Network Pharmacology-based Systematic Analysis of Molecular Mechanisms of Dingji Fumai Decoction for Ventricular Arrhythmia

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Research

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Abstract

**Background:** Dingji Fumai decoction (DFD), a traditional herbal mixture, has been widely used to ventricular arrhythmia (VA) in clinical practice in China. However, research on the bioactive components and underlying mechanisms of DFD in VA is still scarce.

**Methods:** Components of DFD were collected from TCMSP, ETCM, and literature. Then, the chemical structures of each component were obtained from PubChem. Next, SwissADME and SwissTargetPrediction were applied for compounds screening and targets prediction of DFD, meanwhile, targets of VA were collected from DrugBank and OMIM. Then, the H-C-T-D network as well as the PPI network were constructed based on the data obtained above. CytoNCA was utilized to filter hub genes and VarElect was used to analyze the relationship between genes and diseases. At last, Metascape was employed for systematic analysis on the potential targets of herbals against VA, and AutoDock was applied for molecular docking to verify the results.

**Results:** A total of 434 components were collected, 168 of which were qualified, and there were 28 shared targets between DFD and VA. Three function modules of DFD were found from the PPI network. Further systematic analysis of shared genes and function modules explained the potential mechanism of DFD in the treatment of VA, molecular docking has verified the interactions.

**Conclusions:** DFD could be employed for VA through mechanisms, including complex interactions between related components and targets, as predicted by network pharmacology and molecular docking. This work confirmed DFD could apply for the treatment of VA and promoted the explaining of DFD for VA in the molecular mechanisms.

1. Introduction

In recent years, cardiovascular diseases are the leading cause of death in China\(^1\). All cardiac conditions, especially ischemic heart disease, can lead arrhythmias\(^2\). Among all arrhythmias, ventricular arrhythmia (VA) has the highest mortality. VA is a common but life-threatening disease, mainly including ventricular premature contraction, ventricular tachycardia, ventricular flutter, and ventricular fibrillation, with a clinical presentation ranges from no symptoms to cardiac arrest\(^2\). VA is usually generated by all-caused enhanced automaticity or abnormal automaticity, myocardial ischemia, delayed afterdepolarizations, and structural heart disease with reentry\(^3\)–\(^5\). To prevent adverse events of VA, millions of patients are treated with beta-receptor-blockers, \(\text{i}_{\text{Na}}\) antagonists, \(\text{i}_{\text{Kr}}\) antagonists, non-dihydropyridine calcium antagonists, and other drugs suggested by 2017 American Heart Association/American College of Cardiology/Heart Rhythm Society guideline\(^2\), but the control of VA is still far from ideal.

Traditional Chinese medicine (TCM) has a more than 2,000 years clinical-based development history\(^6\),\(^7\). Some researchers mentioned that TCM could be used to treat a variety of diseases, including VA\(^8\)–\(^10\). Besides, related studies suggested that compared with western medicine only, patients suffering from
various diseases can benefit more from a TCM and western medicine combined therapy strategy\textsuperscript{11–22}. Moreover, several TCM, such as Shensong Yangxin Capsule\textsuperscript{23}, Wenxin Keli\textsuperscript{24}, have been transformed into commercial products for the treatment of cardiovascular-related diseases. Convenient ways like these greatly promoted the development of TCM worldwide.

Dingji Fumai Decoction (DFD), consisting of \textit{Chuanxiong Rhizoma} (Chuanxiong), \textit{Jujubae Fructus} (Dazao), \textit{Poria Cocos (Schw.)}, \textit{Wolf (Fuling)}, \textit{Cinnamomi Ramulus} (Guizhi), \textit{Silktree Albizia Bark} (Hehuanpi), \textit{Osdraconis (Fossiliaossiamastodi)} (Longgu), \textit{Ostrea Gigas Thunberg} (Muli), \textit{Ziziphi Spinosae Semen} (Suanzaoren), \textit{Radix Polygalae} (Yuanzhi), and \textit{Licorice} (Gancao), is widely used for VA. Our previous work confirmed its efficacy and safety on VA\textsuperscript{25}, and one of the underlying mechanisms is class I antiarrhythmic property\textsuperscript{26}. To make DFD more recognized, it is essential to make the efficacy and safety of DFD clearly. Since multiple components contained in DFD, it can generate interactions on multiple targets. Based on the theory of network pharmacology, we constructed network relationships between "component-target pathway" to explore the mechanisms of drugs or herbs\textsuperscript{27}. Here, we analyzed the mechanisms of DFD in the treatment of VA systematically (Fig. 1). At first, we obtained its components and potential targets against VA. Then, the protein-protein interaction (PPI) of potential targets against VA was constructed. Next, systematic analysis of potential targets and bio-functional modules were conducted. Meanwhile, the interactions between the components of DFD and key targets were confirmed using molecular docking.

2. Methods

2.1 Chemical structures construction

The traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP)\textsuperscript{28} and the Encyclopedia of Traditional Chinese Medicine (ETCM)\textsuperscript{29} are web-based herb databases, providing comprehensive and standardized information for the commonly used herbs. In this study, the components of each herb in DFD were obtained from TCMSP, ETCM, and published literature. To make the components recognizable for the subsequent analysis work, after deduplicating, the structure of each component was collected from PubChem\textsuperscript{30}.

2.2 Gastrointestinal absorption (GA) and drug-likeness (DL) prediction

Increasingly researches founded that TCM despite of their impressive \textit{in vitro} findings demonstrates less or negligible \textit{in vivo} activity, resulting in poor absorption and hence poor bioavailability\textsuperscript{31}. The absorption, distribution, metabolism, and excretion (ADME) of the drug must be considered by the researcher and developer\textsuperscript{32}. Bioabsorption is highly multifactorial, but is primarily driven by GA\textsuperscript{33}. Besides, DL assesses qualitatively the chance for a molecule to be an oral drug with respect to bioavailability\textsuperscript{32}. It was constructed that the estimation of ADME before the drug development studies reduces the possibility of
failure\textsuperscript{34}. In the mechanism explaining of DFD, GA and DL were evaluated using SwissADME. SwissADME is a free tool to evaluate DL, GA, pharmacokinetics, and medicinal chemistry friendliness of small molecules\textsuperscript{32}. Uploaded the structure of each compound to SwissADME, if the prediction results of the component were shown both “high” GA and got “yes” in more than 2 of 5 filters in DL prediction, it met our inclusion criteria and were adopted to the next screening\textsuperscript{32}.

### 2.3 Target prediction and verification

In the treatment of diseases, not all absorbable components work, therefore, we filtered out the components with bioactive from all absorbable components with SwissTargetPrediction, an online tool which can evaluate compounds with a score by fitting a multiple logistic regression on various subsets of known actives to weight structure similarity parameters\textsuperscript{35}. Here, we uploaded the structure of each component to SwissTargetPrediction to predict potential targets of DFD, and all possible targets were adopted.

Online Mendelian Inheritance in Man (OMIM) is a knowledgebase providing the latest information of human genes\textsuperscript{36}, and DrugBank is a freely available and comprehensive web resource providing drug-target as well as drug interaction information\textsuperscript{37}. Taking “ventricular arrhythmia” or “arrhythmia of ventricular origin” as keywords, we recruited VA related targets from OMIM and DrugBank. Taking the intersection of DFD and VA targets, the common targets between DFD and VA were considered the therapeutic targets of DFD against VA, as described previously\textsuperscript{38}.

Protein-protein interactions (PPI) is one of the cores of cellular processing. The analysis of PPI makes the interactions of proteins clearly and helps to explain the function of possible protein complexes or functional modules\textsuperscript{39}. STRING is a web-database providing online analysis of PPI\textsuperscript{39}. Uploaded common targets to STRING to construct the PPI network. Then, the results were imported to Cytoscape (version 3.8.0)\textsuperscript{40}, and CytoNCA plugin was applied to analyze centrality and evaluate protein interaction networks\textsuperscript{41}.

The VarElect online tool can analyze direct and indirect links between genes and diseases\textsuperscript{42}. In this study, the link relationships of potential targets of DFD against VA were analyzed with VarElect, the results helped determine which targets will be included in the next molecular docking.

### 2.4 Biology functional analysis

Since Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) can contribute to the interpretation of system-level data and enable new discoveries\textsuperscript{43}, it is essential to make gene-related information clearly in the DFD mechanism explain. Metascape is a web-based platform providing gene annotation, functional enrichment, and interactome analysis services, monthly database update could keep our analysis results up to date\textsuperscript{43}. In our work, GO and KEGG terms with $P<0.01$ were considered significantly enrichment analyses.

### 2.5 Molecular docking
Molecular docking was used to assess interactions between components and hub targets, the 4 hub targets connected to VA closely were included in. The structures of these targets were collected from Protein Data Bank. AutoDock and PyMOL were employed for molecular docking, PyMOL was used to remove the water molecules, isolate proteins of each molecular. AutoDock was used to add the nonpolar hydrogen and calculate Gasteiger charges of the molecular and add the nonpolar hydrogen for each ligand. At last, molecular docking was conducted using AutoDock to assess binding energy.

3. Results

3.1 Chemical structures construction

After searching TCMSP, ETCM, and literatures, a total of 434 compounds were collected. Including 92, 73, 76, 38, 19, 70, 12, 36, and 18 compounds in Licorice, Chuanxiong Rhizoma, Jujubae Fructus, Poria Cocos (Schw.) Wolf. Cinnamomi Ramulus, in Silktree Albizia Bark, Ostrea Gigas Thunberg, Ziziphi Spinosae Semen, and Radix Polygalae. Later, the structure of each component was collected from PubChem.

3.2 GA and DL prediction

Uploaded the structures to SwissADME, after screening the GA and DL and deduplicating 168 components qualified, including 80, 20, 27, 11, 4, 19, 4, 10, and 2 in Licorice, Chuanxiong Rhizoma, Jujubae Fructus, Poria Cocos (Schw.) Wolf. Cinnamomi Ramulus, in Silktree Albizia Bark, Ostrea Gigas Thunberg, Ziziphi Spinosae Semen, and Radix Polygalae. Interestingly, several qualified components are owned by more than one herbal, more information about the prediction results was placed in the Supplementary materials. All qualified components were adopted for the next screening.

3.3 Target prediction and verification

After deduplicating, 1096 related targets of DFD were collected from SwissTargetPrediction. Meanwhile, 260 VA related targets were collected from OMIM and DrugBank. Taking the intersection of DFD and VA targets, there were 28 shared targets, based on the data obtained above, the Herb-Compound-Targets-Disease (H-C-T-D) network was constructed. The H-C-T-D network was composed of 147 nodes (DFD, VA, 7 herbals, 110 bioactive compounds, and 28 common targets) and 465 edges (Fig. 2).

In further analysis, all 28 common targets were uploaded to STRING to construct the PPI network, the results were imported to Cytoscape to calculate the degree value of each gene using CytoNCA plugin and reconstruct the PPI network according to the degree value (Fig. 3). There are three possible bio-functional modules divided from the PPI network (Fig. 4). After screening by CytoNCA, the top 10 targets were defined as hub targets, the designations and topological parameters of hub targets were shown in Table 1. Besides, we used VarElect to find, among the 28 common targets, 23 targets were related to VA directly, whereas 5 targets were related to VA indirectly (Table 2). Potassium Voltage-Gated Channel
Subfamily H Member 2 (KCNH2), Sodium Voltage-Gated Channel Alpha Subunit 5 (SCN5A), Troponin T2-Cardiac Type (TNNT2), and Calmodulin 1 (CALM1) are the most related targets.

| Gene symbol | Protein Name                                           | Degree | Betweenness Centrality | Closeness Centrality |
|-------------|--------------------------------------------------------|--------|-------------------------|----------------------|
| KCNH2       | Potassium voltage-gated channel subfamily H member 2   | 10     | 128.5                   | 0.32                 |
| CYP3A4      | Cytochrome P450 3A4                                     | 9      | 166.8                   | 0.35                 |
| CYP2C9      | Cytochrome P450 2C9                                     | 8      | 22.2                    | 0.31                 |
| PIK3R1      | Phosphatidylinositol 3-kinase regulatory subunit alpha  | 8      | 146.7                   | 0.31                 |
| CHRM1       | Muscarinic acetylcholine receptor M1                    | 7      | 19.2                    | 0.27                 |
| CYP2D6      | Cytochrome P450 2D6                                     | 7      | 59.1                    | 0.32                 |
| EGFR        | Epidermal growth factor receptor                        | 7      | 228.3                   | 0.36                 |
| SLC01B1     | Solute carrier organic anion transporter family member 1B1 | 6      | 3.9                     | 0.28                 |
| ADRA1B      | Alpha-1B adrenergic receptor                            | 6      | 11.1                    | 0.27                 |
| ADRB2       | Beta-2 adrenergic receptor                              | 6      | 63.8                    | 0.31                 |
Table 2
The connection information of shared genes.

| GENES       | DESCRIPTION                                   | RELATIONSHIP | SCORE |
|-------------|-----------------------------------------------|--------------|-------|
| SCN5A       | Sodium Voltage-Gated Channel Alpha Subunit 5  | DIRECTLY     | 445.8 |
| KCNH2       | Potassium Voltage-Gated Channel Subfamily H Member 2 | DIRECTLY     | 409.76|
| TNNT2       | Troponin T2, Cardiac Type                     | DIRECTLY     | 293.82|
| CALM1       | Calmodulin 1                                  | DIRECTLY     | 222.12|
| SCN4A       | Sodium Voltage-Gated Channel Alpha Subunit 4  | DIRECTLY     | 158.41|
| SCN10A      | Sodium Voltage-Gated Channel Alpha Subunit 10 | DIRECTLY     | 132.53|
| ADRB1       | Adrenoceptor Beta 1                           | DIRECTLY     | 97.00 |
| ADRB2       | Adrenoceptor Beta 2                           | DIRECTLY     | 75.14 |
| CYP2C9      | Cytochrome P450 Family 2 Subfamily C Member 9 | DIRECTLY     | 53.13 |
| PDGFRB      | Platelet Derived Growth Factor Receptor Beta  | DIRECTLY     | 46.86 |
| CHRM2       | Cholinergic Receptor Muscarinic 2             | DIRECTLY     | 46.86 |
| MAPK1       | Mitogen-Activated Protein Kinase 1            | DIRECTLY     | 43.38 |
| EGFR        | Epidermal Growth Factor Receptor              | DIRECTLY     | 43.38 |
| ADRA1D      | Adrenoceptor Alpha 1D                         | DIRECTLY     | 39.60 |
| ADRB3       | Adrenoceptor Beta 3                           | DIRECTLY     | 39.60 |
| SCN9A       | Sodium Voltage-Gated Channel Alpha Subunit 9  | DIRECTLY     | 35.42 |
| CYP3A4      | Cytochrome P450 Family 3 Subfamily A Member 4 | DIRECTLY     | 30.68 |
| CYP2D6      | Cytochrome P450 Family 2 Subfamily D Member 6 | DIRECTLY     | 25.05 |
| PON1        | Paraoxonase 1                                 | DIRECTLY     | 25.05 |
| PIK3R1      | Phosphoinositide-3-Kinase Regulatory Subunit 1| DIRECTLY     | 17.71 |
| CYP1A1      | Cytochrome P450 Family 1 Subfamily A Member 1 | DIRECTLY     | 17.71 |
| CHRM1       | Cholinergic Receptor Muscarinic 1             | DIRECTLY     | 17.71 |
| CYP1A2      | Cytochrome P450 Family 1 Subfamily A Member 2 | DIRECTLY     | 17.71 |
| ADRA1A      | Alpha-1A adrenergic receptor                  | INDIRECTLY   | 37.78 |
| ADRA1B      | Alpha-1B adrenergic receptor                  | INDIRECTLY   | 36.23 |
| CHRM3       | Muscarinic acetylcholine receptor M3          | INDIRECTLY   | 28.09 |
| SLC01B1     | Solute carrier organic anion transporter family member 1B1 | INDIRECTLY | 13.50 |
The score is an indication of the strength of the connection between the gene and the disease.

### 3.4 Biology functional analysis

The enrichment analysis of GO and KEGG of the 28 common targets were analyzed with Metascape, the results were ranked by $-\log P$, and the top 14 of each enrichment items were shown in Fig. 5, besides, the functional analysis of the three potential bio-function modules divided from PPI network were shown in Table 3.

| Function modules | DESCRIPTION | Log10(Pvalue) |
|------------------|-------------|---------------|
| Module 1         | ko04020,Calcium signaling pathway | -22.3027025 |
|                  | GO:0001996,positive regulation of heart rate by epinephrine-norepinephrine | -12.3777817 |
|                  | ko04810,Regulation of actin cytoskeleton | -11.6704785 |
|                  | GO:0043410,positive regulation of MAPK cascade | -10.5659921 |
|                  | ko04024,cAMP signaling pathway | -9.71878217 |
| Module 2         | GO:0086010,membrane depolarization during action potential | -12.7696584 |
|                  | GO:0006941,striated muscle contraction | -11.8188992 |
|                  | ko04261,Adrenergic signaling in cardiomyocytes | -7.33482642 |
|                  | GO:0019233,sensory perception of pain | -5.53543107 |
|                  | GO:0055080,cation homeostasis | -3.03192747 |
| Module 3         | GO:0016098,monoterpenoid metabolic process | -13.3868655 |
|                  | GO:0008202,steroid metabolic process | -10.1366162 |
|                  | GO:0006690,icosanoid metabolic process | -10.0957724 |
|                  | GO:0008203,cholesterol metabolic process | -9.58364991 |
|                  | GO:0010035,response to inorganic substance | -3.36527057 |

In GO and KEGG enrichment analysis, terms with high enrichment scores suggested that the regulation of musclecontrction, regulation of systemic arterial blood pressure by norepinephrine-epinephine, blood circulation, circulatory system process, adrenergicreceptor activity, calcium signaling pathway, adenylate...
signaling in cardiomyocytes, cGMP-PKG signaling pathway and Neuroactive ligand-receptor interaction could be the most possible ways DFD works. For the 3 protein modules, module 1 can regulate calcium signaling pathway, heart rate as well as cAMP signaling pathway, Module 2 can regulate membrane depolarization during action potential, striated muscle contraction, regulate adrenergic signaling in cardiomyocytes, and cation homeostasis. This work supported that DFD against VA though mechanisms, actually, the mechanisms of DFD against VA has been revealed by this work.

3.5 Molecular docking

Molecular docking was conducted to calculate binding energy between components and hub targets, KCNH2 (PDB ID:1BYW), SCN5A (PDB ID: 4DCK), TNNT2 (PDB ID: 1J1D and CALM1 (PDB ID: 1CDL), the 4 genes connected closely to VA were included in molecular docking. The related information of components docked with key targets were listed in Table 4, and the results suggested that components in DFD are interacting strongly with the hub targets against VA. The top 10 binding energy docking modules were shown in Fig. 6, all 10 components interacted with corresponding targets mainly through hydrogenbond.
Table 4
The related information of components docked with key targets

| GENE  | Component                                                   | PUBCHEM CLD | Origin                  | Binding energy |
|-------|-------------------------------------------------------------|-------------|-------------------------|----------------|
| KCNH2 | Jujubogenin                                                 | 15515703    | Semen                   | -4.85          |
| KCNH2 | acacic acid lactone                                         | 6712546     | Silktree Albizia Bark   | -4.83          |
| KCNH2 | stepharine                                                  | 98455       | Jujubae Fructus         | -4.52          |
| KCNH2 | N-Methylasimilobine                                         | 197017      | Semen                   | -4.29          |
| KCNH2 | Asimilobine                                                 | 160875      | Jujubae Fructus         | -4.2           |
| KCNH2 | Caaverine                                                   | 23335       | Semen                   | -4.16          |
| KCNH2 | Nuciferine                                                  | 10146       | Jujubae Fructus         | -4.07          |
| KCNH2 | 7-Acetoxy-2-methylisoflavone                                | 268208      | Licorice                | -4.02          |
| KCNH2 | (S)-Coclaurine                                              | 160487      | Semen, Jujubae Fructus  | -3.42          |
| KCNH2 | Juzirine                                                    | 3085285     | Semen                   | -3.02          |
| KCNH2 | (2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one | 637112     | Licorice                | -2.96          |
| KCNH2 | zizyphusine                                                 | 102063083   | Semen                   | -2.8           |
| KCNH2 | Senkyunone                                                  | 91726743    | Chuanxiong Rhizoma      | -2.59          |
| KCNH2 | 25-Hydroxy-3-Epidehydotumulosic Acid                       | 10368709    | Poria Cocos (Schw.)     | -2.09          |
| KCNH2 | Ethylpentadecanoate                                         | 38762       | Chuanxiong Rhizoma      | -0.61          |
| KCNH2 | AP1                                                         | 21119850    | Silktree Albizia Bark   | 1.45           |
| TNNT2 | kanzonols W                                                 | 15380912    | Licorice                | -4.62          |
| TNNT2 | Glabridin                                                   | 124052      | Licorice                | -4.02          |
| TNNT2 | Senkyunolide G                                              | 5321250     | Chuanxiong Rhizoma      | -3.23          |
| TNNT2 | Odoratin                                                    | 13965473    | Licorice                | -3.05          |
| GENE   | Component        | PUBCHEM CLD | Origin              | Binding energy |
|--------|------------------|-------------|---------------------|----------------|
| CALM1  | DFV              | 114829      | Licorice            | -4.21          |
| CALM1  | Licochalcone B   | 5318999     | Licorice            | -2.66          |
| SCN5A  | Senkyunone       | 91726743    | Chuanxiong Rhizoma  | -1.59          |

The results show that Jujubogenin has the highest binding energy, connected with HIS70 and ASP67 of KCNH2 (Fig. 6A), acacic acid lactone connected with ARG62 of KCNH2 (Fig. 6B), kanzonols W connected with ASP88 of TNNT2 (Fig. 6C), stepharine connected with LEU86 of KCNH2 (Fig. 6D), N-Methylasimilobine connected with HIS70 of KCNH2 (Fig. 6E), DFV connected with ARG86 and ARG90 of CALM1 (Fig. 6F), Asimilobine connected with LYS101 of KCNH2 (Fig. 6G), Caaverine connected with VAL36 of KCNH2 (Fig. 6H), Nuciferine connected with GLU95 of KCNH2 (Fig. 6I) and 7-Acetoxy-2-methylisoflavone connected with LYS93 of KCNH2 (Fig. 6J).

4. Discussion

Ventricular arrhythmia is a fatal disease, typical drugs may benefit patients, but its side effects such as respiratory diseases, liver and kidney damage as well as bradyarrhythmia can never be ignored. Fortunately, long-time clinical work was told that DFD is an effective herb mixture to against antiarrhythmic. Since its excellent clinical efficacy, we conducted a Real-World Trial to assess the safety and efficacy of DFD for ventricular arrhythmia and the results demonstrate that DFD combined with metoprolol has better efficacy and safety than placebo combined with metoprolol. Besides, we explored the cellular electrophysiological mechanism of DFD, and DFD indeed has anti-arrhythmic effects based on its antioxidant potential, alleviation of Na⁺-K⁺-ATPase and connexin-43, and class I antiarrhythmic properties by suppressing Nav₁.₅ dose-dependently with an IC₅₀ of 24.0 ± 2.4 mg/mL. In this study, the bioactive components and underlying mechanisms of DFD in the treatment of VA were analyzed systematically.

Through related information collection and primary screening, we identified 28 potential targets of DFD in the treatment of VA. A PPI network was constructed with STRING and Cytoscape 3.8.0, the top 10 degree value genes were selected as hub gene and 3 function modules were divided based on its interactions. Analyzed all potential genes with VarElect, all 10 hub genes are directly related to the treatment of VA, and among these genes, KCNH2, TNNT2, CALM1 as well as SCN5A has the highest value of scores, in other words, these are the 4 genes with the highest correlation with the VA. Recently, KCNH2 could be a hot gene in the study of ventricular arrhythmia, it could mediate the rapidly activating component of the delayed rectifying potassium current in heart. A research suggested that pathogenic variants in KCNH2 encoding may result in Long QT syndrome⁴⁷. Meanwhile, another research based on quantitative analysis of consortium disease cohorts and population controls pointed out among patients with long QT syndrome, the mutation probability of KCNH2 gene is greater than 85%⁴⁸. Besides, another research
mentioned the co-expression of CACNA1C and KCNH2 reduces the arrhythmic events\(^4^9\). TNNT2 is another hub gene connected to arrhythmias, a genetic analysis suggested that TNNT2 was co-segregated in ventricular arrhythmias and sudden death\(^5^0\). Wu launched a study based on zebrafish embryos, the result shows that zebrafish embryos exposed to procymidone are more likely alter transcription levels of TNNT2, and resulted in arrhythmia as well as increased heart rate finally\(^5^1\). Raffaele Coppini conducted a cohort study of patients with hypertrophic cardiomyopathy (HCM), the outcome indicated that among patients with HCM, most patients have a mutation in gene TNNT2, and these patients are more likely to suffer from arrhythmias and HCM in the future\(^5^2\). SCN5A plays a vitally important role in the cardiac electrical conduction and arrhythmic risk, a study provided a new effective therapy to reduce arrhythmia through downregulating the expression of SCN5A\(^5^3\). Coincidentally, there is a study reported that a combination of quinidine/mexiletine reduces arrhythmia in patients with SCN5A gene mutation\(^5^4\). CALM1 is a regulator of voltage-dependent L-type calcium channels, its mutations are related to congenital arrhythmia\(^5^5\). Heterozygosity for the CALM1 mutation is causative of an arrhythmia syndrome\(^5^6\). Moreover, it can lead cathecolaminergic polymorphic ventricular tachycardia, idiopathic ventricular fibrillation, long QT syndrome, and even Sudden death\(^5^7\).

In the further GO and KEGG analysis, the results elucidated that the regulation of systemic arterial blood pressure by norepinephrine-epinephrine, musclecontrction, blood circulation, circulatory system process, adrenergic receptor activity, calcium signaling pathway, adenylate signaling in cardiomyocytes, cGMP-PKG signaling pathway and Neuroactive ligand-receptor interaction could be the most possible ways DFD works. Here, the regulation of calcium signaling pathway is affected by more hub genes than other pathways. According to a study based on the genomic, transcriptomic, and proteomic data initiated by Dan E Arking, calcium signaling pathway plays an important role both in the depolarization and repolarization of myocardial, particularly in the repolarization, during the plateau phase of the cardiac action potential, prolonged inward Ca\(^{2+}\) current leads to delays in ventricular myocyte repolarization\(^5^8\). Earlier research mentioned that Ca\(^{2+}\) waves can result when the Ca\(^{2+}\) ion influx into the cell is increased, and Ca\(^{2+}\) waves can generate depolarizations that trigger arrhythmias, It is reasonable to speculate that treatments related to calcium signaling pathways may be effective for arrhythmia\(^5^9\). Reports also suggested that the adrenergic signaling can increase the transmural difference between Ca\(^{2+}\) ion transients duration and action potential duration, finally, promoting the formation of delayed afterdepolarizations, the regulation of adenylate cyclase-activating adrenergic receptor signaling pathway and adrenergic receptor signaling pathway of DFD for VA may work in this way\(^6^0\). Adenylate cyclase-modulating G protein-coupled receptor signaling pathway can result in the regulation of G protein-mediated signaling, which is of great importance for the regulation of heart rate and involved in arrhythmias\(^6^1\). Besides, as we mentioned above, the potential targets were divided into 3 function modules, as shown in Table 3, it is obvious that the enrich analysis results of module 1 can regulate calcium signaling pathway, heart rate as well as cAMP signaling pathway, and it is to say that module 1 has great potential in the anti-arrhythmia. Module 2 can membrane depolarization during action potential, striated muscle contraction, regulate adrenergic signaling in cardiomyocytes and cation homeostasis,
enrichment analysis shows that module 2 also has the possibility of anti-arrhythmia. The enrichment analysis of module 3 may not seem ideal, be careful, and we found 4 genes which connected to VA closest are gathered in function module 3, and it is reasonable to believe module 3 has anti-arrhythmic effects. Furthermore, as is shown in Fig. 5, the mutil-regulation in different aspects may benefit patients suffering from related diseases such as hypertension, cancer, and other diseases.

There are still some limitations in our research, although we tried to find out all components, such as Osdraconis (Fossiliaossiamastodi) (Longgu) and Ostrea Gigas Thunberg (Muli), the shell, mineral drugs, has only several pieces of research, with several components, and excluded for its low possibility and DL, but Osdraconis (Fossiliaossiamastodi) and Ostrea Gigas Thunberg (Muli) played important roles in DFD, according to the theoretical system of traditional Chinese medicine, both Osdraconis (Fossiliaossiamastodi) and Ostrea Gigas Thunberg can tranquilize mind, further studies are needed to confirm the sedative mechanism of Osdraconis (Fossiliaossiamastodi) and Ostrea Gigas Thunberg. Besides, so many components boiled together, researches are needed to determine whether there are some new compounds formed.

5. Conclusion

As mentioned above, DFD could be employed for VA through mechanisms, including complex interactions between related components and targets, as predicted by network pharmacology and molecular docking. This work confirmed DFD could apply for the treatment of VA and promotes the explain of DFD for VA in the molecular mechanisms, similar results can obtain from previous experiments of cellular electrophysiological mechanism. A systematic analysis in this work can provide a comprehensive consideration for further studies.

Abbreviations

DFD: Dingji Fumai decoction
VA: Ventricular arrhythmia
TCM: Traditional Chinese medicine
GA: Gastrointestinal absorption
DL: Drug-likeness
H-C-T-D: herb-compound-target-disease
TCMSP: traditional Chinese medicine systems pharmacology database and analysis platform
ETCM: Encyclopedia of Traditional Chinese Medicine
ADME: absorption, distribution, metabolism, and excretion

PPI: Protein-protein interaction

GO: Gene ontology

KEGG: Kyoto encyclopedia of genes and genomes

KCNH2: Potassium Voltage-Gated Channel Subfamily H Member 2

SCN5A: Sodium Voltage-Gated Channel Alpha Subunit 5

TNNT2: Troponin T2-Cardiac Type

CALM1: Calmodulin 1

HCM: hypertrophic cardiomyopathy

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

Not applicable.

Funding Statement

Not applicable.
Authors' contributions

YL and BL conceived, designed, and planned the study. YL and XRW acquired and analyzed the data. BL, WC and ZLZ interpreted the results. YL and BL drafted the manuscript and BL and ZLZ contributed to critical revision of the manuscript. All authors read and approved the final manuscript.

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**Figures**

- **Targets of DFD**
  - SIB: Swiss Institute of Bioinformatics
  - ETCM: The Encyclopedia of Treacher Collins Medicine
  - LSP: Lab of Systems Pharmacology
  - PubChem

- **VA-related targets**
  - OMIM®: Online Mendelian Inheritance in Man®

- **Candidates of DFD targets against VA**

  - Core targets confirmation
  - GO and KEGG enrichment
  - Molecular docking

- **Sub-network of functional modules**

**Figure 1**

Flow chart of this work.
Figure 1

Flow chart of this work.
Figure 2

The H-C-T-D network of DFD. Red rectangles green octagons indicate DFD and VA, respectively. Green octagons indicate the 7 herbal medicines comprising DFD, respectively. Purple diamonds indicate the 110 active compounds and dark cyan shape V indicate the 28 shard targets, respectively.
**Figure 2**

The H-C-T-D network of DFD. Red rectangles green octagons indicate DFD and VA, respectively. Green octagons indicate the 7 herbal medicines comprising DFD, respectively. Purple diamonds indicate the 110 active compounds and dark cyan shape V indicate the 28 shard targets, respectively.
Figure 3

The H-C-T-D network of DFD. Red rectangles green octagons indicate DFD and VA, respectively. Green octagons indicate the 7 herbal medicines comprising DFD, respectively. Purple diamonds indicate the 110 active compounds and dark cyan shape V indicate the 28 shard targets, respectively.
Figure 3

The H-C-T-D network of DFD. Red rectangles green octagons indicate DFD and VA, respectively. Green octagons indicate the 7 herbal medicines comprising DFD, respectively. Purple diamonds indicate the 110 active compounds and dark cyan shape V indicate the 28 shard targets, respectively.

Figure 4
three possible bio-functional modules divided from the PPI network

Figure 4

three possible bio-functional modules divided from the PPI network

GO and KEGG enrichment analysis of potential targets.

Figure 5
**Figure 5**

GO and KEGG enrichment analysis of potential targets.

**Figure 6**

The top 10 binding energy docking modules
Figure 6

The top 10 binding energy docking modules

Supplementary Files

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