Network pharmacology study on the mechanism of Qiangzhifang in the treatment of panic disorder

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Background: Panic disorder (PD) is a kind of mental illness characterized by the symptom of recurring panic attacks. Qiangzhifang (QZF) is a novel decoction developed by Professor Zhaojun Yan based on a unique system of syndrome differentiation and clinical experience. It has achieved remarkable results after long-term clinical practice, but its mechanism of action is still unclear. This study aims to use network pharmacology and molecular docking to explore the mechanism of QZF in the treatment of PD.

Methods: We used the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), a literature search, and Encyclopedia of Traditional Chinese Medicine (ETCM) to find active ingredients and targets of QZF. We searched for PD targets in GeneCards, Online Mendelian Inheritance in Man (OMIM), the Comparative Toxicogenomics Database (CTD), and DrugBank. We established a PD target database, constructed a protein-protein interaction (PPI) network, and performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis in order to screen possible pathways of action and analyze the mechanism.

Results: This study identified 84 effective components of QZF, 691 potential targets, 357 PD targets, and 97 intersectional targets. Enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) showed that QZF was associated with 118 biological processes (BPs), 18 cellular components (CCs), 35 molecular functions (MFs) [false discovery rate (FDR) <0.01], and 62 pathways (FDR <0.01). QZF mainly acts on its targets AKT1, FOS, and APP through active ingredients such as quercetin, β-sitosterol, 4-(4’-hydroxybenzyl)oxy)benzyl methyl ether, harmine, 1,7-dimethoxyxanthone, and 1-hydroxy-3,7-dimethoxyxanthone to regulate serotonin, gamma-aminobutyric acid (GABA), cyclic adenosine monophosphate (cAMP), and other signal pathways to treat PD.

Conclusions: Through network pharmacology and molecular docking technology, we predicted the possible mechanism of QZF in the treatment of PD, revealed the interaction targets and potential value of QZF, and provided a basis for its clinical application.

Keywords: Qiangzhifang (QZF); network pharmacology; molecular docking; panic disorder (PD)

Submitted Jun 08, 2021. Accepted for publication Aug 16, 2021.
doi: 10.21037/atm-21-4090
View this article at: https://dx.doi.org/10.21037/atm-21-4090
Introduction

Panic disorder (PD) is a common mental illness, also known as acute anxiety attack, which refers to an anxiety disorder in which unexpected panic attacks occur repeatedly. It is clinically characterized by repeated and sudden strong fear, panic, or discomfort and may bring a sense of imminent death or loss of control. PD may manifest as different symptoms such as the cardiovascular system, respiratory system, digestive system, and nervous system (1). The prevalence rate of this disease is 3.6–5.1%. It is more common among young and middle-aged people and more common in women than men. It can easily transform into chronic or even permanent disease if it is not diagnosed and treated in time. Its lifetime prevalence is 3.4–4.7% (2). Prolonged unhealed PD will not only seriously affect the quality of life and social function of its sufferers but even increase the chance of lifelong suicide attempts (3). Therefore, PD should be detected and treated as soon as possible to avoid further detriments to the patient's physical and mental health.

In 2019, novel coronavirus (COVID-19) rapidly spread around the world. The disease was highly infectious, fast transmission, high mortality and poor prognosis, affecting human economic, social and health, causing fear, anxiety and avoidance, and emphasizing the fear of patients with mental illness (4). Traditional Chinese medicine plays an important role in the prevention and control of the epidemic, and has unique advantages in the treatment of fear and anxiety, and other emotional diseases (5).

PD is treated mostly with selective serotonin reuptake inhibitors (SSRIs), benzodiazepine drugs (BZDs) and antidepressants tricyclic antidepressants (TCAs). Although these drugs can relieve panic attacks and improve anxiety, long-term use of these drugs is prone to adverse reactions, such as biological dependence, withdrawal reactions, and gastrointestinal discomfort (6). Therefore, there is an urgent need for safe and effective treatment methods with less side effects. Traditional Chinese medicines have the advantages of multiple components and multiple targets acting in coordination and fewer adverse reactions.

Professor Zhaojun Yan of Shandong Provincial Hospital of Traditional Chinese Medicine has created a unique system of syndrome differentiation for the treatment of mental and behavioral diseases, which has a profound clinical foundation. Qiangzhifang (QZF), invented under the theoretical guidance of Professor Zhaojun Yan, is composed of Morinda officinalis (ba ji tian, BJT), Pinellia (banxia, BX), jujube (da zao, DZ), Poria (fu ling, FL), Chinese yam (shan yao, SY), Gastrodia (tian ma, TM), and Polygala (yuan zhi, YZ). Long-term clinical application has verified its definite curative effect in the treatment of fear, timidity, anxiety, compulsion, insomnia, and restlessness. However, the mechanism of QZF's therapeutic effect remains to be elucidated.

In recent years, network pharmacology has been increasingly applied in the research of traditional Chinese medicine. The comprehensive, systematic and holistic concept of network pharmacology is consistent with the characteristics of traditional Chinese medicine prescription multiple compound, multi-target point and multiple route, and its methods can clearly clarify the molecular mechanism of traditional Chinese medicine. The discovery of new targets of traditional Chinese medicine is a key breakthrough in drug development, and network pharmacology is expected to become the key direction of the research of traditional Chinese medicine prescription agents (7).

This study aims to analyze the multicomponent, multitarget, and multipath mechanism of QZF in the treatment of PD through network pharmacology techniques, in order to reveal the mechanism and potential clinical value of QZF and provide a basis for further in-depth research.

Methods

Schematic diagram

Figure 1 is a schematic diagram of the research methods. We used network pharmacology and molecular docking methods to predict the mechanism of action of QZF in the treatment of PD.

Screening the main active ingredients and targets of QZF

To mix QZF, compounds collected from BJT, BX, DZ, FL, and SY were derived from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (8). The names of the Chinese medicine components were used for the query. Oral availability (OB) and drug-like properties (DL) were included as the screening conditions. OB and DL are important indicators for evaluating pharmacokinetic absorption, distribution, metabolism, and excretion (ADME) (9). OB is the speed and extent to which the active ingredient or active group of the drug is absorbed into the systemic circulation; and DL indicates that the drug contains some specific functional groups or has the same or similar physical characteristics as most drugs (10). The screening value was set to OB ≥30% and DL ≥0.18. We searched for the target proteins of each
molecule in the “related targets” section and imported the results of the active ingredients and target information into WPS Office Excel tables. The UniProt protein database (11) was used to convert the target proteins into the corresponding gene names.

The TM and YZ compounds were collected from a literature search and query of the Encyclopedia of Traditional Chinese Medicine (ETCM) (12). The structure names of the collected compounds were searched in the PubChem database (13), and the chemical structure diagrams of each chemical component were summarized. The obtained 2D structural formula was uploaded to the Swiss ADME database (14) for screening, and the screening parameters were set with gastrointestinal absorption at “high”. The 2D structure formula of the compound after screening was imported into the SwissTargetPrediction database (14) to predict the target sites, and the output was summarized.

**PD disease target collection**

We searched the GeneCards database (15), Online Mendelian Inheritance in Man (OMIM) database (16), Comparative Toxicogenomics Database (CTD) (17), and DrugBank database (18) with “panic disorder” as a keyword, removing duplicate targets, to establish the disease target data.

**Construction of protein-protein interaction (PPI) network**

We compared the target information of QZF and PD and used bioinformatics software (http://www.bioinformatics.com.cn/) to obtain the overlapping genes. The potential targets were imported into the String database (19). Cytoscape software (20) was used to draw the PPI network and calculate the degree value of each node of the PPI network in order to identify the core targets for the treatment of PD by QZF.

**Construction of QZF component-PD-target interaction network**

Cytoscape software (20) was used to draw the QZF component-PD-target interaction network.
Molecular docking verification

We queried the PubChem website (13) to obtain the SDF structure files of the compounds. We then used Open Babel 2.3.2 software (21) to convert the SDF file into a PDB file and retrieved the key targets from the Protein Data Bank (22). PYMOL 2.3.4 software (23) was used to perform operations such as dehydration and ligand removal on the receptor proteins, and AutoDockTools software (24) was used to modify the receptor proteins, such as in their hydrogenation and charge balance. The Grid Box command under the Grid program was used to open the Grid Option tool to process the receptor protein, AutoDock Vina 1.1.2 (25) was used to do molecular docking of receptor proteins and small-molecule ligands, and finally PYMOL software was used to visually analyze the docking results.

Pathway enrichment analysis

The potential targets were imported into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (26), “Homo sapiens” was selected as the species, and KEGG-pathway, GOTERM_BP_DIRECT(BP), GOTERM_CC_DIRECT(CC), and GOTERM_MF_DIRECT(MF) were selected to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the overlapping genes. We obtained the basic data information about the biological processes (BPs), molecular functions (MFs), and cellular components (CCs) and about the involved pathways to draw GO histograms and KEGG enrichment bubble charts (http://www.bioinformatics.com.cn/).

Statistical analysis

Using Windows, WPS Office version 11.1.0.10667-Release for Windows version summary data, protein reciprocal PPI data were analyzed by version Cytoscape 3.7.2, and protein-processed data use AutoDock Vina 1.1.2 for molecular docking. The pathway enrichment visualization analysis is derived from the Database (DAVID) version 6.8. All relevant data analysis results are listed in the Results section below.

Results

Screening the active ingredients and targets of QZF

We input BJT, BX, DZ, FL, and SY into the TCMSP database, setting the parameters to OB ≥30% and DL ≥0.18. The SwissTargetPrediction database was used to predict the target points of TM and YZ, and the setting of “high” gastrointestinal absorption was chosen. The obtained active ingredients numbered 16 for BJT, 11 for BX, 18 for DZ, six for FL, 12 for SY, 12 for TM, and 13 for YZ. A total of 88 compounds were obtained, but after removing duplicate values, 84 remained (Tables 1,2). The 84 compounds were further studied using the UniProt database and SwissTargetPrediction database, which yielded a total of 691 target genes of them.

Identification of disease targets

A total of 133 PD disease targets were collected through the GeneCards website, 118 PD targets were collected through the OMIM website, 195 PD disease targets were collected through the CTD website, and 80 PD disease targets were collected through the DrugBank website. This total of 526 PD targets became 357 after removing duplicates.

Construction of the PPI network

We intersected the targets of the QZF components with the PD disease targets by using bioinformatics software (http://www.bioinformatics.com.cn/). As a result, a total of 97 common targets were obtained (Figure 2). We used Cytoscape software to visualize the PPI network. After discarding unmatched targets, 95 remained (Figure 3). We obtained a total of 95 nodes and 912 edges. The size and the lightness of the color of the node indicate the node degree (number of connections to it), and the thickness and the lightness of the color of each edge indicate the strength of that interaction. The top 10 targets according to node degree were serine/threonine kinase 1 (AKT1), the proto-oncogene (FOS), amyloid beta precursor protein (APP), sodium-dependent serotonin transporter (SLC6A4), catechol O-methyltransferase (COMT), interleukin-6 (IL-6), cell tumor antigen p53, a caspase (CASP3), vascular endothelial growth factor A (VEGFA), and tumor necrosis factor (TNF) (Figure 4).

QZF active ingredient-PD-target interaction network construction

We obtained 155 nodes and 552 edges using Cytoscape software for analysis. As shown in Figure 5, pink indicates the drug QZF, red indicates the disease PD, green indicates the active ingredients of QZF, and purple indicates the common targets of QZF and PD (Figure 5).
| MOLID    | Molecule name                        | OB  | DL  | Herb     |
|----------|--------------------------------------|-----|-----|----------|
| MOL002879 | Diop                                 | 43.59 | 0.39 | BJT      |
| MOL002883 | Ethyl oleate (NF)                    | 32.4  | 0.19 | BJT      |
| MOL000358 | β-sitosterol                          | 36.91 | 0.75 | BJT, BX, DZ |
| MOL000359 | Sitosterol                            | 36.91 | 0.75 | BJT      |
| MOL006147 | Alizarin-2-methylether               | 32.81 | 0.21 | BJT      |
| MOL009495 | 2-hydroxy-1,5-dimethoxy-6-(methoxymethyl)-9,10-anthraquinone | 95.85 | 0.37 | BJT      |
| MOL009496 | 1,5,7-trihydroxy-6-methoxy-2-methoxymethylanthracenequinone | 80.42 | 0.38 | BJT      |
| MOL009500 | 1,6-dihydroxy-5-methoxy-2-(methoxymethyl)-9,10-anthraquinone | 104.54 | 0.34 | BJT      |
| MOL009504 | 1-hydroxy-6-hydroxymethylanthracenequinone | 81.77 | 0.21 | BJT      |
| MOL009513 | 2-hydroxy-1,8-dimethoxy-7-methoxymethylanthracenequinone | 112.3 | 0.37 | BJT      |
| MOL009519 | (2R,3S)-(+-)-3',5-dihydroxy-4,7-dimethoxydihydroflavonol | 77.24 | 0.33 | BJT      |
| MOL009524 | 3β,20(R),5-alkenyl-stigmastol         | 36.91 | 0.75 | BJT      |
| MOL009525 | 3β-24S(R)-butyl-5-alkenyl-cholestol   | 35.35 | 0.82 | BJT      |
| MOL009537 | Americanin A                         | 46.71 | 0.35 | BJT      |
| MOL009551 | Isoprincepin                          | 49.12 | 0.77 | BJT      |
| MOL009562 | Ohiensin-A                           | 38.13 | 0.76 | BJT      |
| MOL001755 | 24-ethylcholest-4-en-3-one           | 36.08 | 0.76 | BX       |
| MOL002670 | Cavidine                             | 35.64 | 0.81 | BX       |
| MOL002714 | Baicalein                            | 33.52 | 0.21 | BX       |
| MOL00449  | Stigmasterol                          | 43.83 | 0.76 | BX, DZ, SY |
| MOL005030 | Gondoic acid                          | 30.7  | 0.2  | BX       |
| MOL00519  | Coniferin                             | 31.11 | 0.32 | BX       |
| MOL006936 | 10,13-eicosadienoic                  | 39.99 | 0.2  | BX       |
| MOL006957 | (3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl)piperazine-2,5-quinone | 46.89 | 0.27 | BX       |
| MOL003578 | Cycloartenol                          | 38.69 | 0.78 | BX       |
| MOL006967 | β-D-ribofuranoside, xanthine-9       | 44.72 | 0.21 | BX       |
| MOL012921 | Stepharine                            | 31.55 | 0.33 | DZ       |
| MOL012946 | Zizyphus saponin I_qt                | 32.69 | 0.62 | DZ       |
| MOL012976 | Coumestrol                            | 32.49 | 0.34 | DZ       |
| MOL012986 | Jujubasaponin V_qt                   | 36.99 | 0.63 | DZ       |
| MOL012992 | Mauritine D                           | 89.13 | 0.45 | DZ       |
| MOL01454  | Berberine                             | 36.86 | 0.78 | DZ       |
| MOL001522 | (S)-coclaurine                        | 42.35 | 0.24 | DZ       |
| MOL00211  | Mairin                                | 55.38 | 0.78 | DZ       |
| MOL004350 | Ruvoside_qt                           | 36.12 | 0.76 | DZ       |

Table 1 (continued)
Table 1 (continued)

| MOLID         | Molecule name                                                                 | OB  | DL  | Herb |
|---------------|-------------------------------------------------------------------------------|-----|-----|------|
| MOL000492     | (+)-catechin                                                                  | 54.83 | 0.24 | DZ   |
| MOL000627     | Stepholidine                                                                   | 33.11 | 0.54 | DZ   |
| MOL007213     | Nuciferin                                                                      | 34.43 | 0.4  | DZ   |
| MOL00787      | Fumarine                                                                       | 59.26 | 0.83 | DZ   |
| MOL02773      | β-carotene                                                                     | 37.18 | 0.58 | DZ   |
| MOL00096      | (−)-catechin                                                                   | 49.68 | 0.24 | DZ   |
| MOL00098      | Quercetin                                                                      | 46.43 | 0.28 | DZ   |
| MOL00273      | (2R)-2-[(3S,5R,10S,13R,14R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid | 30.93 | 0.81 | FL   |
| MOL00275      | Trametenolic acid                                                             | 38.71 | 0.8  | FL   |
| MOL00279      | Cerevisterol                                                                   | 37.96 | 0.77 | FL   |
| MOL00282      | Ergosta-7,22E-dien-3beta-ol                                                    | 43.51 | 0.72 | FL   |
| MOL00283      | Ergosterol peroxide                                                           | 40.36 | 0.81 | FL   |
| MOL00296      | Hederagenin                                                                   | 36.91 | 0.75 | FL   |
| MOL01559      | piperlonguminine                                                              | 30.71 | 0.18 | SY   |
| MOL01736      | (−)-taxifolin                                                                 | 60.51 | 0.27 | SY   |
| MOL00322      | Kadsurenone                                                                   | 54.72 | 0.38 | SY   |
| MOL05430      | Hancinone C                                                                   | 59.05 | 0.39 | SY   |
| MOL05435      | 24-methylcholest-5-enyl-3beta-O-glucopyranoside_qt                           | 37.58 | 0.72 | SY   |
| MOL05438      | Campesterol                                                                   | 37.58 | 0.71 | SY   |
| MOL05440      | Isofucosterol                                                                  | 43.78 | 0.76 | SY   |
| MOL05458      | Dioscoreside C_qt                                                             | 36.38 | 0.87 | SY   |
| MOL00546      | Diosgenin                                                                     | 80.88 | 0.81 | SY   |
| MOL05465      | AIDS180907                                                                    | 45.33 | 0.77 | SY   |
| MOL00953      | CLR                                                                           | 37.87 | 0.68 | SY   |

QZF, Qiangzhifang; OB, oral availability; DL, drug-like properties; BJT, Morinda officinalis (ba ji tian); BX, Pinellia (banxia); DZ, jujube (da zao); SY, Chinese yam (shan yao); FL, Poria (fu ling).

**Molecular docking**

Quercetin, beta-sitosterol, 4-(4′-hydroxybenzoyloxy)benzyl methyl ether, harmine, 1,7-dimethoxyxanthone, and 1-hydroxy-3,7-dimethoxyxanthone were the five active ingredients of QZF with the highest node degrees. We docked the main active ingredients of QZF with the top three core targets AKT1, Fos, and APP one by one (Table 3).

The more stable the binding conformation, the lower the required binding energy. Binding energy ≤−4.25 kcal/mol indicates weaker binding activity, ≤−5.0 kcal/mol indicates good binding activity, and ≤−7.0 kcal/mol indicates strong binding activity. In this study, binding energy ≤−8.5 kcal/mol was chosen as the screening condition, and PYMOL software was used to draw a 3D docking schematic diagram of the docking results. We investigated the binding mode between receptor protein AKT1 and the small-molecule ligand beta-sitosterol and that between receptor protein AKT1 and the small-molecule ligand quercetin. Figure 6 shows the binding mode between the receptor protein...
Table 2 Active ingredients and targets of QZF (YZ and TM)

| ID   | Molecule name                                      | Pharmacokinetics (GI absorption) | Drug likeness | Herb |
|------|----------------------------------------------------|----------------------------------|---------------|------|
| TM1  | Vanillin                                           | High                             | Yes           | TM   |
| TM2  | Cetylic acid, hexadecanoic acid, palmitic acid     | High                             | Yes           | TM   |
| TM3  | p-hydroxybenzaldehyde                              | High                             | Yes           | TM   |
| TM4  | Bis(4-hydroxybenzyl)ether                         | High                             | Yes           | TM   |
| TM5  | Protocatechuic aldehyde                            | High                             | Yes           | TM   |
| TM6  | 4,4’-dihydroxydiphenyl methane                    | High                             | Yes           | TM   |
| TM7  | 4-ethoxymethylphenyl-4’-hydroxybenzylether        | High                             | Yes           | TM   |
| TM8  | Gastrodamine                                       | High                             | Yes           | TM   |
| TM9  | 4-hydroxybenzyl alcohol                            | High                             | Yes           | TM   |
| TM10 | P-hydroxybenzyl ethyl ether                        | High                             | Yes           | TM   |
| TM11 | 4-hydroxybenzyl methyl ether                       | High                             | Yes           | TM   |
| TM12 | 4-(4’-hydroxybenzyloxy)Benzyl methyl ether         | High                             | Yes           | TM   |
| YZ1  | Harman                                             | High                             | Yes           | YZ   |
| YZ2  | Onjixanthone I                                     | High                             | Yes           | YZ   |
| YZ3  | Î’-carboline-1-carboxylic acid, methyl ester      | High                             | Yes           | YZ   |
| YZ4  | 1-ethoxycarbonyl-beta-carboline                   | High                             | Yes           | YZ   |
| YZ5  | 1,7-dimethoxyxanthone                              | High                             | Yes           | YZ   |
| YZ6  | 1-hydroxy-3,7-dimethoxyxanthone                   | High                             | Yes           | YZ   |
| YZ7  | 1-hydroxy-3,6,7-trimethoxy xanthone                | High                             | Yes           | YZ   |
| YZ8  | Norharman                                          | High                             | Yes           | YZ   |
| YZ9  | 1,2,3,6,7-pentamethoxyxanthone                     | High                             | Yes           | YZ   |
| YZ10 | 3,4,5-trimethoxy cinnamic acid                     | High                             | Yes           | YZ   |
| YZ11 | 1-carboethoxy-Î’-carboline                         | High                             | Yes           | YZ   |
| YZ12 | 5,6,7-trimethoxycoumarin                           | High                             | Yes           | YZ   |
| YZ13 | Harmine                                            | High                             | Yes           | YZ   |

QZF, Qiangzhifang; GI, gastrointestinal; TM, Gastrodia (tian ma); YZ, Polygala (yuan zhi).

AKT1 and the small-molecule ligand beta-sitosterol. The amino acid residues Ala230 and Glu228 formed a hydrogen bond with the small-molecule ligand beta-sitosterol. The amino acid residues Tyr229, Ala177, Val164, Lys179, Gly159, Gly162, Leu181, Thr160, Asp292, Glu278, Thr291, and Met281 formed hydrophobic interactions with the small-molecule ligand beta-sitosterol. Taken together, these data suggest that quercetin and beta-sitosterol may be the key active ingredients of QZF in the treatment of PD.

GO function enrichment analysis

GO analysis showed that the 95 targets of QZF in the treatment of PD mainly involved 456 BPs, 55 CCs, and
79 MFs. With P<0.01 and false discovery rate (FDR) <0.01 as the screening criteria, a total of 118 BPs, 18 CCs, and 35 MFs were obtained. We sorted the corrected P values from small to large, and the top 10 targets by P value were analyzed (Figure 8). The BPs of the targets included response to drug, positive regulation of cell proliferation, adenylate cyclase-activating adrenergic receptor signaling pathway, synaptic transmission, and cholinergic synaptic transmission. The CCs included integral component of plasma membrane, postsynaptic membrane, plasma...
Figure 5 QZF component-PD-target interaction network. QZF, Qiangzhifang; PD, panic disorder.

Table 3 Docking results between the main active ingredients and core targets

| Active compound                                           | Target name | PDB ID | Docking score (kcal/mol) |
|-----------------------------------------------------------|-------------|--------|--------------------------|
| 1,7-dimethoxyxanthone                                     | AKT1        | 4EKL   | −8.0                     |
|                                                           | APP         | 1AAP   | −5.5                     |
|                                                           | FOS         | 1FOS   | −5.3                     |
| 1-hydroxy-3,7-dimethoxyxanthone                           | AKT1        | 4EKL   | −7.7                     |
|                                                           | APP         | 1AAP   | −5.6                     |
|                                                           | FOS         | 1FOS   | −5.0                     |
| 4-(4'-hydroxybenzylxyloxy)benzyl methyl ether             | AKT1        | 4EKL   | −7.3                     |
|                                                           | APP         | 1AAP   | −5.1                     |
|                                                           | FOS         | 1FOS   | −4.8                     |
| Beta-sitosterol                                           | AKT1        | 4EKL   | −9.0                     |
|                                                           | APP         | 1AAP   | −6.2                     |
|                                                           | FOS         | 1FOS   | −5.8                     |
| Harmine                                                  | AKT1        | 4EKL   | −7.7                     |
|                                                           | APP         | 1AAP   | −4.9                     |
|                                                           | FOS         | 1FOS   | −4.7                     |
| Paroxetine                                               | AKT1        | 4EKL   | −8.4                     |
|                                                           | APP         | 1AAP   | −4.9                     |
|                                                           | FOS         | 1FOS   | −5.5                     |
| Quercetin                                                | AKT1        | 4EKL   | −8.5                     |
|                                                           | APP         | 1AAP   | −6.2                     |
|                                                           | FOS         | 1FOS   | −5.1                     |
membrane, synapse, and cell junction. The MFs included drug binding, enzyme binding, identical protein binding, extracellular ligand-gated ion channel activity, and dopamine binding.

**KEGG analysis**

The obtained predicted targets were analyzed using DAVID software for the KEGG analysis, and a total of 97 pathways were obtained. Sixty-two pathways had a P value <0.01, as the screening criterion, and the top 20 signal pathways in the KEGG enrichment pathway analysis based on the corrected P value are shown in Figure 9. Each pathway contained different targets (Table 4). Therefore, we speculate that QZF in the treatment of PD mainly acts through neuroactive ligand-receptor interactions, calcium signaling, serotonergic synapses, dopaminergic synapses, cyclic adenosine monophosphate (cAMP) signaling, cholinergic synapses, and GABAergic synapses.

**Discussion**

PD, also known as acute anxiety attack, is a subtype of anxiety disorder. Its symptoms include heart palpitations, chest discomfort, rapid breathing, trembling, nausea, and dizziness, accompanied by a strong sense of imminent death or loss of control. Its onset is sudden, its manifestations last for several or tens of minutes, and it is self-limiting. Modern medicine holds that its pathogenesis is affected by a variety of mechanisms [including genetic factors and the neurotransmitters 5-hydroxytryptamine (5-HT; serotonin), norepinephrine, and gamma-aminobutyric acid (GABA)] (27).

QZF is a novel decoction developed by Professor Zhaojun Yan. The decoction has components of seven sources, BJT, BX, DZ, FL, SY, TM, and YZ. All these medicinal sources have the functions of strengthening
Figure 8 Histogram for the GO enrichment analysis of common targets. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

Figure 9 Bubble plots of the top 20 pathways.
Table 4 KEGG analysis of the top 20 pathways

| ID   | Pathway                              | Gene                                                                                     |
|------|--------------------------------------|-------------------------------------------------------------------------------------------|
| hsa04080 | Neuroactive ligand-receptor interaction | GABRB3, CHRM2, CHRM3, CHRM1, CHRM4, CHRNA7, CHRM5, HTR2B, HTR2C, ADRA1D, ADRB1, ADRB2, HTR2A, ADRA1B, NR3C1, ADRA1A, GRIN2A, HTR6, HTR7, TXA2R, CNR1, DRD1, DRD2, DRD3, DRD4, DRD5, GABRA2, GABRA1, GABRA6, GABRA5, GABRA4, GABRA3, OPR1, GRIN2B, GABRG2, ADRA2A, GRIN1, ADORA2A |
| hsa04020 | Calcium signaling pathway             | CHRM2, CHRM3, CHRM1, CHRNA7, CHRM5, HTR2B, HTR2C, ADRA1D, ADRB1, CACNA1C, ADRB2, HTR2A, ADRA1A, EGFR, GRIN2A, HTR6, HTR7, TXA2R, DRD1, DRD5, PDGFRB, NOS2, GRIN1, ADORA2A |
| hsa05033 | Nicotine addiction                   | GABRB3, GABRA2, GABRA1, GRIN2A, GABRA6, GABRA5, CHRNA7, GABRA4, GABRA3, GRIN2B, GABRG2, GRIN1 |
| hsa04726 | Serotonergic synapse                  | GABRB3, APP, MAOB, MAOA, HTR2B, HTR2C, HTR3A, CACNA1C, HTR2A, CYP2C19, SLC6A4, HTR6, HTR7, CASP3, MAPK1 |
| hsa04728 | Dopaminergic synapse                  | GSK3B, MAOB, MAOA, CACNA1C, FOS, COMT, GRIN2B, SLC6A3, GRIN2A, AKT1, DRD1, DRD2, DRD3, DRD4, DRD5 |
| hsa05014 | Amyotrophic lateral sclerosis        | GRIN2A, CASP3, CAT, BCL2, BAX, TNF, TP53, GRIN2B, GRIN1, SOD1 |
| hsa04024 | cAMP signaling pathway               | CHRM2, CHRM1, ADRB1, CACNA1C, FOS, ADRB2, GRIN2B, GRIN1, HTR6, GRIN2A, ADORA2A, AKT1, MAPK1, DRD1, DRD2, DRD5 |
| hsa04723 | Retrograde endocannabinoid signaling  | GABRB3, GABRA2, GABRA1, FAAH, GABRA6, CNR1, GABRA5, GABRA4, GABRA3, MAPK1, CACNA1C, GABRG2 |
| hsa05031 | Amphetamine addiction               | GRIN2A, MAOB, MAOA, FOS, DRD1, CACNA1C, SIRT1, GRIN2B, SLC6A3, GRIN1 |
| hsa04725 | Cholinergic synapse                  | CHRM2, CHRM3, CHRM1, CHRNA7, CHRM4, CHRM5, BCL2, MAPK1, AKT1, FOS, CACNA1C, JAK2 |
| hsa05133 | Pertussis                            | IL10, IL1A, IL6, NOS2, IL1B, CASP3, NLRP3, MAPK1, FOS, TNF |
| hsa05210 | Colorectal cancer                    | GSK3B, CASP3, BCL2, BAX, MAPK1, CTNNB1, AKT1, FOS, TP53 |
| hsa04727 | GABAergic synapse                    | GABRB3, GABRA2, GABRA1, GABRA6, GABRA5, GABRA4, GABRA3, ABAT, CACNA1C, GABRG2 |
| hsa05215 | Prostate cancer                      | PDGFRB, GSK3B, PTEN, BCL2, MAPK1, CTNNB1, AKT1, TP53, EGFR, FGFR1 |
| hsa05152 | Tuberculosis                         | IL10, NOS2, TNF, IL1A, IL6, IFNG, IL1B, CASP3, BCL2, BAX, AKT1, MAPK1, JAK2 |
| hsa04015 | Rap1 signaling pathway               | PDGFRB, CSF1R, GRIN2B, EGFR, GRIN1, VEGFA, GRIN2A, ADORA2A, CNR1, AKT1, MAPK1, CTNNB1, DRD2, FGFR1 |
| hsa05030 | Cocaine addiction                   | GRIN2A, MAOB, MAOA, DRD1, DRD2, GRIN2B, SLC6A3, GRIN1 |
| hsa05140 | Leishmaniasis                        | IL10, IL1A, IFNG, NOS2, IL1B, MAPK1, FOS, JAK2, TNF |
| hsa05142 | Chagas disease                       | IL10, IL6, ACE, IFNG, NOS2, IL1B, MAPK1, AKT1, FOS, TNF |
| hsa05200 | Pathways in cancer                   | PDGFRB, GSK3B, CSF1R, NOS2, GSTP1, PTEN, FOS, EGFR, VEGFA, IL6, CASP3, BCL2, BAX, AKT1, MAPK1, CTNNB1, TP53, FGFR1 |

KEGG, Kyoto Encyclopedia of Genes and Genomes.

the mind, relieving shock, and nourishing and improving the mind. After long-term clinical practice, it has been found that QZF decoction can effectively alleviate fearful behaviors in patients. It is shown that Inulin starch Morinda officinalis oligosaccharide (IOMO) can shorten the freezing time of shock rats, inhibit fear behavior, and improve anxiety-like behavior (28). Ganmai jujube soup (jujube, licorice, wheat), and Pinellia thick Pu soup (Pinellia, thick Pu, Poria cocos, ginger, Su leaf) two classic prescription are two important prescriptions for the treatment of emotional disease, among which Banellia, jujube, porkahoe, widely used to treat anxiety, fear, depression and other diseases.
Yam can delay the aging process of the tissue structure of the immune organs, protect the tissue structure of the thymus and spleen of the important immune organs in mice, and have the role of immune regulation and treatment of infectious diseases (29). The gastrodia elata extract extends the time on the open arm and enters the percentage of the open arm, increasing the exploratory behavior of rats and plays an anti-anxiety role by regulating the GABA neurotransmitter system (30). The distant active ingredient 3,6'-diaphophol sucrose (DISS) increases the acidification level of cyclic phosphoradenosine reaction element binding protein [cAMP-response element binding protein (CREB)] in the hippocampus of chronic stressed rats, promote transcription of brain-derived neurotrophic factor (BDNF), and then protect neurons and improve depression and stress states (31). Therefore, drug combination can not only improve fear and anxiety symptoms, but also have an anti-inflammatory and immune effect. We found that the total time of freezing posture (such as: static, curl, stagnation, slight swing movement, etc.) was commonly used to detect the fear behavior in rats. Compared with the model group, the freezing time was significantly shortened and dopamine (DA) and tyrosine hydroxylase (TH) content in brain tissue was reduced, we believed that the TH mRNA expression reduced TH content, thus affecting the DA content and thus improving the extraction composite fear behavior in rats (32). It is shown that the cAMP-PKA pathway affects TH acidification, and PKA acts on TH residues to phosphorylation and participates in short- and long-period regulation of TH, leading to an increase in DA synthesis. Therefore, we speculate that QZF intervention in the cAMP-PKA signaling pathway for the treatment of PD (33-35).

This study predicts the potential action mechanism of QZF for PD through network pharmacology and molecular docking methods. First, the screening of QZF active components and action targets revealed that 84 active components, 691 target genes. The PD targets are also collected. Then, the PPI network visualization of the obtained QZF and PD intersection genes was performed and arranged the targets according to the size of the degree value, which showed a total of 95 potential targets for QZF treating PD. Molecular docking shows that the main active components in QZF and the core target have good binding activity, and performs GO enrichment analysis and KEGG pathway analysis on the potential targets, which reflects the multi-component, multi-target and multiple pass complex characteristics of QZF in treating PD.

Figure 1 depicts the drug-component-target relationships, showing that the six ingredients with the highest node degrees were the main active ingredients of quercetin, β-sitosterol, 4-(4′-hydroxybenzoyloxy)benzyl methyl ether, harmine, 1,7-dimethoxyxanthone, and 1-hydroxy-3,7-dimethoxyxanthone. Quercetin has antioxidant, anti-inflammatory and neuroprotective effects. It can increase the mRNA level of BDNF and reduce that of inducible nitric oxide synthase, thereby improving anxiety-like behaviors caused by chronic neuroinflammation and reducing fear memory (36). β-sitosterol is a common component of BJT, BX, and DZ. It is a plant sterol with anti-inflammatory, tumor-inhibiting, and antidepressant effects. It improves the anxiety symptoms of rats by regulating the gene expression of the GABA_A signaling pathway (37). Harmine is a natural alkaloid with anti-inflammatory, antitumor, antianxiety, and immune-promoting effects. Harmine can enhance GABAergic transmission of neurons projecting to mouse basal amygdala by increasing the release of GABA at the presynaptic terminal to improve anxiety and depression (38).

We obtained 357 PD targets. PPI analysis revealed that there were 95 common targets between this Chinese medicine and this disease, with a total of 95 nodes and 912 edges. The top 10 targets are AKT1, FOS, APP, SLC6A4, COMT, IL6, p53, CASP3, VEGFA, TNF. It is shown that AKT1 (also known as protein kinase B or PKB) contains three closely related isoforms AKT1, AKT2 and AKT3 (39). AKT1 are one of them, and that lack of AKT1 increases the behavior of anxiety in mice. However, restoring the expression of AKT1 in the prefrontal cortex (PFC) can improve fear memory, behavior, where the GABA pathway mediated this process (40). In addition to playing an important role in synaptic transmission, memory, and psychosis, AKT1 is also one of the most common highly activated protein kinases in human cancer. Excessive activation of AKT can also affect downstream effecters and mediate a variety of pathways conducive to tumorigenesis (39). FOS is closely associated with a variety of cancers, such as prostate cancer (41), ovarian cancer (42), breast cancer (43), etc. FOS deficiency in prostate cancer cell can increase cell proliferation and induce carcinogenic pathways changes, and the loss of FOS accelerates the progression of advanced latent prostate cancer disease (41). Lateral subnuclear FOS protein expression in the rat dorsal nucleus, GABA receptor antagonist (lwDR) increased significantly and activated GABAergic neurons, hydroxytonergic neurons in lwDR, causing intense panic escape behavior (44). 5-HT (2A) receptor antagonists and 5-HT (2C) agonists to
weaken FOS expression in the dorolateral caudal thalamus (CPu) (45). A trace infusion of TNF-α prevented c-FOS expression in the hippocampus and blocks fear memory and review (46). Fear memory induces the phosphorylation of the transcription factor CREB and regulates the expression of the early gene c-FOS. After fear behavior tests, CREB phosphorylation levels in the amygdala central nucleus and FOS immune response nucleus, and activation of CREB was involved in the formation of long-term fear memory (47). APP is a precursor protein from amyloid β-protein (AB), and studies suggest improved anxiety, depression behavior of amyloid β protein rats by upregulating the BDNF/TrkB pathway and inhibiting hippocampal autophagy (48). IL-6 is a multifunctional, multi-effect, and polytropic cytokine where PD patients are in proinflammatory states and whose severity is positively associated with IL-6 (49). Studies have shown that IL-6 plays an important role in local inflammation, is the most important carcinogenic factor and affects cell proliferation, metastasis and inflammation (50). TNF is an anti-inflammatory cytokine, widely involved in cell differentiation, apoptosis, and immune response, and plays an important role in autoimmune diseases and tumorigenic mechanisms (51,52). Studies have suggested that cAMP plays an important role in regulating TNF-α expression. For example, an increase in cell cAMP inhibited TNF-α production (53). TNF-α plays an important role in the central nervous system as an immune factor, giving multiple TNF-αo simulate chronic inflammatory response states can cause anxiety-like behavior and damage conditional fear memory (51).

Molecular docking further confirmed the screening results. The main active ingredients of quercetin, betasitosterol, 4-(4’-hydroxybenzoyloxy)benzyl methyl ether, harmine, 1,7-dimethoxyxanthone, and 1-hydroxy-3,7-dimethoxyxanthone have good binding activity with the potential targets AKT1, FOS, and APP, and the conformations of the binding complexes are stable, indicating that these are the key active ingredients and targets of QZF in the treatment of PD.

The GO function and KEGG pathway enrichment analyses of the key targets were carried out to further explore the mechanism of QZF in the treatment of PD. QZF may act through pathways involved in the response to drugs, positive regulation of cell proliferation, adenylate cyclase activation of the adrenergic receptor signaling pathway, synaptic transmission, and cholinergic synaptic transmission, and it may act at the plasma membrane, postsynaptic membrane, plasma membrane, synapse, and cell connections to facilitate drug binding, enzyme binding, identical protein binding, extracellular ligand-gated ion channel activity, and dopamine binding, and thereby regulate neuroactive ligand-receptor interactions, serotoninergic synapses, cAMP signaling, GABAergic synapses, and other pathways to improve panic and relieve anxiety.

5-HT pathway

The amygdala is a key brain area for fear processing. This brain area is regulated by the neurotransmitter serotonin. 5-HT neurons in the dorsal raphe nucleus regulate the basolateral amygdala (BLA) circuit by enhancing the release of 5-HT (54). Postplantar electric shock evaluation of mice with 5-HT synthesis deficiency (Tph2-deficient mice) found that the freezing time of mice increased, 5-HT was released rapidly in the amygdala, and the damage to GABAergic synaptic transmission significantly increased the level of c-FOS protein (55). The pairing of a conditioned stimulus and an unconditioned stimulus can enhance the release of 5-HT in the BLA and promote the expression of c-FOS in the dorsal raphe nucleus 5-HT cells, while the optogenetic excitation of 5-HT axons in the BLA may eliminate the effects of 5-HT release during the conditioned stimulus and unconditioned stimulus (56). 5-HTT is a transmembrane protein that controls the duration and degree of 5-HT neurotransmission by removing 5-HT from outside the cell. 5-HTT knockout (5-HTTKO) mice present significantly elevated extracellular 5-HT levels and an abnormal dendritic spine density of the main BLA neurons, which leads to the impairment of the fading of fear memory, and the time for the fading memory of fear is prolonged (57). However, excessive secretion of 5-HT may also lead to increased panic. Patients with anxiety who take 5-HT agonists orally and have acutely elevated 5-HT levels can have anxiety and panic attacks (58). SSRIs are the standard first-line drug treatment for PD (59). Short-term use of SSRIs can increase 5-HT levels and cortisol levels. Patients with PD are more anxious than controls (58), and acute medication with SSRIs will increase fear and panic, but chronic medication does not (54).

GABA pathway

The striatum contains many GABA neurons, and low GABAergic levels can cause panic. Microinjection of GABAergic antagonists into the striatum can cause defensive behavior in rats, showing freezing and evasion.
responses (60). Blocking GABA\(_A\) receptors on the dorsal side of the midbrain can cause panic attacks, and neurons in the deep layer of the superior colliculus that excite the striatum and activate caudate putamen (CPU) play an important role in this process (61). The hippocampus is also involved in the process of panic attacks. In animal models of fear, the administration of tiagabine (GABA reuptake inhibitor) can significantly reduce hippocampal hyperexcitability and abnormal fear circuit activation (62). Stimulation of the BLA-dorsal periaqueductal gray can worsen panic-like attacks (63). If the number of GABA\(_A\) receptors in the prefrontal cortex and hippocampus of PD patients is reduced or their sensitivity is reduced, patients are more prone to anxiety (64). Benzodiazepines can regulate GABA levels, which are related to the pathogenesis of PD, but benzodiazepines have adverse effects (including drug abuse, withdrawal symptoms, and memory defects) (65). PD patients show decreased GABA\(_A\) receptor binding, the main cause of which is to increase GABA brain levels by inhibiting the GABA-decomposing enzyme GABA transaminase (66). In addition to the above Class SSRIs and BZDs drugs, Class TCAs drugs are considered the second selected treatment such as clomipramine is similar to SSRIs, and reduces high responsiveness to CO2, but have less tolerance and safety and antagonistic side effects against muscarinic, \(\alpha_1\)-adrenergic and histaminergic receptors (67).

**cAMP signaling pathway**

cAMP is a second messenger involved in cell growth, neurotransmitter release, gene transcription, and other cellular processes. PKA is an inactive tetrameric holoenzyme that depends on cAMP (68). PKA activity is affected by a variety of neurotransmitters (acetylcholine, dopamine, norepinephrine, and serotonin). These transmitters activate adenylate cyclase activity through G proteins, induce ATP to become cAMP, and participate in the regulation of anxiety and fear emotions. Therefore, the cAMP-PKA signaling pathway plays an important role in fear (69). Injection of a cAMP-like drug (bucaladesine) into the hippocampus of rats can alleviate the fear memory deficits caused by PKA inhibitors (H-89), and the cAMP-PKA signaling pathway plays an important role in memory consolidation in the fear conditioned response model (70). Targeted activation of cAMP-PKA signaling in the lateral amygdala increases the excitability of its neurons, leading to widespread fear. This observation provides new ideas for further research on the role of amygdala cells and pathways in fear (71). Inhibition of phosphodiesterase 4 (PDE4) causes an increase in cAMP, which in turn activates PKA. PDE4 inhibitors activate the cAMP-PKA pathway, which can protect and regenerate nerves, have an anti-inflammatory effect, and enhance memory and cognition. PDE4 inhibitors are used to treat anxiety, depression, and cognitive disorders (72).

In addition, key targets are related to inflammation and tumor response. KEGG pathway analysis of QZF can be effective through cancer pathway, related to colorectal cancer and prostate cancer, reflecting the characteristics of different diseases of traditional Chinese medicine. Because network pharmacology has the characteristics of drug measurement, the mechanism of some TCM treatment diseases may find that the treatment of another disease, before the drug or leading compounds for the new disease, so QZF not only through multiple mechanism treatment of PD can provide certain clues for the treatment of inflammation, tumor and drug use, but still need further clinical and experimental research (73).

**Conclusions**

In this study, we predicted the active ingredients, targets, and mechanisms of action of QZF in the treatment of PD through network pharmacology and molecular docking methods. After analyzing the results, we believe that the 5-HT, GABA, and cAMP signaling pathways are important routes by which QZF treats PD, meaning that QZF might have the characteristics of multicomponent, multitarget, and multipathway synergistic effects in the treatment of PD, providing certain clues for the treatment of inflammatory tumors and drug use.

**Acknowledgments**

**Funding:** Grants supported this study from the National Natural Science Foundation of China (No. 81774249), and the Shandong Science and Technology Development Plan of Traditional Chinese Medicine (No. 2019-0224).

**Footnote**

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4090). All authors report funding support from the National Natural Science Foundation of China (No. 81774249), and Shandong science and
technology development plan of traditional Chinese medicine (No. 2019-0224). The authors have no other conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Zhao R, Liu P, Song A, Liu J, Chu Q, Liu Y, Jiang Y, Dong C, Shi H, Yan Z. Network pharmacology study on the mechanism of Qiangzhifang in the treatment of panic disorder. Ann Transl Med 2021;9(16):1350. doi: 10.21037/atm-21-4090

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