in the UMAP plot refers to the number of neighbors with which a certain individual is compared

fied as the most and least influential controlled DEGs, respectively. The genes are laid out based on outdegree and indegree values, using color to represent the related amounts of the central parameters.

As presented in Figure 6, there are no crucial DEGs that separate the insomnia samples from those in the NI1 group. The central part of the main connected component of the PPI network from the I-NI2 analysis, which is laid out based on outdegree and indegree values (via color and amounts of central parameters), is shown in Figure 7. Thus, *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1* are identified as the principal actors in the analyzed network, with *TP53* being the most influential among them. Finally, *IL10*, *IL6*, *TP53*, *PTGS2*, *ESR1*, *PTEN*, *JUN*, *CREB1*, *CDKN1A*, *CDKN2A*, *CXCR4*, and *GATA3* are the key controlled DEGs.

The network was formed from 6611 recognized significantly DEGs (including 5692 isolated nodes) and 1522 edges. The actor genes and the related outdegree values are shown in green, while the controlled individuals and the associated indegree values are shown in red.

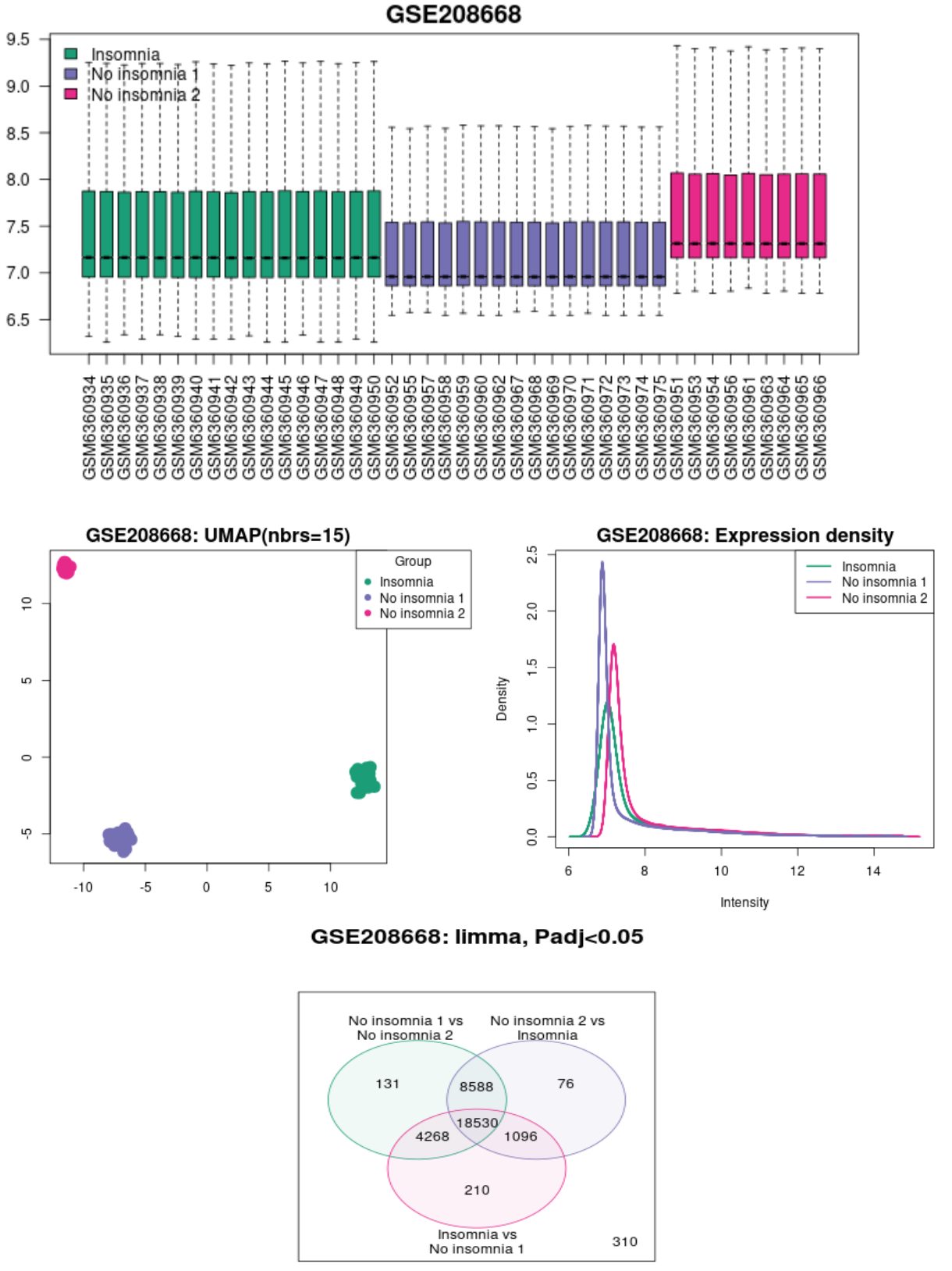

Figure 2. Box plot, UMAP scheme, expression density diagram, and Ven diagram of I-NI1-NI2 groups analysis

Note: "NBRS 15" in the UMAP plot refers to the number of neighbors with which a certain individual is compared.

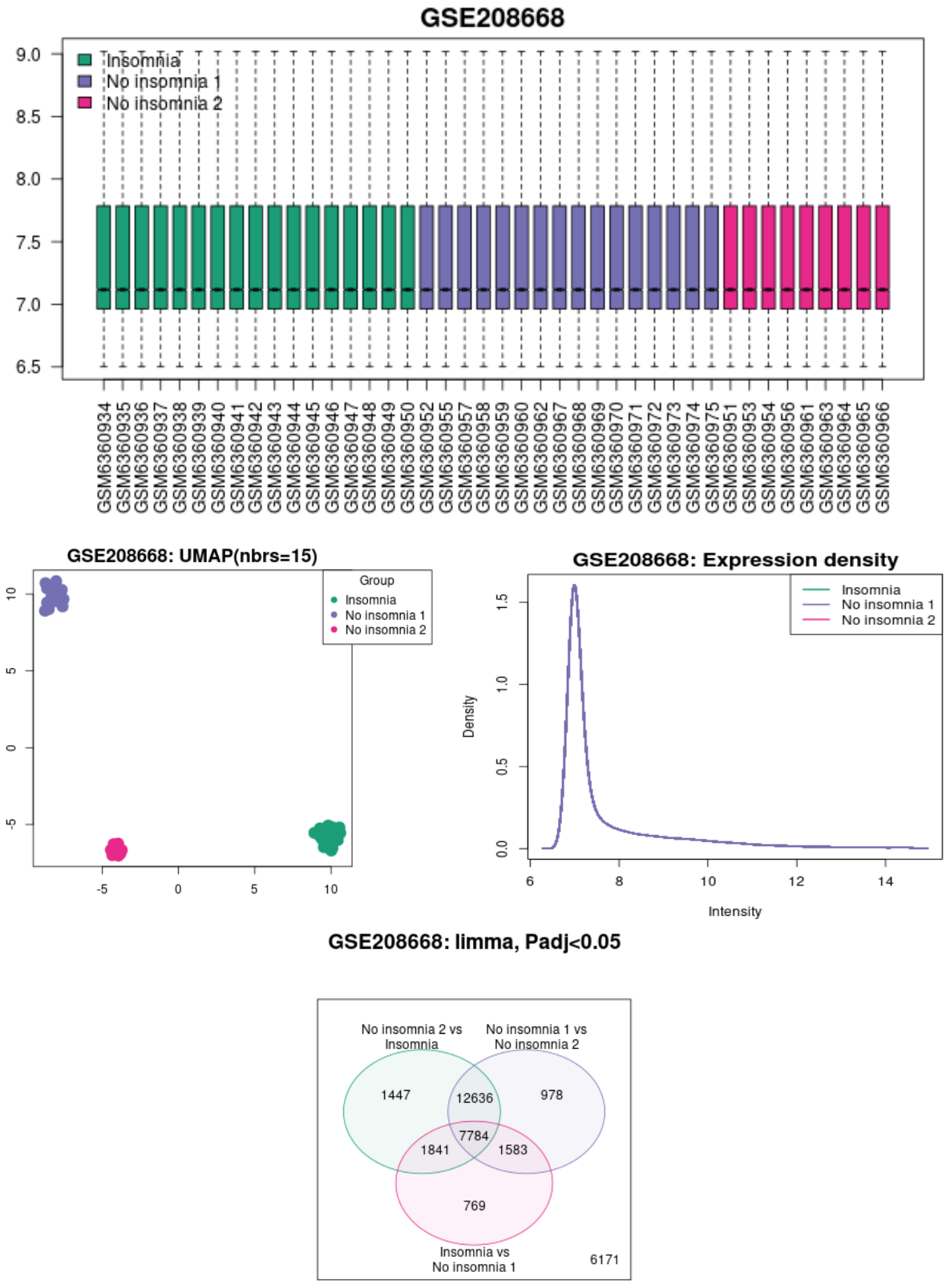

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Figure 3. Box plot, UMAP scheme, expression density diagram, and Ven diagram of I-NI1-NI2 groups analysis after normalization

Note: "NBRS 15" in the UMAP plot refers to the number of neighbors with which a certain individual is compared.

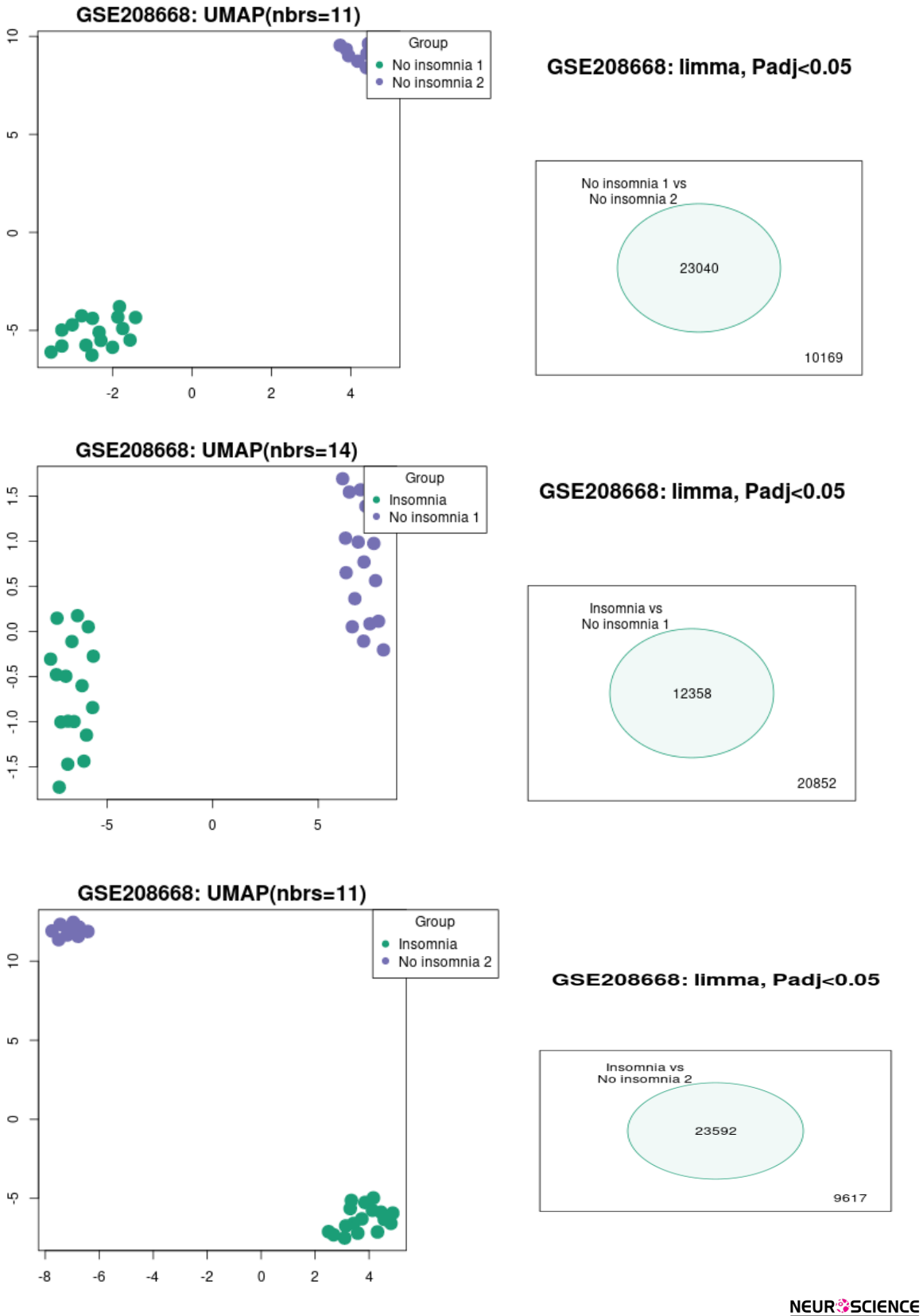

Figure 4. UMAP plot and Ven diagram of NI1-NI2, I-NI1, and I-NI2 groups analyses

Note: "NBRS 11 and 14" in UMAP plots refer to the number of neighbors with which a certain individual is compared.

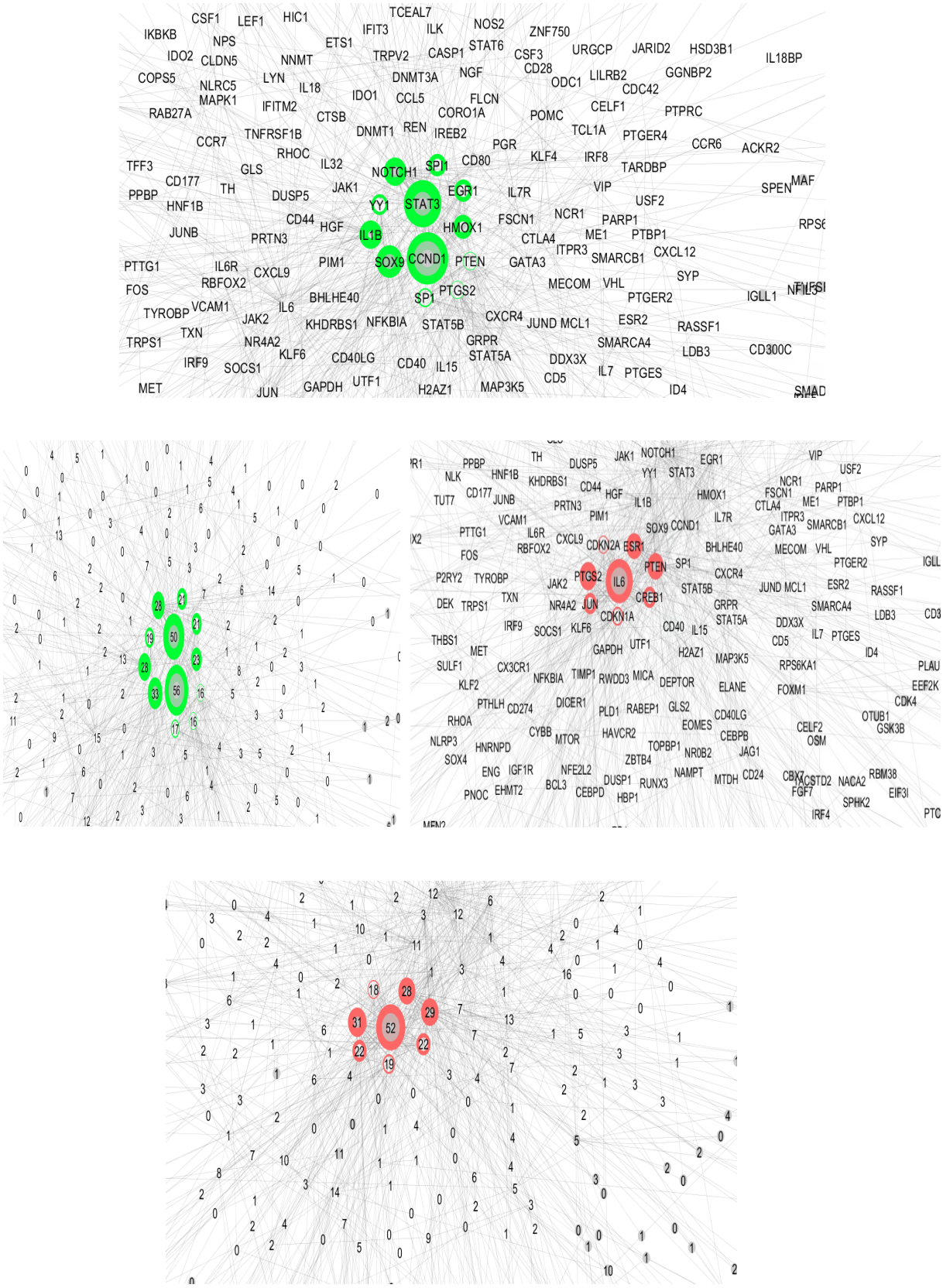

Figure 5. The central part of the main connected component of the directed PPI network for NI1-NI2 gene expression analysis via co-expression interactions

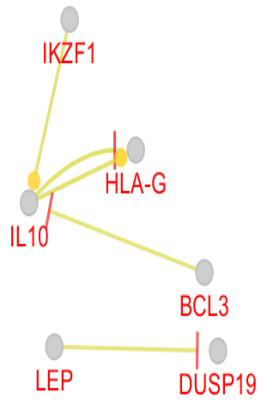

Figure 6. The main connected component of the directed PPI network of I-NI1 gene expression analysis via co-expression action

The network was formed from 6958 recognized significantly DEGs (including 5910 isolated nodes) and 1882 edges. The actor genes and the related outdegree values are shown in green, while the controlled individuals and the associated indegree values are shown in red.

Discussion

Insomnia disorder is reported to be associated with abnormalities in brain function and structure in patients. The investigation has established an association between these abnormalities and gene expression. A study by Zhang et al. revealed the involvement of insomnia disorder-related genes in functions such as brain development, endocrine regulation, and ion transport (Zhang et al., 2024). In the present study, the gene expression of patients was analyzed and compared with that of controls. As depicted in Figures 1, 2, 3 and 4, the analyses revealed heterogeneity in gene expression profiles among the control samples. Although the control samples exhibited heterogeneity, they could be clustered into distinct groups: NI1 and NI2. The issue of sample heterogeneity is addressed in many studies, and various methods have been suggested to resolve it (Feczko & Fair, 2020; Nunes et al., 2020). As shown in Figure 4, the control samples are divided into distinct groups, allowing for comparison with one another.

The genes *CCND1*, *STAT3*, *SOX9*, *NOTCH1*, *IL1B*, *HMOX1*, *EGR1*, *SPI1*, *YY1*, *SP1*, *PTGS2*, and *PTEN* serve as crucial actors, while *IL6*, *PTGS2*, *PTEN*, *ESR1*, *JUN*, *CREB1*, *CDKN1A*, and *CDKN2A* function as key controlled genes that differentiate the two compared groups of control samples (Figure 5). This suggests that the clustered control samples exhibit significant differences, at least at the gene expression level, which cannot be overlooked in the analysis of insomnia disorder samples. Since the categorization of insomnia disorder is considered a "work in progress" (Poon et al., 2021), some control samples are classified as normal but may actually belong to the insomnia patient group. This finding suggests that a portion of the normal samples may include individuals who have insomnia. The results, as illustrated in Figure 6, support this notion. As shown in Figure 6, there are no significant differences between the insomnia group and the NI1 group. The main differences are detected between the insomnia patients and the control group of 9 samples (Figure 7). The genes *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1* are identified as the main actors, while *IL10*, *IL6*, *TP53*, *PTGS2*, *ESR1*, *PTEN*, *JUN*, *CREB1*, *CDKN1A*, *CDKN2A*, *CXCR4*, and *GATA3* are recognized as the key controlled genes that distinguish insomnia disorder patients from normal controls. Liang et al.'s investigation, utilizing PPI network analysis and molecular complex detection, introduced 10 hub genes related to insomnia (Liang et al., 2024). The crucial genes in our study, including *TP53*, *JUN*, *IL6*, and CREB1, are part of this set of hub genes. As depicted in Figure 7, *TP53* is the primary actor in the I-NI2 analysis. Experiments indicate that ketamine has behavioral effects on exposed animals. Exposure of zebrafish larvae to ketamine resulted in difficulty initiating sleep, which was associated with the upregulation of the *TP53* gene, a key component of the apoptotic pathway. After a week of recovery, a shorter duration of sleep (insomnia-like behavior) was observed in the treated zebrafish larvae (Guo et al., 2024). In the present study, TP53 is upregulated twofold in the insomnia group compared to the NI2 group. Several cytokines and immune genes, such as *IL-6*, *IL-10*, and *IL-1β*, have been highlighted in mammals for their association with sleep regulation (Palagini et al., 2014). As mentioned, *IL1B* ranks as the

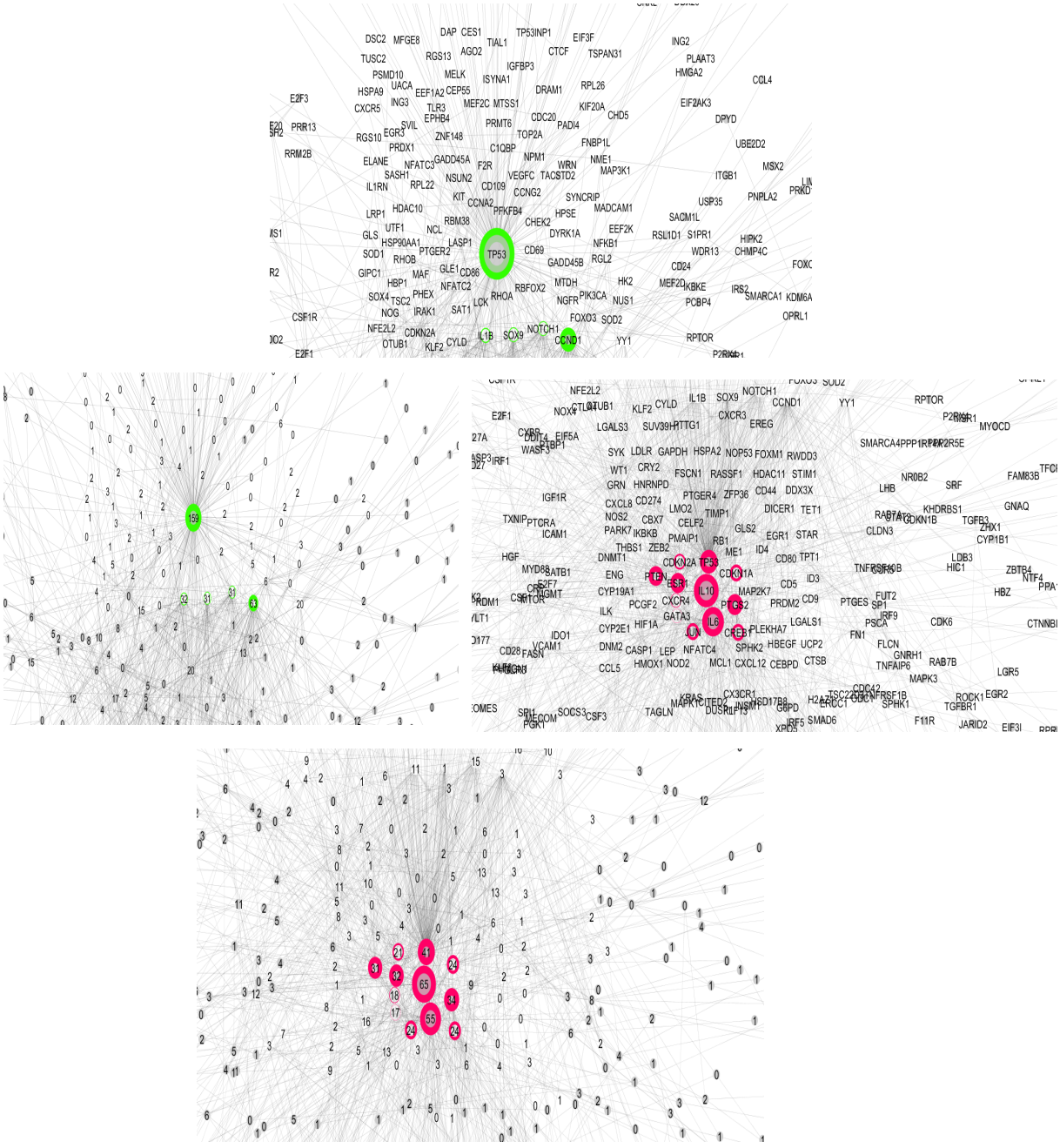

Figure 7. The central part of the main connected component of the directed PPI network for I-NI2 gene expression analysis via co-expression interactions

third most significant actor in our analysis, while *IL10* and *IL6* are the first and second-ranked controlled genes, respectively. *Cyclin D1* (*CCND1*) is an oncogene, and its role in several cancers, including melanoma, breast cancer, and hepatocellular carcinoma, has been highlighted (Ding et al., 2020; González-Ruiz et al., 2021; Valla et al., 2022). *CCND1* appears as the second most significant actor in insomnia. Furthermore, literature suggests a correlation between positive anti-SOX1 in the serum of individuals with fatal familial insomnia, a condition characterized by dysautonomia, motor disorder, and disturbed sleep (Gong et al., 2022). *SOX1* is identified as the fourth actor in insomnia. The last actor introduced is *NOTCH1*, which is regulated by melatonin in rats (Wang et al., 2023). The significant role of melatonin in sleep quality has been both investigated and confirmed (Fatemeh et al., 2022). *PTGS2* and chemokine receptor *CXCR4* are identified as critical genes in the control of

insomnia. As reported in the literature, both *PTGS2* and *CXCR4* are involved in the inflammatory process (Gallego et al., 2021; Martín-Vázquez et al., 2023). These findings, alongside the presence of the other mentioned interleukins, underscore the prominent role of inflammation in insomnia.

A comparison of critical genes between the NI1-NI2 and I-NI2 analyses reveals that *CCND1*, *IL1B*, *NOTCH1*, and members of the *SOX* gene family are common actors in both analyses. This finding suggests that 80% of the actors identified in the I-NI2 analysis overlap with those of the NI1-NI2 analysis. It can be concluded that a significant number of control samples may be involved in insomnia and could develop insomnia disorder soon. The controlled genes further support this idea; 100% of the controlled genes in the NI1-NI2 analysis (*IL6*, *PTGS2*, *PTEN*, *ESR1*, *JUN*, *CREB1*, *CDKN1A*, and *CDKN2A*) are common to the individuals assessed in the I-NI2 analysis. As highlighted in previous investigations, insomnia is categorized as severe, moderate, and mild (Hohagen et al., 1993). Additionally, sleep duration is classified as very short, short, normal, or long (Rhee et al., 2021). Based on these findings, the term "pre-insomnia" is suitable to describe the situation of the NI1 group.

Conclusion

In conclusion, the findings indicate that many people may potentially be involved in an insomnia disorder. It can be concluded that pre-insomnia occurs before the onset of insomnia. There is a close relationship between the molecular mechanisms of pre-insomnia and insomnia. The genes *TP53*, *CCND1*, *IL1B*, *SOX1*, and *NOTCH1* were highlighted as critical actors in insomnia disorder. Apoptosis, inflammation, and immunological responses, as well as changes in sleep quality (resulting from melatonin dysregulation), were highlighted as prominent events in insomnia disorder.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.RETECH.REC.1403.196).

Funding

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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