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SLC26A4 gene polymorphism and late-onset Alzheimer's disease in a Han Chinese population from Qingdao, China☆

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Abstract
In a recent genome-wide association study, the *SLC26A4* gene rs2072064 polymorphism was found to be associated with late-onset Alzheimer's disease in Caucasians. Here, we investigated this association in a large Northern Han Chinese cohort consisting of 599 sporadic late-onset Alzheimer's disease patients and 598 healthy controls matched for sex and age in a Northern Han Chinese population from Qingdao, China. Genotyping by the polymerase chain reaction-ligase detection reaction revealed that there were significant differences in the genotype (P = 0.017) and allele (P = 0.007) frequencies of the rs2072064 polymorphism between late-onset Alzheimer's disease patients and controls. The A allele of this polymorphism was significantly associated with a reduced risk of late-onset Alzheimer's disease (odds ratio (*OR*) = 0.792, 95% confidence interval (*CI*) = 0.670–0.937, P = 0.007). When the data were stratified by the apolipoprotein E ε4 status, there was a significant difference only among apolipoprotein E ε4 non-carriers (genotypic P = 0.001, allelic P = 0.001). Furthermore, the association between rs2072064 and late-onset Alzheimer's disease remained significant by logistic regression analysis after adjustment for age, gender, and the apolipoprotein E ε4 carrier status (dominant model: *OR* = 0.787, 95% *CI* = 0.619–1.000, P = 0.050; recessive model: *OR* = 0.655, 95% *CI* = 0.448–0.959, P = 0.030; additive model: *OR* = 0.792, 95% *CI* = 0.661–0.950, P = 0.012). These findings suggest that SLC26A4 is a susceptibility gene for late-onset Alzheimer's disease in a Northern Han Chinese population from the Qingdao area.

Key Words
neural regeneration; neurodegenerative diseases; late-onset Alzheimer's disease; SLC26A4; rs2072064; polymorphism; genetic association; Han Chinese population; apolipoprotein E; neuroregeneration

Research Highlights
(1) A previous study found a significant association between the *SLC26A4* gene rs2072064 polymorphism and late-onset Alzheimer's disease in Caucasians.
(2) In the present study, we confirmed, for the first time, an association between this polymorphism and late-onset Alzheimer's disease in a Northern Han Chinese population.
(3) *SLC26A4* is a susceptibility gene for late-onset Alzheimer's disease in multiple populations.

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INTRODUCTION

Alzheimer's disease is a common age-associated progressive neurodegenerative disorder[1-2]. Although the etiology of Alzheimer's disease remains poorly understood, genetic factors explain about 60–80% of the heritability of Alzheimer's disease[3]. Several gene mutations, namely β-amyloid precursor protein, presenilin 1, and presenilin 2, have been associated with the early-onset familial form of Alzheimer's disease[4]. In contrast to early-onset Alzheimer's disease, the genetic component of susceptibility to late-onset Alzheimer's disease seems to be more complex. Multiple genetic factors are believed to be involved in the pathogenesis and development of the disorder[5-8]. Only the ε4 allele of apolipoprotein E gene is definitively associated with increased susceptibility to late-onset Alzheimer's disease[9]. However, about half the people carrying at least one ε4 allele do not develop late-onset Alzheimer's disease and 42% of patients with late-onset Alzheimer's disease do not possess an apolipoprotein E ε4 allele[9]. Much effort has been made to search for additional genes that confer late-onset Alzheimer's disease -susceptibility[10-12].

Using linkage studies, researchers have observed several chromosomal regions that are likely to harbor genetic variants associated with Alzheimer's disease, including on chromosome 7[10, 13]. Recently, Naj *et al*[14] reported an intronic single nucleotide polymorphism (single nucleotide polymorphism; rs2072064) in *SLC26A4*, located on chromosome 7q31, which reached statistical significance in its association with late-onset Alzheimer's disease in a genome-wide association study in Caucasians. Because the variants in *SLC26A4* and their population frequencies are different among genetically distinct racial/ethnic groups, replication is needed to confirm the potential effects of *SLC26A4* in other groups, especially in Asians.

In the present study, we assessed the association of the *SLC26A4* single nucleotide polymorphism rs2072064 with late-onset Alzheimer's disease in a large sample of Qingdao residents, China.

RESULTS

Subjects and genotyping

In the present study, we recruited 599 late-onset Alzheimer's disease patients and 598 healthy subjects without any neurodegenerative disorders. The clinical data from the participants are listed in Table 1. Samples in the case and control groups were well matched in terms of age (P = 0.234) and gender (P = 0.193). We genotyped all 1 197 subjects for the rs2072064 single nucleotide polymorphism and the apolipoprotein E ε4 allele by TaqMan assay and/or sequencing. The results were consistent between the two methods.

Table 1 Participant demographics

Item	Alzheimer's disease	Control
n	599	598
Age (mean±SD, year)	80.8±7.3	75.1±6.3
Males [*n*(%)]	291(48.6)	313(52.3)
Body mass (mean±SD, kg)	24.78±1.59	25.43±2.64
Age at onset (mean±SD, year)	75.6±6.2	–
Course of disease (mean±SD, year)	5.7±2.4	–

Association analysis between rs2072064 and late-onset Alzheimer's disease

Both polymorphisms were in Hardy-Weinberg equilibrium in both populations (P > 0.05). As expected, we detected a significant association between apolipoprotein E ε4 status and late-onset Alzheimer's disease, with odds ratio (OR) = 2.915 (95% confidence interval (CI) = 2.202–3.860, P < 0.001) for carrying at least one ε4 allele. The allele and genotype frequencies for rs2072064 are shown in Table 2.

Table 2 Allele and genotype frequencies [*n*(%)] of the rs2072064 polymorphism in subjects before and after stratification by APOE ε4 status

Subject	*n*	Genotype				Allele		
		AA	TA	TT	*P*	A	T	*P*
rs2072064								
Late-onset Alzheimer's disease	599	54(9.0)	286(47.7)	259(43.2)	0.017	394(32.9)	804(67.1)	0.007
Controls	598	78(13.0)	301(50.3)	219(36.6)		457(38.2)	739(61.8)	
APOE ε4 (+)								
Late-onset Alzheimer's disease	204	26(12.7)	94(46.1)	84(41.2)	0.901	146(35.8)	262(64.2)	0.657
Controls	90	10(11.1)	41(45.6)	39(43.3)		61(33.9)	119(66.1)	
APOE ε4 (–)								
Late-onset Alzheimer's disease	395	28(7.1)	192(48.6)	175(44.3)	0.001	248(31.4)	542(68.6)	0.001
Controls	508	68(13.4)	260(51.2)	180(35.4)		396(39.0)	620(61.0)	

APOE: Apolipoprotein E.

Our sample size has the power to detect an OR of at least 0.710, assuming a 5% significance level, a power of 0.80, and an exposure frequency of 0.38 in controls. There were significant differences in both the genotype (P = 0.017) and allele (P = 0.007) frequencies between the late-onset Alzheimer's disease and control groups.

The minor allele (A) of rs2072064 was significantly protective against the risk of developing late-onset Alzheimer's disease (*OR* = 0.792, 95% *CI* = 0.670–0.937; P < 0.05). When stratified by the apolipoprotein E ε4 status, we observed a significant difference in apolipoprotein E ε4 non-carriers (genotypic P = 0.001, allelic P = 0.001), but not in apolipoprotein E ε4 carriers (genotypic P = 0.901, allelic P = 0.657).

Furthermore, by logistic regression analysis we revealed that the rs2072064 single nucleotide polymorphism remained strongly associated with late-onset Alzheimer's disease after adjusting for age, gender, and the apolipoprotein E ε4 status (dominant model: *OR* = 0.787, 95% *CI* = 0.619–1.000, P = 0.050; recessive model: *OR* = 0.655, 95% *CI* = 0.448–0.959, P = 0.030; additive model: *OR* = 0.792, 95% *CI* = 0.661–0.950, P = 0.012; Table 3).

Table 3 Correlation of rs2072064 and late-onset Alzheimer's disease after adjustment for age, gender, and apolipoprotein E ε4 carrier status

Model	*OR* (95% *CI*)	*Wald* test score	*P*
Dom	0.787 (0.619–1.000)	3.828	0.050
Rec	0.655 (0.448–0.959)	4.721	0.030
Add	0.792 (0.661–0.950)	6.349	0.012

Dom: Dominant model; Rec: recessive model; Add: additive model; *OR*: odds ratio; *CI*: confidence interval.

DISCUSSION

We found that the rs2072064 single nucleotide polymorphism in *SLC26A4* was significantly associated with a reduced risk of late-onset Alzheimer's disease in a Northern Han Chinese population. The minor allele frequency in our control group (38.2%) was similar to that in the 45 unrelated Han Chinese in Beijing (42.2%) in the HapMap database (http://www.hapmap.org). Based on the HapMap database, this polymorphism is less frequent in European and Japanese populations than in the Chinese, and is absent in Sub-Saharan African populations. Hence, the rs2072064 polymorphism may have a stronger effect on late-onset Alzheimer's disease in some populations than in others. Replication is needed to test its association with late-onset Alzheimer's disease in other ethnic populations.

SLC26A4 encodes an 86-kDa transmembrane protein named pendrin that has up to 15 predicted membrane-spanning domains[15-16]. Pendrin (also called PDS and *SLC26A4*) is a multifunctional anion exchanger expressed in tissues as diverse as the thyroid gland, inner ear, kidney, and airways, as well as in several other organs[17-19]. Pendrin plays different roles in these various tissues and human pathological conditions are well established (Pendred syndrome, enlarged vestibular aqueduct) or hypothesized (hypertension, chronic obstructive pulmonary disease, asthma) to be associated with loss of function of pendrin or its upregulation, respectively. The biological mechanisms underlying the genetic effect of *SLC26A4* on late-onset Alzheimer's disease risk are beyond the scope of our study, although one hypothesis can be postulated based on the literature. Wall *et al* [20] have reported that pendrin regulates blood pressure by modulating chloride excretion. Moreover, studies have demonstrated that blood pressure is lower in *SLC26A4*-null mice than in wild-type mice[21-22]. Taken together, loss of *SLC26A4* function may protect against hypertension. Therefore, based on the association of late-onset Alzheimer's disease with hypertension[23-25], we hypothesize that reduced *SLC26A4* activity may result in low blood pressure and thus reduce the risk of developing late-onset Alzheimer's disease. Experimental studies are needed to test this hypothesis.

The rs2072064 single nucleotide polymorphism lies within an intron. It is known that intronic region variants can regulate gene expression, thereby affecting disease susceptibility[26-27]. Therefore, the rs2072064 polymorphism may contribute to late-onset Alzheimer's disease vulnerability directly. However, this single nucleotide polymorphism is in linkage disequilibrium with the rs11754661 polymorphism in the *MTHFD1L* gene[14] and it may also be in linkage disequilibrium with variants in other genes. Therefore, we cannot exclude the possibility that rs2072064 is not the causative variant, but rather that it is marker for the true risk factor that lies elsewhere.

In summary, the A allele of the rs2072064 polymorphism is significantly associated with a reduced risk of late-onset Alzheimer's disease in a Han Chinese population. The association was significant only in

apolipoprotein E ε4 non-carriers. Further studies in large cohorts are necessary to clarify the role of *SLC26A4* polymorphisms in the pathological processes of Alzheimer's disease.

SUBJECTS AND METHODS

Design

Genetic association study.

Time and setting

Experiments were performed at the Central Laboratory of Qingdao Municipal Hospital and the Laboratory of Shanghai Genesky Biotechnology Company, China from March to September 2011.

Subjects

We recruited 599 late-onset Alzheimer's disease patients and 598 healthy subjects without any neurodegenerative disorders for our study. All the late-onset Alzheimer's disease patients and control subjects were unrelated Northern Han Chinese residents of Qingdao. The patients were recruited from the Department of Neurology at Qingdao Municipal Hospital, the Affiliated Hospital of the Medical College of Qingdao University, Qingdao Hiser Hospital, and the Qingdao Mental Health Center, in Shandong Province. They had all undergone clinical evaluation and were diagnosed as probable Alzheimer's disease, according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association[28]. All patients were defined as sporadic because none of their first-degree relatives had dementia in their family history. Age- and gender-matched healthy control subjects were collected from the Health Examination Center of each collaborating hospital. The control subjects were confirmed to be healthy and neurologically normal by medical history, general examination, laboratory examination, and Mini Mental State Examination[28]. Subjects with significant illness (such as autoimmune disease, type 2 diabetes mellitus, myocardial infarction, congestive heart failure, asthma, Parkinson's disease, and stroke) were excluded from our study[29-30]. All subjects were ascertained to have parents and grandparents who were of Northern Han Chinese origin to ensure homogeneity of ethnicity. Written informed consent was obtained from each subject or his/her representative, and the study was approved by the Ethics Committee of Qingdao Municipal Hospital, China.

Methods

Genotyping of rs2072064

Genotyping of rs2072064 was performed using PCR-ligase detection reaction (TaqMan assay) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA, USA)[31-32], with technical support from the Shanghai Genesky Biotechnology Company. Genomic DNA was isolated from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The primer sequences used for PCR were: forward: 5'-GCA GGA GAC ACA ATC CCA CCT C-3', reverse: 5'-GTC CAT TCA GGC CAA GCT TCT A-3'. The PCR reaction mixture (10 μL) contained 1 × GC-I buffer (Takara, Dalian, China), 3.0 mM Mg^{2+}, 0.3 mM dNTPs, 1 U HotStarTaq polymerase (Qiagen, Hilden, Germany), 1 μL of sample DNA and 1 μL of each primer. The PCR conditions were: 95°C for 2 minutes; 11 cycles of 94°C for 20 seconds, 65°C for 40 seconds, 72°C for 1.5 minutes; 24 cycles of 94°C for 20 seconds, 59°C for 30 seconds, 72°C for 1.5 minutes; and a final extension at 72 °C for 2 minutes. PCR products were treated with 1 U of Shrimp Alkaline Phosphatase and 1 U of Exonuclease I (Qiagen) to degrade excess dNTPs and primers.

Two allele-specific probes (rs2072064FA and rs2072064FT) and one fluorescently labeled probe (rs2072064FP) were used for the ligase detection reaction. Their sequences are as follows:

Probe	Sequence (5'–3')
rs2072064FA	TCT CTC GGG TCA ATT CGT CCT TGA TTG GTC CAT GTT TCC TGC GAA
rs2072064FT	TGT TCG TGG GCC GGA TTA GTG ATT GGT CCA TGT TTC CTG CGA T
rs2072064FP	GGT AAA TAA CTA GAA GCT TGG CCT GAA TTT

The ligase detection reaction was carried out in 1 μL of 10 × binding buffer, 0.25 μL of thermostable Taq DNA ligase (Takara), 0.4 μL of 1 μM 5' ligation primer, 0.4 μL of 2 μM 3' ligation primer, 2 μL of multiplex PCR product, and 6 μL of double-distilled H_2O. The reaction mixtures were subjected to 38 cycles of 94°C for 1 minute and 58°C for 4 minutes, and then the temperature was maintained at 4°C. Reaction mixture (0.5 μL) was denatured at 95°C for 5 minutes in 9 μL Hi-Di formamide (Qiagen) with 0.5 μL of the LIZ-500 size standard, and run on an ABI 3130XL genetic analyzer (Applied

Biosystems). Data were analyzed using GeneMapper Software v.4.0 (Applied Biosystems). Randomly selected DNA samples from each genotype were also analyzed in duplicate using the ligation detection reaction and sequence analysis.

Statistical analysis

Hardy-Weinberg equilibrium between the expected and observed genotype distributions was assessed using the chi-square test. The genotype and allele frequencies were compared using the chi-square test. Differences in the allele or genotype distribution between cases and controls were analyzed using logistic regression adjusted for age, gender, and apolipoprotein E ε4 status under three genetic models that were defined as 1 (aa + Aa) *versus* 0 (AA) for dominant, 1 (aa) *versus* 0 (AA + Aa) for recessive, and 0 (AA) *versus* 1 (Aa) *versus* 2 (aa) for additive (A: major allele; a: minor allele). The *P* value, *OR*, and 95% *CI* were calculated. The statistical power was estimated with STPLAN 4.3 software (The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA). Data were analyzed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The criterion for a significant difference was $P < 0.05$.

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Conflicts of interest: None declared.

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