

cDNA microarray and bioinformatic analysis for the identification of key genes in Alzheimer's disease

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Abstract. In this study, gene expression profiles in peripheral blood monocytes from patients with Alzheimer's disease (AD) or mild cognitive impairment (MCI) were compared with those of healthy individuals to identify key differentially expressed genes (DEGs), in an effort to broaden our understanding of the pathogenesis of these diseases and identify potential therapeutic targets. The microarray data set GSE18309 was downloaded from Gene Expression Omnibus, including 3 AD, 3 MCI and 3 normal control (NC) samples. Raw data were processed and differential analysis was performed using the *R* multtest package. Two groups of comparisons (AD vs. NC and MCI vs. NC) were conducted and two groups of DEGs were acquired. The common DEGs were selected, for which functional enrichment analysis, as well as pathway enrichment analysis were performed to determine their roles in the development of the diseases in question. A total of 405 DEGs were identified in the AD vs. NC samples and 395 in the MCI vs. NC samples. A total of 60 common DEGs were obtained. Functional enrichment analysis revealed that the most common functions of the DEGs identified were response to nutrients, muscle contraction and cellular homeostasis. As shown by pathway enrichment analysis, the most common pathway associated with the DEGs identified was the neuroactive ligand-receptor interaction pathway. A range of DEGs was identified in the present study, which may help to disclose the molecular mechanisms responsible for AD and may thus provide potential novel therapeutic strategies for AD.

Introduction

Alzheimer's disease (AD) is a type of neurodegenerative disorder. It is characterized by memory loss, cognitive decline and persistent movement disorders, and is also accompanied by a series of psychotic symptoms (1). The increasing number of patients with the disease places a heavy burden on family and society. Therefore, there is an urgent demand for the early diagnosis and prevention of AD.

The pathogenesis of AD is very complex. A variety of factors have been found to be involved in AD, such as cholinergic nerve abnormalities, metabolic disorders, free radicals and apoptosis, excitatory amino acid toxicity and genetic background (2). Despite their multifactorial etiopathogenesis, genetics plays a primary role in the progression of disease. Previous studies have identified four genes that may be linked to autosomal dominant or familial early onset AD. These are amyloid precursor protein (APP) (3), presenilin 1 (PS1) (4), presenilin 2 (PS2) (5) and apolipoprotein E (ApoE) (6). Some of the therapeutic approaches that have progressed to the clinical arena are the use of acetylcholinesterase inhibitors, nerve growth factors, non-steroidal anti-inflammatory drugs, estrogen and compounds, such as antioxidants, neuronal calcium channel blockers or anti-apoptotic agents (7). Moreover, the development of immunotherapeutic strategies may prove to be a therapeutic approach with promising results for the treatment of AD.

Therefore, fundamental studies are required to provide novel and effective biomarkers for AD. Among the various technologies, microarray is a powerful and convenient tool which may aid in the search for biomarkers. Pasinetti (8) adopted cDNA microarray to search for molecular markers involved in the onset of AD dementia. Blalock *et al* (9) discovered major transcriptional and tumor suppressor responses in incipient AD by microarray correlation analyses. Maes *et al* (10) carried out transcriptional profiling of blood mononuclear cells from patients with AD by microarray analysis.

In present study, the cDNA microarray data of peripheral blood mononuclear cells from patients with AD were compared with those of normal control (NC) samples to discover the key genes associated with AD. The data from transcriptome analysis of samples from patients with mild cognitive impairment (MCI) were also compared with those of NC samples, in order to identify early biomarkers for AD.

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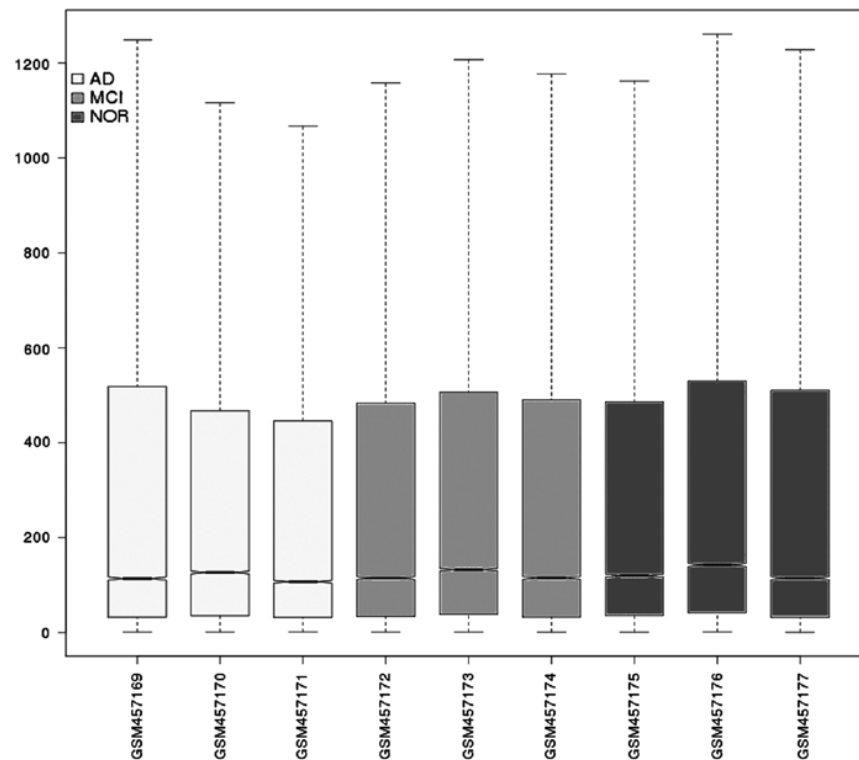


Figure 1. Box plot for normalized gene expression data. Alzheimer's disease (AD) samples are shown in white, mild cognitive impairment (MCI) samples in gray and normal controls in black. The medians (black lines) are almost at the same level, indicating a good performance standards.

Materials and methods

Microarray data. The microarray data set GSE18309 was downloaded from Gene Expression Omnibus, including 3 MCI, 3 AD and 3 NC samples. The following platform was adopted: GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 array to collect raw data.

Conversion of raw data. Raw data were converted into a recognizable format. After the missing values were imputed as previously described (11), the data were normalized, as described in a previous study (12). Two groups of comparisons were carried out: AD vs. NC and MCI vs. NC.

Differential analysis. Differential analysis (AD vs. NC and MCI vs. NC) was conducted with a t-test using the *R* multtest package, as previously described (13). $P < 0.05$ and $|\log FCI| > 1$ were set as the cut-off values to screen for differentially expressed genes (DEGs).

Construction of interaction network. Interactions between the two groups of DEGs were predicted with the STRING database (14), and then were visualized with Cytoscape, as previously described (15).

Functional enrichment analysis. The common DEGs were selected and functional enrichment analysis was then performed for these genes using the FuncAssociate web application, as previously described (16). A hypergeometric distribution-based test was applied and $P < 0.05$ was determined as the threshold.

Pathway enrichment analysis. Pathway enrichment analysis was performed with Fisher's exact test and the Expression Analysis Systematic Explorer (EASE) application, as previously described (17), which provided statistically significant GO terms. $P < 0.05$ was set as the threshold.

Results

Differentially expressed genes. Data normalization was carried out successfully (Fig. 1). A total of 405 DEGs were identified in the AD vs. NC samples and 395 DEGs in the MCI vs. NC samples. A total of 60 DEGs were found to be common in both groups.

Interaction network. Interaction networks for the two groups of DEGs (AD vs. NC; MCI vs. NC) were constructed using the STRING and Cytoscape (Fig. 2). From the network, we were able to easily observe the locations of genes and thus identify key players in the networks. Several genes associated with inflammatory response were identified: chemokine (C-X-C motif) ligand (CXCL)11, CXCL3 and platelet factor 4 (PF4).

Functional enrichment analysis results. Common DEGs for AD and MCI were selected and analyzed using the FuncAssociate web application. The three most common functions of the DEGs identified were response to nutrients, muscle contraction and cellular homeostasis (Table I).

'Response to nutrients' (GO:0007584) was the most significant term (function) and four genes were found to be associated with this term: cholecystokinin A receptor (CCKAR),

Table I. Three significant functional terms revealed by functional enrichment analysis.

Term ID	Function	P-value	Genes
GO:0007584	Response to nutrients	0.006518	CCKAR, IL6ST, CDKN2D, MAP1B
GO:0006936	Muscle contraction	0.008318	P2RX6, SLC6A8, KCNQ1, GJC1
GO:0019725	Cellular homeostasis	0.008333	CCKAR, IL6ST, EGLN2, POU3F2, KCNQ1, KCNMB2

CCKAR, cholecystokinin A receptor; IL6ST, interleukin 6 signal transducer; CDKN2D, cyclin-dependent kinase inhibitor 2D; MAP1B, microtubule-associated protein 1B; P2RX6, purinergic receptor P2X, ligand-gated ion channel, 6; SLC6A8, solute carrier family 6 (neurotransmitter transporter, creatine), member 8; KCNQ1, potassium voltage-gated channel, KQT-like subfamily, member 1; GJC1, gap junction protein, gamma 1, 45 kDa; EGLN2, egl nine homolog 2 (*C. elegans*); POU3F2, POU class 3 homeobox 2; KCNMB2, potassium large conductance calcium-activated channel, subfamily M, beta member 2.

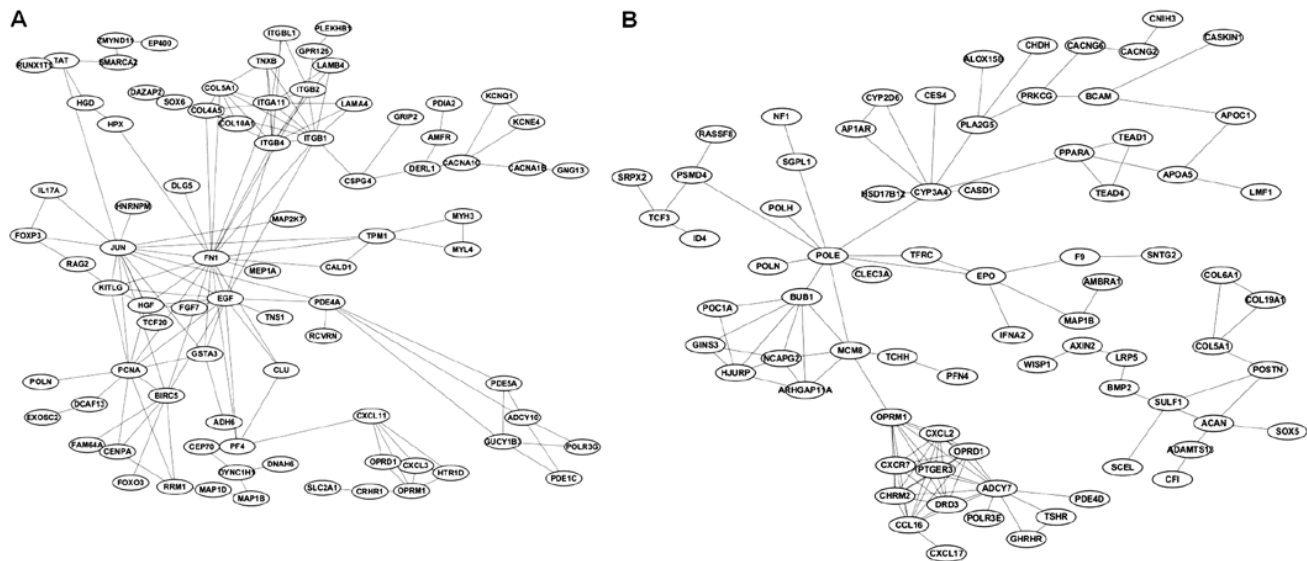


Figure 2. Interaction networks for differentially expressed genes (DEGs) from (A) Alzheimer's disease (AD) vs. normal controls (NC) and (B) mild cognitive impairment (MCI) vs. NC samples.

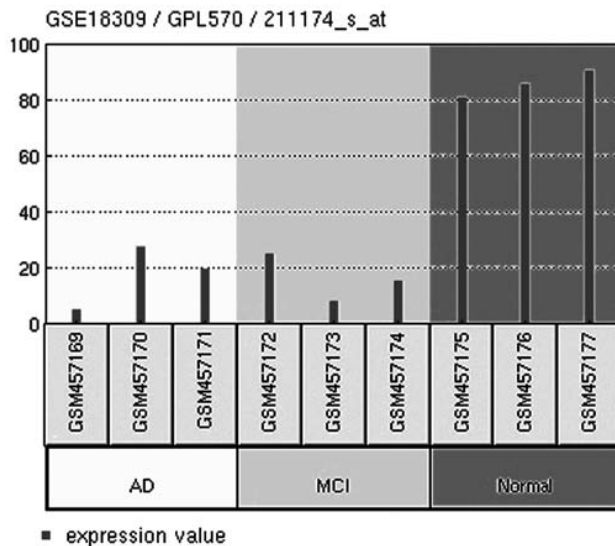


Figure 3. Expression level of cholecystokinin A receptor (CCKAR) in each sample. Alzheimer's disease (AD) samples are shown in white, mild cognitive impairment (MCI) in gray and normal controls (NC) in black.

interleukin 6 signal transducer (IL6ST), cyclin-dependent kinase inhibitor 2D (CDKN2D) and microtubule-associated protein 1B (MAP1B). Previous studies have demonstrated that cognitive impairment is related with a lack of nutrients. The dietary intake of certain nutrients or antioxidant supplements can prevent cognitive decline and the effects of dementia (18).

Pathway enrichment analysis results. The 60 common DEGs between the AD and MCI samples compared with NC samples were analyzed using the EASE application to identify the most common pathways associated with the DEGs identified. The most common pathway associated with the DEGs was the neuroactive ligand-receptor interaction pathway (hsa04080), associated with the opioid receptor, mu 1 (OPRM1), CCKAR, purinergic receptor P2X, ligand-gated ion channel, 6 (P2RX6) and opioid receptor, delta 1 (OPRD1) genes.

The CCKAR gene was found to be associated the most common functional term, as well the most common pathway, suggesting its important role in the development of AD. The downregulation of this gene was observed in the AD and MCI samples (Fig. 3).

Discussion

Early diagnosis and prevention, as well as the development of molecular targeted therapy are of great importance to the treatment of AD. However, they require the identification of effective biomarkers. For this purpose, gene expression profiles in samples from patients with AD and MCI were compared with those of NC (healthy individuals). A total of 405 and 395 DEGs were revealed in the AD and MCI samples, respectively. Two interaction networks were then constructed to identify the key genes involved.

The association between inflammation and AD is very complicated (19). In the present study, several genes related to inflammation were identified. CXCL11 is a CXC member of the chemokine superfamily. It can induce a chemotactic response in activated T-cells and is the dominant ligand for CXC receptor-3. However, controversial roles have been found for the chemokine receptor CXCR3 and its ligands, CXCL9, CXCL10 and CXCL11, in the pathogenesis of neuroinflammatory diseases according to various studies (20). Several interactors of CXCL11 have been found in the network, such as CXCL3 and PF4. Endoplasmic reticulum (ER) stress is a novel neuronal trigger for inflammation and AD (21). The upregulation of CXCL3 has been observed in patients with AD compared with healthy individuals, which can be explained by ER stress (22,23). Platelets also play an important role in inflammatory processes and are thus involved in the development of AD (24). PF4 is one of the inflammatory signaling molecules secreted by platelets. Clusterin (CLU) interacts with PF4 and is a secreted chaperone. Protein misfolding is also closely linked with AD (25). In a genome-wide association study, Harold *et al* (26) found that variants at CLU and phosphatidylinositol binding clathrin assembly protein (PICALM) are associated with the development of AD. Similar findings were reported by Lambert *et al* (27). Braskie *et al* (28) further pointed out that variants within the CLU gene affect white matter microstructure in young adults.

In our study, 60 common DEGs were found in AD and MCI. Three functional terms were significantly over-represented: response to nutrients, muscle contraction and cellular homeostasis. There may be a correlation between diet and the development of AD. Certain studies have suggested that a high intake of vitamins C, E (29), B6 and B12, as well as folate, unsaturated fatty acids and fish are related to a low risk of developing AD; however, the results obtained are inconsistent (30,31). Liu and Ames (32) reported that reducing mitochondrial decay with mitochondrial nutrients can delay and treat AD. Lefebvre *et al* (33) find that dysregulation of the nutrient/stress sensor, GlcNAcylation, is involved in the development of AD. MAP1B is thought to be involved in microtubule assembly, which is an essential step in neurogenesis (34). Aberrantly phosphorylated MAP1B has been detected in sites of neurofibrillary degeneration in the brains of patients with AD (35,36). IL6ST (CD130) is a signal transducer shared by many cytokines, such as interleukin 6 (IL6) (37). Previous studies have indicated that abnormal IL6 activity contributes to the development of AD (38). Therefore, it may be a potential target to modulate signal transduction. In our study, pathway enrichment analysis revealed that the most common pathway associated with the DEGs identified was the neuroactive ligand-receptor interaction pathway.

The CCKAR was found to be associated with this pathway. It encodes a G-protein coupled receptor that binds non-sulfated members of the cholecystokinin (CCK) family of peptide hormones. It appears to be a major physiological mediator. In the central and peripheral nervous system, this receptor regulates satiety and the release of β -endorphin and dopamine (39). Wang *et al* (40) pointed out that there was an association between CCKAR polymorphism and visual hallucinogenesis in patients with Parkinson's disease. P2RX6 (P2X6) belongs to the family of P2X receptors, which are ATP-gated ion channels and mediate rapid and selective permeability to cations. Köles *et al* (41) indicated that P2X receptors are possible targets for therapeutic manipulations in disorders of the central nervous system. A previous study demonstrated blocking the purinergic P2X7 receptor exerts neuroprotective effects in an animal model of AD (42). Therefore, it is possible that P2X6 plays a role in the development of AD. P2X receptor expression, mainly of that of P2X4 and P2X6 subtypes, has been detected in adult brains and during neuronal development. Da Silva *et al* (43) reported the alternative splicing of P2X6 receptors in the developing mouse brain and during *in vitro* neuronal differentiation.

In conclusion, a range of DEGs were identified in AD through this comparative study. These DEGs may play important roles in the development of AD. Further studies on these genes are required in order to fully elucidate their role in the pathogenesis of AD. Additionally, the common DEGs between AD and MCI are worthy of greater attention as they may be early biomarkers for AD.

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