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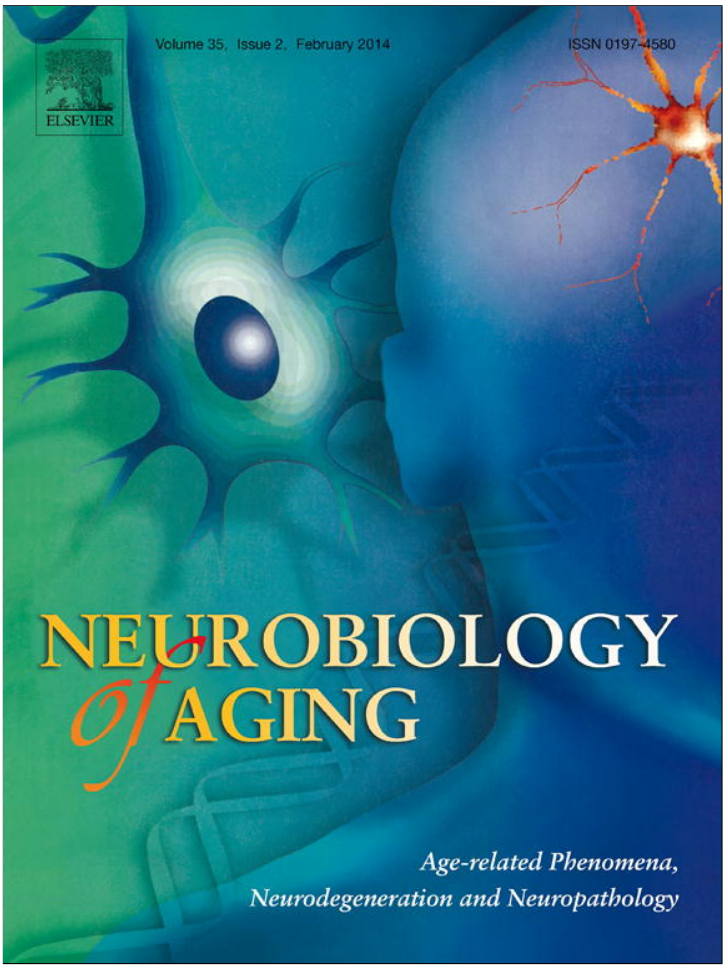

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Negative results

No association of the *LRRK2* genetic variants with Alzheimer's disease in Han Chinese individuals

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ABSTRACT

The leucine-rich repeat kinase–2 (*LRRK2*) gene has been regarded as 1 of the most common genetic causes of Parkinson's disease (PD). We hypothesized that *LRRK2*-susceptible allele(s) for PD might pose a risk for Alzheimer's disease (AD). In this study, we screened 12 *LRRK2* gene variants in 2 independent cohorts from southwestern China (341 AD patients and 435 normal individuals) and eastern China (297 AD patients and 384 normal individuals), to discern the potential association between this gene and AD. No variant was identified to be associated with AD in either case-control sample. As both of the cohorts were of Han Chinese origin, we combined the *LRRK2* variant data for the 2 sample sets together (a total of 638 AD patients and 819 normal individuals) and still found no association between the *LRRK2* gene and AD, suggesting that *LRRK2* gene variants may not affect the development of AD in Han Chinese individuals.
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1. Introduction

Alzheimer's disease (AD) is a prevalent neurodegenerative disease that leads mainly to severe memory loss in elderly persons (>60 years of age) (Querfurth and LaFerla, 2010). The pathogenesis of AD is multifactorial; both environmental and genetic factors can affect the development of AD (Querfurth and LaFerla, 2010).

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have been reported to be responsible for Parkinson's disease (PD) (Kett and Dauer, 2012). Because of the observed tau pathology in patients with *LRRK2* mutation and the very close location between the *LRRK2* gene and the reported AD-associated genomic region (Pericak-Vance et al., 1997; Zimprich et al., 2004), this gene was regarded as a potential candidate for AD in recent studies (Lee et al., 2006; Santos-Reboucas et al., 2008; Tan et al., 2009; Toft et al., 2005; Zhao et al., 2011). However, only 2 independent studies have identified potential risk variants in AD patients (Santos-Reboucas et al., 2008; Zhao et al., 2011), whereas the other studies showed that *LRRK2* mutations are not common in AD patients. As only 1 or 2 *LRRK2* mutations were analyzed in these previous studies, it remains elusive as to whether other genetic variants of the *LRRK2* gene are susceptible to AD.

To clarify the potential association between the *LRRK2* variants and AD, we screened 8 reported PD-associated variants and 4 Tag single nucleotide polymorphisms (SNPs) of the *LRRK2* gene in 341 AD patients and 435 healthy subjects from southwestern China. We did not identify any association of the *LRRK2* variants with AD. This result was further validated in an independent sample set (297 AD patients and 384 normal individuals) from eastern China, indicating that *LRRK2* gene variants may not affect the development of AD in individuals of Han Chinese origin.

2. Methods

Two independent sample sets, 1 from southwestern China and the other from eastern China, were recruited for this study. The

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Table 1
Allele and genotype frequencies of the 12 *LRRK2* SNPs in 341 AD patients and 435 normal controls from southwestern China, and in 297 AD patients and 384 normal controls from eastern China

SNP ID	Populations	Allele	No. of samples		*p* Value[a]	Genotype	No. of samples		*p* Value[a]	Adjusted *p* value[b]	HWE *p* value (control)
			Patients	Controls			Patients	Controls			
Rs732374[c,d]	Southwestern China	A/G	207/473	288/582	0.27	AA/GG/AG	38/171/131	46/193/196	0.18	0.09	0.72
	Eastern China		210/382	252/516	0.33		43/129/124	50/182/152	0.60	0.67	0.05
	Combined		417/855	540/1098	0.94		81/300/255	96/375/348	0.62	0.32	0.27
Rs34594498[c,d]	Southwestern China	C/T	672/8	861/9	0.81	CC/TT/CT	333/1/6	426/0/9	0.60	-	0.83
	Eastern China		588/4	759/9	0.41		292/0/4	375/0/9	0.41	-	0.82
	Combined		1260/12	1620/18	0.72		625/1/10	801/0/18	0.34	-	0.75
Rs4473003[d]	Southwestern China	C/T	245/437	288/582	0.26	CC/TT/CT	46/142/153	42/189/204	0.25	0.23	0.22
	Eastern China		180/412	264/504	0.13		23/139/134	50/170/164	0.09	0.09	0.30
	Combined		425/849	552/1086	0.87		69/281/287	92/359/368	0.98	0.97	0.87
Rs33939927[e]	Southwestern China	C/T	682/0	869/1	—	CC/TT/CT	341/0/0	434/0/1	-	-	-
	Eastern China		594/0	768/0	—		297/0/0	384/0/0	-	-	-
	Combined		1276/0	1637/1	—		638/0/0	818/0/1	-	-	-
Rs33949390	Southwestern China	C/G	15/667	20/850	1.00	CC/GG/CG	0/326/15	0/415/20	1.00	-	0.62
	Eastern China		8/586	7/761	0.45		0/289/8	0/377/7	0.45	-	0.86
	Combined		23/1253	27/1611	0.78		0/615/23	0/792/27	0.77	-	0.63
Rs35801418[e]	Southwestern China	A/G	682/0	870/0	—	AA/GG/AG	341/0/0	435/0/0	-	-	-
	Eastern China		594/0	768/0	—		297/0/0	384/0/0	-	-	-
	Combined		1276/0	1638/0	—		638/0/0	819/0/0	-	-	-
Rs7298930	Southwestern China	A/C	386/296	467/403	0.26	AA/CC/AC	112/67/162	124/92/219	0.43	0.33	0.80
	Eastern China		308/286	427/339	0.15		83/72/142	125/81/177	0.37	0.23	0.22
	Combined		694/582	894/742	0.91		195/139/304	249/173/396	0.95	0.93	0.50
Rs34637584[e]	Southwestern China	A/G	0/682	0/870	—	AA/GG/AG	0/341/0	0/435/0	-	-	-
	Eastern China		0/594	0/768	—		0/297/0	0/384/0	-	-	-
	Combined		0/1276	0/1638	—		0/638/0	0/819/0	-	-	-
Rs35870237[e]	Southwestern China	A/G	682/0	870/0	—	AA/GG/AG	341/0/0	435/0/0	-	-	-
	Eastern China		594/0	768/0	—		297/0/0	384/0/0	-	-	-
	Combined		1276/0	1638/0	—		638/0/0	819/0/0	-	-	-
Rs7307310[c]	Southwestern China	C/T	444/236	588/282	0.36	CC/TT/CT	149/45/146	196/43/196	0.35	0.24	0.55
	Eastern China		406/188	507/261	0.38		137/28/132	168/45/171	0.60	0.50	0.88
	Combined		850/424	1095/543	0.97		286/73/278	364/88/367	0.87	0.71	0.75
Rs34778348	Southwestern China	A/G	18/664	20/850	0.74	AA/GG/AG	0/323/18	0/415/20	0.74	-	0.62
	Eastern China		15/579	35/733	0.06		0/282/15	3/352/29	0.13	-	0.01
	Combined		33/1243	55/1583	0.23		0/605/33	3/767/49	0.31	-	0.03
Rs3761863	Southwestern China	A/G	390/292	484/386	0.57	AA/GG/AG	115/66/160	132/83/220	0.54	0.38	0.61
	Eastern China		324/270	436/332	0.44		90/63/144	126/74/184	0.72	0.47	0.64
	Combined		714/562	920/718	0.91		205/129/304	258/157/404	0.80	0.82	0.96

Key: AD, Alzheimer's disease; HWE, Hardy–Weinberg equilibrium; SNP, single nucleotide polymorphism.
[a] *p* Value was calculated by Fisher's exact test.
[b] Binary logistic regression analysis was performed to assess the association of *LRRK2* SNPs with AD, with an adjustment for APOE4 allele status. The most common genotype of each SNP in the control population was set as reference. Minor allele frequencies of rs34594498, rs33939927, rs33949390, rs35801418, rs34637584, rs35870237, and rs34778348 were too low and these SNPs were not included in the logistic regression analysis.
[c] One patient from southwestern China failed to be genotyped for SNPs rs732374, rs34594498, and rs7307310.
[d] One patient from eastern China failed to be genotyped for SNPs rs732374, rs34594498, and rs4473003.
[e] Minor allele frequencies of SNPs rs33939927, rs35801418, rs34637584, and rs35870237 were less than 0.01, and we did not include these SNPs in the analysis.

cohort from southwestern China was composed of 341 AD patients and 435 normal individuals who were collected at the Mental Health Center of West China Hospital. The cohort from eastern China was composed of 297 AD patients and 384 normal individuals who were collected at the Shanghai Mental Health Center and Tongde Hospital of Zhejiang Province. All subjects were of Han Chinese origin. The diagnosis of AD was performed following the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) and the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria. The healthy controls were confirmed to have normal cognitive function. Written informed consent conforming to the tenets of the Declaration of Helsinki was obtained from all participants before this study. The institutional review board of the Kunming Institute of Zoology, Chinese Academy of Sciences approved this study.

Eight previously reported disease-associated variants and 4 Tag SNPs (HapMap, http://hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) of the *LRRK2* gene were investigated. The information for each SNP is shown in Table S1. All 12 SNPs were detected by SNaPshot assay, which was composed of a multiplex polymerase chain reaction (PCR) of all of the SNPs and followed by a single-base extension process. The SNaPshot assay was

Table 2
Haplotype frequencies of 8 SNPs of the *LRRK2* gene in 341 AD patients and 435 healthy controls from southwestern China, and in 297 AD patients and 384 normal controls from eastern China

Haplotype[a]	Southwestern China		Eastern China		Combined	
	Patients	Controls	Patients	Controls	Patients	Controls
GCCGATGA	222	262	164	253	386	515
ACTGCCGG	167	230	177	199	344	429
GCTGACGA	113	147	108	136	221	283
GCTGCCGG	58	81	64	69	122	150
GCTGCCGA	28	39	17	21	45	60
GCTGACGG	19	18	8	14	27	32
ACTGCCAG	16	20	14	34	30	54
ACTCCCGG	15	18	-	-	15	18
Rare haplotypes[b]	44	55	42	42	86	97
p Value	0.96		0.10		0.88	

Key: AD, Alzheimer's disease; SNP, single nucleotide polymorphism.
[a] Order of SNPs for each haplotype: rs732374-rs34594498-rs4473003-rs33949390-rs7298930-rs7307310-rs34778348-rs3761863.
[b] Haplotypes with a frequency of less than 3% were pooled together.

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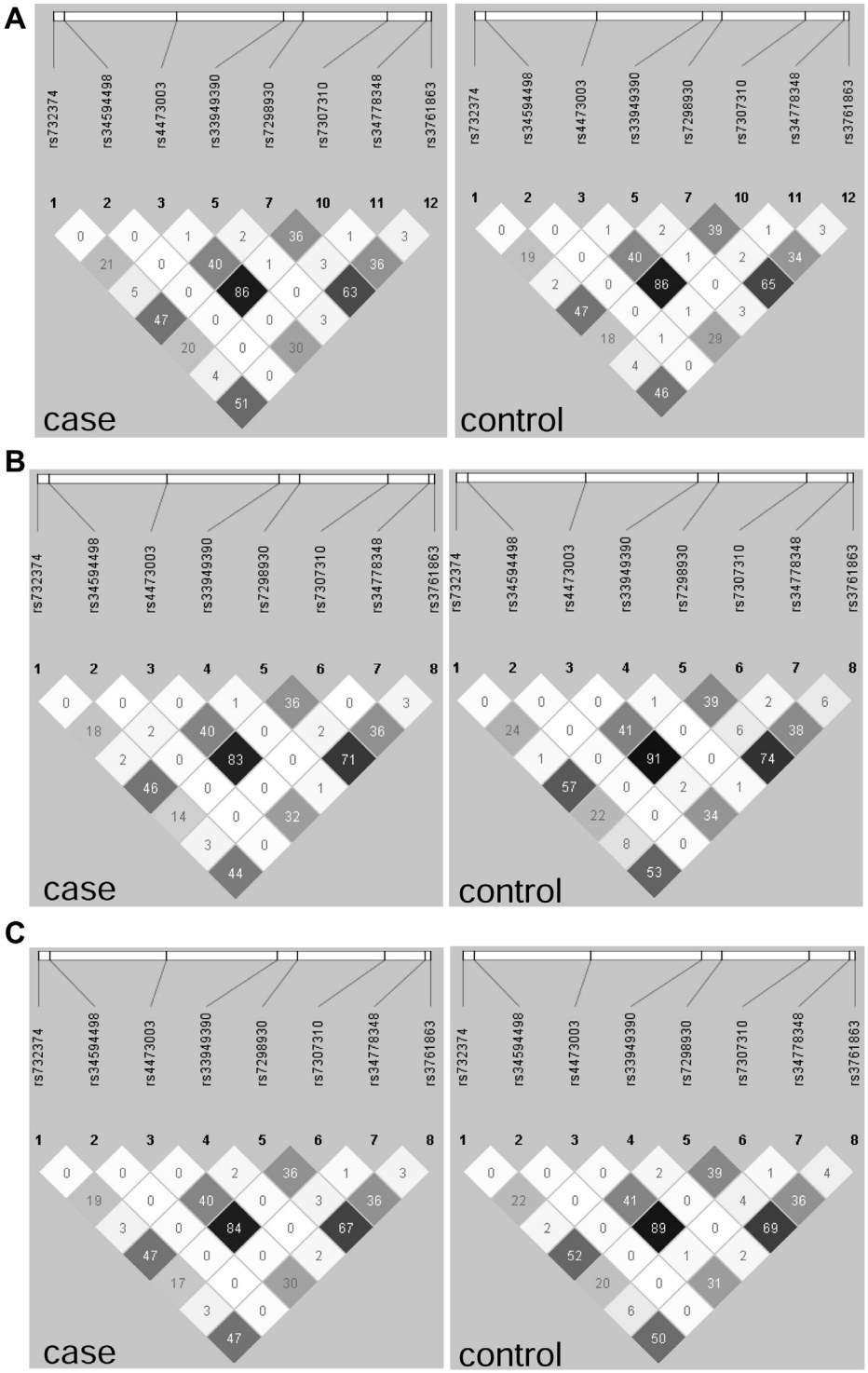

Fig. 1. Linkage disequilibrium (LD) pattern of 8 single nucleotide polymorphisms (SNPS) in the *LRRK2* gene in Alzheimer's disease (AD) patients and controls. Value in each square refers to $r^2 \times 100$. (A) LD pattern of 8 SNPS in the *LRRK2* gene in AD patients and controls from southwestern China. (B) LD pattern of 8 SNPS in the *LRRK2* gene in AD patients and controls from eastern China. (C) LD pattern of 8 SNPS in the *LRRK2* gene in AD patients and controls in the combined populations from southwestern China and eastern China.

performed following the detailed, step-by-step procedure described in our recent study (Wang et al., 2012). The ε4 allele of the apolipoprotein E (*APOE*) gene, which is considered to be a risk factor for AD, was also genotyped by using the SNaPshot assay. Primers for PCR and single-base extension are listed in Table S1. Binary logistic regression analysis was performed to assess the associations of *LRRK2* SNPs with the risk of AD, with adjustment for APOE4 allele status. The statistical power was calculated by using Quanto software (Gauderman, 2002) with log-additive inheritance mode. With a false-positive result rate controlled as 0.05 for minor allele frequency (MAF) from 0.1 to 0.5, the statistical power to detect the odds ratio (OR) as 1.5 for risk alleles was expected to be from 67.6% to 95.8%. A detailed Methods section is available in the online Supplementary data.

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3. Results

In total, we analyzed 12 *LRRK2* SNPs and the *APOE* genotypes by using SNaPshot in 2 independent sample sets from southwestern China (341 AD patients and 435 normal individuals) and eastern China (297 AD patients and 384 normal individuals), respectively (Fig. S1). The *APOE* genotyping result confirmed the previous finding that APOE allele ε4 is a risk factor for AD (Table S2). The allele frequency of ε4 in our patient population from southwestern China (13.3%) was, in general, similar to those in previous studies in a Chinese population (11.8%) (Liu et al., 1999) and a Mongolian population (17%) (Huriletemuer et al., 2010), whereas the patient population from eastern China presented with a higher allele frequency of ε4 (24.6%). Four of the 12 *LRRK2* SNPs (rs33939927, rs35801418, rs34637584, and rs35870237) had a MAF of less than 0.01 (Table 1). The genotype, allele and haplotype frequency of the other 8 SNPs were calculated (Tables 1 and 2). None of these 8 SNPs showed any deviation from Hardy–Weinberg equilibrium in the control population of either sample set (Table 1). In both cohorts, LD structures of the 8 SNPs were similar in the patient and control populations (Fig. 1A and B), and no significant difference was observed between patients and controls for the genotype, allele, and haplotype frequency of the 8 SNPs (Tables 1 and 2). Logistic regression analysis revealed that, after adjustment for APOE4 status, there was still no statistically significant difference for the *LRRK2* SNPs between the patient and control populations in either cohort (Table 1).

As both of the cohorts were of Han Chinese origin, we further pooled the *LRRK2* variant data of the 2 sample sets together (a total of 638 AD patients and 819 normal controls). There was still no variant identified to be associated with AD (Tables 1 and 2, Fig. 1C).

4. Discussion

Previous studies of the *LRRK2* mutations in AD patients have yielded controversial results. Some studies showed an infrequent occurrence of the *LRRK2* mutations in AD (Lee et al., 2006; Tan et al., 2009; Toft et al., 2005), whereas others argued for an important role of the *LRRK2* gene in AD (Santos-Reboucas et al., 2008; Zhao et al., 2011). Specifically, mutation p.G2019S was identified in a Brazilian man who presented with clinical features of both AD and PD (Santos-Reboucas et al., 2008), which suggested that a certain part of the neurodegenerative pathway overlapped in the 2 diseases. Variant p.R1628P was regarded as a risk factor for AD in patients from Singapore (Zhao et al., 2011). It should be noted that most of those studies screened for only 1 or 2 *LRRK2* variants, and a thorough analysis considering more *LRRK2* variants is necessary to clarify the potential association between the *LRRK2* gene and AD.

In this study, we used the SNaPshot assay to genotype 12 *LRRK2* variants (including 8 reported disease-associated variants and 4 Tag SNPs) in 2 independent cohorts from southwestern China and eastern China, respectively. These genetic variants are located in the entire *LRRK2* gene region. Analysis for the matrilineal genetic components in the patient and control populations showed that our samples were well matched, and there was no apparent population stratification in our sample (author's unpublished data). Consistent with some previous studies (Lee et al., 2006; Toft et al., 2005), we found no AD patient with mutations p.R1441C (rs33939927), p.Y1699C (rs35801418), p.G2019S (rs34637584), and p.I2020T (rs35870237), indicating that these PD pathogenic mutations were very rare in Chinese patients with AD. For the other 8 disease-associated SNPs or Tag SNPs with a higher MAF, there was no significant difference between AD patients and controls with regard to the allele, genotype, or haplotype frequency in either sample set. After adjustment for the APOE allele ε4 status, no statistically significantly different result was obtained. Our results indicate that genetic variants in the *LRRK2* gene may not affect AD in Han Chinese individuals.

Our study has 2 limitations. First, the sample size was relatively small, which may preclude us from drawing a firm conclusion. Nonetheless, we remedied this limitation by screening the *LRRK2* genetic variants in 2 independent cohorts. Consistent results were obtained in these 2 independent sample sets in that no *LRRK2* variant was identified to be associated with AD. Moreover, we performed a combined analysis with the *LRRK2* variant data of the 2 cohorts, and still found no positive association between *LRRK2* variants and AD in the combined Han Chinese sample set (composed of 638 AD patients and 819 normal controls). Second, we lacked some demographical data, such as age, sex, and years of education in the study groups (AD and control groups), which prevented us from retrieving more information in the association analyses. Further study with a large sample size and sufficient demographic data shall be carried out to solidify our conclusion.

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.013.

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Supplementary Materials

No association of the *LRRK2* genetic variants with Alzheimer's disease in Han Chinese

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1. Introduction

Alzheimer's disease (AD) is a prevalent neurodegenerative disease, which mainly lead to severe memory loss in elderly people over 60 years old (Querfurth and LaFerla, 2010). The pathogenesis of AD is multifactorial, both environmental and genetic factors can affect the development of AD (Querfurth and LaFerla, 2010).

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have been reported to be responsible for Parkinson's disease (PD) (Kett and Dauer, 2012). Because of the observed tau pathology in patients with *LRRK2* mutation and the very close location between the *LRRK2* gene and the reported AD-associated genomic region (Pericak-Vance et al., 1997; Zimprich et al., 2004), this gene was regarded as a potential candidate for AD in recent studies (Chang et al., 2010; Lee et al., 2006; Li et al., 2012; Santos-Reboucas et al., 2008; Santos-Reboucas et al., 2009; Tan et al., 2009; Tedde et al., 2007; Toft et al., 2005; Zabetian et al., 2006; Zhao et al., 2011). However, only two independent studies had identified potential risk variants in AD patients (Santos-Reboucas et al., 2008; Zhao et al., 2011), whereas most of the other studies showed that *LRRK2* mutations were not common in AD patients. As only one or two *LRRK2* mutations were analyzed in these previous studies, it remains elusive whether other genetic variants of the *LRRK2* gene were susceptible to AD.

In order to clarify the potential association between the *LRRK2* variants and AD, we screened 8 reported PD-associated variants and 4 Tag SNPs of the *LRRK2* gene in 341 AD cases and 435 healthy subjects from Southwest China. We identified no association of the *LRRK2* variants with AD. This result was further validated in an independent sample set (297 AD cases and 384 normal individuals) from East China, indicating that *LRRK2* gene variants may not affect the development of AD in Han Chinese.

2. Materials and methods

2.1. Samples

A total of two independent sample sets, one was from Southwest China and the other one was from East China, were recruited in this study. The cohort from Southwest China was composed of 341 AD patients and 435 normal individuals which were collected at the Mental Health Center of West China Hospital. The cohort from East China, which was composed of 297 AD patients and 384 normal individuals, were collected at the Shanghai Mental Health Center and Tongde Hospital of Zhejiang Province. All subjects were of Han Chinese origin. The diagnosis of AD was performed following the DSM-IV and the NINCDS-ADRDA criteria. The healthy controls were confirmed to have normal cognitive function. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from all participants prior to this study. The institutional review board of the Kunming Institute of Zoology, Chinese Academy of Sciences approved this study.

2.2. SNP selection and genotyping

Eight previously reported disease-associated mutations/variants and four Tag SNPs (HapMap, http://hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) of the *LRRK2* gene were investigated in this study. The information of each SNP is shown in Table S1. All of the 12 SNPs were detected by SNaPshot assay which was composed of a multiplex PCR of all the SNPs and followed with a single-base extension process. The SNaPshot assay was performed following the detailed step-by-step

procedure described in our recent study (Wang et al., 2012). In brief, multiplex PCR was conducted in a volume of 8 μL reaction solution containing 20-50 ng template DNA, 0.4 mM dNTPs, 0.2-0.5 μM of each primer (Table S1), 2.0 mM $MgCl_2$ and 1.0 U of AmpliTaq Gold polymerase (Applied Biosystems). The PCR reaction was performed with the following procedures: a pre-denaturation cycle at 94 ^{o}C for 2 min; 40 amplification cycles of 94 ^{o}C for 30 s, 55 ^{o}C for 30 s and 72 ^{o}C for 1 min. After being cleaned up with 1.0 U of shrimp alkaline phosphatase (SAP) and 0.5 U of Exonuclease I (TaKaRa Biotechnology Co. Ltd., Dalian, China), 4 μL of the PCR products were used as DNA template for the single-base extension reaction, which was further supplemented with 5 μL SNaPshot Multiplex Ready Reaction Mix and 0.4-0.8 μM pooled SNP-specific oligonucleotide primers (Table S1) to a total volume of 10 μL. Twenty five cycles of single-base extension reaction were carried out with the following condition per cycle: 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. Products were purified by SAP (1.0 U) at 37°C for 40 min, followed by a heat inactivation at 75°C for 20 min. Mixture with 4 μL of products and 9 μL of Hi-DiTM formamide was analyzed with capillary electrophoresis on ABI PRISM TM 3730xl DNA analyzer (Applied Biosystems). The GeneMarker software was used to read the genotyping result (Holland and Parson, 2011).

2.3. APOE genotyping

The ε4 allele of the apolipoprotein E (*APOE*) gene, which is considered to be a risk factor for AD (Tsai et al., 1994), was also examined. The first nucleotide in the 112th and 158th amino acid of the APOE protein were analyzed by SNaPshot assay, primers for PCR and single-base extension were listed in Table S1.

2.4. Data analysis

The linkage disequilibrium (LD) structure of 8 SNPs (rs732374, rs34594498, rs4473003, rs33949390, rs7298930, rs7307310, rs34778348 and rs3761863) was constructed by using Haploview software version 4.2 (Barrett et al., 2005). Haplotypes consisting of these 8 SNPs were constructed through PHASE2.0 program (Stephens et al., 2001). The allele frequency, genotype frequency and haplotype frequency were compared between AD cases and controls by Fisher exact test (two tailed) or Pearson's chi-squared test by SPSS16.0 (SPSS Inc., Chicago, Illinois). Chi-square test was utilized to estimate the deviation from the Hardy–Weinberg equilibrium (HWE). Binary logistic regression analysis was performed with SPSS16.0 (SPSS Inc., Chicago, Illinois) to assess the associations of the *LRRK2* SNPs with the risk of AD, with an adjustment for APOE4 status (APOE4+, APOE4-). The statistical power was calculated by using the Quanto software (Gauderman, 2002) with log-additive inheritance mode. With false positive rate controlled as 0.05, for minor allele frequency (MAF) from 0.1 to 0.5, the statistical power to detect the odds ratio (OR) value as 1.5 for risk allele was expected to be from 67.6% to 95.8%.

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Table S1. Primers for genotyping 12 *LRRK2* SNPs and APOE alleles by using SNaPshot assay

SNP ID	Location (in LRRK2) and potential function	Primer (5'-3') [a]
rs732374	intron 7	F: CTATTTACATCAATAATTTTGAATGGTG
	Tag SNP	R: ATTGTCTAAGTAAGATAATTAAAGGACAGC
		E: $(gact)_1$AATTAAAATAGCTTAATATGATTGAGTAAA
rs34594498	c.C1256T, p.A419V	F: TCCATGCTGATGCATTCTT
	Disease associated	R: CCACTGCTTACCATTTTGTTCT
		E: t$(gact)_7$CTTCATCAAAGGAAGTTTTCCAGGCATCTG
rs4473003	intron 15	F: TTAATTATTCAGGATCACTAGTGTAAGG
	Tag SNP	R: AAAATAAGAGATACACCAGCAACTTAG
		E: t$(gact)_5$GACTTTGAAAGGAAAAATAGAAATATTCTC
rs33939927	c.C4321T/G, p.R1441C/G	F: CAGTTTGAAAGCAAACACAAGA
	Disease associated	R: TTGCTTCTCATCAGAAACATCC
		E: ct$(gact)_8$AAGAGGGTTTTGTGTCTTTCCCTCCAGGCT
rs33949390	c.G4883C, p.R1628P	F: TGAAAGTGGAAGGTTGTCC
	Disease associated	R: TGTGACATGTAGTTCTTTGGAAAT
		E: t$(gact)_6$GTCCAAAACACCCTAAGGGCATTATTTCGC
rs35801418	c.A5096G, p.Y1699C	F: TTCCCCATTGTGAGAACTCT
	Disease associated	R: TAATAAGTCTTCATTAGATACTTACCTCTCC
		E: act$(gact)_9$AAATTATCATCCGACTATATGAAATGCCTT
rs7298930	intron 38	F: AAATTAGGTGTATCCTTCTAAAAACATT
	Tag SNP	R: AAATGGAAAGAGGGAAAATTG
		E: $(gact)_4$CAGGTAGCGACTCCAGCATCTTTATATTAG
rs34637584	c.G6055A, p.G2019S	F: TTTTCACACTGTATCCCAATGC
	Disease associated	R: ATAGAATTATGAGACAGACCTGATCAC
		E: $(gact)_{11}$TTTATCCCCATTCTACAGCAGTACTGAGCA
rs35870237	c.C6059T, p.I2020T	F: TTTTCACACTGTATCCCAATGC
	Disease associated	R: ATAGAATTATGAGACAGACCTGATCAC
		E: t$(gact)_{12}$TTTATCCCCATTCTACAGCAGTACTGAGCA
rs7307310	intron 43	F: AAAGAGTATCAAGCACAGTTTAAAAATAC
	Tag SNP	R: TTAAACTGGAACCCAACTAGATCA
		E: act$(gact)_2$ATACATATTTGTCTTTTCATGTAATTTTAT
rs34778348	c.G7153A, p.G2385R	F: TAGCCCTGTTGTGGAAGTGT
	Disease associated	R: AACCACAGAATTTACCTTAAAAAGTG
		E: act$(gact)_{13}$GTGTGGGATAAGAAAACTGAAAAACTCTGT
rs3761863	c.T7190C, M2397T	F: TTCTGAAGGCAGAGGGTTT
	Disease associated	R: TAAATAGCATTTATCTCTTAATTGGTG
		E: TTGATTCCTTGTTTTCTTTTACC
APOE-112-158		F: ACAAATCGGAACTGGAGGAA
		R: GGCCAGGGAGCCCACAGT
		E-112: act$(gact)_3$GCTGGGCGCGGACATGGAGGACGTG
		E-158: t$(gact)_5$CCGCGATGCCGATGACCTGCAGAAG

[a] In the "$(gact)_n$", n means repeats of "gact". F: forward primer; R: reverse primer; E: extension primer.

Table S2. Genotyping results of the *APOE* gene

Haplotype [a]	Isoform	Southwest China		*P*-value	OR (95%CI)	East China		*P*-value	OR (95%CI)
		Case [b]	Control			Case [b]	Control		
CC	ε4	88	86	0.04	1.39 (1.02-1.91)	144	67	$9.24x10^{-14}$	3.20 (2.33-4.37)
TC	ε3	515	716			403	591		
TT	ε2	61	68			39	66		
Total		664	870			586	724		
	ε4+	82	81	0.05	1.43 (1.01-2.03)	124	63	$2.03x10^{-10}$	2.82 (2.03-3.90)
	ε4-	250	354			169	299		
	Total [b]	332	435			293	362		

[a] The order of variants for each haplotype: the first nucleotides of the codon that coded the 112th and 158th amino acid of the APOE protein, respectively.

[b] Nine patient samples from Southwest China, four patient samples and 22 control samples from East China failed to be genotyped.

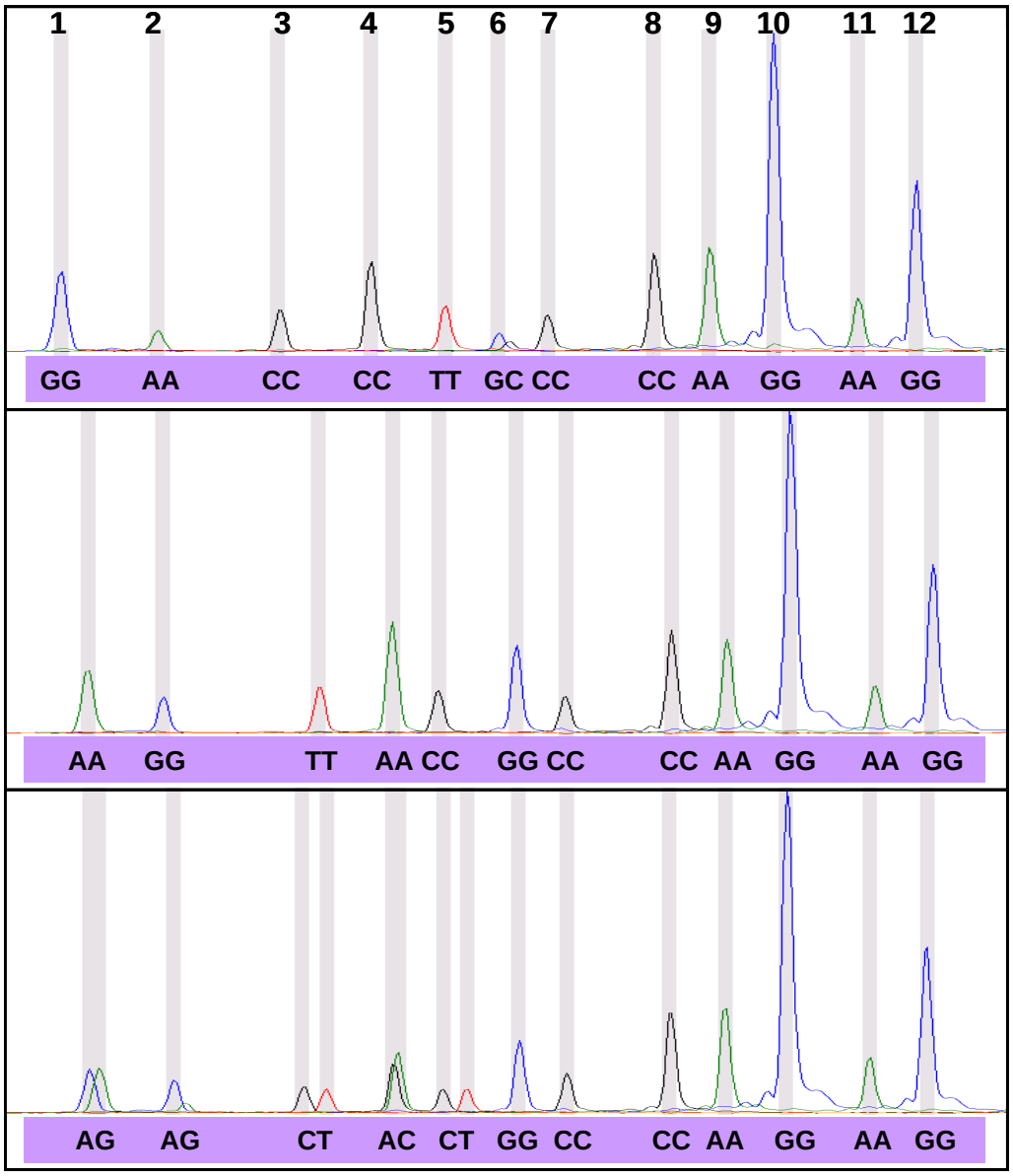

Fig. S1. SNaPshot profile of 12 *LRRK2* SNPs analyzed in this study. 1. rs3761863; 2. rs732374; 3. rs7307310; 4. rs7298930; 5. rs4473003; 6. 33949390; 7. rs34594498; 8. rs33939927; 9. rs35801418; 10. rs34637584; 11. rs35870237; 12. rs34778348.