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ORIGINAL ARTICLE

Association studies of 19 candidate SNPs with sporadic Alzheimer's disease in the North Chinese Han population

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Abstract Genome-wide association studies (GWAS) identified multiple single-nucleotide polymorphisms (SNPs) that are associated with the pathogenesis of Alzheimer's disease (AD). As replication in independent studies remains the only way to validate proposed GWAS signals, we detect SNPs reported in the GWAS, in order to explore their association with sporadic AD (SAD) in the Chinese population. We analyzed genotype and allele distributions of 19 SNPs reported in GWAS in 191 SAD patients and 180 healthy controls. We found that higher frequencies of rs10868366 G and rs7019241 C carriers were observed in SAD patients compared with controls (rs10868366 G: $P = 0.026$, odds ratio (OR) = 1.4, 95% confidence intervals (CI) 1.0–1.9; rs7019241 C: $P = 0.019$, OR 1.4, 95% CI 1.6–1.9). Furthermore, rs10868366 G/T and rs7019241 C/T in GOLPH2 were in strong linkage disequilibrium and formed a relative protective factor rs10868366 T/rs7019241 T and a relative risk factor rs10868366 G/rs7019241 C. For SNP rs3826656 in near gene 5′ region of CD33, the results revealed that in subjects with APOE ε4 alleles, the A allele was associated with a reduced risk of SAD compared with the G allele (OR 0.479; 95% CI 0.263–0.870, $P = 0.015$), and AA genotype was associated with a reduced risk of SAD compared with the genotype AG + GG (OR 0.395; 95% CI 0.158–0.659, $P = 0.008$). Our results support the view that rs10868366 and rs7019241 in GOLPH2 and rs3826656 in near gene 5′ region of CD33 are significantly associated with SAD in the north Chinese Han population.

Keywords Alzheimer's disease · Genome-wide association studies (GWAS) · GOLPH2 · Polymorphisms · rs3826656 · Replication

Introduction

Alzheimer's disease (AD) is the most prevalent form of progressive dementia in the elderly population. Like several other neurodegenerative diseases, AD is influenced by both genetic and environmental factors. Genetic studies have shown that three major genes, APP, PSEN-1, and PSEN-2, are implicated in the familiar early-onset form of AD [1], whereas the apolipoprotein E (APOE) ε4 allele has been reported to be a risk factor for both familial AD (FAD) and sporadic AD (SAD) [2]. However, the APOE ε4 allele only accounts for a fraction of SAD patients, which indirectly suggests that other susceptibility genes may exist.

Genome-wide association studies (GWAS) may be useful for identifying novel genes and single-nucleotide polymorphisms (SNPs) that affect the risk of complex diseases. To identify SAD susceptibility genes, a series of GWAS using Caucasian populations as the study subjects were conducted in recent years, and multiple gene polymorphisms associated with AD have been reported. A GWAS in 2007 found that five loci located in the GALP (rs3745833), TNK1 (rs1554948), PCK1 (rs8192708), and

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PGBD1 (rs3800324) genes and a locus with unknown function in 14q32.13 (rs11622883) were associated with AD pathogenesis in British and American populations [3]. In addition, in 2007 Reiman et al. [4] conducted a GWAS and reported that six loci in the GAB2 gene were associated with the pathogenesis of AD4. Also, a GWAS conducted in 2008 based on Canadian and British populations showed that two polymorphic sites in the GOLPH2 gene, rs10868366 and rs7019241, were associated with onset of SAD [5]. There have been several other GWAS targeting populations of the United States, England, Canada, Belgium, Finland, Italy, and Spain which reported that a few polymorphic sites in the CLU, PICALM, LRAT, PCDH 11X, CD33 genes were also associated with the pathogenesis of AD [6–14]. These reports are breakthrough studies on AD susceptibility genes. However, because different nations and regions have strong heterogeneity in genetics and phenotypes of SAD, as well as the fact that SNPs allele frequency distribution is different among diverse populations, population association studies on these SAD-related loci identified through GWAS have been carried out in different regions and ethnic groups [15–17]. Nevertheless, there have been few reports related to the Asian population [18–21]. As the follow-up and replication of GWAS findings is the only way to validate proposed GWAS signals [22], our study used samples from the North Chinese Han population to detect SNPs reported in the above GWAS (Table 1), in order to explore their association with SAD in the Chinese population.

Materials and methods

Subjects

The study included 191 SAD patients (88 men and 103 women; mean age: 73.1 ± 8.9 years; mean age at onset: 66.7 ± 7.5 years) who were enrolled from the Xuan Wu Hospital of the Capital Medical University and from the Beijing Senile Hospital in Beijing City. Medical interviews, physical examinations, blood tests, brain magnetic resonance imaging (MRI), and neuropsychological assessments, which included the Mini-Mental State Examination (MMSE), Clinical Dementia Rating (CDR), Hachinski Ischemic Scale (HIS), Activity of Daily Living scales (ADL), and Hamilton Depression Scale (HDS), were performed. All patients were diagnosed as having "probable AD" according to the National Institute of Neurologic and Communicative Disorders and Stroke-AD and Related Disorders Association (NINCDS-ADRDA) criteria [23]. None of these patients reported a family history of AD. The control group consisted of 180 healthy subjects (85 men and 95 women; mean age: 74.3 ± 5.6 years) who were recruited from the outpatient department of Xuan Wu Hospital in Beijing. They underwent regular health examinations, and were confirmed to be healthy and neurologically normal by medical history, physical examination, and neuropsychological assessment including the MMSE, a clinical memory scale (CMS), and the HDS to rule out cognitive deficit. All the SAD patients and control subjects were unrelated Han Chinese residents from the North of China. Informed consent was obtained for each subject, either directly or from his or her guardian. The protocol of this study was approved by the Institute Ethical Committee.

SNP selection

We selected for this study AD-associated polymorphic sites reported in recent GWAS. Expecting loci in APOC1, SORL1, and other genes reported by our laboratory previously [24] and loci with a minor allele frequency (MAF) of less than 5% in the Chinese population, we chose a total of 19 loci in this study (Table 1).

Genotyping

Genomic DNA was isolated from peripheral blood samples by a salting-out procedure [25], and all DNA samples were normalized to 50 ng/μl. The genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry(MALDI-TOF MS) assay (Mass ArrayTM, Sequenom Inc., San Diego, CA, USA). Polymerase chain reaction (PCR) and extension primers were designed using the Sequenom MassARRAY Assay Design software (primer sequences shown in Table 1). Genotype calls were made using SEQUENOM Typer 4.0 software with the post-processing calling procedure. Ten percent of randomly selected DNA samples from both patients and controls were sequenced to validate the genotyping by MALDI-TOF MS.

All subjects were genotyped for APOE using methods previously described [26].

Statistical analyses

Hardy–Weinberg equilibrium (HWE) was tested at http://analysis.bio-x.cn/myAnalysis.php. Allelic and genotypic distributions in SAD patients and controls were assessed by chi-square test. Genotype and allele frequencies for each SNP were also stratified by the presence of the APOE ε4 allele. The results were adjusted for age, sex, and APOE ε4 status using multiple logistic regression models. Analyses were performed using the Statistical Package for the Social Science (SPSS) version 13.0. Linkage disequilibrium was checked using EH program and D' and r^2 were calculated at http://analysis.bio-x.cn/myAnalysis.php. The odds ratio

Table 1 General information and primer sequences of the 19 SNPs

SNPs	Ref. SNP	Chromosome and gene	Type	1st sequence of the PCR amplification primers	2nd sequence of the PCR amplification primers	Extension primers sequence
1	rs10868366	Chr9_GOLPH2	Intron	ACGTTGGATGTCACAATCTTCTGCAGCAGG	ACGTTGGATGGTCAGGCATTAAAAAGTTTG	aAGCAGGAGAATGCCT
2	rs7019241	Chr9_GOLPH2	Intron	ACGTTGGATGCAGCCCAGTCCTAGCAAAG	ACGTTGGATGTAGGGATGAGGAACTTGGTC	gcaggCCTAGCAAAGGATCCC
3	rs3745833	Chr19_GALP	Exon	ACGTTGGATGGGAGGACGCAGGAGAAGAC	ACGTTGGATGTTGAGATCCTAGACCTGTGG	CCCCGCTCACTCACC
4	rs1554948	Chr17_TNK1	Exon	ACGTTGGATGACAAAGTCGAAGTGCTCTGG	ACGTTGGATGTCCAGTTGGCCCAGTTTTAC	cccctCCGAGTGACATTAAGCTCCTC
5	rs3800324	Chr6_PGBD1	Exon	ACGTTGGATGGAGAAGTGGTCACATCTGAG	ACGTTGGATGTGAGGCTCATAACTGTCTCC	ggCGGAGGAACCTCTGTG
6	rs11622883	Chr14q32.13Near SERPINA13	Intergenic	ACGTTGGATGTTAGGCTCTTTGCTTCCTCG	ACGTTGGATGAAGGAAGGCTCAACTCGGG	cGCTTCCTCGCATTGAA
7	rs10793294	Chr11_GAB2	Intron	ACGTTGGATGCAGTATTTTGTAGGCAGCCA	ACGTTGGATGCAGAGCCTGTCAGGTTTTGC	AGGCAGCCATTTATCA
8	rs2373115	Chr11_GAB2	Intron	ACGTTGGATGGCTTGTAGACTTATGCGGAC	ACGTTGGATGTCTTTTTTGTGTATGCAAG	GGACATGGATTTATAGTCCG
9	rs1007837	Chr11_GAB2	Intron	ACGTTGGATGTGGAGCAGGTAGCAAGTGTT	ACGTTGGATGTCCTCAAGGAAATCACAGTC	tGCACCATTACTACTTATCTC
10	rs7101429	Chr11_GAB2	Intron	ACGTTGGATGAGCAGGTTGTACTAAAAAG	ACGTTGGATGGCAGGGTGAGTTGAAGTAAC	ACTTGTTTTTCTGCTACTTAC
11	rs4945261	Chr11_GAB2	Intron	ACGTTGGATGTCACAGAATTCCTAGGTTAG	ACGTTGGATGTCATTTTACACTGGGCTCTG	acTTCCTAGGTTAGGATATCTAGTTCA
12	rs1385600	Chr11_GAB2	Exon	ACGTTGGATGACCCTTGGGTTAACCCACAC	ACGTTGGATGGTCTGCTGAATCCATGAGTG	ccgCACCAGGAAAGAGCC
13	rs11136000	Chr8_CLU	Intron	ACGTTGGATGTATTGGGTCAAGTGGCAAGG	ACGTTGGATGGAATGGCAGGCATTCAGCAC	aaggGGCAAGGGCCCGTTAGAGA
14	rs3851179	Chr11_PICALM	5′ region	ACGTTGGATGAGAGCCTGCAAACAATACAC	ACGTTGGATGCCTCAGTGTCAGCAGTCAAC	gcacACAATACACACTTCAGTAAAT
15	rs11610206	Chr12_12q13.11	Intergenic	ACGTTGGATGGATAGAGGTGATCTTTAGCC	ACGTTGGATGTTGCCTTTACTAACTGGCAC	cTGATCTTTAGCCTCCCTA
16	rs201825	Chr4_LRAT	Intron	ACGTTGGATGAAAATTCCCAGTAAGAGGGC	ACGTTGGATGCAACTCTGAAGCACCAACAC	aagcGGGCCCCCCTGGTAG
17	rs727153	Chr4_Near LRAT	Intergenic	ACGTTGGATGATGACTCTGGGCAAAGAGTG	ACGTTGGATGGTTCCTCCTGTAAAACCTGC	tgggtAGTGAAACCCTTCATAGG
18	rs3826656	Chr19_Near CD33	Near gene-5′	ACGTTGGATGCTCTACACACAATAGTATC	ACGTTGGATGTCAAATGAGGATGCAGCTAC	cGATAACTCTTCATTCATCCTTA
19	rs5984894	ChrX_PCDH11X	Intron	ACGTTGGATGTGACCATTCAATATAGTCAT	ACGTTGGATGGAAACTAAATATTTGGATCTC	agtaATAATGTACAATGGATAATCATTC

SNPs 1–2 are from Li et al. [5], SNPs 3–6 are from Grupe et al. [3], SNPs 7–12 are from Reiman et al. [4]

SNPs 13 are from Lambert et al. [6], SNPs 14 are from Harold et al. [7], SNPs 15 are from Beecham et al. [9]

SNPs 16–17 are from Abraham et al. [8], SNPs 18 are from Bertram et al. [10], SNPs 19 are from Carrasquillo et al. [11]

Table 2 Genotype and allele frequencies of three SNPs in SAD cases and controls stratified by APOEε4 status

SNP number	Total	Genotype			*P* value	Allele		*P* value
		G/G (%)	G/T (%)	T/T (%)		G (%)	T (%)	
SNP1								
rs10868366								
AD	191	53 (27.7)	94 (49.2)	44 (23.0)	0.081	200 (52.4)	182 (47.6)	0.026
Control	180	38 (21.1)	83 (46.1)	59 (32.8)		159 (44.2)	201 (55.8)	
APOE ε4 (+)								
AD	71	21 (29.6)	34 (47.9)	16 (22.5)	0.252	76 (53.5)	66 (46.5)	0.09
Control	33	6 (18.2)	15 (45.5)	12 (36.4)		27 (40.9)	39 (59.1)	
APOE ε4 (−)								
AD	120	32 (26.7)	60 (50.0)	28 (23.3)	0.271	124 (51.7)	116 (48.3)	0.119
Control	147	32 (21.7)	68 (46.3)	47 (32.0)		132 (44.9)	162 (55.1)	
SNP number	Total	Genotype			*P* value	Allele		*P* value
		C/C (%)	C/T (%)	T/T (%)		C (%)	T (%)	
SNP2								
rs7019241								
AD	191	50 (26.2)	94 (49.2)	47 (24.6)	0.07	194 (50.8)	188 (49.2)	0.019
Control	180	33 (18.3)	86 (47.8)	61 (33.9)		152 (42.2)	208 (57.8)	
APOE ε4 (+)								
AD	71	19 (26.8)	34 (47.9)	18 (25.4)	0.322	72 (50.7)	70 (49.3)	0.128
Control	33	5 (15.2)	16 (48.5)	12 (36.4)		26 (39.4)	40 (60.6)	
APOE ε4 (−)								
AD	120	31 (25.8)	60 (50.0)	29 (24.2)	0.187	122 (50.8)	118 (49.2)	0.066
Control	147	28 (19.0)	70 (47.6)	49 (33.3)		126 (42.9)	168 (57.1)	
SNP number	Total	Genotype			*P* value	Allele		*P* value
		A/A (%)	A/G (%)	G/G (%)		A (%)	G (%)	
SNP18								
rs3826656								
AD	191	21 (11.0)	83 (43.5)	87 (45.5)	0.427	125 (32.7)	257 (67.3)	0.228
Control	180	23 (12.8)	87 (48.3)	70 (38.9)		133 (36.9)	227 (63.1)	
APOE ε4 (+)								
AD	71	5 (7.0)	36 (50.7)	30 (42.3)	0.016	46 (32.4)	96 (67.6)	0.015
Control	33	9 (27.3)	15 (45.5)	9 (27.3)		33 (50.0)	33 (50.0)	
APOE ε4 (−)								
AD	120	16 (13.3)	47 (39.2)	57 (47.5)	0.244	79 (32.9)	161 (67.1)	0.789
Control	147	14 (9.5)	72 (49.0)	61 (41.5)		100 (34.0)	194 (66.0)	

(OR) and 95% confidence intervals (CI) were calculated as estimates of the strength of the association between genotypes or haplotypes and SAD. A value of $P < 0.05$ on a two-sided test was considered significant.

Results

Eighteen of the 19 SNPs were in HWE. The genotype frequencies of SNP19 (SNP rs5984894 on Xq21.3) in male samples did not conform to the HWE due to its location on the X-chromosome. The control females were in HWE ($\chi^2 = 2.280$, $P = 0.131$), and the case females were in HWE ($\chi^2 = 0.264$, $P = 0.607$). This result is similar to that reported by Wu et al. [18].

As expected, the APOE ε4 allele in the SAD patients was significantly higher than in the controls (22.0 vs. 9.7%; $\chi^2 = 24.708$, $P < 0.01$).

For SNP1 (GOLPH2/rs10868366) and SNP2 (GOLPH2/rs7019241), there were significant differences in allele frequencies between SAD patients and controls (rs10868366: $P = 0.026$, rs7019241: $P = 0.019$), but not in genotype

distribution (rs10868366: $P = 0.081$, rs7019241: $P = 0.07$). Higher frequencies of rs10868366 G and rs7019241 C carriers were observed in patients with SAD compared with control subjects (rs10868366 G: 52.4 vs. 44.2% $P = 0.026$, OR = 1.4, 95% CI: 1.0–1.9; rs7019241 C: 50.8 vs. 42.2% $P = 0.019$; OR = 1.4, 95% CI: 1.6–1.9). However, after stratification by age of onset, gender, and APOE ε4 status, there was no significant difference in the allele or genotype frequencies between the controls and SAD patients for the two SNPs (Table 2). The genetic models of the two SNPs were analyzed and the results remained no significant differences between the SAD patients and controls after gender, age, and APOE adjustment by logistic regression.

Linkage disequilibrium between alleles at the two SNPs were studied and we found rs10868366 and rs7019241 to be in strong linkage disequilibrium ($D' = 0.983$, $r^2 = 0.900$). The rs10868366T allele was in strong linkage disequilibrium with the rs7019241 T allele, and likewise there was strong linkage disequilibrium between the rs10868366 G and rs7019241 C alleles. Furthermore, the rs10868366 T/rs7019241 T haplotype occurred at a lower frequency in the AD patients than in the controls, suggesting that it is a possible protective factor for AD, whereas rs10868366 G/rs7019241 C is a risk factor (Table 3).

For SNP18 (SNP rs3826656 in near gene 5′ region of CD33), there were no significant differences in the distributions of either allele or genotype between SAD patients and controls. However, in subjects with APOE ε4 alleles, a significant difference in the distribution of alleles ($\chi^2 = 5.929$, df = 1, $P = 0.015$) and genotypes ($\chi^2 = 8.324$, df = 2, $P = 0.016$) between the SAD and control groups was seen, with A allele and AA genotype lower in the SAD group (Table 2). The A allele was associated with a reduced risk of SAD compared with the G allele (OR: 0.479; 95% CI: 0.263–0.870, $P = 0.015$), and the AA genotype was associated with a reduced risk of SAD compared with the genotype AG + GG (OR: 0.395; 95% CI: 0.158–0.659, $P = 0.008$).

There were no significant differences in the allele or genotype frequencies between the control and SAD subjects for the other 16 SNPs, even after stratification by age of onset, gender, and APOE ε4 status (data not shown). No statistical interaction was observed between APOE and any of the SNPs examined.

Discussion

Genome-wide association studies have opened a new era for the study of AD, allowing researchers to compare all variations in allele frequencies between patients and controls among the whole genome, and thereby discover sequence variations associated with the disease. However, one of the important issues concerning GWAS is enlarged type I error due to multiple hypothesis testing and false-positive association. Repeat of the GWAS results, especially in other ethnic cohorts, is necessary to identify the true SNPs associated with the disease [22]. Therefore, in this study we used North Chinese Han population samples to detect such loci reported in GWAS, in order to explore the association of SNPs with SAD in the Chinese population.

Using a cohort of 191 LOAD cases and 180 controls, we replicated in North Chinese Han population the association

Table 3 Distribution of haplotype of GOLPH2 in SAD cases and controls stratified by APOE ε4 status

Haplotype	Frequency (%)		χ^2	P	OR (95% CI)	D'	r^2
	SAD	Control					
Total							
rs10868366 G/rs7019241 C	50.3	41.9	5.152	0.023	1.398 (1.046–1.869)	0.983	0.9
rs10868366 G/rs7019241 T	2.1	2.2	0.013	0.908	0.944 (0.351–2.537)		
rs10868366 T/rs7019241 C	0.5	0.3	0.283	0.594	1.888 (0.175–20.384)		
rs10868366 T/rs7019241 T	47.1	55.5	5.284	0.022	0.713 (0.534–0.952)		
APOE(−)							
rs10868366 G/rs7019241 C	50	42.9	2.703	0.1	1.333 (0.946–1.877)	0.912	0.984
rs10868366 G/rs7019241 T	1.7	2	0.093	0.761	0.821 (0.230–2.933)		
rs10868366 T/rs7019241 C	0.8	0	3.609	0.08	–		
rs10868366 T/rs7019241 T	47.5	55.1	2.502	0.114	0.737 (0.523–1.037)		
APOE(+)							
rs10868366 G/rs7019241 C	50.7	37.8	3	0.083	1.690 (0.931–3.069)	0.979	0.871
rs10868366 G/rs7019241 T	2.8	3.1	0.011	0.917	0.913 (0.164–5.071)		
rs10868366 T/rs7019241 C	0	1.6	2.229	0.135	–		
rs10868366 T/rs7019241 T	46.5	57.5	2.201	0.138	0.641 (0.356–1.155)		

of the two SNPs (rs10868366 and rs7019241) in the Golgi phosphoprotein 2 gene (GOLPH2) that were reported to be associated with AD in Caucasians [5]. According to Li et al.'s study, rs10868366 TT + TG genotype and rs7019241 TT + CT genotype decreased the risk of AD when compared with rs10868366 GG genotype and rs7019241 CC genotype. Our results are in partial agreement with results of Li et al.; we found that lower frequencies of rs10868366 T and rs7019241 T carriers were observed in patients with SAD compared with control subjects. The rs10868366 T allele is in strong linkage disequilibrium with rs7019241 T allele, and likewise there is strong linkage disequilibrium between the rs10868366 G and rs7019241 C alleles. Furthermore, the rs10868366 T/rs7019241 T haplotype is a possible protective factor for SAD, while rs10868366 G/rs7019241 C is a risk factor. Our study provides further support for the association of SAD with the common variant rs10868366 and rs7019241 at the GOLPH2 locus. According to our data (Table 2), there was significant difference in allele frequencies at GOLPH2 gene loci rs10868366 and rs7019241 between SAD group and control group, and the frequencies of rs10868366 G and rs7019241 C carriers was higher in SAD group than in control group. Comparison of allele frequencies at the two loci showed no significant difference after stratification by APOE $\varepsilon 4$ status. However, the data (Table 2) showed that, in subjects with APOE $\varepsilon 4$ alleles, the frequencies of loci rs10868366 G and rs7019241 C carriers was higher in SAD group than in control group, demonstrating a trend similar to the overall allele frequency distribution prior to stratification. The relatively fewer number of cases in APOE $\varepsilon 4$ positive group may be responsible for the insignificant statistical difference. In further research, it may be necessary to use larger sample size and stratified comparison.

The GOLPH2 gene is located in 9q21.33 with suggestive linkage to AD [27]. The Golgi phosphoprotein 2 gene is highly expressed in subfields of the hippocampal formation in mouse brain [28]. In addition, Inkster et al. [29] reported that AD patients with the rs10868366 higher risk GG genotype have less left prefrontal cortical gray matter volume relative to AD patients with the lower risk genotype. The SNPs rs10868366 and rs7019241 are located in the intron of the GOLPH2 gene and seems not to be associated with known functional roles, such as splicing or transcriptional regulation. Therefore, we presumed that identified SNPs may be in linkage disequilibrium with some causative loci of the GOLPH2 gene, which might affect GOLPH2 expression and impact the risk of AD. However, two recent studies reported that no significant association existed between GOLPH2 gene polymorphisms and the risk of AD [16, 30]. We used the same type of study subjects as Li et al. [5], i.e., probable AD patients, while Antúnez et al. [30] and Schjeide et al. [16] chose definite, probable, and possible AD patients as their study subjects. In addition, unlike the family-based study by Schjeide et al., Li et al., and our group selected unrelated individuals as study subjects. Also, variable sample sizes and experimental methods may have produced statistical bias. The number of cases in our study is relatively few, so future studies need to use a larger size sample of the Chinese population to detect the polymorphic sites in the promoter region, coding region, and 3′ end of the GOLPH2 gene to confirm the association of this gene with SAD.

In addition, we found that a SNP rs3826656 in near gene 5′ region of CD33 allele A and AA was associated with SAD in APOE carriers, which is different from the conclusion of Bertram et al. [10] in a family-based GWAS study that used the American population. According to Bertram and colleagues's study, the increased risk for AD was found in the dominant model of the minor allele G, but their data were not based on APOE-stratified comparison. However, we found that the rs3826656 A allele and AA genotype may decrease the risk for development of SAD in APOE $\varepsilon 4$ carriers in the north Chinese Han population. Meanwhile, there was also a study reporting that there was no association between SNP rs3826656 and AD in the Belgian population [31]. A possible explanation for the discrepancy might be the genetic heterogeneity of AD. The SNP rs3826656 may have contributed to AD pathology in Americans and Chinese, whereas in Belgians, other genetic factors may play a role in the development of AD.

SNP rs3826656, on 19q13.33, resides less than 2 kb proximal of the transcription initiation site of the CD33 gene. The CD33 gene encodes a cell surface receptor on cells of monocytic or myeloid lineage, and CD33 is a member of sialic-acid-binding immunoglobulin-like lectins [32]. Currently, there is no evidence that the CD33 gene may be involved in the pathogenesis of AD. GWAS of AD may identify more sequence variations in some unknown gene or chromosome regions that are associated with the disease. Such information may help us understand the disease biology in greater detail in the future. Hence, further experimental research is necessary to define the direct functional association between the SNP rs3826656 on chromosome 19q33 and the occurrence of SAD.

In this study, we failed to find a significant association between alleles, genotypes of the other 16 SNPs, and SAD in the North Chinese Han population. Stratification by APOE $\varepsilon 4$ allele status or age at onset did not change the results. According to our data, the association of the 16 previously reported SNPs with AD, if it exists, was not statistically significant in our study population. In view of these inconsistent results, a possible explanation is that if

these SNPs play a role in influencing risk for SAD, it is likely to be small and may only exist in certain defined populations. However, the discrepancies might also be explained by reduced statistical power of our study resulting from moderate sample size. We estimated the power of our study material and found that our sample size had a 87% power to detect a genotypic OR of 2.0 at $P < 0.05$ for a risk allele frequency of 0.20, assuming disease prevalence of 5%. Therefore, further studies in larger samples are necessary to decrease the chance of false negatives.

In summary, through an exploration of 19 candidate SNPs our results indicate that rs10868366 and rs7019241 in GOLPH2 and rs3826656 in near gene 5′ region of CD33 are significantly associated with SAD in the North Chinese Han population. Further studies in larger samples in Chinese populations and meta-analyses of genetic association studies will be the best tools for validating GWAS findings in the future.

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