Network pharmacology-based study on the mechanism of *Schisandra chinensis* for treating Alzheimer’s disease

Cheng Kun, Sun Feiyi¹, Dong Jian², Chen Feng, Wu Guihua, Zhu Jiangping³, Ji Jianwu³, Liu Hong, Han Xiaowei³

**Abstract:**

**BACKGROUND:** Alzheimer’s disease (AD) is a mental illness that poses a serious threat to human health worldwide. *Schisandra chinensis* is a natural herb that can treat the effects of AD, but its specific mechanism is still unclear. The purpose of this study was to explore the potential components and pharmacological pathways of *S. chinensis* in the treatment of AD.

**MATERIALS AND METHODS:** In this study, we investigated the compound of *S. chinensis* and the effects of it on AD by network pharmacology. Meanwhile, the potential mechanism was proved in vitro.

**RESULTS:** The results showed that *S. chinensis* contained 173 compounds. Compound-target network confirmed that (E)-9-Isopropyl-6-Methyl-5,9-Decadiene-2-One, 1-Phenyl-1,3-Butanedion, nootkatone and phenyl-2-Propanone were the main chemical constituents which highly aimed at APOE, CACNA1D, GRIN2A, and PTGS2. KEGG and GO enrichment analysis indicated that the main pathways involved neural-related signaling pathways and functions, such as nicotine addiction, GABAergic synapse, Ca²⁺ signaling pathway, AD, and so on. Validation experiments showed that nootkatone was able to exert anti-apoptotic effects related to Ca²⁺ signaling pathway by inhibiting nitric oxide production, enhancing the activity of antioxidant enzymes, upregulating the expression of anti-oxidation and anti-apoptotic proteins in vitro.

**CONCLUSIONS:** These results illustrated that *S. chinensis* could regulate neuronal apoptosis through the calcium signaling pathway to exert anti-AD by integrating multi-component, multi-target and multi-pathway.

**Keywords:** Alzheimer's disease, network pharmacology, *Schisandra chinensis*

**Introduction**

Alzheimer’s disease (AD) is an irreversible neurodegenerative disease induced by multiple complex mechanisms. Its pathogenesis involves abnormal tau protein expression, nerve cell apoptosis, genetic associations, and abnormal mitochondria. AD can not only seriously jeopardize the health of the elderly but also impose a great burden on families and society and grow with age. The total number of patients with AD worldwide in 2016 was about 35 million, which is expected to reach 65.7 million in 2030 and 115.4 million in 2050. At present, AD can only be treated symptomatically, delaying its development; a treatment strategy to cure AD remains lacking to date. Anti-AD drug N-methyl-D-aspartate receptor (NMDAR) blockers and acetylcholinesterase inhibitors approved by the US Food and Drug Administration can only delay symptoms but cannot alleviate or reverse pathological processes. Therefore, the development of anti-AD drugs still has patient needs and market demand.
Many anti-AD drugs against a single target have failed in phase III clinical trials, indicating that drug research should shift to multi-component, multi-target, and multi-pathway. The pharmacological activity characteristics of multi-target natural products render them uniquely advantageous compared with Western medicine. Schisandra chinensis of the Magnoliaceae family is a type of dry and mature fruit. It is a common Chinese herbal medicine with the bioactivities of astringents, benefiting qi for promoting the production of tranquilizers in official Chinese Pharmacopoeia. The main pharmacologically active ingredients in S. chinensis are dibenzocyclooctadiene lignans. Modern pharmacological studies showed that S. chinensis has multiple bioactivities, such as anti-inflammatory, antioxidative, and anticancer. It can also influence the central nervous system. Related studies confirmed the neuroprotective and anti-AD effects of S. chinensis. However, the active ingredient in S. chinensis and its specific target remain to be determined. To solve these problems, we applied network pharmacology, which has been widely used in the discovery and development of drugs to understand the treatment, the nature of Chinese medicine, and the role of prescriptions.

In summary, the chemical constituents of S. chinensis and their target were analyzed using a network pharmacology method and bioinformatics analysis to explain the potential mechanism by which S. chinensis treats AD. Finally, the results were verified by in vitro cell experiments.

Materials and Methods

Materials
1-Phenyl-1,3-Butanedion and nootkatone (>99% purity) (NOT) were purchased from Sigma-Aldrich (St. Louis, MO, USA), (E)-9-Isopropyl-6-Methyl-5 and 9-Decadiene-2-One (>98% purity) were brought from Servicebio (Hangzhou, China). PC12 cells, dimethyl sulfoxide (DMSO), Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Servicebio (Hangzhou, China). The kits for determining nitric oxide (NO), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were obtained from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China). All antibodies were obtained from Abcam (Cambridge, UK). Other chemicals and materials were purchased from Servicebio (Hangzhou, China).

Database building
The active ingredients and targets of S. chinensis were collected from BATMAN-TCM (http://batman.tcm.org/batman-tcm/). First, we opened the website according to the previous URL and selected a and enter the Latin name of the medicinal material S. chinensis, other settings selected default, then started running the program. Finally, after a few seconds, you would be able to download the compound and target from download all the target prediction results. All setting parameters and operation steps are executed according to the manual.

ADMET and toxicity analysis of the active ingredients
After obtaining the active constituents and targets of S. chinensis in the treatment of nervous system diseases and AD from BATMAN-TCM, we screened compounds that they could regulated nervous system diseases and AD. Then, ADMET and toxicity analysis of these compounds were tested by the discovery studio 4.0. First, we converted all the compounds into sdf format and imported them into the software. Next, we chose the calculate molecular properties module under the small molecule interface, we used the AMET descriptors in it for filtering compounds, and all parameters were default. All setting parameters and operation steps are executed according to the manual.

Bioinformatics analysis
According to the result of ADMET and toxicity analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) enrichment analyses of the targets of these compounds were performed using DAVID 6.7 (https://david.ncifcrf.gov/). First, the obtained targets were imported into the DAVID database, and then the species selected humans. Finally, the obtained data were sequentially analyzed using KEGG and GO options. All setting parameters and operation steps are executed according to the manual.

Network conduction and analysis
To determine the complex relationship between S. chinensis and AD, we constructed a network of compound-target-disease relationships by Cytoscape 3.7.1. First, we processed the target and compound for the disease in excel, then they were imported them into the software, and all parameters used default settings. All setting parameters and operation steps are executed according to the manual.

Protein-protein interaction analysis
To identify key proteins, we further constructed a protein–protein interaction network by STRING 10.5 (https://string-db.org/). First, we selected the multiple proteins option in the URL interface, then entered the protein into the box on the right, and the species selected humans. Finally, all parameters selected the default settings to get the results. All setting
parameters and operation steps are executed according to the manual.

**Cells culture**
PC12 cells were cultured at 5% CO₂ in air and 37°C in 25 cm² flasks containing DMEM supplemented with 15% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin.

**3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay**
Cell viability was analyzed using the MTT assay. First, PC12 cells were seeded at a density of 10⁴ cells/well and then cultured in complete medium for 24 h in 96-well plates. Then, 6.25, 12.5, 25, and 50 μg/mL NOT were incubated for 12 h. After incubation with 20 μM Aβ₁₋₄₂ for 24 h (model group), the cells were added with 50 μg of MTT in each hole and incubated for 4 h at 37°C. Finally, the cells removed from the medium were dissolved in 200 μL of DMSO for 10 min, and the absorbance at 570 nm was recorded with a microplate reader (Infinite M1000, Tecan, Sunrise, Austria). What’s more, we also tested the activity of (E)-9-Isopropyl-6-Methyl-5,9-Decadiene-2-One, 1-Phenyl-1,3-Butanedion and phenyl-2-Propanone by the MTT.

**Lactate dehydrogenase levels, nitric oxide levels and glutathione peroxidase, and superoxide dismutase activities analysis**
PC12 cells were cultured at 10⁴ cells/well in 6-well plates for 24 h. The cells were pre-incubated with or without 25 μg/mL NOT for 12 h, followed by 20 μM Aβ₁₋₄₂ for 24 h. The supernatant was collected for the following experiments. NO levels, GSH-Px activities, SOD activities, and LDH release were analyzed using commercial kits in accordance with the manufacturer’s instructions.

**Western blot assay**
PC12 cells were cultured in 75 cm² flasks. After being incubated with 25 μg/mL NOT and 20 μM Aβ₁₋₄₂, the cells were collected for total protein extraction, and the protein concentration was determined by BCA assay kit. Then, 30 μg protein was separated by 12% sodium dodecyl sulfate poly-acrylamide gel electrophoresis gels. Afterward, they were transferred onto 0.22 μm NC membranes and then blocked with 5% (w/v) nonfat milk in PBST for 2 h. All primary antibodies were incubated at 4°C overnight, and the secondary antibodies were incubated at room temperature for 2 h. Finally, the ECL plus kit was used to detect the protein bands.

**Statistical analysis**
All data were presented as mean ± standard deviation, and each experiment was repeated at least three times.

Significant differences were compared using one-way ANOVA or Dunnett’s test. Statistical significance was considered at P < 0.05.

**Results**

**Collection of compounds and targets from Schisandra chinensis**
Through the BATMAN-TCM database, 175 chemical constituents of *S. chinensis* were collected, but 63 species had no structural information. To further investigate the targets of these compounds, we set the parameters as follows: predicted candidate targets with scores not smaller than score cutoff = 20 for each ingredient, adjusted P ≤ 0.05 were highlighted in the results. Finally, 134 potential targets related to the nervous system and AD were screened in accordance with the disease classification of the medical subject vocabulary. Moreover, we further carried out ADMET and toxicity analysis of the active ingredients related to the 134 targets. As shown in Figure 1, four compounds had no good metabolic activity, they were epiguaipyridine, clupanodonic acid, Vitamin K1 and nonylphenol. Luckily, the four compounds regulated more targets had a good metabolic activity which were marked by yellow. Thus, we finally got 132 targets related to nervous system diseases and AD [Supplementary Material 1].

**Bioinformatics analyses of potential targets**
The 132 targets obtained from the above analysis were imported into the DAVID database for GO annotation and KEGG enrichment analysis. The results of GO annotation indicated that the most relevant functions involved...
in these targets were acetylcholine regulation and ion channel, which are the main pathological processes of AD [Supplementary Material 2]. KEGG analysis showed that the pathways involved are nicotine addiction, retrograde endocannabinoid signaling, GABAergic synapse, and morphine addiction. One of the results was especially noticeable because these proteins can significantly enrich the AD pathway [Figure 2]. As shown in Figure 2, *S. chinensis* can regulate the calcium signaling pathway by controlling Apoe, NMDAR, VDCC, and NOS, thereby exerting anti-oxidative and anti-apoptotic effects, which are important for AD treatment.

**Network construction and analysis**
KEGG and disease enrichment analyses revealed that *S. chinensis* treats AD by controlling 33 key proteins. We also found 39 compounds corresponding to the 33 proteins from the BATMAN-TCM database. A chemical disease–target–compound network was constructed using the Cytoscape 3.7.1 software [Figure 3]. Statistical analysis showed that (E)-9-isopropyl-6-methyl-5,9-decadiene-2-one, 1-phenyl-1,3-butanedione, nootkatone, and phenyl-2-propanone can regulate more targets, and APOE, CACNA1D, GRIN2A, and PTGS2 were the most active proteins. Moreover, protein–protein interaction analysis showed that NOS1 was the most critical protein [Supplementary Material 3].

**Effects of the representative ingredient of Schisandra chinensis on the Aβ1-42-induced PC12 cells injury**
The survival rate and LDH activity of PC12 cells in each group were compared. Compared with that in the control group, the survival rate of PC12 cells in the model group was significantly lower, but the survival rates of the cells in the 12.5, 25, and 50 μg/mL NOT groups were significantly higher. Notably, 25 μg/mL NOT exerted the greatest protective effect [Figure 4a]. LDH is normally present in cells and leaks during necrosis or apoptosis. Compared with that in the control group,
the LDH activity in the model group was significantly higher, whereas that in the 25 μg/mL NOT group was significantly lower [Figure 4b]. Meanwhile, we tested the another three the representative ingredient of *S. chinensis* on AD, the results of MTT showed that they could protect PC12 cells on the Aβ1-42-induced injury [Figure 4c-e].

**Effects of NOT on nitric oxide level, superoxide dismutase activity, and glutathione peroxidase activity in PC12 cells with Aβ1-42-induced injury**

Compared with those in the control group, the SOD and GSH-Px activities in the PC12 cells of the model group significantly decreased [Figure 5b and c], whereas the NO level significantly increased [Figure 5a]. However, the SOD and GSH-Px activities in the 25 μg/mL NOT group significantly increased and the NO levels significantly decreased in the PC12 cells with Aβ1-42-induced injury compared with the model group [Figure 5].

**Effects of NOT regulated HO-1 and cleaved caspase-3 levels in PC12 cells with Aβ1-42-induced injury**

Compared with those in the control group, the expression levels of HO-1 and cleaved caspase-3 protein in the model group were significantly higher [Figure 6a-c]. By contrast, the expression levels of HO-1 and Bax protein in the 25 μg/mL NOT group were significantly lower than those in the model group [Figure 6].

**Discussion**

*S. chinensis* is a member of the Magnoliaceae family, and its main drug site is its mature fruit. *S. chinensis* has diverse activities, such as anti-inflammatory, anti-oxidative, anti-viral, vasodilating, neuroprotective, and anti-ulcer.[8,13,21] It is mainly used to treat nephropathy and encephalopathy in traditional medicine, and its main pharmacodynamic effect is conferred by its complex composition. As a result, the potential mechanisms of *S. chinensis* remain unclear to date. Network pharmacology analyzes the relationship between drugs and targets from a holistic and systematic perspective, and it can predict the pharmacodynamic effects of the active ingredients of traditional Chinese medicine. Therefore, we used network pharmacology to construct a compound–gene–disease network of the main components of *S. chinensis*, analyzed the biological functions of each pathway, and classified it. We formulated a hypothesis about the main pharmacodynamic effects of *S. chinensis* in preventing AD and performed cell experiments to confirm the results in vitro.
Network pharmacology analyzes the relationship among drugs, targets, and diseases by constructing a network model. It is a new model for drug research. On the basis of existing data in the database, a drug–target–disease network for specific drugs is established, and then the drug is analyzed. The intervention and influence of the disease network can predict the effects of pharmacodynamic components on certain key targets and their pathways. The composition of traditional Chinese medicines is diverse, and complex network relationships also exist between diseases and targets. Therefore, network pharmacology is suitable for elucidating the mechanism of traditional Chinese medicine. In the present study, 175 compounds of S.
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Figure 6: Effects of NOT on the expressions of HO-1 and cleaved caspase-3 protein in PC12 cells. (a) HO-1 and cleaved caspase-3 protein expressions. (b) Quantitative analysis of HO-1 level in PC12 cells. (c) Quantitative analysis of cleaved caspase-3 level in PC12 cells. ***P < 0.001 versus control, **P < 0.01 and ***P < 0.001 versus model

Schisandra chinensis and 134 nervous system and AD hippocampal targets were collected from the BATMAN-TCM database, and then, ADMET and toxicity analysis of the potential active ingredient were finished by discovery studio 4.0, the targets related to active ingredient were imported into the DAVID database for GO annotation and KEGG enrichment analysis. Finally, we obtained 39 active components of S. chinensis to regulate 33 related targets for AD. Furthermore, a component–gene–disease network was constructed. Network analysis showed that (E)-9-isopropyl-6-methyl-5,9-decadiene-2-one and NOT were the compounds that regulated the most targets, and CACNA1D and GRIN2A were the most regulated targets. Moreover, protein–protein interaction analysis revealed that NOS1 was the most critical protein. KEGG function enrichment analysis showed that the active ingredients of S. chinensis treat AD by regulating the calcium signaling pathway. This result is consistent with the pathology of AD.[23,24] However, an interesting phenomenon was that we had not enriched the targets regulated by lignin compounds, but there were many reports that lignin compounds in S. chinensis had anti-AD effects, such as schizandrin B, schisantherin A, and lignans. To explain this phenomenon, we further looked at the raw data of the analysis. We found that this was related to the threshold set during protein screening. If we lowered the current standard, lignin compounds in S. chinensis could be enriched to the target related to against AD.

AD is a progressive neurodegenerative disease where progressive decline occurs after intelligence reaches normal levels. Although the molecular biology of AD has been researched since the 1980s, the cause remains unclear. Calcium overload is reportedly the main cause of AD pathogenesis.[23,24] Neurons with near amyloid deposits have higher Ca^{2+} levels than normal resting levels, and the environment of elevated resting Ca^{2+} promotes negative plasticity.[25,26] This mechanism increases the expression and activity of calcineurin (CaN) by elevating intracellular levels. CaN is a Ca^{2+} signaling protein activated by calmodulin (CaM), which is sensitive to subtle rises in intracellular Ca^{2+} levels. Activated CaN activates additional phosphatases,[27] such as PP1. Then, CaMKII holenzymes can be activated upon the binding of Ca2+/CaM. The elevated intracellular Ca^{2+} levels could initiate a series of harmful processes, including further promotion of free radical growth, destruction of cell membranes and skeletons, and sequential activation of Ca^{2+}-dependent kinases, lipases, and proteases, which lead to cell damage and even death.[28] Our experiment showed that NOT reduced apoptosis and LDH level in PC12 cells with Aβ1-42-induced injury, reduced the secretion of NO, enhanced the activities of SOD and GSH-Px, and reversed the expression of HO-1 and cleaved caspase-3, which are closely related to the calcium signaling pathway in AD. The results also corroborated the anti-AD effect of S. chinensis in treating AD and the correctness of previous results.[13,21,29]

However, this study has some limitations. For example, this conclusion needs to be further verified in vivo, and additional inhibitors must be included to reverse verification. In addition, it also requires the addition of pharmacokinetic experiments to confirm the blood components.

Conclusions

To the best of our knowledge, this research provided the first network pharmacology evidence that S. chinensis protects and treats AD through the Ca^{2+} signaling pathway by exerting anti-apoptotic and anti-oxidant activities and lowering NO level. Moreover, network pharmacology in vitro showed that the active ingredients from S. chinensis can protect PC12 cells against...
Aβ$_{1-42}$-induced apoptosis. This study may serve as a reference in developing *S. chinensis* as a complementary and alternative medicine to treat AD.

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Nil.

**Conflicts of interest**
There are no conflicts of interest.

**References**

1. Zhang L, Trushin S, Christensen TA, Bachmeier BV, Gateno B, Schroeder A, et al. Altered brain energetics induces mitochondrial fission arrest in Alzheimer’s Disease. Sci Rep 2016;6:18725.

2. Huang KL, Marcera E, Pimenova AA, Di Narzo AF, Kapoor M, Jin SC, et al. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer’s disease. Nat Neurosci 2017;20:1052-61.

3. Sheng C, Feng W, Chen Z, Cao Y, Song Z, Xia ZA, et al. Impact of 2, 3, 5, 4′-tetrahydroxyflavone-2-O-β-D-glucoside on cognitive deficits in animal models of Alzheimer’s disease: A systematic review. BMC Complement Altern Med 2016;16:320.

4. Mischoulon D, Raab MF. The role of folate in depression and dementia. J Clin Psychiatry 2007;68 Suppl 10:28-33.

5. Berk C, Paul G, Sabbagh M. Investigational drugs in Alzheimer’s disease: Current progress. Expert Opin Investig Drugs 2014;23:837-46.

6. Li L, Zhang L, Yang CC. Multi-target strategy and experimental studies of traditional Chinese medicine for Alzheimer’s disease therapy. Curr Top Med Chem 2016;16:357-48.

7. Yang B, Han W, Han H, Liu Y, Guan W, Kuang H. Lignans from *Schisandra Chinensis* ratten stems suppresses primary Aβ$_{1-42}$-induced microglia activation via NF-kB/MAPK signaling pathway. Nat Prod Res 2019;33:2726-9.

8. Szopa A, Ekiert R, Ekiert H. Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: A review on the bioactive components, pharmacological properties, analytical and biotechnological studies. Phytochem Rev 2017;16:195-218.

9. Hu D, Yang Z, Yao X, Wang H, Han N, Liu Z, et al. Dibenzocyclooctadiene lignans from *Schisandra chinensis* and their inhibitory activity on NO production in lipopolysaccharide-activated microglia cells. Phytochemistry 2014;104:72-8.

10. Thandavarayan RA, Giridharan VV, Arumugam S, Suzuki K, Ko KM, Krishnamurthy P, et al. Schisandrin B prevents doxorubicin induced cardiac dysfunction by modulation of DNA damage, oxidative stress and inflammation through inhibition of MAPK/p53 signaling. PLoS One 2015;10:e0119214.

11. Zhao X, Liu C, Xu M, Li X, Bi K, Jia Y. Total Lignans of *Schisandra chinensis* Ameliorates Aβ$_{1-42}$-Induced Neurodegeneration with Cognitive Improvement in Mice and Primary Mouse Neuronal Cells. PLoS One 2016;11:e0152772.

12. Sa F, Zhang LQ, Chong CM, Guo BJ, Li S, Zhang ZJ, et al. Discovery of novel anti-parkinsonian effect of schisantherin A in *in vitro* and *in vivo*. Neurosci Lett 2015;593:7-12.

13. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. An overview of neuroprotective and cognitive enhancement properties of lignans from *Schisandra chinensis*. Biomed Pharmacother 2018;97:958-68.

14. Zhao X, Liu C, Xu M, Li X, Bi K, Jia Y. Total Lignans of *Schisandra chinensis* Ameliorates Aβ$_{1-42}$-Induced Neurodegeneration with Cognitive Improvement in Mice and Primary Mouse Neuronal Cells. PLoS One 2016;11:e0152772.

15. Song JX, Lin X, Wong RN, Sze SC, Tong Y, Shaw PC, et al. Protective effects of dibenzocyclooctadiene lignans from *Schisandra chinensis* against beta-amyloid and homocysteine neurotoxicity in PC12 cells. Phytother Res 2011;25:435-43.

16. Luo Y, Wang Q, Zhang Y. A systems pharmacology approach to decipher the mechanism of danggui-shaoxyan-san decoction for the treatment of neurodegenerative diseases. J Ethnopharmacol 2016;178:66-81.

17. Yang K, Luo Y, Lu S, Hu R, Du Y, Liao P, et al. Salvianolic acid B and ginsenoside re synergistically protect against Ox-LDL-induced endothelial apoptosis through the antioxidative and antiinflammatory mechanisms. Front Pharmacol 2018;9:662.

18. Liu Z, Guo F, Wang Y, Li C, Zhang X, Li H, et al. BATMAN-TCM: A Bioinformatics Analysis Tool for Molecular mechAnism of Lignans from *Schisandra chinensis*. Sci Rep 2016;6:21146.

19. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44-57.

20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498-504.

21. Zhang M, Xu L, Yang H. *Schisandra Chinensis* fructus and its active ingredients as promising resources for the treatment of neurological diseases. Int J Mol Sci 2018;19:1970.

22. Hopkins AL. Network pharmacology: The next paradigm in drug discovery. Nat Chem Biol 2008;4:682-90.

23. Alzheimer’s Association Calcium Hypothesis Workgroup. Calcium Hypothesis of Alzheimer’s disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis. Alzheimers Dement 2017;13:178-82E+19.

24. Mattson MP. ER calcium and Alzheimer’s disease: In a state of flux. Sci Signal 2010;3:pe10.

25. Kuchibhotla KV, Goldman ST, Lattarulo CR, Wu HY, Hyman BT, Backskai BJ. Abeta plaques lead to aberrant regulation of calcium homoeostasis in vivo resulting in structural and functional disruption of neuronal networks. Neuron 2008;59:214-25.

26. Foster TC. Calcium homeostasis and modulation of synaptic plasticity in the aged brain. Aging Cell 2007;6:319-25.

27. Mulkey RM, Endo S, Shenolikar S, Malenka RC. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 1994;369:486-8.

28. Chadwick W, Mitchell N, Martin B, Maudsley S. Therapeutic targeting of the endoplasmic reticulum in Alzheimer’s disease. Curr Alzheimer Res 2012;9:110-9.

29. Pak ME, Kim YR, Kim HN, Ahn SM, Shin HK, Baek JU, et al. Studies on medicinal herbs for cognitive enhancement based on the text mining of Dongeuibogam and preliminary evaluation of its effects. J Ethnopharmacol 2016;179:383-90.
### Target name

| Target name | CHRNB2 | GRIA4 | KCNJ9 | CHRNA2 | GRK2 | GRIA4 |
|-------------|--------|-------|-------|--------|------|-------|
| ACHER       | CHRNB4 | GRIK2 | KCNQ1 | CHRNA3 | GRN1 | GRIK3 |
| ADRBK1      | CNR1   | GRN2A | MAOA  | CHRNA4 | GRN1 | GRIK4 |
| ALOX15      | COMT   | GRN2B | MAOB  | CHRNA5 | GRN2 | GRIK5 |
| ALOX15B     | CPLX2  | GRN2C | MAP2K5| CHRNA6 | GRN3 | GRIK6 |
| ALOX5       | CRH    | GRN2D | MPO   | CHRNA7 | GRN3 | GRIK6 |
| APOE        | DDC    | GRN3A | NGFR  | ARRB2  | HAP1 |       |
| ARRB2       | DLG4   | GRN3B | NOS1  | BAX    | HOMER1|       |
| BAX         | DNM3   | GSK3A | PPP2CA| BCHE   | DRD1  |       |
| BCHE        | DRD2   | GUCY1B3| PPP2CB| BCL2   | DRD2  |       |
| BCL2        | DRD3   | HAP1  | PPP3CA| BDKRB2 | DRD3  |       |
| BDKRB2      | DRD4   | HOMER1| PPP3CB| CACNA1A| DRD5  |       |
| CACNA1A     | FASLG  | HTR1A | PPP3R1| CACNA1C| GABRA1|       |
| CACNA1C     | GABRA2 | HTR1B | PRKCD | CACNA1D| GABRA2|       |
| CACNA1D     | GABRA3 | HTR2A | PTGS1 | CACNA1S| GABRA4|       |
| CACNA1S     | GABRA5 | HTR2B | PTGS2 | CALM1  | GABRA6|       |
| CALM1       | GABRA6 | HTR2C | RAB3A | CALM2  | GABRB1|       |
| CALM2       | GABRB2 | HTR3A | RYR1  | CALM3  | GABRB3|       |
| CALM3       | GABRB3 | HTR3B | SCN1A | CALML3 | GABRD |       |
| CALML3      | GABRD  | HTR3C | SHANK3| CAMK2D | GABRE |       |
| CAMK2D      | GABRE  | HTR3D | SLC17A7| CAMK2G| GABRG1|       |
| CAMK2G      | GABRG1 | HTR3E | SLC18A2| CDC42  | GABRG2|       |
| CDC42       | GABRG2 | HTR4  | SLC18A3| CHRFAM7A| GABRG3|       |
| CHRFAM7A    | GABRG3 | HTR6  | SLC5A7 | CHRM1  | GABRP |       |
| CHRM1       | GABRP  | HTR7  | SLC6A3 | CHRM2  | GABRQ |       |
| CHRM2       | GABRQ  | ITPR1 | SLC6A4 | CHRM3  | KCND2 |       |
| CHRM3       | KCND2  | ITPR2 | SRC   | CHRM4  | KCNJ3 |       |
| CHRM4       | KCNJ3  | ITPR3 | STX1A | CHRM5  | KCNJ5 |       |
| CHRM5       | KCNJ5  |       | STX3  | CHRNA3 | KCNJ6 |       |
| CHRNA3      | KCNJ6  |       | TH    | CHRNA4 |       |       |
| CHRNA4      |       |       | TPH1  | CHRNA6 |       |       |
| CHRNA6      |       |       |       | CHRNA7 |       |       |
| CHRNA7      |       |       |       |        |       |       |
Supplementary Material 2

| Annotation Cluster 1 | Enrichment Score | Count | P_Value | Benjamiini |
|----------------------|------------------|-------|---------|------------|
| GOTERM_CC_DIRECT      | acetylcholine-gated channel complex | RT    | 12      | 4.3E-18 1.2E-16 |
| GOTERM_MF_DIRECT      | acetylcholine receptor activity | RT    | 12      | 5.4E-18 2.4E-16 |
| GOTERM_MF_DIRECT      | acetylcholine-activated cation-selective channel activity | RT    | 12      | 5.4E-18 2.4E-16 |

| Annotation Cluster 2 | Enrichment Score | Count | P_Value | Benjamiini |
|----------------------|------------------|-------|---------|------------|
| GOTERM_BP_DIRECT      | adenylate cyclase-inhibiting G-protein coupled acetylcholine receptor signaling pathway | RT    | 6       | 5.6E-10 4.1E-8  |
| GOTERM_MF_DIRECT      | G-protein coupled acetylcholine receptor activity | RT    | 6       | 5.6E-10 1.4E-8  |
| GOTERM_BP_DIRECT      | phospholipase C-activating G-protein coupled acetylcholine receptor signaling pathway | RT    | 6       | 1.5E-9 9.9E-8  |

| Annotation Cluster 3 | Enrichment Score | Count | P_Value | Benjamiini |
|----------------------|------------------|-------|---------|------------|
| GOTERM_BP_DIRECT      | regulation of cell communication by electrical coupling involved in cardiac conduction | RT    | 4       | 2.5E-5 6.1E-4  |
| GOTERM_MF_DIRECT      | titin binding | RT    | 4       | 1.6E-4 1.7E-3  |
| GOTERM_BP_DIRECT      | regulation of release of sequestered calcium ion into cytosol by sarcoplasmic reticulum | RT    | 4       | 3.5E-4 6.1E-3  |

| Annotation Cluster 4 | Enrichment Score | Count | P_Value | Benjamiini |
|----------------------|------------------|-------|---------|------------|
| GOTERM_MF_DIRECT      | G-protein activated inward rectifier potassium channel activity | RT    | 4       | 5.4E-5 6.3E-4  |
| GOTERM_MF_DIRECT      | inward rectifier potassium channel activity | RT    | 4       | 4.8E-4 4.5E-3  |
| GOTERM_BP_DIRECT      | potassium ion import | RT    | 4       | 1.3E-3 1.9E-2  |

Supplementary Material 3

[Image of a network diagram]