Research Paper

Study on the Mechanism of Jianpi Shenshi Jiangzhuo Formula in the Treatment of Non-Alcoholic Steatohepatitis Based on Network Pharmacology and Molecular Docking

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Non-alcoholic steatohepatitis is an intermediate link in the transformation from non-alcoholic fatty liver disease to non-alcoholic cirrhosis. Jianpi Shenshi Jiangzhuo formula, an effective prescription for treating non-alcoholic fatty liver disease, is summarized by our research group on the basis of long-term clinical practice. Based on the network pharmacology and molecular docking, this study explores the mechanism of Jianpi Shenshi Jiangzhuo formula in non-alcoholic steatohepatitis treatment. The expression profiles of non-alcoholic steatohepatitis-associated genes were downloaded and the potential disease targets were obtained by differential gene analysis. 11 single drug ingredients of the Jianpi Shenshi Jiangzhuo formula were searched and the active ingredients were screened with oral bioavailability ≥30 % and drug-likeness ≥0.18 as the criteria. The corresponding targets were found and the Kyoto Encyclopedia of Genes and Genomes pathway and Gene Ontology functional pathway enrichment analyses were carried out to identify mitochondrial pathway genes. The drug target genes were then intersected with the differentially expressed genes of the disease to identify the overlapping genes, after which molecular docking between active ingredients and core targets was performed.

Key words: Jianpi Shenshi Jiangzhuo formula, network pharmacology, molecular docking, nonalcoholic steatohepatitis

In case of obesity, the incidence of metabolic diseases has increased exponentially over the past few decades[1]. Insulin resistance, type 2 diabetes, hypertension, dyslipidemia, cardiovascular diseases, and fatty liver have been recognized as obesity-related comorbidities[2]. Non-Alcoholic Fatty Liver Disease (NAFLD) is also a major independent risk factor for the development of cardiovascular diseases[3]. NAFLD is a series of medical conditions associated with fatty degeneration caused by the accumulation of >5 % of triglyceride vesicles in hepatocytes[4]. This process occurs due to the mismatch in the regulatory mechanisms involved in lipid metabolism, with some patients developing into more aggressive disease subtypes characterized by lobular inflammation and hepatic balloon degeneration, with or without fibrosis, called Non-Alcoholic Steatohepatitis (NASH)[5,6]. Approximately 22.5 % of NASH patients will develop hepatocellular carcinoma and 20 % will develop cirrhosis[7,8]. At present, there are no specific drugs to treat NASH.

The pathogenesis of NAFLD is mainly related to mitochondrial dysfunction, insulin resistance, oxidative stress and adipocytokine secretion disorder, etc., but the specific mechanism of action is not yet clear[9]. Therefore, improving the body's lipid metabolism is the key to NAFLD treatment. In addition, mitochondria are the most important organelles for fatty acid Beta (β) oxidation in the liver and play an important role in regulating fatty acid metabolism[10]. Mitochondria have been found to be critical in the pathogenesis of NAFLD[11]. Normal individuals maintain a relative balance between lipid uptake and synthesis through mitochondrial fatty acid β-oxidation and Very Low Density Lipoprotein (VLDL) secretion. Free fatty acids enter mitochondria for β-oxidation or esterification to triacylglycerols, and are eventually secreted in the form of VLDL. However, hindered or excessive oxidation of fatty acids and increased production of VLDL can lead

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to fatty liver[12,13]. Therefore, maintaining the normal function of hepatocyte mitochondria cannot be ignored in the treatment of NASH.

Studies have confirmed that Traditional Chinese Medicine (TCM) has a promising prospect in regulating blood lipids and improving lipid metabolism, and has become an important means of clinical treatment of NAFLD[14]. Jianpi Shenshi Jiangzhuo formula is an effective prescription for treating NAFLD, which is summarized by our research group on the basis of long-term clinical practice. Its main medicinal components include Radix *Pseudostellaria* (Chinese Taizishen), Rhizoma *Atractylodes macrocephala* (Chinese Baizhu), *Poria cocos* (Chinese Fuling), *Magnolia officinalis* (Chinese Houpu), *Tetrapanacis medulla* (Chinese Tongcao), Rhizoma *Pinellia ternata*, talcum powder, *Tetrapanax papyrifer*, Radix *Bupleuri*, *Fructus aurantii*, Radix *Paeonia Rubra* (Chinese Chishao), Radix *Panax notoginseng* (Chinese Sanqi), Rubia *cordifolia* (Chinese Qiancao) and *Broussonetia fructus* (Chinese Chushiziu). All the components are closely associated with the functions of stimulating spleen, promoting diuresis, soothing liver, regulating qi, eliminating dampness, resolving phlegm, promoting qi circulation and reducing turbidity. It has been reported that other decoctions containing the above TCM can play an important role in protecting liver cells[15].

In this study, the Gene Expression Omnibus (GEO) database and network pharmacology method were used to screen the effective components and targets of Jianpi Shenshi Jiangzhuo formula, and their intersections were mapped with the Differentially Expressed Genes (DEGs) of NASH. Moreover, were analyzed the possible pathways to screen the mitochondrial pathways, so as to predict the potential targets and mechanisms of action of Jianpi Shenshi Jiangzhuo formula in treating NASH, providing a theoretical basis for further research.

MATERIALS AND METHODS

NASH-related target screening:

GEO (https://www.ncbi.nlm.nih.gov/geo/) microarray datasets GSE164760[16] and GSE89632[17] of NASH were selected to download the relevant Gene Set Enrichment (GSE) and GEO Platform (GPL) files, which were then sorted out, grouped and imported into R-software for differential analysis using the Limma package, and the DEGs were screened with an adjusted value of $p<0.05$ and Logarithm of Fold Change (logFC)>0.5 as the thresholds. The ggplot package was used to present the DEGs in a volcano plot.

Screening of active ingredients and targets of the Jianpi Shenshi Jiangzhuo formula:

The active ingredients of Radix *Pseudostellaria*, Rhizoma *Atractylodes*, *Poria cocos*, *Magnolia officinalis*, *Tetrapanax papyrifer*, Radix *Bupleuri*, *Fructus aurantii,* Radix *Paeonia Rubra*, Radix *Panax notoginseng*, Radix *Rubia* and *Broussonetia* in Jianpi Shenshi Jiangzhuo formula were searched by using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) platform (https://old.tcmsp-e.com/tcmsp.php). With Druglikeness (DL) ≥0.18 and Oral Bioavailability (OB) ≥30 % as screening parameters, the active ingredients with good drug properties were screened. Universal Protein Resource (UniProt) database (https://www.uniprot.org/) was used to correct the names of target genes of chemical components, so as to normalize the protein target information and remove duplicate genes.

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

The clusterProfiler package of R-software was used to conduct GO functional enrichment and KEGG pathway enrichment analysis of the non-repetitive target genes and DEGs in GEO microarray datasets. "Mitochondria" as keyword, mitochondria-related pathways were screened in the enrichment results in order to analyze the Molecular Function (MF), Cell Components (CC), Biological Processes (BP), and metabolic pathways of genes.

Identification of overlapping genes of drugs and diseases:

The previously obtained genes of the active ingredients and disease-related DEGs were mapped by Venn diagram, and the overlapping genes were obtained.

Molecular docking:

The active ingredients and core target genes of the Jianpi Shenshi Jiangzhuo formula were molecularly docked. Primarily, the structural formula of the active ingredient (MOL2 format) was downloaded from the TCMSP database. Next, the protein conformation of the target genes was searched in the

Protein Data Bank (PDB) (https://www.rcsb.org/). Screening was carried out based on the following criteria; identification of protein structure by X-ray diffraction; crystal resolution of the protein <3 Å; being a well-defined protein. The proteins were then pretreated with Autotools to remove water molecules by hydrogenation. Energy lattice calculation and small molecule-protein docking were then performed using AutoGrid and AutoDock Vina software, respectively, and binding energy (affinity) scores for each docking was studied. The part with a binding energy <-7 was selected for plotting.

RESULTS AND DISCUSSION

GEO differential gene analysis was carried out. The expression profiles of genes namely, GSE164760 and GSE89632 were retrieved from GEO database. Among 6 normal samples and 74 NASH samples which were included in the GSE164760 microarray dataset, 384 DEGs were identified, including 270 down-regulated and 114 up-regulated genes (fig. 1A). Similarly, in the GSE89632 dataset, 24 normal samples and 19 NASH samples were found, from which 2815 DEGs were identified, with 1386 down-regulated and 1429 up-regulated genes (fig. 1B).

Pathway enrichment analysis of DEGs was studied. Subsequently, the GO function and KEGG pathway enrichment analyses of the DEGs found in the GSE164760 and GSE89632 microarray datasets were analyzed (fig. 2). According to KEGG pathway enrichment analysis, DEGs in GSE164760 were dominantly enriched in Alzheimer's disease, Huntington disease, and Hypoxia-Inducible Factor 1 (HIF-1) signaling pathway (fig. 2A). GO function enrichment analysis showed that the DEGs in GSE164760 were mainly enriched in endomembrane system, vesicles, nucleoplasm and other functions (fig. 2B). However, the DEGs in GSE89632 were primarily enriched in cytokine-cytokine receptor interaction pathway (fig. 2C) and response to stress function (fig. 2D). Using the keyword "mitochondria" we searched and screened the mitochondria-related pathways. There was no mitochondrial pathway in GSE89632, while one associated pathway, "mitochondrial electron transport, cytochrome c to oxygen", was found in GSE164760, which includes 5 DEGs namely, Cytochrome C Oxidase subunit 8A (COX8A), COX6C, COX7B, COX7C and COX6A1.

The active ingredients and targets of Jianpi Shenshi Jiangzhuo formula were screened. A total of 1067 related compounds were found from the TCMSP database for 11 single drug ingredients. With OB ≥30 % and DL ≥0.18 as the screening criteria, a total of 93 active ingredients were obtained. In the TCMSP database, the potential targets involved in each active ingredient were obtained, sorted out, and then deduplicated by UniProt database comparison and correction. A total of 224 non-repetitive potential targets were obtained.

Further, functional enrichment analysis of potential targets of active ingredients was studied. A total of 2598 GO pathways (BP: 2332; CC: 70 and MF: 196) and 188 KEGG pathways were acquired through KEGG pathway and GO function enrichment analysis of the 224 potential targets. The top 10 functional pathways have been presented in fig. 3. From these enrichment results, mitochondria-related pathways were further screened by using the keyword "mitochondria". 26 associated pathways were obtained, returning 189 mitochondria-related genes, 31 of which were found after removing duplicate genes (Table 1).

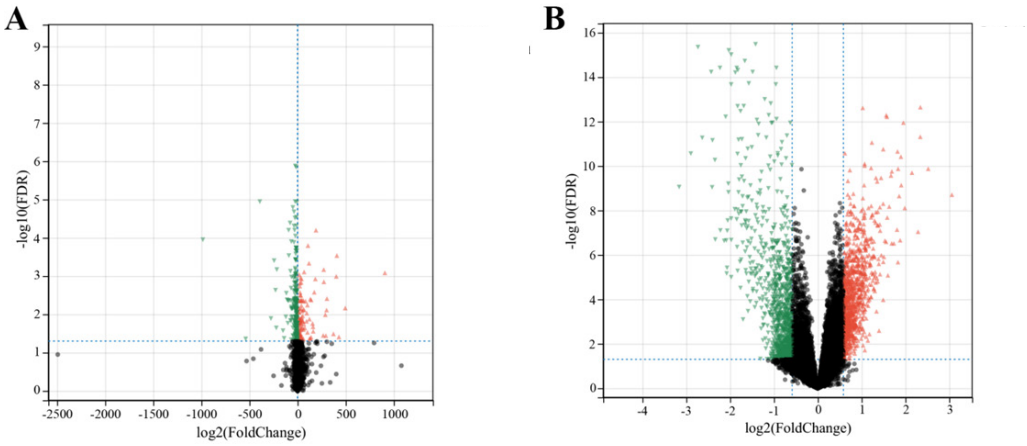

Fig. 1: Volcano plots showing DEGs, (A): GSE164760 and (B): GSE89632
Note: (▼): Down-regulated and (▲): Up-regulated

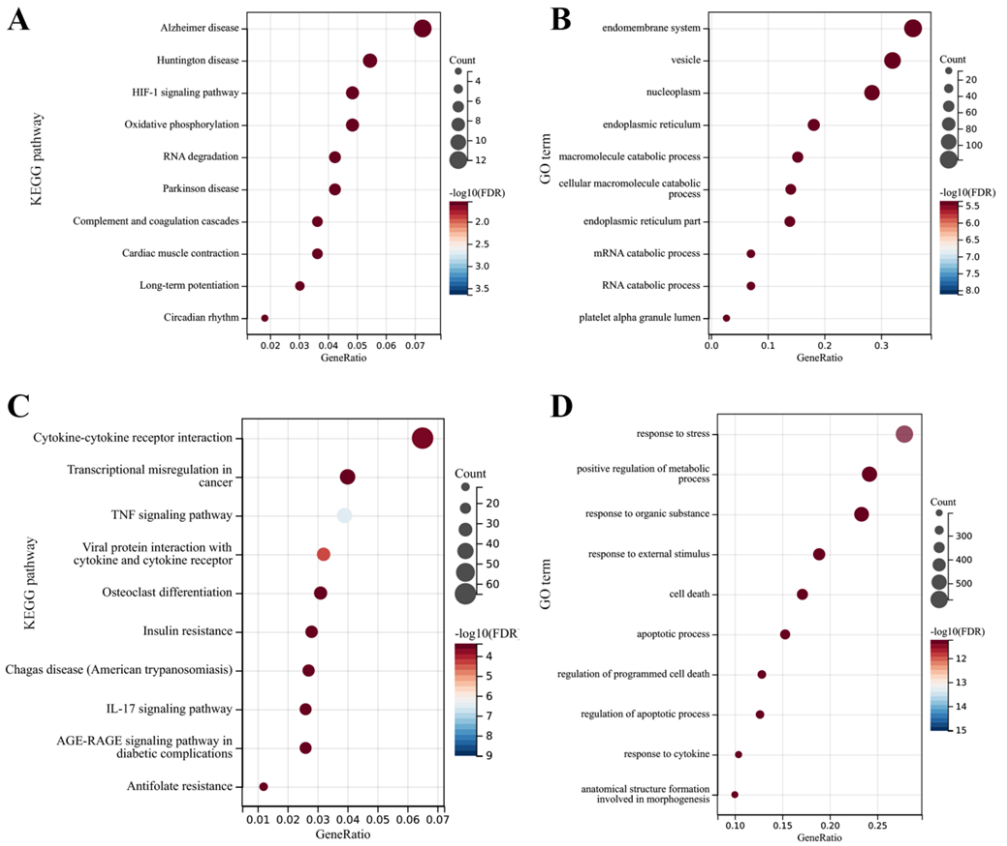

Fig. 2: KEGG and GO enrichment analysis of DEGs, (A): KEGG pathway enrichment analysis in GSE164760; (B): GO function enrichment analysis in GSE164760; (C): KEGG pathway enrichment analysis in GSE89632 and (D): GO function enrichment analysis in GSE89632

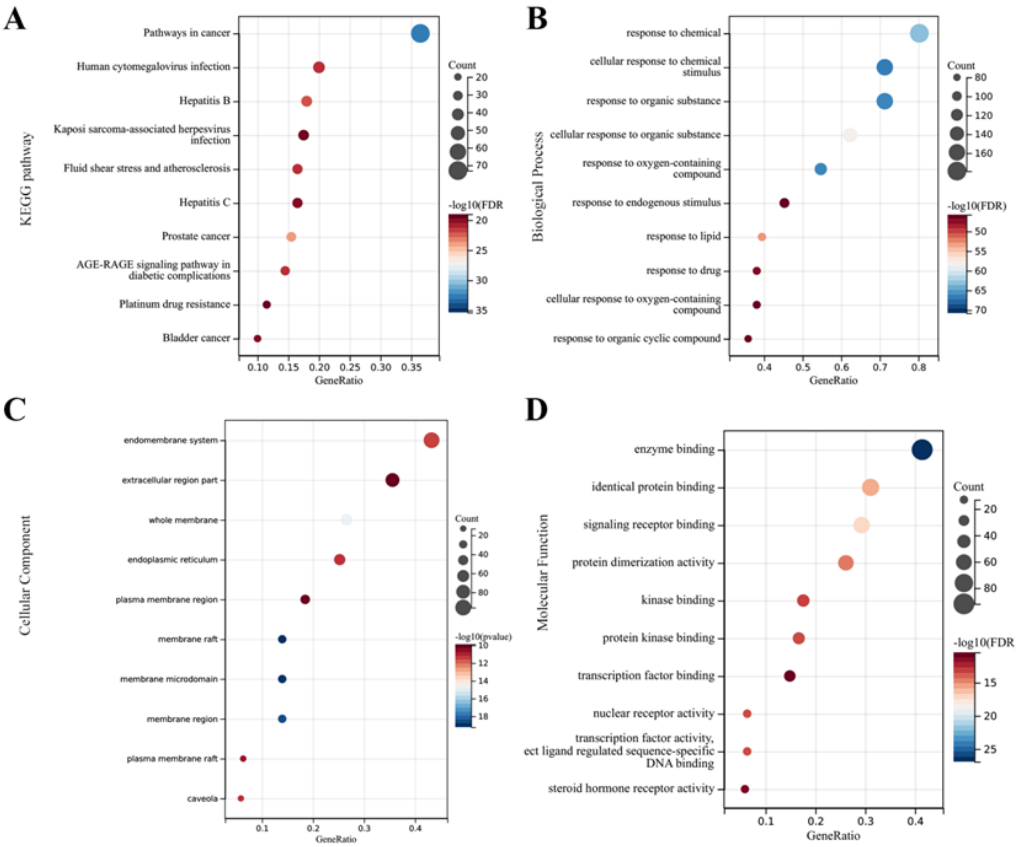

Fig. 3: KEGG pathway and GO function enrichment analysis of 224 potential targets, (A): KEGG pathway enrichment analysis results of 224 potential target genes; (B): BP enrichment analysis results of 224 potential targets; (C): CC enrichment analysis results of 224 potential targets and (D): MF enrichment analysis results of 224 potential targets

TABLE 1: 26 PATHWAYS RELATED TO MITOCHONDRIA

Ontology	ID	Description	Gene ID	Count
CC	GO:0005741	Mitochondrial outer membrane	BCL2L1/MCL1/CASP8/PGR/BAX/BCL2/MAOB/MAOA/GJA1/RAF1/HK2/ACSL4/HK1/ACSL1/MTOR	15
BP	GO:0006839	Mitochondrial transport	TP53/BCL2L1/CASP8/BAX/BCL2/GSK3B/BBC3/HK2/ACACA/E2F1/NPEPPS/MAPK8/SREBF1/SREBF2	14
BP	GO:0008637	Apoptotic mitochondrial changes	AKT1/MMP9/TP53/BCL2L1/CASP8/BAX/BCL2/GSK3B/BBC3/HK2/E2F1/MAPK8	12
BP	GO:0046902	Regulation of mitochondrial membrane permeability	TP53/BCL2L1/CASP8/BAX/BCL2/GSK3B/BBC3/HK2/E2F1/MAPK8	10
BP	GO:0007006	Mitochondrial membrane organization	TP53/BCL2L1/CASP8/BAX/BCL2/GSK3B/BBC3/HK2/E2F1/MAPK8	10
BP	GO:0051881	Regulation of mitochondrial membrane potential	AKT1/BCL2L1/BAX/BCL2/KDR/OPRD1/PARP1/SOD1/CASP1	9
BP	GO:1902108	Regulation of mitochondrial membrane permeability involved in apoptotic process	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:0035794	Positive regulation of mitochondrial membrane permeability	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:1902686	Mitochondrial outer membrane permeability involved in programmed cell death	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:1902110	Positive regulation of mitochondrial membrane permeability involved in apoptotic process	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:0097345	Mitochondrial outer membrane permeability	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:1901028	Regulation of mitochondrial outer membrane permeability involved in apoptotic signaling pathway	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:1901030	Positive regulation of mitochondrial outer membrane permeability involved in apoptotic signaling pathway	TP53/CASP8/BAX/BCL2/GSK3B/BBC3/E2F1/MAPK8	8
BP	GO:0001836	Release of cytochrome c from mitochondria	AKT1/MMP9/TP53/BCL2L1/BAX/BCL2/BBC3	7
BP	GO:0090151	Establishment of protein localization to mitochondrial membrane	TP53/CASP8/BAX/BCL2/BBC3/E2F1/MAPK8	7
BP	GO:0051204	Protein insertion into mitochondrial membrane	TP53/CASP8/BAX/BCL2/BBC3/E2F1/MAPK8	7
BP	GO:0001844	Protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	TP53/CASP8/BAX/BCL2/BBC3/E2F1/MAPK8	7
BP	GO:0090199	Regulation of release of cytochrome c from mitochondria	AKT1/MMP9/TP53/BCL2L1/BAX/BBC3	6
BP	GO:1900739	Regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	TP53/CASP8/BCL2/BBC3/E2F1/MAPK8	6
BP	GO:1900740	Positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	TP53/CASP8/BCL2/BBC3/E2F1/MAPK8	6
BP	GO:0090200	Positive regulation of release of cytochrome c from mitochondria	MMP9/TP53/BAX/BBC3	4

BP	GO:0051882	Mitochondrial depolarization	BCL2/KDR/PARP1/CASP1	4
BP	GO:0051900	Regulation of mitochondrial depolarization	BCL2/KDR/PARP1	3
BP	GO:0000002	Mitochondrial genome maintenance	TP53/PARP1	2
BP	GO:0090201	Negative regulation of release of cytochrome c from mitochondria	AKT1/BCL2L1	2
BP	GO:0032042	Mitochondrial Deoxyribonucleic Acid (DNA) metabolic process	TP53/PARP1	2

Molecular docking of active ingredients with core targets was studied. The target genes of the Jianpi Shenshi Jiangzhuo formula were intersected with the 5 NASH mitochondria-associated pathway genes, and no intersection was found. Therefore, the 31 mitochondria-related genes were used to intersect with DEGs of both the NASH datasets, i.e., GSE89632 and GSE164760. As shown in the fig. 4, 7 of the 31 mitochondria-related genes were found to be associated with NASH DEGs. They are, Gap Junction protein Alpha 1 (GJA1), Sterol Regulatory Element-Binding Protein 1 (SREBF1), Caspase-1 (CASP1), SREBF2, Hexokinase 2 (HK2), Matrix Metalloproteinase-9 (MMP-9), and HK1, corresponding to the 9 active chemical components in Jianpi Shenshi Jiangzhuo formula (Table 2). Finally, the Vina tool was used for batch molecular docking, and the results showed that the genes that docked well with the active ingredients were SREBF1, CASP1, HK1, and HK2 (Table 3). The detailed simulation diagrams of molecular docking between naringenin and SREBF1 and quercetin and SREBF1 are shown in fig. 5. TCM treatment of NAFLD from a holistic perspective, integrates the patient's physique, the causes and laws of the disease to comprehensively regulate the qi-blood and yin-yang imbalance. Treatment based on syndrome differentiation, specific method and prescription for certain illness, experience of renowned doctor, integrated traditional Chinese and Western medicine therapy, and multi-channel and approach treatment of TCM have achieved unique therapeutic advantages in controlling etiology, lowering blood lipid and glucose, promoting the regression of liver fat deposits, reducing hepatocyte necrosis, inflammation and fibrosis[18]. Shenshi Jiangzhuo formula is the experiential effective prescription developed by Chen Qichang, a famous doctor in Henan Province in the late Qing Dynasty and the early Republic of China, which has the effects of inducing diuresis and excreting dampness, harmonizing and promoting blood circulation, removing blood stasis, promoting qi circulation and reducing turbidity.

Network pharmacology is a new data analysis technology emerging in recent years. This study applies this technology and data mining to validate the scientificity and feasibility of Jianpi Shenshi Jiangzhuo formula in the treatment of NASH. Among the TCM components of Jianpi Shenshi Jiangzhuo formula, Rhizoma *Atractylodes* and *Magnolia officinalis* can eliminate dampness to resolve phlegm, improve digestion, relieve chest and diaphragm discomfort caused by gas upwelling[19]; Radix *Bupleuri* and *Fructus aurantii* have the functions of soothing the liver, regulating qi, resolving phlegm, dissipating distention and fullness, promoting blood circulation, removing blood stasis, and eliminating alcohol and facilitating digestion[20,21]. *Poria cocos*, also known as Yuling and Wanlinggui, is sweet and light in taste, with the functions of stimulating spleen, relieving edema, calming the mind, and relieving diarrhea[22]; Rhizoma *Pinellia ternata* is capable of drying dampness to resolve phlegm and removing blood stasis[23]; Radix *Panax notoginseng*, Radix *Paeonia Rubra* and Radix *Rubia* can promote blood circulation, remove blood stasis, nourish blood, promote blood circulation, and dredge menstruation[24-26]; talcum powder and *Tetrapanax papyrifer* have the effects of clearing damp, promoting diuresis, and relieving difficult urination, edema, fullness, and phlegm and retained fluid-induced dizziness[27,28]; *Broussonetia* has the function of nourishing kidney, clearing liver-heat and improving eyesight[29]. The combination of the above drugs can effectively treat NAFLD and give full play to their respective medicinal effects, complementing each other.

Mitochondrial function is closely related to the metabolic function of the three major nutrients in the liver, so it is also important to the pathology of NASH[30]. Lipid deposition in NASH cells is bound to physically destroy the formation of mitochondria in the cells and negatively affect mitochondrial function, suggesting a close pathological relationship

between them. In this study, 93 active ingredients and 224 non-repetitive potential targets were obtained by screening. Subsequently, KEGG pathway and GO function analyses of the 224 potential targets were performed, and mitochondrial pathways were screened, returning 31 target genes. Seven overlapped mitochondrial genes, GJA1, SREBF1, CASP1, SREBF2, HK2, MMP9, and HK1, corresponding to 9 active chemical components (sucrose, quercetin, nobiletin, naringenin, luteolin, ginsenoside Rh2, ellagic acid, β-carotene, and baicalein) in Jianpi Shenshi Jiangzhuo formula, were obtained by combining the DEGs in the NASH-associated datasets. Among them, quercetin is a flavonoid found in the human diet that has been shown to have a wide range of activities in preventing common diseases[31]. In addition, studies in mice showed that quercetin treatment ameliorated inflammation and fibrosis in mice with NASH[32]. Nobiletin improves the efficacy of hypercholesterolemia and NAFLD in mice on a high-cholesterol diet[33]. It may also be involved in the prevention of NAFLD and its associated risk factors by increasing the expression of Peroxisome Proliferator-Activated Receptor (PPAR) target genes such as PPAR-Alpha (α), PPAR-Gamma (γ) and regulating energy homeostasis[34]. The role of luteolin is linked to changes in the gut microbiota that help prevent progression from simple steatosis to NASH[35].

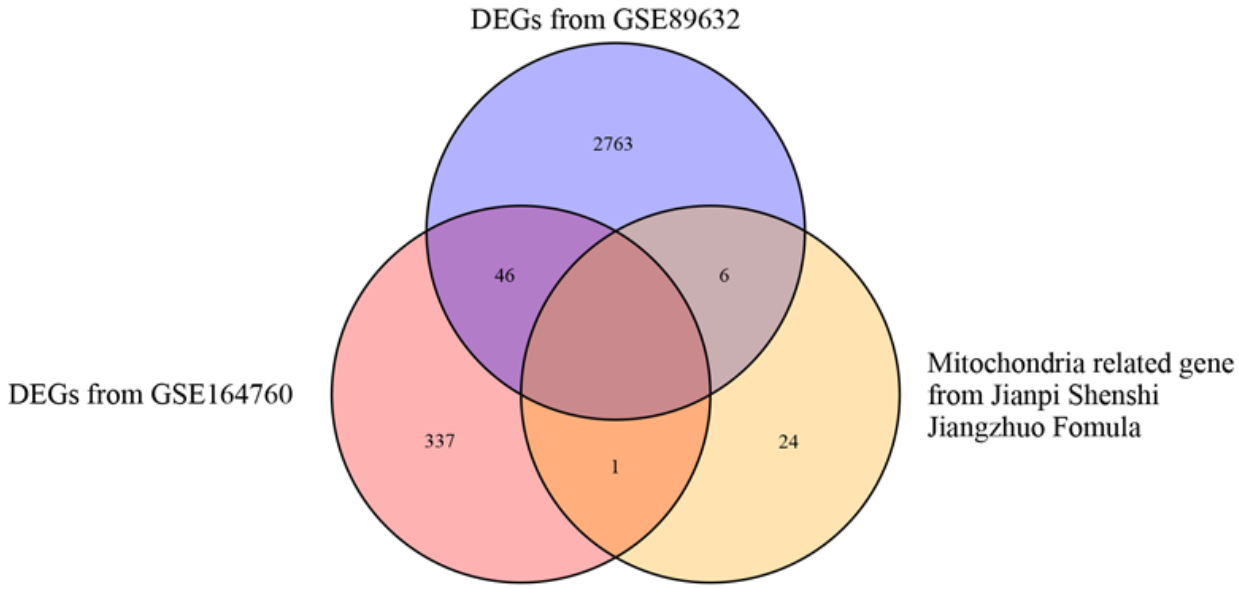

Fig. 4: Venn diagram of intersection between mitochondria-related genes and differentially expressed genes in datasets

TABLE 2: 7 CORRESPONDING GENES TO THE 9 ACTIVE CHEMICAL COMPONENTS OF THE JIANPI SHENSHI JIANGZHUO FORMULA

TCM	Scientific name	Molecular ID	Molecule name	Symbol	OB	DL
Chishao	Radix *Paeonia Rubra*	MOL000842	Sucrose	HK1	7.171	0.227
Chishao	Radix *Paeonia Rubra*	MOL000842	Sucrose	SREBF2	7.171	0.227
Chishao	Radix *Paeonia Rubra*	MOL000842	Sucrose	SREBF1	7.171	0.227
Chaihu	Radix *Bupleuri*	MOL000098	Quercetin	GJA1	46.433	0.275
Chaihu	Radix *Bupleuri*	MOL000098	Quercetin	HK2	46.433	0.275
Chaihu	Radix *Bupleuri*	MOL000098	Quercetin	MMP9	46.433	0.275
Sanqi	*Panax notoginseng* (Burk.) F. H. Chen Ex C. Chow	MOL000098	Quercetin	HK2	46.433	0.275

Sanqi	*Panax notoginseng* (Burk.) F. H. Chen Ex C. Chow	MOL000098	Quercetin	GJA1	46.433	0.275
Sanqi	*Panax notoginseng* (Burk.) F. H. Chen Ex C. Chow	MOL000098	Quercetin	MMP9	46.433	0.275
Zhishi	*Fructus aurantii*	MOL005828	Nobiletin	MMP9	61.669	0.517
Zhishi	*Fructus aurantii*	MOL004328	Naringenin	SREBF1	59.294	0.211
Taizishen	*Pseudostellaria* Radix	MOL000006	Luteolin	MMP9	36.163	0.246
Zhishi	*Fructus aurantii*	MOL000006	Luteolin	MMP9	36.163	0.246
Chushiziu	*Broussonetia*	MOL000006	Luteolin	MMP9	36.163	0.246
Sanqi	*Panax notoginseng* (Burk.) F. H. Chen Ex C. Chow	MOL005344	Ginsenoside Rh2	CASP1	36.32	0.559
Chishao	Radix *Paeonia Rubra*	MOL001002	Ellagic acid	MMP9	43.065	0.434
Chushiziu	*Broussonetia*	MOL002773	ß-carotene	GJA1	37.184	0.584
Chishao	Radix *Paeonia Rubra*	MOL002714	Baicalein	MMP9	33.519	0.209

TABLE 3: MOLECULAR DOCKING OF COMPONENTS AND CORE TARGETS

PDB ID	Gene	PubChem ID	Molecule name	Binding energy (kcal/mol)
Lam9	SREBF1	932	Naringenin	-7.4
Lam9	SREBF1	5988	Sucrose	-5.5
Lam9	SREBF1	5280343	Quercetin	-7.2
5HEX	HK2	5280343	Quercetin	-6.8
5HEX	HK2	932	Naringenin	-7.4
5HEX	HK2	5988	Sucrose	-5.2
Lam9	SREBF1	72344	Nobiletin	-7.8
5HEX	HK2	72344	Nobiletin	-6.8
Lam9	SREBF1	119307	Ginsenoside Rh2	-8
5HEX	HK2	119307	Ginsenoside Rh2	-6.8
Lam9	SREBF1	5280445	Luteolin	-7.4
5HEX	HK2	5280445	Luteolin	-7.2
Lam9	SREBF1	5280489	ß-carotene	-8.2
Lcza	HK1	5280489	ß-carotene	-5.7
2hbq	CASP1	5280489	ß-carotene	-4.2
5HEX	HK2	5280489	ß-carotene	-7.8
Lam9	SREBF1	5281605	Baicalein	-7.5
5HEX	HK2	5281605	Baicalein	-7.2

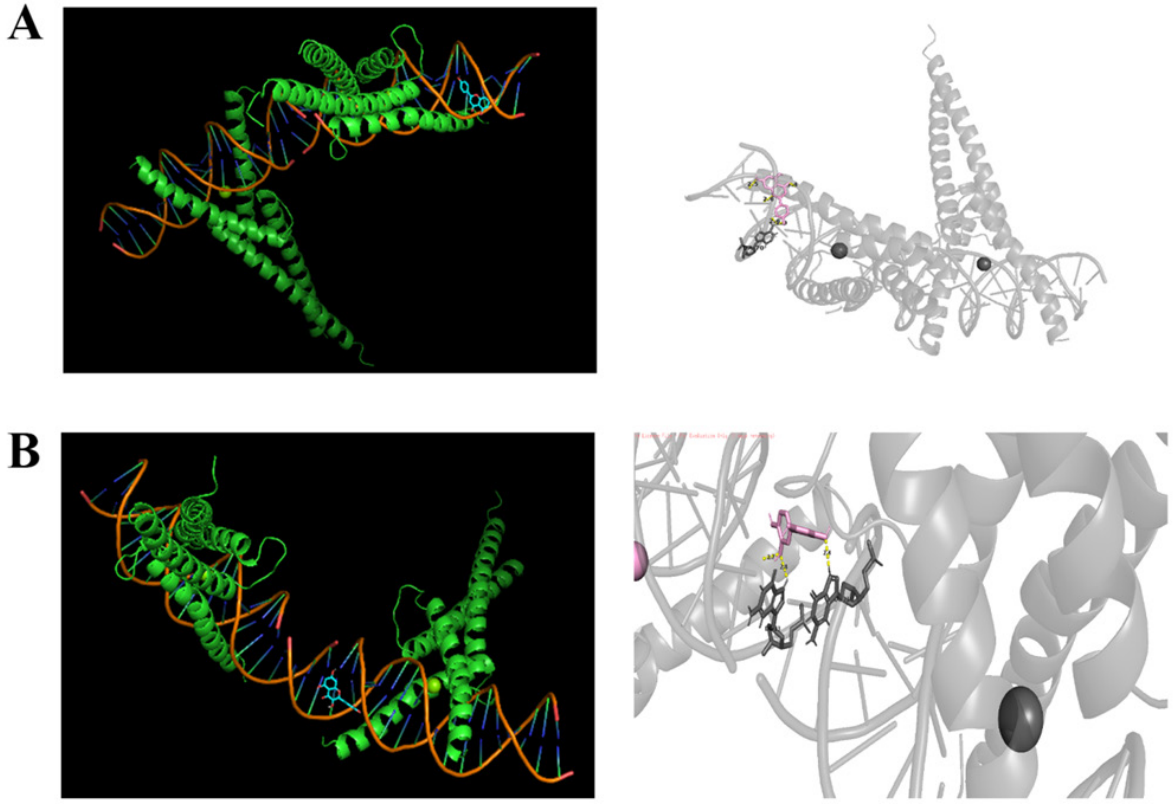

Fig. 5: Molecular docking detail diagram, (A): Docking details of naringenin and SREBF1 and (B): Docking details of quercetin and SREBF1

While ginsenoside Rh2 which is a natural compound, reduces the number and size of lipid droplets in 3T3-L1 adipocytes, which can be used as a natural pharmaceutical approach for NAFLD[36]. Similarly, the use of ellagic acid can activate the liver Adenosine-Monophosphate Activated-Protein Kinase (AMPK) pathway to treat liver diseases and NAFLD and prevent hepatic steatosis[37]. Baicalein, on the other hand, affects the intestinal microbial community structure and liver transcriptome expression through its own metabolism, thus affecting the metabolism of NAFLD by liver fatty acids[38].

Finally, we used the Vina tool to dock molecules in batches. The results showed that SREBF1, CASP1, HK1, and HK2 were the genes that docked well with the active ingredients. SREBF1 belongs to the Sterol Regulatory Element Binding Proteins (SREBPs) family, can stimulate the expression of lipogenic genes such as acetyl-CoA carboxylase and Fatty Acid Synthase (FAS)[39]. There is increasing evidence that hepatic steatosis is associated with increased expression of SREBF1-mediated pathway, and inhibition of SREBF1 can reduce cellular fat accumulation *in vivo* and *in vitro*[40]. In a word, the loss of CASP1 ameliorates the damaging effects of obesity induced by a high-fat diet. CASP1 plays a central regulatory role in high-fat diet-induced NASH, and mice with CASP1 deficiency are protected from high-fat induced hepatic steatosis, inflammation, and early fibrosis[41]. Studies have shown higher expression of HK1 in liver samples from patients with NASH compared to samples from patients with NAFLD[42]. All the selected target genes mentioned above belong to mitochondria-related genes. It also means that these active ingredients of Jianpi Shenshi Jiangzhuo formula may have therapeutic effects on NASH by regulating these genes and pathways.

However, our study has some limitations. First, the results need to be validated by further experiments. Second, a more comprehensive database of target genes of TCM is needed to make the results of network pharmacology analysis more reliable. Third, even combining the results of network pharmacology and molecular docking, the exact therapeutic mechanism of Jianpi Shenshi Jiangzhuo formula and NASH it is still not completely comprehensible, which relies on multidisciplinary co-development.

In summary, this study focused on the active ingredients of Jianpi Shenshi Jiangzhuo formula and explored the mechanism of action of this formula in the treatment of NASH through combining differential analyses of NASH datasets in GEO and network

pharmacology to reveal the potential therapeutic role of the mitochondrial pathway in NASH, providing a new reference for the application of Jianpi Shenshi Jiangzhuo formula in the treatment of NASH.

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Conflict of interests:

The authors declared no conflict of interests.

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