Potential therapeutic targets for Alzheimer's disease and their association with Helicobacter pylori were analyzed based on the data of H. pylori-positive miRNA and Alzheimer's disease mRNA chips in the database

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Abstract

Background: Although H. pylori infection has previously been reported to be linked with Alzheimer's disease(AD), the pathogenesis between the two is unclear. Dysregulation of miRNA in AD in Helicobacter pylori(H. pylori) infection have been implicated in the development and progression of AD. The purpose of this study is to analyze and screen the relevant and promising molecular markers between H. pylori infection and AD. Method：In the present study, differentially expressed genes (DEGs) in AD samples (gray matter) of GSE37263 dataset and in H pylori-positive of GSE19769 collected by NCBI Gene Expression Omnibus (GEO) were analyzed utilizing the bioinformatics approach (R software and related packages), The MiRNA target gene was predicted by miRDB. Then intersected them and common genes were obtained,The interaction between genes was analyzed by STRING.WebGestalt was used for GO and KEGG analysis. Moreover, the MCODE of Cytoscape software was employed to uncover the protein–protein interaction (PPI) network and the matching hub gene.

Result: The gene set of H. pylori infection was crossed with that of AD acquired from GEO to obtain 48 common genes. Among them, 13 genes were interacted with each other and were key genes in AD regulatory network. After the establishment of PPI network, 9 pivot genes related to AD were retrieved, among which the most critical genes were GAD2, GABRG2, SLC32A1 and GABRA1，which are regulated by mirNA-650, mirNA-206, mirNA-142-3p and mirNA326. To reveal functional enrichment assessment of 13 cross genes, which were abundant in biological regulation and cell communication, and participates in nervous system regulation and neurotransmitter transmission. KEGG cascade analysis revealed three main pathways，KEGG cascade analysis revealed five pathways associated with the Key genes: GABA A receptor activation, GABA synthesis, release, Nicotine addictio, GABAergic synapse, and Neurotransmitter release cycle.

Conclusion: The establishment of these candidate key genes and their enriched signal transduction cascades provide promising molecular markers for H. pylori infection associated AD, which may contribute to the diagnosis and future treatment of patients with AD.

Intriduction

AD is a neurodegenerative disorders distinguished by memory difficulty, daily activity dysfunction, and cognitive decline, with Neuropathy and neuronal loss in the brain[1,2]. The pathological character of AD are extracellular amyloid plaques and intraneuronal neurofibrillary tangles, whose building modules are amyloid-β (Aβ) peptides and phosphorylated tau.There have not been effective pharmacotherapeutic options for the prevention and treatment of AD yet[3,4].Helicobacter pylori is a gram-negative microbe with a spiral shape in the stomach,even both innate and acquired immune responses are activated,when individuals infected with H. pylori，but it cannot be cleared by the host, resulting in chronic lifelong infection[5,6].In addition to gastritis, peptic ulcer disease, adenocarcinoma，and mucosa-associated lymphoid tissue (MALT) -lymphoma, H. pylori infection has also been associated with neurological diseases.

Although the pathology of H. pylori infection is not limited to gastric ulcers, it is also associated with a range of diseases, such as neurological disorders (Parkinson's disease and AD), the specific mechanism may be that H. pylori enters the central nervous system through the oral-naso-olfactory pathway or the gastral-cerebral nerve pathway[7,8].The mini-Mental State Examination scores of AD patients infected with Helicobacter pylori were lower, and the corresponding cognitive impairment was more serious[9]. Recently study on APP transgenic mice has showed an increase in the level of Helicobacter and Odoribacter and a decreased abundance of Prevotella[9]. Besides,Helicobacter pylori induces tau hyperphosphorylation through activation of glycogen synthase kinase 3β (GSK-3β)[10].However, in a recent population-based cohort including 4215 participants, the association between H. pylori serology and dementia risk was not confirmed[11].Althouth there are Clinical Datas above on the H. pylori in AD, it is still unclear which specifc gene targets are involved.

As the threat of AD to the elderly becomes greater and greater, there is an urgent need to identify the etiology and molecular characteristics of AD.Currently, high-throughput sequencing technology is increasingly considered to have important clinical significance in disease research by evaluating gene expression differences and possible splicing variations, especially in molecular diagnosis, prognosis assessment and drug target discovery.Gene Expression Database (GEO) is a public website supported by the National Center for Biotechnology Information (NCBI). There are dozens of basic experimental disease gene expression patterns, which are widely used to explore pivot genes and expected mechanisms of disease onset and development[12].This research attempted to screen key genes for the occurrence and development of AD from the perspective of differential mirnas in H pylori infection, so as to learn more about the pathological mechanism associated with H pylori infection and AD, and provides a new direction for exploring the pathogenesis and treatment of AD.

Data Abstraction

We retrieved gene expression chip data GSE37263 and GSE19769 Respectively about H. pylori infection and AD ,GSE37263 contains 8 control and 8 AD samples,which are subjects recuited into OPTIMA(Oxford Project to Investigate Memory and Ageing).while,the miRNA dataset GSE19769 of H. pylori infection was obtained from ten H. pylori-negative and nine H. pylori-positive patients' subjects .

Prediction of miRNA target genes

miRNA target gene of data GSE37263 prediction was performed using miRWalk (http://mirwalk.umm.uni-heidelberg.de/) web-based online analysis tool. MiRWalk was developed by Sticht et al[13]. and can be used by users to provide a more comprehensive relationship between miRNA and target genes.

Variance analysis

Te core R package was employed to process the abstracted matrix fles. Following the normalization, we determined the diferences between AD and the control group,H. pylori-negative and nine H. pylori-positive

via truncation criteria (|log fold change (FC)|≥1, adjusted P<0.05), and determined the signifcant DEGs for subsequent analyses.Te intersection of the two sets of mrna was obtained by VENN diagram.

Construction of protein interaction networks

The interaction between proteins was analyzed using STRING(https://string-db.org/) database[14] Based on the prediction results of STRING and miRWalk, Cytoscape software was used to draw the interaction network.

Assessment of the PPI network of the DEGs

We used the STRING online search tool to analyze the PPI data encoded by DEGs[15], and only the combination score > 0.7 was considered signifcant. Then,the PPI network was analyzed and visualized using Cytoscape, and the frst four hub genes were determined as per the connectivity between DEGs. Te standard default setting of the mcode parameter. Te function enrichment of DEGs of each module was analyzed by adjusted P < 0.05 as the cutof standard.

Functional enrichment analysis of genes

Using WebGestalt analyses function of enrichment (http://www.webgestalt.org/)[16]. The WebGestalt database can be used for GO analysis (Gene Ontology) and KEGG analysis (Kyoto Encyclopedia of Genes and Genomes) and their graphs.

Result

DEGs identifcation

Firstly, we selected 89 DEGs from AD samples and healthy controls in the GSE37263 data set via limma package screening of R software,Of these, we selected 12 upregulated genes and 77 downregulated genes. At the same time,71 DEGs consisting of 19 upregulated genes and 52 downregulated genes, were uncovered via analysis of the H. pylori-positive samples in the miRNA dataset GSE19769,the frst 12 DEGs of two dataset were represented by volcano map, and heat map respectively (Fig. 1a–d), using |log FC|≥ 1 criteria and adjusted P < 0.05.

target genes of differential miRNA and Screening key genes for interaction network

a total of 10424 miRNA-binding genes were obtained from H. pylori-positive DEGS by using MiRWalk,There were 48 differentially overlapping genes with Alzheimer's disease (fig.2)and STRING was used to analyze the protein interaction of these 48 co-genes,among the rest,13 genes interact with each other,We believe that these 13 genes are key genes in the regulatory network of Alzheimer's disease(fig.3)

Module screening from the PPI network

Based on 13 co-genes,the Cytoscape publicly available platform and the STRING resource were employed to develop the PPI network, perform module analysis, as well as visualization. Thus, we developed a PPI network bearing 15 crosstalk based on 7 integrated DEGs related to AD,We employed the MCODE algorithm to ensure highly interconnected subnets, which are usually protein complexes, as well as components of cascades as per the topological structure.We selected only one module from the entire network for Subsequent analysis(Fig.4) .

Functional enrichment analysis of 13 DEGS in Alzheimer's disease regulatory network

WebGestalt was used for enrichment analysis of 13 genes in the regulatory network,GO term assessment illustrated that these genes, which were mainly related to biological regulation and cell communication, and participates in the molecular functions of nervous system regulation and neurotransmitter transmission through the combination of proteins or ion receptors with cell membranes and vesicles(Fig.5a). KEGG cascade analysis identifed first 5 pathways associated with the Key genes:GABA A receptor activation,GABA synthesis,release,Nicotine addictio,GABAergic synapse,Neurotransmitter release cycle (Fig.5b).

Discussion

The study showed that through network analysis of GO, KEGG and PPI, four pivot genes (GABA、GABRA1、GABRG2、SLC32A1) were screened out,which are respectively regulated by mirNA-650, mirNA-206, mirNA-142-3p and mirNA326.Moreover, GO term “cell communication, protein binding, ion binding and transporter activity ”and KEGG term "GABA A receptor activation,GABA synthesis,release" was obtained.All of these have potential therapeutic effects on AD.

The four mrnas and KEGG term are commonly involved in GABAergic signal transduction system.GABA synthesis occurs via the α-decarboxylation of L-glutamate by the enzyme glutamic acid decarboxylase (GAD).GABA is then recruited into synaptic vesicles via the action of vesicular GABA transporter (vGAT). Following membrane depolarization, GABA is released into the synapse and can bind to GABAA receptors, lead to inhibition of the post-synaptic neuron.

GABA is widely spreaded over in the brain, its receptors show a high diversity of conformations. Thus, the GABAergic system has been related to a wide range of behavioral and cognitive functions encompassing the regulation of vigilance, anxiety, learned fear and memory[17-20].Furthermore,GABA signaling is considered to be the underlying mechanism for a lot of diseases, including schizophrenia, anxiety disorders, depression, bipolar disorder, autism, and others[21,22].Pathological markers of Alzheimer's disease have been found to be associated with changes in GABA signal transduction in extensive animal model studies.recently in vitro experiments, however, have revealed that Aβ neurotoxicity weakens GABAergic neuron activity and impairs inhibitory postsynaptic potentials、 due to downregulating postsynaptic GABAA receptors[23,24]. As well, TgCRND8 mice, which represent early Aβ deposition,exhibit a loss of GABAergic neurons at 6 months of age[23]. Similarly, a 50–60% reduction in the number of GABAergic interneurons coexpressing SOM and NPY is showed up in APP/PS1 mice a at 6 months,

preceding pyramidal cell loss, which suggests that GABAergic dysfunction may be an early performance of pathology in AD.In term of tau pathology, a significant reduction in the number of GAD-, SOM- and PV-positive cells in the hippocampus is showed in JNPL3(P301L) mice, which expresses human tau at twice endogenous levels. [25]. Furthermore, tau proteins co-localize with these populations of interneurons,illustrating that tau possiblely promote to a loss of GABA neurotransmission in the hippocampus[25,26].

Joan Jiménez-Balado etal. hypothesize that The effect of age on GABAergic level may be influenced by some factors such as female, APOE ε4 and cerebrovascular disease, which cripple GABAergic function either independently or interactively. These factors may decrease GABA levels via impairing interneurons or weekening GABAergic function Which overexcites the hippocampal circuitry. Sustained exposition to hippocampal hyperactivity result in episodic memory loss, accumulation and atrophy of Aβ/tau , Ultimately increases the likelihood of dementia.in line with the hypothesis, GABAergic dysfunction might anterior to both the clinical symptoms of cognitive disorder, and tau and Aβ accumulation, and playing a critical role between risk factors and episodic memory impairment.Further research for the GABAergic system in aging may be useful for understanding of age-related cognitive decline and AD. furthermore , GABA emerged as a potential pharmacological target,as previous clinical trials have reported positive effects of levetiracetam on cognitive decline[27,28].

GABAA receptors is an ionic channel in the central nervous system[29], Contextual learning not only induces synaptic delivery of AMPA receptors but also strengthens GABAA receptor-mediated inhibitory synapses onto CA1 neurons[30] Since Aβ weakened GABAA receptor-mediated synaptic inhibition, GABAA receptor agonists may improve either symptoms or progression of AD. A human AD patient showed several alterations in GABAA receptor subunits including α1, α2, α5, β2, β3 and γ2[31].GABAA α5 receptors are particularly included in tonic inhibition, and their selective reduction has been shown to lead to network hyperactivity in the hippocampus[32]. for the same reason, novel benzodiazepine-like ligands, targeting GABAA α receptors, have been known to reverse working memory deficits in aged (21–22 month old) C57BL/6 mice[33]More over,GABRG2 gene has be known as the most common epileptic gene in GABA A R subunit.If GABRG2 gene is mutated, neurons have different responses and metabolic abilities to abnormal mRNA and mutated subunits as temporal and spatial specificity of NMD and individual differences in ERAD efficiency. Impaired expression of GABAA receptors in the postsynaptic membrane results in a decrease in the inhibitory function of GABA in the brain.studies indicated that GABAergic dysfunction causes neural overactivity, which ultimately leads to AD seizures[34].Therefore,GABAA receptors are a promising potential therapeutic target due to its high expression in the hippocampus and its significant role in memory.

H. pylori has evolved several strategies, including controling innate immune receptors and inhibiting effector T cell responses,for purpose of evading the host's immune response and surviving in the adverse conditions found in the stomach[35]. The host-induced immune response can result in the local secretion of various inflammatory mediators,such as interleukin (IL) 8, -6, -1b, -10, and -12, tumor necrosis factor (TNF) and interferon (IFN) , which enter the circulation causing systemic effects and inducing

neuroinflammation and toxicity[36].Meanwhile, H. pylori infection contribute to the release of various neurotransmitters, such as acetylcholine, adrenaline, noradrenaline, serotonin, and dopamine[37,38]. In addition, H. pylori infection may cause damage to axons/neurons, produce free radicals, and alter neuropeptide expression , such as vasoactive intestinal peptide (VIP) and c-fos . Lastly, H. pylori infection is related to changes in the group of the gastrointestinal microbiome and can possibly change the prognosis of neurological disorders[39].The specific mechanism may be due to changes in gastrointestinal pH or Inflammatory cells secrete inflammatory cytokines caused by helicobacter pylori infection[40,41].

A recent review[42]highlighted the important role of the microbiome - gut-brain (MGB) axis disorder in the development of AD . Alteration in the constituent of intestinal flora lead to impaired blood-brain barrier (BBB) function due to increased intestinal barrier permeability and activation of immune cells, which promotes neuroinflammation, neuron loss, nerve damage, and finally AD. The gut and brain are connected bidirectionally by multiple pathways, including neural, immune, metabolic and endocrine pathways[43].Bacteria can produce a variety of neurotransmitters or similar substances. Some strains of gut bacteria have the ability to produce and release neurotransmitters, such as GABA, serotonin, catecholamine and histamine. Neurotransmitters produced by these bacteria transmit signals to the central nervous system by intestinal chromaffin cells and intestinal nerve receptors . In the animal studies of Gao Yong et al.,They found that GABA derived from gut bacteria crosses the BBB and enters the central nervous system. It was found that Lactobacillus rhamnosus could reduce anxiety and depression-related behaviors in mice and increase the concentration of GABA in the hippocampus[44]. And this effect only occurs when the vagus nerve is intact, so it is believed that intestinal microbes may indirectly regulate GABA signal via the vagus nerve . Gut microbes affect the formation, absorption and transport of serotonin and GABA in the brain. more over, some bacterial species influence amyloid plaque formation and trigger an inflammatory cascade that leads to the development of AD[45]. Therefore we will open up new therapeutic pathway for the treatment of AD depend on reshaping the intestinal microbiota and the anti-AD action focused on the microbiota guide the development of effective therapeutic methods in the future by exploring the intestinal flora associated with H. pylori.

Evidence[46]suggests that miRNAs can be transmitted between cells, even over long distances, which showing that these small RNAs can deliver physiological states and change the function of cells throughout the body, it is obviously that they can control various aspects of AD consider that mirnas have important intra- and intercellular roles.A research shows that low level of miR-650 was a risk factor for developing AD and was particularly pronounced in severe dementia and correlated with cognitive functions[47].miR-326 as a proinflammatory factor has been implicated in MS pathology. miR-326 expression in leukocytes correlated with disease severity in MS patients and in mice with EAE[48].In contrast, Other research demonstrate that Mir-326 improves cognitive function of AD mice and inhibits neuron apoptosis in AD mice through inactivation of the JNK signaling pathway by targeting VAV1[49].Aidan Kenny et al[50]suggests that Mirna-206 in the peripheral plasma may be elevated in the prodromal and presymptomatic stages of AD and it can be used as an economical and effective biomarker.lastly,It is wellknown that miRNAs critically contribute to immune function and

homeostasis[51-53]. A study of T1D[54]identified the mir142-3p /Tet2/Foxp3 axis in mouse and human CD4+ T cells, which interferes with effective induction of tregs and leads to them during islet autoimmunity Treg stability is impaired, allowing islet autoimmunity to activate and progress,and suggest that targeting miR142-3p could contribute to the development of intervention strategies.All of these results indicate that these small molecules have great clinical potential. Thus, revealing therapy-related immunomodulatory miRNAs may lead to new ones therapies that inhibit neuroinflammation and improve AD outcomes.

Conclusions

In conclusion, we defined the core function of key candidate genes, including GABA、GABRA1、GABRG2、SLC32A1,mirNA-650、mirNA-206、mirNA-142-3p and mirNA326, by using a sequence of bioinformatics tools for gene expression profling. In addition,the enriched signaling cascades constituting the GABAergic signal transduction system pathways in the molecular modulation network of cognitive decline via integrated bioinformatic analysis. Trough the above Analysis , we found that there may be a signifcant correlation between H. pylori infection and AD. This provides a series of possible therapeutic targets for AD in patients with H. pylori infection in the future. However, in vitro and in vivo studies should be conducted to verify our findings.

Abbreviations

AD
Alzheimer’s disease
H. pylori
Helicobacter pylori
DEGs
Differentially expressed genes
PPI
Protein–protein interaction
GEO
Gene Expression Omnibus
BBB
Blood-brain barrier
IL
Interleukin
TNF
Tumor necrosis factor
IFN
Interferon
VIP

Vasoactive intestinal peptide
MGB
Microbiome - gut-brain

Declarations

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Not applicable.

Authors' contributions

Meijun Wu: performed data collection, developed the web tool, and wrote and edited the manuscript;Bin Chen: performed data collection and developed the web tool;Yue Gao: performed data analysis and study design.All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., et al., 2017. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell, 169(7):1276–1290 e17.
2. Kukull, W.A. and Bowen, J.D., 2002. Dementia epidemiology. Medical Clinics of North America, 86(3):573–590.
3. Yiannopoulou, K.G. and Papageorgiou, S.G., 2013. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord, 6(1):19–33.
4. Michaud, J.P., Halle, M., Lampron, A., Theriault, P., Prefontaine, P., Filali, M., et al., 2013. Toll-like receptor 4 stimulation with the detoxified ligand monophosphoryl lipid A improves Alzheimer's disease-related pathology. Proc Natl Acad Sci U S A, 110(5):1941–6.
5. Abadi, A.T.B., 2017. Strategies used by helicobacter pylori to establish persistent infection. World J Gastroenterol, 23(16):2870–2882.
6. Robinson, K., Kaneko, K. and Andersen, L.P., 2017. Helicobacter: Inflammation, immunology and vaccines. Helicobacter, 22 Suppl 1(https://doi.org/ 10.1111/hel.12406
7. Doulberis, M., Kotronis, G., Thomann, R., Polyzos, S.A., Boziki, M., Gialamprinou, D., et al., 2018. Review: Impact of Helicobacter pylori on Alzheimer's disease: What do we know so far? Helicobacter, 23(1).
8. Kountouras, J., Boziki, M., Gavalas, E., Zavos, C., Deretzi, G., Grigoriadis, N., et al., 2009. Increased cerebrospinal fluid Helicobacter pylori antibody in Alzheimer's disease. Int J Neurosci, 119(6):765–77.
9. Shen, L., Liu, L. and Ji, H.F., 2017. Alzheimer's Disease Histological and Behavioral Manifestations in Transgenic Mice Correlate with Specific Gut Microbiome State. J Alzheimers Dis, 56(1):385–390.
10. Wang, X.L., Zeng, J., Yang, Y., Xiong, Y., Zhang, Z.H., Qiu, M., et al., 2015. Helicobacter pylori filtrate induces Alzheimer-like tau hyperphosphorylation by activating glycogen synthase kinase–3beta. J Alzheimers Dis, 43(1):153–65.
11. Fani, L., Wolters, F.J., Ikram, M.K., Bruno, M.J., Hofman, A., Koudstaal, P.J., et al., 2018. Helicobacter pylori and the risk of dementia: A population-based study. Alzheimers Dement, 14(10):1377–1382.
12. Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., et al., 2013. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res, 41(Database issue):D991–5.
13. Sticht, C., De La Torre, C., Parveen, A. and Gretz, N., 2018. miRWalk: An online resource for prediction of microRNA binding sites. PLoS One, 13(10):e0206239.
14. Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res, 47(D1):D607-D613.
15. Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., et al., 2013. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids

Res, 41(Database issue):D808–15.

16. Wang, J., Vasaikar, S., Shi, Z., Greer, M. and Zhang, B., 2017. WebGestalt 2017: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. Nucleic Acids Res, 45(W1):W130-W137.
17. Chapouthier, G. and Venault, P., 2002. GABA-A receptor complex and memory processes. Curr Top Med Chem, 2(8):841–51.
18. Heaney, C.F. and Kinney, J.W., 2016. Role of GABA(B) receptors in learning and memory and neurological disorders. Neurosci Biobehav Rev, 63(1–28.
19. Nuss, P., 2015. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. Neuropsychiatr Dis Treat, 11(165–75.
20. Makkar, S.R., Zhang, S.Q. and Cranney, J., 2010. Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology, 35(8):1625–52.
21. Egerton, A., Modinos, G., Ferrera, D. and McGuire, P., 2017. Neuroimaging studies of GABA in schizophrenia: a systematic review with meta-analysis. Transl Psychiatry, 7(6):e1147.
22. Engin, E., Benham, R.S. and Rudolph, U., 2018. An Emerging Circuit Pharmacology of GABAA Receptors. Trends Pharmacol Sci, 39(8):710–732.
23. Krantic, S., Isorce, N., Mechawar, N., Davoli, M.A., Vignault, E., Albuquerque, M., et al., 2012. Hippocampal GABAergic neurons are susceptible to amyloid-beta toxicity in vitro and are decreased in number in the Alzheimer's disease TgCRND8 mouse model. J Alzheimers Dis, 29(2):293–308.
24. Letouzey, V., Ulrich, D., Balenbois, E., Cornille, A., de Tayrac, R. and Fatton, B., 2015. Utero-vaginal suspension using bilateral vaginal anterior sacrospinous fixation with mesh: intermediate results of a cohort study. Int Urogynecol J, 26(12):1803–7.
25. Levenga, J., Krishnamurthy, P., Rajamohamedsait, H., Wong, H., Franke, T.F., Cain, P., et al., 2013. Tau pathology induces loss of GABAergic interneurons leading to altered synaptic plasticity and behavioral impairments. Acta Neuropathol Commun, 1(34.
26. Najm, R., Jones, E.A. and Huang, Y., 2019. Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. Mol Neurodegener, 14(1):24.
27. Cumbo, E. and Ligori, L.D., 2010. Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. Epilepsy Behav, 17(4):461–6.
28. Bakker, A., Krauss, G.L., Albert, M.S., Speck, C.L., Jones, L.R., Stark, C.E., et al., 2012. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron, 74(3):467–74.
29. Li, J., Chen, L., Guo, F. and Han, X., 2020. The Effects of GABAergic System under Cerebral Ischemia: Spotlight on Cognitive Function. Neural Plast, 2020(8856722.
30. Sakimoto, Y., Oo, P.M., Goshima, M., Kanehisa, I., Tsukada, Y. and Mitsushima, D., 2021. Significance of GABAA Receptor for Cognitive Function and Hippocampal Pathology. Int J Mol Sci, 22(22).

31. Kwakowsky, A., Calvo-Flores Guzman, B., Pandya, M., Turner, C., Waldvogel, H.J. and Faull, R.L., 2018. GABAA receptor subunit expression changes in the human Alzheimer's disease hippocampus, subiculum, entorhinal cortex and superior temporal gyrus. J Neurochem, 145(5):374–392.
32. Glykys, J. and Mody, I., 2006. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. J Neurophysiol, 95(5):2796–807.
33. Prevot, T.D., Li, G., Vidojevic, A., Misquitta, K.A., Fee, C., Santrac, A., et al., 2019. Novel Benzodiazepine-Like Ligands with Various Anxiolytic, Antidepressant, or Pro-Cognitive Profiles. Mol Neuropsychiatry, 5(2):84–97.
34. DiFrancesco, J.C., Tremolizzo, L., Polonia, V., Giussani, G., Bianchi, E., Franchi, C., et al., 2017. Adult-Onset Epilepsy in Presymptomatic Alzheimer's Disease: A Retrospective Study. J Alzheimers Dis, 60(4):1267–1274.
35. Mejias-Luque, R. and Gerhard, M., 2017. Immune Evasion Strategies and Persistence of Helicobacter pylori. Curr Top Microbiol Immunol, 400(53–71.
36. Alvarez-Arellano, L. and Maldonado-Bernal, C., 2014. Helicobacter pylori and neurological diseases: Married by the laws of inflammation. World J Gastrointest Pathophysiol, 5(4):400–4.
37. Budzynski, J. and Klopocka, M., 2014. Brain-gut axis in the pathogenesis of Helicobacter pylori infection. World J Gastroenterol, 20(18):5212–25.
38. Meng, W.P., Wang, Z.Q., Deng, J.Q., Liu, Y., Deng, M.M. and Lu, M.H., 2016. The Role of H. pylori CagA in Regulating Hormones of Functional Dyspepsia Patients. Gastroenterol Res Pract, 2016(7150959.
39. Engstrand, L. and Lindberg, M., 2013. Helicobacter pylori and the gastric microbiota. Best Pract Res Clin Gastroenterol, 27(1):39–45.
40. Noto, J.M. and Peek, R.M., Jr., 2017. The gastric microbiome, its interaction with Helicobacter pylori, and its potential role in the progression to stomach cancer. PLoS Pathog, 13(10):e1006573.
41. Cremer, J., Arnoldini, M. and Hwa, T., 2017. Effect of water flow and chemical environment on microbiota growth and composition in the human colon. Proc Natl Acad Sci U S A, 114(25):6438–6443.
42. Megur, A., Baltriukiene, D., Bukelskiene, V. and Burokas, A., 2020. The Microbiota-Gut-Brain Axis and Alzheimer's Disease: Neuroinflammation Is to Blame? Nutrients, 13(1).
43. Novotny, M., Klimova, B. and Valis, M., 2019. Microbiome and Cognitive Impairment: Can Any Diets Influence Learning Processes in a Positive Way? Front Aging Neurosci, 11(170.
44. Janik, R., Thomason, L.A.M., Stanisz, A.M., Forsythe, P., Bienenstock, J. and Stanisz, G.J., 2016. Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. Neuroimage, 125(988–995.
45. de, J.R.D.-P.V., Forlenza, A.S. and Forlenza, O.V., 2018. Relevance of gutmicrobiota in cognition, behaviour and Alzheimer's disease. Pharmacol Res, 136(29–34.
46. Gaudet, A.D., Fonken, L.K., Watkins, L.R., Nelson, R.J. and Popovich, P.G., 2018. MicroRNAs: Roles in Regulating Neuroinflammation. Neuroscientist, 24(3):221–245.

47. Prendecki, M., Florczak-Wyspianska, J., Kowalska, M., Ilkowski, J., Grzelak, T., Bialas, K., et al., 2019. APOE genetic variants and apoE, miR–107 and miR–650 levels in Alzheimer's disease. Folia Neuropathol, 57(2):106–116.
48. Honardoost, M.A., Kiani-Esfahani, A., Ghaedi, K., Etemadifar, M. and Salehi, M., 2014. miR–326 and miR–26a, two potential markers for diagnosis of relapse and remission phases in patient with relapsing-remitting multiple sclerosis. Gene, 544(2):128–33.
49. He, B., Chen, W., Zeng, J., Tong, W. and Zheng, P., 2020. MicroRNA–326 decreases tau phosphorylation and neuron apoptosis through inhibition of the JNK signaling pathway by targeting VAV1 in Alzheimer's disease. J Cell Physiol, 235(1):480–493.
50. Kenny, A., McArdle, H., Calero, M., Rabano, A., Madden, S.F., Adamson, K., et al., 2019. Elevated Plasma microRNA–206 Levels Predict Cognitive Decline and Progression to Dementia from Mild Cognitive Impairment. Biomolecules, 9(11).
51. Serr, I., Furst, R.W., Ott, V.B., Scherm, M.G., Nikolaev, A., Gokmen, F., et al., 2016. miRNA92a targets KLF2 and the phosphatase PTEN signaling to promote human T follicular helper precursors in T1D islet autoimmunity. Proc Natl Acad Sci U S A, 113(43):E6659-E6668.
52. Serr, I., Scherm, M.G., Zahm, A.M., Schug, J., Flynn, V.K., Hippich, M., et al., 2018. A miRNA181a/NFAT5 axis links impaired T cell tolerance induction with autoimmune type 1 diabetes. Sci Transl Med, 10(422).
53. Snowhite, I.V., Allende, G., Sosenko, J., Pastori, R.L., Messinger Cayetano, S. and Pugliese, A., 2017. Association of serum microRNAs with islet autoimmunity, disease progression and metabolic impairment in relatives at risk of type 1 diabetes. Diabetologia, 60(8):1409–1422.
54. Scherm, M.G., Serr, I., Zahm, A.M., Schug, J., Bellusci, S., Manfredini, R., et al., 2019. miRNA142–3p targets Tet2 and impairs Treg differentiation and stability in models of type 1 diabetes. Nat Commun, 10(1):5697.

Figures

Figure 1

a(AD,volcano map)

b(Twelve DEGs were identifed by analysis of AD gene expression datasets.Each column represents a sample and each row represents the expression level of a given gene. The color scale represents the raw Z score ranging from blue (low expression) to red (high expression). Dendrograms by heatmap correspond to the hierarchical clustering by expression of the 12 genes.)

c(H. pylori infection,volcano map)

d(Twelve DEGs were identifed by analysis of H. pylori-positive DEGS gene expression datasets)

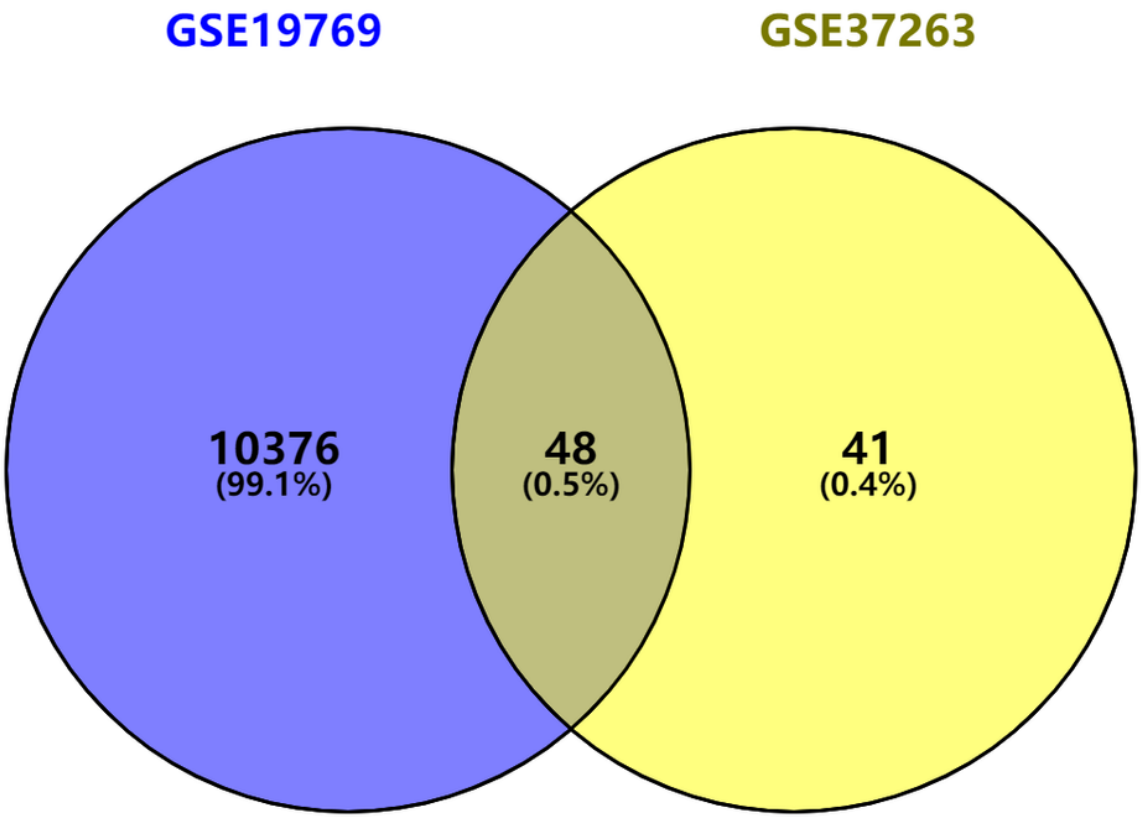

Figure 2

VENN diagram

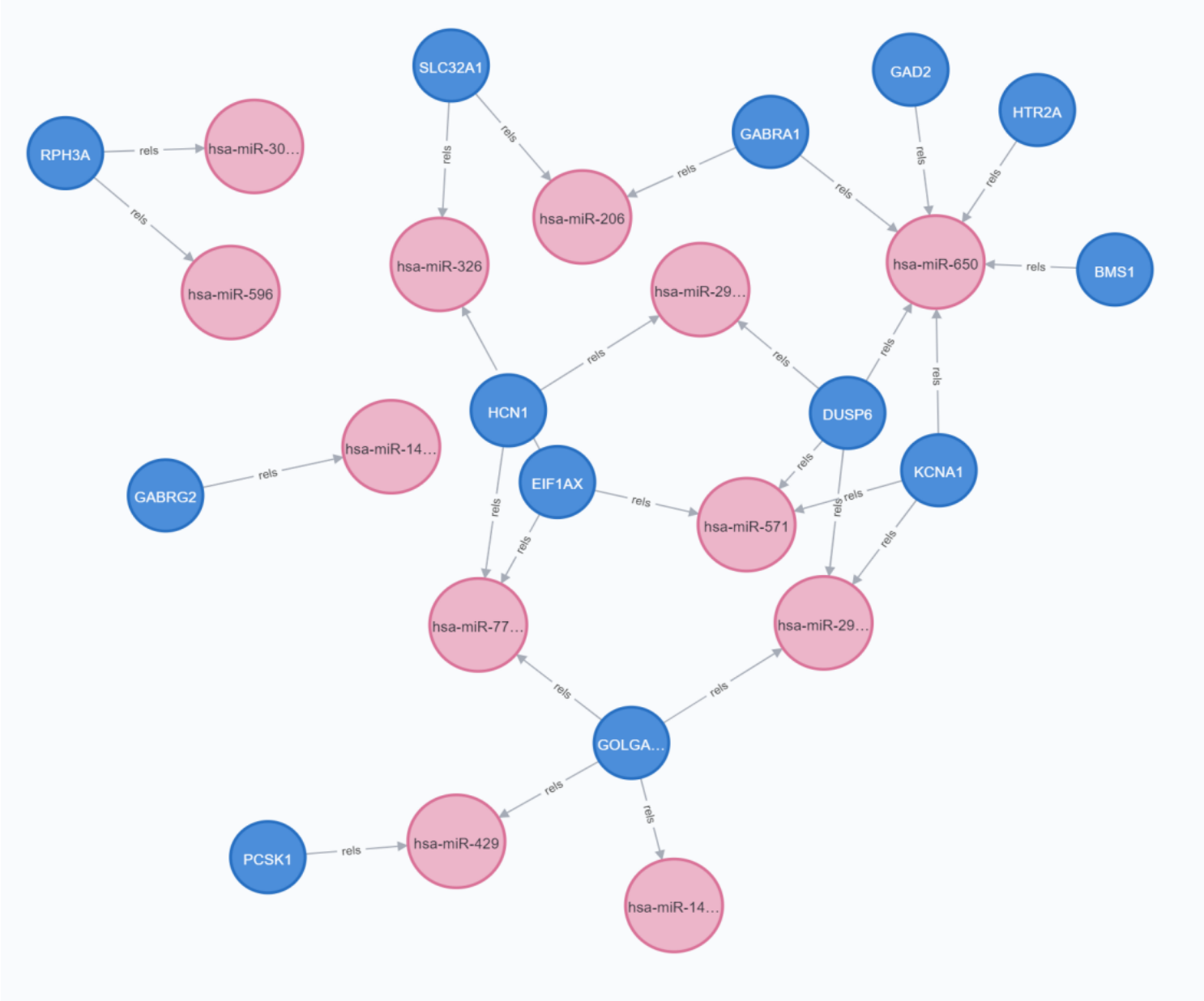

Figure 3

An interaction network constructed by H pylori-positive differential miRNAs and 13 key genes in AD regulatory network

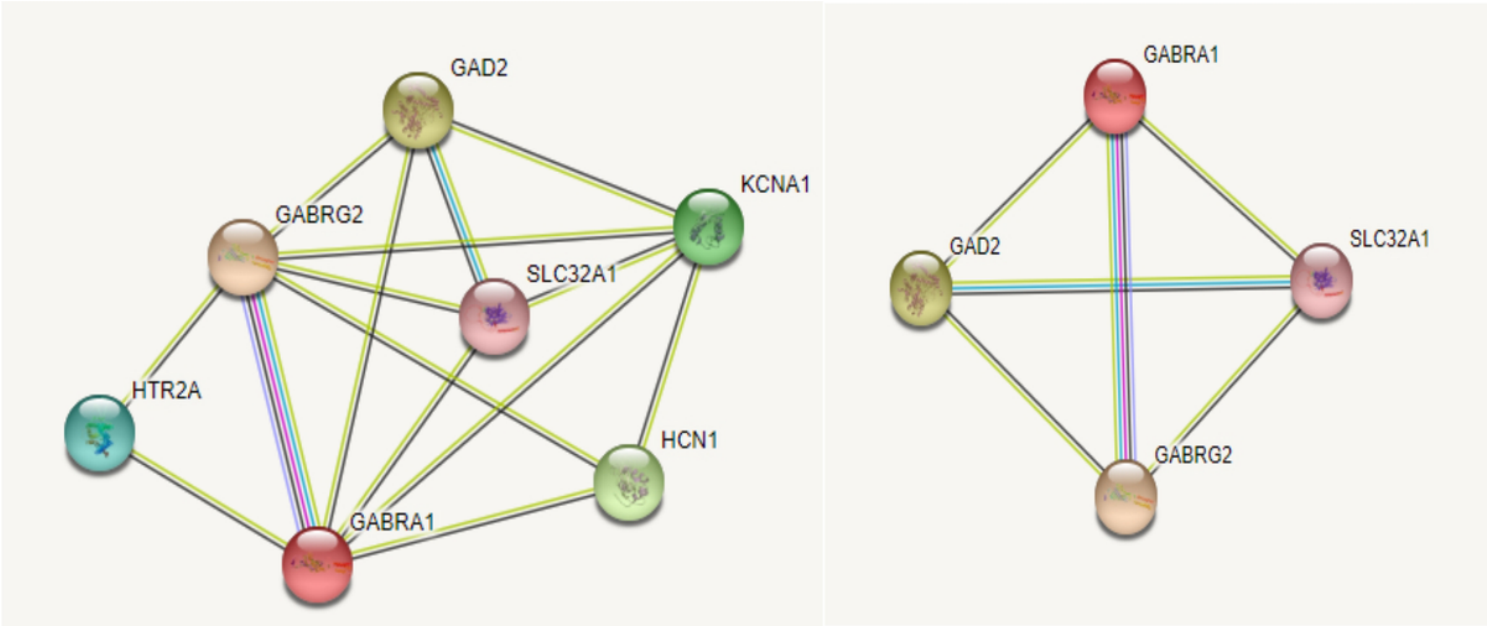

Figure 4

The protein–protein interaction (PPI) networks construction and signifcant gene modules analysis. a Based on the STRING online database, 7 common genes were fltered into common genes PPI network. b The most signifcant module from the PPI network

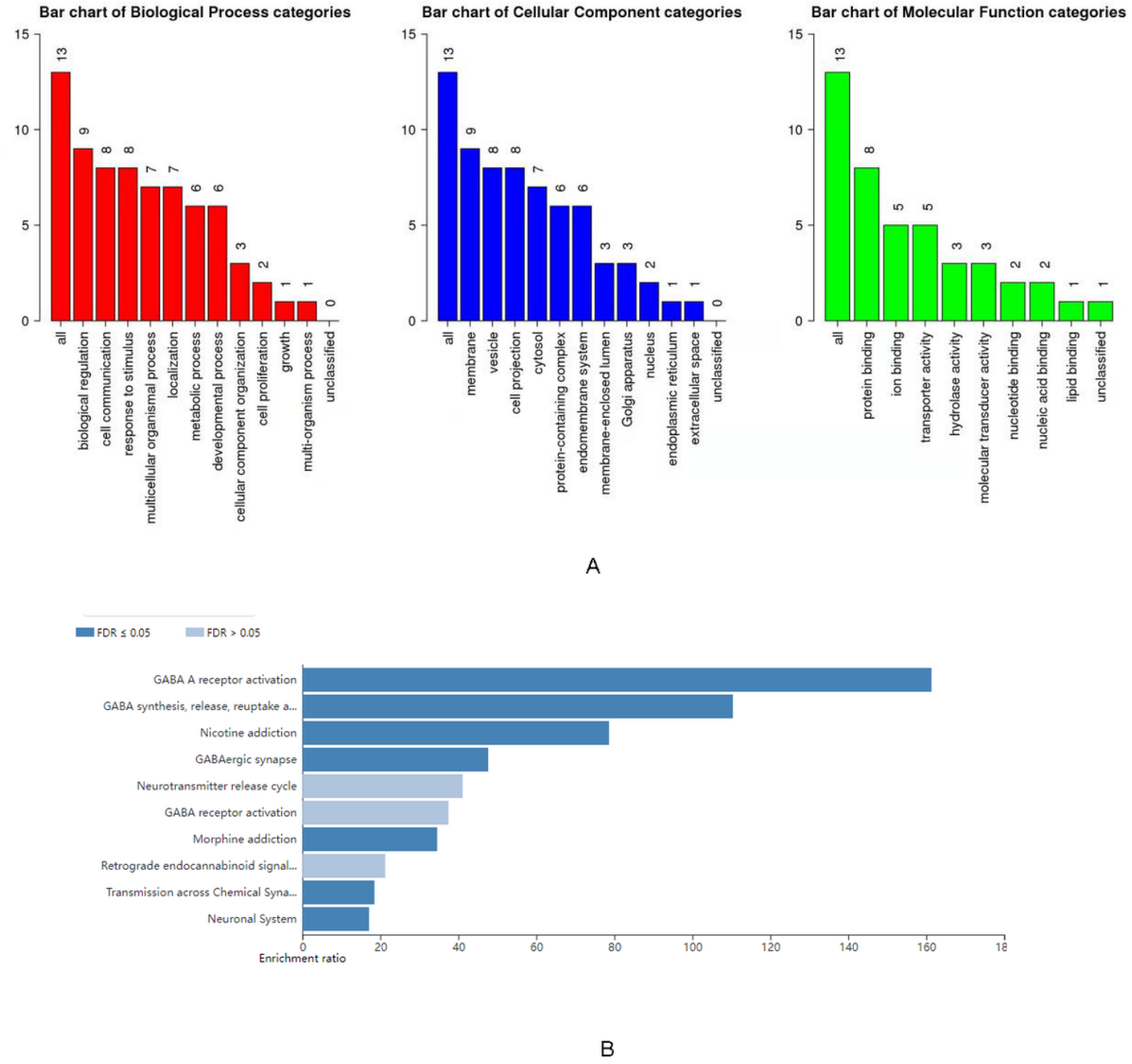

Figure 5

a(GO analysis results of 13 key genes in AD regulatory network)

b(KEGG analysis results of 13 key genes in lung cancer regulatory network)