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Analysis of the Mechanism of Intervention of Fangxiangxiaozhi Prescription on Dyslipidemia Based on Network Pharmacology and Molecular Docking

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Abstract: *Objective:* To explore the mechanism of intervention of Fangxiangxiaozhi prescription on dyslipidemia by using network pharmacology and molecular docking. *Methods:* The traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Pubchem, Uniprot, and other databases were adopted to screen the active ingredients and the corresponding targets of Fangxiangxiaozhi prescription. Dyslipidemia-related targets were identified using the databases of Disgenet and GeneCards. Then, the intersection target of drugs and diseases was demonstrated via a Venn diagram. Cytoscape3.7.2 was used to construct a "drugs-active ingredients-intersection targets" network map and the key active ingredients with the top 7-degree values were determined. The protein interaction network and topology analysis of the intersection target genes were carried out by combining STRING11.0 and Cytoscape3.7.2. Moreover, the gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the intersection target genes were carried out using the Metascape database. Lastly, the key active ingredients and targets were molecular docked by AutoDockTools, Pymol, and other software. *Results:* There were 51 active components and 509 target genes of which 74 intersect with dyslipidemia. The key targets included tumor necrosis factor (TNF), interleukin-6 (IL-6), AKT1, PPAR gamma (PPARG), VEGFA, and PPARα. GO enrichment analysis obtained 1040 biological processes, 33 cell components, and 84 molecular functions; KEGG enrichment analysis obtained 148 pathways. The molecular docking results showed that the key targets and compounds exhibited good binding force. *Conclusion:* The active ingredients of Fangxiangxiaozhi prescription regulated several pathways through multiple targets to intervene in dyslipidemia. This study can serve as a foundation for further research.

Keywords: Fangxiangxiaozhi prescription; Dyslipidemia; Network pharmacology; Molecular docking

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1. Introduction

Dyslipidemia usually refers to the increase in total serum cholesterol and/or triglyceride, and various abnormal

plasma lipoprotein levels including low-density (LDL) and high-density lipoprotein (HDL) cholesterol are also found in clinical practice. Dyslipidemia is a high-risk factor of atherosclerotic cardiovascular disease, which can trigger various cardiovascular and cerebrovascular end events such as hemorrhagic stroke and acute myocardial infarction. The existing Western medicine treatment schemes are mainly statins and cholesterol absorption inhibitors, which have adverse reactions such as elevated transaminase, myositis, rhabdomyolysis, and digestive tract symptoms. Traditional Chinese medicine (TCM) has unique advantages in regulating dyslipidemia. Relevant meta-analysis showed that Chinese medicine is more effective and safer in improving clinical symptoms and blood lipid levels [1].

Fangxiangxiaozhi prescription (FXXZ) was developed by Yalin Qian, director of our department. It is composed of various ingredients including *Huoxiang, yinchen, baishao, danggui, fuling, diercao, yuzhizi,* and *liuyuexue*. In this paper, network pharmacology and molecular docking technology were used to construct the correlation of drug-active ingredient-target gene-disease, explore the effective active ingredients in FXXZ, the target, and pathway of the predicted mechanisms, to provide a new direction for further experimental and clinical research in related fields.

2. Materials and methods

2.1. Collection and screening of active ingredients of FXXZ

In the traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), we adopted the Chinese names of each herb in the prescription respectively for the keyword and retrieved its active composition. Then, according to oral bioavailability (OB) ≥ 30%, drug-likeness (DL) ≥ 0.18, and biological half-life (HL) ≥ 4.00, components with higher activity that can easily enter the bloodstream were screened.

2.2. The search for the target genes of FXXZ and dyslipidemia

The Pubchem database was used to query the SMILE name of each active ingredient, which was then input into the SWISSTargetPrediction database to obtain the corresponding target genes. If the above method fails, the target genes are obtained through the Uniprot database. “Dyslipidemia” was used as a keyword in the Disgenet database and checked using the GeneCards database. The target genes of the disease were obtained by deleting the duplicate targets between the two databases.

2.3. Target genes of drug intersected with disease and its Venn map

Through the online bioinformatics platform, the target genes of drugs that intersected with the disease were presented as a Venn diagram.

2.4. Network map of drugs-active ingredients-intersection targets

The drug names, active ingredients, and intersecting genes were imported into Cytoscape3.7.2 software and the network map of drugs-active ingredients-target genes was constructed.

2.5. Construction of protein interaction networks

The intersecting genes were imported into the STRING11.0 online platform for preliminary analysis, and the results were then imported into Cytoscape3.7.2 to construct the protein interaction (PPI) network for further analysis.

2.6. GO functional enrichment and KEGG pathway enrichment analysis of target genes

The Metascape database was used to process the intersecting target genes to predict the biological functions and pathways in the treatment of dyslipidemia with FXXZ. Meanwhile, the key results were visualized by an online platform to explore the possible mechanisms.

2.7. Molecular docking of active ingredients and key targets

The small molecule structure of the active component was obtained from the TCMSP analysis platform as a ligand, and the three-dimensional structure of the protein macromolecule was obtained from the RSCB PDB database as a receptor. The PyMOL software was used to remove water and solvent molecules from protein, and AutoDockTools software was used to add total hydrogen to the acceptor. Total hydrogen was added to the ligand in AutoDockTools software, and a torsion bond was detected and set. AutoDockTools software was used to set the docking box and perform molecular docking. The heat map was drawn in an Excel table according to the binding energy and some molecular docking results were visualized by PyMOL software.

3. Result

3.1. Information on the active ingredients in FXXZ

The TCMSP analysis platform was used to retrieve all kinds of ingredients in FXXZ. None of the active ingredients of Liu yue xue was found in the database and was removed. Among the others, 59 active ingredients met the screening conditions of OB, DL, and HL. The active ingredients of corresponding target sites were deleted because they were not found in the SWISSTargetPrediction and Uniprot databases. A total of 51 active ingredients were selected. Among them, 7 were “Huoxiang”, 12 were Fuling, 13 were Yinchen, 8 were in Baishao, 2 were in Danggui, 5 were in Diercao, and 4 were Yuzhizi, as shown in **Table 1**. Five of these active ingredients are shared by different drugs. Among them, beta-sitosterol was shared by Baishao, Danggui, Diercao, Yinchen, and Yuzhizi. Kaempferol was shared by Baishao and Diercao, and mairin was shared by Baishao and Diercao. Quercetin was shared by Diercao, Huoxiang and Yinchen. Genkwanin was shared by Huoxiang and Yinchen.

Table 1. Information on the active ingredients in FXXZ

Ingredient	Mol ID	Molecule Name	OB (%)	DL	HL
Huoxiang	MOL005923	3,23-dihydroxy-12-en-28-oic acid	30.86	0.86	15.5
	MOL005573	Genkwanin	37.13	0.24	16.1
	MOL005916	Irisolidone	37.78	0.3	15.41
	MOL005918	Phenanthrone	38.7	0.33	15.98
	MOL000098	Quercetin	46.43	0.28	14.4
	MOL005921	Quercetin 7-O-β-D-glucoside	49.57	0.27	14.57
	MOL005911	5-Hydroxy-7,4’-dimethoxyflavanon	51.54	0.27	16.16
Fuling	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	6.81
	MOL000275	Trametenolic acid	38.71	0.8	7.78
	MOL000279	Cerevisterol	37.96	0.77	5.31
	MOL000282	Ergosta-7,22E-dien-3beta-ol	43.51	0.72	5.11
	MOL000283	Ergosterol peroxide	40.36	0.81	3.43

Table 1 (Continued)

Ingredient	Mol ID	Molecule Name	OB (%)	DL	HL
	MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.7	0.81	6.59
	MOL000289	Pachymic acid	33.63	0.81	9.27
	MOL000290	Poricoic acid A	30.61	0.76	8.26
	MOL000291	Poricoic acid B	30.52	0.75	8.67
	MOL000292	Poricoic acid C	38.15	0.75	7.73
	MOL000296	Hederagenin	36.91	0.75	5.35
	MOL000300	Dehydroeburicoic acid	44.17	0.83	7.04
Yinchen	MOL007274	Skrofulein	30.35	0.3	15.8
	MOL000358	Beta-sitosterol	36.91	0.75	5.36
	MOL005573	Genkwanin	37.13	0.24	16.1
	MOL008041	Eupatolitin	42.55	0.37	14.31
	MOL008040	Eupalitin	46.11	0.33	14.25
	MOL000098	Quercetin	46.43	0.28	14.4
	MOL004609	Areapillin	48.96	0.41	16.52
	MOL000354	Isorhamnetin	49.6	0.31	14.34
	MOL008046	Demethoxycapillarisin	52.33	0.25	16.67
	MOL008039	Isoarcapillin	57.4	0.41	15.08
	MOL008043	Capillarisin	57.56	0.31	16.09
	MOL008047	Artepillin A	68.32	0.24	5.96
	MOL008045	4’-methylcapillarisin	72.18	0.35	16.31
Baishao	MOL000359	Sitosterol	36.91	0.75	5.37
	MOL000358	Beta-sitosterol	36.91	0.75	5.36
	MOL000422	Kaempferol	41.88	0.24	14.74
	MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53	4.34
	MOL001921	Lactiflorin	49.12	0.8	7.26
	MOL001924	Paeoniflorin	53.87	0.79	13.88
	MOL000211	Mairin	55.38	0.78	8.87
	MOL001918	Paeoniflorgenone	87.59	0.37	7.45
Danggui	MOL000358	Beta-sitosterol	36.91	0.75	5.36
	MOL000449	Stigmasterol	43.83	0.76	5.57
Diercao	MOL000211	Mairin	55.38	0.78	8.87
	MOL000358	Beta-sitosterol	36.91	0.75	5.36
	MOL000422	Kaempferol	41.88	0.24	14.74
	MOL007879	Tetramethoxyluteolin	43.68	0.37	15.45
	MOL000098	Quercetin	46.43	0.28	14.4
Yuzhizi	MOL010929	Glyceryl linolenate	38.14	0.31	6.05
	MOL002882	[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate	34.13	0.3	5.19
	MOL000358	Beta-sitosterol	36.91	0.75	5.36
	MOL008121	2-Monoolein	34.23	0.29	4.41

3.2. The search for the target genes of FXXZ and dyslipidemia

A probability lesser than 0 was used as the screening condition to predict the targets of the 51 active components, and 509 target genes were obtained. By querying the Disgenet and GeneCards databases, 478 disease-related targets were obtained after excluding items with relevance score < 5 in the GeneCards database and 28 duplicate items between both databases.

3.3. Target genes of drug intersecting with the disease and its Venn map

The Venn map of target genes of drugs and diseases was drawn by an online platform (**Figure 1**). According to the intersection of Venn diagram, it could be seen intuitively that 74 target genes in FXXZ interfere with dyslipidemia.

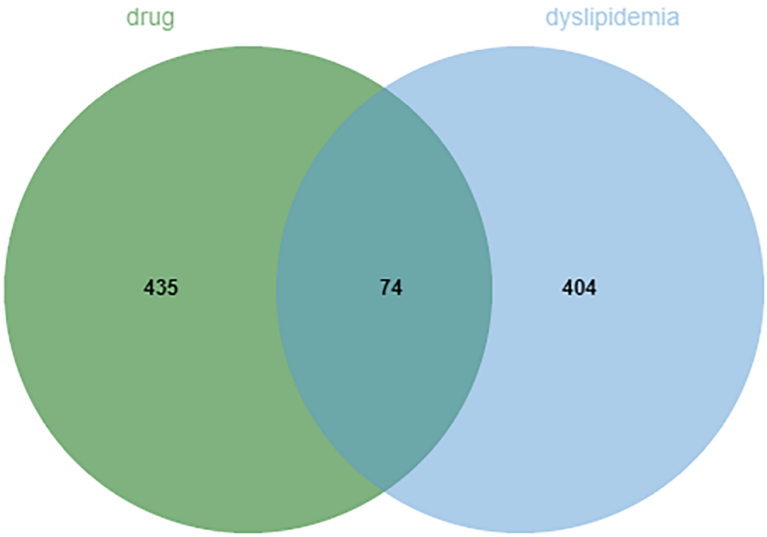
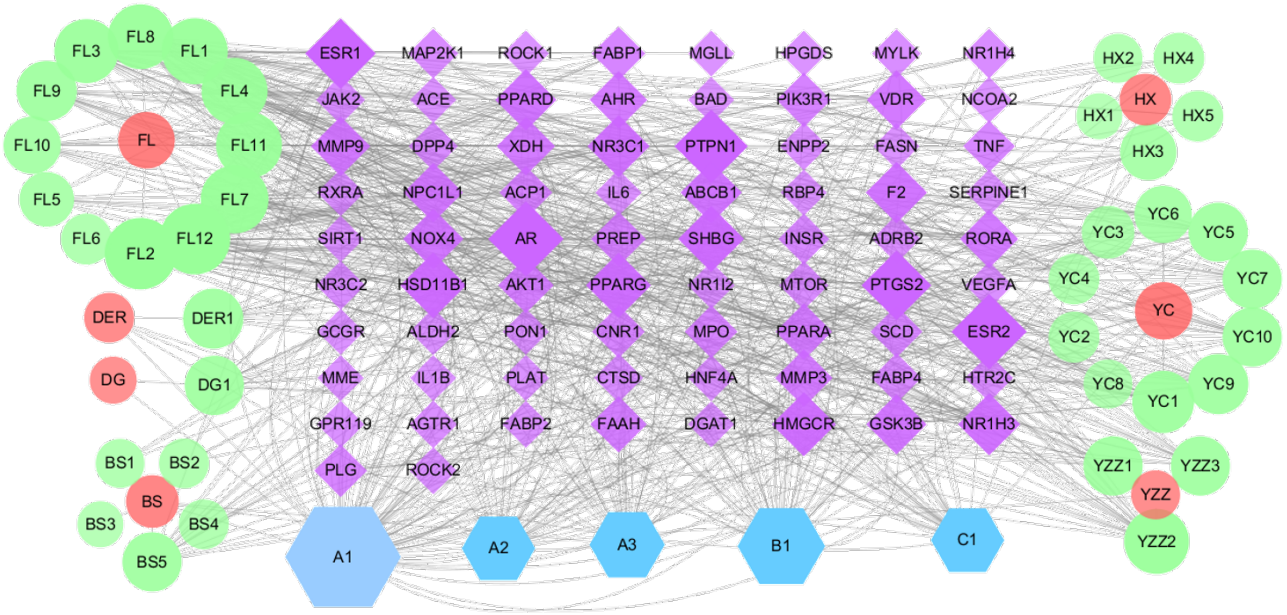


Figure 1. Venn diagram of the target gene of FXXZ interfering with dyslipidemia

3.4. Network map of drugs-active ingredients-intersection targets

The network diagram of FXXZ for the intervention of dyslipidemia was drawn with Cytoscape3.7.2 software (**Figure 2**), which contains 7 drug nodes, 41 active ingredient nodes, and 74 intersecting target gene nodes. The red circles represent the drug, the cyan circles represent the active ingredients unique to a drug, the blue hexagons represent the active ingredients common to some drugs, and the purple diamonds represent the intersecting target genes. The specific active ingredient nodes corresponding to the 7 drug nodes were also arranged according to the degree value. The drug-active ingredients-intersection targets network diagram showed that FXXZ acts on dyslipidemia in multiple ways. The network analyzing the function of the software was used to determine the top 7 active ingredients by degree value. This is shown in **Table 2**.



Note：FL：Fuling；DER：Diercao；DG：Danggui；BS：Baishao；HX：Huoxiang；YC：Yinchen；YZZ：Yuzhizi；A1：beta-sitosterol；A2：kaempferol；A3：Mairin；B1：quercetin；C1：Genkwanin；BS1：(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione；BS2：Lactiflorin；BS3：paeoniflorgenone；BS4：paeoniflorin；BS5：sitosterol；DG1：Stigmasterol；DER1：Tetramethoxyluteolin；FL1：(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid；FL2：3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid；FL3：Cerevisterol；FL4：dehydroeburicoic acid；FL5：ergosta-7,22E-dien-3beta-ol；FL6：Ergosterol peroxide；FL7：hederagenin；FL8：pachymic acid；FL9：Poricoic acid A；FL10：Poricoic acid B；FL11：poricoic acid C；FL12：trametenolic acid；HX1：3,23-dihydroxy-12-oleanen-28-oic acid；HX2：5-Hydroxy-7,4'-dimethoxyflavanon；HX3：irisolidone；HX4：phenanthrone；HX5：quercetin 7-O-β-D-glucoside；YC1：4'-Methylcapillarisin；YC2：Areapillin；YC3：Artepillin A；YC4：capillarisin；YC5：Demethoxycapillarisin；YC6：Eupalitin；YC7：Eupatolitin；YC8：Isoarcapillin；YC9：isorhamnetin；YC10：Skrofulein；YZZ1：[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate；YZZ2：2-Monoolein；YZZ3：glyceryl linolenate

Figure 2. Network map of drugs-active ingredients-intersection targets in FXXZ

Table 2. The top 7 active ingredients by degree value

Mol ID	Molecule Name	Degree
MOL000358	Beta-sitosterol	75
MOL000098	Quercetin	45
MOL000211	Mairin	32
MOL005573	Genkwanin	30
MOL000422	Kaempferol	30
MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	28
MOL000275	Trametenolic acid	27

3.5. Construction of PPI networks

On the STRING online platform, the species was set as “Homo sapiens” to process the 74 intersecting genes. The confidence level was set as 0.4 and the free target genes were eliminated. The data above were imported into Cytoscape3.7.2 software for analysis, and the protein interaction network was constructed (**Figure 3**). There were 74 nodes representing proteins and 646 edges representing protein-protein interactions. A larger node and a darker red color indicated a higher degree and a thicker edge represented a higher combined score. Network Analysis and cytoNCA were used to conduct topology analysis. The analysis showed that the median degree in the network was 17 and the target genes with more than twice the median degree were screened out. These included TNF, IL6, AKT1, PPARG, VEGFA, IL1B, and PPARα. The degree value of a node was on

behalf of the number of nodes connected. If the degree value of a node is more than 2 times the median of all nodes, it is often categorized as a key target.

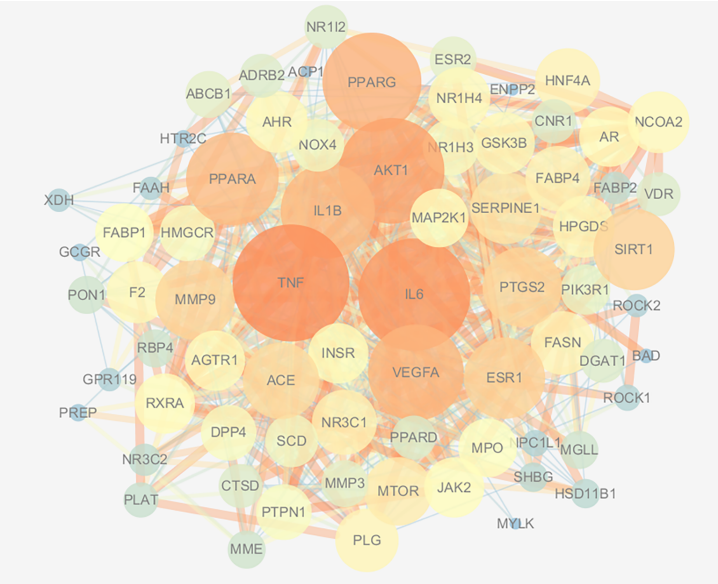


Figure 3. PPI diagram

3.6. GO and KEGG pathway enrichment analysis of drug-disease intersecting target genes

To better grasp the mechanism of intersecting target genes, the Metascape database was applied to conduct a GO enrichment analysis on the 74 target genes of FXXZ in dyslipidemia. 1157 GO entries were obtained, including 1040 biological processes (BP), 33 cellular components (CC), and 84 molecular functions (MF). The top 10 in each category were drawn in bar charts (**Figure 4**). BP enrichment results showed that response to hormones, intracellular receptor signaling pathway, cellular response to lipid, cellular response to hormone stimulus, and response to steroid hormones played a prominent role in the intervention of FXXZ in dyslipidemia.

After enrichment analysis of KEGG pathways, 148 pathways were obtained. The first 20 pathways were made into a bubble diagram (**Figure 5**). These pathways and their corresponding genes were visualized through Cytoscape3.7.2 (**Figure 6**), with purple diamonds representing genes and yellow triangles representing pathways. The signaling pathways with a high correlation with dyslipidemia are lipid and atherosclerosis, adipocytokine signaling pathways, and fluid shear stress and atherosclerosis. The intersection targets of these pathways are shown in **Table 3**. AKT1, BAD, GSK3B, IL1B, IL6, JAK2, MMP3, MMP9, PIK3R1, PPARG, RXRA, TNF, ROCK2, MTOR, PPARA, PLAT, and VEGFA simultaneously exist in these three pathways, containing 7 key targets obtained from PPI data analysis.

3.7. Molecular docking of active ingredients and key targets

The top 7 active ingredients (beta-sitosterol, quercetin, mairin, genkwanin, kaempferol, 3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid, trametenolic acid), and 7 key targets (TNF, IL6, AKT1, PPARG, VEGFA, IL1B, PPARA) were docked. Molecular docking is a computer simulation method to study the interaction between molecules and predict the binding enerfy and affinity between them.

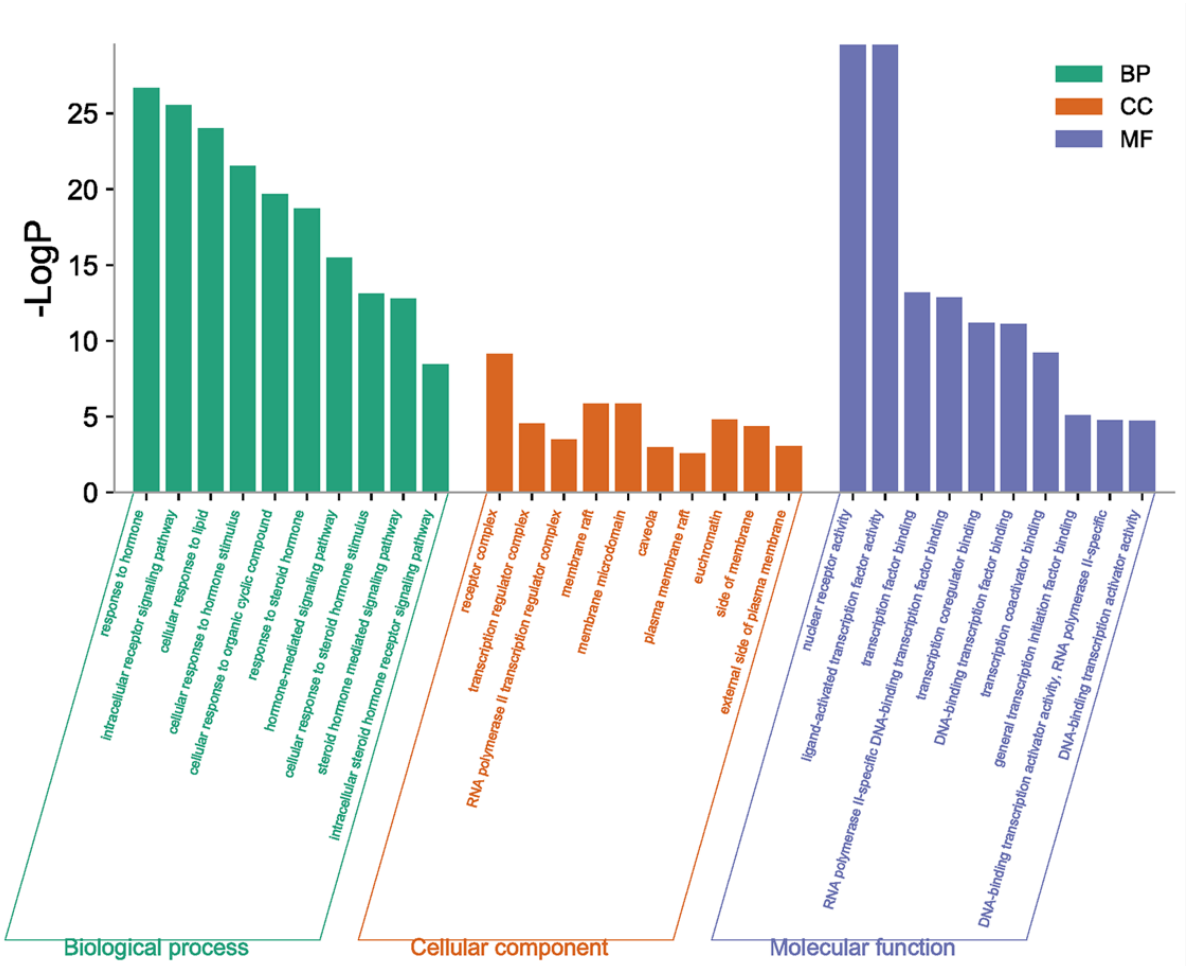


Figure 4. GO enrichment analysis of intersecting target genes

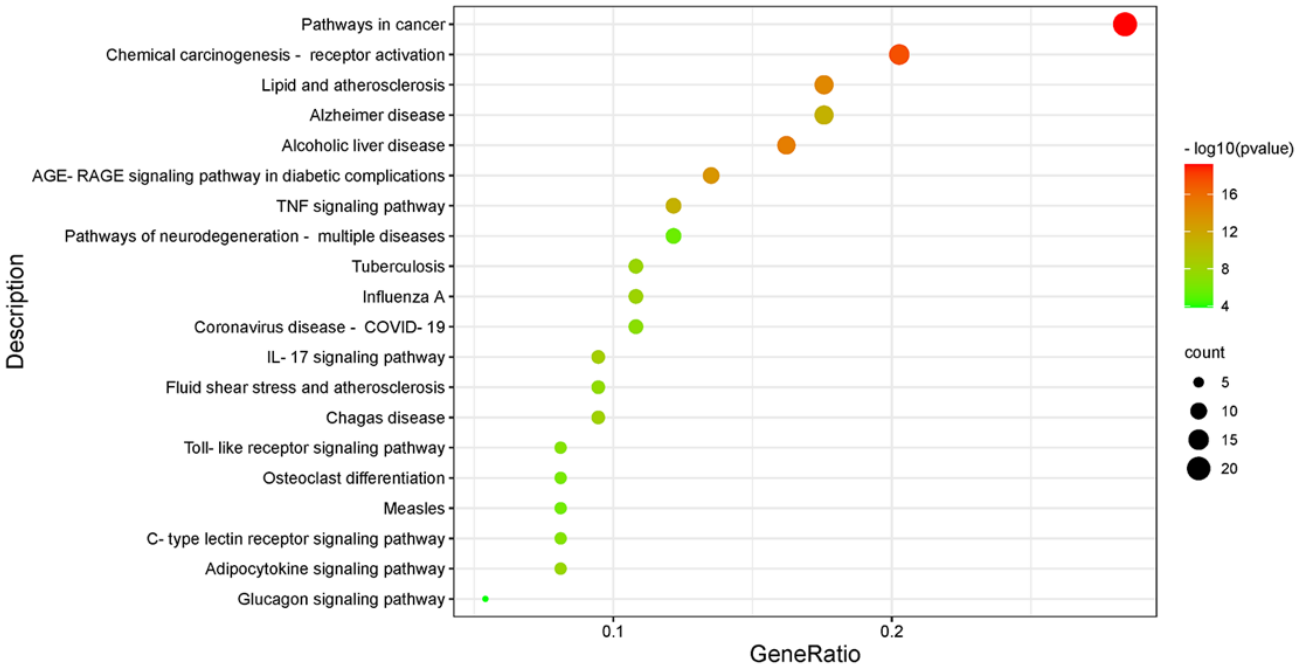


Figure 5. Bubble diagram of the top 20 pathways for KEGG enrichment analysis of intersecting target genes

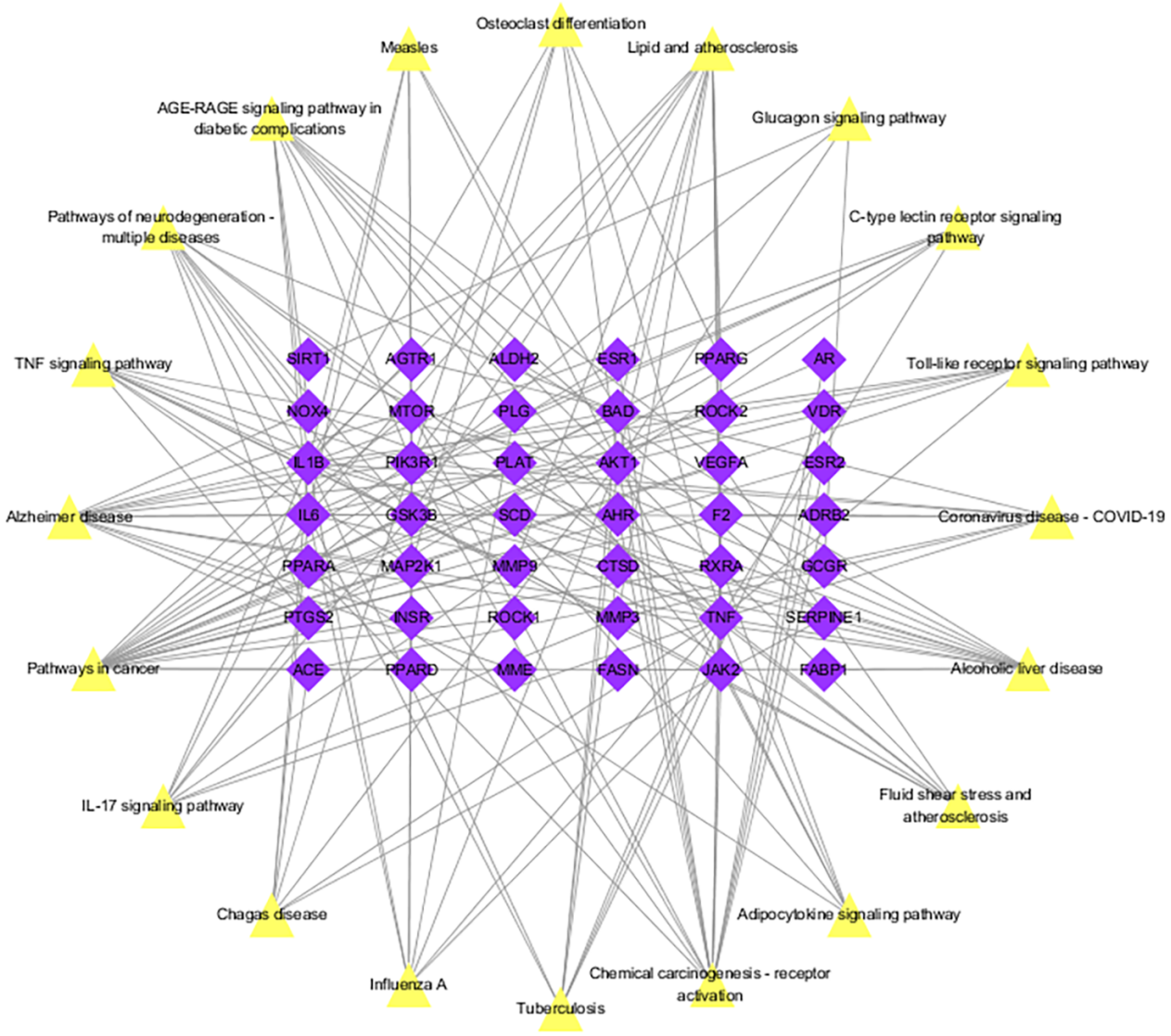


Figure 6. Diagram of signaling pathway and target genes about FXXZ intervention in dyslipidemia

Table 3. Target genes in key pathways

Pathways	Target genes
Lipid and atherosclerosis	AKT1, BAD, GSK3B, IL1B, IL6, JAK2, MMP3, MMP9, PIK3R1, PPARG, RXRA, TNF, ROCK2
Adipocytokine signaling pathway	AKT1, MTOR, JAK2, PPARA, RXRA, TNF
Fluid shear stress and atherosclerosis	AKT1, IL1B, MMP9, PIK3R1, PLAT, TNF, VEGFA

The binding energy was negatively correlated with the binding force between the receptor ligands (**Figure 7**). The results showed that beta-sitosterol had the strongest binding force with AKT1, PPARG, and PPARα. Trametenolic acid had the strongest binding force with TNF, IL6, and IL1B. Mairin had the strongest binding force with VEGFA. The molecular docking pattern between the above receptors and ligands was visualized (**Figure 8**).

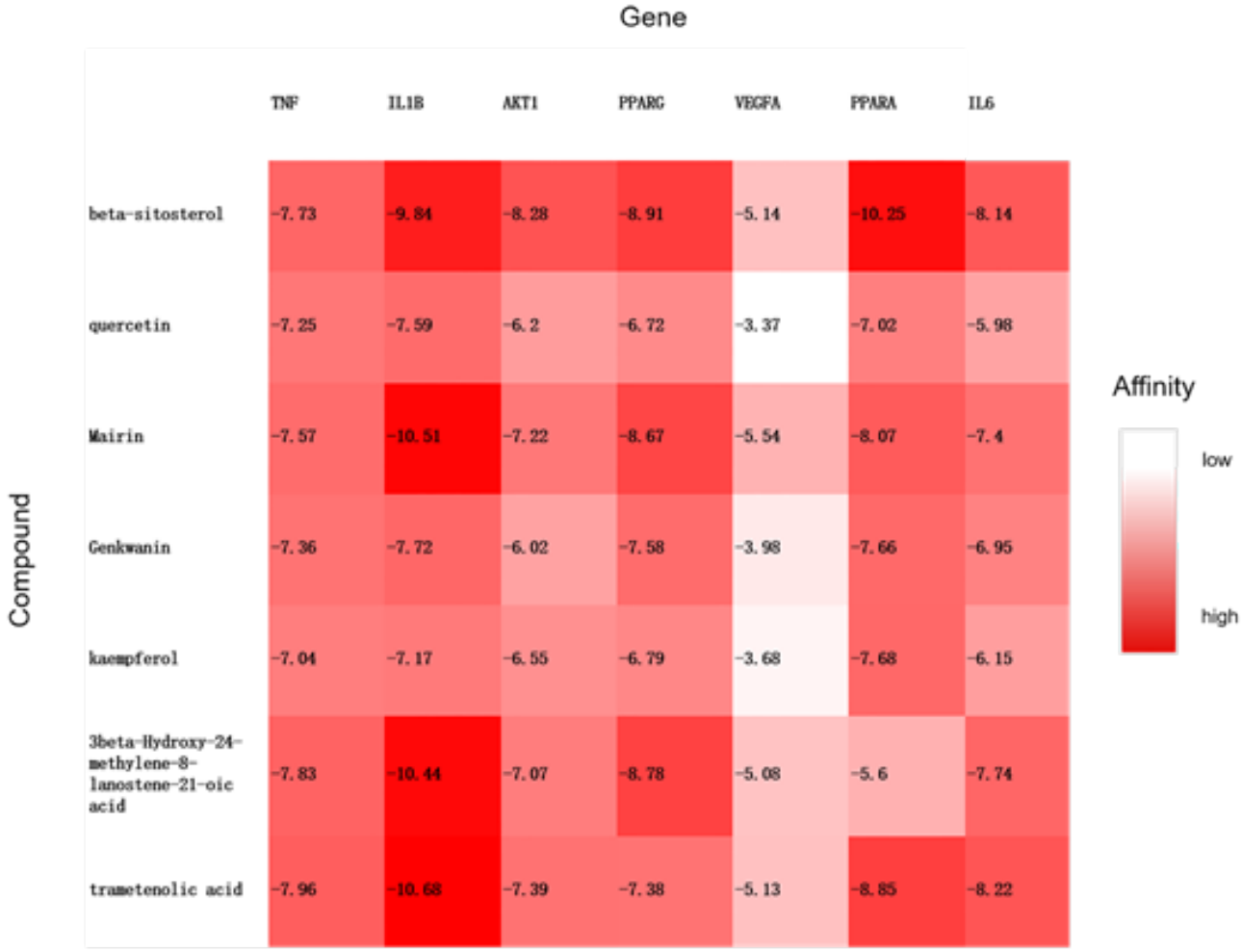


Figure 7. Heat map of affinity and binding energy between core active components and key targets

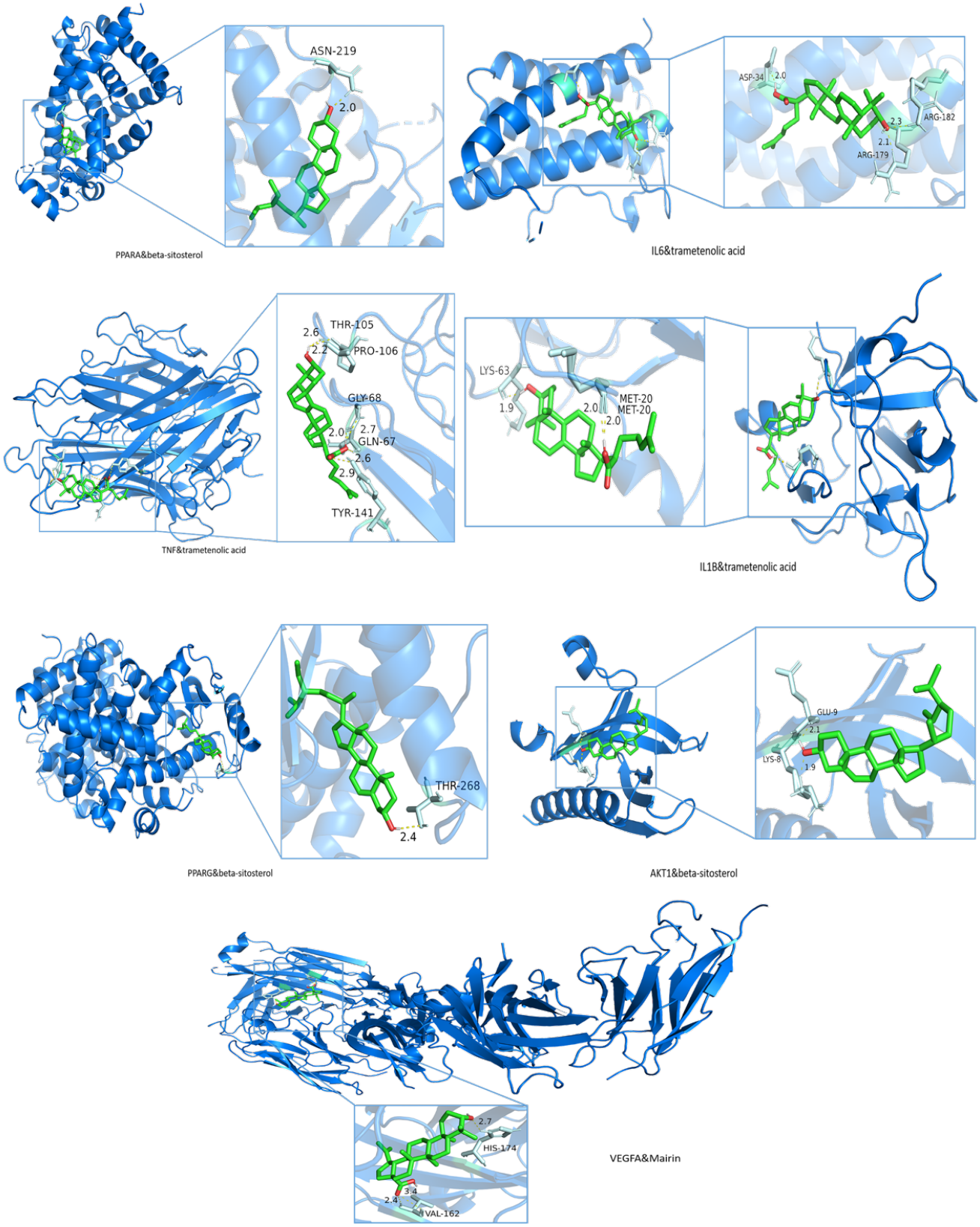


Figure 8. Molecular docking pattern diagram of several core active components and key targets

4. Discussion

Dyslipidemia is a disorder of lipid metabolism with a high prevalence. Commonly used Western drugs to treat dyslipidemia such as statins are usually associated with adverse side effects. TCM has the characteristics of multi-targeting, integrity, and minimal side effects in the regulation of dyslipidemia. Previous studies have confirmed that FXXZ can significantly regulate triglyceride (TG), total cholesterol (TC), HDL, and LDL.

During the collection of active ingredients, the relevant information of Liu yue xue could not be found in the TCMSP database and was omitted. In this study, the active ingredients of FXXZ in the intervention of dyslipidemia were screened from the database as beta-sitosterol, quercetin, Mairin, Genkwanin, kaempferol, 3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid, and trametenolic acid.

Beta-sitosterol can lower lipid levels and its artificial preparation has demonstrated a good effect in mouse models [2]. Various animal experiments have verified that quercetin can improve metabolic syndrome including dyslipidemia through various mechanisms [3,4]. Both *in vivo* and *in vitro* studies have shown that kaempferol reduced levels of LDL and TG [5,6]. 3beta-Hydroxy-24-methylene-8-en-21-oic acid is a compound isolated from the ethyl acetate component of medicinal mushrooms, which exhibits an anti-lipid peroxidation effect in mice [7].

Topological analysis of PPI showed that TNF, IL6, AKT1, PPARG, VEGFA, IL1B, and PPARα were key target genes, especially TNF, IL6, and AKT. IL-6 acts as an adipocytokine to affect visceral obesity, leading to various metabolic syndromes [8]. TNF-α is responsible for maintaining lipid homeostasis, reducing the uptake of free fatty acids, promoting adipogenesis and decomposition, inhibiting enzymatic activities of lipid metabolism, regulating cholesterol metabolism, and adjusting adipokine levels [9]. As a transcriptional regulator, PPARG encodes lipid metabolism. The gene expression ratio between PPARG and other transcription factors is of great significance for predicting the development of dyslipidemia and insulin resistance in obese people [10]. VEGFA reflects single nucleotide polymorphisms, which are associated with individual genetic differences in lipid profile and response to lipid-lowering therapy [11]. PPARα can reduce the level of TG by controlling the expression of various genes involved in human metabolism, alter the levels of HDL in plasma by increasing its apolipoprotein component, and regulate the metabolism of lipoprotein by upregulation of specific target genes [12].

The GO enrichment analysis showed that the intervention of FXXZ on dyslipidemia was mainly through the biological process of response to hormone, cellular response to hormone stimulus, cellular response to lipid, response to steroid hormone, intracellular receptor signaling pathways, and so on. Not only do glucocorticoids act directly on adipose tissue but they also lead to dyslipidemia and visceral obesity through the mediated function of insulin [13]. Insulin resistance alters the systemic lipid metabolism by inhibiting lipid oxidation to some extent, resulting in the classic dyslipidemia of elevated TG and LDL, and decreased HDL. All of these factors combined with endothelial dysfunction could result in atherosclerotic plaques [14].

The KEGG enrichment analysis proved that the key signaling pathways include lipid and atherosclerosis, adipocytokine signaling pathway, fluid shear stress and atherosclerosis. FXXZ could play a part in the intervention of lipid levels by regulating these pathways, as evidenced by the enrichment of key targets in related pathways.

Dyslipidemia can trigger or accelerate all stages of atherosclerosis, while endothelial dysfunction and damage caused by atherosclerosis can exacerbate dyslipidemia [15]. Adipose tissue, an important endocrine organ in the human body, has various signal pathway networks, whose function depends on the synthesis and release of cytokines [16]. The adipocytokine signaling pathway belongs to the signaling pathways related to dyslipidemia [17]. A study has shown that the fluid shear stress of arteries directly regulated the expression of interferon regulatory factors in arterial endothelial cells and downstream inflammatory response, which is an important mediator of dyslipidemia and atherosclerosis [18]. The key target was selected as the ligand and the active component

was the receptor. Next, semi-flexible docking was carried out for each receptor and ligand. It turned out that the receptors and ligands were stably bound by hydrogen bonding, which confirmed the intervention effect of FXXZ on dyslipidemia in terms of molecular recognition.

5. Conclusion

FXXZ regulated lipid levels through multiple active components, target genes, and pathways. This prescription demonstrated positive outcomes in the treatment of dyslipidemia.

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Disclosure statement

The authors declare that no conflict of interest.

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