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Systems pharmacology to decipher the combinational

anti-migraine effects of Tianshu Formula

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Abstract:

Migraine is the most common neurovascular disorder that imparts a considerable burden to health care system around the world. However, currently there are still no effective and widely applicable pharmacotherapies for migraine patients. Herbal formulae, characterized as multiple herbs, constituents and targets, have been acknowledged with clinical effects in treating migraine, which attract more and more researchers' attention although their exact molecular mechanisms are still unclear. In this work, a novel systems pharmacology-based method which integrates pharmacokinetic filtering, target fishing and network analysis was developed and exemplified by a probe, i.e. Tianshu formula, a widely clinically used anti-migraine herbal formula in China which comprises of *Rhizoma chuanxiong* and *Gastrodia elata*. The results exhibit that 20 active ingredients of Tianshu formula possess favorable pharmacokinetic profiles, which have interactions with 48 migraine-related targets to provide potential synergistic therapeutic effects. Additionally, from systematic analysis, we speculate that *Rhizoma chuanxiong* as the monarch herb mediates the major targets like PTGS2, ESR1, NOS2, HTR1B and NOS3 to regulate the vascular and nervous systems, as well as the inflammation and pain-related pathways to benefit migraine patients. Meanwhile, as an adjuvant herb, *Gastrodia elata* may not only assist the monarch herb to improve the outcome of migraine patients, but also regulate multiple targets like ABAT, HTR1D, ALOX15 and KCND3 to modify migraine accompanying symptoms like vomiting, vertigo and gastrointestinal disorders.

Keywords: systems pharmacology; Tianshu formula; migraine; pharmacokinetics

1 Introduction

Migraine is a common, chronic and paroxysmal neurovascular disorder with complex pathophysiology that has fascinated researchers and physicians for several centuries (Monteith and Goadsby, 2011). Typical attack of migraine is featured by throbbing headache, which is usually unilateral, and is normally associated with nausea, vomiting, or sensitivity to sound and light, or exacerbation with head movement (Bussone, 2004; Hargreaves and Shepheard, 1999). Due to the throbbing nature of headache, migraine has long been considered as a vascular event. However, subsequent research data show that, when the headache starts, the blood flow of patients with migraine with aura is still reduced (Cutrer et al., 1998). Thus, vascular theory cannot sufficiently explain the pathophysiology of migraine (Bussone, 2004). Soon afterwards, researchers prove that migraine attacks are usually accompanied by repeated vascular inflammation, and various cytokines and neuropeptides are involved in neuroinflammatory process (Mohr, 2004). Recently, convincing evidences have led to the neural basis of migraine (Bussone, 2004), for the reason that numerous clinical and experimental results support that activating trigeminal system is crucial in migraine pathogenesis (Tajti et al., 2011). However, despite these significant progresses, the exact pathogenesis of migraine has not been fully elucidated (Bussone, 2004), which complicates the development of effective anti-migraine agents.

However, currently, there already have some pharmacologic treatments of migraine which comprise non-specific and relatively specific agents (Monteith and Goadsby, 2011). The non-specific drugs like NSAIDs (such as aspirin) and acetaminophen are effective (Evers et al., 2009), but their side effects on gastrointestinal tract often limit their use (Monteith and Goadsby, 2011). The second kind of non-specific agents, opioids, may be useful for some migraine patients, however, owing to the risk of medication-overuse headache or addiction, their use is restricted (Monteith and Goadsby, 2011). The specific migraine agents consist of triptans (serotonin 5-HT$_{1B/1D}$ receptor agonists) and ergot alkaloids. Beginning in the 1990s, triptans have been developed into relatively effective prescription medications which are used to abort migraines through blocking the release of vasoactive peptides and neurotransmitters (Loder, 2010). Nevertheless, the results of phase I clinical trials show that only one third of migraine patients are pain-free two hours after treatment with a triptan (DeMaagd, 2008). Ergot alkaloids like ergotamine and dihydroergotamine are the first specific anti-migraine agents available (DeMaagd,

2008). However, their applications are still greatly limited by their severe side effects, which include muscle cramps, nausea and vomiting, depression, cheat discomfort, together with nasal congestion and fatigue (DeMaagd, 2008). Therefore, developing more effective and safe anti-migraine agents is still a pressing task.

Traditional Chinese Medicine (TCM) formulae which usually comprise multiple herbs, are expected to exert therapeutic effects through chemical, pharmacological and pharmacokinetic synergistic effects of multi-herbs (Kiyohara et al., 2004), which may show some advantages in developing anti-migraine agents in the following two ways: 1) containing multiple active compounds which may mediate various biological pathways for providing synergistic and/or additive action to benefit those patients with migraine; 2) being natural and therefore may be of low toxicity and side effects (Zhang et al., 2014). Fortunately, some herbal formulae like Tou Feng Yu Pill (Li et al., 2011), Chuanxiong Ding Tong herbal formula granule (Fu et al., 2012) and Tianshu formula have been proved with powerful anti-migraine functions. For example, Tou Feng Yu Pill which is composed of *Radix angelicae dahuricae*, *Rhizoma chuanxiong* and *Folium camelliae sinensis* exerts remarkable therapeutic effects on migraine, and its possible mechanism may be through increasing the cerebral blood flow, relieving neurogenic inflammation, as well as regulating the neurotransmitters, vasoactive substances and neuropeptides, together with increasing the pain threshold (Li et al., 2011). However, for most TCM formulae, owning to their inherent characteristics, i.e. multiple herbs, constituents, targets and mechanism, their exact mechanisms of actions are still unclear.

Among these herbal formulae with effective anti-migraine functions, Tianshu formula which was originated from Da Chuan Xiong Fang and described in Xuan Ming Lun Fang, a well-known formulae book in China that was edited by Liu Wan-su in 1172 (Liang et al., 2014), has attracted our special interests. This formula is composed of two herbs, *Rhizoma chuanxiong* (*R. chuanxiong*, Chuan-Xiong) and *Gastrodia elata* (*G. elata*, Tian-Ma) in the mass ratio of 4:1. Among them, herb *R. Chuanxiong* has been used to promote blood circulation, to expel wind and to alleviate pain (Wagner et al., 2011), and exhibits haemodynamic and analgesic effects (Yan et al., 2005), which is treated as monarch drug in Tianshu formula to benefit the patients with migraine. The other herb, *G. elata*, has the functions of calming the liver, extinguishing wind and alleviating pain (Wagner et al., 2011), and is used as auxiliary drug to ameliorate the migraine syndrome which may function through its sedative, anticonvulsant and antivertigo effects (Cao et al., 2001). Moreover, several active

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constituents of these two herbs are documented to exhibit various biological activities, which may be contributed to the migraine patients. For examples, the major active ingredient of *R. chuanxiong*, ligustilide, has vasodilatation, antiplatelet aggregation, analgesic, neuroprotection and antithrombotic effects (Yan et al., 2008); the active molecule of *G. elata*, gastrodin, has long been used for treating epilepsy, dizziness, dementia and stroke (Zeng et al., 2006). Therefore, in this work, Tianshu formula is selected as a typical probe formula to decipher the molecular mechanism of its anti-migraine property.

Being the basic entity of herbal formulae, herbal medicines usually contain multiple bioactive ingredients that are implicated in multiple targets and pathways, which make the process of decoding the molecular mechanism of herbal formula extremely difficult. Fortunately, as an emerging field, systems pharmacology provides new avenues to decipher the complex pharmacological problems (Berger and Iyengar, 2009). Systems pharmacology integrates pharmacokinetic data, together with targets, pathways and network analyses to explore the drug actions from molecular and cellular levels to tissue and organism levels, which also provides an analysis platform for decoding molecular mechanisms of herbal formula (Zhang et al., 2014). Recently, being a major tool of systems pharmacology, network analysis has also been used to integrate abundant high-dimensional biological data (Zhang et al., 2015), and then to systematically decipher the relationships among drugs, targets, pathways and diseases. Therefore, considering the herbal ingredients and their related targets in the context of systems pharmacology analysis can help us to have a better understanding of the molecular mechanisms of herbal formula (Zhang et al., 2014).

In the present work, in order to dissect the underlying anti-migraine function of Tianshu formula, a novel systems pharmacology-based method was developed. Specifically, we firstly employed four pharmacokinetic models, including drug-likeness (DL), oral bioavailability (OB), Caco-2 permeability and blood-brain barrier (BBB), to screen the active constituents with favorable ADME profiles from Tianshu formula. Then, the biological targets of these active ingredients were identified and validated via an integrated approach which combined the biological, pharmacological and conformational methods. Finally, by virtue of the systematic pharmacology and network analyses, we deciphered the underlying anti-migraine effect of Tianshu formula.

2 Materials and methods

2.1 Ingredients database

The constituents of medicinal herbs *Rhizoma chuanxiong* and *Gastrodia elata* were extracted from the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP, http://sm.nwsuaf.edu.cn/lsp/tcmsp.php), and then manually supplemented through a wide-scale text-mining method. Subsequently, all structures of these ingredients were optimized using Sybyl 6.9 (Tripos Associates, St. Louis, MO) with the same parameters as our previous work (Li et al., 2012). Considering the fact that glycoside could be hydrolyzed to its aglycone before being absorbed (Zhang et al., 2014), in this section, 9 compounds containing glycosyls were further deglycosylated based on the ruler of glycosidase hydrolysis reaction and their corresponding aglycone chemicals were also added to the database of Tianshu formula for further research.

2.2 Active ingredients screening

The primary cause of costly late-stage failures in drug development is the poor pharmacokinetic profiles (Wang and Urban, 2004). Therefore, an early pharmacokinetic evaluation of the candidate compounds is extremely important in the development phase. For herbal medicine, an accurate identification of the active ingredients that possess pharmacokinetic properties is a fundamental step to assess the therapeutic mechanism of herbs. However, owing to the disadvantages of biological experiments as high-cost and time-consuming, identifying the pharmacokinetic properties of candidate compounds by *in silico* methods becomes an accessible approach in drug development. Here, for filtering the active ingredients from herbal medicines, we employed four ADME parameters including drug-likeness, oral bioavailability, Caco-2 and blood-brain barrier penetrations.

Drug-likeness evaluation. Drug-likeness generally means "molecule which holds functional groups and/or has physical properties consistent with the majority of known drugs" (Walters and Murcko, 2002). In our present work, we employed an *in silico* model which was described in our previous work (Liu et al., 2013) to calculate the drug-likeness index for each compound. This model was constructed based on all the drugs and drug-likeness molecules of DrugBank database (http://www.drugbank.ca/), i.e. 6511 structurally diverse compounds. All descriptors that were calculated by Dragon software had been introduced to this model. The classifications of these parameters range from constitutional parameters (such as molecular weight), one-dimensional descriptors (like log$P$, numbers of H-donors and

H-acceptors), two-dimensional profiles (for instance, polarity number, global topological charge index), three-dimensional factors (for example, average geometric distance degree and radius of gyration), and other parameters (such as total positive and negative charges). After removing the descriptors without significant differences, 1533 parameters were finally employed to build this model, based on Tanimoto coefficient (as displayed in Eq.1).

$$T(A,B) = \frac{A \cdot B}{|A|^2 + |B|^2 - A \cdot B} \tag{1}$$

where A is the molecular properties of herbal ingredients, and B shows the average molecular properties of 6,511 compounds in DrugBank database based on Dragon soft descriptors. The higher DL index an herbal ingredient has, the larger possibility it may possess certain biological properties. Finally, the ingredient with suitable DL index (DL $\geqslant$ 0.18) was chosen as the candidate compound for further research, for the reason that the average DL index of these 6511 molecules is 0.18,.

Oral bioavailability. The oral bioavailability is a crucial criterion for judging the delivered capability of a given compound to the systemic circulation by oral administration. In the present work, a robust in-house model OBioavail 1.1 (Xu et al., 2012) that integrated the metabolism and transport information was employed to calculate the OB values of all herbal ingredients. The optimal model was built based on 805 structurally diverse drugs and drug-like molecules, and shows high predictability (with $R_{training}$ = 0.89 and R_{test} = 0.85 for internal training and external test sets, respectively, and the standard errors of estimate $SEE_{training}$ = 0.35, SEE_{test} = 0.42, respectively). Finally, herbal ingredients having suitable OB values (OB $\geqslant$ 30%) were filtered as the candidate compounds for subsequent research.

Caco-2 cell permeability. Caco-2 cell lines have been extensively used to predict the oral absorption properties of drugs across the intestinal epithelial cell barrier. Thus, the *in silico* Caco-2 permeability model constructed in our previous work (HU et al., 2009) was employed to select compounds that are more likely to possess good permeabilities. This model was constructed by 100 drugs with satisfactory statistical results (R^2 > 0.8). Eventually, by considering the fact that molecule with Caco-2 value less than -0.4 is not permeable, the threshold of Caco-2 permeability is set to -0.4.

BBB penetration. High BBB permeability is a necessary condition for CNS (central

nervous system)-active drugs to pass the blood-brain barrier and then to deliver therapeutics into the brain (Gabathuler, 2010). Therefore, we introduced a reliable BBB model described in our previous works (Gabathuler, 2010) to evaluate the abilities of compounds which can diffuse through the BBB to reach CNS. In this model, the dataset was made up of 190 related but chemically diverse molecules which are either penetrating or non-penetrating across the BBB. Due to the fact that molecules with BBB values less than -0.3 are considered as non-penetrating, the threshold of BBB is set to -0.3.

2.3 Target fishing

Identifying the molecular targets is an essential step following the discovery of active molecules that elicit biological phenotypes (Nidhi et al., 2006). Thus, after screening the active ingredients of herbs, several approaches enriched with chemometric, information integration, chemogenomic method and data-mining were implemented to identify the molecular targets. The detailed identification procedures are as follows. Firstly, we obtained the biological targets of active ingredients from three molecular target predicted models, including Similarity Ensemble Approach (SEA, http://sea.bkslab.org/) (Keiser et al., 2007), STITCH (http://stitch.embl.de/) (Kuhn et al., 2012) and a chemogenomic integrated model (Yu et al., 2012) which was constructed by our previous work. Among them, the chemogenomic integrated method effectively combines the chemical, genomic and pharmacological information based on support vector machine (SVM) and random forest (RF), and the optimal model displayed impressive performance of predicting the compound-target interactions, with concordance of 85.83%, sensitivity of 79.62%, and specificity of 92.76%, respectively. Here, we extract the compound-target interactions with SVM score $\geqslant$ 0.8 and RF score $\geqslant$ 0.7 for further research. Secondly, all active compounds were sent to databases of HIT (Herbal Ingredients' Targets database, http://lifecenter.sgst.cn/hit/) (Ye et al., 2011), TTD (Therapeutic Targets Database, http://bidd.nus.edu.sg/group/ttd/) (Zhu et al., 2012) and DrugBank to mine the literature-supported compound-target interactions. Finally, for better dissecting the role of Tianshu formula in migraine treatment, we sent the target information of these ingredients obtained from the previous two steps to TTD, PharmGKB (http://www.pharmgkb.org) (Whirl-Carrillo et al., 2012) and the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) to mine target-related diseases. As mentioned above, the pathophysiology of migraine is implicated in inflammation, vascular and neural systems, and migraine is normally accompanied with nausea,

vomiting and pain. Therefore, according to the target-related diseases as well as the tissue distribution of these diseases, those targets which were implicated in vascular and neural systems, as well as inflammation, nausea, vomiting and pain were retained, and other ones were eliminated.

In order to improve the reliability of obtained models, we implemented the molecular docking method to validate those predictive compound-target interactions in program GOLD5.1. Being an automated ligand docking program, GOLD software uses a genetic search algorithm method to explore the full range of ligand conformational flexibility and the rational flexibility of receptor (Jones et al., 1997; Perola et al., 2004). The 3D crystallographic structures of these proteins were downloaded from RCSB Protein Data Bank (http://www.pdb.org/) or built by the Swiss-Mode Automated Mode (http://swissmodel.expasy.org/) if the 3D crystal structures were not available. The ratio of PDB to homology models is 2:1. In details, 30 targets were obtained from the predictive models, and only 10 homology models were built by the Swiss-Mode Automated Mode. For PDB models, before docking simulations were performed, those co-crystal ligands were extracted and then mixed into the docking database for re-dock. For homology models, their active sites were built by the residues, atoms or the atomic coordinates which were already identified from corresponding experimental literatures. Taking PTGS1 as an example, the major residues of its active site included His89, Leu92, Met112, Val115, Arg119, Gln357 and Glu523 (Gierse et al., 1996) which were employed to explore the active site of PTGS1 based on the module of list of atoms or residues in GOLD software. Before docking simulations were performed, hydrogen atoms were added. The docking optimized parameters were set as default, and ligand-receptor interactions were analyzed using GoldScore scoring functions. Finally, the models possessing high compound-target binding affinities (when GoldScore $\geq$ 40) were selected for further research.

2.4 Network construction

For better deciphering the underlying molecular mechanism of Tianshu formula, in this section, we constructed three corresponding networks: 1) Compound-target network (C-T network). Active ingredients of Tianshu formula and their corresponding targets were employed to generate the C-T network in which a compound and a target are connected with each other if this protein is a known or validated target of this molecule. 2) Target-disorder network (T-D network). We firstly

mined the related disorders of all targets from the databases of TTD and PharmGKB, and then all targets and their corresponding disorders were employed to build a bipartite graph of target-disorder network. 3) Target-pathway network (T-P network). We extracted the exact pathway information of targets from the database of KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/), and then built a target-pathway bipartite graph which is composed of targets and their corresponding canonical pathways. All visualized network graphs were constructed by Cytoscape 3.1.0, an open software package project for visualizing, integrating, modeling and analyzing the molecular and genetic interaction networks (Smoot et al., 2011).

3 Results and discussion

TCM formula, being characterized as using multiple herbs together, acts on multiple targets to balance the whole body system and then achieves the therapeutic effects. Compared with the mono-therapeutic agent, TCM formula possessing multiple active ingredients not only strengthens the therapeutic efficacy of single drugs, but also attenuates the side effect of its major components through *in vivo* drug and drug interactions (Tian, 2011). Nevertheless, there are two sides to every coin. It is just due to the same characteristics of multi-components, multi-targets and multi-pharmacologic effects of TCM formulae, that the exact molecular mechanisms of most TCM formulae still remain unclear. Thus, in this work, we employed bioinformatics resources and methodologies which integrated the active components filtering, target fishing and network analysis to decipher the active ingredients, targets and pathways of Tianshu formula, and then to systematically decode its therapeutic mechanism of actions.

3.1 Active ingredients of Tianshu formula

Normally, the pharmacological activity of herbal medicine is achieved when its active components reach and sustain proper levels at action sites. The pharmacodynamic processes of active constituents in the body govern their concentrations at the target sites after administration of an herbal product, thus influencing the therapeutic responses (Lu et al., 2008). Consequently, screening those active ingredients with favorable pharmacokinetic properties is essential for us to understand the therapeutic effectiveness of herbal medicines. In the present work, we employed four classical ADME parameters to filter active compounds from Tianshu formula. Finally, from the 256 compounds of Tianshu formula (as shown in

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Supporting information Table S1), a total of 20 active chemicals (as displayed in Table 1) are identified with favorable pharmacokinetic profiles.

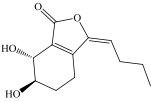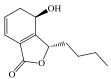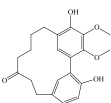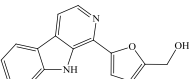Table 1 Active ingredients and ADME parameters of Tianshu formula.
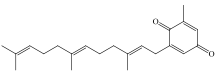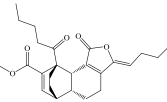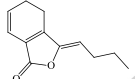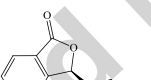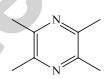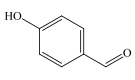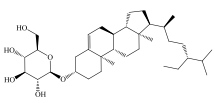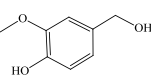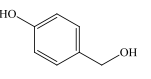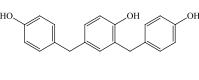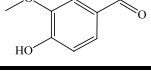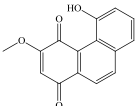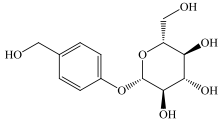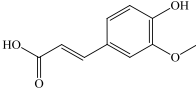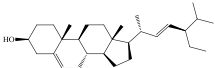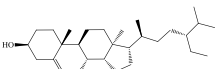
No.	Name	Structure	OB	Caco-2	DL	BBB	Herbs
M005	Senkyunolide-I		21.38	1.00	0.08	0.90	*R. chuanxiong*
M006	Senkyunolide-K		61.80	0.52	0.08	0.30	*R. chuanxiong*
M045	Myricanone		57.61	0.67	0.51	-0.08	*R. chuanxiong*
M051	Perlolyrine		67.82	0.88	0.27	0.15	*R. chuanxiong*
M062	Senkyunone		15.20	1.15	0.24	0.50	*R. chuanxiong*
M068	Wallichilide		5.73	0.82	0.71	0.73	*R. chuanxiong*
M122	Ligustilide		51.30	1.31	0.07	1.28	*R. chuanxiong*
M129	Senkyunolide-A		65.15	1.30	0.07	1.35	*R. chuanxiong*
M141	Ligustrazine		29.64	1.19	0.03	1.06	*R. chuanxiong*
M160	4-Hydroxybenzaldehyde		18.85	0.80	0.02	0.65	*G. elata*
M162	Daucosterol		20.63	0.03	0.63	-0.69	*G. elata, R. chuanxiong*
M165	Vanillyl alcohol		37.70	0.56	0.03	0.24	*G. elata*
M170	4-Hydroxybenzyl alcohol		55.21	0.60	0.02	0.34	*G. elata*
M177	2,4-Bis(4-hydroxybenzyl) phenol		30.49	1.00	0.28	0.08	*G. elata*
M203	Vanillin		69.24	0.67	0.03	0.56	*G. elata, R. chuanxiong*

Table 1: Continued

No.	Name	Structure	OB	Caco-2	DL	BBB	Herbs
M208	Cymbinodin A		50.26	0.71	0.21	0.28	*G. elata*
M214	Gastrodin		7.56	-1.21	0.17	-1.86	*G. elata*
M233	Ferulic acid		55.14	0.41	0.06	0.00	*R. chuanxiong*
M250	Stigmasterol		43.83	1.31	0.76	0.77	*G. elata*
M256	β-Sitosterol		36.91	1.33	0.75	0.88	*G. elata, R. chuanxiong*

3.1.1 *Gastrodia elata*

After ADME screening, 10 out of 72 ingredients with satisfactory pharmacokinetic profiles are extracted from herb *G. elata*, and many of them exert excellent pharmacological effects. Taking stigmasterol (M250) as an example, this sterol compound possesses satisfactory ADME profiles (OB = 43.83%, Caco-2 = 1.31, DL = 0.76 and BBB = 0.77), and exhibits significant analgesic effects against both acetic acid- and formalin- induced nociception in mice model (Santos et al., 1995). Additionally, β-sitosterol (M256) with appropriate pharmacokinetic parameters (OB = 36.91%, Caco2 = 1.33, DL = 0.75 and BBB = 0.88) shows potent anti-inflammatory activity against carrageenin-induced oedema in rat model (Gupta et al., 1980), and also exhibits antipyretic effect against aconitine-induced writhing in mice model (Gupta et al., 1980).

In order to avoid omitting active ingredients, we also reviewed abundant related articles to manually add a few literature-based active ingredients. For example, gastrodin (M214), being the major bioactive ingredient of *G. elata,* exhibits the sedative, anti-inflammatory, anti-convulsive and neuroprotective effects, although its DL index is extremely low (0.02). Analogously, vanillin (M203) which is the overlapping compound of herbs *G. elata* and *R. chuanxiong*, also exerts desirable biological activities like antiepileptic (Zhao, 1985), anti-oxidant and anticonvulsant effects (Ojemann et al., 2006), although it has a low DL index (0.03). These two compounds are manually added into the active ingredients database.

3.1.2 *Rhizoma chuanxiong*

For herb *R. chuanxiong*, only 13 ingredients pass through these strict filtering criteria, and most of them exhibit biological activities, such as perlolyrine (M051), myricanone (M045), daucosterol (M162), and senkyunolide-I (M005). For examples, the indole alkaloid perlolyrine (OB = 67.82%, Caco-2 = 0.88, DL = 0.27 and BBB = 0.15) possesses powerful protective effect on injured human umbilical vein endothelial cells (LIU et al., 2003); myricanone (OB = 57.61%, Caco-2 = 0.67, DL = 0.51 and BBB = -0.08) shows significant inhibitory effect in an *in vivo* two-stage carcinogenesis test on mouse skin tumor promotion (Ishida et al., 2000). According to these filtering criteria, we also obtain some other potential bioactivity components such as wallichilide (M068) and senkyunone (M062) which have been unconfirmed until now.

Additionally, although compound like ferulic acid (M233, DL = 0.06) holds low DL value, it exhibits remarkable pharmacological effect. For instance, ferulic acid possesses powerful antioxidant activities through scavenging the hydroxyl radical, superoxide, hydrogen peroxide and nitrogen dioxide free radicals (Ou and Kwok, 2004), and also shows the thromboxane A2 (TXA2) synthetase inhibitory activity (Xu et al., 1984; Zhang et al., 1994), all of which make ferulic acid to be a potential chemopreventive drug against coronary heart disease (Ou and Kwok, 2004). Therefore, for the reasons mentioned above, ferulic acid is also added into the active ingredient database for further research.

Despite of the fact that this research system for screening active ingredients is capable of effectively narrowing down the candidate active compounds, it still has some space for further optimization. The incorporation of the consideration of the contents of active ingredients in herbs, for instance, is just an interesting research direction. However, due to the diverse experimental measurement criteria and herbal samples, a quantification and comparison of the concentration of ingredients of herbs based on the quantitative references is extremely difficult currently. Even though, we have still conducted a wide-scale text mining of PubMed and CNKI (China National Knowledge Infrastructure) databases for quantifying these potential active ingredients of *R. Chuanxiong* and *G. elata*. As a result, however, only a small amount of quantitative data (data not shown) can be obtained based on a unified measurement criteria and sample, which makes it impossible for quantitative analysis of potential active ingredients with the available experimental data. But from these results, we pay

more attention to those ingredients with abundant contents in herbs for avoiding the risk of omitting possible active ingredients. For those ingredients that have both high contents in *R. Chuanxiong* and/or *G. elata*, and experimentally proved pharmaceutical activities, we also add them into corresponding candidate compound pools. Take senkyunolide-I as an example. This compound, as a component of *R. chuanxiong*, displayed analgesic and anti-migraine activities through improving the neurotransmitter levels in rat model, as well as decreasing the levels of nitric oxide in the blood and brain of rats (Wang et al., 2011), thus also has potential to be a novel pharmaceutical drug in treating migraine pain. However, it only obtains low OB value (21.38%). We assume it is the high content of senkyunolide-I, the average of which reaches 1.73mg/g (Wang et al., 2011) in the herb that may significantly increase its absolute OB value. Thus, senkyunolide-I is also added into the active ingredient database.

3.2 Target proteins of Tianshu formula

In order to decipher the underlying molecular mechanism as well as the combinatorial rule of Tianshu formula, it is indispensable to identify the target proteins of active ingredients. However, experimental methods for searching targets of drugs are challenging and time-consuming (Lomenick et al., 2010), and many drugs may not be predicted precisely due to their multiple physiological targets (Wang et al., 2013). Thus, in the present work, we introduced an integrated *in silico* approach which contains the predictive models including SEA, STITCH and chemogenomic model, and mining the databases of HIT, TTD and DrugBank to respectively identify the predicted and known target proteins of active ingredients of Tianshu formula, and molecular docking was also employed to validate those predictive compound-target interactions. Finally, 48 targets were confirmed possessing interactions with the active ingredients of Tianshu formula, with the detailed information of these target proteins displayed in Table 2.

Table 2 Target information of Tianshu formula

ID	Target	Uniprot ID	Gene name	Related diseases
T_01	Prostaglandin G/H synthase 2	P35354	PTGS2	Stroke, Inflammation, Nervous system diseases, Myocardial infarction, pain
T_02	Estrogen receptor	P03372	ESR1	Nervous system diseases, Hyperlipidemia, Coronary artery diseases

Table 2: Continued

ID	Target	Uniprot ID	Gene name	Related diseases
T_03	Inositol-1(or 4)-monophosphatase 1	P29218	IMPA1	Mental disorders
T_04	Nitric oxide synthase, brain	P29475	NOS1	Migraine, Mental disorders
T_05	5-hydroxytryptamine receptor 1B	P28222	HTR1B	Migraine, Mental disorders
T_06	Nitric oxide synthase, inducible	P35228	NOS2	Ischemia reperfusion injuries
T_07	Transcription factor AP-1	P05412	JUN	Immune system diseases, Cancer, Vascular diseases
T_08	5-hydroxytryptamine receptor 2B	P41595	HTR2B	Migraine, Mental disorders
T_09	4-aminobutyrate aminotransferase, mitochondrial	P80404	ABAT	Nervous system diseases, Convulsion
T_10	Apoptosis regulator Bcl-2	P10415	BCL2	Immune system diseases, Neoplasms, Cardiovascular diseases
T_11	Transcription factor p65	Q04206	RELA	Embolic focal cerebral ischemia, Ischemic renal injury, Thrombosis, Inflammation, Atherosclerosis
T_12	Apoptosis regulator BAX	Q07812	BAX	Neoplasms
T_13	Caspase-3	P42574	CASP3	Venous thrombosis
T_14	Tumor necrosis factor	P01375	TNF	Asthma, Brain diseases, Diabetes, Inflammation
T_15	Sodium/potassium-transporting ATPase subunit alpha-2	P50993	ATP1A2	Hemiplegia, Migraine
T_16	Nitric-oxide synthase, endothelial	P29474	NOS3	Angina, Inflammation, Cardiovascular diseases, Mental disorders
T_17	Mitogen-activated protein kinase 1	P28482	MAPK1	Nervous system diseases, Mental disorders
T_18	Matrix metalloproteinase-9	P14780	MMP9	Atherosclerosis, Multiple sclerosis, Coronary artery disease, Heart failure
T_19	Prostaglandin G/H synthase 1	P23219	PTGS1	Inflammation, Cardiovascular disease
T_20	Prothrombin	P00734	F2	Myocardial infarction, Thromboembolism
T_21	Glycogen synthase kinase-3 beta	P49841	GSK3B	Diabetes, Ischemia, Brain injury, Nervous system diseases
T_22	Acetylcholinesterase	P22303	ACHE	Brain ischemia, Nervous system diseases, Cognitive deficits

Table 2: Continued

ID	Target	Uniprot ID	Gene name	Related diseases
T_23	Alcohol dehydrogenase 1C	P00326	ADH1C	Neoplasms
T_24	Alpha-1D adrenergic receptor	P25100	ADRA1D	Hypertension
T_25	Beta-3 adrenergic receptor	P13945	ADRB3	Hypertension, Cardiac arrhythmias, Diabetes mellitus
T_26	Arachidonate 15-lipoxygenase	P16050	ALOX15	Atherosclerosis, Vascular diseases
T_27	Arachidonate 5-lipoxygenase	P09917	ALOX5	Asthma, Gastrointestinal disease, Cerebrovascular disorders, Inflammation, Cerebral vasospasm,
T_28	Caspase-8	Q14790	CASP8	Nervous system diseases
T_29	Caspase-9	P55211	CASP9	Nervous system diseases, Brain injury
T_30	Choline O-acetyltransferase	P28329	CHAT	Mental disorders
T_31	Serine/threonine-protein kinase Chk1	O14757	CHEK1	Neoplasms
T_32	Coagulation factor VII	P08709	F7	Thromboembolism, Cardiovascular diseases
T_33	Hypoxia-inducible factor 1-alpha	Q16665	HIF1A	Stroke, Cardiovascular diseases, Lung diseases
T_34	5-hydroxytryptamine receptor 1D	P28221	HTR1D	Migraine, Vomiting, Vascular headache
T_35	5-hydroxytryptamine receptor 1F	P30939	HTR1F	Migraine
T_36	5-hydroxytryptamine receptor 7	P34969	HTR7	Migraine
T_37	Inositol-1(or 4)-monophosphatase 2	O14732	IMPA2	Mental disorders
T_38	Integrin alpha-IIb	P08514	ITGA2B	Cardiovascular diseases, Thrombosis, Myocardial infarction
T_39	Potassium voltage-gated channel subfamily D member 3	Q9UK17	KCND3	Migraine, Pain, Vascular disease, Vertigo
T_40	Amine oxidase [flavin-containing] B	P27338	MAOB	Nervous system diseases
T_41	Serum paraoxonase/arylesterase 1	P27169	PON1	Cardiovascular disease
T_42	Tyrosine-protein phosphatase non-receptor type 1	P18031	PTPN1	Diabetes mellitus, Neoplasms
T_43	Glycogen phosphorylase, muscle form	P11217	PYGM	Diabetes mellitus
T_44	Superoxide dismutase [Cu-Zn]	P00441	SOD1	Ischemic injury, Neoplasms
T_45	Thromboxane-A synthase	P24557	TBXAS1	Asthma, Cerebral circulatory disorders, Immune system diseases, Vascular diseases, Thromboembolic disorders

Table 2: Continued

ID	Target	Uniprot ID	Gene name	Related diseases
T_46	Transforming growth factor beta-1	P01137	TGFB1	Multiple sclerosis, Neoplasms
T_47	Transient receptor potential cation channel subfamily V member 1	Q8NER1	TRPV1	Migraine, Pain
T_48	Vascular endothelial growth factor A	P15692	VEGFA	Neoplasms, Ischemic heart disease

3.2.1 *Rhizoma chuanxiong*

For *R. chuanxiong*, 42 targets are confirmed to have 78 interactions with 13 active ingredients of this herb. And the results show that active ingredients of *R. chuanxiong* mediate 9 therapeutic targets of migraine, including serotonin receptors (HTR1B, HTR1D, HTR1F, HTR2B and HTR7), as well as ATR1A2, NOS1, TRPV1 and KCND3. Taking serotonin receptors as examples, several compounds, like β-sitosterol, senkyunone and daucosterol, display high affinities with serotonin receptors, such as HTR2B, HTR1B and HTR1F, and these actions may provide therapeutic effects for migraine patients through causing cranial vasoconstriction or blocking the release of vasoactive peptides from the perivascular trigeminal (Arulmani et al., 2004). Among them, HTR1B mediates vasoconstriction both in the cranial circulation and in the heart (Olesen and Ashina, 2011) and also controls the release of both substance P and CGRP (calcitonin gene-related peptide) (Waeber and Moskowitz, 2005); 5HT1F is expressed on trigeminal neurons, thereby hindering the release of the major transmitters (like CGRP) in trigeminal nociception and of the neuropeptide co-transmitters (Waeber and Moskowitz, 2005); additionally, in human pulmonary artery endothelial cells, stimulating HTR2B can cause intracellular Ca^{2+} release and potent contraction (Hannon and Hoyer, 2002). Therefore, we speculate that the powerful anti-migraine effects of Tianshu formula are closely related with these 9 therapeutic targets of migraine which are mediated by *R. chuanxiong*.

Besides these therapeutic targets of migraine, *R. chuanxiong* can regulate two major categories of proteins including nitric oxide synthase (NOS) enzymes and inflammatory mediators (like TNF and TGFB1). Specifically, some active constituents such as senkyunolide-I, ferulic acid, ligustilide (M12) and β-sitosterol may mediate the NOS enzymes (NOS1, NOS2 or NOS3) to inhibit the nitric oxide biosynthesis, thereby possibly acting at peripheral sites to suppress the neurogenic dural vasodilation and at the endothelial level to hinder CGRP-induced dilation (Goadsby, 2005). It deserves to note that NSAIDs and selective COX2

(cyclooxygenase 2, PTGS2) inhibitors are by far the most frequently-used drugs for the acute treatment of migraine headaches (Farinelli et al., 2009; Pardutz and Schoenen, 2010), which inhibit the COXs (PTGS1 and/or PTGS2) activities and then decrease the prostaglandin synthesis to relieve the migraine-related inflammation and pain (Farinelli et al., 2009). Fortunately, 5 compounds of *R. chuanxiong*, including M005, M006, M062, M122 and M233, may have interactions with PTGS2 and/or PTGS1, which are expected to offer the same therapeutic benefits to migraine patients as the NSAIDs or selective COX2 inhibitors. Additionally, other mediators involved in the inflammatory process, like cytokines TNF and TGFB1, are also regulated by the active constituents of *R. chuanxiong*, which might afford additional anti-inflammation opportunities in migraine treatment.

It's worth noting that, most of the targets regulated by herb *R. chuanxiong* like PTGS2, ESR1 and NOS2, are implicated in the vascular and nervous system diseases, and in this way, *R. chuanxiong* may offer synergistic and/or additive effects to regulate the vascular and nervous systems. Taking ESR1 as an example, 4 compounds including M045, M051, M062 and M068 all have the potential to interact with proteins ESR1, which may mediate the vascular system to regulate the lipid and cholesterol levels and to promote the functional recovery of vascular injury (Deroo and Korach, 2006). Additionally, some major targets, like HTR1B, PTGS2, NOS3 and NOS1, are also involved in the pain-related diseases, which may contribute to the anti-migraine effect of *R. chuanxiong* through relieving the migraine pain. All above phenomena may well explain why *R. chuanxiong* can be used as traditional Chinese herb to promote the blood circulation and to relieve pain in clinical practice for thousands of years (Li and Bi, 2003).

3.2.2 *Gastrodia elata*

For *G. elata*, after target fishing, 10 active ingredients are validated to bind with 31 target proteins which contain 8 therapeutic targets of migraine. Additionally, there are also plenty targets that are implicated in inflammatory, vascular and nervous system diseases. For example, gastrodin (M214), the major active constituent of *G. elata*, may have the potential to interact with 8 targets including ABAT, PTGS2, NOS2, TNF, IL1B, ESR1, NOS1 and ATP1A2, which are all associated with vascular and nervous system diseases. Actually, gastrodin was identified as an inhibitor of ABAT (Tao et al., 2006), which might contribute to its therapeutic application in nervous system diseases (Tao et al., 2006). Besides, gastrodin also significantly

attenuates the levels of neurotoxic proinflammatory mediators and cytokines like NOS2, PTGS2, TNF and IL1B (Dai et al., 2011). All these findings may be conducive to better explaining the pharmacological effects of gastrodin on the nervous systems in several animal models (Ojemann et al., 2006; Xu et al., 2007). Analogously, being the common active compound of these two herbs, vanillin may have interactions with 5 potential targets including PTGS2, ABAT, JUN, MMP9 and MAPK1. And all these targets are relevant to nervous system and/or vascular diseases, which could be used to explain why vanillin shows many related pharmacologic actions such as anticonvulsant, antioxidant and anti-mutagenic properties (Tai et al., 2011).

Additionally, there are also many targets which are involved in pain-related disorders, like NOS1, HTR1B, PTGS2, HTR2B and ESR1. 4 herbal ingredients including M162, M214, M250 and M256 all have the potential to target on NOS1 to regulate NO production, which may offer synergistic effects to relieve neuropathic pain (Pardutz et al., 2000). As we all know, besides headache, most patients who experience migraine have vertigo, gastrointestinal disturbances and vomit as the major symptoms. Fortunately, 4 targets of *G. elata* including ABAT, HTR1D, ALOX15 and KCND3 are found to be implicated in vertigo, vomit or gastrointestinal -related diseases. For instances, M160 (4-hydroxybenzaldehyde) and M170 (4-hydroxybenzyl alcohol) may have interactions with target ABAT to regulate the brain GABA concentration, which may contribute to the anti-vertigo activity of *G. elata* (Hayashi et al., 2002). And these findings may explain why *G. elata* exhibits the sedative and anticonvulsant functions (Cao et al., 2001).

3.2.3 Target analysis to decipher the combination rule of Tianshu formula

To facilitate the visualization and further interpretation of these results, all active ingredients and targets of Tianshu formula were employed to generate a bipartite graph of compound-target network (as displayed in Figure 1). Subsequently, the topological analysis of this network was engaged to reveal the compounds and proteins properties and to explore the crucial therapeutic mechanism of Tianshu formula. From this network, two characteristics of Tianshu formula are observed: 1) the promiscuous properties of its active ingredients. Most active ingredients in this formula are multi-target compounds, with the potential to become the promiscuous drugs. As displayed in this network, 19 out of 20 compounds are associated with more than one target, indicating the multi-target features of active herbal ingredients. 2) the presence of highly interconnected compounds. Among these multi-target ingredients,

those ones with high degrees, especially for compounds β-sitosterol (degree = 13), ligustilide (degree = 11) and gastrodin (M02, degree = 10), are responsible for the high interconnectedness of the C-T network. For example, β-sitosterol has interactions with 13 targets like NOS1, HTR2B and KCND3, showing non-selective for special targets, which may contribute to its analgesic and anti-inflammatory activities (Nirmal et al., 2012).
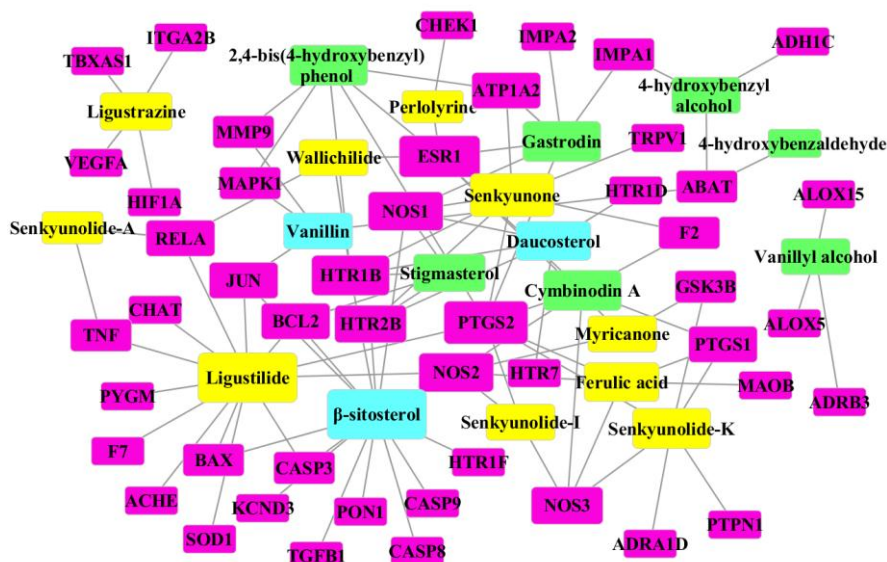

Figure 1 Compound-target network of Tianshu formula. The fuchsia nodes represent the targets of Tianshu formula, the yellow ones are the particular compounds of *R. Chuanxiong*, the green ones indicate the particular molecules of *G. elata*, and cyan nodes represent the shared compounds of *R. Chuanxiong* and *G. elata*. Node size is proportional to its degree.

Furthermore, from the topological features of the compounds in this network and the functional properties of the proteins, we observed that this formula modulates the multiple systems to achieve the treatment effects of migraine, which is the typical pattern of the TCM formula treatment. As shown in the C-T network, benefits of this formula are not only concentrated on modulating several crucial targets, such as target proteins in the nervous and vascular systems (like ESR1, HTR1B and NOS3), but also, more essentially, focus on the regulation of other accompanying changes that prolong the healing process, including mediating inflammation, vertigo and pain-related proteins, like PTGS2, TNF, NOS1, ABAT and NOS2. For instance, 7 ingredients like 2,4-bis(4-hydroxybenzyl) phenol (M177), gastrodin (M214) and cymbinodin A (M208) have the potential to interact with ESR1 simultaneously, and may

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synergistically regulate this protein. Indeed, ESR1 G594A polymorphism has been implicated in migraine susceptibility, which positively interrelates with an increased incidence of migraine (Colson et al., 2004). Additionally, HTR1B, the other high-degree target protein, is linked with 5 constituents such as stigmasterol (M250) and senkyunone (M062), thus becoming the other core target of Tianshu formula. It has been reported that 5-HT$_{1B/1D}$ receptor agonists triptans are one type of the most effective medications for migraine, and their beneficial effects are associated with the multiple mechanisms of action, including causing cranial vasoconstriction, inhibition of nociceptive neurotransmission and peripheral trigeminal inhibition (Tepper et al., 2002). Furthermore, several active ingredients, especially for compounds gastrodin (Dai et al., 2011), ligustilide (Chung et al., 2012) and vanillin (Kim et al., 2010), significantly attenuate the expression level of neurotoxic proinflammatory mediator PTGS2, exhibiting enhanced anti-inflammatory effects. Therefore, we conclude that the reason Tianshu formula exhibits potent anti-migraine effect may be through the regulation of nervous and vascular systems, as well as of those inflammation, pain and vertigo- related proteins.

In order to further decipher the action mechanism and combination rule of Tianshu formula, we constructed the T-D network based on all targets and their corresponding diseases (as displayed in Figure 2). As shown in this figure, both herbs of Tianshu formula can regulate vascular and nervous systems, as well as migraine, inflammation and pain-related proteins. However, compared with *G. elata*, the target scope of herb *R. chuanxiong* is much broader, which is supported by the fact that 42 (pink and red nodes in Figure 2) out of 48 targets of Tianshu formula have 78 interactions with active ingredients of this herb. And these phenomena may be used to explain why *R. chuanxiong* is the monarch drug in Tianshu formula. As to herb *G. elata*, bedsides acting on those targets with the same pharmacological mechanisms as the ones of *R. chuanxiong*, its active ingredients also mediate several targets like ABAT, HTR1D, ALOX15 and KCND3, which may provide pharmacological effects to reduce the accompanying symptoms of migraine like vomit, vertigo and gastrointestinal disorders. And this phenomenon fully manifests the auxiliary role of *R. chuanxiong* in Tianshu formula. Therefore, we conclude that in the treatment of migraine by Tianshu formula, herb *R. chuanxiong* as the monarch drug may improve the vascular and nervous systems, and exert anti-inflammatory and analgesic effect, while herb *G. elata* as the adjuvant component may not only assist the monarch drug to improve the outcome of the patients with migraine, but also produce pharmacologic

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effects to prevent the migraine accompanying symptoms like vomiting, vertigo and gastrointestinal disorders.

Figure 2 Target-disorder of Tianshu formula.

3.3 Pathway analysis to explore the underlying action mechanism of Tianshu formula

For further deciphering the underlying therapeutic mechanisms of Tianshu formula, we extracted the canonical pathways that may be related to the migraine treatment and prophylaxis from KEGG database, which ends up with 20 KEGG pathways, like the PI3K-Akt signaling pathway, serotonergic synapse, neuroactive ligand-receptor interaction, and TNF signaling pathway. For examples, PI3K-Akt signaling pathway modulates the expression of angiogenic factors like nitric oxide (Karar and Maity, 2011), and also suppresses the LPS-induced inflammation and coagulation (Schabbauer et al., 2004); additionally, the pathway of serotonergic synapse modulates the peripheral and cerebral vascular systems, as well as the platelet function, and is also involved in the pathophysiology of mood disorders, emesis and migraine (Mohammad-Zadeh et al., 2008).

In order to systematically analyze the underlying mechanism, all targets are

mapped onto these 20 pathways. Results show that besides targets KCND3 and PYGM, other targets are also implicated in these pathways, thus all of them are employed to generate the T-P network (as displayed in the Figure 3).
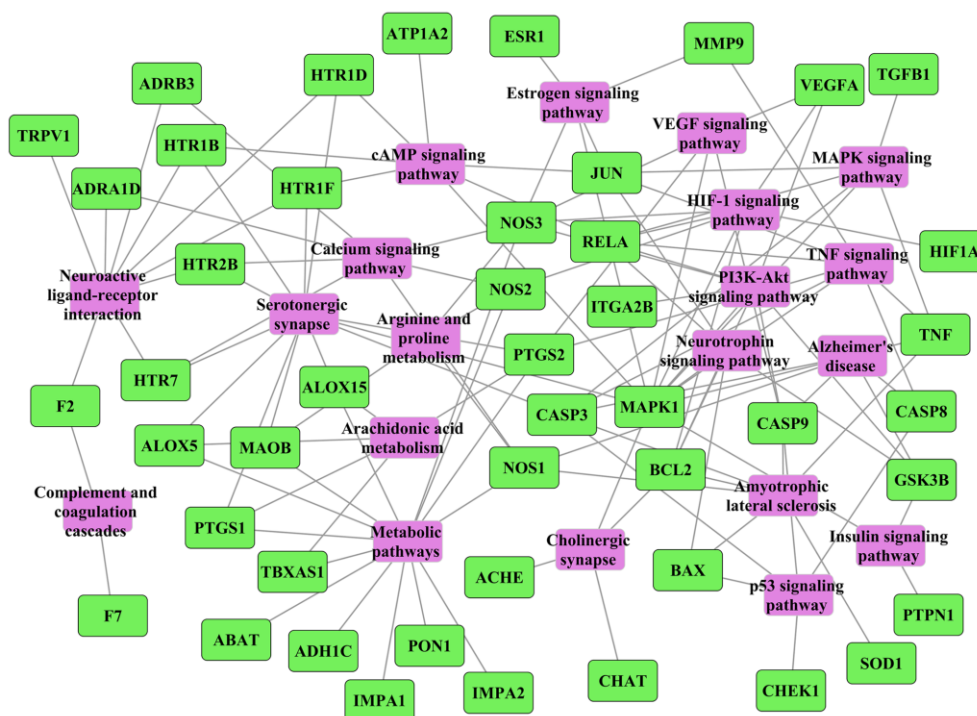

Figure 3 Target-pathway network of Tianshu formula where green nodes represent the targets and purple nodes pathways.

In T-P network, 8 therapeutic targets of migraine for Tianshu formula are mainly implicated in 4 pathways including neuroactive ligand-receptor interaction, serotonergic synapse, cAMP and calcium signaling pathways. Although the precise mechanisms of action underlying the pathogenesis of migraine are unclear, this disease is deemed to be of combined neuronal and vascular origins (Gasior et al., 1999). Thus, modulating the neuroactive receptors, which are contained in the pathway of neuroactive ligand-receptor interaction, with proper ligands may provide therapeutic benefits for migraine patients. Factually, triptans like frovatriptan and rizatriptan exert anti-migraine effects through mediating the neuroactive receptors (HTR1D and HTR1B). Fortunately, the active constituents of Tianshu formula are implicated in mediating the major components of neuroactive ligand-receptor interaction such as HTR1D and HTR1B, which offer the potential therapeutic benefits to patients with migraine. Therefore, we speculate that the anti-migraine effect exerted by Tianshu formula occurs, at least partially, through regulating the neuroactive ligand-receptor interaction, serotonergic synapse, cAMP and calcium

signaling pathways.

Additionally, several high-degree pathways such as PI3K-Akt (degree = 8), TNF, cAMP (degree = 8), HIF-1 (degree = 7) and calcium (degree = 7) signaling pathways which are responsible for the high interconnectedness of T-P network pathways are found to be closely related to the vascular and nervous systems, as well as the inflammation and pain diseases. For example, in endothelial cell model, PI3K-Akt signaling pathway can be triggered by many angiogenic growth factors, and then mediates the downstream effector molecules that may be implicated in the blood vessel homeostasis and growth (Shiojima and Walsh, 2002), and also negatively regulate the expression of the LPS-induced acute inflammatory mediators (Guha and Mackman, 2002). Fortunately, 14 compounds of Tianshu formula, especially for compounds ligustilide, ligustrazine (M141), senkyunolide-K (M006), vanillin and β-sitosterol, are implicated in regulating the major constituents of PI3K-Akt signaling pathway (like NOS3, RELA, MAPK1 and BCL2) to show potential therapeutic benefits in vascular system and inflammatory diseases. Therefore, we conclude that the active constituents of Tianshu formula may, mainly by mediating the PI3K-Akt, TNF, cAMP, HIF-1 and calcium signaling pathways, regulate the vascular and nervous systems, as well as the inflammation and pain-related proteins, and then exert therapeutic effects in the treatment of those patients with migraine.

In summary, we speculate that Tianshu formula exerting anti-migraine effect may mainly through the regulation of neuroactive ligand-receptor interaction, serotonergic synapse, cAMP and calcium signaling pathways. Besides, as a holistic medicine, Tianshu formula may be also implicated in PI3K-Akt, TNF, cAMP, HIF-1 and calcium signaling pathways to regulate the vascular and nervous systems, as well as the inflammation and pain-related proteins to benefit those patients with migraine.

4 Conclusions

The lack of effective and widely applicable pharmacotherapies for migraine patients leads to an increasing attention in traditional herbal formulae (like Tianshu formula), for which abundant clinical data for migraine patients have been accumulated in the past hundred years. Nevertheless, the exact active ingredients and molecular mechanism of Tianshu formula are not well understood. Thus, in this text, a systems pharmacology-based method which combines the computational models and wide-scale text-mining methods is employed to dissect the underlying molecular mechanism of Tianshu formula for migraine treatment. The major findings are

summarized as follows:

1) After ADME filtering, 20 active ingredients with favorable pharmacokinetic profiles are extracted from Tianshu formula, which have the potential to participate in migraine treatment. And the systematic use of these active ingredients may provide useful clues on the combination therapies for migraine treatment.

2) The results of target fishing exhibit that Tianshu formula probably acts on 48 targets, and then displays potential synergetic therapeutic effects in migraine treatment through following multiple ways: regulating the therapeutic targets of migraine (like HTR1B, HTR1D, ATR1A2, NOS1 and TRPV1), mediating the vascular and nervous systems, together with those pain and inflammation-related proteins (such as PTGS2, ESR1 and NOS2).

3) The C-T and T-D networks of Tianshu formula demonstrate that, in migraine treatment, herb *R. chuanxiong*, as the monarch drug, benefits migraine patients probably through the regulation of the vascular and nervous systems, as well as of inflammation and analgesia-related proteins, while herb *G. elata*, as the adjuvant component, may not only assist *R. chuanxiong* to improve the outcome of the patients with migraine, but also offer therapeutic effects to modify the migraine accompanying symptoms like vomiting, gastrointestinal disorders and vertigo.

4) The results of T-P network indicate that Tianshu formula displays the anti-migraine effects may mainly through the regulation of neuroactive ligand-receptor interaction, serotonergic synapse, cAMP and calcium signaling pathways. Moreover, this formula may also mediate PI3K-Akt, TNF, cAMP, HIF-1 and calcium signaling pathways to regulate vascular and nervous systems, as well as the inflammation and pain-related proteins to benefit the patients with migraine.

Acknowledgement

Conflict of Interest

All the authors do not have any conflicts of interest.

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