Original Article

Pharmacological mechanism of JiaWeiSiWu granule in the treatment of hypertension based on network pharmacology

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Background: JiaWeiSiWu granule (JWSWG) has been applied clinically for more than a decade, and the preliminary results show that blood pressure can be reduced while protecting the target organ at the same time. The purpose of this research is to study the pharmacological mechanism of JWSWG in treating hypertension using network pharmacology.

Methods: The chief active components, relevant targets, and the target genes of JWSWG were retrieved by the databases TCMSP and UniProt. The GeneCards database was used to obtain target genes of hypertension. Then, the target genes of hypertension and active components were intersected to discover the potential targets by which JWSWG acts on hypertension. Cytoscape software was employed to construct the "medicine-compound-target-disease" network. The STRING database was used to construct the protein-protein interaction network in order to screen the key target genes. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were analyzed by RGUI and org.Hs.eg.db.

Results: By intersecting 102 compound target genes with 6,732 target genes of hypertension, 88 action target genes were obtained, thereby screening out the key compounds and key targets. The results of GO enrichment showed the main molecular functions, biological processes, and cellular components. The main pathways of JWSWG in treating hypertension were revealed by KEGG pathway enrichment.

Conclusions: This research clarified the mechanism of JWSWG in the treatment of hypertension systematically, providing new potential ideas and a theoretical foundation for further experimental and clinical research.

Keywords: JiaWeiSiWu granule (JWSWG); hypertension; network pharmacology; protein-protein interaction network; multiple component; multiple target

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Introduction

Hypertension is a type of vascular disease that seriously affects human health. If blood pressure is at a high level for a long time, it can cause serious complications such as stroke, heart failure, and renal failure (1). Currently, there are a wide variety of anti-blood pressure drugs, but the occurrence rate of the complications cannot be reduced. The side effects of certain drugs can also influence the metabolism, and blood pressure is not only needed to be actively reduced for the treatment of hypertension, but also, the protection of the target organ is taken into account (2). Some treatments lower patient treatment compliance, resulting in a low control rate and low treatment rate (3). The traditional Chinese medicine (TCM) treatment of hypertension has multi-target functions, and can reduce the adverse reactions caused by taking other medicines (4,5).

JiaWeiSiWu granule (JWSWG) was devised by our hospital after the long-term accumulation of clinical experience and screening effective prescriptions (6,7). JWSWG has been applied clinically for more than a decade, and the preliminary results show that blood pressure can be reduced, while also protecting the target organ at the same time. JWSWG is composed of 7 Chinese medicines, namely DiHuang, ChiShao, DangGui, ChuanXiong, GouQi, DiGuPi, and DiLong. DiHuang functions to nourish blood and nourish yin, as well as cooling blood and generating jin. ChiShao functions to cool blood, clear heat, and regulate qi. DangGui functions to replenish blood circulation and activate blood, as well as nourishing the liver and regulating blood. ChuanXiong functions to activate qi, activate blood, and relieve pain. GouQi can treat vertigo, nourish the liver, nourish yin, and nourish essence. DiGuPi can clear heat, cool blood, and clear collaterals. DiLong has the effect of clearing away heat and clearing collaterals. The combination of multiple Chinese medicines is effective in nourishing qi and activating blood circulation as well as nourishing the liver and kidneys. It has been confirmed by clinical and scientific research for many years that JWSWG can accelerate symptom relief, control blood pressure, regulate blood lipids, and prevent the damage of target organs (8-10).

A major feature of Chinese medicine is that the components are various and complex. Up to now, the research on TCM has been limited to explaining its mechanisms and pathways for a target gene, and has lacked a whole view and syndrome differentiation view of TCM (11). TCM is characterized by its multi-component, multi-target, and multi-pathway mechanisms, which fits well with the network-oriented and multi-component research methods that network pharmacology focuses on. Therefore, network pharmacology is now widely applied in pharmacological studies of TCM (12,13).

At present, there is a lack of research on the multi-target and multi-pathway mechanisms of JWSWG. The mechanism of JWSWG in treating hypertension needs to be further studied. In this study, the relationship and connection of biological network nodes were analyzed with the technology and methods of TCM network pharmacology, which revealed the mechanism of JWSWG in treating hypertension. Subsequently, the multi-level network of "molecule-target-pathway-disease" was employed to accomplish the comprehensive network analysis of molecular action. Finally, the binding of the target and its corresponding components was verified by molecular docking. The mechanism of JWSWG in treating hypertension was eventually expounded from the overall view, providing novel research directions for further experimental and clinical studies.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1140).

Methods

Materials

The following programs and databases were used: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php); UniProt (http://www.uniprot.org/); STRING database (https://string-db.org/); Cytoscape software (Version 3. 6. 0) and the tool Network Analyzer and its apps: ClueGO, CluePedia; Bioconductor (https://bioconductor.org/bioLite.R) and its packages: org. Hs. eg.db, clusterProfiler; The R programming language (RGUI); KEGG PATHWAY Database (https://www.kegg.jp); GeneCards database (https://www.genecards.org/).The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All these databases are publicly available, and ethical approval was unnecessary.

Study methods

Selecting active components and targets of JWSWG

The effective chemical components of JWSWG (DiHuang,

ChiShao, DangGui, ChuanXiong, GouQi, DiGuPi) were obtained by using the TCMSP database (DiLong is not a herbal medicine, so we did not analyze it) (14). TCMSP is a distinctive pharmacology platform for Chinese herbal medicines which can reflect the relationships and connections among drugs, targets, and diseases. Two important indicators of pharmaceutically active ingredients in researching and developing drugs are oral bioavailability (OB) and drug-likeness (DL). OB reflects the absorption and distribution of drugs in the human body, and DL reflects the similarity between the components and existing drugs, as well as the possibility of developing drugs. Therefore, the screening conditions of the main active components and relevant targets of JWSWG from TCMSP were OB ≥30% and DL ≥0.18. Human gene codes were screened out with the UniProt knowledge base, retrieval form: [Homo sapiens (organism)]. All the searched genes were corrected to their official names.

Screening out action targets

The corresponding targets of hypertension were searched with the keyword "hypertension" in the GeneCards database. GeneCards is one of the most comprehensive websites for human genetic information, providing integrative, user-friendly information on all annotated and predicted human genes. The targets which related to both the drug JWSWG and the disease hypertension were the possible action targets of JWSWG in treating hypertension.

Constructing and analyzing the "medicine-compound-target-disease" network

According to the compounds and targets screened, the "medicine-compound-target-disease" network was constructed. Then, this network was analyzed by the Cytoscape software which has the function of "Network Analyzer". Nodes represented the herbal medicine of JWSWG, compounds, action targets, and disease, while edges represented the relationships among them. Therefore, the key compounds of JWSWG in treating hypertension were selected in accordance with the junctions of the compound and target.

Constructing the protein-protein interaction (PPI) network and selecting the key targets

The STRING database was employed to construct the PPI network. The genes which were screened out from medicine-disease interaction target proteins were introduced into STRING, the research species was defined as "Homo sapiens", 0.4 was set as the minimum interaction score, and default settings were applied as for other parameters to acquire the PPI network of JWSWG on hypertension. Then, the PPI network was subjected to topology analysis by virtue of the "Network analyzer" function of Cytoscape software, and the key targets with advanced degrees were determined.

Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The entrezIDs of action targets were obtained by RGUI and org.Hs.eg.db. GO functional enrichment including molecular function (MF), biological process (BP), and cellular component (CC) as well as KEGG pathway enrichment were obtained by RGUI and clusterProfiler. Furthermore, by enriching the GO functions and KEGG pathways by inputting action targets into ClueGO, and employing CluePedia to edit the networks, the connection between action targets and pathways were displayed more directly.

Verification of molecular docking

The binding of the target proteins and their corresponding components was verified by molecular docking. The 2D and 3D structures of the small-molecule compounds were obtained from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) and the macromolecular protein target receptors were obtained from the RCSB PDB database (http://www.rcsb.org/). Molecular docking simulations of macromolecular protein target receptors and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock Vina software, and further demonstration was using the PyMOL Molecular Graphics System (Version 2.4.0).

Screening results of candidate compounds and targets

Under the conditions of OB ≥30% and DL ≥0.18, a total of 87 possible compounds of JWSWG were obtained using the TCMSP. The essential information of all possible compounds is displayed in *Table 1*. The TCMSP was adopted to obtain the relevant targets of the 87 compounds. Then, Homo sapiens search was employed to screen out corresponding human gene codes in the UniProt knowledge base, and 102 target genes were selected as a result (*Table 2*).

Action targets acquisition

By intersecting 102 compound target genes with 6,732

Table 1 Candidate compounds of JWSWG with OB and DL paraments

MOL ID	Molecule name	OB (%)	DL	Source
MOL000359	sitosterol	36.91	0.75	ShuDi; ChiShao; ChuanXiong
MOL000449	Stigmasterol	43.83	0.76	ShuDi; ChiShao; DangGui; GouQi; DiGuPi
MOL001002	Ellagic acid	43.06	0.43	ChiShao
MOL001918	Paeoniflorgenone	87.59	0.37	ChiShao
MOL001921	Lactiflorin	49.12	0.8	ChiShao
MOL001924	paeoniflorin	53.87	0.79	ChiShao
MOL001925	Paeoniflorin_qt	68.18	0.4	ChiShao
MOL002714	Baicalein	33.52	0.21	ChiShao
MOL002776	Baicalin	40.12	0.75	ChiShao
MOL000358	Beta-sitosterol	36.91	0.75	ChiShao; DangGui; GouQi; DiGuPi
MOL004355	Spinasterol	42.98	0.76	ChiShao
MOL000492	(+)-catechin	54.83	0.24	ChiShao
MOL006990	(1S,2S,4R)-trans-2-hydroxy-1,8-cineole-B-D-glucopyranoside	30.25	0.27	ChiShao
MOL006992	(2R,3R)-4-methoxyl-distylin	59.98	0.3	ChiShao
MOL006994	1-o-beta-d-glucopyranosyl-8-o-benzoylpaeonisuffrone_qt	36.01	0.3	ChiShao
MOL006996	1-o-beta-d-glucopyranosylpaeonisuffrone_qt	65.08	0.35	ChiShao
MOL006999	Stigmast-7-en-3-ol	37.42	0.75	ChiShao
MOL007003	Benzoyl paeoniflorin	31.14	0.54	ChiShao
MOL007004	Albiflorin	30.25	0.77	ChiShao
MOL007005	Albiflorin_qt	48.7	0.33	ChiShao
MOL007008	4-ethyl-paeoniflorin_qt	56.87	0.44	ChiShao
MOL007012	4-o-methyl-paeoniflorin_qt	56.7	0.43	ChiShao
MOL007014	8-debenzoylpaeonidanin	31.74	0.45	ChiShao
MOL007016	Paeoniflorigenone	65.33	0.37	ChiShao
MOL007018	9-ethyl-neo-paeoniaflorin A_qt	64.42	0.3	ChiShao
MOL007022	evofolinB	64.74	0.22	ChiShao
MOL007025	Isobenzoylpaeoniflorin	31.14	0.54	ChiShao
MOL002883	Ethyl oleate (NF)	32.4	0.19	ChiShao
MOL005043	campest-5-en-3beta-ol	37.58	0.71	ChiShao
MOL001494	Mandenol	42	0.19	ChuanXiong; GouQi
MOL002135	Myricanone	40.6	0.51	ChuanXiong
MOL002140	Perlolyrine	65.95	0.27	ChuanXiong

Table 1 (*continued*)

Table 1 (*continued*)

MOL ID	Molecule name	OB (%)	DL	Source
MOL002151	Senkyunone	47.66	0.24	ChuanXiong
MOL002157	Wallichilide	42.31	0.71	ChuanXiong
MOL000433	FA	68.96	0.71	ChuanXiong
MOL001323	Sitosterol alpha1	43.28	0.78	GouQi
MOL003578	Cycloartenol	38.69	0.78	GouQi
MOL001495	Ethyl linolenate	46.1	0.2	GouQi
MOL001979	LAN	42.12	0.75	GouQi
MOL005406	Atropine	45.97	0.19	GouQi
MOL005438	Campesterol	37.58	0.71	GouQi
MOL006209	Cyanin	47.42	0.76	GouQi
MOL007449	24-methylidenelophenol	44.19	0.75	GouQi
MOL008173	daucosterol_qt	36.91	0.75	GouQi
MOL008400	Glycitein	50.48	0.24	GouQi
MOL010234	Delta-Carotene	31.8	0.55	GouQi
MOL000953	CLR	37.87	0.68	GouQi DiGuPi
MOL009604	14b-pregnane	34.78	0.34	GouQi
MOL009612	(24R)-4alpha-Methyl-24-ethylcholesta-7,25-dien-3beta-ylacetate	46.36	0.84	GouQi
MOL009615	24-Methylenecycloartan-3beta,21-diol	37.32	0.8	GouQi
MOL009617	24-ethylcholest-22-enol	37.09	0.75	GouQi
MOL009618	24-ethylcholesta-5,22-dienol	43.83	0.76	GouQi
MOL009620	24-methyl-31-norlanost-9(11)-enol	38	0.75	GouQi
MOL009621	24-methylenelanost-8-enol	42.37	0.77	GouQi
MOL009622	Fucosterol	43.78	0.76	GouQi
MOL009631	31-Norcyclolaudenol	38.68	0.81	GouQi
MOL009633	31-norlanost-9(11)-enol	38.35	0.72	GouQi
MOL009634	31-norlanosterol	42.2	0.73	GouQi
MOL009635	4,24-methyllophenol	37.83	0.75	GouQi
MOL009639	Lophenol	38.13	0.71	GouQi
MOL009640	4alpha,14alpha,24-trimethylcholesta-8,24-dienol	38.91	0.76	GouQi
MOL009641	4alpha,24-dimethylcholesta-7,24-dienol	42.65	0.75	GouQi
MOL009642	4alpha-methyl-24-ethylcholesta-7,24-dienol	42.3	0.78	GouQi
MOL009644	6-Fluoroindole-7-Dehydrocholesterol	43.73	0.72	GouQi
MOL009646	7-O-Methylluteolin-6-C-beta-glucoside_qt	40.77	0.3	GouQi
MOL009650	Atropine	42.16	0.19	GouQi

Table 1 (*continued*)

Table 1 (*continued*)

MOL ID	Molecule name	OB (%)	DL	Source
MOL009651	Cryptoxanthin monoepoxide	46.95	0.56	GouQi
MOL009653	Cycloeucalenol	39.73	0.79	GouQi
MOL009656	(E,E)-1-ethyl octadeca-3,13-dienoate	42	0.19	GouQi
MOL009660	methyl (1R,4aS,7R,7aS)-4a,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopenta[d]pyran-4-carboxylate	39.43	0.47	GouQi
MOL009662	Lantadene A	38.68	0.57	GouQi
MOL009664	Physalin A	91.71	0.27	GouQi
MOL009665	Physcion-8-O-beta-D-gentiobioside	43.9	0.62	GouQi
MOL009677	lanost-8-en-3beta-ol	34.23	0.74	GouQi
MOL009678	lanost-8-enol	34.23	0.74	GouQi
MOL009681	Obtusifoliol	42.55	0.76	GouQi
MOL000098	Quercetin	46.43	0.28	GouQi
MOL001552	OIN	45.97	0.19	DiGuPi
MOL001645	Linoleyl acetate	42.1	0.2	DiGuPi
MOL001689	Acacetin	34.97	0.24	DiGuPi
MOL001790	Linarin	39.84	0.71	DiGuPi
MOL002218	Scopolin	56.45	0.39	DiGuPi
MOL002219	Atropine	34.53	0.21	DiGuPi
MOL002222	Sugiol	36.11	0.28	DiGuPi
MOL002224	Aurantiamide acetate	58.38	0.59	DiGuPi
MOL002228	Kulactone	45.44	0.82	DiGuPi
MOL000296	Hederagenin	36.91	0.75	DiGuPi

OB, oral bioavailability; DL, drug-likeness.

hypertension target genes, 88 action target genes were obtained from the GeneCards database (*Table 3*).

Network construction and analysis results

Cytocsape software was employed to construct the "medicine-compound-target-disease" interaction network on the basis of the 88 action target genes. The network showed 146 nodes (51 compound nodes, 88 target gene nodes, 6 herbal medicine nodes, and 1 disease node) and 395 edges (*Figure 1*). From the figure, it was found that the most valuable compounds were quercetin, beta-sitosterol, baicalein, stigmasterol, acacetin, ellagic acid, and glycitein (*Table 4*). These high-value compounds are the possible key compounds in treating hypertension with JWSWG.

Constructing the PPI network and screening the key targets

The STRING database was employed to construct the PPI network by analyzing the 88 intersection target genes for the purpose of exploring the mechanism of JWSWG in treating hypertension. The lowest interaction score was set to 0.4, then 87 targets in this network showed protein interaction (one target did not have protein interaction), and 787 edges indicated the interaction between proteins (*Figure 2*). The figure showed that the high-degree targets were interleukin 6 (IL6), caspase 3 (CASP3), epithelial growth factor receptor (EGFR), proto-oncogene myc (MYC), vascular endothelial growth factor A (VEGFA), estrogen receptor α (ESR1), cyclin

Table 2 The corresponding targets of candidate compounds

Target	Symbol	Compounds
Progesterone receptor	PGR	Sitosterol; Stigmasterol; ellagic acid; beta-sitosterol; Spinasterol; stigmast-7-en-3-ol; campest-5-en-3beta-ol; Sitosterol alpha1; LAN; Campesterol; 24-methylidenelophenol; daucosterol_qt; CLR; 14b-pregnane; 24-ethylcholest-22-enol; 24-ethylcholesta-5,22-dienol; 24-methyl-31-norlanost-9(11)-enol; 24-methylenelanost-8-enol; Fucosterol; 31-norlanost-9(11)-enol; 31-norlanosterol; 4,24-methyllophenol; Lophenol; 4alpha,14alpha,24-trimethylcholesta-8,24-dienol; 4alpha,24-dimethylcholesta-7,24-dienol; 4alpha-methyl-24-ethylcholesta-7,24-dienol; 6-Fluoroindole-7-Dehydrocholesterol; lanost-8-en-3beta-ol; lanost-8-enol; Obtusifoliol; hederagenin
Nuclear receptor coactivator 2	NCOA2	Sitosterol; Stigmasterol; Baicalein; beta-sitosterol; Spinasterol; (+)-catechin; Ethyl oleate (NF); Mandenol; Wallichilide; Ethyl linolenate; LAN; 24-methylidenelophenol; daucosterol_qt; CLR; 14b-pregnane; Fucosterol; 31-norlanosterol; 4alpha,24-dimethylcholesta-7,24-dienol; 7-O-Methylluteolin-6-C-beta-glucoside_qt; (E,E)-1-ethyl octadeca-3,13-dienoate; Obtusifoliol; Quercetin; Linoleyl acetate; Acacetin; hederagenin
Mineralocorticoid receptor	NR3C2	Sitosterol; Stigmasterol; Spinasterol; Wallichilide; Sitosterol alpha1; Cycloartenol; LAN; 24-methylidenelophenol; CLR; 24-ethylcholesta-5,22-dienol; Fucosterol; 31-norlanosterol; 4alpha,24-dimethylcholesta-7,24-dienol; 6-Fluoroindole-7-Dehydrocholesterol; lanost-8-en-3beta-ol; lanost-8-enol; Obtusifoliol
Nuclear receptor coactivator 1	NCOA1	Stigmasterol; Baicalein; Myricanone; Glycitein; acacetin
Prostaglandin G/H synthase 1	PTGS1	Stigmasterol; Baicalein; beta-sitosterol; (+)-catechin; (2R,3R)-4-methoxyl-distylin; Mandenol; Myricanone; Ethyl linolenate; Glycitein; Quercetin; Linoleyl acetate; acacetin; hederagenin
Aldose reductase	AKR1B1	Stigmasterol; quercetin
Urokinase-type plasminogen activator	PLAU	Stigmasterol; quercetin
Chymotrypsinogen B	CTRB1	Stigmasterol
Muscarinic acetylcholine receptor M3	CHRM3	Stigmasterol; beta-sitosterol; atropine; Atropine; OIN; Atropine Sugiol; hederagenin
Muscarinic acetylcholine receptor M1	CHRM1	Stigmasterol; beta-sitosterol; atropine; Atropine; OIN; Atropine; Sugiol; Hederagenin
Alpha-1A adrenergic receptor	ADRA1A	Stigmasterol; beta-sitosterol; atropine; Atropine; OIN; Atropine Sugiol;
Muscarinic acetylcholine receptor M2	CHRM2	Stigmasterol; beta-sitosterol; atropine; Atropine; OIN; Atropine; Sugiol; hederagenin
Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1	Stigmasterol; Paeoniflorigenone; beta-sitosterol; Sitosterol alpha1; Atropine; Atropine; Quercetin; OIN; Atropine; hederagenin
Estrogen receptor	ESR1	Ellagic acid; (+)-catechin; (2R,3R)-4-methoxyl-distylin; Myricanone; glycitein
Androgen receptor	AR	Ellagic acid; baicalein; Myricanone; Glycitein; Quercetin; acacetin

Table 2 (*continued*)

Table 2 (*continued*)

Target	Symbol	Compounds
Transcription factor p65	RELA	Ellagic acid; baicalein; quercetin; acacetin
Vascular endothelial growth factor A	VEGFA	Ellagic acid; baicalein; quercetin
NF-kappa-B inhibitor alpha	NFKBIA	Ellagic acid; quercetin
Glutathione S-transferase P	GSTP1	Ellagic acid; quercetin
Insulin-like growth factor II	IGF2	Ellagic acid; baicalein; quercetin
Glutathione S-transferase Mu 1	GSTM1	Ellagic acid; quercetin
Glutathione S-transferase Mu 2	GSTM2	Ellagic acid; quercetin
Interleukin-6	IL6	Paeoniflorin; quercetin
Trypsin-1	PRSS1	Baicalein; Glycitein; Quercetin; Acacetin; aurantiamide acetate
Apoptosis regulator Bcl-2	BCL2	Baicalein; beta-sitosterol; quercetin; acacetin
Proto-oncogene c-Fos	FOS	Baicalein; quercetin
Caspase-3	CASP3	Baicalein; beta-sitosterol; Quercetin; acacetin
Cellular tumor antigen p53	TP63	Baicalein; Quercetin; acacetin
Hypoxia-inducible factor 1-alpha	HIF1A	Baicalein; quercetin
Fos-related antigen 1	FOSL1	Baicalein
G2/mitotic-specific cyclin-B1	CCNB1	Baicalein; quercetin
Aryl hydrocarbon receptor	AHR	Baicalein; quercetin
Cytochrome c	CYCS	Baicalein
NADPH oxidase 5	NOX5	Baicalein
Apolipoprotein D	APOD	Baicalein
Muscarinic acetylcholine receptor M4	CHRM4	Beta-sitosterol; atropine; Atropine; OIN; Atropine; sugiol
Neuronal acetylcholine receptor subunit alpha-2	CHRNA2	Beta-sitosterol
Caspase-9	CASP9	Beta-sitosterol; quercetin
Caspase-8	CASP8	Beta-sitosterol; quercetin; acacetin
Protein kinase C alpha type	PRKCA	Beta-sitosterol; quercetin
Serum paraoxonase/arylesterase 1	PON1	Beta-sitosterol; quercetin
Peroxisome proliferator activated receptor gamma	PPARG	Myricanone; Glycitein; quercetin; quercetin
Coagulation factor VII	F7	Myricanone; quercetin
Estrogen receptor beta	ESR2	Myricanone; glycitein
Glycogen synthase kinase-3 beta	GSK3B	Myricanone; FA; glycitein
Serine/threonine-protein kinase Chk1	CHEK1	Myricanone; Glycitein; acacetin
Glucocorticoid receptor	NR3C1	Wallichilide; 6-Fluoroindole-7-Dehydrocholesterol
Muscarinic acetylcholine receptor M5	CHRM5	Atropine; Atropine; OIN; Atropine; sugiol
Alpha-2C adrenergic receptor	ADRA2C	Atropine; Atropine; OIN

Table 2 (*continued*)

Table 2 (*continued*)

Target	Symbol	Compounds
D(2) dopamine receptor	DRD2	Atropine; Atropine; OIN; Atropine; sugiol
Amyloid beta A4 protein	APP	Glycitein
Acetylcholinesterase	ACHE	Quercetin; sugiol
Epidermal growth factor receptor	EGFR	Quercetin
G1/S-specific cyclin-D1	CCND1	Quercetin
Eukaryotic translation initiation factor 6	EIF6	Quercetin
Retinoblastoma-associated protein	RB1	Quercetin
Activator of 90 kDa heat shock protein ATPase homolog 1	AHSA1	Quercetin
ETS domain-containing protein Elk-1	ELK1	Quercetin
NADPH--cytochrome P450 reductase	POR	Quercetin
RAF proto-oncogene serine/threonine-protein kinase	RAF1	Quercetin
Protein CBFA2T1	RUNX1T1	Quercetin
Receptor tyrosine-protein kinase erbB-2	ERBB2	Quercetin
Acetyl-CoA carboxylase 1	ACACA	Quercetin
Cytochrome P450 3A4	CYP3A4	Quercetin
Caveolin-1	CAV1	Quercetin
Myc proto-oncogene protein	MYC	Quercetin
Cytochrome P450 1A1	CYP1A1	Quercetin
Intercellular adhesion molecule 1	ICAM1	Quercetin
E-selectin	SELE	Quercetin
Vascular cell adhesion protein 1	VCAM1	Quercetin
Prostaglandin E2 receptor EP3 subtype	PTGER3	Quercetin
Baculoviral IAP repeat-containing protein 5	BIRC5	Quercetin
Dual oxidase 2	DUOX2	Quercetin
Nitric oxide synthase, endothelial	NOS3	Quercetin
Heat shock protein beta-1	HSPB1	Quercetin
Maltase-glucoamylase, intestinal	MGAM	Quercetin
Cytochrome P450 1B1	CYP1B1	Quercetin
Arachidonate 5-lipoxygenase	ALOX5	Quercetin
Nuclear factor erythroid 2-related factor 2	NFE2L2	Quercetin
NAD(P)H dehydrogenase [quinone] 1	NQO1	Quercetin
Poly [ADP-ribose] polymerase 1	PARP1	Quercetin
26S proteasome non-ATPase regulatory subunit 3	PSMD3	Quercetin

Table 2 (*continued*)

Table 2 (*continued*)

Target	Symbol	Compounds
Solute carrier family 2, facilitated glucose transporter member 4	SLC2A4	Quercetin
Collagen alpha-1(III) chain	COL3A1	Quercetin
DDB1- and CUL4-associated factor 5	DCAF5	Quercetin
Nuclear receptor subfamily 1 group I member 3	NR1I3	Quercetin
Serine/threonine-protein kinase Chk2	CHEK2	Quercetin
Heat shock factor protein 1	HSF1	Quercetin
C-reactive protein	CRP	Quercetin
Runt-related transcription factor 2	RUNX2	Quercetin
Ras association domain-containing protein 1	RASSF1	Quercetin
Cathepsin D	CTSD	Quercetin
Insulin-like growth factor-binding protein 3	IGFBP3	Quercetin
Interferon regulatory factor 1	IRF1	Quercetin
Receptor tyrosine-protein kinase erbB-3	ERBB3	Quercetin
Type I iodothyronine deiodinase	DIO1	Quercetin
Puromycin-sensitive aminopeptidase	NPEPPS	Quercetin
Hexokinase-2	HK2	Quercetin
Ras GTPase-activating protein 1	RASA1	Quercetin
Fatty acid synthase	FASN	Acacetin
Glutamate receptor 2	GRIA2	Hederagenin
Alcohol dehydrogenase 1B	ADH1B	Hederagenin

Table 3 Intersection action target genes

Symbol	EntrezID
PGR	5241
NCOA2	10499
NR3C2	4306
NCOA1	8648
PTGS1	5742
AKR1B1	231
PLAU	5328
CHRM3	1131
CHRM1	1128
ADRA1A	148

Table 3 (*continued*)

Table 3 (*continued*)

Symbol	EntrezID
CHRM2	1129
ESR1	2099
AR	367
RELA	5970
VEGFA	7422
NFKBIA	4792
GSTP1	2950
IGF2	3481
GSTM1	2944
GSTM2	2946

Table 3 (*continued*)

Table 3 (*continued*)

Symbol	EntrezID
IL6	3569
BCL2	596
FOS	2353
CASP3	836
TP63	8626
HIF1A	3091
CCNB1	891
AHR	196
CYCS	54205
NOX5	79400
APOD	347
CHRNA2	1135
CASP9	842
CASP8	841
PRKCA	5578
PON1	5444
PPARG	5468
F7	2155
ESR2	2100
GSK3B	2932
NR3C1	2908
ADRA2C	152
DRD2	1813
APP	351
ACHE	43
EGFR	1956
CCND1	595
RB1	5925
ELK1	2002
POR	5447
RAF1	5894
ERBB2	2064
ACACA	31
CYP3A4	1576
CAV1	857

Table 3 (*continued*)

Table 3 (*continued*)

Symbol	EntrezID
MYC	4609
CYP1A1	1543
ICAM1	3383
SELE	6401
VCAM1	7412
PTGER3	5733
BIRC5	332
DUOX2	50506
NOS3	4846
HSPB1	3315
MGAM	8972
CYP1B1	1545
ALOX5	240
NFE2L2	4780
NQO1	1728
PARP1	142
SLC2A4	6517
COL3A1	1281
NR1I3	9970
CHEK2	11200
HSF1	3297
CRP	1401
RUNX2	860
RASSF1	11186
CTSD	1509
IGFBP3	3486
IRF1	3659
ERBB3	2065
DIO1	1733
HK2	3099
RASA1	5921
FASN	2194
ADH1B	125

Table 3 (*continued*)

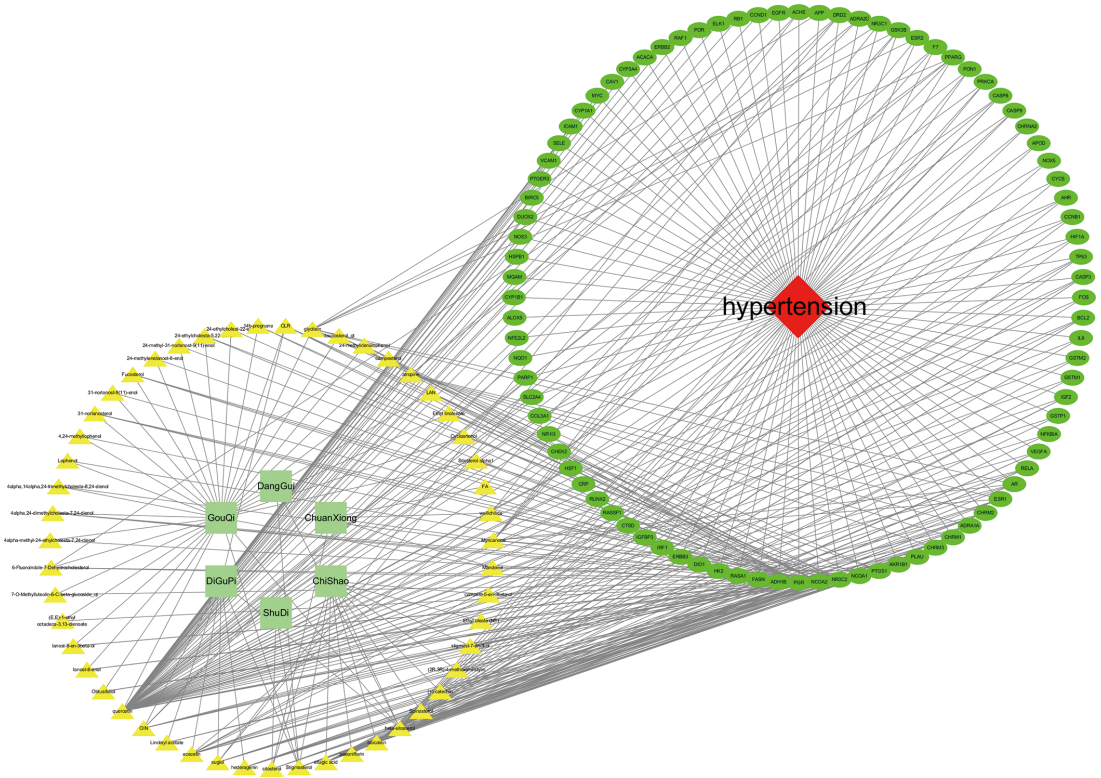

Figure 1 The "medicine-compound-target-disease" network. A total of 146 nodes (51 compound nodes, 88 target gene nodes, 6 herbal medicine nodes, and 1 disease node) and 395 edges were shown in the network.

Table 4 Key compounds of JWSWG in the treatment of hypertension

Compound	Degree	Herbal medicine
Quercetin	70	GouQi
Beta-sitosterol	18	ChiShao; DangGui; GouQi; DiGuPi
Baicalein	18	ChiShao
Stigmasterol	16	ShuDi; ChiShao; DangGui; GouQi; DiGuPi
Acacetin	11	DiGuPi
Ellagic acid	11	ChiShao
Glycitein	9	GouQi
Myricanone	9	ChuanXiong

JWSWG, JiaWeiSiWu granule.

D1 (CCND1), proto-oncogene c-Fos (FOS), tyrosine kinase receptor 2 (ERBB2), and androgen receptor (AR) (*Table 5*, *Figure 3*). The above-mentioned targets are therefore the possible key targets for JWSWG in treating hypertension.

GO functional enrichment analysis

GO functions were enriched by RGUI and clusterProfiler, and the results of GO-MF, GO-BP, and GO-CC were as follows.

The 88 intersection target genes influenced 107 MF (P value <0.05, q value <0.05). The P value ranking was adopted as the screen condition and the MF information of the top 20 was obtained. The intersection genes of JWSWG in treating hypertension were principally involved in nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, steroid binding, proximal promoter sequence-specific DNA binding, DNA-binding transcription activator activity, RNA polymerase II-specific, protein heterodimerization activity, RNA polymerase II proximal promoter sequence-specific DNA binding, cofactor binding, heme binding, tetrapyrrole binding, ubiquitin-like protein ligase binding, ubiquitin protein ligase binding, Hsp90 protein binding, RNA polymerase II transcription factor binding, chromatin binding, ammonium ion binding, G protein-coupled amine receptor

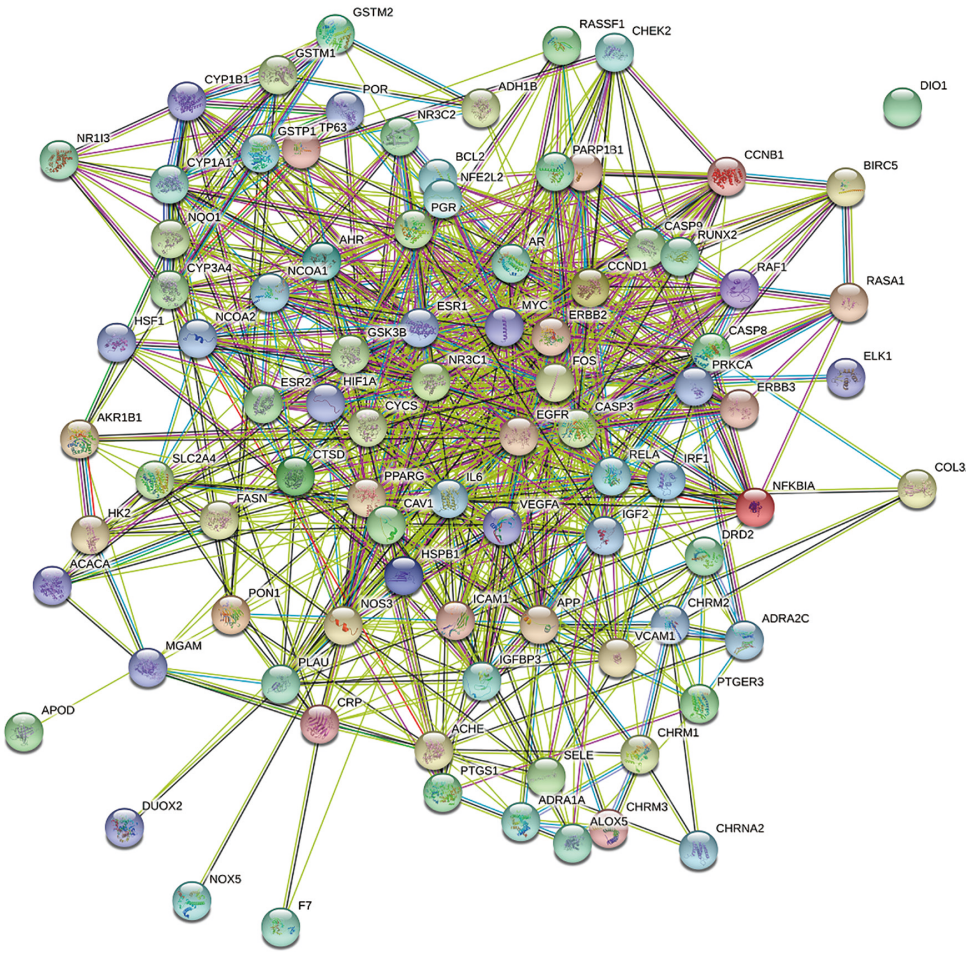

Figure 2 PPI network of JWSWG in treating hypertension. The lowest interaction score was set to 0.4, then 87 targets in this network showed the protein interaction (one target did not), and 787 edges indicated the interaction between proteins. PPI, protein-protein interaction; JWSWG, JiaWeiSiWu granule.

Table 5 Key genes of JWSWG in treating hypertension

Gene	Full name	Degree
IL6	Interleukin 6	55
CASP3	Caspase 3	50
EGFR	Epithelial growth factor receptor	49
MYC	proto-oncogene myc	49
VEGFA	Vascular endothelial growth factor A	48
ESR1	Estrogen receptor α	45
CCND1	Cyclin D1	41
FOS	proto-oncogene c-Fos	41
ERBB2	Tyrosine kinase receptor 2	38
AR	Androgen receptor	37

JWSWG, JiaWeiSiWu granule.

activity, nuclear hormone receptor binding, and activating transcription factor binding (*Table 6*, *Figure 4*).

The 88 intersection target genes influenced 1,331 BP (P value <0.05, q value <0.05). The P value ranking was adopted as the screen condition and the BP information of the top 20 was obtained. The intersection genes of JWSWG in treating hypertension were chiefly enriched in response to steroid hormone, response to ketone, response to xenobiotic stimulus, cellular response to xenobiotic stimulus, response to toxic substance, regulation of body fluid levels, response to oxidative stress, response to metal ion, response to antibiotic, response to oxygen levels, cellular response to steroid hormone stimulus, response to hypoxia, cellular response to drug, response to decreased

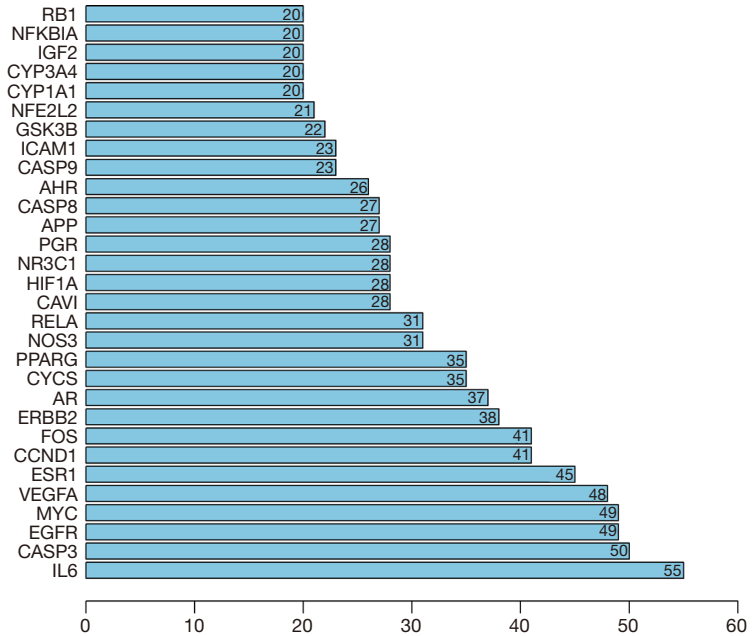

Figure 3 Key genes revealed by the PPI network. This figure showed that the high-degree targets were IL6, CASP3, EGFR, MYC, VEGF A, ESR1, CCND1, FOS, ERBB2, and AR. PPI, protein-protein interaction; IL, interleukin; CASP, caspase; EGFR, epithelial growth factor receptor; MYC, proto-oncogene myc; VEGF, vascular endothelial growth factor; ESR1, estrogen receptor α; CCND1, cyclin D1; FOS, proto-oncogene c-Fos; ERBB, tyrosine kinase receptor; AR, androgen receptor.

oxygen levels, reactive oxygen species metabolic process, cellular response to oxidative stress, gland development, response to radiation, intracellular receptor signaling pathway, and response to acid chemical (*Table 7*, *Figure 5*).

The 88 intersection target genes influenced 61 CC (P value <0.05, q value <0.05). The P value ranking was adopted as the screen condition and the CC information of the top 20 was obtained. The intersection genes of JWSWG in treating hypertension were primarily enriched in membrane raft, membrane microdomain, membrane region, nuclear chromatin, nuclear chromosome part, chromatin, axon terminus, integral component of postsynaptic membrane, neuron projection terminus, intrinsic component of postsynaptic membrane, integral component of presynaptic membrane, transcription factor complex, axon part, intrinsic component of presynaptic membrane, receptor complex, integral component of synaptic membrane, intrinsic component of synaptic membrane, presynapse, distal axon, and glutamatergic synapse (*Table 8*, *Figure 6*).

KEGG pathway enrichment analysis

RGUI and clusterProfiler were applied to KEGG pathway enrichment. The analysis of KEGG pathway enrichment indicated that the 88 intersecting target genes were observably enriched in 107 pathways (P value <0.05, q value <0.05). Among which, the top 20 pathways included prostate cancer, Kaposi sarcoma-associated herpesvirus infection, hepatitis B, human cytomegalovirus infection, fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, colorectal cancer, hepatocellular carcinoma, proteoglycans in cancer, platinum drug resistance, bladder cancer, apoptosis, breast cancer, thyroid hormone signaling pathway, p53 signaling pathway, hepatitis C, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, legionellosis, and endometrial cancer, suggesting that JWSWG plays a crucial role in treating hypertension by working on the above-mentioned multiple pathways (*Table 9*, *Figure 7*). The chief pathways are displayed in *Figures 8* and *9*.

Molecular docking

We obtained the 3D structures of the small-molecule compounds from the PubChem Database and the macromolecular protein target receptors from the RCSB PDB database. Then, molecular docking simulations of potential targets and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock

Table 6 GO-MF of drug-disease intersection genes (from clusterProfiler)

ID	Description	P value	Count
GO:0004879	Nuclear receptor activity	8.4E-11	8
GO:0098531	Transcription factor activity, direct ligand regulated sequence-specific DNA binding	8.4E-11	8
GO:0003707	Steroid hormone receptor activity	3.24E-10	8
GO:0005496	Steroid binding	4.29E-10	9
GO:0000987	Proximal promoter sequence-specific DNA binding	1.23E-09	16
GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	1.39E-09	15
GO:0046982	Protein heterodimerization activity	4.79E-09	16
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	5.58E-09	15
GO:0048037	Cofactor binding	5.34E-08	14
GO:0020037	Heme binding	7.54E-08	8
GO:0046906	Tetrapyrrole binding	1.52E-07	8
GO:0044389	Ubiquitin-like protein ligase binding	2.61E-07	11
GO:0031625	Ubiquitin protein ligase binding	1.33E-06	10
GO:0051879	Hsp90 protein binding	1.47E-06	5
GO:0001085	RNA polymerase II transcription factor binding	1.96E-06	7
GO:0003682	Chromatin binding	2.56E-06	12
GO:0070405	Ammonium ion binding	4.8E-06	5
GO:0008227	G protein-coupled amine receptor activity	1.08E-05	4
GO:0035257	Nuclear hormone receptor binding	1.12E-05	7
GO:0033613	Activating transcription factor binding	2E-05	5

GO-MF, Gene Ontology molecular function.

Vina software. Finally, the binding of the target and its corresponding component was verified by molecular docking and demonstrated by the PyMOL Molecular Graphics System. We selected IL6-beta-sitosterol to demonstrate. In the molecular docking simulations of IL6-beta-sitosterol, minimum affinity was −7.0 kcal/mol, grid center was −0.585, 0.365 and 0.253, dist from best mode was 0.000 rmsd l.b. and 0.000 rmsd u.b. (*Figure 10*).

Statistical analysis

A part of the statistical analysis was simultaneously conducted with the biotechnology add-ons of the software and platforms that have been disclosed in previous sections. In the GO function and KEGG pathway enrichment, an adjusted P (adj. P) value was used and adj. P<0.05 was perceived as statistically significant.

Discussion

In recent years, with the changes of people's diets and lifestyles, the incidence of hypertension has increased year by year, becoming a serious public health problem. Persistent hypertension not only increases the risk of cardiovascular and cerebrovascular adverse events, but also causes damage to target organs such as the heart, brain, and kidneys, among others, which seriously threatens the health and safety of patients (15). Renal injury is particularly common, as the kidney can be regulated by water and sodium metabolism and secretory pressure, and antihypertensive substances affect the fluctuation of blood pressure. Essential hypertension can also cause renal arteriosclerosis, resulting in renal function damage. After renal function is damaged, the hypertension will be further aggravated, thus forming a vicious circle (16).

JWSWG was formed with the background of several

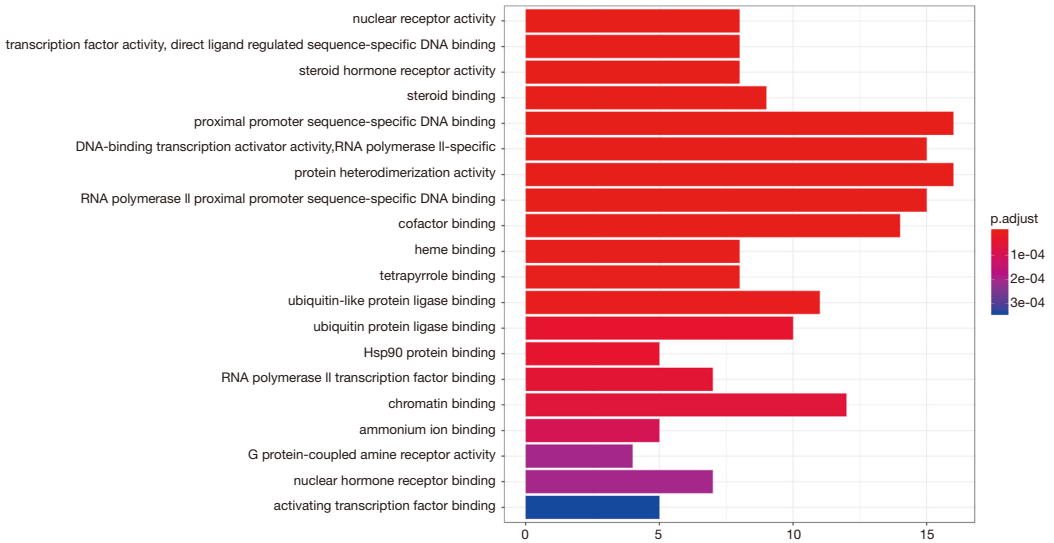

Figure 4 Histogram of GO-MF enrichment analysis (from clusterProfiler). The intersection genes of JWSWG in treating hypertension primarily converged on nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, steroid hormone receptor activity, steroid binding, proximal promoter sequence-specific DNA binding, DNA-binding transcription activator activity, RNA polymerase II-specific, protein heterodimerization activity, RNA polymerase II proximal promoter sequence-specific DNA binding, cofactor binding, heme binding, tetrapyrrole binding, ubiquitin-like protein ligase binding, ubiquitin protein ligase binding, Hsp90 protein binding, RNA polymerase II transcription factor binding, chromatin binding, ammonium ion binding, G protein-coupled amine receptor activity, nuclear hormone receptor binding, and activating transcription factor binding. GO-MF, Gene Ontology molecular function; JWSWG, JiaWeiSiWu granule.

years of clinical practice. The original basic prescription includes DiHuang, DangGui, ChuanXiong, GouQi, DiGuPi, and DiLong. Modern pharmacological studies of DiHuang have shown that it can significantly reduce blood pressure, improve renal function, and reduce blood glucose. Studies also showed that the indexes of hemorheology in the model group of blood stasis syndrome were markedly higher than those in the normal group, and the indexes of hemorheology in the low dose group were significantly lower than those in the model group. Therefore, DiHuang can also significantly improve microcirculation (17). The pharmacological effects of ChiShao include anticoagulation and antithrombosis. ChiShao could significantly reduce blood viscosity, fibrin content, erythrocyte aggregation index, and hematocrit in rats with blood stasis (18,19). Also, ChiShao could significantly improve microcirculation (20). ChiShao has also been shown to decrease the viscosity of serum and plasma, inhibit platelet aggregation, prolong prothrombin time and activated partial thromboplastin time, and had protective effects on renal ischemia, cerebral ischemia, and myocardial ischemia (21-23). Modern pharmacology shows that DangGui has the effects of dilating blood vessels, reducing vascular resistance, improving organ blood flow, reducing platelet aggregation and antithrombosis, increasing low shear whole blood viscosity, enhancing erythrocyte aggregation, promoting platelet aggregation, increasing cardiac blood supply, reducing myocardial oxygen consumption, and protecting cardiomyocytes (24-26). ChuanXiong can dilate the coronary artery, increase coronary flow, inhibit aortic smooth muscle contraction, and antagonize the pressor effects of methoxyamine, phenylephrine, and epinephrine. It can also inhibit platelet aggregation and inhibit thrombosis (27-29). GouQi has the functions of enhancing immunity, reducing blood lipids, lowering blood glucose, lowering blood pressure, protecting the liver, and preventing radiation damage, and also has anti-hypoxia, anti-tumor, anti-aging, and anti-fatigue effects, among others. GouQi could protect against cerebral ischemia-reperfusion injury in mice (30-32). Modern pharmacological studies have revealed that DiGuPi has the activities of lowering blood pressure, regulating blood lipids, and lowering blood sugar, and also has anti-pyretic, antibacterial, and antiviral properties (33,34).

Table 7 GO-BP of drug-disease intersection genes (from clusterProfiler)

ID	Description	P value	Count
GO:0048545	Response to steroid hormone	1.68E-20	24
GO:1901654	Response to ketone	6.40E-20	19
GO:0009410	Response to xenobiotic stimulus	1.02E-19	21
GO:0071466	Cellular response to xenobiotic stimulus	3.36E-18	17
GO:0009636	Response to toxic substance	1.25E-17	24
GO:0050878	Regulation of body fluid levels	2.14E-15	22
GO:0006979	Response to oxidative stress	2.34E-15	21
GO:0010038	Response to metal ion	2.44E-15	19
GO:0046677	Response to antibiotic	2.55E-14	18
GO:0070482	Response to oxygen levels	3.67E-14	19
GO:0071383	Cellular response to steroid hormone stimulus	4.87E-14	16
GO:0001666	Response to hypoxia	9.46E-14	18
GO:0035690	Cellular response to drug	1.39E-13	18
GO:0036293	Response to decreased oxygen levels	1.60E-13	18
GO:0072593	Reactive oxygen species metabolic process	2.36E-13	16
GO:0034599	Cellular response to oxidative stress	1.02E-12	16
GO:0048732	Gland development	1.86E-12	18
GO:0009314	Response to radiation	3.09E-12	18
GO:0030522	Intracellular receptor signaling pathway	4.21E-12	15
GO:0001101	Response to acid chemical	5.35E-12	16

GO-BP, Gene Ontology biological process.

A number of clinical studies have demonstrated that JWSWG has a remarkable effect on treating hypertension and its complications. JWSWG can effectively reduce blood pressure, has obvious curative effect on hypertension of yin deficiency and yang hyperactivity, and can effectively reduce the level of serum CRP (7). JWSWG can effectively reduce urinary albumin in patients with hypertension while controlling blood pressure to achieve a protective effect on the kidneys (6,8). JWSWG is safe and reliable in the treatment of stable angina pectoris complicated with dyslipidemia in patients with stable angina pectoris caused by phlegm and blood stasis. It can not only reduce angina pectoris attack, improve ischemic changes of electrocardiogram, reduce nitroglycerin dosage, and improve TCM symptoms, but also improve blood lipid levels (10). JWSWG can reduce the increase of serum total cholesterol and low density lipoprotein cholesterol in patients with mild to moderate hypertension with yin deficiency and yang hyperactivity, and can improve many indexes of hemorheology in patients with mild to moderate hypertension (35). JWSWG can treat sleep disorders after cerebral infarction, which can dispel blood stasis, promote new blood generation, smooth qi, unobstruct the heart and brain choroid, restore heart spirit, and recover sleep (9). JWSWG can not only effectively improve visual acuity, retinal circulation time, and TCM syndrome, but can also help to improve the hemorheological indexes of patients (36).

The ingredients of Chinese medicine are various and complex. In the study of TCM, there are currently still some problems, including the complex compositions and unclear mechanisms of action. Most of the current research has the limitation of explaining mechanisms and pathways of Chinese medicine for a certain target gene, and the lack of holistic studies on multi-component, multi-target, and multi-pathway

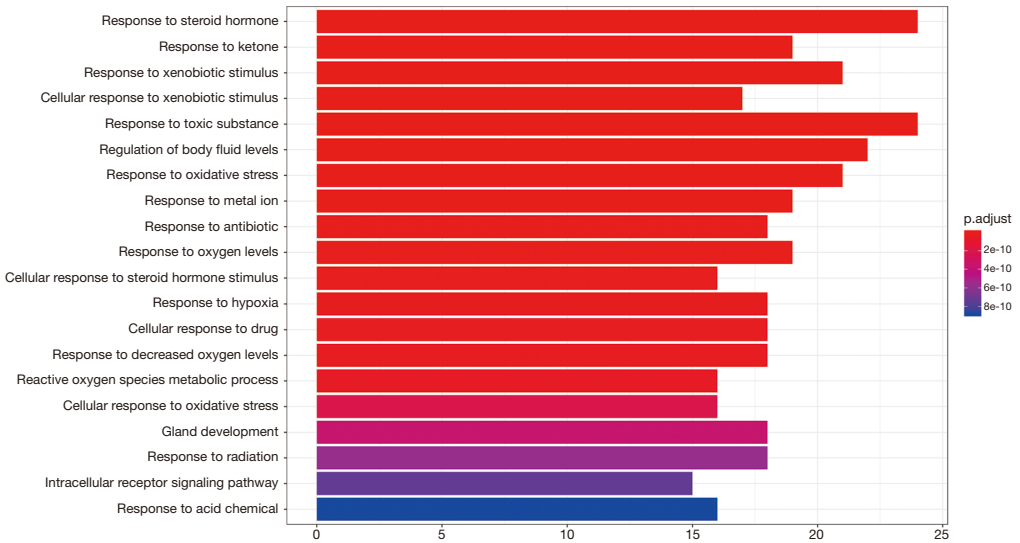

Figure 5 Histogram of GO-BP enrichment analysis (from clusterProfiler). The intersection genes of JWSWG in treating hypertension chiefly converged on response to steroid hormone, response to ketone, response to xenobiotic stimulus, cellular response to xenobiotic stimulus, response to toxic substance, regulation of body fluid levels, response to oxidative stress, response to metal ion, response to antibiotic, response to oxygen levels, cellular response to steroid hormone stimulus, response to hypoxia, cellular response to drug, response to decreased oxygen levels, reactive oxygen species metabolic process, cellular response to oxidative stress, gland development, response to radiation, intracellular receptor signaling pathway, and response to acid chemical. GO-BP, Gene Ontology biological process; JWSWG, JiaWeiSiWu granule.

aspects of TCM. Nowadays, network pharmacology is well developed, and utilizes the research methods of network goal and multi-component therapy, which is in accordance with the feature of "multi-component, multi-target, and multi-pathway" of TCM, and is widely applied to pharmacological research on TCM (37,38).

In this study, the whole view of TCM and syndrome differentiation were combined with the method of network pharmacology analysis. With the support of the corresponding databases and software, the network was constructed and the pathway enrichment of the targets was analyzed. The mechanism of JWSWG in the treatment of hypertension was systematically discussed.

In this study, quercetin, beta-sitosterol, baicalein, stigmasterol, acacetin, ellagic acid, and glycitein were the key compounds of JWSWG in treating hypertension. It has been shown that quercetin can decrease blood pressure and heart rate in elderly hypertensive rats (39). Quercetin can inhibit the proliferation of aortic wall fibroblasts, smooth muscle cells, and the synthesis and secretion of collagen, and delay the process of arteriosclerosis (40). Beta-sitosterol has the effects of reducing blood lipids, and has anticancer and anti-inflammation effects (41). Baicalin has a protective effect on rat cardiomyocytes damaged by ischemia in vivo or oxidative injury in vitro (42). Baicalin has a protective effect on myocardial ischemia and reperfusion injury (43,44). Stigmasterol lowers cholesterol and reduces the risk of cardiovascular disease (45). Acacetin has a protective effect on blood lipid metabolism and atherosclerosis in mice (46-48). Ellagic acid can ameliorate cerebral ischemia or reperfusion injury and has an effect on antioxidant and antimicrobial activity (49,50).

The key targets for JWSWG in the treatment of hypertension were IL6, CASP3, EGFR, MYC, VEGFA, ESR1, CCND1, FOS, ERBB2, and AR. GO enrichment analysis suggested that the target genes related to JWSWG in the treatment of hypertension were related to a variety of molecular functions, biological processes, and cell compositions. We used ClueGO and CluePedia analysis to express this more intuitively (*Figure 11*). These target genes play important roles in the regulation of endothelial function and the neuroendocrine system, and are also involved in anti-inflammatory and antioxidative effects.

KEGG pathway enrichment analysis indicated that multiple pathways were involved in the pathogenic mechanisms of hypertension. The chief pathways included prostate cancer,

Table 8 GO-CC of drug-disease intersection genes (from clusterProfiler)

ID	Description	P value	Count
GO:0045121	Membrane raft	2.19E-07	11
GO:0098857	Membrane microdomain	2.27E-07	11
GO:0098589	Membrane region	3.21E-07	11
GO:0000790	Nuclear chromatin	1.92E-06	10
GO:0044454	Nuclear chromosome part	1.99E-06	12
GO:0000785	Chromatin	3.85E-06	12
GO:0043679	Axon terminus	8.15E-06	6
GO:0099055	Integral component of postsynaptic membrane	1.72E-05	6
GO:0044306	Neuron projection terminus	2.19E-05	6
GO:0098936	Intrinsic component of postsynaptic membrane	2.19E-05	6
GO:0099056	Integral component of presynaptic membrane	2.58E-05	5
GO:0005667	Transcription factor complex	4.07E-05	8
GO:0033267	Axon part	4.36E-05	9
GO:0098889	Intrinsic component of presynaptic membrane	4.5E-05	5
GO:0043235	Receptor complex	7.06E-05	8
GO:0099699	Integral component of synaptic membrane	8.23E-05	6
GO:0099240	Intrinsic component of synaptic membrane	0.000125	6
GO:0098793	Presynapse	0.000219	9
GO:0150034	Distal axon	0.000238	7
GO:0098978	Glutamatergic synapse	0.000255	8

GO-CC, Gene Ontology cellular component.

Kaposi sarcoma-associated herpesvirus infection, hepatitis B, human cytomegalovirus infection, fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, colorectal cancer, hepatocellular carcinoma, proteoglycans in cancer, platinum drug resistance, bladder cancer, apoptosis, breast cancer, thyroid hormone signaling pathway, p53 signaling pathway, hepatitis C, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, legionellosis, and endometrial cancer, suggesting that JWSWG plays a crucial role in treating hypertension by working on the above-mentioned pathways. The main pathways showed that the key nodes of these pathways were closely related to hypertension (51-55) (*Figures 8*,*9*,*12*).

The inadequacies of this study are as follows: First, network pharmacology is supported by data, the data collection process may not inclusive. Furthermore, the setting of screening criteria for effective active components was not completely accurate. Second, in this study, only 6 main herbs were analyzed. Lastly, the effect of dosage of Chinese medicine on treatment results was not taken into account.

Conclusions

To summarize, this research elaborated on the connections among the active components, targets, and pathways of JWSWG in treating hypertension based on network pharmacology. We also determined the characteristic of "multi-component, multi-target, and multi-pathway" of JWSWG. Pathway enrichment analysis revealed that the mechanisms of JWSWG in treating hypertension may include inhibiting the secretion of inflammatory factors, participating in anti-inflammatory responses, inhibiting oxidative stress, regulating vascular endothelial function, and regulating the neuroendocrine system to reduce blood

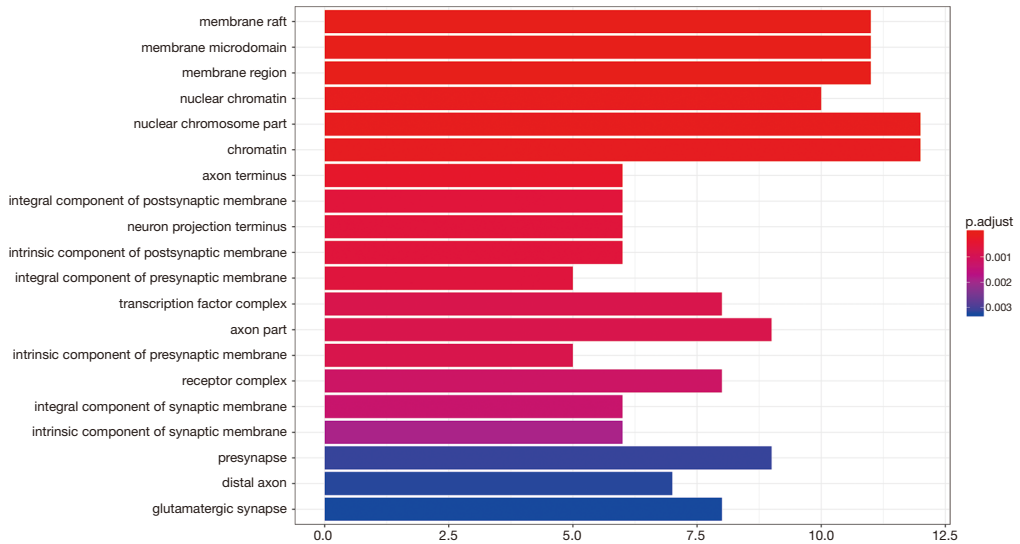

Figure 6 Histogram of GO-CC enrichment analysis (from clusterProfiler). The intersection genes of JWSWG in the treatment of hypertension chiefly converged on membrane raft, membrane microdomain, membrane region, nuclear chromatin, nuclear chromosome part, chromatin, axon terminus, integral component of postsynaptic membrane, neuron projection terminus, intrinsic component of postsynaptic membrane, integral component of presynaptic membrane, transcription factor complex, axon part, intrinsic component of presynaptic membrane, receptor complex, integral component of synaptic membrane, intrinsic component of synaptic membrane, presynapse, distal axon, and glutamatergic synapse. GO-CC, Gene Ontology cellular component; JWSWG, JiaWeiSiWu granule.

Table 9 Pathway information of drug-disease intersection genes

ID	Description	P value	Count
hsa05215	Prostate cancer	1.34E-11	13
hsa05167	Kaposi sarcoma-associated herpesvirus infection	4.73E-11	16
hsa05161	Hepatitis B	7.15E-11	15
hsa05163	Human cytomegalovirus infection	8.61E-11	17
hsa05418	Fluid shear stress and atherosclerosis	1.06E-10	14
hsa04933	AGE-RAGE signaling pathway in diabetic complications	3.29E-10	12
hsa05210	Colorectal cancer	9.47E-10	11
hsa05225	Hepatocellular carcinoma	1.34E-09	14
hsa05205	Proteoglycans in cancer	1.86E-09	15
hsa01524	Platinum drug resistance	2.92E-09	10
hsa05219	Bladder cancer	6.68E-09	8
hsa04210	Apoptosis	1.18E-08	12
hsa05224	Breast cancer	2.85E-08	12
hsa04919	Thyroid hormone signaling pathway	3.1E-08	11
hsa04115	p53 signaling pathway	4.33E-08	9
hsa05160	Hepatitis C	5.16E-08	12

Table 9 (*continued*)

Table 9 (*continued*)

ID	Description	P value	Count
hsa04151	PI3K-Akt signaling pathway	8.58E-08	17
hsa01521	EGFR tyrosine kinase inhibitor resistance	9.87E-08	9
hsa05134	Legionellosis	1.01E-07	8
hsa05213	Endometrial cancer	1.16E-07	8

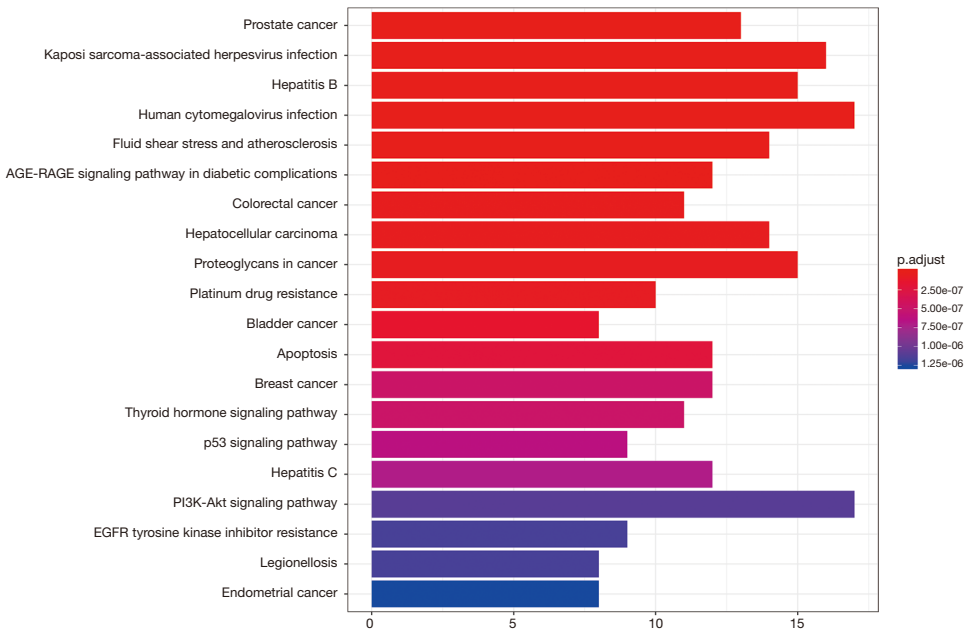

Figure 7 Histogram of KEGG pathway enrichment analysis. The top 20 pathways were prostate cancer, Kaposi sarcoma-associated herpesvirus infection, hepatitis B, human cytomegalovirus infection, fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, colorectal cancer, hepatocellular carcinoma, proteoglycans in cancer, platinum drug resistance, bladder cancer, apoptosis, breast cancer, thyroid hormone signaling pathway, p53 signaling pathway, hepatitis C, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, legionellosis, and endometrial cancer, suggesting that JWSWG plays a crucial role in treating hypertension by working on the above-mentioned multiple pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes; EGFR, epithelial growth factor receptor; JWSWG, JiaWeiSiWu granule.

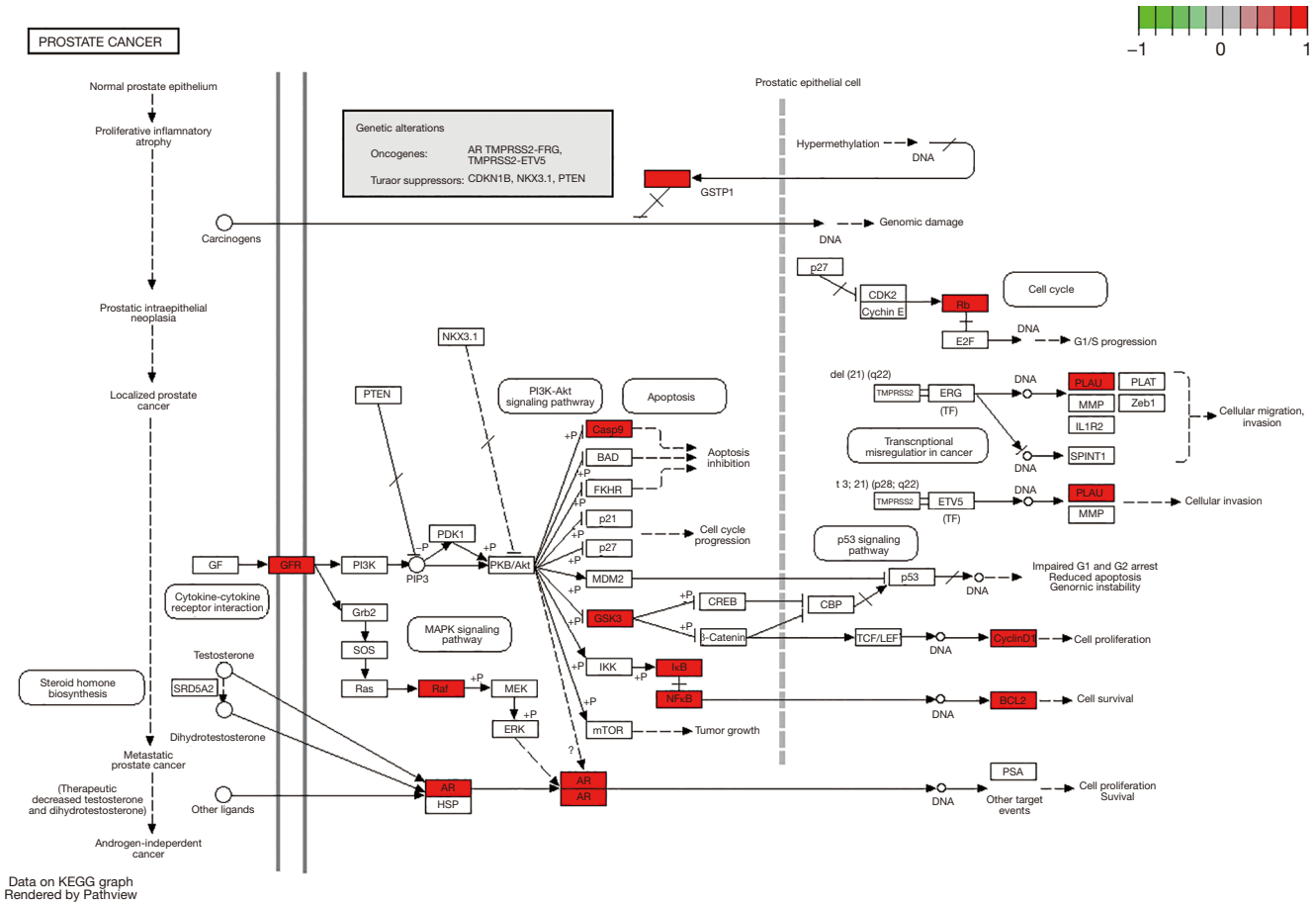

Figure 8 Prostate cancer pathway.

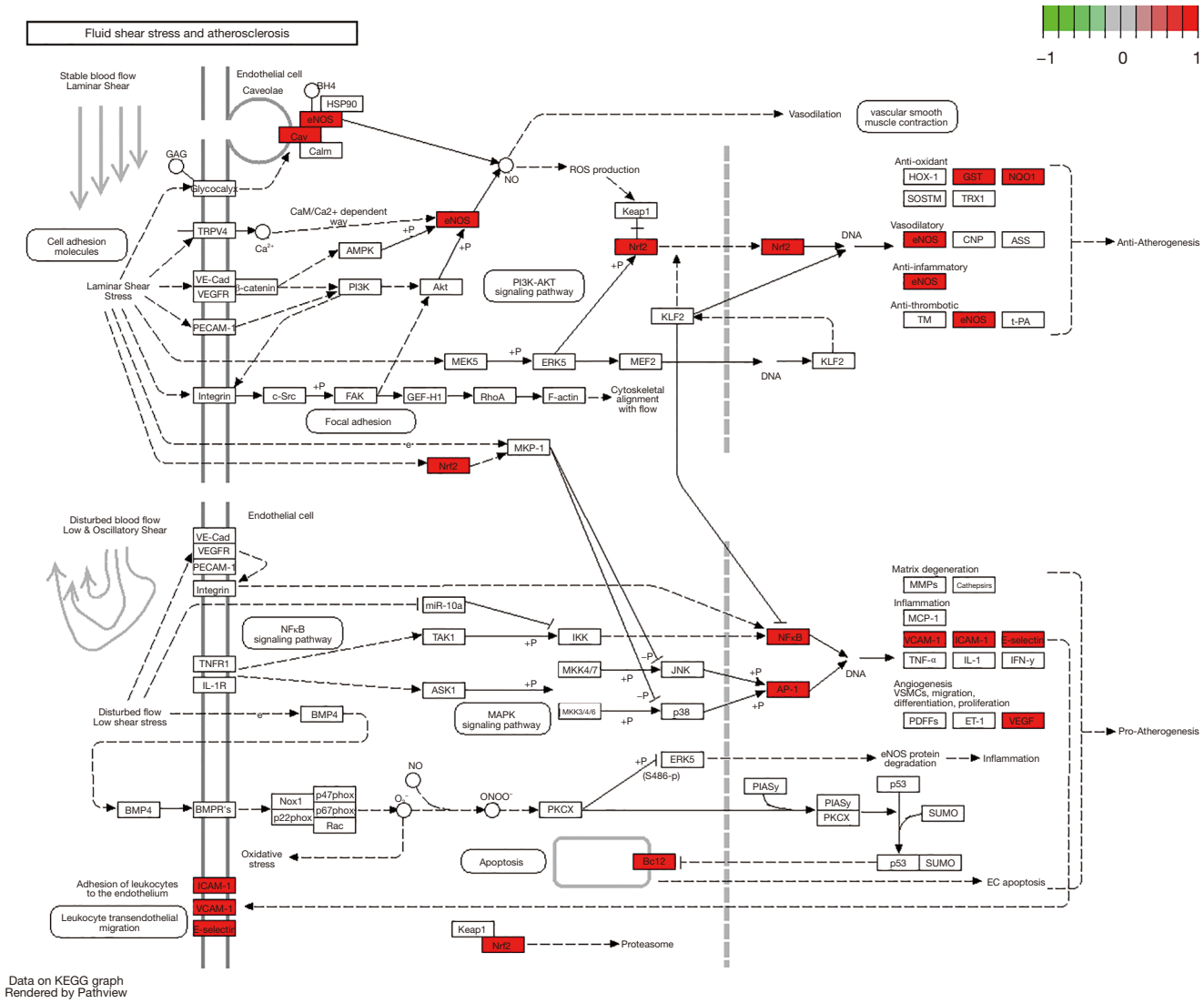

Figure 9 Fluid shear stress and atherosclerosis pathway.

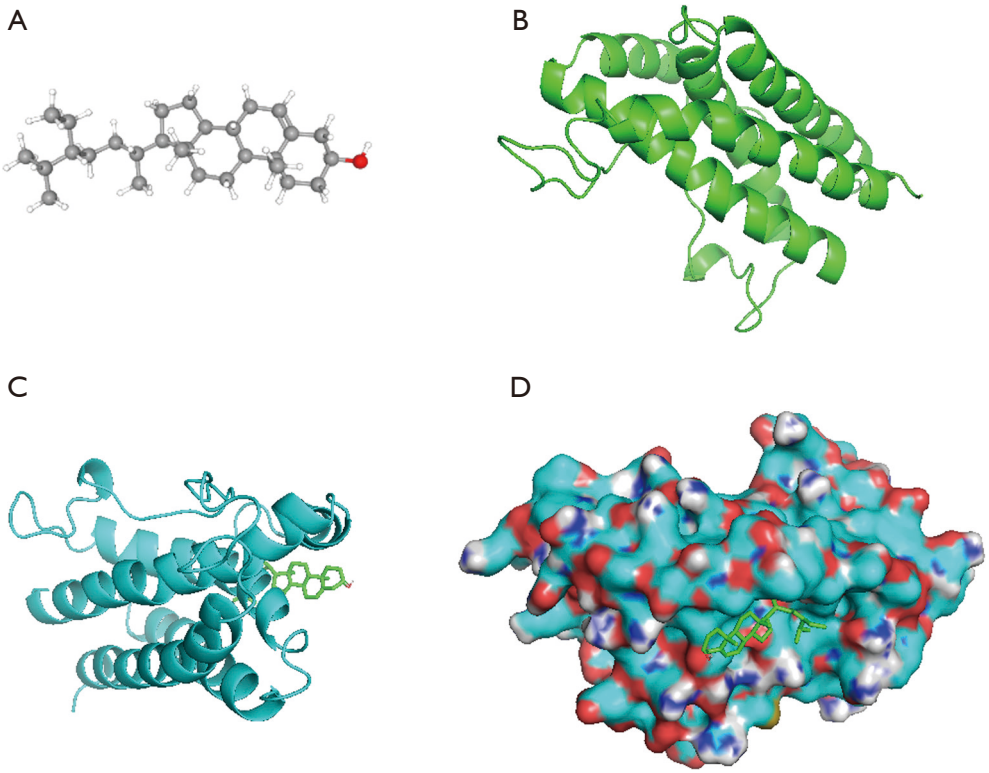

Figure 10 IL6-beta-sitosterol molecular docking. (A) 3D structures of beta-sitosterol; (B) 3D structures of IL6; (C) molecular docking simulation; and (D) molecular docking simulation (display protein surface). IL6, interleukin 6.

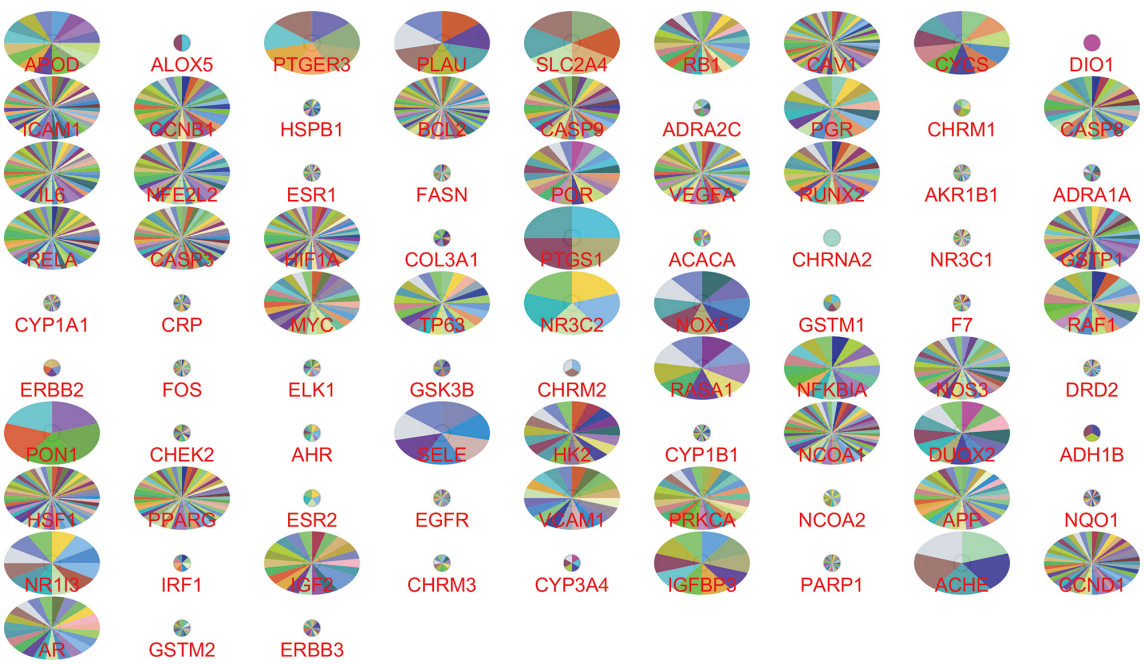

Figure 11 GO enrichment analysis (from ClueGO). GO, Gene Ontology.

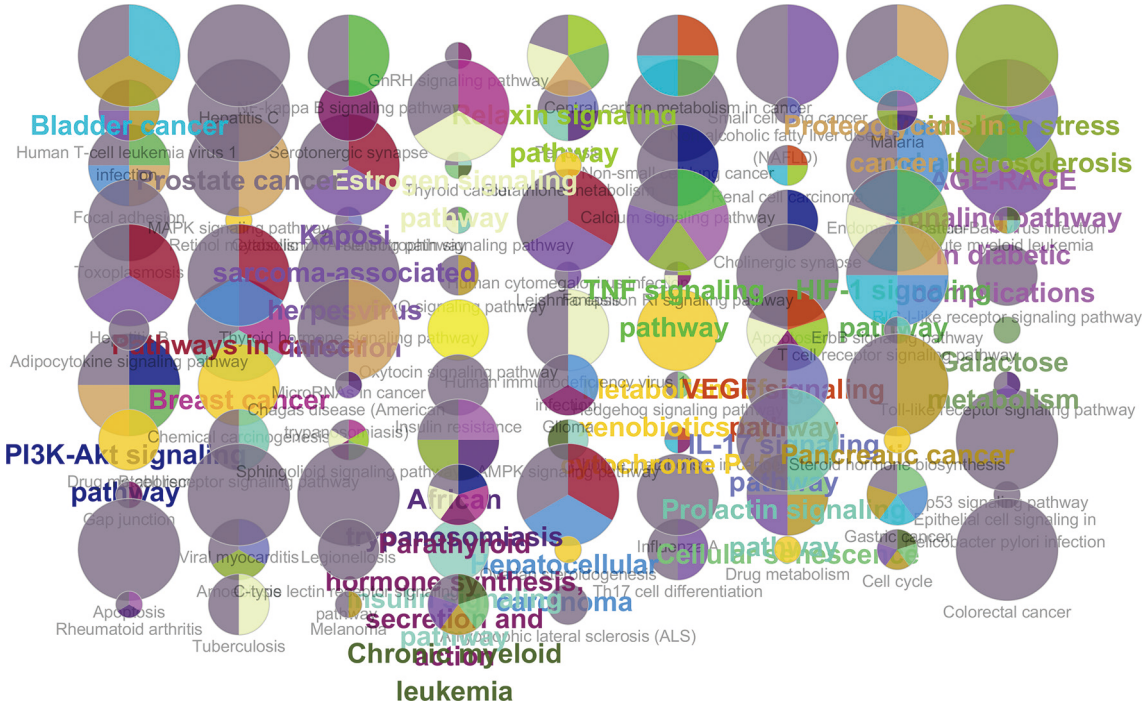

Figure 12 KEGG enrichment analysis (from ClueGO). KEGG, Kyoto Encyclopedia of Genes and Genomes.

pressure and protect target organs.

In this study, the connections and relationships of biological network nodes were obtained to analyze the mechanism of JWSWG in treating hypertension, and to perform comprehensive network analysis of molecular action. Overall, the mechanism of JWSWG in treating hypertension was revealed, providing a new direction for research on the pharmacology of TCM, and providing fresh research ideas, methods, and a theoretical foundation for future experimental and clinical research.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All these databases are publicly available, and ethical approval was unnecessary.

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