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Original Article

Correlation of polymorphism of APOE and LRP genes to cognitive impairment and behavioral and psychological symptoms of dementia in Alzheimer's disease and vascular dementia

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Abstract: Objective: to discuss the correlation of polymorphism of APOE and LRP genes to cognitive impairment and behavioral and psychological symptoms of dementia (BPSD) in Alzheimer's disease (AD) and vascular dementia (VD). Method: AD cases, VD cases and healthy control cases totaling 237, 255 and 234 were recruited, respectively. The mini-mental state examination (MMSE) was performed to evaluate cognitive impairment. Hamilton Depression Rating Scale (HAMD) and Hamilton Anxiety Scale (HAMA) were adopted to evaluate BPSD. Apolipoprotein E (APOE) and Low-density lipoprotein receptor-related protein gene (LRP) genotyping was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: (1) Frequencies of APOEε4 allele in AD group and VD group were significantly higher than that of the control ($P<0.05$); (2) MMSE scores of APOEε4 carriers in AD group and VD group were lower than that of non-APOEε4 carriers in the same group ($P<0.05$); (3) The proportion of APOEε4 carriers presenting with BPSD in AD group was considerably higher that of non-APOEε4 carriers ($P<0.05$). Conclusion: APOEε4 may be the common risk factor for cognitive impairment in AD and VD and the risk factor for BPSD in AD.

Keywords: Alzheimer's disease (AD), vascular dementia (VD), APOE gene, LRP gene

Introduction

Alzheimer's disease (AD) is a degenerative disease of the central nervous system. Many studies argue that vascular risk factor is not only associated with cognitive impairment and vascular dementia (VD), but also with the onset and progression of AD [1, 2]. AD and VD are the most common types of senile dementia, both presenting with the symptoms of cognitive impairment, decline in basic self-care ability and behavioral and psychological symptoms of dementia (BPSD) [3, 4]. The two were once strictly distinguished from each other, but recent studies show that microvascular lesions also represent the pathological basis of AD. Vascular risk factors can increase the risk of AD. APOEε4 allele was discovered in 1993 as the susceptibility allele of AD and is also associated with coronary heart disease. APOEε4 provides convincing evidence for vascular hypothesis of AD [5, 6].

Low density lipoprotein receptor-related protein (LRP) is a 600 kDa transmembrane glycoprotein, the largest of the low density lipoprotein receptor family. It has multiple functions: transportation of cholesterol, recognition of at least 30 structurally diverse ligands, transcytosis of ligands across the blood-brain-barrier (BBB), and transmembrane and nuclear signaling. LRP may play an important role in AD. The correlations between some cardiovascular risk factors and AD and VD in previous studies have been reported [7].

The present study aimed to clarify the correlation of polymorphism of APOE and LRP genes to cognitive impairment and BPSD in AD and VD.

Table 1. Distribution of genotype and allele of APOE

Group	N	Genotype						Allele		
		ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
AD group	237	0	19 (8.0)*	14 (5.9)	135 (56.9)	44 (18.6)*	25 (10.6)	33 (6.96)	333 (70.25)*	108 (22.79)*
VD group	255	0	22 (8.6)*	18 (7.1)	148 (58.0)	53 (20.8)*	14 (5.5)	40 (7.84)	371 (72.74)*	99 (19.42)*
Control group	234	0	38 (16.2)	8 (3.4)	160 (68.4)	21 (8.9)	7 (2.8)	46 (9.82)	379 (80.98)	43 (9.20)

Compare to control group, *P<0.05.

Table 2. Distribution of genotype and allele of LPR

Group	N	Genotype (N, %)			Allele (N, %)	
		CC	CT	TT	C	T
AD group	237	214 (90.3)	18 (7.6)	5 (2.1)	446 (94.1)	28 (5.9)
VD group	255	231 (90.6)	16 (6.3)	8 (3.1)	478 (93.7)	32 (6.3)
Control group	234	206 (88.1)	20 (8.5)	8 (3.4)	432 (92.3)	36 (7.7)

Subjects and methods

From May 2002 to December 2014, 237 AD patients and 255 VD patients treated at our hospital were included. All patients were of Han Nationality and conformed to the diagnostic standards laid down by National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders (NINCDS/ADRDA) and Diagnostic and Statistical Manual of Mental Disorders (Fourth Version).

They were divided into the following group: (1) AD group: 120 males and 117 females, aged 65-81 years old (73.8±9.1), with course of disease of 2.5-10.0 years (median 4 years) and no family history of AD; (2) VD group: 151 males and 104 females, aged 62-82 years old (73.1±8.3), with course of disease of 1.5-7.0 years (media 4.5 years), complicated by atherosclerotic cerebrovascular diseases but no hereditary vasculopathy or history of hypoxic-ischemic encephalopathy; (3) Healthy control group: 139 males and 95 females, aged 64-82 years old (73.1±9.1), with no history of cerebrovascular diseases and diabetes and confirmed with normal cognitive function by MMSE and BPRS. There were not differences between these three groups in age and gender (P>0.05).

Exclusion criteria included the following: (a) cerebrovascular disorders, intracranial mass or hydrocephalus documented by CT or MRI within the past 12 months; (b) history of schizophrenia, delusional disorder, mood disorder with psychotic features or mental retardation according to DSM-IV criteria; (c) abnormalities in syphilis serology, serum folate, vitamin B12, or thyroid hormone levels; (d) history of traumatic brain injury, Parkinson's or Huntington's disease, and (e) lack of a knowledgeable subject who was able to report on the patient's behavior.

Reagents and equipment

PCR System 9700 (ABI PE), vertical electrophoresis apparatus (Beijing Liuyi Instrument Factory), gel imaging system (Biorad); protease K and Taq DNA polymerase (Beijing Xinjingke Biotechnology Co., Ltd), dNTP (Pharmacia), restriction endonucleases Hha I and Rsa I (Promega), HpaI (Takara, Dalian).

Genomic DNA extraction

Under sterilized conditions 5 mL of venous blood was collected from each subject and placed into a tube containing EDTA as an anticoagulant. Genomic DNA was extracted conventionally from the peripheral white blood cells for polymerase chain reaction (PCR).

PCR amplification of target fragments of APOE and LRP genes and identification of PCR products

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was carried out according to literature [8]. According to the results of APOE genotyping, AD cases and VD cases were divided into APOEε4 carriers and non-APOEε4 carriers, respectively. APOEε4 carriers were subdivided into ε2/ε4, ε3/s4 and ε4/ε4 genotypes; non-APOEε4 carriers were subdivided into ε2/ε2, ε2/ε3 and ε3/ε3 genotypes. According to LRP genotyping, AD cases and VD cases were divided into LRP T carriers and non-LRP T carriers, respectively. LRP T carriers were subdivided into LRP C/T and T/T genotypes, and non-LRP T carriers had LRP C/C genotype only.

Table 3. MMSE and BPSD score between different genotypes

Group	n	MMSE (point)	BPSD (N, %)
AD group			
APOEε4 (+)	80	12.424 (%)	64 (80.0)*
APOEε4 (-)	157	15.62±3.6	86 (54.7)
RLP T allele (+)	22	14.30±2.6	14 (63.6)
RLP T allele (-)	215	15.01±3.4	106 (49.3)
VD group			
APOEε4 (+)	85	14.21±2.8*	42 (49.4)
APOEε4 (-)	170	16.11±3.9	78 (45.9)
RLP T allele (+)	25	17.01±4.1	13 (52.0)
RLP T allele (-)	230	16.02±3.4	113 (49.1)

Compared to APOEε4 (-) group, *P<0.05.

Evaluation of cognitive ability and mental state

Standards for screening cognitive impairment in MMSE were: illiteracy ≤17 points, primary school ≤20 points, middle school and above ≤24 points, on a range of 0-30 points. Mental symptoms were measured with the Brief Psychiatric Rating Scale (BPRS), HAMD (24-item version) and HAMA for all AD cases. Scoring standards for HAMD were: <8 points, no depression; 8-17 points, mild depression; 18-35 points, moderate depression; >35 points, severe depression. The scoring standards for HAMA provided by China Scale Coordination Group (1993) were: <15 points, no depression; 15-21 points, mild depression; 22-29 points, moderate depression; >29 points, severe depression. BPRS was adopted to evaluate the psychotic symptoms. No BPSD was defined for cases scoring normally in all these scales; abnormal scores in any of these scales indicated the presence of BPSD.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. Whether the Hardy-Weinberg equilibrium was obeyed was analyzed by X^2 test. Binary variables such as genotypes, allele frequencies and presence and absence of BPSD were compared within AD group and VD group and across the groups using X^2 test. Continuous variables (eg., age and MMSE scores) were expressed as M ± SD. The data conformed to normal distribution according to P-P plot. Homogeneity of variance was confirmed using F-test. For intergroup comparison two-sided t-test was applied at a=0.05.

Results

Testing for Hardy-Weinberg equilibrium

APOE and LRP allele frequencies of each group were analyzed with X^2 test. APOE and LRP genotypes obeyed Hardy-Weinberg equilibrium in the control group, respectively. APOEε4 allele frequencies of AD group and VD group were both higher than that of the control (P<0.01); APOEε3 allele frequencies of AD group and VD group were lower than that of the control (P<0.01) (**Table 1**). Compared with the control, APOEε2 allele frequencies of AD group and VD group showed no significant difference (P>0.05) (**Table 1**). LRP C allele and LRP T allele frequencies did not show significant differences between the two groups either (P>0.05) (**Table 2**).

Comparison of cognitive ability between different genotypes in AD group and VD group

MMSE scores of APOEε4 carriers in AD group were considerably lower than those of non-APOEε4 carriers (P<0.01); MMSE scores of APOEε4 carriers in VD group were much lower than those of non-APOEε4 carriers (P<0.01); MMSE scores of LRP T carriers and non-LRP T carriers in AD group and VD group showed no significant difference (**Table 3**).

Comparison of BPSD between APOEε4 carriers and non-APOEε4 carriers and between LRP T carriers and non-LRP T carriers in AD group and VD group

The proportion of APOEε4 carriers presenting with BPSD was obviously higher than that of non-APOEε4 carriers in AD group. However, the difference was not significant for VD group. The proportions of LRP T carriers and non-LRP T carriers presenting with BPSD were not markedly different in either AD group or VD group (**Table 3**).

Discussion

APOE is a gene that encodes for apolipoprotein E (apoE), which has neuroprotective effect and neural repair effect in the brain. APOE polymorphism can lead to functional changes of apoE or even aggravate AD-like lesions including the formation of senile plaques and neurofibrillary tangles, with the following mechanisms: (1)

Promoting the deposition of fibrillar amyloid-β protein (Aβ); (2) Affecting the clearing of A8 by astrocytes; (3) Facilitating the formation of neurofibrillary tangles. Studies have demonstrated the association between LRP and AG generation and clearing. Two common polymorphic loci of LRP gene are tetranucleotide polymorphism at 5' end and C766T polymorphism of the third exon. The correlation between tetranucleotide polymorphism at 5' end and AD is much disputed, while the correlation between C766T polymorphism of the third exon and AD has been demonstrated by several laboratories [9, 10].

In the present study, APOEε4 frequencies of AD group and VD group were higher than that of the control. MMSE scores of APOEε4 carriers in AD group and VD group were considerably lower than those of non-APOEε4 carriers in the two groups. This finding points to the similarities in pathogenesis of AD and VD, which agrees with previous results [11]. Psychotic symptoms are the core symptoms of dementia, showing substantial correlation with APOEε4 in AD according to many reports. However, the role of APOEε4 in VD has not been intensively studied. We found that the proportion of APOEε4 carriers presenting with BPSD was much higher than that of non-APOEε4 in AD group, but the difference was not significant for VD group. AD usually presents as extensive cognitive impairments, which are associated with cerebral cortex and limbic system atrophy, formation of senile plaques and neurofibrillary tangles. VD mainly presents as fluctuating, focal cognitive impairment, the severity of which is related to the position and severity of the lesions. However, it is still unknown whether the psychotic symptoms of VD are related to the position of the lesions. We found no significant differences in LRP C and T allele frequencies between AD group and VD group or in MMSE scores and BPSD occurrence of LRP T carriers and non-LRP T carriers for AD group and VD group. It is generally believed that LRP C766T polymorphism is not correlated with AD because the former does not change the first-level structure of the encoded products, but only causes a silencing of nucleotide sequence. We found that LRP C766T polymorphism was related to the differences in MMSE scores, but this needs to be verified by future studies given the limited sample size of the present paper.

In conclusion, APOEε4 may be the common risk factor for cognitive impairment in AD and VD and also the risk factor for BPSD in AD.

Disclosure of conflict of interest

None.

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