

To Investigate the Molecular Mechanism of Wugedan Pill in Improving Insulin Resistance Based on Network Pharmacology

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Abstract: Objective To explore the network pharmacological mechanism of Wugedan Pill in improving insulin resistance (IR). **Methods** The main active ingredients and corresponding target genes of Wugedan Pill were retrieved from the Traditional Chinese Medicine System Pharmacology Technology Platform (TCMSP), and the related targets of insulin resistance were obtained through the Human Gene Database (GeneCards) and the Human Online Mendelian Inheritance Database (OMIM). Gene, the active ingredient target of the drug is mapped with the insulin resistance target, and the intersection target is obtained, which is the predicted target of Wugedan Pill acting on insulin resistance. The "pharmaceutical active ingredient-target" network model was constructed by Cytoscape 3.7.0 software, and the key active ingredients were selected. The STRING database was used to construct an intersecting target-protein interaction network (PPI), and key proteins were selected. Intersection targets were subjected to Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the Metascape platform. **Results** There are 94 active ingredients and 163 related targets of Wugedan Pill acting on insulin resistance. The key active ingredients are quercetin, β -sitosterol and kaempferol, and the key proteins are estrogen receptor 1 (ESR1), aldose reductase (AR), nuclear receptor coactivator protein 1 (NCOA1), Fos protein et al.; GO enrichment analysis showed that Wugedan Pill could affect the activities of oxidoreductase, tyrosine kinase-binding protein, steroid-binding protein and other proteins in improving insulin resistance; KEGG pathway enrichment analysis showed that the effect was the most Notably lipid and atherosclerotic pathways, also significantly affected diabetes complications AGE-RAGE signaling pathway, PI3K-Akt signaling pathway, neuroactive ligand-receptor interactions, alcoholic liver disease, prostate cancer, cancer miRNAs expression, pancreatic cancer, thyroid hormone signaling and other pathways. **Conclusion** Wugedan Pill may play a role in improving insulin resistance, regulating lipid metabolism, reducing blood glucose and preventing complications by regulating the above targets and pathways with the effective ingredients quercetin, β -sitosterol and kaempferol.

1. Introduction

Insulin resistance (IR) is a decrease in the metabolic response of adipocytes, muscle cells and hepatocytes to normal concentrations of insulin, that is, the down-regulation of the hypoglycemic function of insulin. IR is affected by dietary patterns, obesity, long-term sedentary and other factors, and its pathological pathogenesis mainly includes: abnormal insulin quality and quantity, abnormal insulin receptor, abnormal insulin signal transduction pathway, complex molecular action, etc.[1]. As a common pathophysiological mechanism of various metabolic diseases, it is commonly found in type 2 diabetes, obesity, Alzheimer's disease, cancer and other diseases[2,3]. Studies have confirmed that insulin resistance is related to a variety of signal transduction, inflammatory responses, changes in cytokine secretion levels, and oxidative stress responses[4-7].

This process belonged to the categories of "consumptive thirst" and " spleen pyretic abundance" in traditional Chinese medicine. Chief physician Bo Lu summarized its pathogenesis as cold-heat complex, Phlegm and Blood Stasis Syndrome, and set the method of "the method pungent and bitter acidizing". According to the theory of "Plum Pill" laying for Wugedan Pill, after five years of clinical and basic research, confirmed that the party can lower in patients with type 2 diabetes, metabolic syndrome, 2h postprandial blood glucose, fasting glucose, glycosylated hemoglobin, total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL - C), It can control body weight and improve insulin resistance[8] with good safety, but its mechanism of action is still unclear.

Due to the diversity of the components of compound Chinese medicine and the complex interaction mechanism in the human body, this study aimed to find the core target of action, taking Wugedan Pill as an example, and using the relevant principles and methods of network pharmacology, to explore its mechanism of action in improving insulin resistance, so as to provide a reference for future in-depth research.

2. Data and Methods

2.1. Active ingredients and target mining of Wugedan Pill

Obtain the chemical components of various traditional Chinese medicines (Wumei, Gegen, Danshen, Danggui, Ganjiang, Guijanyu, Huanglian, Huangqin) in Wugedan Pill in the Traditional Chinese Medicine System Pharmacology Technology Platform (TCMSP) (<http://tcmsp.com/tcmssp.php>), and set the oral bioavailability (OB) \geq 30%, Drug-Likeness(DL) \geq 0.18, screen out qualified candidate compounds and their corresponding targets, and then use the Uniprot database to convert the targets into corresponding genes to obtain the gene target of Wugedan Pill point.

2.2. Acquisition of insulin resistance-related targets and prediction of drug targets

Using "insulin resistance" as the search term, the GeneCards database (<https://www.genecards.org/>) and the OMIM database (<https://omim.org/>) were searched for insulin resistance-related targets, and then the resulting diseases were the target and the target of Wugedan Pill are intersected by the R language program to obtain the common target, and the Venn diagram is drawn at the same time to obtain the common drug-disease target.

2.3. Construction and analysis of the network of "chemical components of traditional Chinese medicine-targets"

The "Network Analyzer" function in Cytoscape 3.7.0 software was used to analyze the chemical

composition-target network of traditional Chinese medicine. The nodes represent the medicinal chemical components contained in Wugedan Pill and their potential targets; the edges represent the links between the components of traditional Chinese medicine and their targets. According to the connection between the compounds and the target, the key compounds of Wugedan Pill acting on insulin resistance were screened out.

2.4. Construction of protein-protein interaction (PPI) network and screening of key targets

The drug-disease intersection targets were imported into the STRING database (<https://string-db.org/>) for PPI analysis, the study species was limited to "human" (Homo Sapiens), and the lowest interaction score was set to the highest confidence ["HighestConfidence (0.900)"], set the hidden free point at the same time, and keep the default settings for the rest of the parameters. Download the PPI network pictures and TSV format files of Wugedan Pill acting on insulin resistance. The downloaded TSV files in STRING were screened with R language, and the top 30 proteins with the greatest correlation were selected.

Use the "CytoNCA" tool in the Cytoscape 3.5.1 software to perform network topology analysis on the TSV result file, according to the average of the degree centrality (Degree), betweenness centrality (Betweenness), and closeness (Closeness) of each gene Perform 2 screenings to screen out target genes that satisfy the average of correlation, centrality, and intimacy at the same time, and further construct a disease-component-target core network.

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

Using the Metascape (<https://metascape.org/>) platform to conduct GO function and KEGG pathway enrichment analysis on the intersection targets of Wugedan Pill and insulin resistance, with $P < 0.01$ as the screening condition, the eligible GO biological processes were selected and KEGG pathways, and selected the pathways with the top 20 p-values for visualization.

3. Result

3.1. Drug - disease target prediction results

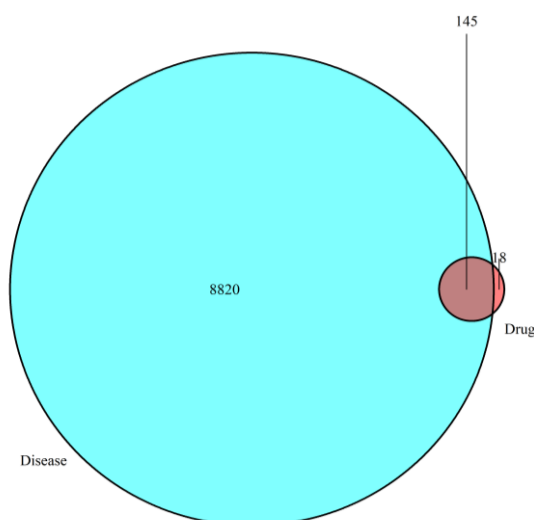


Figure 1: Venn diagram of Wugedan Pill and insulin resistance targets

In TCMSP, $OB \geq 30\%$ and $DL \geq 0.18$ were set, and 164 effective compounds were obtained, including Wumei(10), Gegen(6), Danshen(55), Danggui(5), Ganjiang(11), Guijianyu(11), Huanglian(20), Huangqin(46). Corresponding targets one by one, removing non-target compounds and removing duplicates, a total of 94 targets were obtained; a total of 163 potential targets were obtained after removing duplicate values. Insulin resistance-related target genes were retrieved from the GeneCards database and OMIM database, and a total of 8820 targets were obtained after removing duplicate values. Finally, the Venn diagram was made using R language, and 145 common drug-disease targets were obtained. As shown in figure 1.

3.2. Network construction and analysis results

The traditional Chinese medicine compound-target gene interaction network was constructed using Cytoscape 3.7.0 software, as shown in Figure 2. The network contains 409 nodes and 1822 edges, of which 54 nodes represent active components, 306 nodes represent target genes related to active components, one component corresponds to multiple targets, and one target also corresponds to multiple the composition reflects the multi-component and multi-target characteristics of the traditional Chinese medicine compound. After analysis of Figure 2 by Cytoscape 3.7.0 software, in the figure: the compound with the most potential targets is quercetin, followed by β -sitosterol and kaempferol. It is speculated that these compounds may be the key compounds for Wugedan Pill to improve insulin resistance.

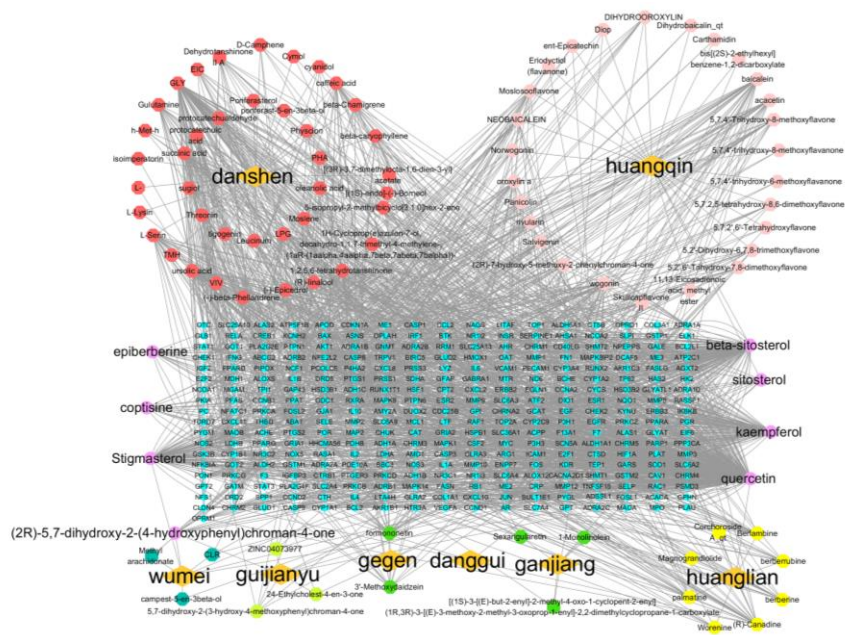


Figure 2: The "active ingredient-target" interaction network of Wugedan Pill

3.3. Construction of PPI network and its core genes between Wugedan Pill and insulin resistance

Enter the 145 drug-disease common targets obtained from the Venn diagram into the STRING database for analysis to obtain PPI (Figure 3), set highest confidence ≥ 0.9 , hide free points, which contain 145 nodes, 225 edges, average the node degree value is 3.1, and the PPI enrichment P value is less than $1.0e-16$. The nodes represent proteins, and each edge represents the interaction between proteins and proteins. The more lines, the greater the degree of association.

Save the above results as a TSV format file; open the TSV result file in Cytoscape software, use

the cytoNCA plug-in for topological analysis, and obtain core proteins in PPI, such as estrogen receptor 1 (ESR1), aldose reductase (AR), nuclear receptor coactivator protein 1 (NCOA1), Fos protein (Fos), etc., Figure 4. It is speculated that these proteins may be the key proteins for Wugedan Pill to improve insulin resistance.

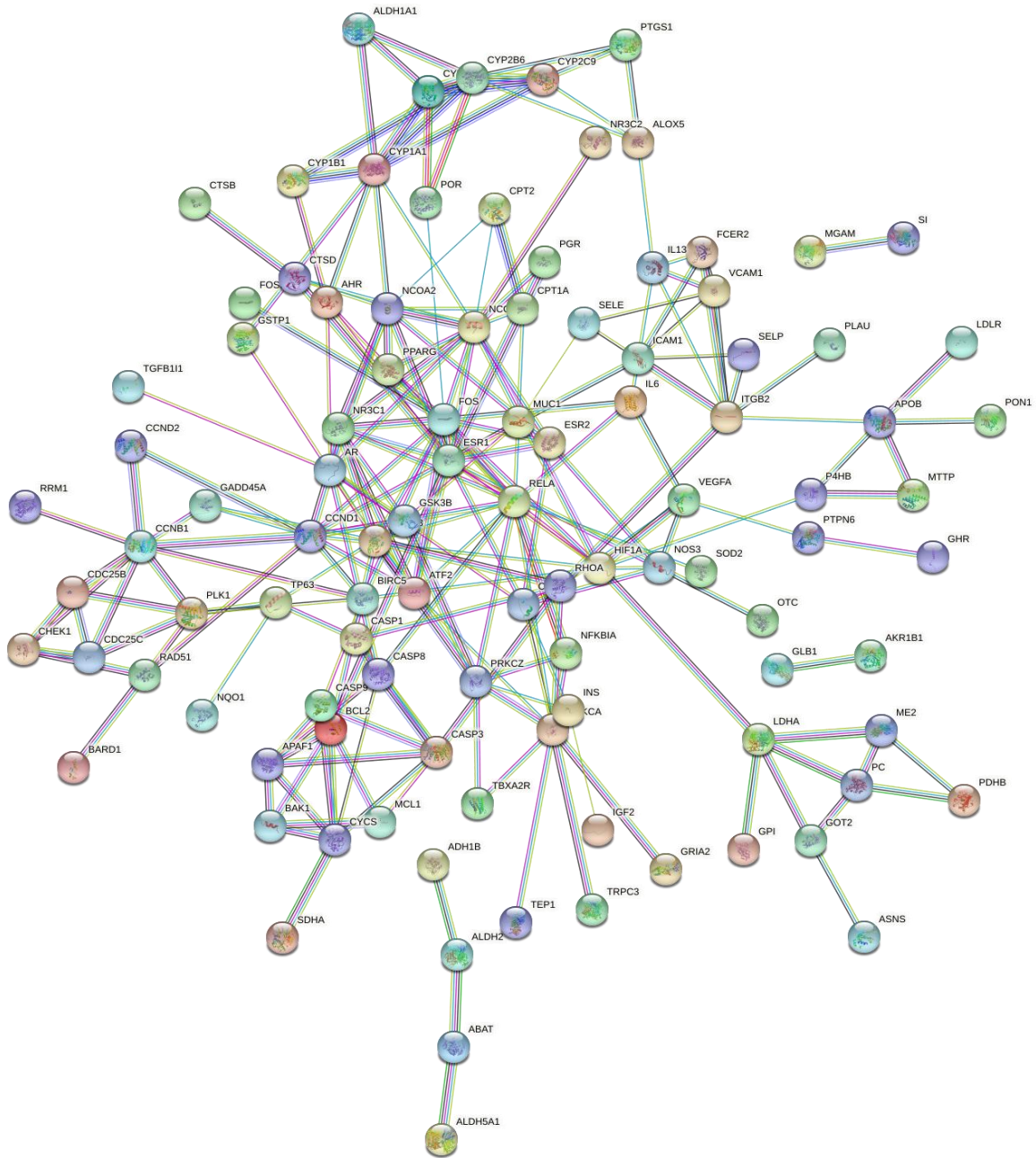


Figure 3: Wugedan Pill improve insulin resistance target protein network

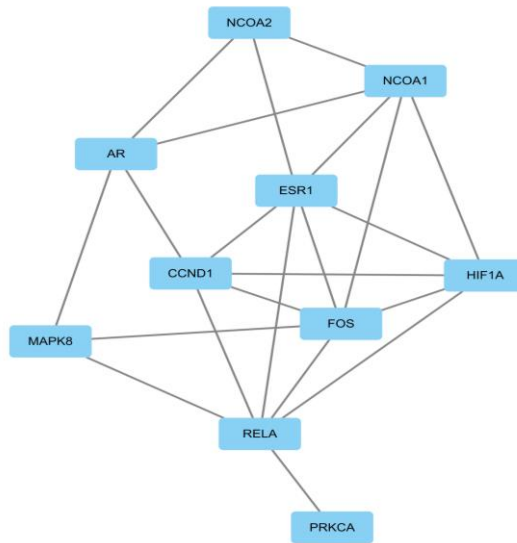


Figure 4: Core genes in PPI

3.4. GO functional enrichment analysis

Through Metascape platform analysis, 145 Wugedan Pill-insulin resistance intersection genes were found to affect a total of 380 biological processes ($P < 0.01$, $FDR < 0.01$). The P-value represents the significance of enrichment, and the top 20 functional information with the smallest P-value was selected to make a bar chart of GO functional enrichment (Figure 5). According to the analysis results, Wugedan Pill may be involved in the regulation of blood circulation, circulatory system, endocrine hormone metabolism and other biological processes. It plays a role in cell components such as membrane side, postsynaptic membrane and mitochondrial envelope. Molecular functions such as oxidoreductase, tyrosine kinase binding protein and steroid-binding protein were activated. It is speculated that Wugedan Pill may improve insulin resistance by improving the above biological processes.

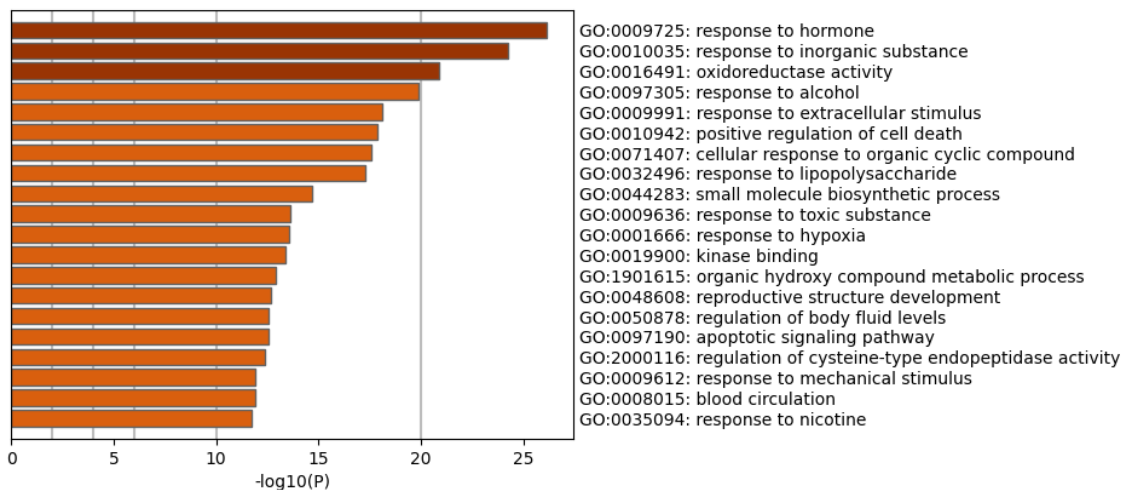


Figure 5: GO function enrichment bar graph of Wugedan Pill improving insulin resistance

Note: The right side represents the name of the biological process, the abscissa represents the number of targets, and the color represents the P value. The smaller the P value, the more yellow the color is, and the larger the P value, the more red it is.

3.5. KEGG pathway enrichment analysis

The KEGG pathway enrichment analysis was performed on the Metascape platform, and it was found that 145 intersecting target genes affected a total of 158 pathways, and the top 20 pathways with the most significant results were made into a bar graph (Figure 6). The results show that the top 10 pathways most significantly affected by Wugedan Pill include lipid and atherosclerosis, chemical carcinogenesis-receptor activation, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, neuroactive ligand-receptor interaction, alcoholic liver disease, Prostate cancer, microRNAs in cancer, pancreatic cancer, thyroid hormone signaling pathway, suggesting that Wugedan Pill may improve insulin resistance through these pathways.

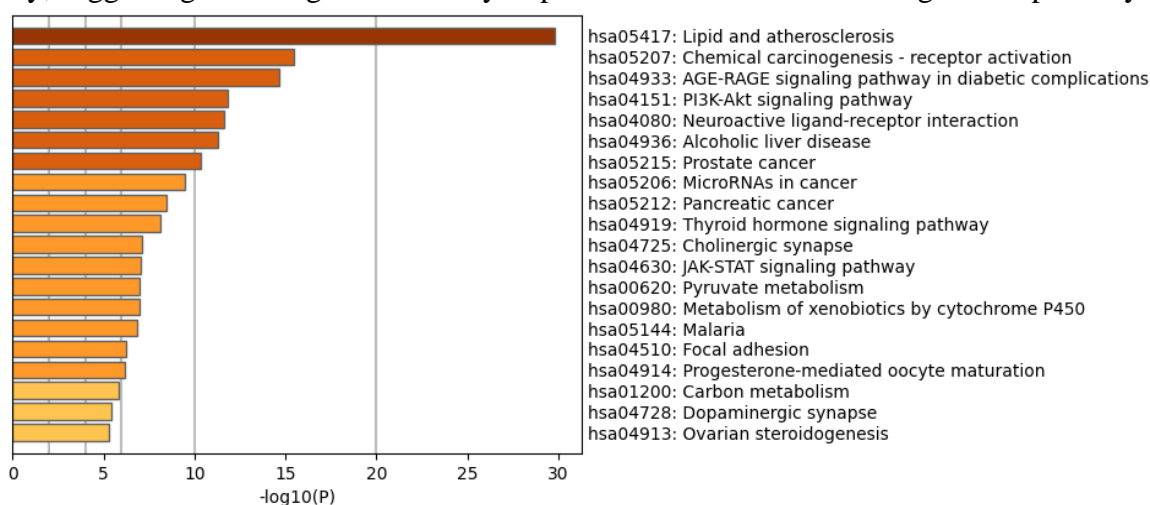


Figure 6: KEGG pathway enrichment bar graph of Wugedan Pill improving insulin resistance

Note: The right side represents the name of the biological process, the abscissa represents the number of targets, and the color represents the P value. The smaller the P value, the more yellow the color is, and the larger the P value, the more red it is.

4. Discussion

In this study, 164 active ingredients of Wugedan Pill were selected through screening conditions in the TSMCP database. After intersecting the corresponding targets of the candidate ingredients in Wugedan Pill and the corresponding targets of insulin resistance, a common drug-disease target was obtained. 145 points. From the drug-target interaction network, it can be seen that the key compounds of Wugedan Pill to improve insulin resistance are quercetin, kaempferol and β -sitosterol. Quercetin and kaempferol are common flavonol monomers in nature. Studies[9] have shown that kaempferol and quercetin have similar hypoglycemic and blood lipid effects in mice to Xiaohe Pill, and hypoglycemic effects., The blood lipid lowering effect is obvious. Quercetin may play a hypoglycemic effect by reducing the level of oxidative stress in the body and reducing islet cell apoptosis[10]; kaempferol can reverse glucose transporter 4 (GLUT4) and adenosine in mouse muscle cells and adipose tissue. Impaired expression of acid-activated protein kinase (AMPK) improves blood glucose and the sensitivity of surrounding tissues to insulin[11,12], and can also inhibit Type 2 Diabetes through the antioxidant, anti-inflammatory, and aldose reductase pathways to a certain extent. The occurrence of chronic complications of diabetes[13]; β -sitosterol may improve glucose and lipid metabolism by activating AMPK[14]. It is suggested that the key compounds in Wugedan Pill can improve insulin resistance through various ways, that is to say, there are various components in Wugedan Pill to improve insulin resistance.

By analyzing the PPI network, it was found that the key receptors for Wugedan Pill to improve insulin resistance may be ESR1, AR, NCOA1, Fos and so on. Activation of ESR1 can regulate hepatocyte factor (HGF) and hepatocyte growth factor receptor (MET), thereby improving hepatic steatosis and insulin resistance[15]; AR gene may reduce lipolysis by affecting the metabolism of fat in the intestine and liver Adipocyte hypertrophy, free fatty acid (FFA), tumor necrosis factor alpha (TNF- α), resistin (Resistin), etc. produced by hypertrophic adipocytes increase, while the decrease of adiponectin secretion affects the sensitivity of peripheral tissues to insulin. Insulin resistance. It is suggested that Wugedan Pill can improve insulin resistance by activating multiple targets.

By analyzing the GO enrichment results, it can be seen that Wugedan Pill can affect various biological processes to improve insulin resistance to varying degrees, such as affecting endocrine metabolism, oxidoreductase, tyrosine kinase-binding protein, steroid-binding protein, etc. Activation of estrogen receptors can effectively inhibit the vascular endothelial dysfunction caused by diabetes, and can prevent cardiovascular damage in diabetes[16]. The results of the KEGG pathway showed that the most significant pathways for Wugedan Pill to improve insulin resistance were lipid and atherosclerosis pathways. It has been confirmed to be closely related to diabetes complications. The PI3K/Akt signaling pathway is mainly involved in cell differentiation, proliferation, apoptosis and migration[17]. Current in vitro and in vivo studies have shown that the occurrence of diabetic nephropathy is related to the activation of the PI3K/Akt signaling pathway[18,19], and excessive activation of MAPK will cause Accelerates diabetic atherosclerosis[20,21]. Wugedan Pill can also affect neuroactive ligand-receptor interaction, alcoholic liver disease, prostate cancer, miRNA expression in cancer, pancreatic cancer, thyroid hormone signaling pathway and other pathways. Studies [22] have shown that the AGE-RAGE pathway is also closely related to diabetic macroangiopathy. In vascular endothelial cells, the combination of AGE and RAGE can accelerate the process of atherosclerosis by inducing oxidative stress and inflammatory response. It is speculated that Wugedan pill may improve insulin resistance and reduce the incidence of complications such as atherosclerosis and coronary heart disease. However, there is still a lack of relevant literature to provide theoretical reference for expanding the scope of Wugedan Pill in the future.

In summary, this study is based on the technology of network pharmacology, with the help of the corresponding database and software, the network is constructed and the target is enriched and analyzed, and the key compounds and key proteins of Wugedan Pill to improve insulin resistance are described., the relationship between the target and the pathway of action. The results suggest that Wugedan Pill may improve insulin resistance, regulate lipid metabolism, lower blood sugar and prevent complications by regulating the above targets and pathways with the active ingredients quercetin, kaempferol and β -sitosterol. This study provides a reference for further experimental study of Wugedan Pill, but there are still some problems that need to be solved. For example, the screening criteria of active ingredients are not completely accurate, and the data collection relies on database, which has certain limitations and cannot reveal the complete pharmacological action. In addition, this study focuses on the theory, and more clinical trials and animal experiments are needed for further verification.

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