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Integrated Analysis of Alzheimer's Disease and Schizophrenia Dataset Revealed Different Expression Pattern in Learning and Memory

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Abstract. Alzheimer's disease (AD) and schizophrenia (SZ) are both accompanied by impaired learning and memory functions. This study aims to explore the expression profiles of learning or memory genes between AD and SZ. We downloaded 10 AD and 10 SZ datasets from GEO-NCBI for integrated analysis. These datasets were processed using RMA algorithm and a global renormalization for all studies. Then Empirical Bayes algorithm was used to find the differentially expressed genes between patients and controls. The results showed that most of the differentially expressed genes were related to AD whereas the gene expression profile was little affected in the SZ. Furthermore, in the aspects of the number of differentially expressed genes, the fold change and the brain region, there was a great difference in the expression of learning or memory related genes between AD and SZ. In AD, the CALB1, GABRA5, and TAC1 were significantly downregulated in whole brain, frontal lobe, temporal lobe, and hippocampus. However, in SZ, only two genes CRHBP and CX3CR1 were downregulated in hippocampus, and other brain regions were not affected. The effect of these genes on learning or memory impairment has been widely studied. It was suggested that these genes may play a crucial role in AD or SZ pathogenesis. The different gene expression patterns between AD and SZ on learning and memory functions in different brain regions revealed in our study may help to understand the different mechanism between two diseases.

Keywords: Alzheimer's disease, gene expression, learning or memory, schizophrenia

INTRODUCTION

Alzheimer's disease (AD) is a progressive and ultimately fatal neurodegenerative disease and mostly affects the elderly population. AD is characterized by progressive memory loss and disordered cognitive function, altered behavior including paranoia, delusions, withdrawal from work or social activities, and

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a degradation of language function [1, 2]. Previous studies showed the widespread loss of neurons and their synapses in the cerebral cortex, entorhinal area, hippocampus, ventral striatum, and basal forebrain in the autopsy brain [3]. AD is now known to disrupt normal thinking and memory by blocking the transmission of complex messages between brain cells [4], although the exact cause of the disorder remains the subject of ongoing debate and investigation. The vast majority of AD cases are late onset (LOAD), and the amyloid-β (Aβ), microtubule-associated protein tau (MAPT), lipid transport proteins apolipoprotein E (APOE), and presynaptic protein alpha-synuclein have been irrefutably recognized as the major risk factors for LOAD [5, 6]. In addition, new researches showed many potential genetic and epigenetic factors may contribute to the process of AD [7–9].

Schizophrenia (SZ) is a common and severe, often disabling psychiatric illness of unknown etiology that is characterized by extreme disturbances of cognition and thought, affecting language, perception, and sense of self [10]. SZ is suggested to be a disorder of developmental maturation rather than one of neurodegeneration. A combination of genetic and environmental changes during the prenatal or perinatal period of life may result in structural changes that primarily affect the medial temporal lobe [11]. This brain region is crucial for the processing and integration of information received from the association cortex, and its dysfunction appears to contribute to the clinical symptoms that typically emerge during late adolescence or early adulthood [11]. Additionally, memory impairment is one of the more severe and well-documented cognitive deficits associated with SZ and numerous genetic studies have led to the identification of a large number of genes that may contribute to SZ [12–14].

Furthermore, a large number of animal experiments and clinical trials have attempted to develop new drugs that target the main biomarkers of AD and SZ. The role of serotonin (5-hydroxytryptamine, 5-HT) receptors and histamine receptors in cognitive functions are widely studied. 5-HT is a major neurotransmitter and is known to interact with multiple receptors in both the central and peripheral nervous system [15]. Serotonin 5-HT_4 and 5-HT_6 receptors are both belong to the G-Protein-Coupled Receptors family (GPCR) [16]. It is widely recognized that ligands of 5-HT_6 receptor can be used as potential therapeutic drugs for cognitive dysfunction (including AD), obesity, and SZ. Many clinical studies have confirmed that the serotonergic 5-HT_6 antagonists as important agents for the palliative treatment for AD and correcting the memory deficits for SZ [17, 18]. Histamine receptors (H1R, H2R, H3R, and H4R) belong to the rhodopsin-like family of G protein-coupled receptors (GPCRs). H3R as a presynaptic autoreceptor in the brain, inhibits the release of histamine and modulates the release of other neurotransmitters [15, 19]. Researchers showed that H3 antagonists enhance dopamine release, counteract the effects of dopamine on D2 receptor containing neurons, and potentiate the effects of dopamine on D1 receptors containing neurons [20]. H3 antagonists/inverse agonists have been reported to improve cognitive function, spatial orientation, attention, memory, and learning in a variety of *in vivo* models [15]. Although the treatment effect of H3 antagonists/inverse agonists on SZ remains controversial in animal models, more studies support the view of these drugs can promote cognitive function [20].

These reports indicate that the pathogenesis of AD or SZ may be both related to impairment of learning and memory functions. Based on their different pathological mechanisms, we hypothesized that the gene expression profiles (especially learning or memory-related genes) between AD and SZ have a vast difference. The aim of this study was to investigate the difference of whole gene expression profiles between AD and SZ, and we mainly focus on the expression difference of learning or memory related genes between these two diseases.

MATERIALS AND METHODS

Microarray data collection

AD and SZ datasets were downloaded from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo) in May 2015. The data selection criteria were: (1) the series type in GEO was expression profiling by array; (2) the organism was *Homo sapiens* (human); (3) the samples in each study should including cases (AD patients or SZ patients) and controls; (4) the brain tissue must be contained; and (5) the dataset must provide the original data. We used "Alzheimer" and "schizophrenia" as keywords to search the datasets both from GEO and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). The filters we selected in GEO were "Expression profiling by array" and "Homo sapiens", and in ArrayExpress were "Human" and "Array assay". Next, according to the data selection criteria, we remove some search results that did not include case and controls, brain tissue, or

the original data. Finally, we selected 10 datasets of AD including 633 samples (285 AD patients and 348 controls), and 10 datasets of SZ including 478 samples (243 SZ patients and 235 controls). No dataset include mixed samples of AD and/or SZ patients and also did not include the sample of Down's syndrome. The sources of the datasets that we selected in ArrayExpress were all from GEO, therefore, we have downloaded all datasets from GEO. For the details of datasets, see Supplementary Tables 1 and 2.

Data preprocessing and integration

The R programming language v3.2.0 normalized the raw expression data using quartile normalization methods and the Robust Multichip Average (RMA) algorithm to generate normalized gene expression intensity. Gene annotation and integration of different datasets was carried out using custom written Python code. Our datasets contained seven different microarray platforms and these platforms use their own probe IDs. To perform integration, we choose to match probe IDs from different platforms to the unique official gene symbol. When multiple probes (or probe sets) matched an official gene symbol, we took the average expression value across multiple probe IDs to represent the expression of the corresponded gene symbol. Next, we include all genes appearing in each study. Because the number of genes in different platforms was different and this may have some empty value,s we used "NA" to represent these empty values and excluded these values in the subsequent analysis.

Gene expression value renormalization

Because the distribution of gene expression values in different datasets had a large deviation, we carried out a global renormalization of all gene expression values in each dataset before performed differential expression analysis. The formula for renormalization was: $u_{ijk} = v_{ijk} * t_{ij},\ t_{ij} = \bar{x}_j/\bar{x}_{ij}$. Where u_{ijk} means the renormalized gene expression value for gene j in sample k of study i, and v_{ijk} represents the RMA processed gene expression value. Where $\bar{x}_j$ is the mean expression value for gene j of control groups in all studies, and $\bar{x}_i\, j$ is the mean expression value for gene j of control groups in study i. The distribution of RMA processed and renormalized gene expression values across all studies are shown in Supplementary Figures 1–4.

Bioinformatics analysis

The R v3.2.0 and Bioconductor Library were used to analyze the microarray data. The comparison of gene expression values between cases and controls was performed by empirical Bayes algorithm (the function "eBayes" in R) with false discovery rate (FDR) for p values adjustment, and using "topTable" function to show the analysis results. Statistically significant genes in differential expression (up- or downregulation) were considered as a FDR adjusted p value of 0.05 or lower and a $\geq$2-fold change between cases and controls. Next, we conducted gene enrichment analysis of these above differentially expressed genes in "GO Processes" of MetaCore^{TM} (https://portal.genego.com). The original input file was the gene symbols list and a FDR adjusted p value ≤ 0.05 was considered to be significant enrichment. In addition, the learning or memory related genes (GO term: 0007611) were downloaded from MetaCore^{TM}. We mapped the genes that related learning or memory to the integrated dataset and differentially expressed genes with written R code, and used "plot" and other functions in R to show these results.

RESULTS

Overview of differentially expressed genes

Microarray datasets in this study contained 12 brain partitions of AD patients (entorhinal cortex, frontal cortex, hippocampus, hippocampal gray matter, medial temporal gyrus, neocortex, parietal lobe, postcentral gyrus, posterior cingulated, primary visual cortex, superior frontal gyrus, and temporal cortex) and 5 brain partitions of SZ patients (hippocampus, parietal cortex, prefrontal cortex, superior temporal cortex, superior temporal gyrus) (Supplementary Tables 1 and 2). In order to compare the gene expression profile between patients with AD or SZ, we conducted a classification of these microarray data. The data was divided into four sub-datasets with different brain regions that including frontal lobe, parietal lobe, temporal lobe, and hippocampus (the temporal lobe area in this study did not include hippocampus, Supplementary Table 3). Each subset contained at least one dataset of AD and SZ (Supplementary Table 3). The overview of differentially expressed genes in AD patients and SZ patients is shown in Table 1. Because our datasets were screened from different platforms, this caused the different

Table 1
Differentially expressed genes profile in Alzheimer's disease and schizophrenia

Brain Partition	Cases/ Controls	Mapped Genes	Upregulated	Downregulated
Alzheimer's Disease				
Brain	285/348	22663	6	19
Frontal Lobe	65/85	22623	24	3
Parietal Lobe	29/47	20331	0	0
Temporal Lobe	26/31	22599	357	260
Hippocampus	80/83	20331	1	11
Schizophrenia				
Brain	243/235	23038	0	0
Frontal Lobe	137/131	20366	0	0
Parietal Lobe	51/50	9910	0	0
Temporal Lobe	40/36	21653	0	0
Hippocampus	15/18	20307	5	6

number of genes in each dataset of AD and SZ. In AD, we mapped 22,663 genes in whole brain and found 6 upregulated and 19 downregulated genes. However, the gene expression profiles in parietal lobe seemed to not be affected. It is worth noting that we found 617 differentially expressed genes in the temporal lobe. In SZ, we mapped 23,038 genes in whole brain and found only 11 affected genes in the hippocampus, while no differentially expressed gene was observed in the whole brain or other brain partitions.

Biological process enrichment analysis of differentially expressed genes

We conducted biological process enrichment analysis of the differentially expressed genes in AD and SZ, respectively. The results showed that the biology processes, such as multicellular organismal response to stress (including: fear response, general adaptation syndrome, and response to pain) (GO term: 0033555), calcium ion homeostasis (GO term: 0055074), regulation of synaptic transmission, GABAergic (GO term: 0032228), and tachykinin receptor signaling pathway (GO term: 0007217) were significantly enriched in the whole brain of AD (adjusted p values were: 9.262E-07, 2.883E-05, 6.804E-07, and 5.115E-04, respectively). Furthermore, for AD, the process of learning or memory (GO term: 0007611) was significantly enriched in the whole brain (adjusted $p = 6.049E-06$), temporal lobe (adjusted $p = 9.410E-09$), and hippocampus (adjusted $p = 4.975E-02$). In addition, this process was also significantly enriched in hippocampus (adjusted $p = 8.204E-03$) of SZ. However, the differentially expressed genes in the frontal lobe in AD seemed to not be related to learning or memory.

Different gene expression patterns between AD and SZ

We calculated the fold-change between cases and controls of all genes in AD and SZ (Fig. 1). The total number of combination genes of AD and SZ was 24,093. Figure 1A shows the logarithmic transformed fold-change of all genes. The red and blue points are expressed as log2(FC) of genes in AD and SZ, respectively. The result showed that the gene expression

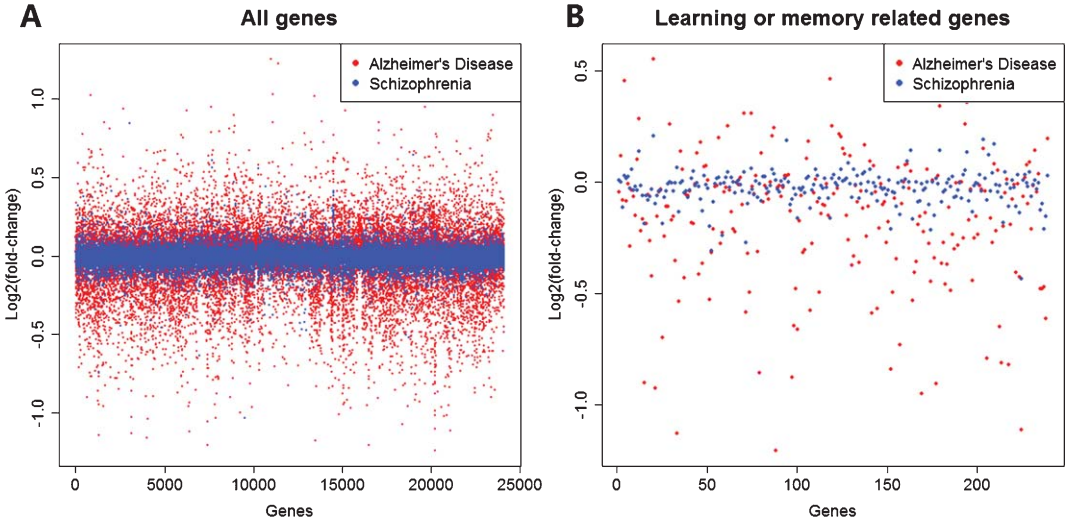

Fig. 1. Distribution of the logarithmic transformed fold-change of the gene expression values. Panel A shows the log2(FC) of all genes in Alzheimer's disease and schizophrenia. Panel B shows the log2(FC) of learning or memory-related genes in Alzheimer's disease and schizophrenia.

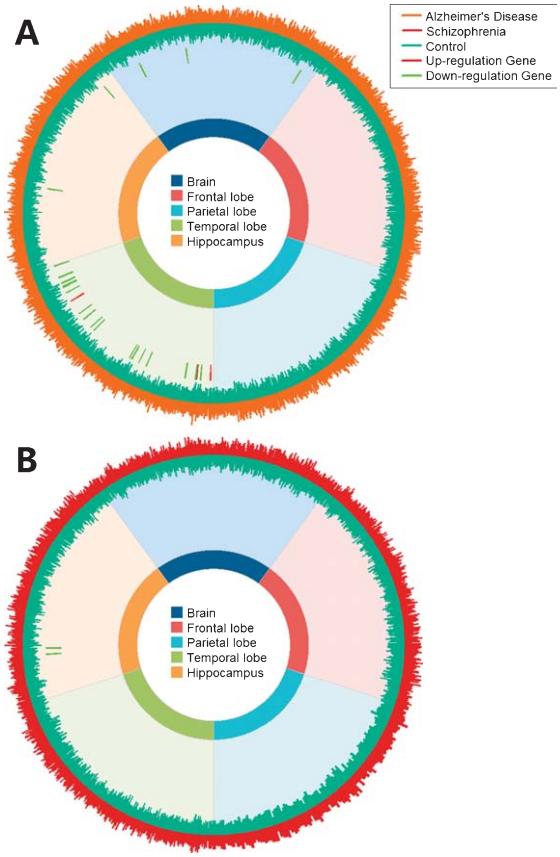

Fig. 2. Expression profiles of learning or memory related genes in different brain partitions. Panel A shows the gene expression profiles in Alzheimer's disease. Panel B shows the gene expression profiles in schizophrenia.

profile was little affected in SZ whereas numerous genes were up- or downregulated in AD. In addition, we obtained 248 genes related to learning or memory on MetaCore^{TM} (GO term: 0007611) and 239 genes were mapped to our datasets. Figure 1B shows the log2(FC) of learning or memory related genes in AD and SZ. It also shows a trend that the expression profiles of learning or memory genes were more affected in AD than SZ.

Expression profiles of learning or memory related genes in different brain regions

The details of learning or memory related gene expression profiles in different brain partitions of AD and SZ are shown in Fig. 2. The length of the first layer lines outside the circle represents the expression value in AD and SZ, respectively. The length of the second layer lines within the circle represents the expression value in controls. Furthermore, the up- and down-regulated genes are marked as red and green lines in the third layer. For AD, the CALB1, GABRA5, and TAC1 were downregulated in the total brain, and the GABRA5 and TAC1 were downregulated in hippocampus (Fig. 3). Additionally, there were 17 downregulation genes and 3 upregulation genes in temporal lobe. However, for SZ, we found no differentially expressed gene in brain, frontal lobe, parietal lobe, or temporal lobe. Only CRHBP and CX3CR1 were downregulated in hippocampus. The total 22 differentially expressed genes that relate to learning and memory functions in AD or SZ are shown in Supplementary Figure 5.

Because there was a great difference in the age distribution between AD and SZ datasets (Supplementary Figures 6 and 7), we have reanalyzed these datasets grouped by age. We defined those individuals aged $\geq$60 years as the elderly population, and those aged <60 years as the non-elderly population. The age-stratified gene expression profiles of learning and memory functions in different brain partitions of AD and SZ are shown in Supplementary Figures 8–10. There were only 7 non-elderly AD patients in our datasets; therefore, we did not analyze the

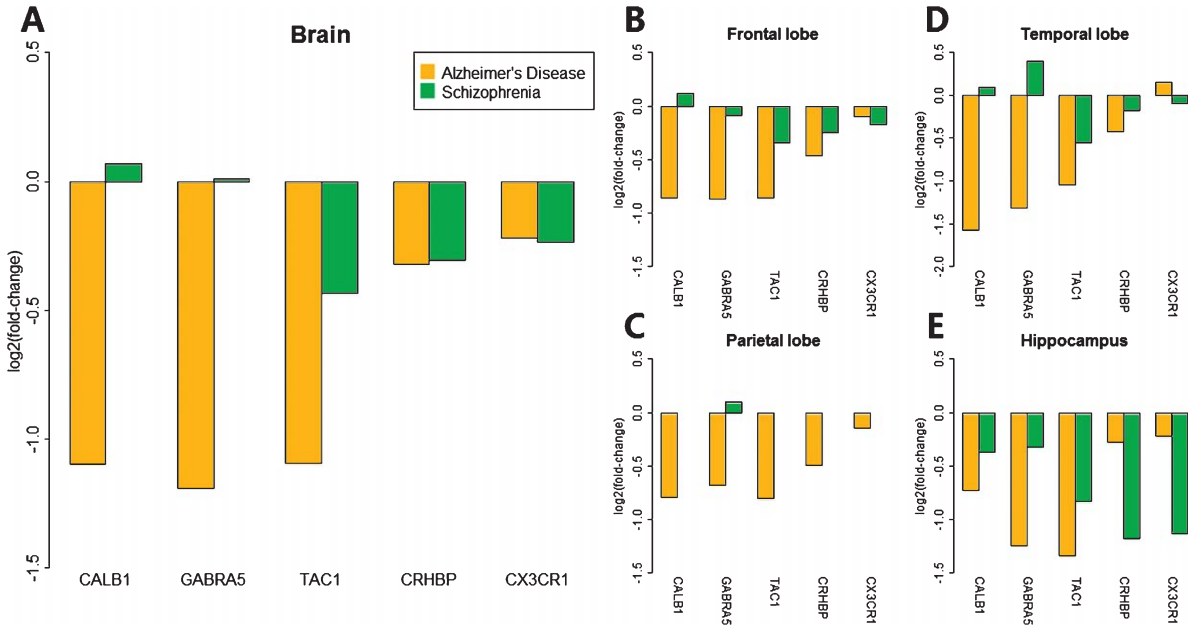

Fig. 3. Comparison of logarithmic transformed fold-change of CALB1, GABRA5, TAC1, CRHBP, and CX3CR1 between Alzheimer's disease and schizophrenia. Panels A-E shows the log2(FC) of the five genes in the whole brain, frontal lobe, parietal lobe, temporal lobe, and hippocampus, respectively.

gene expression profiles in non-elderly AD patients. For elderly AD patients, GABRA5 and TAC1 were downregulated in the total brain. Furthermore, the hippocampus and temporal lobe also presented with affected learning and memory functions (Supplementary Figure 8). However, in elderly SZ patients we did not find any affected gene in whole brain or other brain partitions (Supplementary Figure 9). In addition, the same as the results in all SZ datasets, we found CRHBP and CX3CR1 were downregulated in hippocampus in non-elderly SZ patients (Supplementary Figure 10). The age-stratified analysis results provide strong evidence that AD and SZ patients showed a greater difference of the impairment of learning and memory functions.

Fold change of the main differential expressed genes in AD and SZ

We compared the logarithmic transformed fold-change of CALB1, GABRA5, TAC1, CRHBP, and CX3CR1 between AD and SZ (Fig. 3). For SZ, the expression data of CALB1, TAC1, CRHBP, and CX3CR1 was absent in the parietal lobe and was not shown in Fig. 3. In whole brain, the CALB1, GABRA5, and TAC1 were doubly downregulated in AD, whereas there were no expression differences in SZ. Furthermore, the expression values of CALB1, GABRA5, and TAC1 in AD were all lower than controls in the frontal lobe, parietal lobe, temporal lobe, and hippocampus. However, for SZ, the expression profiles of these genes in different brain partition were different and all these did not reach significance compared with controls. In addition, the expression profiles of CRHBP and CX3CR1 are similar in the whole brain between AD and SZ. However, in SZ patients, CRHBP and CX3CR1 were significantly downregulated in hippocampus whereas there was no expression difference in AD.

DISCUSSION

In this study, we revealed great differences in gene expression profiles between AD and SZ. Particularly for the influences on the learning or memory related genes, patients with AD showed a large number of downregulated genes in the whole brain and other brain partitions compared with controls.

Based on our data, there was a wide distribution of logarithmic transformed fold-change (log2(FC)) of gene expression values (–1 to 1) in the whole brain in AD. However, the distribution of log2(FC) in the whole brain in SZ was mainly between –0.2 to 0.2 (Fig. 1). These results showed that the influences of gene expression profiles by SZ may relatively weak compared with AD. Furthermore, from the

perspective of the gene expression profiles in the different brain regions, AD patients exhibited a greater number of differentially expressed genes in multiple brain regions than SZ patients, especially in the temporal lobe (based on our criteria that adj p value <0.05 and $\log2(FC) >= 1$). Under this criterion, the influence of SZ on the gene expression profiles is mainly located in the hippocampus (Table 1). According to some neuroimaging studies, the brain structural abnormalities in SZ mostly appear in the left superior temporal gyrus and hippocampus [21, 22]. However, in addition to the regional brain abnormalities in AD patients, studies showed that individuals with AD have disruptive neuronal integrity in large-scale structural and functional brain systems underlying high-level cognition, as demonstrated by a loss of small world network characteristics [23, 24]. These results further confirmed our findings that there were more serious brain lesions in AD patients than SZ patients.

We identified three genes (CALB1, GABRA5, and TAC1) that were significantly downregulated in whole brain in AD patients. Further, we found CRHBP and CX3CR1 showed lower expression in hippocampus in SZ patients. The deficiency of these genes and the effect of them on learning or memory impairment have been widely studied. CALB1, as a member of the calcium-binding protein family, has a critical role in preventing neuronal death and maintaining calcium homeostasis [25]. Previous studies reported that there was disrupted calcium homeostasis in the brains of AD patients and normal aged subjects [26, 27]. Riascos et al. showed that age-related loss of the CALB1 from basal forebrain cholinergic neurons has a harmful role in AD pathogenesis [28]. A recent study reported that CALB1 had a crucial role in maintaining neuronal survival, preventing cell apoptosis, improving mitochondrial functions, and modifying learning and memory function [29]. In our study, AD patients showed a lower expression of CALB1 in the whole brain and other brain partitions. Therefore, we speculate that the depletion of CALB1 may be one of the causes of the AD pathogenesis.

$GABA_A$ receptors that contain the α5 subunit ($\alpha5GABA_AR$ encoded by GABRA5) were widely reported to influence cognitive processes such as hippocampal-dependent learning and memory functions [30, 31]. A recent study showed genetic deletion or pharmacological inhibition of $\alpha5GABA_AR$ markedly reduces the threshold for the induction of long-term potentiation mainly via independently synaptic inhibition, and this action correlated with improved memory performance [32, 33]. Additionally, other studies reported the alpha subunits of the $GABA_A$ receptor also affected panic disorder [34] and epilepsy treatment response [35]. Our results showed the GABRA5 gene was significantly downregulated in a variety of brain partitions in AD patients, suggesting it may play a crucial role in AD pathogenesis.

Tachykinin precursor 1 (TAC1) gene encodes four tachykinins that are substance P (SP), neurokinin A (NKA), neuropeptide K (NPK), and neuropeptide γ (NPγ) [36]. The TAC1 gene expression profile was shown to have an important role in modulating short-term working memory in an animal model [37]. Our results showed the TAC1 gene expression was significantly downregulated in AD patients, suggested that the tachykinin system was important in the development of dementia in AD. The biological functions of SP and NKA have been widely studied. Schlesinger et al. first reported that SP administration improves learning and memory in mice models (passive and active avoidance tests) in a dose-dependent manner [38]. Similarly, SP-treated mice performed better than controls in appetite-motivated learning test [39]. Furthermore, the memory-promoting and reinforcing effect of SP has been widely demonstrated in various brain regions [40]. Additionally, the neuroprotective effect of NKA and NPK had also been reported [41, 42]. Recently, a study showed that treatment with tachykinin peptides reduced amyloid-β (1–40) neurotoxicity in cells, and this may be a strategy for developing anti-AD compounds [43].

CRHBP is a modulator of corticotropin releasing hormone activity [44]. The most important role of CRHBP was the inhibitory effect on CRH-induced adrenocorticotropic hormone secretion [44, 45]. A previous study showed the cognition enhancing effect of selective CRHBP ligands in models of learning and memory in animals [46]. Furthermore, Herringa et al. showed that reduced basolateral amygdala and lateral amygdala CHRBP might be a risk factor for males that relates to the development or maintenance of bipolar disorder and SZ [47]. Similarly, De Luca et al. found CRHBP mutation was significantly related to suicidal behavior in SZ patients [48]. Based on these reports and our findings, we believe that the downregulation of CHRBP might impair learning or memory function and relate to the pathogenesis of SZ.

CX3C chemokine receptor 1 (CX3CR1) and its ligand CX3CL1 play an important role in regulating a variety of physiological functions

throughout the lifespan. The homeostasis of CX3CL1/CX3CR1 signaling has been associated with several neuroprotective effects whereas the disruption of CX3CL1/CX3CR1 signaling showed various metabolic and behavioral abnormalities, especially on cognitive function impairment [49, 50]. Recently, a study indicated that CX3CR1 plays a physiological role in normal hippocampal-dependent cognitive function and synaptic plasticity [51]. Similarly, Ali et al. showed an important role of CX3CL1/CX3CR1 signaling pathways in limbic seizure-induced hippocampal pathology including microglia activation, neurodegeneration, and neuroblast production [52]. Our present results showed that CX3CR1 was significantly downregulated in hippocampus of SZ patients. Combining the above studies, we suggested that CX3CR1 is a key modulator in hippocampus-related cognitive functions.

In conclusion, there were great differences in gene expression profiles between AD and SZ in the whole brain and other brain partitions. In particular, patients with AD showed severe impairment of learning and memory functions, whereas SZ patients showed a relatively slight damage of cognitive function in hippocampus. Future studies are needed to clarify these mechanisms.

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SUPPLEMENTARY MATERIAL

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