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Review Article

Network pharmacology implicates traditional Chinese medicine in regulating systemic homeostasis to benefit Alzheimer's disease

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Abstract

Traditional Chinese medicine (TCM) has the characteristics of multi-component, multi-target, and biological systems coordination, which meet the criteria of the network pharmacological application. Therefore, using network pharmacology to discover the relationship between TCM, diseases, and cellular responses is easily achievable. Aging-induced imbalanced homeostasis is a risk factor for Alzheimer's disease (AD), a neuronal disease regulated by multiple genes. Meta-analysis of TCM in metabolic regulation to improve symptoms of AD helps understand the pharmacological effects. The drug targets of TCM can be investigated using a holistic network pharmacology approach to find potential modulators involved in AD-related metabolic pathways. Based on the theoretical prediction of TCM for AD, experimental validation is needed to develop pure compounds for specific treatments.

Keywords: *Alzheimer's disease, Network pharmacology, Traditional Chinese medicine*

Introduction

Neurodegenerative diseases are a broad definition of neurological defects based on loss of neuronal function. Alzheimer's disease (AD) is one of the major types of neurodegenerative diseases [1]. AD is a complex neurological disease associated with memory and cognition impairment caused by a variety of physiological dysfunctions. Gene mutations are present in a small subset of AD patients, leading to the early onset. However, metabolic disturbances and environmental factors are key risks for AD in most sporadic patients. These disturbances induce elevated oxidative stress, accumulation of abnormal protein aggregates, cerebrovascular dysfunction, and neuroinflammation, resulting in neuronal damage and disease onset [2]. The high bioenergetic demand of neurons makes them susceptible to damage caused by metabolic stress.

During aging, dysregulation of glucose and lipid metabolism leads to energy deficits and mitochondrial dysfunction in AD [3]. The energy utility of the brain depends on allostasis in response to lifestyle challenges [4]. A healthy brain adapts to acute ectopic load, but a pathological brain loses adaptability due to accumulated chronic allostatic load. Long-term allostatic overload caused by environmental stressors impairs brain architectures and induces aberrant epigenetic regulation [5]. The consequence is permanent cognitive impairment in the brain. Notably, the brain exhibits neuroplasticity in the early stage of AD, which provides a time window for interventional therapy to reverse or redirect the imbalanced systemic homeostasis. Brain energetics reprogramming restores the neuron to a vibrant state [6]. Furthermore, recent papers report that stress-induced epichaperome, disease-associated scaffolds, or chaperones adapt the brain to environmental stressors [7]. However, persistent stressors fail to adjust homeostasis and instead use these protein connections to demolish neuronal structure. Pharmacological manipulation can rearrange these protein-protein interactions (PPIs) before disease progression. Therefore, in the search for new compounds, analysis of protein-protein or protein-drug interactions using network pharmacology is increasingly becoming an attractive tool for developing drugs for neurodegenerative diseases.

Allopathic therapy, also known as conventional Western medicine, has been a central concept in modern medicine for decades, treating these diseases with evidence-based diagnosis and scientifically approved therapeutic strategies. However, systemic dysregulation of homeostasis appears to be the predominant pathology in patients with sporadic AD.

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The risk of systemic dysfunction increases with age, implying that these multiple high-stress effectors associated with aging impair these known aging-related neurodegenerative diseases. Therefore, one molecule targeting one symptom is not enough to defeat the disease because the causes of damage are diverse.

Traditional Chinese medicine (TCM) has a long history of application, with two core concepts, including Qi (referring to the vital energy of the body) reconciliation and stress alleviation, making TCM a systemic medicine different from Western medicine [8]. It is increasingly convincing that sporadic AD is a systemic metabolic disorder [9]. Alterations in the expression of numerous genes are associated with abnormalities in various tissues or organs, triggering functional defects and accelerating disease progression. TCM is a herbal prescription that contains more than one plant used to treat diseases. Several TCMs have been shown to improve neuronal function in cognition [10-14]. Some of these TCMs have exerted functions on cardiovascular disease, inflammation, diabetes, obesity, and other metabolic disorders [15-18]. Studies of defined TCMs have been shown to modulate levels of blood lipid, cholesterol, glucose, metabolites, and antioxidants, which are metabolic risks of neurodegenerative diseases. Few studies have clearly described the role of TCM in the relationship between metabolism and neurodegeneration. Due to the complexity of these prescription extracts, it is not easy to prove the pharmacological effects of TCM. The alternative research approach is to use systemic pharmacology that integrates chemical analysis, pharmacokinetics, target screening, and pathway network interactions to elucidate the therapeutic mechanism of TCM. Based on this holistic approach, the distinct modulation of metabolic dysfunction, inflammation, and neurodegeneration by compounds extracted from TCM shows synergistic effects ranging from the molecular level to cellular level even biological organism.

In this review, the relationship between metabolism and AD is summarized from systematic network pharmacology studies of documented TCM formulas, focusing on target molecules and pathway regulation.

Metabolic dysfunction in Alzheimer's disease

As an energy-intensive organ, the brain requires tight regulation on metabolic homeostasis. Imbalances in glucose and lipid metabolism and an unhealthy lifestyle affect insulin response and lead to insulin resistance (IR) in the brain, thereby increasing the risk of AD during aging [Figure 1]. A number of papers classify AD as type 3 diabetes, which means that patients with AD and metabolic disorder share similar pathological symptoms and abnormal signaling pathways [19]. Chronic hyperinsulinemia due to IR alters the permeability of the brain barrier, resulting in the transport of inflammatory cytokines, oxidative substances, and molecules that induce lipotoxicity and glucotoxicity from the blood to the brain [20]. These toxic molecules trigger aberrant amyloid peptide (Aβ) aggregation and tau phosphorylation, which disrupt the endothelial structure of small vessels and induce neuronal apoptosis. IR reduces PI3K-AKT activity through insulin signaling that preserves the activity of glycogen synthase kinase-3 beta (GSK3β) to phosphorylate tau protein, leading to its accumulation in the brain. Insufficient insulin-degrading enzyme (IDE) levels caused by IR cannot remove excess Aβ and insulin, so maintaining IDE levels in the brain is a consideration in AD treatment [21]. These

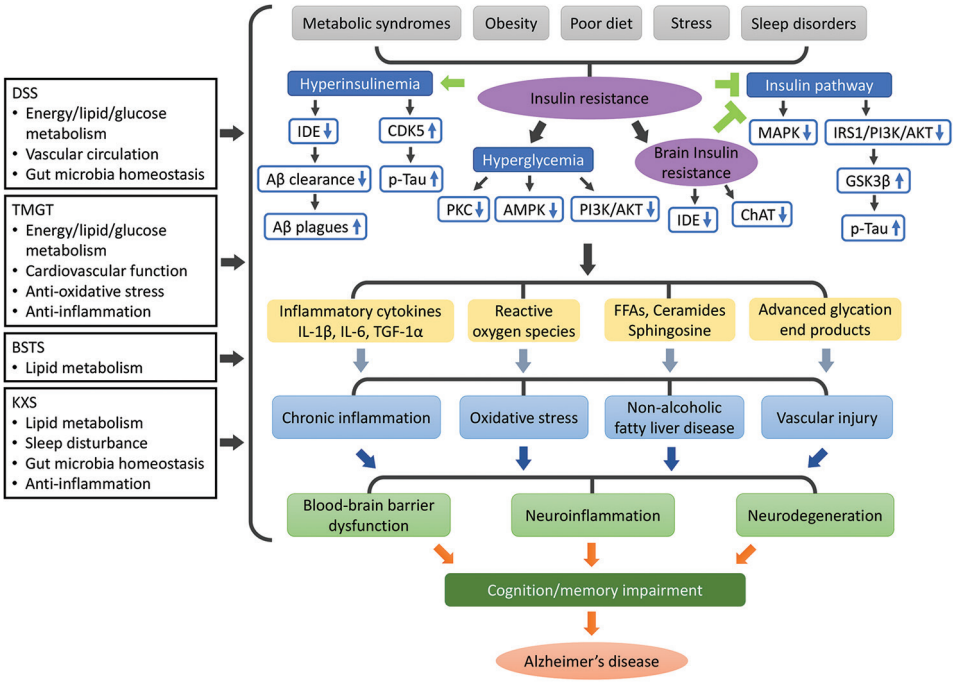


Figure 1: Major metabolic pathways and related genes involved in AD. Molecules in blue line boxes are known targets of TCMs listed in the left panel. AD: Alzheimer's disease, TCMs: Traditional Chinese medicine

dysregulated metabolic pathways promote the accumulation of aggregated proteins and harmful substances in the brain, which induce neuronal damage and ultimately burst into irreversible AD neurodegeneration.

Several commonly used TCM prescriptions for AD, including Danggui-Shaoyao-San (DSS), Tianma-Gouteng (TMGT), Bushen Tiansui (BSTS), and Kai-Xin-San (KXS), have been reported to modulate the AD-like symptoms. The main functions of these TCM in metabolism are documented, and some of them share the same regulatory nodes in the pathological pathways of AD. DSS regulates lipid homeostasis and glycolysis to promote the gut health, as disturbances in the gut environment are highly associated with AD [22]. DSS attenuates neuroinflammation via nuclear factor-kappa B (NF-κB) pathway, which is also involved in Aβ production [10,23]. Docking analysis of targets in cerebral ischemia studies reveals that MAPK1, AKT, and SRC kinases are regulated by DSS [24], and the abnormal insulin-PI3K-Akt signaling observed in AD suggests that this pathway can be targeted by DSS in AD [25]. TMGT has antihypertensive effects, including reduction of oxidative substances and inflammatory cytokines, and modulation of peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC1α)-peroxisome proliferator-activated receptor-γ (PPARγ) signaling pathway to improve vascular function in angiotensin II-induced hypertensive rats [26]. Molecular docking analysis discovers that TMGT ingredients inhibit arachidonate 15-lipoxygenase and mitigate lipid peroxidation to prevent neuronal loss [27]. BSTS rescues synaptic loss and induces neurotrophic factor release to activate the TrkB-PI3K-Akt pathway in AD [28]. KXS accelerates the clearance of excess Aβ by inducing IDE expression to prevent pathological changes in the hippocampus [29]. After KXS treatment, mice with depressive symptoms have increased tight junction-related proteins and decreased inflammatory cytokines in the gut and brain [30]. Experimental evidence provides insight into the modulation of metabolic pathways involved in AD as possible targets for TCM. To accelerate drug development, systematic network analysis is required to mine potential TCM targets that may act as undiscovered or unvalidated regulators in AD models, followed by experimental validation.

Network of pharmacology

Advances in genetic bioinformatics, systemic biology, and polypharmacology have accelerated the development of network pharmacology to integrate information from these systems. Due to the synergistic effects, systematic analytical tools must be developed to study the multi-component, multi-rule, and multi-target characteristics of TCM. Network-based approaches in TCM use computational algorithms to elucidate the underlying mechanisms of bioactive compounds and identify the underlying synergistic effects [31]. Commonly used TCM ingredient databases, including TCMSP (https://tcmsp-e.com/), TCMID (http://bidd.group/TCMID/), and ETCM (http://www.tcmip.cn/ETCM/), collect information on target validation or prediction [32]. Using the established database, we can construct a "disease-gene/target-drug" flowchart to mine potential TCM with synergistic effects on AD [Figure 2]. Based on disease gene-related information obtained from OMIM (https://omim.org/), HPO (https://hpo.jax.org/), and DisGeNET (https://www.disgenet.org/), AD-related genes are analyzed by KEGG (https://www.genome.jp/) and Gene Ontology (http://geneontology.org/) to reveal enriched signaling pathways involving in different functions. These biological databases provide

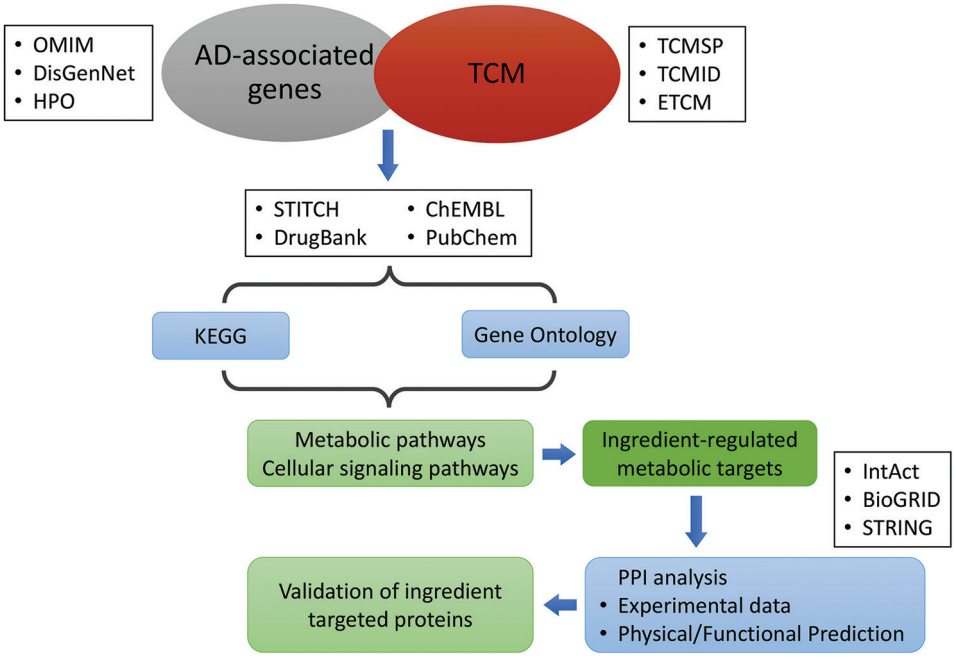


Figure 2: A network pharmacology flowchart for investigating candidate TCM targets that modulate metabolic pathways responsible for AD pathogenesis. KEGG: Kyoto encyclopedia of genes and genomes, PPI: Protein-protein interaction, AD: Alzheimer's disease, TCMs: Traditional Chinese medicine

researchers with a network of comparison and interaction between "compound-gene" and "gene-disease" to investigate the detailed relationships between TCM and AD. Several commonly used tools, such as STITCH (http://stitch.embl.de/), DrugBank (https://go.drugbank.com/), ChEMBL (https://www.ebi.ac.uk/chembl/), and PubChem (https://pubchem.ncbi.nlm.nih.gov/), are drug-based databases that provide drug target information. IntAct (https://www.ebi.ac.uk/intact/home), BioGrid (https://thebiogrid.org/), and STRING (https://string-db.org/) can delineate network connections between drug-targeted proteins or their related proteins predicted from PPI databases to identify previously unrevealed disease-related drug targets. Furthermore, system analysis requires computational network algorithms to demonstrate key nodes in distinct clusters. Drug-target interaction algorithms for predicting novel associations from drug and gene/protein databases are developed to improve the establishment of network topology information [33]. Therefore, the development of network pharmacology diminishes the cost, reduces the risk, and saves time in researching new bioactive compounds for disease treatment. Researchers can conduct these tools and experimental knowledge to determine effective substances in TCM for AD.

Danggui-Shaoyao-San

DSS consists of six Chinese herbs, including *Angelica sinensis* (Oliv.) Diels (DangGui), *Paeonia lactiflora* Pallas (BaiShao), *Atractylodes macrocephala* Koidz. rhizoma (BaiZhu), *Ligusticum chuanxiong* Hort. rhizoma (ChuanXiong), *Alisma orientalis* (Sam.) Juzep. rhizoma (ZeXie), and *Poria cocos* (Schw.) Wolf (FuLing), traditionally used for menorrhagia in women and to regulate the hypothalamic–pituitary–ovarian axis [34]. DSS has been widely used in recent decades for cognitive impairment based on its antioxidant activity and anti-inflammatory effects [10]. DSS also regulates the production of neurotrophic factor and neurotransmitter production to prevent age-induced cellular damages in the brain. DSS improves glucose metabolism and blood lipid homeostasis in diabetes-induced cognitive dysfunction mice and increases neurotrophic factors against neuronal damage [35]. DSS alleviates cognitive deficits by increasing antioxidant levels to reduce mitochondrial damage caused by galactosemia [36,37]. Cognitively impaired mice receiving DSS have reduced AD-like symptoms and improved barrier function in the hippocampus and gut [22,38]. DSS modulates lipid metabolism pathways by increasing PPARγ/LXR expression in the hippocampus and gut, suggesting a role for DSS in the AD gut-brain axis. Based on experimental knowledge, growing data are derived from network pharmacology analysis that helps to find possible DSS regulatory nodes to understand the interplay between AD and metabolism. A network construct of DSS has been computerized to reveal the compound with anti-AD effects that modulate multiple biological processes associated with AD pathogenesis [39,40]. According to large-scale pharmacological experimental data, several compounds are beneficial for AD, such as quercetin, apigenin, luteolin, kaempferol, and γ-aminobutyric acid [41,42]. Apigenin exhibits antidiabetic and anti-oxidative effects and stimulates nitric oxide release, which prevents microvessel damage from hyperglycemia [42]. Luteolin modulates the inflammation and oxidation stress in AD, and also reduces Aβ deposit and increases insulin utility in the brain [43]. Kaempferol is a polyphenol whose primary function is to scavenge free radicals and activate anti-oxidative enzymes to prevent neuronal degeneration [44]. Table 1 summarizes previous DSS network studies targeting AD and metabolic pathways. The dataset obtained from overlapping analyses of the AD-related genes and DSS targets provides predicted molecules involved in metabolic pathways [39]. Metabolic experiments in rodents have shown that the DSS-regulated genes are involved in lipid metabolism in the liver and brain [22,45,46]. These metabolism-related targets, including TNF involved in hepatic lipid homeostasis, AKT1, PPARG, HSP90AA1, EGFR, ESR1, and MAPK14 involved in nonalcoholic fatty liver diseases, and ALOX15 and iPLA2 involved in docosahexaenoic acid metabolism in the brain, are AD-related DSS targets. Neuronal lipid metabolism is essential for energy production, cellular structure, and signaling molecules. For example, binding the lipid hormone 17β-estradiol to the estrogen receptor encoded by ESR1 can promote blood–brain barrier (BBB) function [47]. PPARG not only regulates lipid homeostasis in the brain but also regulates mitochondrial function, Aβ metabolism, and neuronal inflammation [48]. As DSS primarily regulates lipid metabolism in the liver, how DSS affects the expression of these genes and their function in the brain remains to be elucidated.

We performed a Venn analysis using AD-associated genes (Phenotype MIM ID: 104300 in OMIM; MCID: ALZ065 in the MalaCards database) and DSS targets from Wu *et al*. [39] to further analyze their cross-targets with metabolic diseases. We obtained 32, 29, and 8 AD-related DSS targets associated with diabetes (MCID: TYP009 in the MalaCards database), hypertension (MCID: HYP595 in the MalaCards database), and atherosclerosis (MCID: ATH013 in MalaCards database), respectively. Figure 3 shows the genes of intersection between drugs and diseases, and the hub genes are listed in the table.

Tianma-Gouteng

The formula of TMGT is widely used in patients with hypertension and cerebral ischemia, indicating its primary function is to modulate vascular healthy [49,50]. TMGT is composed of 11 herbs, including *Gastrodia elata* Blume rhizoma (Tianma), *Uncaria rhynchophylla* (Miq.) Jacks (GouTeng), *Concha Haliotidis* (ShiJueMing), *Gardenia Jasminoides* J. Ellis (ZhiZi), *Scutellaria baicalensis* Georgi (HuangQin), *Achyranthes bidentata* Blume radix (HuaiNiuXi), *Eucommia ulmoides* Oliv (DuZhong), *Leonurus Artemisia* (Laur.) S. Y. Hu F (YiMuCao), *Loranthus parasiticus* (L.) Merr (SangJiSheng), *Polygonum multiflorum* Thunb. (ShouWuTeng), and *Poria cocos* (Schw.) Wolf (FuLing). TMGT extracts reduce the secretion of vasoconstrictors thromboxane A2 and angiotensin II to lower blood pressure. TMGT contributes to reverse vascular remodeling which can improve cardiac function. The anti-apoptotic effect of TMGT exerts cardiovascular protection in regulating the caspase pathway. TMGT attenuates the immune response of neuroglial cells and preserves cell viability.

Table 1: The summary of Traditional Chinese medicine targets involved Alzhermer's disease and metabolic pathways

	Main active compound	Drug-likeness ingredient	AD-related targets	Metabolism-related target
DSS Angelica sinensis (Oliv.) Diels Atractylodes macrocephala Koidz. Poria cocos (Schw.) Wolf Alisma orientale (Sam.) Juzep. Paeonia lactiflora Pall. Ligusticum chuanxiong Hort.	Quercetin, Apigenin, Luteolin, Kaempferol, Caffeic acid, Gallic acid, DL-glutamic acid, Scopoletin Emodin, Gamma-aminobutyric acid, [39]	Ferulic acid, Atractylenolide I, Ligustilide, Tetramethylpyrazine, Senkyunolide I, Senkyunolide A, Gallic acid, Butylphthalide, Butylidenephthalide, Cnidilide, Albiflorin, Alisol B acetate, Paeoniflorin [22,39]	Network pharmacology (Top 30 genes of 299 AD-related genes) IGF1R, CDH2, ESR1, INSR, ADAM10, APOE, CDK5, GRIN2A, PPARG, PSEN1, ACE, ADAM17, APP, CTSB, CTSD, CXCR4, GRIN2B, HMOX1, MAPK1, MPO, NOS3, PLAU, TNF, ATP1A1, BCHE, BCL2, CAT, DYRK1A, GAD1, GRIN1 [39] Mice study GJA1, ARC, CREB1, EID1, MGAT3, RGS4, RHOA, ST8SIA1, POU3F4, PTMS [40]	Hepatic lipid homeostasis HSD17B7, HSD17B13, TNF, NLRP12, IL-33 [22] Non-alcoholic Fatty Liver Disease ALB, AKT1, PPARG, CASP3, HSP90AA1, EGFR, ESR1, MAPK14, MAPK8, MEM2 [46] DHA metabolism ALOX15, iPLA2 [45]
TMGT Gastrodia elata Blume. Uncaria rhynchophylla F. C. How. Concha haliotidis Gardenia jasminoides J. Ellis Scutellaria baicalensis Georgi. Achyranthes bidentata Blume. Eucommia ulmoides Oliv. Leonurus japonicus (Lour.) S. Y. Hu. Loranthus parasiticus (L.) Merr. Caulis polygoni multiflora Poria Cocos (Schw.) Wolf.	Gastrodin, Parishin A, Parishin B, Gastrodigenin, Chlorogenic acid, Ferulic acid, Quercetin Parishin C [50]	Angustidine, Methyleugenol, Benzoylacetone, Benzophenone, Questin, Dauricine, Neral, Squalene, Skatole, Apigenin, 3-acetyl-2,6-dihydroxy- 4-methoxybenzaldehyde, Questinol [52]	Network pharmacology (Nodes degrees ≥5 among 24 genes) ABCG2, ACHE, BACE1, MAPT, PTGS1 [52]	Network pharmacology (Top 30 hypertension related genes) ACHE, ADRA2A, ADRA2B, ADRA2C, ALOX5, AR, CHRM2, CHRM3, COL1A1, CYP3A4, DRD2, DRD4, ESR1, HIF1A, HTR3A, IFNG, CHUK, IL1B , LPL, MMP9, MPO, OPRM1, PRKCA, PRKCB, PTGER3, PTGS1, PTGS2, RXRA, SERPINE1, SLC6A4 [50] Network pharmacology (hypertension related AD genes based on PharmGKB) ABCB1, AHR, APOE, BCL2, CAT, CRP, ESR1, F2, GPX2, GSK3B, IL1B, LEP, MME, MTOR, NOS2, NOS3, PON1, SOD1, SOD2 [52]
BSTS Epimedium acuminatum Franch. Fallopia multiflora (Thunb.) Harald. Polygala tenuifolia Willd. Acorus tatarinowii Schott. Plastrum testudinis Ossa draconis	Stilbene glycoside, Epimedin A1, Epimedin B, Epimedin C, Icariin [28]	Top 10 of 37 active compounds compounds (OB ≥30%; DL ≥0.18) Icariside A7, Cycloartenol, Yinyanghuo A, Clionasterol, Sitosterol, sitosterol, 20-Hexadecanoylingenol, Anhydroicaritin, Dihydrobrassicasterol, 6-Methoxyl-2-acetyl-3-methyl-1, 4-naphthoquinone-8-O-beta-D-glucopyranoside [59]	Network pharmacology (Nodes degrees ≥5 in PPI analysis) MAPK8, CCND1, IL6, EGFR, VEGFR, APP, CASP3, AR, ESR1, FOS, PPARG, RELA, ERBB2, MCL1, MDM2, RB1, ICAM1, PGR, CCNB1, AHR, CASP9, BCL2, CHRM2, CYP1A1, CYP3A4, ESR2, GSK3B, PCNA, VCAM1 [59]	Rat study CYP1A1, CYP3A4, ALOX5, GRIA2 [59]

Contd...

Table 1: Contd...

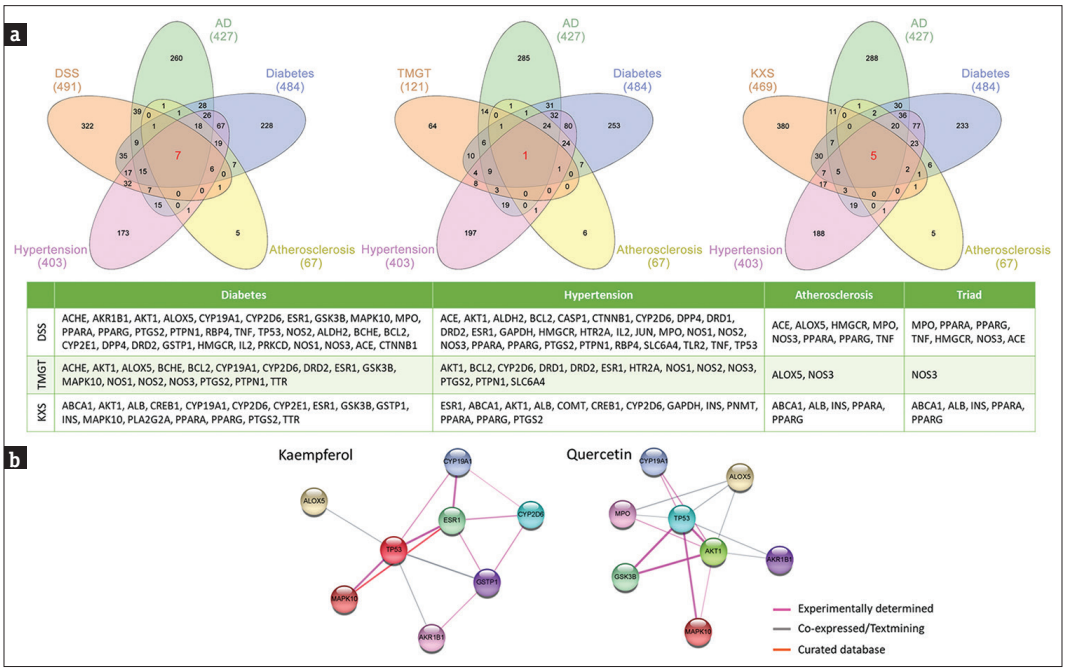
	Main active compound	Drug-likeness ingredient	AD-related targets	Metabolism-related target
KXS Panax ginseng C.A. Meyer Poria Cocos (Schw.) Wolf. Polygala tenuifolia Willd. Acorus tatarinowii Schott	Ginsenoside Rb1, Ginsenoside Rg1, Ginsenoside Re, Ginsenoside Rd, Onjisaponin B, Tenuifoliside A, Tenuifoliside C, Sibiricaxanthone B	Top 10 of 31 active compounds (OB ≥30%; DL ≥0.18) Fumarine, Dehydroeburicoic acid, Gomisin B, Peroxyergosterol, Deoxyharringtonine, Eburicoic Acid, Trametenolic acid, Ginsenoside Rg5, Panaxadiol, Cycloartenol [Kong *et al*. preprint data]	Network pharmacology (Top 39 genes of 447 AD related genes) ACHE, BACE1, BCHE, BCL2, CALM1, CASP2, CDK5, CDK5R1, CHRNB2, CTSB, CTSD, DPP4, DYRK1A, ESR1, F2, FOXO1, GABRA1, GABRA6, GABRG2, GAPDH, GSK3B, HBB, HPRT1, HSD17B10, MAOB, MAPK1, MAPT, NOS1, NOX4, NQO1, PPARG, PRNP, RBP4, SIGMAR1, SLC6A4, TNF, TNFRSF1A, VCP, VEGFA [65]	Network pharmacology (Genes in glucose metabolism, AchE/Aβ/ Tau pathways, TNF inflammation) FOXO1, GSK3B, CALM1, ACHE, CHRN2B, BCL2, MAPK1, BACE1, NOS1, HSD17B10, CDK5R1, CDK5, MAPT, TNF, TNFRSF1A [65]

DSS: Danggui-Shaoyao-San, TMGT: Tianma-Gouteng, BSTS: Bushen Tiansui, KXS: Kai-Xin-San, AD: Alzhermer's disease

Recently, TMGT molecular docking analysis discovers that ALOX15 may be a target for reducing lipid peroxidation and improved pathological symptoms in neurodegenerative disease [27]. Increased ALOX15-encoded 12/15-lipoxygenase in Swedish familial AD mutations regulates amyloid plague production and tau phosphorylation [51]. Experimental data indicate that TMGT can alleviate vascular diseases caused by hypertension or lipid metabolism. The construction of the TMGT computational network based on ingredient analysis, molecular docking, and PPIs provides a broad perspective for searching details and unexpected key nodes between AD and metabolism [52]. Twelve AD-associated TMGT compounds target 11 genes with the most promising nodes (node degree >5), of which 5 genes (ABCG2, ACHE, BACE1, MATP, and PTGS1) are reported to be associated with AD [Table 1]. Based on the target analysis of AD-related TMGT compounds, predicted genes are enriched onto KEGG pathways, including serotonergic synapse, dopaminergic synapse, arachidonic acid metabolism, linoleic acid metabolism, steroid hormone biosynthesis, arginine and proline metabolism, tryptophan metabolism, retinol metabolism, and metabolism of xenobiotics by cytochrome P450. Most of these pathways are associated with lipid or hormone biosynthesis, suggesting the effects of TMGT on lipid metabolism. Notably, network analysis of TMGT reveals that two drug-resistant transporters, ABCG2 and ABCB1, are hypertension-related genes. The role of ABCG2 on urate elimination has been experimentally documented [53]. Both ABCG2 and ABCB1 are located on the luminal membrane of BBB endothelial cells to help exclude Aβ [54]. TMGT has been used in patients with renal hypertension [55] and rats with Aβ deposition in the retina [56]; accordingly, it is speculated that TMGT contributes to Aβ metabolism in the brain. These data allow researchers to find other unreported metabolic targets associated with AD, but further experiments are needed. The roles of TMGT targeting AD-related genes in free radical scavenging, inflammation, and vascular function have been implicated in hypertensive pathology. The synergistic effects of TMGT on metabolic disorders are further analyzed using the predicted targets from the study of Wang *et al*. [52] A summary of disease-related genes is listed [Figure 3].

Bushen Tiansui

BSTS is improved and derived from the old prescription Kong-sheng-zhen-zhong-dan, which has been included in the "Qianjin Fang" by the pharmacologist Sun Simiao. This formula is composed of six herbs, including *Epimedium acuminatum* Franch. (YinYangHuo), *Fallopia multiflora* (Thunb.) Harald. (HeShouWu), *Polygala tenuifolia* Willd. (YuanZhi), *Acorus tatarinowii* Schott. (ShiChangPu), *Plastrum Testudinis* (GuiBan), and Ossa draconis (LongGu). The traditional medical theory holds that nourishing the kidney and replenishing the essence of blood is the key to treat dementia. In Aβ-induced dementia rats, BSTS regulates the synaptic function to protect memory deficits in AD animals [28]. Modern pharmacological analysis of the composition of BSTS compounds shows several bioactive compounds that benefit neurological function. Icariin, rich in BSTS extract, has been shown to inhibit β-secretase



	Diabetes	Hypertension	Atherosclerosis	Triad
DSS	ACHE, AKR1B1, AKT1, ALOX5, CYP19A1, CYP2D6, ESR1, GSK3B, MAPK10, MPO, PPARA, PPARG, PTGS2, PTPN1, RBP4, TNF, TP53, NOS2, ALDH2, BCHE, BCL2, CYP2E1, DPP4, DRD2, GSTP1, HMGCR, IL2, PRKCD, NOS1, NOS3, ACE, CTNNB1	ACE, AKT1, ALDH2, BCL2, CASP1, CTNNB1, CYP2D6, DPP4, DRD1, DRD2, ESR1, GAPDH, HMGCR, HTR2A, IL2, JUN, MPO, NOS1, NOS2, NOS3, PPARA, PPARG, PTGS2, PTPN1, RBP4, SLC6A4, TLR2, TNF, TP53	ACE, ALOX5, HMGCR, MPO, NOS3, PPARA, PPARG, TNF	MPO, PPARA, PPARG, TNF, HMGCR, NOS3, ACE
TMGT	ACHE, AKT1, ALOX5, BCHE, BCL2, CYP19A1, CYP2D6, DRD2, ESR1, GSK3B, MAPK10, NOS1, NOS2, NOS3, PTGS2, PTPN1, TTR	AKT1, BCL2, CYP2D6, DRD1, DRD2, ESR1, HTR2A, NOS1, NOS2, NOS3, PTGS2, PTPN1, SLC6A4	ALOX5, NOS3	NOS3
KXS	ABCA1, AKT1, ALB, CREB1, CYP19A1, CYP2D6, CYP2E1, ESR1, GSK3B, GSTP1, INS, MAPK10, PLA2G2A, PPARA, PPARG, PTGS2, TTR	ESR1, ABCA1, AKT1, ALB, COMT, CREB1, CYP2D6, GAPDH, INS, PNMT, PPARA, PPARG, PTGS2	ABCA1, ALB, INS, PPARA, PPARG	ABCA1, ALB, INS, PPARA, PPARG

Figure 3: Co-regulated genes involved in AD and metabolic disorders. (a) The intersections in TCM targets, AD-associated genes, and metabolic disease-associated genes are shown in red. The hub genes are listed in the tables. (b) PPI analysis of selected compound targets is shown, with edges between proteins presenting different weights of relationships. PPI: Protein-protein interaction, AD: Alzheimer's disease, TCMs: Traditional Chinese medicine

activity, reduce Aβ deposition in the hippocampus, prevent tau hyperphosphorylation, and promote antioxidant activity and Sirt1 function and is therefore considered a candidate for AD treatment [57]. Active components such as kaempferol, luteolin, and quercetin present in the extract are screened from Epimedium, the main herbal component in BSTS, which has the neuroprotective effect of BSTS against AD [58]. This old formula has been modified based on theoretical and clinical studies. Still it is a time-consuming work, so network pharmacology analysis is required to determine the effective BSTS [59]. Metabolite analysis in the blood of AD rats treated with BSTS reveals increases in serotonin, linoleic acid, and α-linolenic acid, partially regulated by BSTS target genes, including CYP1A1, CYP3A4, ALOX5, HTR3A, and GRIA2 [Table 1]. Some predictive BSTS targets associated with AD are not directly involved in metabolic pathways but tend to response to intermediate metabolites. BSTS administration balances dysregulated amino acid metabolism, and recovers abnormal lipidomic profiles, including sphingolipid metabolism, glycerophospholipid metabolism, and linoleic acid metabolism in cerebral cortex of AD rats [60]. Previous network pharmacology studies of BSTS on AD and metabolism are limited; however, a significant role for BSTS in lipid metabolism is confirmed, guiding the experimental design for further investigation.

Kai-Xin-San

The four components in KXS, including *Panax ginseng* C. A. Mey (RenShen), *Polygala tenuifolia* Willd. (YuanZhi), *Acorus tatarinowii* Schott. (ShiChangPu), and *Poria cocos* (Schw.) Wolf (FuLing) are well-known medical herbs commonly used for neurological disorders such as depression and dementia [Table 1]. Modulation of inflammation, neurotransmitter, and neurotrophic secretion is the primary function of KXS in neurological dysfunction [61,62]. Recently, two independent laboratories investigate metabolic profiling in AD models to reveal the metabolic pathways regulated by KXS [63,64]. KXS reverses changes in lipid metabolites and downregulated the expression of apolipoproteins and phospholipid transfer proteins in AD animals to improve cognitive function. These studies aim to search for metabolic biomarkers of KXS to assess the therapeutic efficacy but have not yet demonstrated how KXS modulates disease-related genes to affect metabolic changes. Network pharmacology analysis of KXS discloses the interconnections between ingredients and AD-related genes [65]. The integration of pathway analysis classifies KXS-targeted AD-related genes and reveals their potential locations in metabolic processes. Luo *et al*. have elucidated five regulatory modules of KXS, and the coordination among the pathway modules highlights the switching nodes of multiple functions of KXS in AD pathology [65]. These KXS target AD-related genes involved in glucose metabolism, Aβ-related pathways, tau protein-related pathways, cholinergic system pathways, and TNF-mediated inflammation are summarized in Table 1. Jiao *et al*. provide KXS network analysis and detailed experiments to validate the predicted pathway of tau hyperphosphorylation activated by GSK-3β/cyclin-D kinase 5 (CDK5) in senescence-accelerated mice [66]. KXS inhibits Toll-like receptor 4/myeloid differentiation factor 88/NF-κB signaling and reduces inflammatory cytokines and nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome to attenuate neuroinflammation and neuronal apoptosis in the aged brain. KXS reduces NLRP3 expression and lowers the immune responses to aggregated Aβ and hyperphosphorylated

tau, thereby reducing inflammatory cytokine maturation and secretion. KXS targets disclosed in preprint data by Yang *et al*. [67] (Preprint, https://doi.org/10.21203/rs.3.rs-966634/v1) are used to discover the intersection of genes associated with AD and metabolic disorders [Figure 3]. Vann diagrams show that 17 diabetes, 13 hypertension, and 5 atherosclerosis-related KXS targets overlap with AD-related genes, respectively.

Traditional Chinese medicine targets in metabolic pathways

Network pharmacology of TCM provides a broad perspective on "drug-disease-gene" associations. We summarize these genes based on reported data and genetic databases and obtained some noteworthy information. Among the TCM prescriptions reviewed here, many targeted genes are involved in lipid metabolism (ABCA1, ALDH2, ESR1, HMGCR, PPARA, PPARG, and RBP4). Several other genes regulate glucose metabolism (DPP4), oxidative stress (NOS1/2/3), tau metabolism (PTPN1), inflammation (TNF, PTGS2), and drug metabolism. Cytochrome P450 enzymes (CYP19A1, CYP2D6, and CYP2E1) are involved in drug metabolism and regulate levels of biological molecules, such as neurotransmitters and steroids, and neurotoxins [68]. Due to polymorphisms in the promoters of CYPs, patients have different levels of CYPs and are known to have different susceptibilities to neurotransmission, neurotoxicity, and drugs. Previous studies have reported that DSS and TMGT extracts regulate CYP activity and expression in blood and hepatocytes [69,70]. Elevated CYP activity can lead to adverse reactions to other drugs caused by drug-drug interaction. Therefore, how TCM administration affects the CYPs to modulate neuronal activation, receptor activation, and drug response remains to be investigated in AD.

Defects in fatty acid sensors encoded by PPARA and PPARG result in inefficient lipid oxidation, reduced lipid storage, and low glucose utilization leading to lipotoxicity, which is a risk for AD [71]. Transcriptomics analysis of PPARA and PPARG reveals their regulation on metabolic pathways, choline/dopamine signaling pathways, and Aβ metabolism [48]. Numerous active compounds extracted from herbs have been discovered as PPAR activators [72]. Some of these compounds, such as quercetin, linolenic acid, kaempferol, and ginsenoside, are present in the TCM prescriptions discussed here. Researchers can use the analytic information from database to further predict unknown links between biological regulation and AD-associated pharmacology.

DSS drug-target network analysis data show that kaempferol binds to PPARγ and has other 47 targets (target degree D = 47), 8 (AKR1B1, ALOX5, CYP19A1, CYP2D6, ESR1, GSTP1, MAPK10, and TP53) of which are associated with diabetes and AD [Figure 3]. The connections of these 8 genes are revealed by weighing each protein using PPI analysis. Inhibition of aldose reductase encoded by AKR1B1 prevents memory loss in diabetic rats [73]. It is attributed to attenuating the conversion of glucose to fructose, thereby reducing the consumption of antioxidants, inflammation, and the production of oxidative stress. Triple transgenic AD mice have reduced inhibition on CDK5 activity due to low levels of GSTP1-encoded glutathione S-transferase P1, resulting in increased phosphorylated tau [74].

Quercetin, a bioactive compound widely distributed in many herbal medicines (including herbal constituents in DSS and TMGT), inhibits the activities of acetylcholinesterase, beta-secretase-1, and GSK3β. As a result, Aβ deposition and tauopathies are reduced, and synaptic function is improved in AD brains [41]. PPI network analysis reveals that the interactions between quercetin-targeted AD-related genes are also implicated in diabetes [Figure 3]. Quercetin targets MPO-encoded myeloperoxidase, whose plasma levels are elevated in AD patients [75]. When myeloperoxidase is absent in a 5xFAD transgenic mouse model of AD, the mice present improved cognitive behavior, reduced inflammation, and APOE expression in the brain [76]. The potent peroxidative activity of myeloperoxidase is essential for defense against infection, but oxidative metabolites also increase, which is a risk for AD. Herbs are a rich source of MPO inhibitors developed for AD treatment. Not only quercetin but also other flavonoids, polyphenols, alkaloids, and anthraquinones were found as potential MPO inhibitors [77]. While experimental data on drug-targeted MPO have been determined, direct evidence of the "drug-MPO-AD" interaction remains to be completed.

Following this analytic procedure [Figures 2 and 3], predicted AD-related metabolic genes could be selected to match the corresponding bioactive compounds obtained from TCM databases. The shared targets of different compounds, such as ALOX5 for kaempferol and quercetin shown in Figure 3, can be pointed out to act as potential targets of TCM for AD treatment. Therefore, PPI analysis uncovers hidden or indirect targets of drugs and reveals relationships or interactions between TCM compounds. It provides a reasonable basis for researchers to analyze the synergistic effects of TCM on AD.

There are some limitations in predicting active compounds and underlying mechanisms targeting AD-related genes using network pharmacology. Target prediction may be inaccurate due to intermediate metabolites of the drug. The theoretical basis for molecular docking does not provide sufficient information about the effects of the drugs on target activity. Highly bypassed and complex regulation in metabolism may impact functional predictions and neglect drug side effects. Therefore, further experimental validation of potential active ingredients is required to demonstrate their theoretical functions in metabolic pathways related to AD pathology.

Conclusion

A systemic approach for identifying the meta-interaction of drugs with genes is a powerful tool for studying the various compounds in herbal medicines. Based on chemical analysis and disease gene databases, we can make connections based on computational algorithms, group these disease-related drug targets, and then perform enrichment pathway analysis to demonstrate the likely functions of compounds in organisms. This approach is a broad, rapid, and cost-effective assay for acquiring primary information on drug-regulated metabolic

pathways relevant to disease pathogenesis. Experimental designs based on this prediction can be more efficient and reveal more detailed information about disease pathogenicity.

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Conflicts of interest

There are no conflicts of interest.

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