SNCA Gene Polymorphism may Contribute to an Increased Risk of Alzheimer's Disease

Quanbao Wang, Qian Tian, Xiaojie Song, Yunyong Liu, and Wei Li*

Department of Neurology, Linyi People's Hospital, Linyi, Shandong Province, China

Background: The purpose of this study is to elucidate the association between α -synuclein (SNCA) polymorphisms and the risk of Alzheimer's disease (AD). **Methods:** The PCR-RFLP was applied to detect SNCA gene rs6532190, rs3775430, and rs10516846 polymorphisms in 98 AD patients and 105 healthy elderly. **Results:** The GG frequency of rs10516846 was evidently increased in AD group than control group ($P < 0.05$). There was a significant difference in SNCA level between the AD and control groups ($P < 0.01$). In the AD group, the SNCA level in cerebrospinal fluid of GG (rs10516846) carriers was increased as compared with AA carriers ($P < 0.05$). The GG (rs10516846) fre-

quency of the early-onset AD group is significantly higher than that of the late-onset AD group ($P < 0.05$). The frequency of rs3775430 GG was lower in the early-onset group than that in the late-onset group (0% vs. 16.7%). The SNCA level in cerebrospinal fluid of GG (rs10516846) carriers in the early-onset AD group is higher than that of AA carriers ($P < 0.05$). **Conclusion:** SNCA gene polymorphism may be associated with an increased risk of AD and GG genotype of rs10516846 and elevated SNCA level in CSF may increase the risk of early-onset AD. J. Clin. Lab. Anal. 30:1092–1099, 2016.

© 2016 Wiley Periodicals, Inc.

Key words: Alzheimer's disease; Polymorphism; Risk; rs10516846; SNCA

INTRODUCTION

Alzheimer's disease (AD), also called primary degenerative dementia of the Alzheimer's type or senile dementia of the Alzheimer type, is a degenerative disorder of the central nervous system (1). The major clinical manifestations are progressive decline in memory, cognitive impairment, and neuropsychological disorders (2). AD patients commonly divided into early-onset patients aged 65 years or late-onset patients older than 66, which accounts for 94% of total AD patients (3). Genetic factors or environmental factors were reported to be involved in the formation of AD. So far, there were many genes related to AD widely reported, such as Cathepsin D Ala224Val gene, Apolipoprotein E (4,5). It is of great clinical diagnostic significance of early AD to find more new genes or genetic mutation sites.

The SNCA gene encodes α -synuclein, located on chromosome 4q21–23, to some extent, to presynaptic terminals that modulates vesicle trafficking as well as neurotransmitter release (6). In 1997, an association between α -synuclein and Parkinson's disease (PD) was initially reported when a missense mutation (A53T) in

SNCA caused autosomal dominant Parkinsonism (7). Subsequently, triplication of the SNCA gene was revealed to result in dominant early-onset PD, implying that overexpression of wild-type α -synuclein could cause neurodegenerative disease (8,9). Shortly thereafter, interest in the link between SNCA gene polymorphism and PD increased (10,11). At the same time, many researchers discovered that substantial aggregation of SNCA participated in the pathogenesis of several neurodegenerative diseases, such as PD, AD, multiple systems atrophy (MSA), and dementia with Lewy bodies (DLB) (12,13). Thus, here we put forward a hypothesis that SNCA gene polymorphism is associated with the susceptibility to AD. In this study, we investigated the association between SNCA single-nucleotide polymorphisms (SNPs), rs6532190, rs3775430, and rs10516846, and the risk of AD.

*Correspondence to: Dr. Wei Li, Department of Neurology, Linyi People's Hospital, No. 27 Jiefang Road, Linyi 276000, Shandong Province, China. E-mail: liwei_lwlw@yeah.net

Received 4 December 2015; Accepted 31 March 2016

DOI 10.1002/jcla.21986

Published online in Wiley Online Library (wileyonlinelibrary.com).

MATERIALS AND METHODS

Study Subjects

This study included 98 AD patients as an AD group from the outpatients and inpatients in Linyi People's Hospital between January 2014 and November 2014. The 98 AD patients were subgrouped as an early-onset AD group (<65 years old) and a late-onset AD group (≥65 years old). Inclusion criteria: (a) the age of patients are above 60 years old and all are Han nationality; (b) patients meet the criteria for AD diagnosis set by CCMD-3 and DSM-IV (14); (c) MMSE was applied to evaluate cognitive ability with scores less than 24 points (15); (d) patients whose immediate relatives do not have dementia or mental illness. Exclusion criteria: (a) vascular dementia; (b) systemic disease and dementia caused by substance intoxication; (c) pseudodementia and other senile dementia; (d) co-existed nervous system disorders; (e) patients in the acute stage of heart, vessels, lung, liver, and kidney, etc.; (f) medical history of sudden onset, early epilepsy, dystrophy, and focal neurological symptoms and early extrapyramidal symptoms; (g) other diseases causing hypomnesia or similar symptoms. At the same time, we selected 105 healthy elderly individuals as a control group from Han nationality without a family medical history of dementia, cognitive impairment, serious physical diseases, not being in the acute stage of heart, vessels, lung, liver kidney disorders, depression, and anxiety as control group with MMSE scores more than 28 points. This study was approved by the ethics committee of our hospital and all participants or their families in this study agreed and signed the informed consents.

Sample Collection

Venous blood (10 ml) was collected from the elbow of each subject after fasting for 10–12 hr in the early morning. Venous blood (5 ml) was anticoagulated by using ethylenediaminetetraacetic acid (EDTA) and then stored at -80°C . One hour later, samples were centrifuged (3,000 rpm) at room temperature for 10 min

and peripheral blood mononuclear cells were separated. We extracted genome DNA from leukocyte in peripheral blood using genomic DNA extraction kit (DP318-03) (Tiangen Biotech Co., Ltd, Beijing, China) (16). Other samples were also centrifuged (3,000 rpm) at room temperature for 10 min and stored at -80°C for further use. Lumbar puncture was performed to collect cerebral spinal fluid (CSF). And we vertically injected 0.5% lidocaine into the skin from the point of puncture to anesthetize. We withdrew the needle after reaching supraspinous ligament; after anaesthetization, we vertically pierced the needle into the back from the point of puncture and CSF flew out of the body after withdrawing the core needle when the resistance sharply reduced. We extracted three tubes (2–3 ml) of CSF (after extracting CSF, we inserted the core needle and withdrew the puncture needle. We used the antiseptic gauze to cover the punctured skin and slightly pressed the skin to prevent bleeding and taped the gauze and asked patients to lie down for 4–6 hr). CSF (if it was bloody, put it in a 2,500 rpm centrifuge for 3 min) was stored at -20°C for further use (<http://www.biomart.cn/experiment/648/651/43761.htm>).

SNP Detection

We chose SNP rs6532190, rs3775430, and rs10516846 of *SNCA* gene as the research target. PCR-RFLP method was used to test allelic polymorphism and genotype frequency distribution in different sites between AD group and control group. PCR primers were designed by Premier 5.0 and primers were synthesized by Sangon, Shanghai. Amplified loci, primer sequences, fragment length, annealing temperature, times of cycles were shown in Table 1. PCR reaction system contains 2 μl of $10 \times$ PCR reaction buffer, 2 μl of dNTP (2.5 mmol/L each), 0.5 μl of upstream primer (10 pmol/ μl), and 0.5 μl of downstream primer (10 pmol/ μl), 2.5U Platinum Taq DNA polymerase (Invitrogen, Shanghai) and 50 ng of genome DNA. PCR reaction condition: initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing for 30 s with annealing temperature of 58°C ,

TABLE 1. Primer sequences of three SNPs (rs65324190, rs3775430, and rs10516846) of *SNCA* gene

SNP	Primer	Sequence length (bp)	Annealing temperature ($^{\circ}\text{C}$)	Cycles
rs65324190	5'-TGAATGTCTACTTCTTTGTCTT-3'	345	58	32
	5'-GTGGTTTCCAGGAACCTTT-3'			
rs3775430	5'-CTTCATTACACCTGAAG-3'	232	58	32
	5'-AAAACACATAGAAATTTTA-3'			
rs10516846	5'-ATTGGGAGAGAGTGTA-3'	220	57	32
	5'-GGTCAGTTCTCAGGCA-3'			

SNP, single nucleotide polymorphism.

58°C, and 57°C, and an extension at 72°C for 40 s, total 32 cycles and a final extension at 72°C for 10 min. Restriction enzyme reaction system (total 20 µl) was as follows: PCR product (17 µl), BlnI (10 U/µl) (Takara Biotechnology (Dalian) Co., LTD., China)/Bse8I (10 U/µl) (MBI Fermentas, Hanover, MD)/BtrI (5 U/µl) (NEB Company, Beverly, MA) 1 µl, buffer 2 µl. After mixing well, the reaction system was subject to water bath for 6 hr, followed by the addition of 2 µl 10× loading buffer to stop reaction. Amplified products were electrophoresed on 3% agarose gel. Amplified products were sent to Invitrogen for sequencing and SNP genotype was identified according to the sequencing results.

Protein Detection

We determined the SNCA level in CSF of all subjects using the SNCA ELISA kit (YS01449B, Shanghai Yaji Biological Technology Co., Ltd., Shanghai, China). We determined the OD value of each hole with a wavelength of 450 nm and used the concentration of standard products and OD value to calculate the linear regression equation of standard curve and finally calculated the concentration of samples. All the samples were determined twice with the average obtained.

Statistical Analysis

All data were analyzed by using SPSS.18.0 (IBM Corporation, Somers, NY, USA). Measurement data were presented with mean ± standard deviation (mean ± SD); comparisons between two groups were conducted using *t*-test or variance analysis. Count data were presented with percentage and tested by chi-square test; genotype frequency distribution of different SNP was evaluated whether they corresponded to Hardy–Weinberg equilibrium by chi-square test. Difference on genotype frequency between two groups were analyzed using unconditional logistic regression to calculate odds ratio (OR) and 95% confidence interval (CI). Binary logistic regression analysis was employed to elucidate the association between age, SNCA level in CSF, gender, three SNPs, and the risk of AD. *P* < 0.05 was considered as statistically significant.

RESULTS

Baseline Information

The AD group covered 98 patients consisting of 31 males and 67 females, who aged 61–90 years with a

TABLE 2. Baseline information of subjects in the early-onset AD and late-onset AD groups

	Early-onset AD (n = 26)	Late-onset AD (n = 72)	<i>t</i> / χ^2	<i>P</i>
Gender (M/F)	8/18	23/49	0.01	0.912
Age	68.4 ± 3.7	76.8 ± 6.4	6.31	<0.01
Course of disease	6.3 ± 2.7	5.0 ± 2.9	1.99	0.049
MMSE score	16.6 ± 4.3	17.7 ± 4.1	1.16	0.25

AD, Alzheimer's disease; M, male; F, female.

mean age of 74.5 ± 6.9 years. Their courses of diseases were between 1 and 10 years with mean of 5.3 ± 2.9 years. The control group included 105 subjects (39 males and 66 females) whose age ranged from 60 to 88 years, with a mean age of 73.9 ± 7.5 years. No significant difference was observed between the AD group and control group in terms of gender ($\chi^2 = 0.681$), age (*t* = 1.304) and systemic diseases (all *P* > 0.05). The AD group was subgrouped as an early-onset AD group (<65 years old) and a late-onset AD group (≥65 years old), and these two subgroups exhibited significant differences in age and course of disease (both *P* < 0.05), but not in the gender and MMSE score (both *P* > 0.05) (Table 2).

SNCA SNPs Between the AD Group and Control Group

The genotype frequency distribution of SNCA SNP rs6532190, rs3775430, and rs10516846 in the AD group and control group corresponded to Hardy–Weinberg equilibrium (all *P* > 0.05), which showed that genotype frequency of three SNPs were at equilibrium and representative. As shown in Table 3, the differences in genotype distribution frequency of rs10516846 between AD group and control group were statistically significant (*P* < 0.05); mutant genotype frequency in AD group (42.9%) was significantly higher than control group (29.5%) (*P* < 0.05). The differences were not statistically significant between genotype distribution frequency of rs10516846 and rs3775430 between AD group and control group (both *P* > 0.05). Furthermore, the differences in recessive models of rs10516846 were statistically significant between two groups (GG vs. AG + AA, *P* = 0.048, OR = 1.790, 95% CI = 1.003–3.197); the differences in rs10516846 allele distribution were statistically significant (G vs. A, *P* = 0.022, OR = 1.587, 95% CI = 1.068–2.358).

Comparison of SNCA Expression Levels

The SNCA expression levels were higher in the AD group (95.1 ± 8.3 ng/ml) than those in the control

TABLE 3. Comparison of genotype frequency among three SNPs (rs65324190, rs3775430, and rs10516846) of *SNCA* gene in the control and AD groups

Genotype	Control group (n = 105) (%)	AD group (n = 98) (%)	P	OR	95% CI
rs65324190 (A > T)					
AA	54 (51.4)	56 (57.1)	Ref		
AT	39 (37.1)	37 (37.8)	0.766	0.915	0.510–1.642
TT	12 (11.4)	5 (5.1)	0.099	0.402	0.133–1.217
Dominant	AA	56 (57.1)	Ref		
	AT + TT	42 (42.9)	0.415	0.795	0.4569–1.382
Allele	A	147 (70.0)	Ref		
	T	47 (24.0)	0.173	0.737	0.474–1.144
rs3775430 (A > G)					
AA	57 (54.3)	49 (50.0)	Ref		
AG	39 (37.1)	37 (37.8)	0.743	1.104	0.612–1.991
GG	9 (8.6)	12 (12.2)	0.360	1.551	0.603–3.991
Recessive	AG + AA	86 (87.8)	Ref		
	GG	12 (12.2)	0.391	1.488	0.598–3.706
Allele	A	153 (72.9)	Ref		
	G	61 (31.1)	0.378	1.213	0.790–1.863
rs10516846 (A > G)					
AA	29 (27.6)	18 (18.4)	Ref		
AG	45 (42.9)	38 (38.8)	0.408	1.360	0.656–2.823
GG	31 (29.5)	42 (42.9)	0.040	2.183	1.032–4.618
Recessive	AG + AA	56 (57.1)	Ref		
	GG	12 (12.2)	0.048	1.790	1.003–3.197
Allele	A	103 (49.0)	Ref		
	G	107 (51.0)	0.022	1.587	1.068–2.358

AD, Alzheimer's disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Fig. 1. Comparison of SNCA level in CSF between the AD group and control group. Note: AD, Alzheimer's disease; CSF, cerebral spinal fluid.

group (67.4 ± 7.8 ng/ml) ($P < 0.01$) (Figure 1). In the AD group, the SNCA level in CSF of rs10516846 genotype carriers was significantly higher than that of genotype AA carriers ($P < 0.05$), while the SNCA level in CSF of rs10516846 genotype AG carriers and AA carriers had no statistical significance ($P > 0.05$). In the AD group, the SNCA level in CSF of rs6532190 mutant AT and TT carriers and rs3775430 mutant AG and GG carriers had no statistical significance as

TABLE 4. Comparison of SNCA level of cerebrospinal fluid of three SNPs (rs65324190, rs3775430, and rs10516846) of *SNCA* gene (ng/ml)

Genotype	SNCA level	P	t	95% CI
rs65324190				
AA (n = 56)	94.2 ± 8.9	Ref		
AT (n = 37)	96.4 ± 7.7	0.222	1.230	−1.354 to 5.754
TT (n = 5)	95.2 ± 4.8	0.806	0.247	−7.110 to 9.110
rs3775430				
AA (n = 49)	94.2 ± 9.1	Ref		
AG (n = 37)	96.2 ± 8.0	0.291	1.062	−1.745 to 5.745
GG (n = 12)	95.3 ± 5.6	0.691	0.399	−4.415 to 6.615
rs10516846				
AA (n = 18)	91.9 ± 8.6	Ref		
AG (n = 38)	95.3 ± 9.0	0.186	1.339	−1.692 to 8.492
GG (n = 42)	96.3 ± 7.3	0.047	2.027	0.056 to 8.744

t, Student's *t*; CI, confidence interval; SNP, single nucleotide polymorphism.

compared with those of AA carriers (all $P > 0.05$), as shown in Table 4.

Association Between SNCA SNPs and Early-Onset and Late-Onset AD

As shown in Table 5, the differences were statistically significant in the recessive model (GG vs.

TABLE 5. Comparison of genotype frequency of three SNPs (rs10516846, rs6532190, and rs3775430) in the late-onset AD and early-onset AD groups

Genotype	Late-onset AD (n = 72)	Early-onset AD (n = 26)	<i>P</i>	OR	95% CI
rs10516846					
AA	22 (30.6%)	5 (19.2%)	Ref		
AG	25 (34.7%)	4 (15.4%)	0.630	0.704	0.168–2.955
GG	25 (34.7%)	17 (65.4%)	0.0561	2.992	0.947–9.452
AG + AA	47 (65.3%)	9 (34.6%)	Ref		
GG	25 (34.7%)	17 (65.4%)	0.007	3.551	1.383–9.115
A	69 (47.9%)	14 (26.9%)	Ref		
G	75 (52.1%)	38 (73.1%)	0.008	2.497	1.247–5.002
rs6532190					
AA	42 (58.3%)	14 (53.8%)	Ref		
AT	26 (36.1%)	11 (42.3%)	0.615	1.269	0.501–3.214
TT	4 (5.6%)	1 (3.9%)	0.804	0.750	0.077–7.287
AT + AA	68 (94.4%)	25 (96.1%)	Ref		
TT	4 (5.6%)	1 (3.9%)	0.734	0.680	0.072–6.383
A	110 (76.4%)	39 (75.0%)	Ref		
T	34 (23.6%)	13 (25.0%)	0.841	1.078	0.516–2.252
rs3775430					
AA	34 (47.2%)	15 (57.7%)	Ref		
AG	26 (36.1%)	11 (42.3%)	0.928	0.959	0.378–2.433
GG	12 (16.7%)	0 (0.0%)	0.027	0.089	0.005–1.602
AG + AA	60 (83.3%)	26 (100%)	Ref		
GG	12 (16.7%)	0 (0.0%)	0.026	0.091	0.005–1.601
A	94 (65.3%)	41 (78.8%)	Ref		
G	50 (34.7%)	11 (21.2%)	0.070	0.504	0.239–1.067

AD, Alzheimer's disease; OR, odds ratio; CI, confidence interval; Ref, reference; SNP, single nucleotide polymorphism.

AG + AA) and allele (G vs. A) at rs10516846 between the two subgroups (both $P < 0.01$). The frequency of rs3775430 GG was lower in the early-onset group than that in the late-onset group (0% vs. 16.7%, $P = 0.027$), and recessive model of rs3775430 (GG vs. AG + AA) also exhibited significant difference between the two subgroups ($P = 0.026$). However, there was no significant difference in the genotype frequency of rs6532190 between the two subgroups (both $P > 0.05$). As shown in Table 6, in the early-onset AD group, the SNCA level in CSF of rs10516846 GG carriers was significantly higher than that of AA carriers ($P < 0.05$); the differences were not statistically significant between AG carriers and AA carriers ($P > 0.05$). In late-onset AD group, the differences were not statistically significant among AG carriers and GG carriers and AA patients (both $P > 0.05$). In these two subgroups, the differences in the SNCA level in CSF between carriers of different genotype were not statistically significant (all $P > 0.05$). Further, there was no significant difference in the SNCA protein expressions between different genotypes of rs65324190 and rs3775430 in the early-onset AD and late-onset AD groups (all $P > 0.05$).

Logistic Regression Analysis of Risk Factors of AD

We used as the dependent variable, either AD patients or non-AD patients, and age, SNCA expression level in CSF, gender, rs6532190 AA and non-AA genotype, rs3775430, GG and non-GG genotype and rs3775430 as independent variables, and conducted duality regression analysis. The result showed that rs10516846 GG genotype ($P = 0.041$, OR = 1.858, 95% CI = 1.027–3.364), and elevated SNCA expression level in CSF ($P < 0.01$, OR = 1.617, 95% CI = 1.333–1.962) were risk factors of AD (Table 7).

DISCUSSION

Previous studies on SNCA gene polymorphism paid more attention to its correlation with PD (11,17). In this study, we explore the association of SNCA gene polymorphisms (rs6532190, rs3775430, and rs10516846) and the risk of AD. The present study results revealed that the correlation between rs10516846 and AD was evidently noted in the polymorphism screening of SNCA in AD patients. And

TABLE 6. Comparison of SNCA level of cerebrospinal fluid of three SNPs (rs10516846, rs6532190, and rs3775430) (ng/ml)

SNP	AD type	Genotype	SNCA level	<i>P</i>	<i>t</i>	95% CI
rs10516846	Late-onset AD	AA (n = 22)	93.7 ± 8.5	Ref		
		AG (n = 25)	95.7 ± 8.7	0.431	0.795	−3.068 to 7.068
		GG (n = 25)	96.6 ± 7.4	0.218	1.251	−1.770 to 7.570
	Early-onset AD	AA (n = 5)	85.7 ± 8.1	Ref		
		AG (n = 4)	92.0 ± 12.6	0.5	0.712	−11.150 to 20.750
		GG (n = 17)	95.9 ± 7.5	0.034	2.274	0.720–16.680
rs6532190	Late-onset AD	AA (n = 42)	95.9 ± 8.3	Ref		
		AT (n = 26)	95.0 ± 8.6	0.67	0.429	−5.092 to 3.292
		TT (n = 4)	96.5 ± 3.5	0.888	0.142	−7.904 to 9.104
	Early-onset AD	AA (n = 14)	93.7 ± 8.8	Ref		
		AT (n = 11)	95.8 ± 8.4	0.552	0.604	−5.092 to 9.292
		TT (n = 1)	81.6 ± 0	–		
rs3775430	Late-onset AD	AA (n = 34)	95.3 ± 8.1	Ref		
		AG (n = 26)	95.8 ± 8.6	0.818	0.231	−3.838 to 4.838
		GG (n = 12)	96.1 ± 8.0	0.769	0.295	−4.664 to 6.264
	Early-onset AD	AA (n = 15)	93.4 ± 10.2	Ref		
		AG (n = 11)	93.9 ± 6.6	0.888	0.142	−6.774 to 7.774
		GG (n = 0)	0.0 ± 0.0	–		

SNP, single nucleotide polymorphism; AD, Alzheimer's disease; *t*, Student's *t*; CI, confidence interval; Ref, reference; SNP, single nucleotide polymorphism.

TABLE 7. Logistic regression analysis of risk factors of Alzheimer's disease

Dependent variable	SE	Wald	df	Sig	Exp (B)	95% CI
Age	0.020	0.368	1	0.544	1.012	0.973–1.053
Gender	0.308	0.954	1	0.329	1.350	0.739–2.467
(rs65324190 non-AA) genotype	0.292	1.118	1	0.290	0.734	0.414–1.302
(rs3775430 GG) genotype	0.482	0.934	1	0.334	1.593	0.620–4.096
(rs10516846 GG) genotype	0.303	4.188	1	0.041	1.858	1.027–3.364
SNCA level	0.099	23.785	1	0.000	1.617	1.333–1.962

SE, standard deviation; df, degrees of freedom; Sig: *P* value; Exp(B), adjusted odds; CI, confidence interval.

GG genotype was evidently associated to SNCA level in CSF.

Notably, our study found that the SNCA expression levels in CSF were higher in the AD group than those in the control group. The possible reasons about abnormalities of SNCA leading to neurodegeneration are as follows: α-synuclein localizes to the presynaptic terminal and binds to lipid membranes, which suggests that this protein is implicated in the function of synapses or synaptic turnover. Thus, the aggregation of SNCA or amyloid fibrils in the disease state results in the loss of normal functions; moreover, these effects could be amplified if other synaptic proteins interacting with α-synuclein are also sequestered or trapped in Lewy bodies and Lewy neurites, thereby disabling it

from performing its synaptic functions (18). Consistent with our study, Rockenstein et al. discovered that compared to controls, levels of α-synuclein were increased in cases of diffuse LBD and AD, suggesting that a critical balance among products of the synuclein gene is significant to maintain normal brain function and that changes in this balance were possibly associated with neurodegenerative disorders (19). Also, our results showed that mutation AA, mutation GG of rs10516846 was evidently different from mutation AA and significantly correlated with AD. And the difference was more evident in early AD. Compared with the expression level of AA genotype, the expression level of GG genotype was significantly increased in early or late AD in terms of expression of SNCA. And in early AD, the difference was even more significant. Thus, the mutation of rs10516846 may enhance the expression of SNCA. Zhang et al. showed that some sequences of introns can function like enhancers and cis-regulatory elements. Grundemann et al. also supported that the mutation of the introns will influence some proteins related to transcription initiation and extension and open functional chromosome domain and then finally enhance the expression of genes (20,21).

To the best of our knowledge, abnormally excessive accumulation of SNCA is one of the causes of AD because the protein concentration of SNCA had some influences on neurons. It would protect neurons at low concentrations but would contribute to neurovirulence and neuronal apoptosis at high concentration (22). Early reports of SNCA focused on its

correlation with PD, and demonstrated that excessive expression of SNCA often occurs in the prefrontal area midbrain of PD patients (21,23). While current studies exemplified by Spillantini et al., confirmed that the evidently high transcription level of SNCA, especially which appeared more evidently in LBV/AD patients, is consistent with the results of our study. SNCA is one of main elements of SNCA so the formation of AD may be decided by SNCA's influence on Lewy bodies (LB) (24). Excessive expression of SNCA contributes to AD because of the interaction of SNCA and LRRK (25). According to the description of previous studies, Spillantini et al. and Fujiwara et al. found that, during the process of LBV/AD occurrence and development, the expression level of SNCA protein was abnormally enhanced with unusual phosphorylation, which were evidenced by the fact that serine 129 was phosphorylated (25,26). The phosphorylation was caused by the effect of LPRK and was related with the excessive expression with LPRK in AD and PD (27). Therefore, in the brains of AD patients, SNCA protein was abnormally excessively accumulated, and LBV-related symptoms and AD-related symptoms were produced.

In summary, our study demonstrated that *SNCA* gene polymorphism may be associated with an increased risk of AD and GG genotype of rs10516846 and elevated SNCA level in CSF may increase the risk of early-onset AD. Therefore, this study exhibits some medical value for the diagnostic index of early AD for reference. Further studies with larger sample size are needed to elucidate the association between *SNCA* gene and the occurrence and development of AD on the basis of missense mutation, and duplication or triplication of *SNCA* gene.

ACKNOWLEDGMENTS

We would like to acknowledge the helpful comments from our reviewers on this paper.

REFERENCES

- Liu S, Zeng F, Wang C, et al. The nitric oxide synthase 3 G894T polymorphism associated with Alzheimer's disease risk: a meta-analysis. *Sci Rep* 2015;5:13598.
- Joshi YB, Giannopoulos PF, Pratico D. The 12/15-lipoxygenase as an emerging therapeutic target for Alzheimer's disease. *Trends Pharmacol Sci* 2015;36:181–186.
- van der Flier WM, Pijnenburg YA, Fox NC, et al. Early-onset versus late-onset Alzheimer's disease: the case of the missing APOE varepsilon4 allele. *Lancet Neurol* 2011;10:280–288.
- Paz YMC, Garcia-Cardenas JM, Lopez-Cortes A, et al. Positive association of the Cathepsin D Ala224Val gene polymorphism with the risk of Alzheimer's disease in Ecuadorian population. *Am J Med Sci* 2015;350:219–231.
- Al-Asmary SM, Kadasah S, Arfin M, et al. Apolipoprotein E polymorphism is associated with susceptibility to schizophrenia among Saudis. *Arch Med Sci* 2015;11:869–876.
- Cheng F, Vivacqua G, Yu S. The role of alpha-synuclein in neurotransmission and synaptic plasticity. *J Chem Neuroanat* 2011;42:242–248.
- Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045–2047.
- Singleton AB, Farrer M, Johnson J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 2003;302:841.
- Farrer M, Kachergus J, Forno L, et al. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann Neurol* 2004;55:174–179.
- Miyake Y, Tanaka K, Fukushima W, et al. SNCA polymorphisms, smoking, and sporadic Parkinson's disease in Japanese. *Parkinsonism Relat Disord* 2012;18:557–561.
- Guo XY, Chen YP, Song W, et al. SNCA variants rs2736990 and rs356220 as risk factors for Parkinson's disease but not for amyotrophic lateral sclerosis and multiple system atrophy in a Chinese population. *Neurobiol Aging* 2014;35(2882):e2881–e2886.
- Shannon KM, Keshavarzian A, Mutlu E, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord* 2012;27:709–715.
- Tateno F, Sakakibara R, Kawai T, et al. Alpha-synuclein in the cerebrospinal fluid differentiates synucleinopathies (Parkinson Disease, dementia with Lewy bodies, multiple system atrophy) from Alzheimer disease. *Alzheimer Dis Assoc Disord* 2012;26:213–216.
- Sarazin M, deSouza LC, Lehericy S, et al. Clinical and research diagnostic criteria for Alzheimer's disease. *Neuroimaging Clin N Am* 2012;22:23–32, viii.
- Neri AL, Ongaratto LL, Yassuda MS. Mini-Mental State Examination sentence writing among community-dwelling elderly adults in Brazil: text fluency and grammar complexity. *Int Psychogeriatr* 2012;24:1732–1737.
- Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. *Lancet* 1991;337:1158–1159.
- Simon-Sanchez J, van Hilten JJ, van de Warrenburg B, et al. Genome-wide association study confirms extant PD risk loci among the Dutch. *Eur J Hum Genet* 2011;19:655–661.
- Lee VM, Trojanowski JQ. Mechanisms of Parkinson's disease linked to pathological alpha-synuclein: new targets for drug discovery. *Neuron* 2006;52:33–38.
- Rockenstein E, Hansen LA, Mallory M, et al. Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease. *Brain Res* 2001;914:48–56.
- Zhang Y, Ma K, Song S, et al. Peroxisomal proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyl-transferase I (CPT-I alpha). *J Biol Chem* 2004;279:53963–53971.
- Grundemann J, Schlaudraff F, Haeckel O, et al. Elevated alpha-synuclein mRNA levels in individual UV-laser-microdissected dopaminergic substantia nigra neurons in idiopathic Parkinson's disease. *Nucleic Acids Res* 2008;36:e38.
- Cabeza-Arvelaiz Y, Fleming SM, Richter F, et al. Analysis of striatal transcriptome in mice overexpressing human wild-type alpha-synuclein supports synaptic dysfunction and suggests mechanisms of neuroprotection for striatal neurons. *Mol Neurodegener* 2011;6:83.
- Chiba-Falek O, Lopez GJ, Nussbaum RL. Levels of alpha-synuclein mRNA in sporadic Parkinson disease patients. *Mov Disord* 2006;21:1703–1708.
- Spillantini MG, Schmidt ML, Lee VM, et al. Alpha-synuclein in Lewy bodies. *Nature* 1997;388:839–840.

25. Fujiwara H, Hasegawa M, Dohmae N, et al. alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002;4:160–164.
26. Walker DG, Lue LF, Adler CH, et al. Changes in properties of serine 129 phosphorylated alpha-synuclein with progression of Lewy-type histopathology in human brains. *Exp Neurol* 2013;240:190–204.
27. Qing H, Wong W, McGeer EG, et al. Lrrk2 phosphorylates alpha synuclein at serine 129: Parkinson disease implications. *Biochem Biophys Res Commun* 2009;387:149–152.