RESEARCH PAPER

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Study on the molecular mechanism of Guipi decoction against sleep deprivation based on integrated pharmacology analysis and gene expression profiling

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Sleep disorder is a puzzling and complex health problem, and sleep deprivation (SD) may be a window for studying sleep disorder. Guipi decoction (GPD) is a classic Chinese prescription for the treatment of sleep disorder. However, the mechanism of GPD remains puzzling. In this paper, integrated pharmacological analysis and gene expression profiling were introduced to study the mechanism of GPD in treatment with SD. Firstly, the integrative pharmacology-based research platform of traditional Chinese medicine (TCMIP) was applied to collect chemical compounds and corresponding targets for GPD. Secondly, SD-related targets were obtained by gene expression profiling (GSE56931) from Gene Expression Omnibus (GEO) database. The String database screened the core targets according to protein-protein interaction (PPI) network. Furthermore, kyoto encyclopedia of genes and genomes (KEGG) pathways were carried out based on the Database for Annotation, Visualization and Integrated Discovery (DAVID) database. Conclusively, the "formula-herbs-compounds-targets-pathways" network was established to explore the mechanism of GPD in the treatment of SD. In addition, molecular docking was carried out to verify the connection between hub compounds and targets. The results showed that GPD was mainly linked to 44 compounds, 19 targets and 5 pathways. GPD in the treatment of sleep deprivation through metabolic pathways and cAMP signaling pathway, which were related to NR3C1, MAPK3, PPARA and core compounds such as adenosine. This study preliminarily revealed the molecular mechanism of GPD for SD, and lays a foundation for the study of the mechanism against SD for GPD.

Key words: sleep deprivation, Guipi decoction, gene expression profiling, integrated pharmacology, molecular mechanism

INTRODUCTION

Sleep remains a major mystery of biology, with little understood about its basic function. Sleep deprivation (SD) is a window for sleep research. Worldwide, about one-third of people were suffers from sleep problems according to the World Health Organization. White paper on sleep and exercise 2021 reported that more than 300 million Chinese individuals were troubled with sleep, the prevalence up to 38%. It's suggested that there is a bidirectional relationship between sleep disorders and mental disorders (Goldstein and Walker, 2014; Freeman et al., 2020), and sleep disruption results in worse mental health, such as schizophrenia, depression, and post-traumatic stress disorder (PTSD) (Schrimpf et al., 2015; Reeve et al., 2018). Considering the effect of SD on our quality of life and social function, understanding the mecha-

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nism and molecular pathways affected by SD is clearly of clinical and social importance. In terms of treatment for sleep disorders, oral medicine is still the major measure in clinic. However, due to its adverse reactions such as dependence, withdrawal, and amnesia, an increasing number of people cannot benefit from this treatment. Thus, TCM has attracted interest as an alternative treatment, owing to multi-component and multi-target synergism characteristics. TCM emerges with the advantages components safety, high efficiency and lower side effects (Chen et al., 2014; Shi et al., 2016).

GPD, is one of the prescriptions that was used most commonly for sleep therapy. It was first recorded in Ji Sheng Fang, and was a classic prescription created by Yan Yonghe in the Song dynasty. GPD has the effect of tonify qi and replenish blood, and nourish the heart to tranquilize, which showed a remarkable therapeutic effect in the treatment of sleep deprivation. This formula was composed of Atractylodis macrocephalae rhizoma, Ginseng radix et rhizoma, Astragali radix, Angelicae sinensis radix, Glycyrrhizae radix et rhizoma, Poria, Polygalae radix, Ziziphi spinosae semen, Aucklandiae radix, Longan arillus, Zingiberis rhizoma recens and Jujubae fructus. The results of a randomized controlled meta-analysis containing 705 patients with the treatment of SD by GPD showed that the effective rate of GPD was more than 91% (Zhao and Bai, 2018). Recent research showed that GPD combined with auricular beans therapy improved sleep quality via regulating the level of serum TNF-α (Zhao et al., 2016). Although both clinical and basic studies have proved that GPD has great potential for sleep deprivation, the literature reports are mostly limited to clinical research, and the specific molecular mechanism is still unclear. Therefore, it is of great significance to analyze the molecular mechanism of GPD for its material basis and further research on sleep deprivation.

Thus, this study is based on integrated pharmacological analysis and gene expression profiling, aiming to reveal the material basis and molecular mechanism of GPD in the treatment of sleep deprivation (the whole flowchart has been shown in Fig. 1). It provides a theoretical basis for further research on the molecular mechanism of GPD in the treatment of sleep deprivation.

METHODS

Identification of candidate compounds for GPD

The candidate compounds were screened by using TCMIP v2.0 (Zhao et al., 2016), which contained over 400 herbs of the Chinese Pharmacopoeia (2015). The chemical components of every single herb were obtained to assemble the chemical component database of GPD.

Target fishing of the chemical compounds for GPD

The target fishing was performed in terms of MedChem Studio (version 3.0) software embedded in the TCMIP platform (Wishart et al., 2018; Xu et al., 2019a). The software is an efficient drug similarity search tool that aims to identify known drugs with high structural similarity to herbal ingredients (Wishart et al., 2018). The Tanimoto score is in the range of [0,1], where "0" represents completely different structures between ingredients and known drugs, and "1" indicates the same structures of two components. It is considered that the targets of the known drugs are in accordance with the targets of the test compounds, when the Tanimoto score is higher than 0.6 between the known drugs and the test compounds.

Targets recognition of sleep deprivation by gene expression profiling

GEO database (https://www.ncbi.nlm.nih.gov/geo/) is the largest and most comprehensive public gene expression data resource which consists of gene chip sequencing, single cell sequencing and omics data of clinical, animal or cell samples (Barrett et al., 2013). The candidate targets of SD were predicted based on gene expression profiling by the GEO database. And GSE56931 was determined as a clinical sample of SD. It includes blood gene expression of 249 samples by using HuRSTA-2a520709 microarray collected from 7 sleep deprivation resident individuals and 7 sleep deprivation sensitive patients (Arnardottir et al., 2014).

In this session, 249 samples (126 sensitive samples, 123 resident samples) were used to identify differentially expressed genes (DEGs) of SD. Firstly, the probes set were annotated as gene symbols based on GPL10379 platform. Further, the whole genes of SD related were screened out by comparing gene expression between SD sensitive group and the control group in the use of gene expression profiling in R studio (version 3.2.3, https://www.r-project.org/). Conclusively, the DEGs were identified with a P value<0.05. Genes with log fold change (log FC) > 0 were regarded as up-regulated genes (sleep deprivation sensitive showed higher expression levels), while those with log FC<0 were regarded as down-regulated genes (sleep deprivation resident group showed higher expression levels).

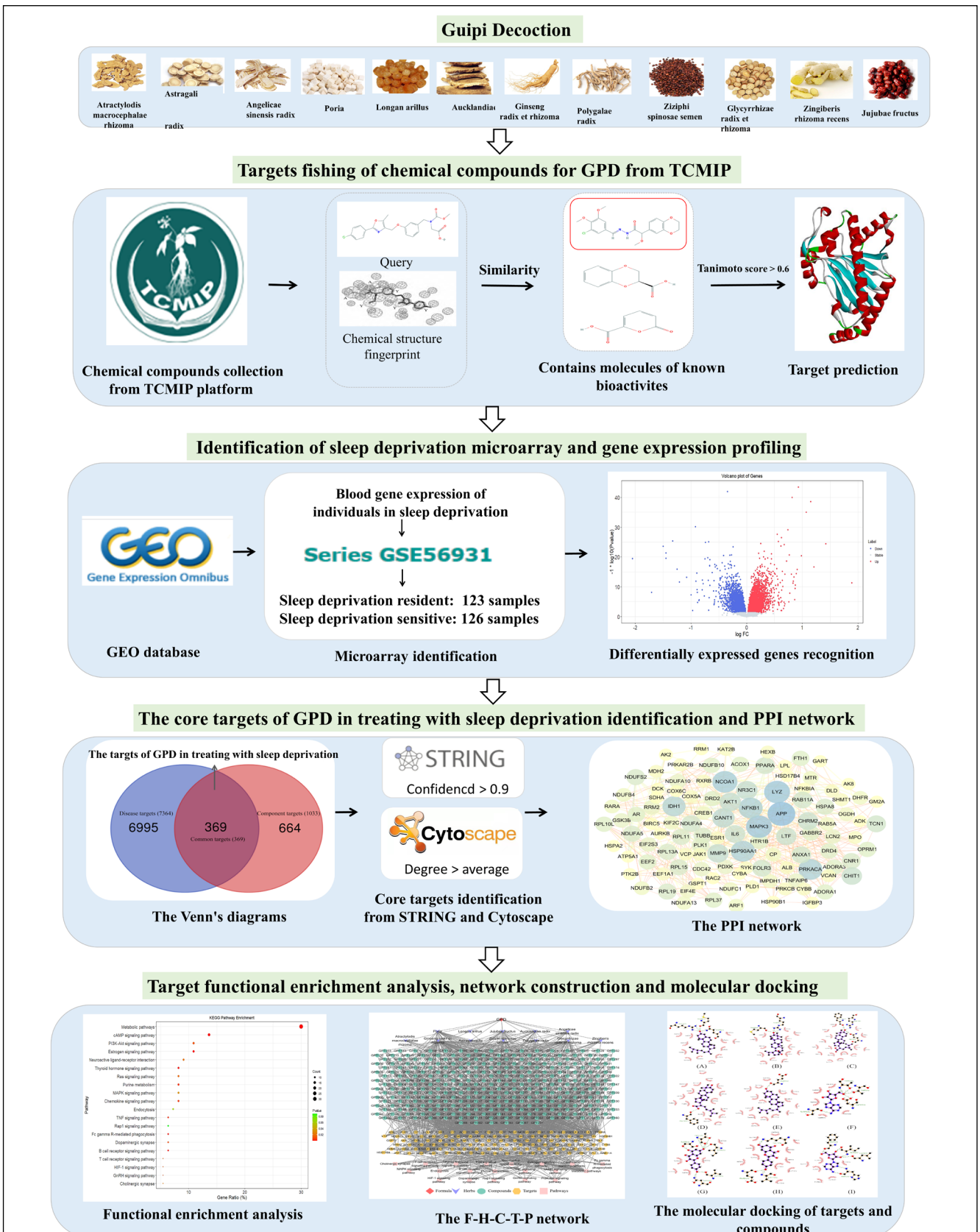

Fig. 1. The flowchart of GPD in the treatment of sleep deprivation.

The common targets of GPD targets and sleep deprivation targets

The common targets of disease-causing genes and compound targets for GPD were shifted out by Venn online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Subsequently, the common targets were considered potential targets of GPD in the treatment of SD.

The core targets of GPD in the treatment of sleep deprivation were determined by the protein-protein interaction (PPI) network

The protein-protein interaction (PPI) relationship was constructed by String v11.0 (https://STRING-db.org/) (Szklarczyk et al., 2019). Then, the core targets were determined by setting the minimum interaction threshold with the highest confidence 0.9. Afterward, the interactions were introduced to Cytoscape 3.8.2 to construct PPI networks and screen out the core targets by calculating the topological properties using the Network analyzer plug-in of Cytoscape (Kohl et al., 2011). Centrality is a common concept in evaluate topological properties, and degree centrality (DC), closeness centrality (CC) and betweeness centrality (BC) were three important properties in it. Ultimately, the proteins with a higher average of DC were regarded as core targets of GPD in the treatment of sleep deprivation.

Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis of GPD in the treatment of sleep deprivation

The hub targets were introduced to the enrichment database DAVID (Huang et al., 2007) for exploring biological function of GPD in the treatment of sleep deprivation. The top 20 pathways were screened out according to *P* value and mapped as bubble plots by using R studio.

Formula-herbs-compounds-targets-pathways (F-H-C-T-P) network construction of GPD in the treatment of sleep deprivation

The data of herbs, compounds, targets, and pathways were input into Cytoscape to construct the F-H-C-T-P network for GPD in the treatment of sleep deprivation. In addition, the hub network was identified with topological properties.

Molecular docking prediction of GPD in the treatment of sleep deprivation

Molecular docking was used to explore the directive interactions between crucial targets and compounds. The structures of the targets were obtained from Protein Data Bank (PDB, http://www.rcsb.org), and the structures of compounds were obtained from the PubChem database. Further, Pymol and Auto Dock tools was used to delete water and heteroatom, add polar hydrogens, and compute gasteiger charge of protein (Seeliger and De Groot, 2010; Morris et al., 2009). The processed protein and components were introduced into AutoDock Vina for molecular docking in the end. The active binding pocket was determined according to the binding site between the protein and the corresponding known ligand in the PDB database. Following, the binding energy was calculated by Iterated local search global optimizer. The smaller the binding energy was illustrated as the more stable the complex formed.

RESULTS

Collection of GPD compounds from TCMIP

A total of 302 compounds of GPD were screened out in TCMIP, including 8 ingredients in Atractylodis macrocephalae rhizoma, 94 ingredients in Ginseng radix et rhizoma, 11 ingredients in Astragali radix, 20 ingredients in Angelicae sinensis radix, 55 ingredients in Glycyrrhizae radix et rhizoma, 31 ingredients in Poria, 16 ingredients in Polygalae radix, 22 ingredients in Ziziphi spinosae semen, 23 ingredients in Aucklandiae radix, 6 ingredients in Longan arillus, 18 ingredients in Zingiberis rhizoma recens and 49 ingredients in Jujubae fructus, these compounds were renumbered as GPT001 to GPT302 (Supplementary Table 1). The ingredient of GPD was mainly related to Astragaloside IV, Longan polysaccharide, ginsenoside, atractylodes macrocephala polysaccharide, ferulic acid, jujuboside, poria cocos polysaccharide, polygala saponins, costunolide and glycyrrhizic acid (Elmenhorst et al., 2017) of which scopoletin both related to angelicae sinensis radix and Atractylodis macrocephalae rhizoma. It indicated that different herbs shared the same components, reflecting the characteristics of multi-component in TCM.

It's obvious that Ginseng radix et rhizoma contained the most components, its compounds found in most ginseng varieties are known to include ginsenosides, polysaccharides, peptides, alkaloids, polyacetylene, and phenolic compounds. Studies have confirmed that

Ginsenoside Rg1 promoted sleep in rats by depressing extracellular norepinephrine concentrations in both locus coeruleus (LC), dorsal raphe nucleus (DRN), and in other sleep-regulating brain regions of which functions can be modulated by monoaminergic neurotransmitters discharged from projecting noradrenergic and serotonergic neurons (Xu et al., 2019b). Similarly, Ginsenoside Rh2 reversed spatial and non-spatial memory impaired by sleep deprivation probably through preventing oxidative stress damage in the body, including the serum and brain during sleep deprivation (Lu et al., 2018).

The targets recognition for GPD in the treatment of sleep deprivation

Overall, 1033 potential targets of GPD were predicted with the help of drug likeness method based on the TCMIP platform.

GSE56931 was determined to identify the DEGs between SD-sensitive individuals and SD resident individuals. As a result, 52378 probes set with 21105 gene symbols were revealed. And 7364 DEGs were identified with a *P* value<0.05 through R studio using Limma package and Bioconductor package (Fig. 2).

Consequently, 369 common targets were regarded as the potential targets of GPD in the treatment of sleep deprivation in use of Venn's diagrams (Fig. 3).

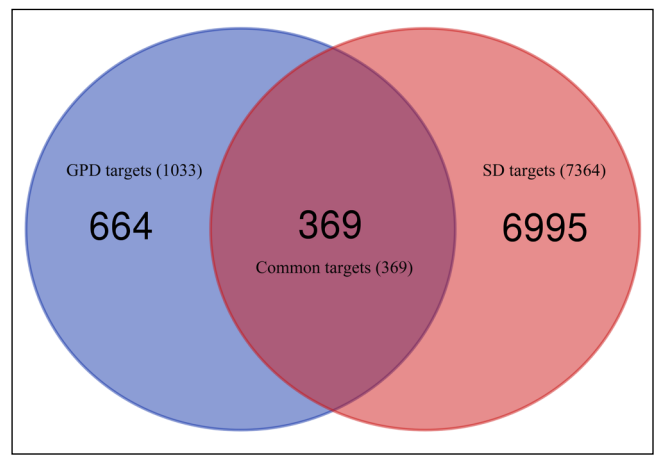

Fig. 3. The Venn's diagrams between compound targets and disease targets of GPD in the treatment of sleep deprivation. The blue circle illustrated the target number of the GPD, pink circle illustrated the target number of sleep deprivation, and the red circle illustrated the targets of GPD in the treatment of sleep deprivation.

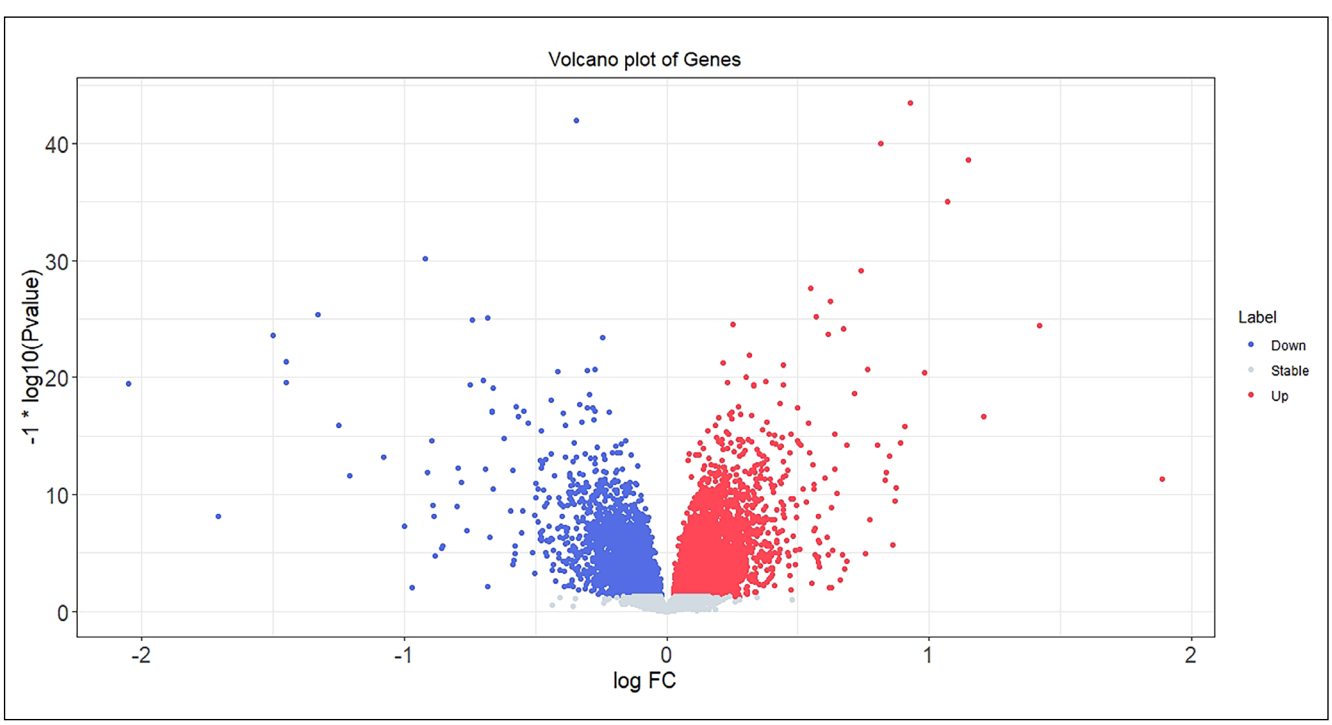

Fig. 2. The volcano plot of the differentially expressed genes in microarray data sets of GSE56931. The horizontal axis represents log 2 FC (fold change) and the vertical axis represents the *P* value. Blue represented down-regulated genes, red represented up-regulated genes, and gray represented genes without significantly different expression.

Protein-protein interaction (PPI) network construction and identification of core targets

As a result, 229 targets with confidence level of 0.9 have been considered as potential targets in use of String. Further, 110 targets and 433 edges were screened out with higher than the average DC in use of network analyzer (Fig. 4). In Fig. 4, the larger the nodes and the darker the color were illustrated as the more significant role in the network.

KEGG pathway enrichment analysis of GPD in the treatment of sleep deprivation

A total of 78 pathways were carried out from the DAVID database. The unrelated pathways were removed, such as "non-alcoholic fatty liver disease", "pathways in cancer" and "viral carcinogenesis". The bubble diagram of the top 20 KEGG pathways was mapped by R studio (Fig. 5). Metabolic pathways, cAMP signaling pathway, estrogen signaling pathway and PI3K-Akt signaling pathway are may the significant pathways of GPD for treating SD. Among these pathways, metabolic pathways contained the most targets, which suggested a significant close connection of GPD in the treatment of sleep deprivation.

The F-H-C-T-P network construction of GPD in the treatment of sleep deprivation

The F-H-C-T-P network was constructed to investigate the core network of GPD in the treatment of sleep deprivation by Cytoscape (Fig. 6). The network consisted of 421 nodes (1 formula, 12 herbs, 278 compounds,

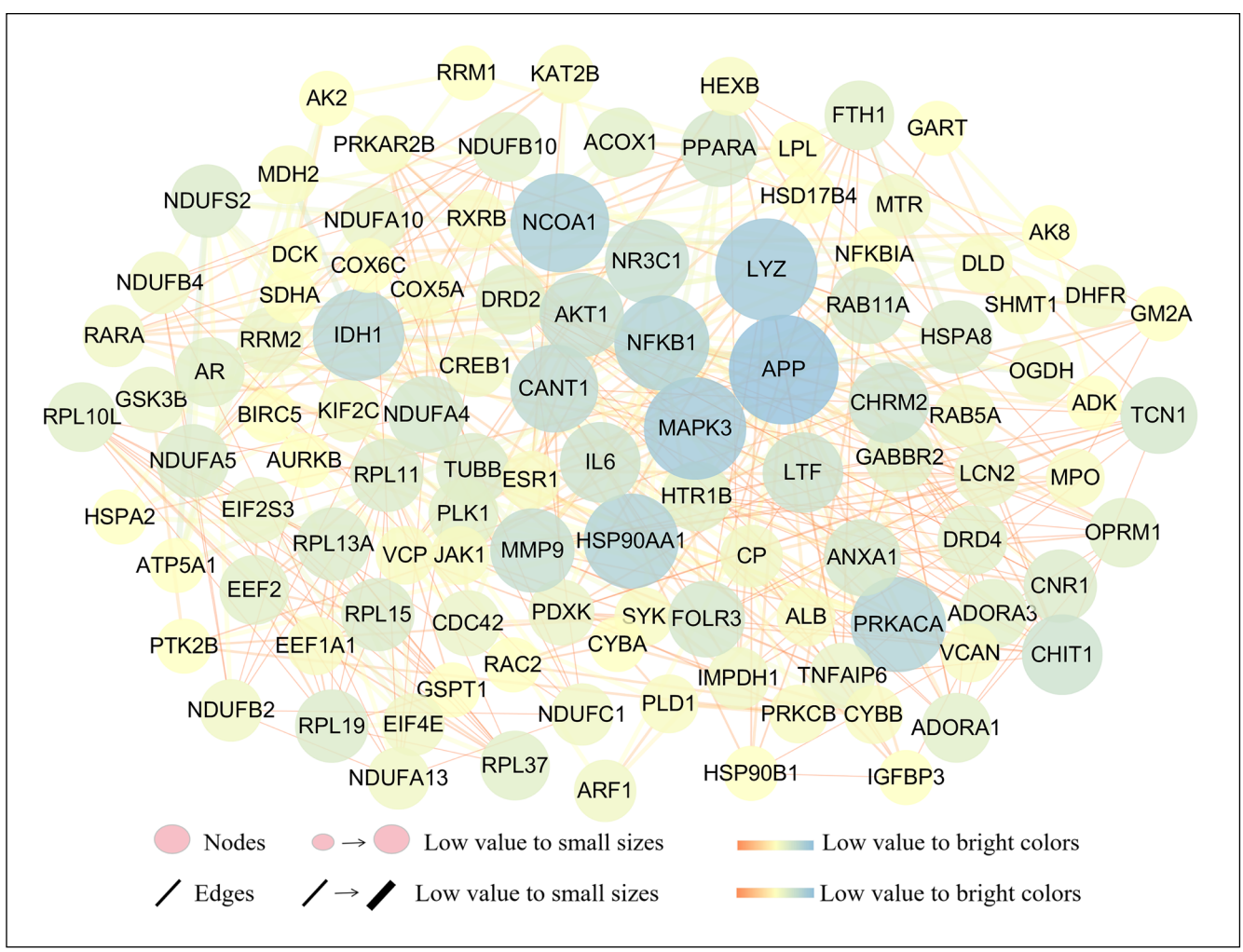

Fig. 4. The protein-protein interaction (PPI) network for GPD in treatment with sleep deprivation. The nodes represent targets and the edges represent the interaction between two nodes. Low value to small size and light colors, and the larger the nodes, the darker the color, the more important the target was.

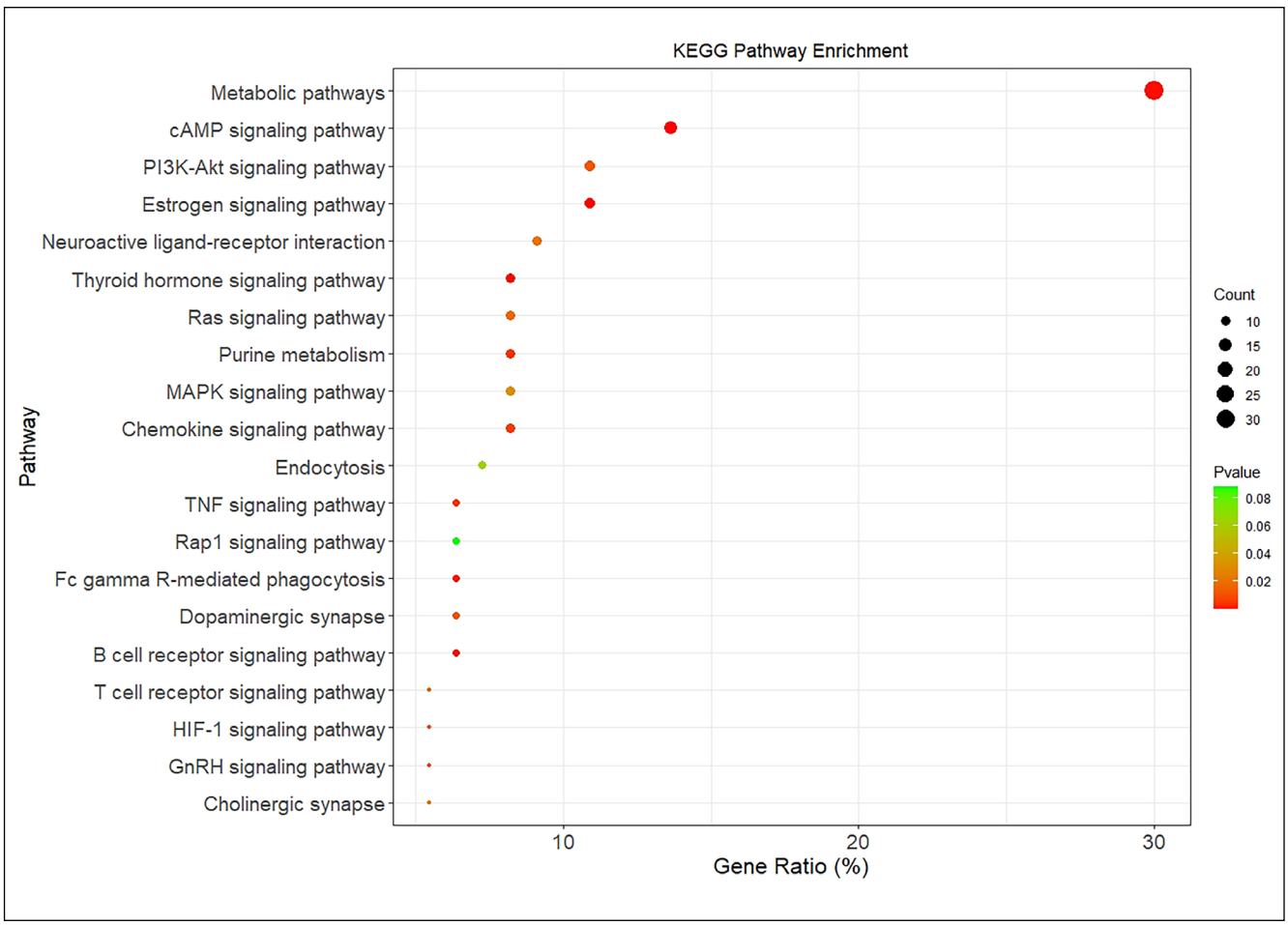

Fig. 5. The KEGG pathway enrichment of GPD in the treatment of sleep deprivation. Bubble size depends on the target's number of pathway, and *P* value decides color, red represents low value, green represents high value.

110 targets, and 20 pathways) and 1576 edges. In Fig. 6, the formula nodes are shown in red diamond, the blue arrow represented herb nodes, the compound nodes are shown in the green ellipse, the targets nodes are shown in orange hexagon, and the pink rectangle represented the pathway nodes. The gray edges represented the relationships among the formula, herbs, compounds, targets, and pathways. Furthermore, 44 compounds (Table 1), 19 targets (Table 2), and 5 pathways (Table 3) were comprised of a hub network according to topological properties. The compounds greater than the average of DC, BC, and CC were considered important components, the targets and pathways which higher than the average of DC were regarded as core targets and pathways.

Molecular docking of GPD in the treatment of sleep deprivation

In further to reflect the relationship between core targets and compounds, molecular docking was introduced in the study. The cAMP signaling pathway is one of the common and significant pathways of neuropathic regulation and proved to affect sleep deprivation in our study. Hence, the core targets that belonged to the cAMP pathway and their corresponding compounds were selected to simulate docking. Targets including NR3C1 (PDB ID: 1P93), MAPK3 (PDB ID: 6GES), PPARA (PDB ID: 3ET1), PRKACA (PDB ID: 4O21), and AKT1 (PDB ID: 3O96), and compounds consisted of adenosine (GPT049), kaempferol (GPT173), eburicoic

acid (GPT094), betulinic acid (GPT064) and ergosterol (GPT095). The molecular docking result are displayed in Table 4. The results showed that most of the binding abilities of key ingredients and corresponding targets showed strong binding activity. Here, we exhibited the interaction between core compounds and targets (Fig. 7). As known, the hydrogen bond is a strong non-bonded interaction, as one of the four non-covalent binding processes between small drug molecules and biological macromolecules, it played a significant role for protein molecule stability and recognition with ligands. The results showed that all complex with hydrogen bonds and has stable structures, for instance, 3 hydrogen bonds in the complex NR3C1-eburicoic acid, 2 hydrogen bonds in the complex NR3C1-ergosterol, 3 hydrogen bonds in the complex NR3C1-betulinic acid. Docking results indicated that hydrogen may provide a big contribution to binding energy.

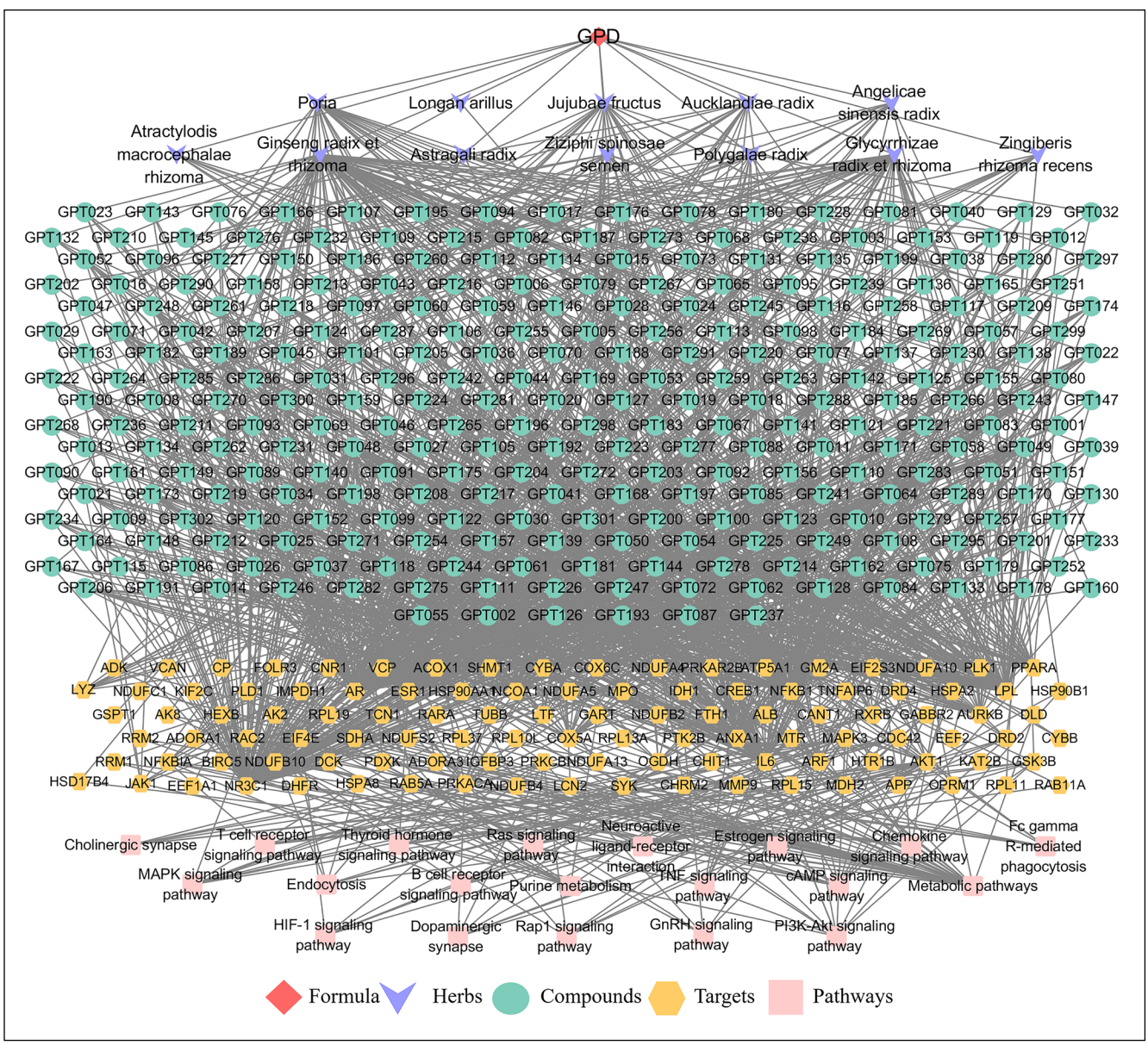

Fig. 6. The formula-herbs-compounds-targets-pathways network for GPD in the treatment of sleep deprivation. The formula nodes are shown in red diamond, the blue arrow represented herb nodes, the compound nodes are shown in green ellipse, the targets nodes are shown in orange hexagon, and pink rectangle represented the pathway nodes. The gray edges represented the relationships among the formula, herbs, compounds, targets, and pathways.

Table 1. The degree centrality (DC), betweenness centrality (BC), closeness centrality (CC) values of the key compounds for GPD in the treatment of sleep deprivation.

No.	Compound ID	Compound name	Source	CC	DC	BC
1	GPT048	Adenosine Triphosphate	Ginseng radix et rhizoma	0.3382	42	0.0985
2	GPT049	Adenosine, Adenine Nucleoside	Ginseng radix et rhizoma	0.3226	26	0.0544
3	GPT173	Kaempferol	Ginseng radix et rhizoma	0.3535	12	0.0146
4	GPT075	Cetylic Acid, Hexadecanoic Acid, Palmitic Acid	Ginseng radix et rhizoma	0.3437	11	0.0100
5	GPT084	Dehydroeburicoic Acid	Poria	0.3797	10	0.0059
6	GPT094	Eburicoic Acid	Poria	0.3797	10	0.0059
7	GPT080	Cis-9, Cis-12-Linoleic Acid, Inositol, Linoleic, Linoleic Acid	Ginseng radix et rhizoma	0.3415	9	0.0078
8	GPT006	(S)-5,7-Dihydroxy-2-Phenylchroman-4-One, Pinocembrin	Glycyrrhizae radix et rhizoma	0.3297	9	0.0043
9	GPT018	3,3'-Dimethylquercetin	Glycyrrhizae radix et rhizoma	0.3297	9	0.0043
10	GPT021	3',7-Dihydroxy-4',6-Dimethoxyisoflavone	Glycyrrhizae radix et rhizoma	0.3297	9	0.0043
11	GPT190	Liquiritigenin	Glycyrrhizae radix et rhizoma	0.3297	9	0.0043
12	GPT028	3-O-Acetyl-Glycyrrhetinic Acid	Glycyrrhizae radix et rhizoma	0.3590	9	0.0064
13	GPT137	Glycyrrhetinic Acid	Glycyrrhizae radix et rhizoma	0.3770	9	0.0056
14	GPT072	Catechin	Jujubae fructus	0.3172	9	0.0044
15	GPT064	Betulinic Acid	Jujubae fructus	0.3832	9	0.0068
16	GPT015	25-Hydroxy-3-Epidehydrotumulosic Acid	Poria	0.3784	9	0.0048
17	GPT022	3-Epidehydrotumulosic Acid	Poria	0.3784	9	0.0048
18	GPT025	3Î'-Hydroxylanosta-7,9(11), 24-Trien-21-Oic Acid	Poria	0.3784	9	0.0049
19	GPT085	Dehydrotumulosic Acid	Poria	0.3784	9	0.0048
20	GPT279	Trametenolic Acid	Poria	0.3784	9	0.0049
21	GPT281	Tumulosic Acid	Poria	0.3784	9	0.0048
22	GPT248	Protopanaxadiol	Ginseng radix et rhizoma	0.4054	8	0.0086
23	GPT249	Protopanaxatriol	Ginseng radix et rhizoma	0.4054	8	0.0086
24	GPT221	Oleanolic Acid	Jujubae fructus	0.3818	8	0.0058
25	GPT024	3Î'-Hydroxy-16Î'-Acetoxy-Lanosta-7,9(11), 24-Trien-21-Oic Acid	Poria	0.3627	8	0.0030
26	GPT226	Pachymic Acid	Poria	0.3627	8	0.0030
27	GPT241	Polyporenic Acid C	Poria	0.3627	8	0.0030
28	GPT242	Poricoic Acid A	Poria	0.3627	8	0.0030
29	GPT243	Poricoic Acid B	Poria	0.3627	8	0.0030
30	GPT244	Poricoic Acid D	Poria	0.3627	8	0.0030
31	GPT246	Poricoic Acid G	Poria	0.3627	8	0.0030
32	GPT247	Poricoic Acid H	Poria	0.3627	8	0.0030
33	GPT052	Alphitolic Acid	Ziziphi spinosae semen	0.3646	8	0.0044
34	GPT017	2Î',3Î'-Dihydroxyolean-12-En-28-Oic Acid	Jujubae fructus	0.3559	7	0.0046
35	GPT030	3-O--Maslinic Acid	Jujubae fructus	0.3355	7	0.0029

No.	Compound ID	Compound name	Source	CC	DC	BC
36	GPT059	Atractylenolide III	Atractylodis macrocephalae rhizoma	0.3541	6	0.0061
37	GPT010	2,4,4'-Trihydroxychalcone	Glycyrrhizae radix et rhizoma	0.3246	6	0.0047
38	GPT155	Isoliquiritigenin	Glycyrrhizae radix et rhizoma	0.3246	6	0.0047
39	GPT027	3-O-[Î'-D-Glucuronopyranosyl-(1â†'2)-O-Î'-D -Glucuronopyranosyl]-24-Hydroxyglabrolide	Glycyrrhizae radix et rhizoma	0.3187	6	0.0056
40	GPT095	Ergosterol	Poria	0.3578	6	0.0031
41	GPT055	Arbusculin A	Aucklandiae radix	0.3471	5	0.0039
42	GPT069	Campesterol, M-Cresol	Ginseng radix et rhizoma	0.3911	5	0.0053
43	GPT261	Sitosterol, Î'-Sitosterol	Ginseng radix et rhizoma	0.3911	5	0.0053
44	GPT264	Stigmasterol	Ginseng radix et rhizoma	0.3911	5	0.0053

Table 2. The degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) values of the key targets for GPD in the treatment of sleep deprivation.

No.	Gene symbol	Gene name	CC	DC	BC
1	NR3C1	nuclear receptor subfamily 3 group C member 1	0.3914	132	0.1469
2	NFKB1	nuclear factor kappa B subunit 1	0.3959	117	0.1342
3	AR	androgen receptor	0.3747	111	0.1915
4	LPL	lipoprotein lipase	0.3451	89	0.0352
5	ESR1	estrogen receptor 1	0.3599	72	0.0764
6	ANXA1	annexin A1	0.3401	68	0.0281
7	IL6	interleukin 6	0.3417	64	0.0209
8	NCOA1	nuclear receptor coactivator 1	0.3462	52	0.0468
9	AURKB	aurora kinase B	0.2939	33	0.0115
10	PPARA	peroxisome proliferator activated receptor alpha	0.3310	30	0.0224
11	COX5A	cytochrome c oxidase subunit 5A	0.3299	27	0.0190
12	COX6C	cytochrome c oxidase subunit 6C	0.3299	27	0.0190
13	AKT1	AKT serine/threonine kinase 1	0.3412	27	0.0353
14	PRKACA	protein kinase cAMP-activated catalytic subunit alpha	0.3127	23	0.0201
15	MAPK3	mitogen-activated protein kinase 3	0.3037	16	0.0070
16	ARF1	ADP ribosylation factor 1	0.3002	16	0.0215
17	RXRB	retinoid X receptor beta	0.3041	13	0.0072
18	CNR1	cannabinoid receptor 1	0.2952	12	0.0028
19	LTF	lactotransferrin	0.2750	12	0.0018

Table 3. The degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) values of the key pathways for GPD in the treatment of sleep deprivation.

No.	Pathway ID	Pathway name	Targets	CC	DC	BC
1	hsa01100	Metabolic pathways	NDUFA13, PDXK, NDUFB10, HEXB, SHMT1, NDUFB4, NDUFA10, ATP5A1, ADK, AK2, NDUFB2, HSD17B4, MTR, PLD1, COX5A, AK8, RRM1, RRM2, NDUFA5, NDUFA4, MDH2, IDH1, NDUFC1, COX6C, SDHA, DCK, DHFR, IMPDH1, ACOX1, OGDH, NDUFS2, DLD, GART	0.2975	33	0.0603
2	hsa04024	cAMP signaling pathway	CHRM2, GABBR2, HTR1B, PLD1, NFKB1, NFKBIA, CREB1, ACOX1, ADORA1, RAC2, AKT1, DRD2, PPARA, PRKACA, MAPK3	0.3448	15	0.0213
3	hsa04915	Estrogen signaling pathway	GABBR2, HSPA8, HSP90AA1, CREB1, AKT1, HSPA2, OPRM1, PRKACA, ESR1, MMP9, HSP90B1, MAPK3	0.3052	12	0.0127
4	hsa04151	PI3K-Akt signaling pathway	CHRM2, GSK3B, IL6, HSP90AA1, CREB1, SYK, AKT1, EIF4E, NFKB1, HSP90B1, JAK1, MAPK3	0.3191	12	0.0109
5	hsa04080	Neuroactive ligand-receptor interaction	CHRM2, GABBR2, CNR1, ADORA3, ADORA1, HTR1B, OPRM1, DRD2, NR3C1, DRD4	0.3271	10	0.0163

Table 4. The docking results of the complex between key targets and the key compounds for GPD in treatment with sleep deprivation.

Protein name	Gene name	PDB ID	Ligand name	Binding energy (kcal/mol)
protein kinase cAMP-activated catalytic subunit alpha	PRKACA	4O21	Kaempferol	-6.81
AKT serine/threonine kinase 1	AKT1	3O96	Adenosine	-7.11
AKT serine/threonine kinase 1	AKT1	3O96	Kaempferol	-4.98
nuclear receptor subfamily 3 group C member 1	NR3C1	1P93	Eburicoic acid	-7.10
nuclear receptor subfamily 3 group C member 1	NR3C1	1P93	Betulinic acid	-8.45
nuclear receptor subfamily 3 group C member 1	NR3C1	1P93	Ergosterol	-11.53
peroxisome proliferator activated receptor alpha	PPARA	3ET1	Eburicoic acid	-8.50
peroxisome proliferator activated receptor alpha	PPARA	3ET1	Ergosterol	-11.4
mitogen-activated protein kinase 3	MAPK3	6GES	Adenosine	-5.12

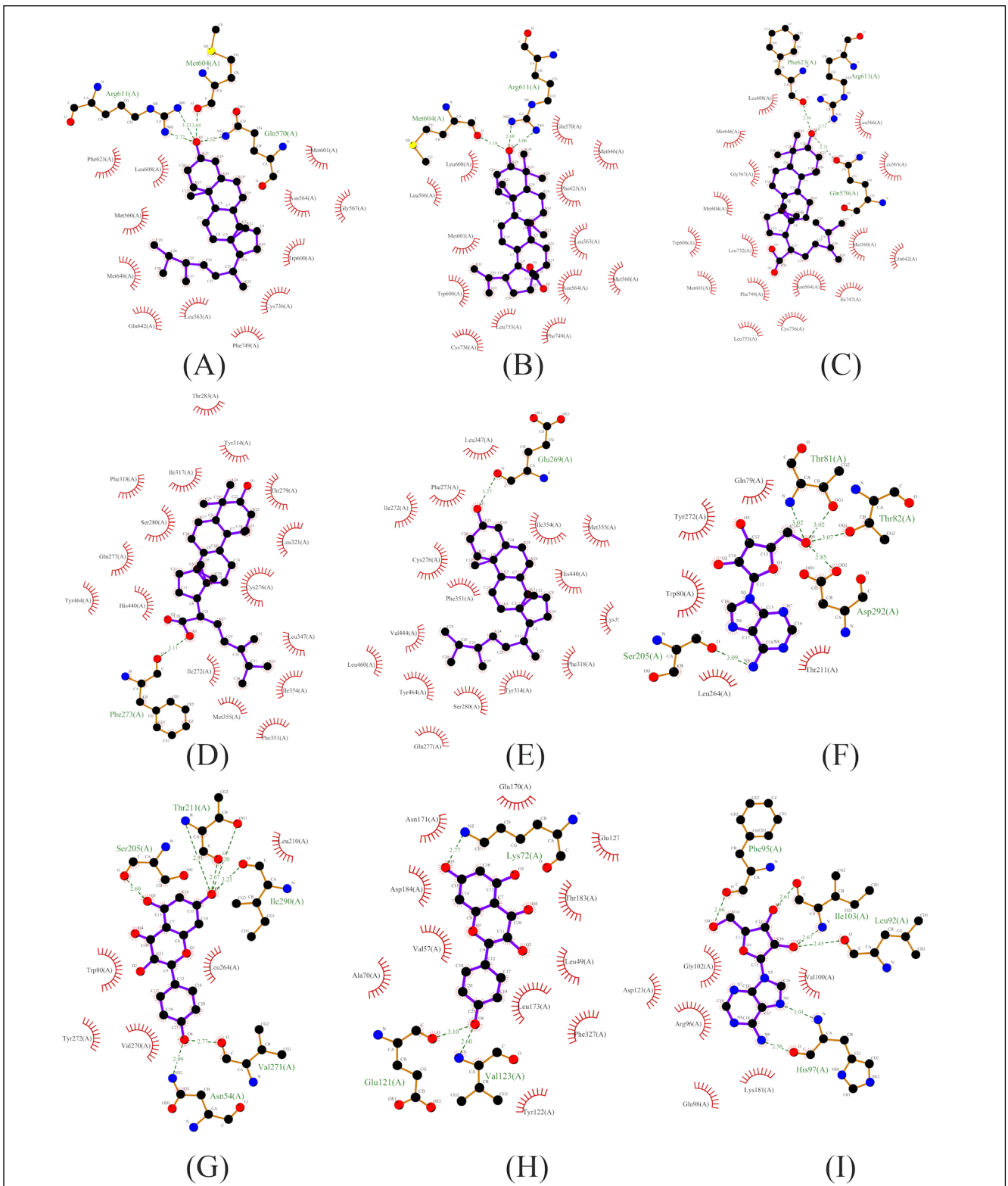

Fig. 7. The interaction graphics result between core targets and compounds of GPD in the treatment of sleep deprivation. (A) The interaction graphics between NR3C1 and eburicoic acid, (B) The interaction graphics between NR3C1 and ergosterol, (C) The interaction graphics between NR3C1 and betulinic acid, (D) The interaction graphics between PPARA and eburicoic acid, (E) The interaction graphics between PPARA and ergosterol, (F) The interaction graphics between AKT1 and adenosine, (G) The interaction graphics between AKT1 and kaempferol, (H) The interaction graphics between PRKACA and kaempferol, (I) The interaction graphics between MAPK3 and adenosine.

DISCUSSIONS

It's found in this research that adenosine triphosphate (ATP, DC=42, BC=0.0985, CC=0.3382) and adenosine (DC=26, BC=0.0544, CC=0.3226) were two of the core compounds with highest degree value among compounds, which belongs to Ginseng radix et rhizoma. Adenosine triphosphate was released and when in the extracellular space it is hydrolyzed to adenosine diphosphate (ADP), adenosine monophosphate (AMP) and eventually to adenosine. It was demonstrated that ATP/adenosine signaling has long been considered to be important in sleep regulation (Blutstein and Haydon, 2013). As an experiment shown in cats and rats, the extracellular concentration of adenosine fluctuates in many brain regions (such as the basal forebrain), increases in the cortex and basal forebrain during prolonged wakefulness and decreases during the sleep recovery period (Porkka-Heiskanen et al., 2002). These changes suggested that adenosine may act as a homeostatic regulator of sleep and be a link between the humoral and neural mechanisms of sleep-wake regulation (Huang et al., 2011). Similarly, research showed that the changes in extracellular adenosine concentration regulated sleep homeostasis-related slow wave activity (SWA), promote SWA by acting on neuronal adenosine-related receptors, and be metabolized by adenosine kinase found in glial cells (Greene et al., 2017). *In vitro*/*ex vivo* experiments indicated that adenosine attenuates light-induced phase shifts under conditions of sleep deprivation by inhibiting the effects of light on the circadian clock, corresponding to a high adenosinergic tone (Greene et al., 2017). Studies containing 49 healthy volunteers' attention in the psychomotor vigilance test (PVT) showed a strong significant correlation existed between the performance impairments induced by ethanol and sleep deprivation, and this association may relate to adenosine (Elmenhorst et al., 2017). In addition, the study also found that individuals with a significant increase in adenosine receptor A1AR availability were better adaptable to the effects of sleep loss than those with a slight increase, by measuring A1AR availability by positron emission tomography (PET). Of note, this phenomenon indicated that the difference in the utilization of endogenous adenosine and A1AR may be the consequence of individual's response to sleep deprivation.

Of the targets, NR3C1 (nuclear receptor subfamily 3 group C member 1, DC=132, BC=0.1469, CC=0.3914) has the greatest degree of properties, which was known as the glucocorticoid receptor (GR) and has a great influence on the hypothalamic-pituitary-adrenal axis (HPA) (Vitellius et al., 2016). Research indicated that GR predicted a diurnal cortisol slope, at which level changes along with the circadian rhythm (Lewis et al. 2020). Namely, GR may affect the HPA axis by regulating cortisol levels to counteract sleep deprivation. Besides, reliable evidence supports the integral role of GR in circadian biology (So et al., 2009). The study revealed GR's basic functions in the rhythmic orchestration of hepatic metabolism via mapping GR's chromatin distribution of the diurnal cycle in mouse livers (Quagliarini et al., 2019). It also showed that GR interacts with core Clock factors to enlarge and stabilize the downstream genes' amplitude, and synchronizes 24-hour rhythms by integrating circadian clock loops with hormone release. It emphasized the domination of synchronizing circadian amplitudes as most oscillating genes are constrained by and depend on GR.

MAPK3 (mitogen-activated protein kinase 3, DC=16, BC=0.0070, CC=0.3037), also name ERK1 (extracellular signal-regulated kinase 1) which acts as an essential component of the MAP kinase signal transduction pathway. Evidence showed that systematic and cortical ERK phosphorylation increased and decreased with wakefulness and sleep in mice. Further, the specific deletion of ERK1 or ERK2 significantly increased the awakening duration, and inhibition of ERK phosphorylation in wild-type animals strongly decreased the sleep duration (Mikhail et al., 2017). In other words, ERK phosphorylation showed a strict association with sleep duration. Research suggested that sleep deprivation impaired memory via reducing ERK1/ERK2 expression in the hippocampus (Guan et al., 2004; Wang et al., 2019).

PPARA (peroxisome proliferator activated receptor alpha, DC=30, BC=0.0224, CC=0.3310) is a ligand-activated transcription factor, which may be required for the propagation of clock information to metabolic pathways, and participated in the regulation of circadian rhythm. A sample estimated total sleep time (TST) and wake after sleep onset (WASO) of 289 individuals with HIV/AIDS, indicating that there was a correlation between energy homeostasis genes PPARA and poor sleep (Jansen et al., 2019). Given the unknown mechanism of the role of PPARA on sleep modulation, an injection of PPARA agonist and PPARA antagonist were introduced to rats in trahypothalamic (Mijangos-Moreno et al., 2016). Specifically, it showed that PPARA agonist enhanced wakefulness and decreased slow wave sleep and rapid eye movement sleep whereas PPARA antagonist promoted opposite effects. What's more, PPARA agonists increased contents of dopamine, serotonin, and adenosine, which accumulated from the nucleus accumbens. It emphasized that PPARA may be related to adenosine in regulating sleep. Previous research found that oleoy-

lethanolamide in cerebrospinal fluid (CSF) after 24 h of sleep deprivation by detecting CSF samples of 20 healthy volunteers before and after sleep deprivation (Mijangos-Moreno et al., 2016). Notably, oleoylethanolamide is an endogenous lipid messenger that is released after neural injury and activates PPARA with nanomolar potency, which implied that PPARA was considered related to the increase in oleoylethanolamine level caused by sleep deprivation. Additionally, light pollution led to a high risk of obesity for the influence of altered circadian rhythm where PPARA was implicated (Mijangos-Moreno et al., 2016).

Metabolic pathway (DC=33, BC=0.0603, CC=0.2975) was one of the most significant pathways of GPD in the treatment of sleep deprivation according to degree value. Growing evidence suggested a bidirectional relationship existence between metabolism and rhythm (Panda, 2016; Reynolds et al., 2017). Unfortunately, experimental animal models and epidemiological data indicate the risk of metabolic diseases was increased due to chronic circadian rhythm disruption (Arble et al., 2015). In addition, sufficient evidence has proved that the cAMP signaling pathway (DC=15, BC=0.0213, CC=0.3448) plays a unique role in the occurrence and treatment of sleep deprivation. cAMP signaling pathway undergoes a circadian oscillation in the hippocampus with maximal activation during REM sleep (Xia and Storm, 2017). Early studies have shown that sleep deprivation significantly reduces the number of spine and dendrite length of hippocampal CA1 neurons in vivo rescue experiments. And it was associated with the cAMP/PKA/LIMK/cofilin pathway, leading to memory impairment. On the contrary, enhancing the cAMP level of excitatory neurons in this region prevented the cognitive deficits caused by sleep deprivation in the object recognition experiment in rats (Havekes et al., 2014; 2016). Likewise, sleep deprivation reduced cAMP signaling by specifically impairing synaptic plasticity that cAMP/PKA-dependent formed in the hippocampus, and by increasing activity and protein levels of a cAMP degrading enzyme (PDE4, phosphodiesterase-4) (Vecsey et al., 2009).

In particular, it has been reported that cross-talk between cAMP/PKA and ERK1/2 pathways in the human adrenal NCI-H295R cell line (Sewer and Waterman, 2003). Moreover, the core compound adenosine that we mentioned above has an inhibitory connection with ERK1/2 by activating the adenosine A1 receptor in the striatum and mPFC neurons (Mao and Wang, 2019). Besides, docking result showed that there was a directly interaction between MAPK3 (ERK1) and adenosine. Taken together, our study revealed that MAPK may be a pivot target linked to adenosine and participated in the cAMP signaling pathway.

In general, GPD in the treatment of sleep deprivation through metabolic pathways and cAMP signaling pathway, which were related to NR3C1, MAPK3, PPARA and core compounds such as adenosine.

CONCLUSION

In conclusion, an integrated approach based on bioinformatic analysis that combined with clinical data mining of gene expression profiling, pharmacological network method, and molecular docking was introduced to explore the mechanism of GPD in the treatment of sleep deprivation. Our current study found that 44 chemical components, 19 targets, and 5 pathways were attributed to the mechanisms of GPD in the treatment of sleep deprivation. Furthermore, core compound adenosine and hub targets NR3C1, and MAPK3 played a crucial role in the treatment of sleep deprivation via metabolic pathways and cAMP signaling pathway. Generally, our study provided a theoretical basis for further research on the molecular mechanism of GPD in the treatment of sleep deprivation. However, there are some limitations in our present study, more experimental verification and research should be devoted to the molecular mechanisms for GPD in treatment with sleep deprivation in the future.

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