A Network Pharmacology Approach to Explore the Mechanisms of Qishen Granules in Heart Failure

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Data Collection B
Statistical Analysis C
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Source of support: This work was financially supported by the Grants from the National Natural Science Foundation of China (No. 81530100 and 81673712), Significant New Drugs Creation (No.2018ZX09201008-001-011), Fok Ying Tung Education Foundation (No. 151044), Beijing Nova program (Z171100001117028), Talent Young Scientist of China Association for Science and Technology (No.CACM-2017-QNRC2-C13), and excellent young scientist foundation of BUCM (No. 2016-JYB-XJ003)

Background: This study aimed to investigate the intrinsic mechanisms of Qishen granules (QSG) in the treatment of HF, and to provide new evidence and insights for its clinical application.

Material/Methods: Information on QSG ingredients was collected from Traditional Chinese medicine systems pharmacology (TCMSP), TCM@Taiwan, TCMID, and Batman, and input into SwissTargetPrediction to identify the compound targets. HF-related targets were detected from Therapeutic Target Database (TTD), Disgenet-Gene, Drugbank database, and Online Mendelian Inheritance in Man (OMIM) database. The overlap targets of QSG and HF were identified for pathway enrichment analysis by utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The protein-protein interaction (PPI) network of QSG-HF was constructed, following by the generation of core targets, construction of core modules, and KEGG analysis of the core functional modules.

Results: There were 1909 potential targets predicted from the 243 bioactive compounds in QSG which shared 129 common targets with HF-related targets. KEGG pathway analysis of common targets indicated that QSG could regulated 23 representative pathways. In the QSG-HF PPI network analysis, 10 key targets were identified, including EDN1, AGT, CREB1, ACE, CXCR4, ADRBK1, AGTR1, BDKRB1, ADRB2, and F2. Further cluster and enrichment analysis suggested that neuroactive ligand-receptor interaction, cGMP-PKG signaling pathway, renin secretion, vascular smooth muscle contraction, and the renin-angiotensin system might be core pathways of QSG for HF.

Conclusions: Our study elucidated the possible mechanisms of QSG from a systemic and holistic perspective. The key targets and pathways will provide new insights for further research on the pharmacological mechanism of QSG.

MeSH Keywords: Heart Failure • Medicine, Chinese Traditional • Pharmacologic Actions

Abbreviations: QSG – Qishen granules; TCM – traditional Chinese medicine; HF – heart failure; KEGG – Kyoto Encyclopedia of Genes and Genomes; PPI – protein-protein interaction; TAMP – TCM systems pharmacology database; TIMED – TCM information database; TTD – Therapeutic Target Database; OMAN – Online Mendelian Inheritance in Man

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/919768

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Background

Heart failure (HF), a clinical syndrome that symbolizes the final stage of cardiovascular diseases, has posed a huge threat to global public health [1,2]. Despite the therapeutic developments that have been made in the past few decades, the prevalence of HF continues to increase [3,4]. HF has extremely complex pathophysiological mechanisms, and the drugs recommended in current guidelines, such as angiotensin-converting enzyme inhibitors, beta-blockers, and spironolactone, tend to directly target limited molecules and pathways. Combinations of these drugs are often prescribed to achieve better effect, however, can cause high medical burden and side effects, and increase the economic burden to patients [5,6]. Hence, potential strategies for HF are extremely necessary.

Traditional Chinese medicine (TCM) has been used in the management of cardiovascular diseases for more than 2000 years [7]. Due to its multiple components, targets, and pathways, TCM treatment of HF is drawing more and more attention [8]. The combination of TCM and modern medicine is widely used in China, and it has shown some advantages such as fewer side effects and better efficacy [9].

Qishen granules (QSG), consists of 6 TCM herbs, and is a Chinese herbal formula that has been widely prescribed to treat HF for decades [10]. Several studies have suggested that the possible mechanisms of QSG for treatment of HF are mainly mediated via exerting anti-myocardial fibrotic, anti-apoptotic, and anti-inflammatory effects [11–13]. Nevertheless, the exact mechanisms that underlie the effects of QSG on HF are elusive. Owing to the complexity of the compounds in QSG, its features pose a huge challenge to comprehend and illustrate the internal molecular mechanisms [14,15].

Although the development and application of analytical chemistry and chemical biology have made it possible to identify the bioactive ingredients and biological targets for herbal formulae, it is still difficult to systematically elucidate the various components of herbal medicines and their roles in complex diseases [16]. Fortunately, the rapid development of bioinformatics provides a solution for this. Network pharmacology which can make the drug discovery process predictable and systematic study of herbal formulae achievable, has been employed frequently to uncover the underlying molecular mechanism of herbal formulations from a holistic level [17,18]. For Chinese herbal formulas, it can interpret the overall regulatory role and the relationship between components by integrating information from different databases [19].

Therefore, we applied network pharmacology analysis to in-depth clarify the intrinsic mechanisms of QSG acting on HF in the present study. We first screened several databases for bioactive components of QSG and targets prediction, followed by retrieval of known HF-related targets, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the overlap targets between QSG and HF, the QSG-HF protein-protein interaction (PPI) network construction, and the KEGG pathway enrichment analysis of the core targets in QSG-HF PPI network, in turn. The workflow is illustrated in Figure 1.

Material and Methods

Identification of bioactive QSG components

QSG, also published under the name of Yixin Jiedu Decoction, Qishenkeli and Qishenyiqi in the past, consists of 6 species of medicinal herbs: *Hedysarum Multijugum Maxim* (Huangqi), *Radix Salviae* (Danshen), *Lonicerae Japonicae Flos* (Jinyinhua), *Figwort Root* (Xuanshen), *Aconiti Lateralis Radix Praeparata* (Fuzi), and *licorice* (Gancao). The compounds of the 6 herbs identified in QSG were obtained from the TCM systems pharmacology database and analysis platform (TCMSP, *http://ibts.hkbu.edu.hk/LSP/tcmsp.php*), the TCM Database@Taiwan (*http://tcm.cmu.edu.tw*), the TCM information database (TCMID, *http://bidd.nus.edu.sg/group/TCMsite/*), and the Bioinformatics analysis tool for molecular mechanism of TCM (Batman, *http://bionet.ncpsb.org/batman-tcm/*). They contain comprehensive information of all herb ingredients for drug screening and evaluation [20]. Oral bioavailability and drug-likeness were selected for identification of bioactive ingredients. The common screening criteria are oral bioavailability ≥30% and drug-likeness ≥0.18 [21]. Additionally, the names of compounds were standardized according to PubChem CIDs (*https://pubchem.ncbi.nlm.nih.gov/*).

Potential QSG-related targets and known HF-related targets

Here, SwissTargetPrediction (a tool for target prediction according to 2-dimensional and 3-dimensional similarity measures with known ligands) was selected to predict potential targets for QSG bioactive ingredients [22,23]. Thereafter, the protein names of the QSG bioactive ingredients were converted to gene names using the UniProt Knowledgebase (UniProtKB, *http://www.uniprot.org/*) and species was restricted to "Homo sapiens" so that name standardization and deduplication could be achieved based on the UniProt number.

Known targets related to HF were screened using "heart failure" as the keyword from the Therapeutic Target Database (TTD, *http://systemsdock.unit.osit.jp/iddp/home/index*), the Disgenet-Gene, the Drugbank database (*https://www.drugbank.ca*), and the Online Mendelian Inheritance in Man (OMIM, *http://omim.org/*). Deduplication was performed, after normalizing the targets numbers according to UniProt Knowledgebase.
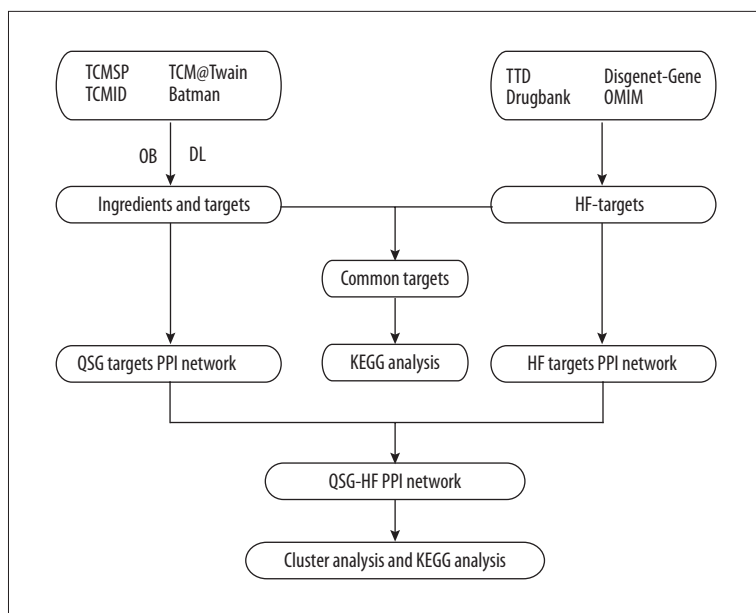
Figure 1. Diagram of the network pharmacology-based strategies for determining the pharmacological mechanisms of the QSG on heart failure through cluster and pathway analysis.

PPI network construction

Three PPI networks were constructed for the purpose of further exploration of pharmacological mechanisms including: 1) QSG targets PPI network, 2) HF targets PPI network, and 3) QSG-HF PPI network. First, the predicted QSG-targets and screened HF-targets were utilized as hub proteins and submitted to String (https://string-db.org/), with species limited to "Homo sapiens" and a confidence score ≥0.7 [24]. Second, the 2 PPI interactive networks were constructed and visualized by Cytoscape 3.2.1 (http://www.cytoscape.org/). Finally, after merging these 2 networks as a candidate network according to the intersection of PPI data, the QSG-HF PPI network was built. Topological features of these PPI networks were analyzed mainly based on degree which can reflect the importance of nodes' biological function [25,26].

Cluster analysis

Network modules or clusters refer to sets of highly interconnected nodes which can help discover and reveal hidden biological information within the network [27]. Module identification which can reduce the complexity of complex networks and avoid information loss during network integration, has been considered as one of the key factors in understanding biological systems [28]. Core modules were identified by finding modules that consist of closely linked, biologically similar targets in the QSG-HF PPI network, using ClusterONE in Cytoscape 3.2.1 [29].

Enrichment analysis

KEGG signaling pathway analysis was performed on the overlap targets of QSG and HF and the identified core functional modules of QSG-HF PPI network respectively, using ClueGO plug-in in Cytoscape 3.2.1. *P*<0.01 was regarded as threshold.

Results

Bioactive components in QSG

Although there are thousands of ingredients in TCM prescriptions, only a few are in accord with satisfactory pharmacokinetic and pharmacodynamic characteristics that ultimately determine efficacy [30]. There were 259 bioactive components from QSG collected, including 28, 71, 26, 10, 29, and 95 from Huangqi, Danshen, Fuzi, Xuanshen, Jinyinhua, and Gancao, respectively. After excluding duplicates, 243 candidate components were selected for further analysis.

Potential targets of QSG and known HF-related targets

A total of 2751 corresponding potential targets of these 243 bioactive components were explored. After the repetition was removed, 1909 potential targets were retained. HF-related gene and protein targets were obtained from 4 databases, including 5 targets from TTD, 73 targets from the Disgenet-Gene, 84 targets from the Drugbank, and 121 targets from the OMIM. After removing duplicates, 262 HF-related targets were collected. Among these, 129 common targets were shared between potential targets of QSG and known HF-related targets (Figure 2).

KEGG pathway enrichment analysis of common targets

Through a KEGG pathway enrichment analysis of these 129 common targets, 58 pathways of significance were identified.
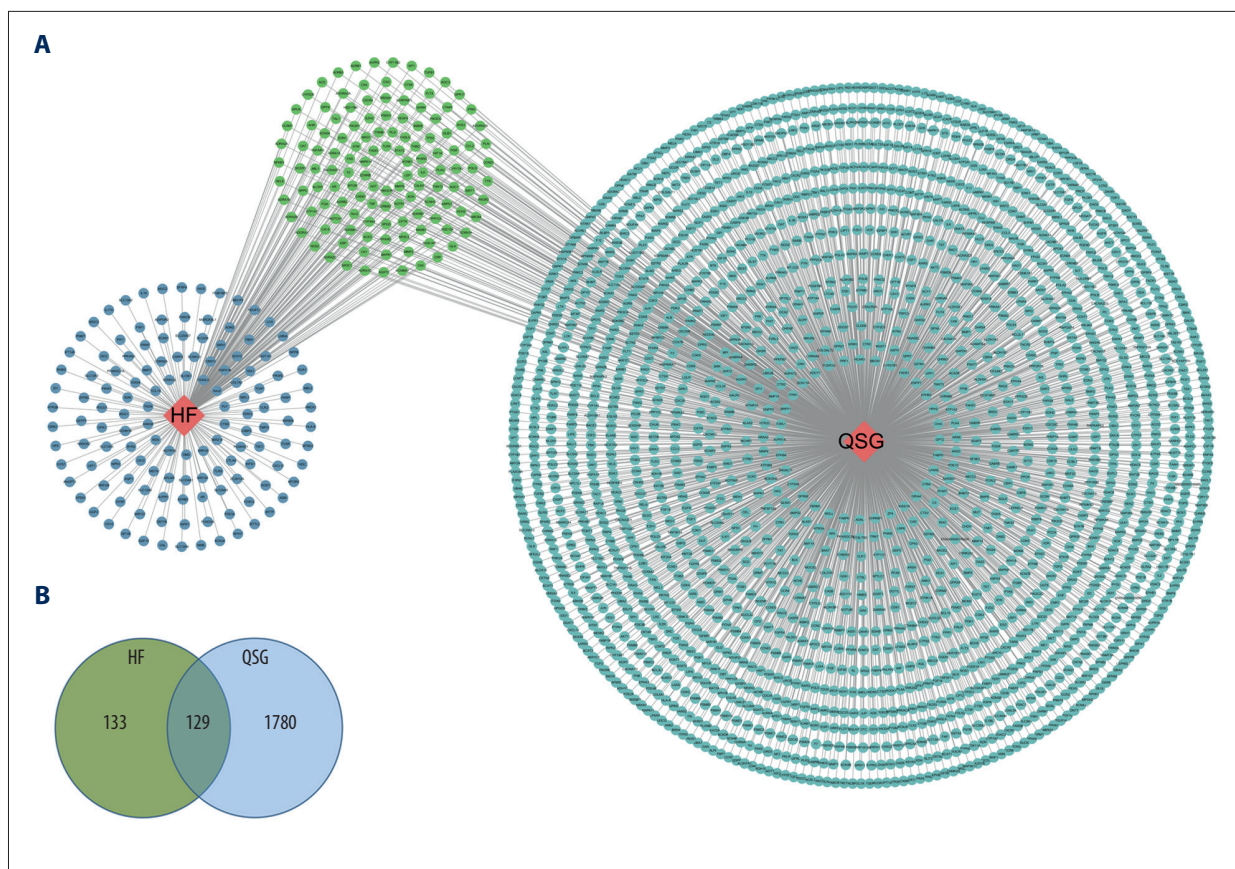
Figure 2. QSG and heart failure (HF) related targets and overlapping targets. (**A**) The network of QSG and HF related targets. The circle on the left represents HF-related targets from 4 databases, the circle on the right indicate associated targets of QSG, and the circle in the middle indicates the potential targets of QSG in the treatment of HF. (**B**) The number of QSG and HF related targets were shown in Venn diagram. There were 1909 targets related to QSG and 262 targets related to HF, and 129 targets shared by both.

After ranking by gene count, a total of 23 representative pathways were screened (Table 1, Figure 3). Four pathways were mainly associated with cardiovascular functions: cGMP-PKG signaling pathway, fluid shear stress and atherosclerosis, vascular smooth muscle contraction, and Apelin signaling pathway. Four pathways were mainly related to inflammation: TNF signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, and IL-17 signaling pathway. Four pathways were mainly involved in glycolipid energy metabolism: AMPK signaling pathway, insulin resistance, and regulation of lipolysis in adipocytes. Nine pathways were mainly relevant to neuro-humoral factor: neuroactive ligand-receptor interaction, adrenergic signaling in cardiomyocytes, calcium signaling pathway, cAMP signaling pathway, renin secretion, thyroid hormone signaling pathway, neurotrophin signaling pathway, aldosterone-regulated sodium reabsorption, and renin-angiotensin system, Two pathways were mainly associated with cell proliferation, differentiation, apoptosis: FoxO signaling pathway and apoptosis.

PPI network analysis

PPI network can visualize and quantify the function of specific proteins in cells at the systematic level [31]. We constructed QSG and HF-related targets network with PPI databases (Figure 4A, 4B). Further, the interactive QSG-HF PPI network was finally obtained after merging these 2 PPI networks (Figure 4C). The results suggested that the QSG-HF interactive PPI network consisted of 57 nodes and 299 edges. Among the 57 QSG core targets, the top 10 targets and corresponding herbs and ingredients were generated and summarized according to degree in Table 2. Furthermore, the herb-compound-target network was established based on the top 10 targets in Figure 5.

KEGG pathway enrichment analysis of core modules

In order to identify the potential mechanism of the 57 key targets, the final central PPI network was divided into 5 clusters. KEGG pathway enrichment analysis were performed on

Table 1. 23 representative pathways according to gene count.

GO ID	Pathway	P-value	Gene count	Associated genes
KEGG: 04022	cGMP-PKG signaling pathway	1.90E-13	22	ADORA1, ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, AGTR1, ATP1A1, CALM1, CREB1, EDNRB, KCNMA1, MAPK1, NPR1, PDE3A, PIK3CG, PLN, PPP1CA
KEGG: 05418	Fluid shear stress and atherosclerosis	1.11E-13	21	CALM1, CCL2, CTNNB1, EDN1, GSTP1, HMOX1, IFNG, IKBKB, MAPK14, MMP9, NFKB1, NPPC, PIK3R1, PIK3R2, PIK3R3, SELE, THBD, TNF, TP53, VCAM1, VEGFA
KEGG: 04080	Neuroactive ligand-receptor interaction	3.92E-08	21	ADORA1, ADORA2A, ADORA2B, ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, AGTR1, AVPR2, BDKRB1, EDNRB, F2, HTR2B, LEP, NR3C1, PLG
KEGG: 04261	Adrenergic signaling in cardiomyocytes	1.47E-11	19	ADRA1A, ADRA1B, ADRA1D, ADRB1, ADRB2, AGTR1, ATP1A1, CACNA2D1, CALM1, CREB1, KCNE1, MAPK1, MAPK14, PIK3CG, PLN, PPP1CA, RYR2, SCN5A, TNNT2
KEGG: 04668	TNF signaling pathway	8.87E-13	18	CCL2, CREB1, EDN1, FADD, FAS, IKBKB, IL6, MAPK1, MAPK14, MMP9, NFKB1, PIK3R1, PIK3R2, PIK3R3, SELE, TNF, TNFAIP3, VCAM1
KEGG: 04020	Calcium signaling pathway	6.25E-09	18	ADORA2A, ADORA2B, ADRA1A, ADRA1B, ADRA1D, ADRB1, ADRB2, ADRB3, AGTR1, BDKRB1, CALM1, EDNRB, ERBB2, HTR2B, NOS2, PLN, RYR1, RYR2
KEGG: 04024	cAMP signaling pathway	2.38E-08	18	ADORA1, ADORA2A, ADRB1, ADRB2, ATP1A1, CALM1, CFTR, CREB1, MAPK1, NFKB1, NPR1, PDE3A, PIK3R1, PIK3R2, PIK3R3, PLN, PPP1CA, RYR2
KEGG: 04066	HIF-1 signaling pathway	3.38E-12	17	EDN1, ERBB2, HIF1A, HMOX1, IFNG, IL6, MAPK1, MTOR, NFKB1, NOS2, PIK3R1, PIK3R2, PIK3R3, SERPINE1, STAT3, TLR4, VEGFA
KEGG: 04924	Renin secretion	9.19E-12	14	ACE, ADORA1, ADRB1, ADRB2, ADRB3, AGT, AGTR1, AQP1, CALM1, CREB1, KCNMA1, NPR1, PDE3A, REN
KEGG: 04620	Toll-like receptor signaling pathway	5.72E-08	13	CTSK, FADD, IKBKB, IL6, MAPK1, MAPK14, NFKB1, PIK3R1, PIK3R2, PIK3R3, TLR4, TLR9, TNF
KEGG: 04152	AMPK signaling pathway	3.50E-07	13	ADRA1A, CCND1, CFTR, CREB1, GYS1, HMGCR, LEP, MTOR, PIK3R1, PIK3R2, PIK3R3, PPARG, SIRT1
KEGG: 04068	FoxO signaling pathway	9.63E-07	13	ATM, CCND1, FASLG, IKBKB, IL6, MAPK1, MAPK14, PIK3R1, PIK3R2, PIK3R3, SIRT1, STAT3, TGFB1
KEGG: 04210	Apoptosis	1.60E-06	13	ATM, CTSK, FADD, FAS, FASLG, IKBKB, MAPK1, NFKB1, PIK3R1, PIK3R2, PIK3R3, TNF, TP53
KEGG: 04931	Insulin resistance	6.33E-07	12	CREB1, GYS1, IKBKB, IL6, MTOR, NFKB1, PIK3R1, PIK3R2, PIK3R3, PPP1CA, STAT3, TNF
KEGG: 04919	Thyroid hormone signaling pathway	1.52E-06	12	ATP1A1, CCND1, CTNNB1, HIF1A, MAPK1, MTOR, NOTCH1, PIK3R1, PIK3R2, PIK3R3, PLN, TP53
KEGG: 04722	Neurotrophin signaling pathway	2.00E-06	12	ABL1, CALM1, FASLG, IKBKB, MAPK1, MAPK14, NFKB1, PIK3R1, PIK3R2, PIK3R3, PSEN1, TP53
KEGG: 04657	IL-17 signaling pathway	1.11E-06	11	CCL2, FADD, IFNG, IKBKB, IL6, MAPK1, MAPK14, MMP9, NFKB1, TNF, TNFAIP3
KEGG: 04270	Vascular smooth muscle contraction	1.49E-05	11	ADORA2A, ADORA2B, ADRA1A, ADRA1B, ADRA1D, AGTR1, CALM1, KCNMA1, MAPK1, NPR1, PPP1CA

Table 1 continued. 23 representative pathways according to gene count.

| GO ID	Pathway	P-value	Gene count	Associated genes
KEGG: 04371	Apelin signaling pathway	5.11E-05	11	AGTR1, APLN, CALM1, CCND1, MAPK1, MTOR, NOS2, PIK3CG, RYR1, RYR2, SERPINE1
KEGG: 04211	Longevity regulating pathway	4.71E-05	9	CREB1, MTOR, NFKB1, PIK3R1, PIK3R2, PIK3R3, PPARG, SIRT1, TP53
KEGG: 04923	Regulation of lipolysis in adipocytes	6.28E-06	8	ADORA1, ADRB1, ADRB2, ADRB3, NPR1, PIK3R1, PIK3R2, PIK3R3
KEGG: 04960	Aldosterone-regulated sodium reabsorption	4.51E-06	7	ATP1A1, MAPK1, NR3C2, PIK3R1, PIK3R2, PIK3R3, SCNN1A
KEGG: 04614	Renin-angiotensin system	3.03E-06	6	ACE, ACE2, AGT, AGTR1, MME, REN
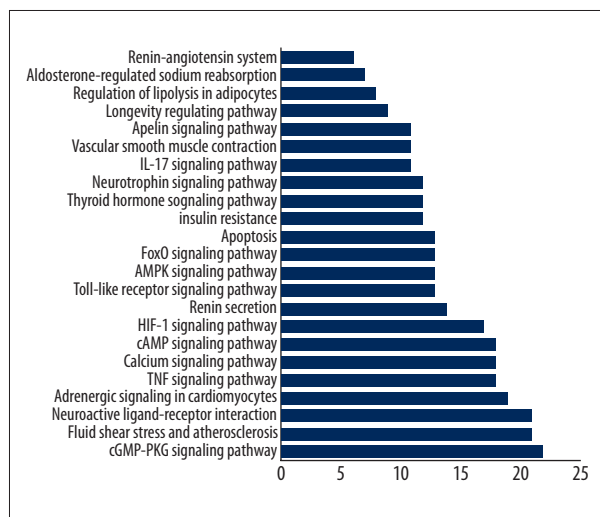
Figure 3. 23 pathways screened after sorting by gene count. The abscissa indicates the number of genes associated.

3 clusters which $P<0.01$ (Figure 6A). Through KEGG pathway enrichment of the 3 modules, after sorting by gene count, the top 5 were collected. The top 5 KEGG pathways were neuroactive ligand-receptor interaction, cGMP-PKG signaling pathway, renin secretion, vascular smooth muscle contraction, and renin-angiotensin system (Figure 6B, Table 3).

Discussion

Preliminary analysis based on common targets

This is the first time for us to systematically explore the internal mechanism of QSG on HF via network pharmacology method, which can provide direction and insights for subsequent basic and clinical researches. With the strategy of database mining, we identified 243 candidate ingredients and their 1909 targets by integrating the most widely used databases. Among these 1909 targets, 129 shared targets of QSG and HF were identified and implemented for KEGG analysis. The enriched pathways confirmed the role of QSG in multi-channel and multi-target regulation. It's worth noting that among the 23 pathways screened, the regulation of QSG on vascular smooth muscle contraction [32], renin secretion or renin-angiotensin system [12,33,34], calcium signaling pathway [35], apoptosis and apoptosis related Bcl-2, Bax, P53, caspase-3 [11], inflammation related TNF signaling pathway, NF-κB, IL-6 [34] have been validated in our previous studies. However, the remaining pathways have yet to be confirmed. This means that the mechanisms of QSG at the system level are still under investigation.

Further analysis based on core targets

In the core QSG-HF PPI network, 57 QSG core targets were obtained and the top 10 targets were screened according to degree. Among the 10 genes, EDN1, a peptide that involves in maintaining vascular tone and cardiovascular system homeostasis [36,37], had the largest value of degree, implicating its critical role in the QSG-HF PPI network. It was targeted by 7 molecules which originated from 4 herbs including Dihydrokaranone (CID177072) from Danshen, 3-methyl-6,7,8-trihydropyrrolo[1,2-A]pyrimidin-2-one (CID 5319799) from Gancao, FA (CID 6037) and canavanine (CID 46224610) from Huangqi, Beta-Pinene (CID14896), 1-hexene (CID 11597) and stigmasterol (CID 5280794) from Jinyinhua. Different components derived from different herbs can target common targets, indicating that QSG can regulate disease targets through synergistic effects of multiple components. On the other hand, the same molecule can act on different targets. For example, Miltirone originating from Danshen, can work on targets that participate in multiple pathways, such as AGT, AGRI, ACE, ADOBE, and FT. This suggests that QSG interferes with HF through a multi-target approach. From the perspective of

Figure 4. Protein-protein interaction (PPI) networks of QSG and heart failure (HF). (**A**) Green represents QSG-related targets PPI network with 890 nodes and 7121 edges. (**B**) Red represents HF-related targets PPI network with 199 nodes and 1015 edges. (**C**) Interactive PPI network of QSG and HF with 57 nodes and 299 edges: nodes indicate target proteins or genes; edges represent correlations between targets; the size of the nodes indicates the value of degree.

network pharmacology, these 10 genes are the key targets for the treatment of HF at the molecular level, and the key for uncovering the pharmacological mechanisms of QSG.

Enrichment analysis based on core modules

We applied module partitioning and analysis to the 57 targets to understand their biological mechanisms. KEGG analysis of the core modules screened 5 key signaling pathways. The neuroactive ligand receptor interaction signaling pathway, which covers a quantity of genes, can mediate cardio-protection [38]. Research has reported that regulation of neuroactive ligand-receptor interaction may protect patients' damaged cardiac function after coronary artery bypass grafting [39]. cGMP is a second messenger widely present in cells, and its stimulation can alleviate myocardial ischemia-reperfusion injury during the process of myocardial infarction [40,41]. The protective role of inhibiting renin secretion or renin-angiotensin system

during HF has been recognized, and relevant drugs have been widely used in clinical practice [42,43]. Vascular smooth muscle contraction pathway that is closely associated with vascular remodeling, may play a significant role in regulating hemodynamics [44,45]. Furthermore, genes identified by network pharmacology analysis that may be involved in the corresponding pathway are also listed in the tables. These genes will be the key points of interest for follow-up researches.

Limitations

In this study, we confirmed the effect of QSG on HF at the molecular level by means of network pharmacology, and systematically expounded its possible mechanism. However, there are still some limitations that exist in this study. First, the acquisition of bioactive ingredients is based on existing databases and literature, rather than expanding the database by liquid chromatography, mass spectrometry, or other new methods for

Table 2. Top 10 genes of QSG-HF PPI network according to degree.

Gene	Degree	Herbs	Molecule (PubChem CIDs)
EDN1	30	Danshen	CID 177072
		Gancao	CID 5319799
		Huangqi	CID 6037; CID 46224610
		Jinyinhua	CID14896; CID 11597; CID 5280794
AGT	26	Danshen	CID 160142; CID 3082765
		Gancao	CID 5319799; CID 72301
		Jinyinhua	CID 1549018
CREB1	24	Huangqi	CID 190
ACE	23	Danshen	CID 19010403222; CID 11683160; CID 160142; CID 5320066; CID 3082765; CID 5320113; CID 5319835; CID 5320114
		Fuzi	CID 20055981
		Gancao	CID 5319013; CID 5417
CXCR4	22	Gancao	CID 5318999
		Huangqi	CID 71448940; CID 13943299; CID 71448939; CID 441905; CID 5988; CID 14241100
ADRBK1	21	Danshen	CID 177072
		Gancao	CID 5319799
		Jinyinhua	CID 244; CID 6054; CID 1549018
AGTR1	19	Danshen	CID 6709746; CID 44425165; CID 160142; CID 3082765
		Fuzi	CID 76963334; CID 138111911
		Gancao	CID 5319013; CID 5317478; CID 503731; CID 5481949; CID 5322053
		Huangqi	CID 73299
		Jinyinhua	MOL003128
BDKRB1	17	Danshen	CID 124268; CID 3083515; CID 3083514
		Gancao	CID 442411
ADRB2	17	Danshen	CID 94162; 5318290; 94162; 11600642; CID 160142
		Fuzi	CID 441737; CID 441742; CID 165581; CID 91588; CID 4076
		Gancao	CID 5481948; CID 5281789; CID 5481234; CID 5317300
		Xuanshen	CID 6992089
F2	17	Danshen	CID 126071; CID 3083515; CID 160142; CID 3082765
		Fuzi	MOL002434; CID 6324887
		Gancao	CID 5318679; CID 10881804; CID 637112; CID 5281619;CID 5317479; CID 14604077; CID 14604081; CID 503731; CID 5316900; CID 5481949
		Huangqi	CID 5280343; CID 5318869; CID 5281654; CID 15689655; CID 108213
		Jinyinhua	MOL003108; MOL003117; CID 334457
		Xuanshen	CID 6450157

The names of all molecules were represented as PubChem CID numbers. The molecules without PubChem CID were represented as the MOL number.
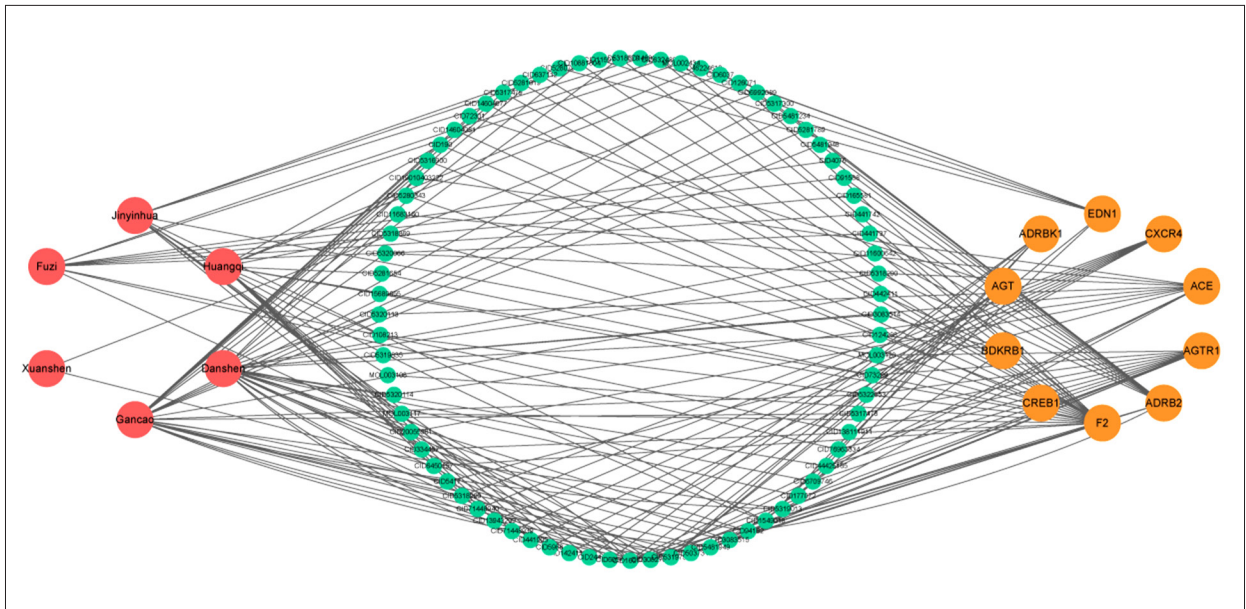
Figure 5. The herb-compound-target network was established based on the top 10 genes of QSG-heart failure (HF) protein-protein interaction (PPI) network. The red represents 6 herbs in QSG; yellow represents the top 10 target genes; green indicates active compounds. The link represents the interaction among compounds, genes and herbs.
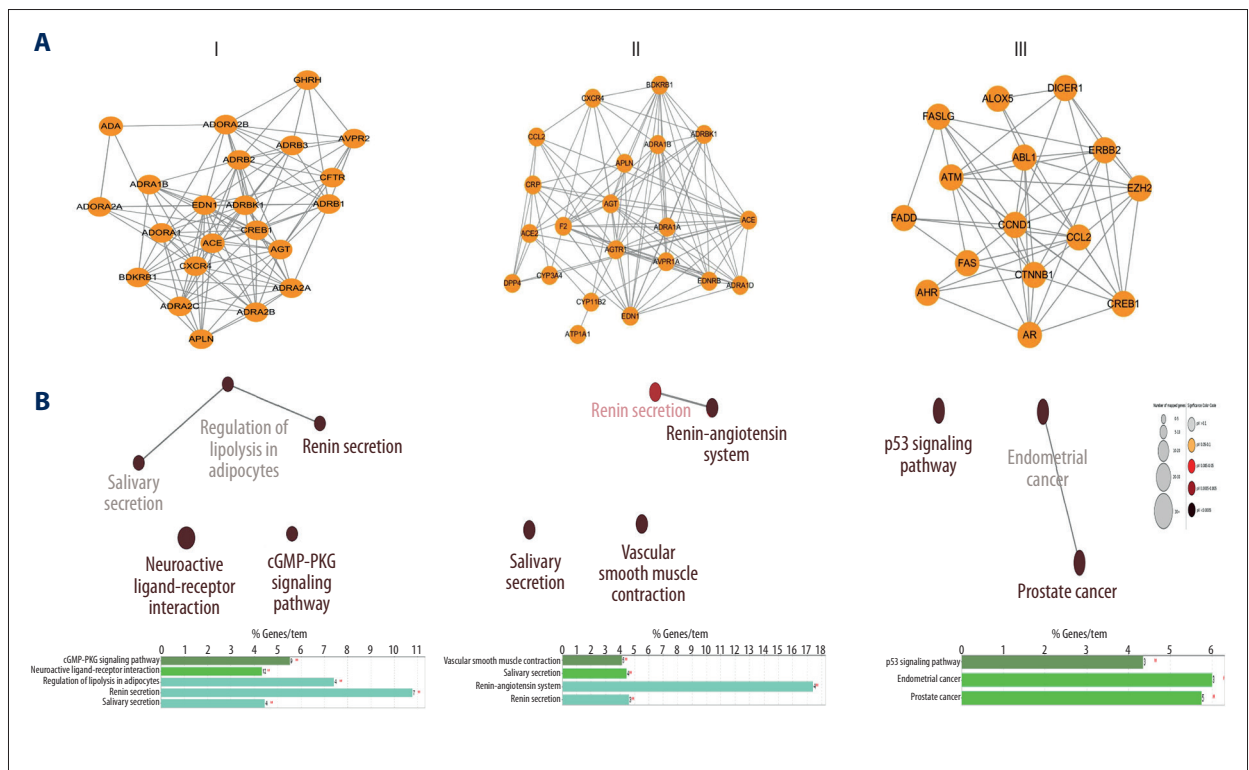

Figure 6. Clusters of core targets protein-protein interaction (PPI) network. (**A**) 3 core clusters of the final central PPI network. (**B**) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the core clusters. The size of the nodes and depth of color is proportional to the number of mapped genes and significance, respectively.

Table 3. Top 5 KEGG pathways among the core modules according to gene count.

GO ID	Pathway	P-value	Gene count	Associated Genes
KEGG: 04080	Neuroactive ligand-receptor interaction	1.89E-12	12	ADORA1, ADORA2A, ADORA2B, ADRA1B, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, AVPR2, BDKRB1
KEGG: 04022	cGMP-PKG signaling pathway	2.94E-10	9	ADORA1, ADRA1B, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, CREB1
KEGG: 04924	Renin secretion	3.72E-10	7	ACE, ADORA1, ADRB1, ADRB2, ADRB3, AGT, CREB1
KEGG: 04270	Vascular smooth muscle contraction	1.57E-05	5	ADRA1A, ADRA1B, ADRA1D, AGTR1, AVPR1A
KEGG: 04614	Renin-angiotensin system	3.72E-07	4	ACE, ACE2, AGT, AGTR1

detecting drug ingredients. Second, although some of the key targets and pathways identified based on network topology parameter analysis have been verified in our previous studies, most of the remaining results are still to be validated, which will be our future research direction.

Conclusions

Our study systematically elaborated the possible mechanisms of QSG, and predicted, screened and analyzed the genes, proteins and pathways that might play a vital role in the biological process. Most importantly, these results provide evidence and new insights for further researches on the pharmacological mechanism of QSG.

Conflict of interest

None.

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