Comparing the mechanism of four classic Gualou-Xiebai prescriptions for cardiovascular diseases with phlegm and blood stasis syndrome based on molecular network modeling

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Author contributions
Bo Zhang and Hua Zhong designed this study and performed the online database search. Jia-Wei Chen contributed to the data collection and data analysis. Ya-Rong Liu and Hong-Fei Wu prepared the original draft. Bo Zhang finished the revision of the manuscript. All authors have read and approved the final manuscript.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
GLXBBX, Gualou Xiebai Banxia decoction; ZSBXGZ, Zhishi Xiebai Guizhi decoction; DL, Danlou prescription; PBSS, phlegm and blood stasis syndrome; TCM, Traditional Chinese Medicine; GLXB, Gualou-Xiebai; GLXBBX, Gualou Xiebai decoction; KEGG, Kyoto encyclopedia of genes and genomes; MCC, Minuta Cylinder Code algorithm.

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Abstract

Background: Four classical Traditional Chinese Medicine prescriptions, namely Gualou Xiebai Baijiu decoction, Gualou Xiebai Banxia decoction (GLXB), Zhishi Xiebai Guizhi decoction (ZSBXGZ) and Danlou prescription (DL), have been frequently used for treatment of phlegm and blood stasis syndrome (PBSS)-related cardiovascular diseases. However, its therapeutic mechanism has not been clearly elucidated. This study aimed to explore PBSS and its molecular mechanism, clarify and compare the mechanisms of four prescriptions in treating PBSS-related diseases. Method: In this study, we collected four prescriptions’ compounds, predicted therapeutic targets, and enriched pathways which were based on network pharmacology. Then, we analysed the common and different mechanisms by combing the network of components, targets and pathways. Finally, molecular docking was engaged to assess the binding potential of key compounds and hub targets. Results: We showed that four prescriptions’ intersection genes (VEGFA, SRC, EGFR, etc.) were commonly enriched in PI3K-AKT signaling pathway, HIF-1 signaling pathway, etc. In addition, platelet activation and cAMP signaling pathway were singly enriched from the GLXXBX through unique compounds 12,13-epoxy-9-hydroxynonadeca-7,10-dienoic acid and Cyclo (L-tyrosyl-L-phenylalanyl). These bioactive compounds may exert GLXXBX’s unique pharmacological pathways via involving in mediating PPARA, PTGER3, etc. Sphingolipid signaling pathway was singly enriched from the ZSBXGZ through unique compounds tetramethoxylyuteolin, ergosterol peroxide, etc. These bioactive compounds could mediate ADORA1, ADORA2 and TNFRSF1A to regulate ZSBXGZ’s unique pharmacological pathways. AMPK signaling pathway was singly enriched from the DL through unique compounds kaempferol, evofolin, ethyl acid and aureusidin. These bioactive compounds were involved in mediating the main targets of AMPK signaling pathway, such as TNF, TNFRSF1A, etc. Conclusions: Our research demonstrated that GLXXBX-prescriptions involved in almost all pathological stages of PBSS-related cardiovascular diseases by modulating high-frequency shared pathways and targets mainly through key compounds (quercetin, mandelon, sitosterol acetate and luteolin, etc.), for example, participate in the process of atherosclerosis, lipid metabolism, inflammation, immune response, thrombosis, inhibit inflammatory factors and platelet aggregation, regulate immune function, vascular function, oxidative stress. In addition to common pharmacological efficacies, there could also be specificities among GLXXBX prescriptions due to different compounds. For example, GLXXBX tends to regulate the function of vascular and endothelial barrier, prevent thrombosis. ZSBXGZ tends to regulate lipid metabolism and protect the heart from lipid accumulation. DL tends to maintain energy homeostasis and improve inflammation.

Keywords: Gualou Xiebai Baijiu decoction; Gualou Xiebai Banxia decoction; Zhishi Xiebai Guizhi decoction; Danlou prescription; phlegm and blood stasis syndrome; network pharmacology analysis; molecular docking.
Introduction

Traditional Chinese medicine (TCM) prescriptions have evolved through thousands of years of clinical observation and refinement, rooted in the unique system of syndrome differentiation and treatment. Phlegm and blood stasis syndrome (PBSS) is recognized as a significant manifestation of elevated blood lipid and blood viscosity levels due to abnormal metabolism of body fluids and blood. It generally encompasses a cluster of conditions marked by microcirculation disturbance, metabolic imbalance, bodily pain, inflammation, and coagulation [1, 2]. Ultimately, elevated blood lipid and viscosity levels not only impact blood transport and organ function, but also predispose to vascular damage and thrombus formation. Thankfully, TCM prescriptions play a crucial and positive role in PBSS therapy. However, there exist differing interpretations and understandings of the scientific essence of PBSS. Recently, more researchers have focused on TCM syndromes to elucidate their molecular mechanisms and the mechanisms of drug treatments for associated diseases [3, 4].

Gualou-Xiebai (GLXB) is a traditional Chinese herb pair that features in classical prescriptions documented in the “Synopsis of the Golden Chamber” by Chinese physician Zhang Zhongqing during the Han Dynasty [5]. Clinically used for thousands of years in China to treat PBSS, GLXB prescriptions include Gualou Xiebai decoction (GLXBBD), Gualou Xiebai Banxia decoction (GLXBBDX), Zhishi Xiebai Guizhi decoction (ZXSBGZ), and Danlou prescription (DL). This herbal combination comprises Gualou (Trichosanthis Fructus) and Xiebai (Allii Macrostemonis Bulbus). Apart from GLXBBD, the other three prescriptions incorporate additional herbs. For instance, GLXBBD incorporates Arum Ternatum Thumb; ZXSBGZ includes Auranti Fructus Immaturus, Magnolia Officinalis Rehd Et Wils, and Cinnamonomum Ramulus; DL comprises Radix Aconiti, Radix Paeoniae Rubra, Chuanxiong Rhizoma, Hedyssum Multijugum Maxim, Radix Puerariae, Drynariae Rhizoma, Curcumae Radix, and Alisma Orientale (Sam.) Jus.

Modern medicine has demonstrated the close association of PBSS with numerous cardiovascular diseases, such as hyperlipidemia, arteriosclerosis, coronary heart disease, and myocardial infarction, posing significant threats to both physical and mental well-being in recent years [6, 7]. In essence, addressing PBSS can help prevent or mitigate the onset and progression of PBSS-related chronic ailments. In contemporary clinical practice, GLXB prescriptions exert a positive influence on PBSS-related cardiovascular diseases and their clinical syndromes [8–11]. Given the pivotal role of GLXB prescriptions in PBSS-related cardiovascular diseases, conducting relevant research becomes imperative. In our prior studies, we initially identified 43 compounds in GLXB, including steroid saponins, flavonoids, organic acids, tetracyclic triterpenoids, amino acids, and nucleosides, using HPLC-Q-TOF-MS. Building on this, we further established that GLXB effectively combats atherosclerosis by ameliorating blood lipid levels, quelling inflammation, and regulating vascular endothelial growth/function [12]. Through metabolomics analysis, we also discerned GLXB’s potential therapeutic role in modulating pathways like glycerophospholipid and sphingolipid metabolism [13]. In combination, classical GLXB prescriptions confer several advantages in addressing PBSS-related cardiovascular diseases. Nonetheless, the shared and distinct mechanisms of the four GLXB prescriptions in treating these diseases remain uncertain, impeding their effective clinical application.

Network pharmacology, a burgeoning discipline grounded in systems biology, bridges the concepts of TCM and modern pharmacology at a systemic level. The salient features of TCM (holistic perspective and syndrome differentiation and treatment) align well with the principles of network pharmacology [14–16]. Molecular docking, a theoretical simulation method, predicts molecular interaction binding patterns and affinity, serving as a key tool to assess the interaction between TCM prescriptions and disease targets [17]. The objective of this study is to delve into PBSS and its molecular mechanism, distinguish the four GLXB prescriptions at a molecular level, elucidate and compare the mechanisms of these prescriptions in treating PBSS-related cardiovascular diseases. This comprehensive approach, leveraging network pharmacology analysis, aids in facilitating a more accessible understanding of PBSS and offers a theoretical foundation for the potential attributes of GLXB prescriptions.

Materials and methods

Related molecules of GLXBBD, GLXBBX, ZXSBGZ and DL

Using the TCMSP database, which serves as a dedicated platform for system pharmacology of Chinese herbal medicines, we compiled the active ingredients of GLXB, GLXBBX decoction, ZXSBGZ decoction, and DL [18]. In order to enhance our study and establish a foundation for potential future drug development, we considered two parameters during the active ingredient selection process. The first parameter, drug-likeness evaluation, plays a crucial role in determining a compound’s chemical suitability for drug application. The second parameter, oral bioavailability, gauges the extent to which a bioactive compound can enter the systemic circulation after oral administration. Specific parameters impact the absorption, distribution, metabolism, and excretion (ADME) properties of each compound. Notably, oral bioavailability should exceed 30% and drug-likeness should be above 0.18, as these factors significantly influence pharmacodynamics and pharmacokinetic profiles. The assessment of oral bioavailability and drug-likeness was conducted through two ADME-related models, as documented in the literature. The application of these models aimed to identify potential bioactive compounds within the four formulas. By inputting compound data from these prescriptions into the SEA database and utilizing Swiss Target Prediction, with “Homo sapiens” specified as the species parameter, researchers obtained the corresponding molecular information [19, 20].

PBSS-related cardiovascular diseases molecules acquisition and network construction

Drawing inspiration from the identification of targets related to conditions like blood stasis syndrome and hyperviscosity syndrome, we utilized the TTD database to collect information on pathological characteristics linked with PBSS and target genes associated with PBSS-related cardiovascular diseases [21–24]. The search terms employed for target identification encompassed “cardiovascular diseases”, “atherosclerosis”, “coronary heart disease”, “hyperlipidemia”, “thrombus”, “inflammation”, “pain” and “insulin resistance”. Ultimately, we compiled targets linked with the pathophysiology and clinical manifestations of PBSS-related cardiovascular diseases. These related genes were then input into the STRING database to create an interaction network (confidence > 0.9). The resulting protein–protein interaction network was visually represented using Cytoscape (version 3.8.0), and central hub genes were pinpointed using cytoHubba. This process yielded the network for PBSS-related cardiovascular diseases.

Comparison of the four prescriptions in treating PBSS-related cardiovascular diseases

Hub genes analysis. The relevant hub genes from GLXBBD, GLXBBX, ZXSBGZ, and DL were correlated with the network of PBSS-related cardiovascular diseases. An examination of the shared and distinct hub genes across the four prescriptions was conducted, facilitating a comparison of the similarities and differences among them.

Functional enrichment analysis. In the subsequent analysis, all genes were presented in the HGNC gene symbol format to prevent confusion across databases and platforms [25]. For a comprehensive understanding of the biological implications underlying a large number of genes, the DAVID database offers an array of functional annotation tools [26]. In order to glean pathway information, all genes were input into the DAVID database for KEGG and GO encyclopedia of genes and genomes (KEGG) pathway enrichment analysis [27–29]. Additionally, the scope was limited to “Homo sapiens” to identify the KEGG pathways. A significance level of P < 0.05 was deemed
indicative of statistically significant differences in the annotation categories. Following this, the R software’s ggplot2 package (Version 4.1.0) was utilised to visualize the data from the KEGG pathway enrichment analysis.

Molecular docking

Molecular docking was performed to predict the interaction of the hub genes with the major compounds. Constructed compound-pathway network, the top three compounds were screened as ligands for molecular docking based on the Minuitia Cylinder-Code algorithm (MCC). In order to investigate the possible binding conformation of key compounds to the active site of the hub targets, molecular docking studies were conducted using the AutoDock (version 1.1.2). Docking of quercetin to EGFR molecules was used as an example. The crystal structures of human EGFR (PDB ID: 6ICG, resolution = 1.15 Å) used for docking were obtained from the RCSB Protein Data Bank [30]. Water molecules and other heteroatoms were removed from the protein and hydrogen atoms were added subsequently by Pymol (version 1.8). The 3D structure of quercetin was prepared in MOL2 format and docked into the protein after minimizing energy by Chem 3D (version 14.0.0.117). In addition, both ligand and protein add Gasteiger charge. Both protein and ligand have been saved in PDB format for molecular docking by AutoDock. The conformation with the highest score was selected to study the interaction between EGFR and quercetin. Finally, the remaining key compounds and key targets apply the same methods to complete molecular docking verification.

Results

Related compounds and genes of the four prescriptions

Using TCMSP, we gathered data for four prescriptions: GLXBBJ, GLXBBX, ZSXBGZ, and DL. We identified 22 correlated compounds for GLXBBJ, 35 for GLXBBX, 53 for ZSXBGZ, and 189 for DL. (Supplementary Tables S1–S4). Once the names of these compounds were cross-referenced in the SEA and Swiss Target Prediction databases, we compiled 308 related genes for GLXBBJ, 501 for GLXBBX, 418 for ZSXBGZ, and 646 for DL (Supplementary Tables S5–S8). Among the four prescriptions, there were 308 genes that overlapped, 125 unique genes in GLXBBX, 2 unique genes in ZSXBGZ, and 183 unique genes in DL (Figure 1a).

Related genes and networks of the four prescriptions and PBSS-related cardiovascular diseases

Using the TTD database, we acquired 543 genes related to PBSS-pathological features and PBSS-related cardiovascular diseases (Supplementary Table S9) and identified 362 genes (score ≥0.9) within the network. Upon comparing the genes associated with the four prescriptions to those linked with PBSS-pathological features and PBSS-related cardiovascular diseases, we identified 123 therapeutic targets (Figure 1b). Consequently, our focus could be directed towards these 123 genes, allowing us to analyze the relationship between the four prescriptions and the network of PBSS-related cardiovascular diseases. In the comparison of genes from the four prescriptions with those of the PBSS-related cardiovascular diseases network, we discovered 57 shared therapeutic targets among the four prescriptions, 8 shared therapeutic targets between GLXBBX and DL, 15 shared therapeutic targets between ZSXBGZ and DL, and 3 shared therapeutic targets encompassing GLXBBX, ZSXBGZ, and DL. Furthermore, there were 17 unique therapeutic targets within GLXBBX, 1 unique therapeutic target within ZSXBGZ, and 25 unique therapeutic targets within DL (Figure 1b).

Comparison of genes in the network of the four prescriptions and PBSS-related cardiovascular diseases

In the PBSS-related cardiovascular diseases network, the pharmacological targets of GLXBBJ, GLXBBX, ZSXBGZ, and DL were plotted (Figure 2a–d). The four prescription groups exhibited both similarities and differences in the drug-target range, indicated by the changing node colors in the network model. To enhance clarity in identifying the drug-target range, colourful nodes were introduced to distinguish overlapping and isolated targets (Figure 2e). Subsequently, we obtained several findings related to the four prescriptions: (1) The drug-target range of GLXBBX and ZSXBGZ displayed more similarities; (2) The targets from GLXBBJ were entirely encompassed by those from GLXBBX, ZSXBGZ, and DL; (3) GLXBBX, ZSXBGZ, and DL each possessed their distinctive drug targets.

Based on the findings extracted from the STRING database, the pertinent target protein encompassed a total of 362 targets. This was reflected in the PBSS-related cardiovascular diseases network, which consisted of 362 nodes (Figure 3). Subsequently, utilizing the MCC criterion, a selection was made of 13 key hub nodes with notably high values from the PBSS-related cardiovascular diseases network. Among these top hub nodes were STAT3, VEGFA, TP53, INS, IL1B, SRC, BCL2L1, EGFR, MYC, HIF1A, TNF, IL6, and IGF1. These outcomes underscored the pivotal role these genes play in the development of PBSS-related cardiovascular diseases.

Figure 1 The related molecules of the four prescriptions and PBSS-related cardiovascular diseases. (a) The overlapping molecules among the four prescriptions and PBSS-related cardiovascular diseases. (b) The overlapping molecules among the four prescriptions and the network of PBSS-related cardiovascular diseases. GLXBBX, Gualou Xiebai Banxia decoction; ZSXBGZ, Zhishi Xiebai Guizi decoction; DL, Danlou prescription; PBSS, phlegm and blood stasis syndrome; GLXB, Gualou-Xiebai.
Figure 2 The network of PBSS-related cardiovascular diseases intervened by the four prescriptions. (a–d) Marked with red, yellow, orange, and blue nodes indicate the molecules intervened by the GLXBBJ, GLXBBX, ZSXBGZ and DL, respectively. (e) The overlapping and unique molecules in the PBSS-related cardiovascular diseases network intervened by the four prescriptions. GLXBBX, Gualou Xiebai Banxia decoction; ZSXBGZ, Zhishi Xiebai Guizhi decoction; DL, Danlou prescription; PBSS, phlegm and blood stasis syndrome; Gualou Xiebai decoction.

Figure 3 The PBSS-related cardiovascular diseases network of 362 nodes (genes). The black part represents all nodes. The green part represents more important hub nodes. PBSS, phlegm and blood stasis syndrome; MCC, Minutia Cylinder-Code algorithm.
Derived from the PBSS-related cardiovascular diseases network, common target hubs, including VEGFA, SRC, EGFR, and TNF, were identified across all four prescriptions. Within the realm of GLXXBX, ZSXXBGZ, and DL, IL6 stood out as a shared target hub, whereas HIF1A emerged as a shared target hub specifically between ZSXXBGZ and DL. Furthermore, within the DL prescription, exclusive hubs were STAT3 and IL1B (Figure 4).

Functional enrichment analysis of the four prescriptions
For the KEGG pathway enrichment analysis, the related genes were entered into the DIVID software (P < 0.05), leading to the identification of 49, 55, 67, and 90 pathways associated with GLXXBX, GLXXBX, ZSXXBGZ, and DL, respectively (Supplementary Tables S10-S13). From these, a selection of the top 20 pathways was made (Figure 5), enabling a comparison of the convergences and divergences in the pharmacological processes of the four prescriptions. The pathway analysis of the common intersection target genes for the four prescriptions revealed shared enrichment in pathways such as the PI3K-AKT signaling pathway, HIF-1 signaling pathway, and Insulin resistance. The GLXXBX prescription exhibited singular enrichment in the Prolactin signaling pathway and T cell receptor signaling pathway. In the case of GLXXBX, it was the platelet activation and cAMP signaling pathway that saw exclusive enrichment. The ZSXXBGZ prescription showed singular enrichment in the sphingolipid signaling pathway, while the DL prescription saw singular enrichment in the AMPK signaling pathway.

Molecular Docking Analysis
Utilizing molecular docking, we conducted assessments to verify the significant regulatory role of compounds from the four prescriptions on hub targets. For each of the four prescriptions, we established a compound-target interaction network (Supplementary Figure S1). Through MCC analysis, we identified the top three major compounds (such as quercetin, mandenol, sitosterol acetate, and luteolin) from the four prescriptions. Molecular docking was then employed to examine the interaction of these key compounds with hub targets. The outcomes revealed that the compounds exhibited strong affinity with EGFR, SRC, TNF, VEGFA, IL6, HIF1A, STAT3, and IL1B, with most of their binding forces being below –1.2 (Supplementary Figure S2). The binding forces and relevant hydrogen bond parameters for all key compounds are presented in Supplementary Tables S14–20 [31]. These results affirm the robust affinity between the major compounds and the key targets within the network.

Additionally, taking quercetin as an example, we proceeded to discuss the binding site and geometric characteristics of ligands in complex with the receptor as obtained from the molecular docking, as illustrated in Figure 6. In Figure 6a, hydrogen bond conjugation between quercetin and amino acids (SER75 and ARG142) occurs within the binding pocket. The –OH group of quercetin forms an H-bond (–OH...O–) with SER75 and ARG142, demonstrating hydrophobic interactions. Similarly, in Figure 6b, hydrogen bond conjugation between quercetin and key amino acids (ALA-300, TRP-20, and TRP-111) is observed in the binding pocket. Here, the –OH group of quercetin engages in H-bonds (–OH...O–) with ALA-300, TRP-20, and TRP-111, with accompanying hydrophobic interactions. In Figure 6c, quercetin interacts with the amino acid THR-230 through hydrogen bond conjugation within the binding pocket. The –OH group of quercetin forms an H-bond (–OH...O–) with THR-230, accompanied by hydrophobic interactions. Similarly, in Figure 6d, quercetin interacts with key amino acids (GLN-87, HIS-86, LYS-48, and GLY-88) through hydrogen bond conjugation within the binding pocket. The O atom of the carbonyl group in quercetin forms an H-bond (–O...HN–) with LYS48, while the –OH group forms H-bonds (–OH...O–) with GLN-87, HIS-86, and GLY-88, accompanied by hydrophobic interactions. Furthermore, in Figure 6e, key amino acids (LYS-27 and LYS-217) interact with quercetin through hydrogen bond conjugation in the binding pocket. The O atom of quercetin’s carbonyl group forms an H-bond (–O...HN–) with LYS27, and the –OH group forms H-bonds (–OH...O–) with LYS-27 and LYS-217, with the aromatic ring side chain of LYS-217 also participating in hydrophobic interactions. Similarly, in Figure 6f, key amino acids (AGR-322 and TYR-329) interact with quercetin through hydrogen bond conjugation within the binding pocket. The –OH group of quercetin forms an H-bond (–OH...O–) with AGR-322 and TYR-329, accompanied by hydrophobic interactions. In Figure 6g, the key amino acid GLY-220 interacts with quercetin through hydrogen bond conjugation within the binding pocket. The –OH group of quercetin forms an H-bond (–OH...O–) with GLY-220 and TYR-329, alongside hydrophobic interactions. Finally, in Figure 6h, quercetin interacts with the key amino acid ARG-111 through hydrogen bond conjugation within the binding pocket. The O atom of quercetin’s carbonyl group forms an H-bond (–O...HN–) with ARG-111, and the –OH group forms H-bonds (–OH...O–) with ARG-111, accompanied by hydrophobic interactions. Importantly, these amino acids also exert a pharmacological role in the treatment of PBSS-related cardiovascular diseases.

Discussion
In TCM, deviations in the blood element have a direct impact on PBSS, a crucial pathophysiological concept that was initially documented in the huangdi neijing and has been the focus of numerous research studies. In the realm of modern medicine, PBSS is evidently linked to microangiopathy, which can subsequently lead to conditions like hyperlipidemia, arteriosclerosis, coronary heart disease, and myocardial infarction. However, a clear understanding of the scientific essence of PBSS and the shared underlying molecular mechanism of the four classical prescriptions concerning PBSS-related cardiovascular diseases has yet to be established. Consequently, our network analysis has indicated that lipid metabolism, inflammation response, vascular wall function, platelet aggregation, and thrombosis contribute to the potential shared comprehensive mechanisms of the four classical prescriptions and PBSS-pathological features. Furthermore, the integration of target and pathway networks has effectively illuminated and compared the disparities and parallels in the molecular synergistic actions of the four GLXXBX prescriptions within a holistic framework.
Figure 5 KEGG pathways enrichment. (a–d) The KEGG pathways among the GLXBBJ, GLXBBX, ZSXBGZ, DL. (e) The overlapping pathways among the four prescriptions. GLXBBX, Gualou Xiebai Banxia decoction; ZSXBGZ, Zhishi Xiebai Guizhi decoction; DL, Danlou prescription; GLXB, Gualou-Xiebai; GLXBBJ, Gualou Xiebai decoction; KEGG, Kyoto encyclopedia of genes and genomes.
Figure 6 Binding of quercetin interacting with targets. The binding targets, including: (a) EGFR, (b) SRC, (c) TNF, (d) VEGFA, (e) IL6, (f) HIF1A, (g) STAT3, and (h) IL1B. (i) The scoring of binding force between quercetin and protein.
Decoding signaling pathways of the network model in the four GLXB prescriptions

Signaling pathways serve as potent mechanisms for the action of drugs. Following the pathway enrichment analysis of the four prescriptions, a total of 13 shared as well as several distinctive signaling pathways associated with PBSS-related cardiovascular diseases were identified. In this context, the prescriptions aiming to reverse the PBSS-related pathological stages would likely prioritize the frequently shared signaling pathways, which could be categorized into the following primary groups.

**Shared signaling pathways of pharmacogenomics.** Dysregulation of lipolysis and insulin resistance in metabolism, along with the PI3K/Akt signaling pathway, FOXO signaling pathway, Rap1 signaling pathway, HIF-1 signaling pathway, and the progesterone pathway in endocrinology, as well as cancer-related pathways, emerge as crucial pathways for the treatment of PBSS-related cardiovascular diseases through the employment of the four classical prescriptions.

1. Metabolism-related pathways: Regarding metabolism, the disruption of lipolysis in adipocytes and insulin resistance can give rise to metabolic irregularities. Adipocyte lipolysis constitutes the fundamental process of hydrolyzing triacylglycerol into fatty acids, intended for internal or systemic energy utilization [32]. Dysregulated lipolysis can prompt lipid buildup in adipose tissue and the release of pro-inflammatory cytokines, thereby contributing to an increased incidence of hyperlipidemia and coronary heart disease [33]. Insulin resistance operates as a pathway that impacts multiple organs, playing a role in safeguarding vascular endothelium and enhancing hemodynamics to reverse the pathology in individuals with PBSS-related cardiovascular diseases. Research has demonstrated that the onset of insulin resistance can induce a systemic disruption in glucose homeostasis, heightening the susceptibility to hyperlipidemia and its associated cardiovascular disorders [34].

2. Signal transduction-related pathways: Regarding signal transduction, the calcium signaling pathway is interconnected with vascular endothelial dysfunction, vascular tension, and microcirculation, as well as platelet aggregation and thrombosis, all of which are implicated in various stages of cardiovascular disease pathology [35, 36]. As a pivotal intracellular signal transduction pathway, activation of the PI3K/Akt signaling pathway can mitigate levels of reactive oxygen species and lipid deposition, inhibit plaque formation, and reverse the progression of atherosclerosis [37]. Concurrently, serving as a central pathway, the PI3K/Akt signaling pathway acts as a crucial regulator, governing macrophage proliferation, migration, and cell survival, while also exerting influence on cell metabolism and the secretion and release of inflammatory factors [38]. Oxidative stress triggers a response in endothelial cells, where FOXO functions to inhibit the transcription of endothelial nitric oxide synthase and induce the expression of inducible Nos. Consequently, peroxynitrite is generated, leading to endothelial dysfunction development [39]. Deficiency in endothelial Rap1 results in heightened leukocyte accumulation and elevated expression of cell adhesion molecules in atheroerone regions [40]. By facilitating the activation of Erk, Akt, Rac1, and Araf1, Rap1 governs pivotal signaling pathways that regulate endothelial cell proliferation, migration, and tubule formation [41]. The regulatory influence of HIF-1 over target genes encompasses inflammation, vascular remodeling, and angiogenesis, thereby contributing to cardiovascular dysfunction. In these set in motion, these mechanisms can culminate in severe cardiovascular conditions [43].

3. Endocrine-related pathways: Progesterone is a steroid hormone endowed with potent anti-inflammatory and antioxidant properties [44]. Its significance extends beyond pregnancy maintenance, as it also exerts influence on various non-reproductive tissues, including the cardiovascular and central nervous systems. Through numerous mechanistic studies, protective effects of estrogen have been elucidated across diverse human cardiovascular cell types, encompassing endothelial cells, smooth muscle cells, and cardiomyocytes. Progesterone enhances eNOS levels and NO production in human endothelial cells, boosts basal insulin levels, and facilitates insulin release. However, progesterone deficiency correlates with diminished vascular dilation, hypertension, and heightened atherosclerosis risk [45].

4. Cancer-related signaling pathways: Apart from the signaling pathways associated with PBSS pathology, multiple pathways related to cancer were also enriched. Through a combined analysis of cancer and PBSS-related cardiovascular diseases, it was revealed that these conditions share several similar biological mechanisms, including vascular endothelial function, inflammation, disrupted lipid metabolism, obesity, and smooth muscle cell function [46]. Consequently, targeting cancer-related pathways could potentially offer a promising avenue for treatment.

**Unique signaling pathways of pharmacogenomics.** Unique signaling routes, as opposed to shared signaling pathways, enable us to make the most appropriate choices for the characteristics of drugs in the treatment of diseases (Figure 4).

1. Unique signaling pathways of GLXB: In the molecular network of GLXB, the CAMP signaling pathway, platelet activation, and cocaine addiction are several discriminating signaling pathways. Increasing levels of CAMP can trigger the Epac-Rap1 signaling pathway, which reduces vascular permeability, stabilizes endothelial barrier function, and reduces inflammation to treat cardiovascular system disorders [51]. Additionally, CAMP performs antagonistic actions on platelet activation through the calcium signaling pathway, which has proven to be a therapeutic potential for platelet activation-mediated diseases, such as thrombosis and atherosclerosis [52]. The cocaine signaling pathway can induce a heightened inflammatory state of the immune system with decreased basal anti-inflammatory markers and increased pro-inflammatory cytokines, all of which contribute to cardiovascular disease [53]. In this study, we found that the regulation of these unique pathways was mainly attributed to specific constituents in GLXB. For instance, 12,13-epoxy-9-hydroxyxodacatcanol, an epoxy-9,10-dienic acid is involved in mediating PPARA and PTGER3, the primary targets in the CAMP signaling pathway. Cyclo(L-tyrosyl-L-phenylalanyl) is involved in mediating ITGB3 and ITGB2A, the main targets in platelet activation [54]. These bioactive compounds may serve as the foundation for GLXB to exert its distinctive pharmacological effects.

2. Unique signaling pathway of ZSXBZ: In the molecular network of ZSXBZ, there is one unique signaling pathway, which is sphingolipid metabolism. Sphingolipid metabolism plays a crucial role in protecting the heart from lipid accumulation, thereby reducing the incidence of atherosclerosis [55]. It is widely acknowledged that sphingolipid metabolites serve an indispensable role in inflammatory signaling [56]. Previous research studies have consistently demonstrated the ability of these sphingolipids to trigger inflammation in human coronary artery smooth muscle cells [57]. In fact, the inhibition of serine palmitoyltransferase, the enzyme responsible for de novo synthesis of sphingolipids, through pharmacological means, effectively halted the occurrence of atherosclerosis in apoE knockout mice [58]. The results of network pharmacology indicated that four compounds (tetramethoxyleutolin, 5,7,4′-Trimethylapigenin, didymin, and ergosterol peroxide) were associated with ADORA1, ADORA3, and TNFRSF1A, which are the main targets involved in sphingolipid metabolism. Ergosterol peroxide is one of the most important active compounds, as it has been demonstrated to inhibit differentiation and lipid accumulation in metabolic diseases [59]. Therefore, these bioactive compounds may constitute the material basis for ZSXBZ to exert its unique pharmacological effects.

3. Unique signaling pathway of DL: In the molecular network of DL, there is one unique signaling pathway, which is the AMPK signaling pathway. The AMPK signaling pathway plays a crucial role in maintaining energy homeostasis and improving inflammation [60]. In atherosclerotic mice, regulating the AMPK signaling pathway can inhibit the expression of NF-kB, IL-1β, and IL-6 at the lesion site, while reducing the infiltration of macrophages in atherosclerotic plaques [61]. The study found that many compounds in DL, especially kaempferol, eflovlolin, ethyl acid, and aureusidin, were involved in

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mediating the main targets of the AMPK signaling pathway, such as TNF, TNFRSF1A, AKT1, PI3KCA, PI3K3CGC, and ADORAI. These targets represent a group of potential drug targets with important physiological functions and pathological significance, indicating that these bioactive compounds may constitute the material basis for DL’s unique pharmacological effects.

Decoding hub nodes of the network model in the four prescriptions

The degree value is an important characterization parameter of TCM in the biological network. Nodes with high values typically play a significant role in various biological processes, such as immune function and lipid metabolism in the cardiovascular diseases network [62]. In this study, we have listed the top 13 nodes with the highest degree in the PBSS-related cardiovascular diseases network model (Figure 3), and these nodes are associated with four prescriptions. In contrast to the pathway analysis results, DL stands out more prominently in terms of targeting, regulating 8 out of 13 targets, namely STAT3, VEGFA, IL1B, SRC, EGFR, HIF1A, TNF, and IL6.

**Shared target hubs of pharmacogenomics.** According to the four colors of lines in Figure 3, shared target hubs of the four prescriptions (VEGFA, SRC, EGFR, and TNF) are exactly unique in the GLXBBJ.

VEGF-A is part of the VEGF family, which promotes endothelial growth, accompanied by higher vascular permeability, and therefore represents an important factor for angiogenesis and vascularization [63]. Experimental evidence supports a positive connection between serum VEGF-A levels and increased microvessel density in the infarcted area [64, 65]. Trials conducted on rabbit models have confirmed VEGF-A as a marker of atherosclerosis [66]. The regulation of angiogenesis by VEGF-A is of paramount importance in the potential treatment of cardiovascular diseases, particularly through strategies targeting the blocking of intraplaque angiogenesis.

SRC is classified as a non-receptor tyrosine kinase that falls under the category of SRC family kinases. A wide range of investigations has attested to SRC’s involvement in various immunologic processes, such as immune cell adhesion, development, migration, phagocytosis, proliferation, chemotaxis, as well as survival [67]. It is the activity of SRC that engages in cardiovascular homeostasis maintenance by regulating important processes, including effecrocytosis, foam cell formation, and proinflammatory cytokine expression, instability, and lesion development in a mouse model of atherosclerosis [68]. The concentration of SRC activation lies in diverse constituents of the reperfusion injury salvage kinase pathway, which has been confirmed as an untapped therapeutic target for safeguarding the heart against ischemic/reperfusion injury [69]. Flavonoids have garnered attention in cardiovascular disease research due to their potential to reduce risks. They achieve this through anti-coagulant and anti-platelet actions, as well as anti-inflammatory effects, by interacting with specific residues in the catalytic site of kinases and ultimately inhibiting SRC activity [70].

The EGFR family and its ligands are important hubs that regulate various cellular processes [71]. Since EGFR ligands are associated with human atherosclerosis, EGFR is considered to be an important mediator in the pathogenesis of human atherosclerosis. LOX-1 expression was reduced when EGFR phosphorylation inhibitors were used, suggesting that EGFR may be involved in foam cell transformation, cell dysfunction, and smooth muscle cell proliferation during atherosclerosis [72]. Transactivation of EGFR signaling is also observed in several cardiovascular diseases, including hypertension, heart failure, etc [73]. Statin-induced cholesterol depletion also modestly activates EGFR, leading to the downregulation of EGFR expression as an important target of statins [74].

TNF plays a significant role as a mediator and regulator of mammalian immune responses in both healthy and pathological conditions. It promotes immune system development, initiates critical signaling pathways for cell survival, orchestrates cellular proliferation, and regulates metabolic activities. Elevated plasma-soluble TNF concentrations, as well as its soluble receptors, are thought to predict cardiovascular events. TNF may have an important role in the etiology of cardiovascular illnesses due to its proinflammatory qualities as well as its ability to cause dyslipidemia and insulin resistance. TNF levels in the blood have been found to be elevated in people with early coronary artery disease and in atherosclerotic plaques [75]. Plasma TNF levels are linked to atherosclerosis, cancer, inflammatory diseases, insulin resistance, lipolysis, and plasma triglyceride and very low-density lipoprotein concentrations [76].

IL6 is the shared target hub among GLXBBJ, ZSXBGZ, and DL. Furthermore, IL6 operates as an intermediary in the acute phase response, serving as a versatile cytokine involved in a variety of activities including inflammation, metabolic regulation, brain functions, cellular development, and hypertrophy. IL-6 dysregulation has been linked to disorders such as insulin resistance, blood pressure irregularities, vascular hypertrophy, and autoimmune diseases [77, 78].

HIF1A is the shared target hub between ZSXBGZ and DL. HIF-1A is a transcriptional factor that acts as a transcription factor, regulating the expression of over 100 target genes, including VEGF, NOS2, and several genes encoding antioxidant enzymes [79]. Notable study findings demonstrate increased expression of HIF-1A in people with coronary artery disease, implying a link between HIF-1A levels, atherosclerosis severity, and increased coronary collaterals [80]. HIF-1A activation causes an increase in VEGF production, which increases insulin resistance and regulates intimal angiogenesis. As a result, mural bleeding, plaque rupture, and the onset of acute coronary syndrome occur [81].

**Unique target hubs of pharmacogenomics.** STAT3 and IL1B are the exclusive hubs of DL. STAT3 plays a central role in transmitting extracellular signals from the plasma membrane to the nucleus, where it regulates various biological processes. A growing body of evidence suggests that STAT3 not only plays an important role in cardiac preservation but also in the regulation of inflammation associated with the heart. Notably, studies have shown that activating STAT3 helps maintain capillary integrity, protects the heart from ischemia and reperfusion injury in vivo, and improves myocardial proinflammatory signaling and subsequent recovery [82]. Another study demonstrated that inhibiting STAT3 can reduce VSMC migration and proliferation, as well as inflammation in macrophages, attenuating nicotine-induced atherogenesis [83]. Among the IL-1 family members, interleukin-1B (IL-1B) is mainly synthesized by mononuclear phagocytes and smooth muscle cells. Its production is triggered by multiple factors, including microbes, uric acid, or cholesterol crystals, due to their stimulatory effects. The induction of an inflammatory response in endothelial cells by IL1B facilitates the recruitment of inflammatory cells into blood vessels and their subsequent invasion into the local intima, exerting multiple effects at every stage of atherosclerosis [84]. Clinically relevant recent surveys show that targeted therapy directly aimed at IL-1B can significantly ameliorate inflammatory damage and reduce vascular events [85, 86].

**Conclusions**

Based on network pharmacology and pathology, this study initially explores the scientific significance of PBSS and the mechanisms underlying the treatment of PBSS-related cardiovascular diseases by four prescriptions. This allows us to understand why these prescriptions have been prominently featured in the first-line clinical practice of TCM. GLXBB-prescriptions are involved in nearly all pathological stages of PBSS-related cardiovascular diseases by modulating high-frequency shared pathways and targets, primarily through key compounds such as quercetin, maodenol, sitosterol acetate, and luteolin. For example, they participate in the processes of atherosclerosis, lipid metabolism, inflammation, immune response, thrombosis, inhibit inflammatory factors and platelet aggregation, and regulate immune functional activity, vascular function, and oxidative stress. Consequently, we have discovered that the four GLXBB-prescriptions can treat PBSS through multiple components, multiple targets, and multiple pathways. In addition to their common pharmacological...
characteristics, high-frequency crosstalk is an important feature of the signaling pathways regulated by GLXBBX. In particular, the cAMP signaling pathway is considered to be a bridge connecting multiple signaling pathways, which could be related to intercellular communication among cardiomyocytes, vascular endothelial function, and thrombosis. ZXSBGZ focuses on the regulation of sphingolipid metabolism, while DL concentrates on maintaining energy homeostasis and regulating oxidative stress through the AMPK signaling pathway and STAT3 target.

In summary, network pharmacology has enhanced our understanding of the scientific significance of PBSS and revealed both similarities and differences in the mechanisms underlying the treatment of PBSS-related cardiovascular diseases by four different prescriptions. Furthermore, these findings may provide a theoretical foundation for the distinctive characteristics of GLXB prescriptions, guiding their clinical use in the treatment of PBSS and its associated cardiovascular diseases.

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