Research on Potential Mechanism of Shenwu Capsule on Treating Alzheimer's Disease Based on Network Pharmacology

Y. XING, N. CHEN[1], DINGGUO WANG, XUEYING LIN, T. LIN, Y. SONG[1], J. ZHUO*

Department of Traditional Chinese Medicine, Hainan Hospital of Traditional Chinese Medicine, Haikou 570203, China, [1]Department of Pharmacology, School of Pharmaceutical Sciences, Hainan University, Haikou 570228, China

The etiology and pathogenesis of Alzheimer's disease are extremely complicated and there is no effective cure available currently. Previous studies have proved the effectiveness of Shenwu capsule in treatment of Alzheimer's disease. However, its mechanism of action has not been systematically elucidated. Here, network pharmacology approach was used to revealing the underlying mechanism of Shenwu capsule in the treatment of Alzheimer's disease. The compound database of Shenwu capsule was constructed, and their potential targets were obtained. Alzheimer's disease gene set was acquired from databases. Subsequently, the potential therapeutic targets of Shenwu capsule on Alzheimer's disease by overlapping analysis, which validated by disease ontology and tissue enrichment analysis. Biological process and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis were performed to find the core biological processes and signaling pathways regulated by Shenwu capsule in treating Alzheimer's disease. By constructing and analyzing the protein-protein interaction network and compound-target network, the core targets and key components were identified. A total of 798 compounds and 1105 potential Shenwu capsule targets, 2566 Alzheimer's disease-related genes and 453 Shenwu capsule therapeutic targets were identified. 3102 biological processes were significantly enriched, such as membrane potential, oxidative stress, synaptic transmission, and neuronal death. 194 pathways of significance were identified, including neuroactive ligand-receptor interactions, lipid-atherosclerosis, Alzheimer's disease, cyclic adenosine 3′,5′-monophosphate signaling and calcium signaling. 35 core therapeutic targets and 55 key components were screened out. Network pharmacology provides an effective way to elucidate the mechanism of action and identify the core targets and key compounds of traditional Chinese medicine. Further studies are needed to validate the validity of this prediction.

Key words: Traditional Chinese medicine, Shenwu capsule, Alzheimer's disease, network pharmacology, therapeutic mechanisms

Alzheimer's Disease (AD) is a highly complex and progressive neurodegenerative disease, which is characterized by memory loss and progressive neurocognitive dysfunction, accompanied by various neuropsychiatric symptoms and behavioral disorders[1,2]. It is one of the leading causes of dementia cases globally[3]. By 2050, it is estimated that a new case of AD will appear every 33 sec, resulting in nearly 1 million new cases each year, with an estimated prevalence between 11 and 16 million[2,3]. There are many hypotheses about the pathogenesis of AD, including cholinergic hypothesis, Amyloid β (Aβ) protein production and metabolism disorder hypothesis, τ-protein abnormal phosphorylation hypothesis, oxidative stress and free radical damage hypothesis, inflammation hypothesis, metal ion metabolism hypothesis and so on. However, at present, there is no hypothesis that can accurately and comprehensively explain the pathological characteristics of AD, which may be the result of multiple mechanisms[2-6]. Due to the lack of

***Address for correspondence**
E-mail: zhuojinsheng@hainmc.edu.cn

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a full understanding of the pathophysiology of AD, especially the initial stage of the disease[7], there is no effective cure available currently[2]. Existing drugs usually target the symptoms, not the underlying pathology[8].

For the first time, our team put forward the biological basis of "Tonifying kidney and filling marrow" in Chinese medicine, including promoting energy metabolism and utilization, activating production of endogenous neurotrophic factor and promoting neuron survival and regeneration. Based on the Chinese medicine theory of "Tonifying kidney and filling marrow, supplemented by relieving phlegm and removing blood stasis", Shenwu Capsule (SWC) was developed and has obtained the national invention patent in China. It is a prescription for the treatment of AD and Mild Cognitive Impairment (MCI) with syndrome differentiation of kidney and spleen deficiency, phlegm turbidity and blood stasis. Like other Traditional Chinese Medicine (TCM) prescriptions, SWC is a complex system with multiple components, multiple targets, and multiple mechanisms. It is composed of six herbs, including *Radix polygoni multiflori preparata* (Chinese Pinyin: Zhi Shou Wu (ZSW)), *Panax ginseng* C. A. Mey (Chinese Pinyin: Ren Shen (RS)), *Acorus tatarinowii* (Chinese Pinyin: Shi Chang Pu (SCP)), *Epimedium brevicornu* Maxim. (Chinese Pinyin: Yin Yang Huo (YYH)), *Pueraria lobate* (Chinese Pinyin: Ge Gen (GG)), *Rhizoma chuanxiong* (Chinese Pinyin: Chuan Xiong (CX)). In the previous studies, SWC showed protective effect on peripheral blood lymphocyte DNA[9], retard aging process of the spinal cord through elevating the expression of neurotrophic factors in the lumbar spinal cord of aged rats[10]. In addition, SWC indicated effectiveness in AD-like mitochondrial deficiency model rats, suggesting its application in the treatment of AD[11]. However, the basic mechanism of action at the molecular level has not been systematically studied. The bioactive compounds, the potential targets and the related pathways of SWC remain unknown.

Network pharmacology, as a new emerging field that interprets the occurrence and development of diseases from the perspective of biological network balance[12,13], provides a new research strategy to explore TCM spanning multiple scales from the molecular and cellular level to the tissue and organism level. In recent years, Chinese scholars have made some important achievements and progress in the establishment of network pharmacology research methods and the application of them to study the scientific connotation of TCM, which has received great international response[14-19]. Therefore, we in this study employed network pharmacology methods to probe into mechanisms underlying curative effects of SWC on AD and mine the material basis, including the core targets and hub components.

MATERIALS AND METHODS

These have six main steps for the integrated system-based network pharmacology approach to disclose the curative effects of SWC as follows (fig. 1). Construction of molecular database for all six herbs in SWC from public databases, then the potential target data of the components were obtained, including known targets and putative targets. Acquisition of AD gene set from databases and then identification and validation of potential therapeutic targets of SWC on AD. Identification of core biological processes and signaling pathways regulated by SWC in treating AD based on enrichment analysis results, identification of core targets and key components through integrating network analysis. To better elucidate the holistic mechanisms of SWC using Alzheimer pathway as an example.

Identification of candidate targets of SWC in treating AD

Construction of a database of the SWC ingredients: In order to obtain the compound information as comprehensively as possible, we manually collected ingredients of SWC from TCM_ID[20], TCM_Mesh[21], TCMGeneDIT[22], TCMID[23,24], ETCM[25], TM_MC[26], TcmSP[27], BATMAN-TCM database[28]. Finally, the ingredients information was normalized through the compound identification number in PubChem database[29].

Acquisition of potential targets of SWC components: It is very important to obtain the interaction profiles between components in SWC and their potential targets to elucidate the mechanism of SWC[30]. In order to ensure the reliability of the target of the ingredients in SWC, we integrated the data from ten sources, which can be divided into four categories (Table 1). Marketed drug databases such as DrugBank[31] and TTD[32], activity assay databases such as ChEMBL[33] and PubChem[34], literature mining databases such as

STITCH[35] and CTD[36], target prediction tools such as TargetNet[37], SwissTargetPrediction[38], ChEMBL_prediction tool[39] and BATMAN-TCM[28]. All the known targets obtained from DrugBank, TTD and ChEMBL, PubChem source was kept. Only the putative targets which can be predicted in at least two prediction models and validated by literature mining source at the same time were preserved as the potential targets of SWC. All the targets were normalized by UniProt database (http://www.uniprot.org)[40] and only the targets belonging to "*Homo sapiens*" were reserved for further analysis.

Acquisition of the AD gene set. Based on current researches about the pathology of AD, the causal genes closely related to it were collected from MetaCore (https://portal.genego.com/), DisGeNET[41], Open Target Platform[42], MalaCards[43], OMIM[44], GeneCards[45], and CTD database[36]. The gene name from different source was standardized based on the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.abcc.ncifcrf.gov/)[46]. To ensure the credibility of AD gene set, we only retained genes that appeared in at least three data sources.

Identification of therapeutic targets of SWC in treating AD: Based on the potential targets of SWC components and AD gene set, we obtained their overlaps. This part can be regarded as the target set of SWC in the treatment of AD.

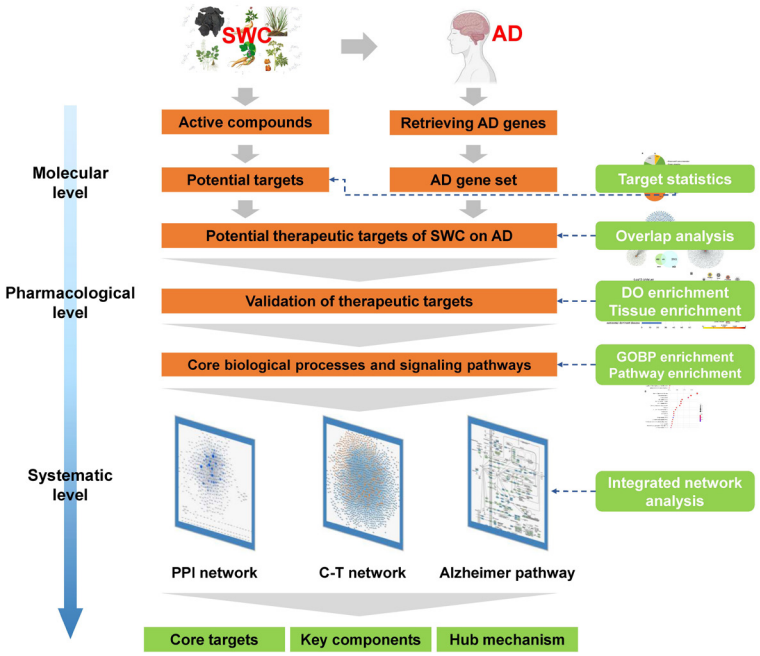

Fig. 1: Schematic illustration of network pharmacology study of SWC in the treatment of AD. SWC: Shenwu capsule; AD: Alzheimer's disease

TABLE 1: 10 TARGET SOURCES OF THE INGREDIENTS IN SWC

#	Target_Source category	Target_Source	Interaction and target type	Selection criteria
1	Marketed drug database	DrugBank	Known	All
2		TTD database	Known	All
3	Activity assay database	ChEMBL	Known	All
4		PubChem	Known	All
5	Literature mining database	STITCH	Text-mining	Score≥0.9
6		CTD	Text-mining	All
7	Target prediction tool	TargetNet	Putative	Score=1
8		SwissTargetPrediction	Putative	Score≥0.9
9		ChEMBL prediction tool	Putative	Confidence 90 % and active
10		BATMAN-TCM	Putative	Score≥0.48

Enrichment analysis of SWC therapeutic targets in treating AD:

To verify whether the SWC target set in the treatment of AD is reliable, two kinds of enrichment analysis were performed. One is Disease Ontology (DO) enrichment, which checks whether the target set can be significantly enriched in AD or AD related diseases. clusterProfiler Version 4.0.3, an R Bioconductor package[47] was used to perform DO enrichment based on the disease annotation data in DO database (http://disease-ontology.org) [48]. The other is tissue enrichment, which examines whether currently identified SWC targets are over-represented by enriched expression in the disease-relevant tissue. Tissue Specific Expression Analysis (TSEA)[49] was taken to perform the tissue enrichment analysis by using Specificity Index thresholds (pSI) R package function to calculate pSI of varying stringency.

For the sake of interpreting the mechanisms of SWC for AD from a systematic perspective, clusterProfiler mentioned above was used to perform functional annotation and pathway enrichment analysis of the potential targets of SWC in the treatment of AD. The Gene Ontology (GO) (http://geneontology.org/), including terms of biological processes, cellular components and molecular functions, which could identify the possible biological mechanism of SWC targets[50]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/), providing the molecular interaction/reaction network diagram, is a comprehensive knowledge base for functional interpretation from a systematic view[51].

Genes or proteins shared by different pathways may play an important role in cross-talk between different signaling pathways[52]. Therefore, in this study, we conducted frequency statistics on targets enriched into these differential pathways as one of the factors for core therapeutic target screening.

Construction and analysis of network:

In order to identify the hub targets and to explore its pharmacological mechanism, two kinds of networks were established.

Protein-Protein Interaction (PPI) network: The potential SWC targets in treatment of AD were submitted to STRING database (https://string-db.org/) to construct PPI network[53] with the highest confidence (0.9)[54]. To verify that the interactions between them were statistically valid, PPI interaction enrichment tests were conducted. Whole genome was assumed as the statistical background.

Compound-Target (C-T) network: The relationships between the compounds and targets were used to build C-T network. If a compound potentially acts on a target, they are connected by an edge. Cytoscape v3.8.1 was used to visualize the above two networks and compute the topological parameters[55]. In addition, NetworkAnalyzer plug-in of Cytoscape v3.8.1 was used to compute topological parameters. Hub nodes in the network were evaluated by the degree parameter, that is, the number of its adjacent nodes[56,57].

In order to identify the core target of SWC in the treatment of AD based on comprehensive consideration of multiple factors, a Combination Score (CS) formula for evaluating the importance of targets is defined as follow:

$$CS=D\_1*0.2+D\_2*0.4+N*0.3+T*0.1 \quad (1)$$

In this formula, D1 and D2 indicates the degree value computed in PPI network and C-T network, respectively. N represents the number of significantly enriched pathways in which a target participates. T is the target type score. Here, the target type scores of known targets (T Known target) and putative targets (T Putative target) are set to 15 and 5 respectively. CS greater than 2.5 times the average CS was used as the threshold to screen the core therapeutic targets. We screen the key components based on the C-T network and take the degree greater than 2.5 times the mean as the threshold

Evaluation of Drug-Likeness (DL) of compounds in SWC:

To screen out the potential active compounds with favorable physicochemical properties and pharmacokinetics properties, SwissADME (http://www.swissadme.ch) was used to evaluate pharmacokinetics, DL and medicinal chemistry friendliness of compounds in SWC[58]. Six physicochemical properties were considered for a rapid appraisal of DL and the optimal range for each property was given below.

Lipophilicity: XLOGP3 between −0.7 and +5.0;

size: MW between 150 and 500 g/mol; polarity: TPSA between 20 and 130 Å; solubility: log S no higher than 6; flexibility: no more than 9 rotatable bonds and saturation: fraction of carbons in the sp3 hybridization no less than 0.25[59,60]. Compounds fully meet the above screening criteria to be considered showing DL. This means that these compounds have potentially excellent Absorption, Distribution, Metabolism, Excretion (ADME) properties with a high development success rate and can be prioritized in subsequent studies. In this study, the predicted ADME parameters were only considered as one of the factors for screening potential active compounds.

RESULTS AND DISCUSSION

After standardization and de duplication, a total of 798 compounds from six herbs of SWC were collected by comprehensive retrieval and integration of compound information from eight public databases; 75 in ZSW, 379 in RS, 123 in SCP, 78 in YYH, 74 in GG, and 206 in CX.

To ensure the accuracy and reliability of SWC targets, ten sources in four categories was used to obtain the corresponding target data for 798 compounds. According to the strict screening criteria, a total of 1105 SWC potential targets were obtained. Through standardization, deduplication, screening, and integration of AD gene information from seven databases, the AD gene set was constructed, which contained 2566 genes in total. The common gene shared between SWC potential targets and AD genes was considered as therapeutic targets of SWC on AD, and a total of 453 targets were obtained for subsequent analysis (fig. 2).

For the 453 therapeutic targets of SWC on AD, there are 12 184 compound-target interaction relationships, including 9893 known interactions (~81 %) and 2291 putative interactions (~19 %) (fig. 3A). There are 342 known targets and 111 (~75 %) putative targets (~25 %) respectively (fig. 3B). This target set can be divided into two types; 342 known targets and 111 (~75 %) putative targets (~25 %) (fig. 3B).

In order to verify that the above 453 therapeutic targets are indeed highly reliable and can be used for subsequent analysis, DO enrichment and tissue enrichment were performed (fig. 4A). The DO enrichment result showed that AD (p=1.88E-57) was significantly enriched. In addition, Tauopathy (p=5.92E-57) and brain disease (p=1.91E-33) ranked second and third, respectively, were both associated with AD (fig. 4A). Tissue enrichment results displayed that SWC targets in the treatment of AD could be enriched into brain tissues at different thresholds (fig. 4B). The analysis results from both angles show that 453 potential targets of SWC for the treatment of AD are highly reliable and can be used for subsequent analysis.

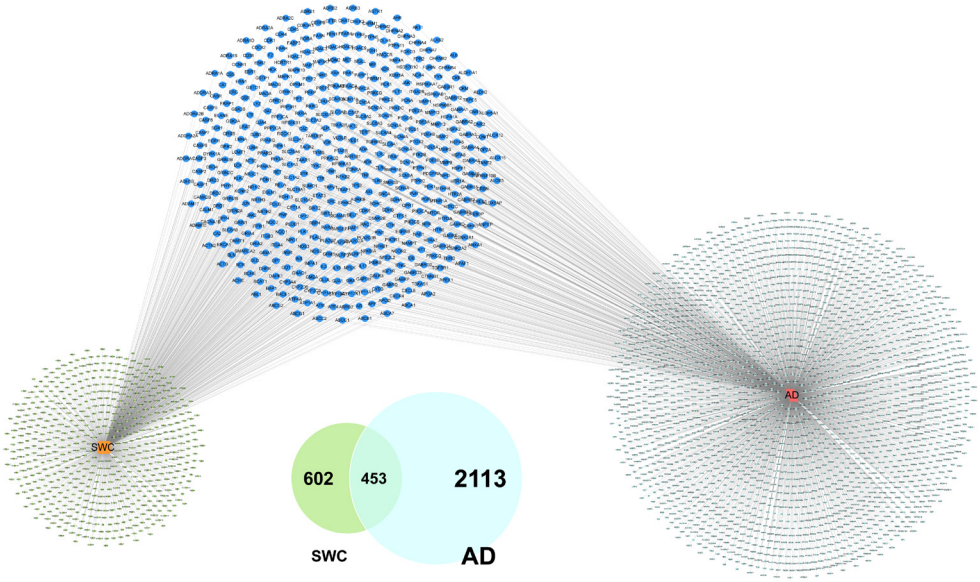

Fig. 2: Identification of potential targets of SWC in the treatment of AD. The network was taken to represent the relationship between them, and the numbers were shown in Venn diagram. The circle on the left represents 1105 related targets of SWC from 10 sources, the circle on the right indicates 2566 associated genes of AD from 7 databases, and the circle in the middle shows the potential targets of SWC in the treatment of AD; SWC: Shenwu capsule; AD: Alzheimer's disease

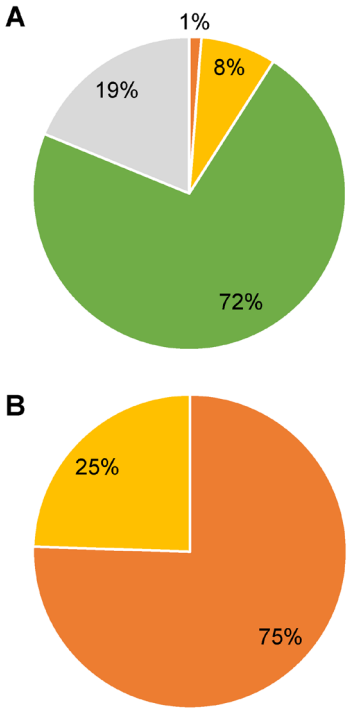

Fig. 3: Statistics on 453 potential targets of SWC in the treatment of AD based on data source; (A): Four kinds of compound-target interaction type; (B): Two types of targets; SW: Shenwu capsule; AD: Alzheimer's disease; (A) ( ): Known and putative interaction; ( ): Known interaction; ( ): Known, putative and text-mining interaction; ( ): Putative and text-mining interaction; (B) ( ): Known target; ( ): Putative target

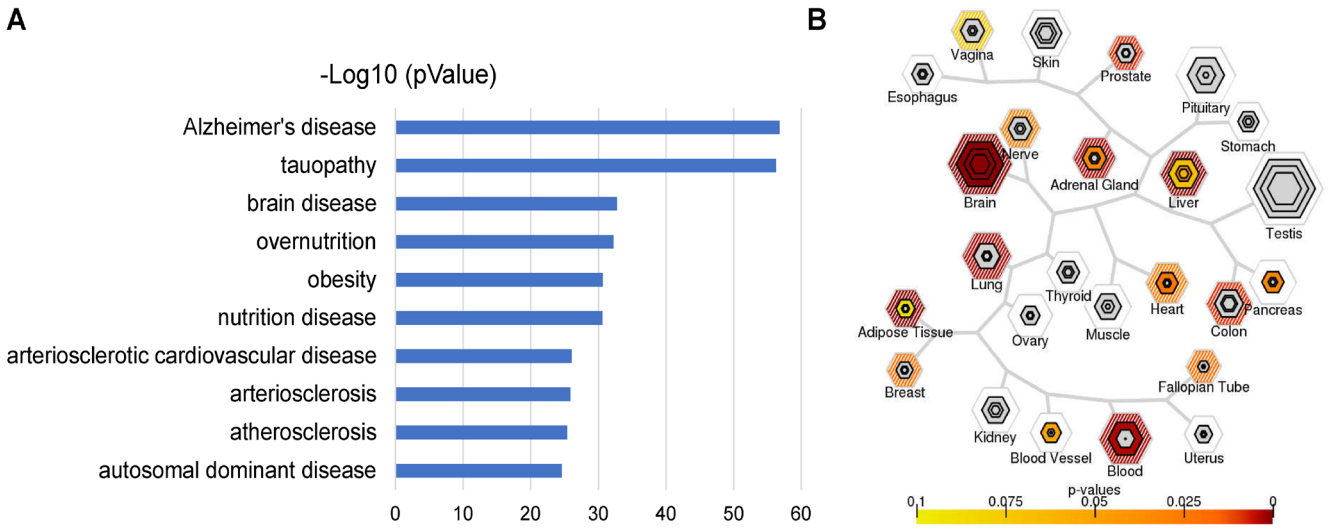

Fig. 4: Enrichment analysis results of DO and TSEA for 453 potential targets of SWC in the treatment of AD; (A): Top 10 terms of DO enrichment results; (B): Bullseye of TSEA results; Different layers represent the significance of enrichment results under different pSI thresholds for 0.05, 0.01, 0.001, and 0.0001; The darker the color is the smaller the p value is at this threshold; SWC: Shenwu Capsule; AD: Alzheimer's Disease; DO: Disease Ontology; TSEA: Tissue Specific Expression Analysis and pSI: Specificity Index thresholds

Through biological process and KEGG pathway enrichment analysis of these 453 common targets, 3102 biological processes and 194 pathways of significance were identified. The representative Gene Ontology Biological Process (GOBP) and KEGG pathway were visualized in fig. 5. The GOBP results indicated that most of these targets were associated with a variety of biological processes, such as membrane potential, oxidative stress, synaptic transmission, neuron death, hypoxia, etc. (fig. 5A). The top 5 significantly enriched KEGG pathway were neuroactive ligand-receptor interaction (p=7.37E-30), lipid and atherosclerosis (p=3.12E-34), AD (p=1.30E-15), cyclic Adenosine 3′,5′-Monophosphate (cAMP) signaling pathway (p=1.09E-19), human cytomegalovirus infection (p=1.22E-17), and calcium signaling pathway (p=1.92E-16) (fig. 5B).

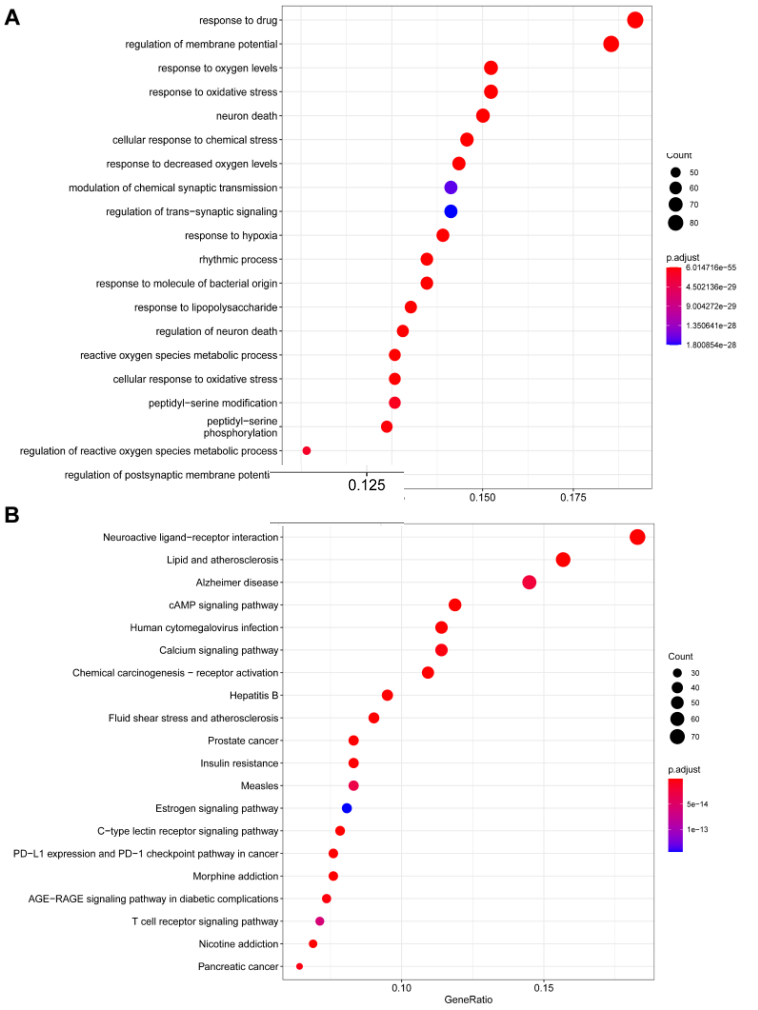

Fig. 5: Key biological functions and KEGG pathways for 453 potential targets of SWC in the treatment of AD; (A) Top 20 biological process (BP) and (B) top 20 KEGG pathways calculated by clusterProfiler package in R (version 4.1); GeneRatio represents the ratio of target genes in the term and background genes of the term. The size of the dot is proportional to the number of genes enriched in the term; SWC: Shenwu Capsule and AD: Alzheimer's Disease and BP: Biological Proces

The PPI network of SWC targets in the treatment of AD was developed to quantify the function of specific proteins and identify the hub targets at the systematic level. The PPI network consisted of 453 nodes and 1982 edges. The average node degree and the average local clustering coefficient were 8.75 and 0.397, respectively. PPI interaction enrichment result indicated that the PPI network has significantly more interactions than what would be expected for a random set of proteins of similar size, drawn from the genome ($p<1.0e-16$). This means that these proteins are closely connected biologically as a group. The degree of nodes in the network was calculated.

The relationships between 453 therapeutic targets of SWC on AD and the compounds in SWC were used to construct C-T network. There are 1224 nodes (including 453 targets and 771 compounds) and 12 184 edges. The degree of nodes in C-T network was calculated. In this network, the average number of targets per compound is 15.8, indicating the multi-target property of SWC ingredients.

Based on the definition of Combination Score (CS), we ranked and identified the core target of SWC in the treatment of AD. The mean value of CS is 16.7. According to the threshold, 35 core therapeutic targets were screened (Table 2), including 32 known targets and 3 putative targets. The average of degree of compounds in C-T network is 15.8. According to the threshold, we screened 55 key components and the top 30 compounds were displayed in Table 3.

Interpretation of the mechanism of SWC intervention in AD pathway:

Based on the results of pathway enrichment, 61 therapeutic targets enriched in the AD pathway

(hsa05010) were displayed (fig. 6). We found that targets were widely distributed in this pathway and were involved in regulating multiple biological processes, such as neuronal injury, apoptosis, energy production, synaptic dysfunction, reactive oxygen species, mitochondrial dysfunction, neurofibrillary tangles, long-term potentiation, autophagy and axonal transport defects, ultimately affecting the survival of neurons. In addition, we also found the cross-talk effect between this pathway and other pathways, such as calcium signaling pathway (p=1.92E-16), apoptosis (p=1.92E-16), Wnt signaling pathway (p=0.00097), AGE-RAGE signaling pathway in diabetic complications (p=1.31E-16), insulin signaling pathway (p=5.06E-07), autophagy (p=1.13E-05). Nine of the 35 core targets are distributed in this pathway, all of which are known targets, including RELA, MAPK1, CASP9, APP, AKT1, PIK3CA, TNF, PIK3R1 and CASP3 (Table 2). Moreover, all the 30 key components in SWC have potential regulatory effects on this pathway (Table 3).

In this study, the key biological processes and KEGG signaling pathways, core target set and key active ingredients of SWC in the treatment of AD were identified based on the network pharmacology research strategy, and the regulatory mechanism of SWC was illustrated by the AD pathway. These results provide the possibility to understand the complex mechanism of TCM from the perspective of system and provide the direction for further research.

TABLE 2: 35 CORE TARGETS OF SWC IN THE TREATMENT OF AD BASED ON CS

Gene Symbol	Gene ID	D1	D2	N	T	CS	Target type	In AD pathway
HTR3A	3359	0	280	2	15	114.1	Known target	FALSE
TLR9	54106	1	231	7	15	96.2	Known target	FALSE
MGLL	11343	2	232	2	5	94.3	Putative target	FALSE
MIF	4282	2	226	2	15	92.9	Known target	FALSE
RELA	5970	51	142	73	15	90.4	Known target	TRUE
PTGS1	5742	7	209	4	15	87.7	Known target	FALSE
ALOX15	246	8	189	4	15	79.9	Known target	FALSE
NOS3	4846	17	165	18	15	76.3	Known target	FALSE
ESR1	2099	43	156	7	15	74.6	Known target	FALSE
MAPK1	5594	59	45	116	15	66.1	Known target	TRUE
ITGA4	3676	6	146	10	15	64.1	Known target	FALSE
HDAC4	9759	8	147	5	15	63.4	Known target	FALSE
ABCG2	9429	2	151	3	15	63.2	Known target	FALSE
CASP9	842	8	123	32	15	61.9	Known target	TRUE
APP	351	20	138	3	15	61.6	Known target	TRUE
AHR	196	7	143	4	15	61.3	Known target	FALSE

MAOA	4128	6	134	11	15	59.6	Known target	FALSE
AKT1	207	64	36	97	15	57.8	Known target	TRUE
HSP90AA1	3320	67	97	13	15	57.6	Known target	FALSE
CYP17A1	1586	4	137	4	5	57.3	Putative target	FALSE
AR	367	27	117	3	15	54.6	Known target	FALSE
PIK3CA	5290	51	31	101	15	54.4	Known target	TRUE
CYP19A1	1588	4	126	1	15	53	Known target	FALSE
ALPL	249	0	128	0	15	52.7	Known target	FALSE
MAOB	4129	4	115	11	15	51.6	Known target	FALSE
TNF	7124	27	69	56	15	51.3	Known target	TRUE
PIK3R1	5295	53	21	100	15	50.5	Known target	TRUE
DNMT1	1786	10	116	1	15	50.2	Known target	FALSE
SLC5A2	6524	1	118	0	15	48.9	Known target	FALSE
TP53	7157	68	47	47	15	48	Known target	FALSE
ESR2	2100	6	108	6	15	47.7	Known target	FALSE
CAT	847	6	108	7	5	47	Putative target	FALSE
FAAH	2166	3	109	1	15	46	Known target	FALSE
PGR	5241	8	98	5	15	43.8	Known target	FALSE
CASP3	836	24	63	40	15	43.5	Known target	TRUE

TABLE 3: TOP 30 KEY COMPONENTS IN SWC WITH THERAPEUTIC EFFECTS ON AD

CID	Name	D2	Regulate AD pathway	Herb
6623	Bisphenol A	279	TRUE	RS
5280343	Quercetin	251	TRUE	ZSW; YYH; GG; CX
445154	Resveratrol	228	TRUE	ZSW
5957	Adenosine-5'-triphosphate	216	TRUE	RS
5280961	Genistein	162	TRUE	GG
985	Palmitic acid	122	TRUE	RS; SCP; YYH; GG; CX
261	Butyraldehyde	111	TRUE	CX
8343	Bis(2-ethylhexyl) phthalate	110	TRUE	GG

446220	Cocaine	110	TRUE	CX	TRUE
611	DL-Glutamic acid	101	TRUE	SCP	
445639	Oleic acid	101	TRUE	RS; CX	
5280445	Luteolin	99	TRUE	YYH	
135398658	Folic acid	98	TRUE	RS; CX	
5280443	Apigenin	95	TRUE	CX	
119	gamma-Aminobutyric acid	87	TRUE	SCP	
5281672	Myricetin	83	TRUE	ZSW	
264	Butyric acid	82	TRUE	CX	TRUE
5280863	Kaempferol	82	TRUE	ZSW; RS; YYH	
60961	Adenosine	78	TRUE	RS	
5281707	Coumestrol	78	TRUE	GG	
6654	alpha-Pinene	72	TRUE	RS; SCP; CX	
3220	Emodin	67	TRUE	ZSW; SCP	
753	Glycerol	66	TRUE	ZSW	
1110	Succinic acid	65	TRUE	RS; YYH	TRUE
3893	Lauric acid	65	TRUE	RS	
5281708	Daidzein	65	TRUE	YYH; GG	
9064	Cianidanol	62	TRUE	ZSW; YYH	
370	Gallic acid	61	TRUE	ZSW; YYH; CX	
7127	Methyleugenol	61	TRUE	SCP; CX	
5280450	Linoleic acid	61	TRUE	RS; SCP; YYH; CX	

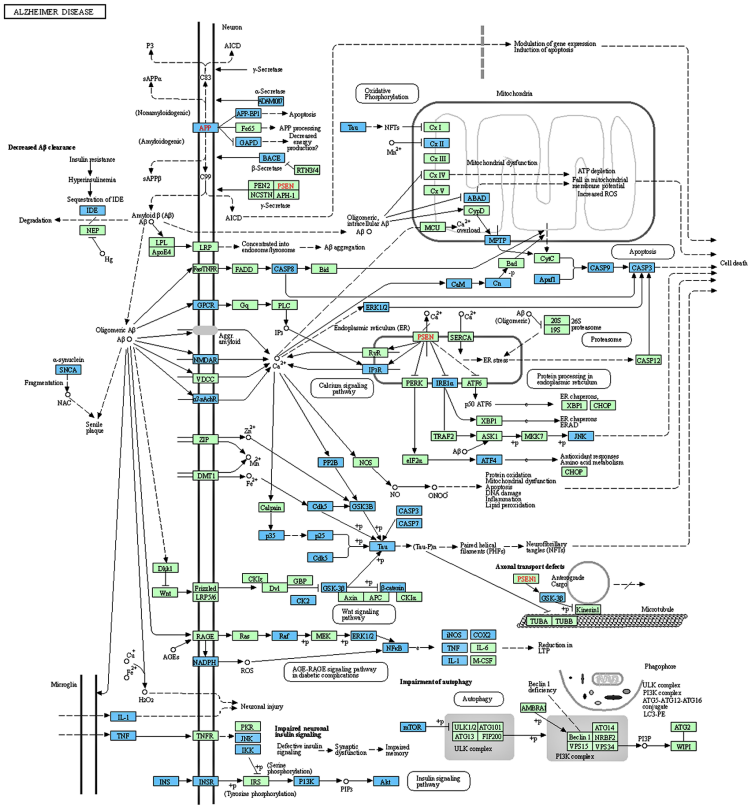

Fig. 6: Regulatory effect of SWC on AD pathway; Rectangular nodes represent AD related genes or proteins on the pathway and blue nodes represent targets regulated by SWC. The solid line represents the direct regulatory effect, and the dotted line indicates the indirect effect. The line with arrow indicates activation. The line with small line segments represents inhibition

In the process of obtaining the therapeutic targets of SWC for AD, we used the data of 10 sources in 4 categories for integration, and adopted very strict screening criteria for the data of predicted sources before integration. Of the 453 targets, ~75 % were known targets, and the other ~25 % predicted targets were verified by data from the two text mining sources, which ensured the reliability of subsequent analysis results. In addition, we performed DO and tissue enrichment analysis to further verify and ensure the reliability of 453 therapeutic targets used for mechanism interpretation. It is worth mentioning that the definition of known targets in this study is those verified by relevant activity tests, among which activity tests at the biochemical level account for a large proportion. Therefore, verification can be carried out on corresponding cells or animal models according to actual needs in subsequent studies.

In order to elucidate the main regulatory pathways of SWC in the treatment of AD from a systematic perspective, biological processes and signaling pathways were enriched. The therapeutic targets are mainly enriched in membrane potential[61], oxidative stress[62], synaptic transmission[63], neuron death[64], hypoxia[65] are biological processes closely related to the physiological process of AD. SWC may exert pharmacological effects on AD by integrating and regulating these biological processes.

According to CS definition and screening threshold, a total of 35 core targets were obtained. The first two known targets are HTR3A and Toll-Like Receptors (TLR)9. As we know, 5-Hydroxytryptamine (5-HT) is primarily involved in the regulation of learning and memory. HTR3A (CS=114.1) was found to be the highest ranked, which has been found that relative mRNA expression of HTR2A, HTR3A (CS=114.1) and MAOA (CS=59.6) were significantly lower in PBMCs of patients with Late-Onset AD (LOAD) compared with controls[66]. At present, 5-HT receptor antagonists as a symptomatic treatment for cognitive and/or behavioral symptoms of AD have been studied[67,68]. TLR signaling pathway(s) may be involved in clearance of Aβ-deposits and direct or indirect activation of specific TLR such as TLR7, TLR8, and TLR9 (CS=96.2), can induce Aβ uptake or inflammatory response, which can be a therapeutic target for AD[69]. MGLL (CS=94.3), a putative target in this study, can regulate lipid metabolism and influence adult neurogenesis, thereby predisposing to AD during aging. It has been treated as a potential target for neurodegenerative diseases[70]. MIF (CS=92.9), a pro-inflammatory cytokine, was upregulated in the brain of AD patients. Its overexpression can significantly protect neuronal cells from Aβ-induced cytotoxicity[71]. RELA (CS=90.4) participates in the AD pathway, and is associated with Inflammatory process[72]. We will not discuss the relationship between these core targets and AD one by one. The above analysis proves that the core targets of SWC are highly reliable and representative, and that SWC treatment of AD is achieved through multiple targets and processes.

As for the key components obtained by the degree parameter, bisphenol A (D2=279) from *Panax ginseng* C.A.Mey (RS) ranked highest. It can mediate AD-like neurotoxicity by affecting brain insulin resistance[73]. Quercetin (D2=251) from four herbs possesses anti-Alzheimer, anti-diabetic and anti-obesity effects with clinical evidence and has been suggested as a promising therapeutic agent[74]. Several studies have shown that resveratrol (D2=228) from *Radix polygoni multiflori preparata* (ZSW) regulates many aspects of AD, such as neuro-inflammation, adaptive immunity, autophagy, antioxidants and estrogen and has excellent therapeutic potential[75,76]. Genistein (D2=162), a representative compound from *Pueraria lobate* (GG), has been found to target directly the Aβ and tau to control the intracellular signaling pathways responsible for neurons death in the AD brain[77]. Part of Chinese herbal medicine prescription toxic side effects, and under the guidance of the theoretical system of TCM compatibility application can reduce the toxic side effects, at the same time to achieve synergistic effect[78]. In this study, we can explain the theory of synergism and toxicity reduction at the molecular level. As for the synergy mechanism, quercetin, resveratrol, apigenin (D2=95), and kaempferol (D2=82) are flavonoids have shown the potential activity against AD. They possess anti-amyloidogenic and fibril-destabilization activity, as well as being able to act as metal chelators and to suppressing oxidative stress[79]. For mechanism of toxicity reduction, quercetin can abrogate bisphenol A induced altered

neurobehavioral response and oxidative stress in zebrafish by modulating brain antioxidant defense system[80]. In addition, resveratrol can prevent bisphenol A-induced autism, type 2 diabetes mellitus, and metabolic syndrome by augmenting brain-derived neurotrophic factor synthesis and action[81]. Through the above analysis, we can identify the active ingredients in SWC, both toxic and beneficial, in a relatively reliable manner. This not only provides the material basis for explaining the mechanism of TCM action, but also provides the possibility for understanding the compatibility theory of TCM from the molecular level, such as the theory of synergism and toxicity reduction.

Data analysis and mining in this study provide many verifiable directions for subsequent research. Although there are few studies on SWC and AD at present, a series of studies can be carried out based on the highly reliable findings of this study in the future, such as the relationship between some core targets and key components, the synergistic mechanism of key components, and the regulatory effects of key targets in a specific signaling pathway.

The purpose of this study is to elucidate the mechanism of SWC in the treatment of AD from a systematic perspective and explore the key material basis through network pharmacology technology. The results shows that SWC exerted its pharmacological effects on AD by regulating a variety of biological processes, such as membrane potential, oxidative stress, synaptic transmission, neuron death, hypoxia, etc., and multiple pathways, including neuroactive ligand-receptor interaction, lipid and atherosclerosis, AD, cAMP signaling pathway, human cytomegalovirus infection, and calcium signaling pathway, etc. HTR3A, TLR9, MGLL, MIF, RELA, PTGS1, ALOX15, NOS3, ESR1, MAPK1, ITGA4, HDAC4, ABCG2, CASP9, APP, AHR and so on are the main therapeutic targets of SWC. The key regulatory and pharmacological components of SWC include quercetin, resveratrol, genistein, palmitic acid, butyraldehyde, bis (2-ethylhexyl) phthalate, cocaine, DL-Glutamic acid, oleic acid, luteolin, folic acid, apigenin, gamma-aminobutyric acid, etc. This study provides a theoretical basis and research paradigm for elucidating the synergistic effects of multiple components, multiple targets, and multiple pathways in TCM treatment of diseases from a systematic perspective, which will undoubtedly promote the modernization of TCM. Although the data used in this study is highly reliable, it is only based on data mining and data analysis. Therefore, further clinical verification studies on the role of SWC in AD should be carried out, and multi-level verification should be carried out in combination with actual research needs, such as cell models and animal models.

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Author Contributions:

Yitao Xing and Nianhong Chen conducted and completed the data analysis and manuscript writing and contributed equally to this work. Dingguo Wang, Xueying Lin and Tiandong Lin contributed to the data of predictive target for components. Yanting Song contributed to the systematic search and study selection. Jinsheng Zhuo provided some good suggestions and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interests:

The authors declare no competing interests.

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