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Original Article

Whether Alzheimer's diseases related genes also differently express in the hippocampus of Ts65Dn mice?

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Abstract: Background: Down syndrome is a condition which extra genetic material causes delays in child development, both mentally and physically. Strengthening the study of the neural defects of DS is of great significance. Methods: Ts65Dn mice were used in this study. We removed the brain and isolated their hippocampus. We customized 54 genes in one PCR arrays, included some important genes related to Alzheimer's disease. The expression of genes were detected by RT-PCR. Results: PCR arrays contained 54 genes related to Alzheimer's disease. After real-time PCR, three genes (Nae1, APP and Mapt) expressed differently in the hippocampus of Ts65Dn, compared with the normal mice. Nae1 was decreased significantly, while APP and Mapt were increased obviously. The levels of fold-changes of Nae1, APP and Mapt were 86.19, 4.49 and 2.89 respectively. Significantly different levels of expression were found in the Ts65Dn mice compared with the normal control group (P=0.00 for Nae1, P=0.02 for APP, P=0.01 for Mapt respectively). Conclusions: There are differential expressed genes in the hippocampus of Ts65Dn mice that may be closely related to Alzheimer's disease. PCR array technology was used in the screening and identification of these genes.

Keywords: Down syndrome, hippocampus, PCR array, Alzheimer's disease, Ts65Dn

Introduction

Down syndrome (DS) is one of the most common gross chromosomal abnormalities and birth defect. It is well-known that DS is a condition which extra genetic material causes delays in child development, both mentally and physically. Intellectual disabilities are the foremost and most debilitating trait, which causes the loss of cognitive abilities and the development of early onset Alzheimer's disease (AD) [1]. The survival rate of people with DS has greatly improved in the past decades, with a current life expectancy of 60 years or longer [2]. However, the problems of mental retardation of Down syndrome patients appeared more and more prominent. Even with effective medical intervention and skills training, the IQ of DS patients could be significantly improved. However, their memory, athletic ability, and learning ability still have great defects [3, 4]. It prevents the ability of language, learning and memory of DS patients, and thus restricts their life and social skills. Therefore, problems concerning cognitive mental retardation become more important to those with DS.

Alzheimer's disease (AD) is a clinically heterogeneous neurodegenerative disease with a strong genetic component [5]. Several genes have been associated with AD risk for nearly 20 years, mainly including APP, PSEN-1, PSEN-2, ApoE, ACT, TREM2, SUMO1 [6]. Some genes were considered as a major genetic risk factor, some were used as biomarkers in AD diagnosis. They might be related to the occurrence and development of AD.

At the same time, it is well known that Down syndrome patients will also get serious problem by AD. DS patients are characterized by the early appearance of neurodegenerative diseases such as AD: 11% of DS patients have AD pathology at 40 years of age and 100% have AD

lesions at >70 years of age [7]. On the other hand, it is well known that Alzheimer's occurs in trisomy 21 since the gene encoding amyloid is in this chromosome. Thus these individuals have a higher load of amyloid that in turn results in a higher incidence of developing cognitive decline and Alzheimer's dementia with aging [8]. So, whether Alzheimer's diseases related genes also have to do with Down syndrome?

In present study, a target PCR array related to the Alzheimer's diseases was constructed, and we observed the changes of gene expression in Ts65Dn mice.

Materials and methods

This study was conducted in the Changzhou Women and Children Hospital of Nanjing Medical University (Changzhou City, Jiangsu Province, China). The animals were bred in the Animal center of Jiangsu University (Zhenjiang City, Jiangsu Province, China). All efforts were made to minimize the suffering and number of animals used.

Animals

Five Ts65Dn mice carrying a partial trisomy of chromosome 16 were purchased from the Jackson Laboratories (Bar Harbor, ME, USA). Five normal C57BL/6JEi mice were used as the normal control group. Both groups were matched by age which were more than 14 weeks old. The animals' health and comfort were monitored by the veterinary service. The animals had access to water and food according to the routine methods of animal breeding in the Animal center of Jiangsu University.

Methods

Sample collection: Anesthetized animals were euthanized using 10% chloral hydrate (Changzhou First People's Hospital, Changzhou, China). We removed the brain and isolated the hippocampus.

Total RNA extraction: Total RNA was isolated by TRIZOL (Invitrogen) extraction. After homogenization of tissue samples, insoluble material was removed from the homogenate by centrifugation at 12000× g for 10 minutes at 2 to 8°C The homogenized samples were incubated for 5 minutes at room temperature, and 0.2 ml chloroform per 1 ml of trizol reagent was added. The tubes were shaken vigorously by hand for 15 seconds and incubated at 15 to 30°C for 2 to 3 minutes. Then, the samples were centrifuged at no more than 12000× g for 15 minutes at 2 to 8°C, and the colorless upper aqueous phase was transferred to a fresh tube. The RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol (0.5 ml isopropyl alcohol per 1 ml trizol reagent). Samples were incubated at 15 to 30°C for 10 minutes and centrifuged at no more than 12000× g for 10 minutes at 2 to 8°C. The supernatant was removed, and the RNA pellet was washed once with 75% ethanol, adding at least 1 ml 75% ethanol per 1 ml trizol Reagent. The samples were mixed by vortex and centrifuged at no more than 7500× g for 5 minutes at 2 to 8°C. Finally, the RNA pellets were dried by air or vacuum for 5-10 minutes, and RNA was dissolved in RNase-free water.

PCR arrays customization: PCR arrays contained 54 genes, 5 housekeeping genes, PPC and GDC. 54 genes were related to Alzheimer's disease. The housekeeping genes were B2M, ACTB, GAPDH, RPL27, HPRT1 and OAZ1. PPC contains synthetic DNA fragments which have no homology with detected species and amplification primers, which was used as quality control. GDC was used to detect the residual genomic DNA.

PCR array experiment: Total RNA from each sample was used for reverse transcription with an RT-PCR Kit (catalog#CTB101; CT biosciences, China) on an ABI 9700 thermo cycler (ABI, Foster City, CA). For reverse transcription, 4 μl total RNA was mixed with 10 μl OligodT Primer (10 μM), and the solution was incubated at 70°C for 10 minutes and then quickly cooled on ice for 2 minutes. The cooled solution was mixed with 4 μl 5× reverse transcription buffer, 1 μl dNTP (10 mM), 0.5 μl RNasin (40 U/μl), and 0.5 μl reverse transcriptase (200 U/μl). Reverse transcription was performed at 42°C for 1 hour, followed by an inactivation reaction at 70°C for 15 minutes. The resulting cDNA was stored at -20°C until it had been used. PCR arrays were performed with customized PCR containing pre-dispensed primers (CT biosciences, China) on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) using SYBR Master Mix ((catalog#CTB101; CT biosciences, China). The thermo

Table 1. Differential apoptotic gene expressions in the Hippocampus of Ts65Dn

Gene symbol	Description of genes	Chromosome		Ts65Dn/Control group	
		Human	Mouse	Fold changes	*P* value
Nae1	NEDD8 activating enzyme E1 subunit 1	16	8	-86.19	0.000245
App	amyloid beta (A4) precursor protein	21	16	4.49	0.021300
Mapt	microtubule-associated protein tau	17	11	2.89	0.010808
Snca	synuclein, alpha	4	6	6.66	0.0593829
Mme	membrane metallo endopeptidase	3	3	2.87	0.0663999
Cdk5	cyclin-dependent kinase 5	7	5	2.79	0.0699807
Ncstn	nicastrin	1	1	3.26	0.0712695
Psen1	presenilin 1	14	12	11.62	0.0743482
Nos1	nitric oxide synthase 1	5	12	2.77	0.0841564
Bace1	beta-site APP cleaving enzyme 1	11	9	2.96	0.0868817
Apoe	apolipoprotein E	19	7	2.52	0.103259
Adam10	a disintegrin and metallopeptidase domain 10	15	9	2.52	0.103259
Atp2a1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	16	7	2.52	0.103259
Capn1	calpain 1	11	19	2.52	0.103259
Eif2ak3	eukaryotic translation initiation factor 2-alpha kinase 3	6	2	2.52	0.103259
Gnaq	guanine nucleotide binding protein, alpha q polypeptide	9	19	2.52	0.103259
Bace2	beta-site APP-cleaving enzyme 2	21	16	2.52	0.103259
Ern1	endoplasmic reticulum (ER) to nucleus signalling 1	17	11	2.52	0.103259
Tnfrsf1a	tumor necrosis factor receptor superfamily, member 1a	12	6	2.52	0.103259
Apaf1	apoptotic peptidase activating factor 1	12	10	2.52	0.103259
Casp8	caspase 8	2	1	2.52	0.103259
Casp9	caspase 9	1	4	2.52	0.103259
Fadd	Fas (TNFRSF6)-associated via death domain	11	7	2.52	0.103259
Casp3	caspase 3	4	8	2.52	0.103259
Il1a	interleukin 1 alpha	2	2	2.52	0.103259
Casp7	caspase 7	10	19	2.52	0.103259
Cdc2a	cyclin-dependent kinase 1	10	20	2.52	0.103259
Aph1a	anterior pharynx defective 1a homolog (C. elegans)	1	3	-59.69	0.1262045
Il1b	interleukin 1 beta	2	2	6.75	0.1390537
Mapk3	mitogen-activated protein kinase 3	16	7	3.21	0.1722344
Ide	insulin degrading enzyme	10	19	2.98	0.1858611
Hsd17b10	hydroxysteroid (17-beta) dehydrogenase 10	X	X	6.04	0.216086
Ryr3	ryanodine receptor 3	2	15	4.59	0.2207425
Plcb1	phospholipase C, beta 1	20	2	4.81	0.222985
Cox4i1	cytochrome c oxidase subunit IV isoform 1	16	8	5.86	0.2703607
Cycs	cytochrome c, somatic	7	6	-3.16	0.2720677
Mapk1	mitogen-activated protein kinase 1	22	16	6.59	0.2937304
Bid	BH3 interacting domain death agonist	22	6	-2.03	0.3028315
Calm1	calmodulin 1	14	12	-5.31	0.3548665
Psen2	presenilin 2	1	1	2.01	0.4103427
Fas	Fas (TNF receptor superfamily member 6)	10	19	3.23	0.4264696
Casp4	caspase 4	11	9	1.7	0.4756739
Tnf	tumor necrosis factor	6	17	2.01	0.5039548
Cdk5r1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	17	11	1.1	0.6050327
Atf6	activating transcription factor 6	1	1	1.43	0.6661326
Lpl	lipoprotein lipase	8	8	-1.87	0.6721144
Gsk3b	glycogen synthase kinase 3 beta	3	16	1.78	0.6980795
Ppp3ca	protein phosphatase 3, catalytic subunit, alpha isoform	4	3	-1.09	0.7517183
Ppp3cc	protein phosphatase 3, catalytic subunit, gamma isoform	8	14	1.58	0.8033197
Itpr1	inositol 1,4,5-trisphosphate receptor 1	6	3	1.19	0.8720633
Ppp3r1	protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)	2	11	1.22	0.8949331
Bad	BCL2-associated agonist of cell death	11	19	1.15	0.901663
Apbb1	amyloid beta (A4) precursor protein-binding, family B, member 1	11	7	1.1	0.9243038
Lrp1	low density lipoprotein receptor-related protein 1	12	10	1.02	0.9865195

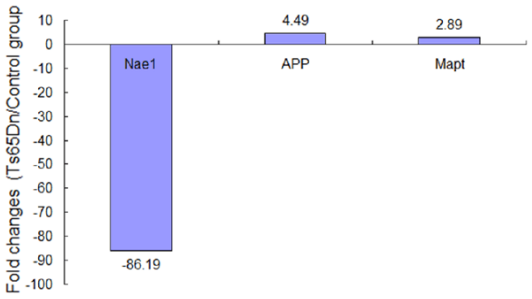

Figure 1. Fold change expressions of genes between Ts65Dn and control group. Compared with control group, the expressions of Nae1 were decreased and App, Mapt were increased in the hippocampus tissues of Ts65Dn.

cycler parameters consisted of an initial denaturation at 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 10 s and annealing at 60°C for 20 s. Relative changes in gene expression were calculated using the ΔΔCt (threshold cycle) method. The housekeeping genes (B2M, ACTB, GAPDH, RPL27, HPRT1 and OAZ1) were used to normalize the amount of RNA. Fold change values were calculated using the formula: 2-ΔΔCt.

Statistical analysis

All data were collected and statistically analyzed using SPSS 13.0 software. Results of parameters were expressed as median (M), 2.5th percentile (P2.5) and 97.5th percentile (P97.5). Non-parametric tests (Mann-Whitney U test) was employed to compare differences for CP (median level) between two groups. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

In present study, PCR arrays contained 54 genes which were related to Alzheimer's disease. After real-time PCR, three genes (Nae1, APP and Mapt) expressed differently in the hippocampus of Ts65Dn, compared with the normal mice (**Table 1**). Nae1 was decreased significantly, while APP and Mapt were increased obviously. The old-changes levels of Nae1, APP and Mapt were 86.19, 4.49 and 2.89 respectively (**Figure 1**). The gene levels were compared by the value of 2-ΔΔCt for target genes to housekeeping genes expression in two groups. We found significantly different levels of expression in the Ts65Dn mice compared with the

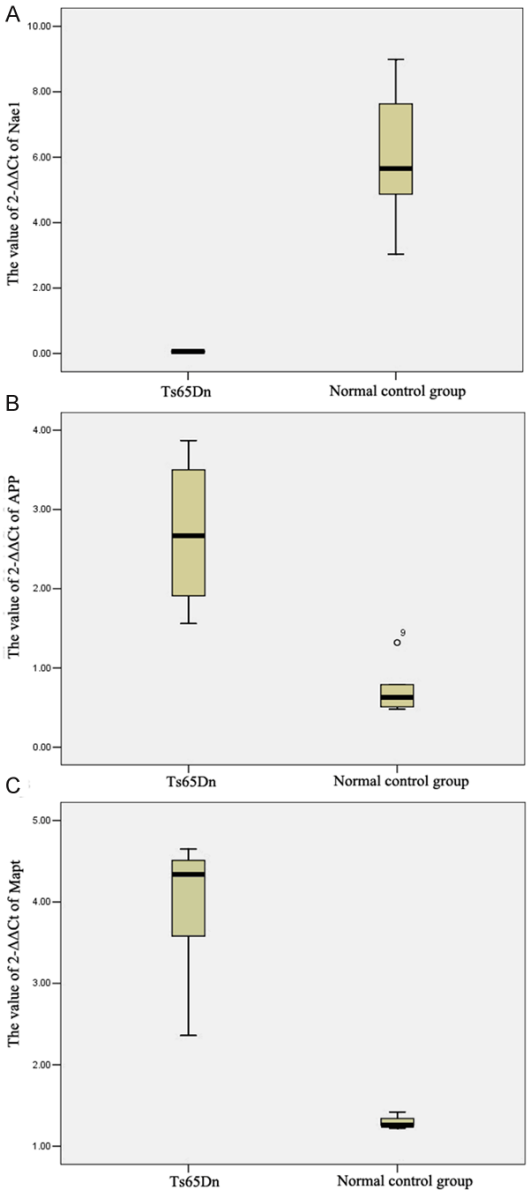

Figure 2. Comparison of the expressions of Nae1, APP and Mapt by Box plots. Box plots show the comparison of the value of 2-ΔΔCt of Ts65Dn and normal control group. Data were compared using the t-test. A: Nae1, B: APP, C: Mapt

normal control group (P=0.00 for Nae1, P=0.02 for APP, P=0.01 for Mapt respectively). We compared the significant differences in the expression of Nae1, APP and Mapt using Box plots, as shown in (**Figure 2**).

In present study, we also detected the genes of ApoE, which were more than a contributing factor to neurodegeneration [9, 10]. Some reports also showed that they have been linked to early onset of Alzheimer's disease. However, it had

no significant differences in expression levels. Compared with normal control group, ApoE was increased in Ts65Dn mice ant its levels of fold-changes was 2.52 (P=0.103259) (**Table 1**).

Discussion

In the study, we discovered three genes were differently expressed in the hippocampus of Ts65Dn by a target PCR array, which included 54 genes related to Alzheimer's disease.

Down syndrome is characterized by the early appearance of neurodegenerative diseases such as Alzheimer's disease. Cognitive disabilities in DS from appearance to result mainly two pathological processes neurogenesis impairment and Alzheimer-like degeneration [8]. On the other hand, it is well known that Alzheimer's occurs in trisomy 21 since the gene encoding amyloid is in this chromosome. Therefore, they might have similar pathological processes between the two kinds of disease. Recently, some genes have been associated with AD risk were paid more and more attention, such as APP, ApoE, PSEN-1, PSEN-2, ACT, TREM2, SUMO1[6]. So, whether they also have to do with Down syndrome? In this study, we costumed them in one chip and detected their expression by PCR.

There are so many molecules involved in the process of Alzheimer's disease that real-time quantitative PCR may not be suitable for observing them. A high-throughput platform with efficient detection capacity is needed to address this issue. Recently, PCR arrays have been considered the most reliable tools for analyzing the expression for a focused panel of genes, especially in signal transduction pathways, biological process or disease related gene networks [11]. PCR arrays have been used for cancer, immunology, stem cells, toxicology, and many other areas of biological and medical research [12, 13].

In PCR arrays, we found that the Nae1, APP and Mapt genes showed significantly altered expression in Ts65Dn mice. They might be play important roles in the early appearance of Alzheimer's disease in Down syndrome patients. Nae1 encodes the protein NEDD8-activating enzyme E1 regulatory subunit, which is bound to the beta-amyloid precursor protein. Beta-amyloid precursor protein is a cell surface protein with signal-transducing properties, and it is thought to play a role in the pathogenesis of Alzheimer's disease [14]. However, there has been no reports about Nae1 and Down syndrome until now. APP is a familiar gene. It encodes a cell surface receptor and transmembrane precursor protein, which is the major component of amyloid plaques found in the brains of Alzheimer's patients [15]. Mutations in APP have been linked is ralative to early onset Alzheimer's disease [16]. As a cell surface receptor, it performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis. Furthermore, it has been associated with other diseases including Lewy body dementia, inclusion body myositis and cerebral amyloid angiopathy. Mapt gene encodes the microtubule-associated protein tau which is differentially expressed in the nervous system, depending on is relative to the stage of neuronal maturation and neuron type. Its main functions are promoting microtubule assembly and stability, and may be involved in the establishment and maintenance of neuronal polarity. According to the current reports, Mapt gene mutations have been associated with several neurodegenerative disorders [17] such as Alzheimer's disease, Parkinson's disease [18], frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

In present study, we did a interesting but preliminary research. We found three gene which were related to Alzheimer's disease also differently expressed in the brain of Down syndrome mice. The functions of three genes played a important role in several neurodegenerative disorders. We need further research to prove whether they also have to do with Down syndrome.

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Disclosure of conflict of interest

None.

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