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Genetic variation in *BIN1* gene and Alzheimer's disease risk in Han Chinese individuals

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ABSTRACT

Genome-wide association studies have identified the bridging integrator 1 (*BIN1*) gene as the most important genetic susceptibility locus in late-onset Alzheimer's disease (LOAD) after apolipoprotein E for individuals of European ancestry. To further characterize this association and to isolate the variants within *BIN1* contributing to LOAD in Han Chinese individuals, we conducted a 2-step design study in our cohort of 1133 LOAD patients and 1159 control subjects. Sequencing analysis identified 44 variants within *BIN1*. Follow-up genotyping analysis revealed that a novel missense mutation P318L appeared to exert risk effect for development of LOAD; and rs67327804 was also significantly associated with LOAD risk even after adjusting for age, gender, and apolipoprotein E ε4 status. Haplotype analysis confirmed that the "GA" haplotype derived from single-nucleotide polymorphisms in rs67327804 and rs1060743 showed a 1.4-fold increased risk of LOAD. Our findings provided the first independent evidence that variants in *BIN1* were significantly associated with LOAD in Han Chinese individuals.

1. Introduction

Alzheimer's disease (AD) is a complex, multifactorial neurodegenerative disease that is the leading cause of dementia in the elderly individuals. Genetic susceptibility at multiple loci and interactions among these genes influence the risk of developing AD; recent estimates of heritability range from 58%–79% (Gatz et al., 2006). For many years, amyloid precursor protein and the presenilin genes 1 and 2 (*PSEN1*, *PSEN2*) have been the only unequivocally established susceptibility genes for early-onset familial AD, and apolipoprotein E (*ApoE*) the only confirmed susceptibility gene for common late-onset AD (LOAD). In recent years, large genome-wide association studies (GWAS) have identified 9 other genes and/or loci (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011; Seshadri et al., 2010) that, along with *ApoE* ε4, contribute to a high proportion of genetic risk for LOAD. Among them, the bridging integrator 1 (*BIN1* or amphiphysin 2/*AMPH2*) gene, located on chromosome 2q14.3, is currently identified as the most important genetic susceptibility locus in LOAD after *ApoE*, according to the continuously updated Alzgene database (http://www.alzgene.org/) (Bertram et al., 2007).

As an important AD candidate gene, *BIN1* is expressed in many tissues and overexpressed *BIN1* has been found in AD brains (Chapuis et al., 2013). It has been reported that higher *BIN1* expression was associated with later age at onset and shorter disease duration in AD patients (Karch et al., 2012). In addition, our study has recently observed that *BIN1* expression was also increased in peripheral blood of AD patients; and plasma *BIN1* might become potential biomarker for AD diagnosis (Sun et al., 2013). Similar to *ApoE*, numerous studies have presented compelling evidence implicating *BIN1* in AD pathogenesis (Tan et al., 2013a, 2013b). Besides its potential interaction with tau pathology (Chapuis et al., 2013), *BIN1* might also be involved in regulating endocytosis and trafficking, immune, and inflammation, calcium transients, and apoptosis (Carter, 2011; Elliott et al., 2000; Hong et al., 2012; Tjondrokoesoemo et al., 2011). All these observations suggest that the *BIN1* gene could be considered a functional candidate gene for AD susceptibility.

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Initially, *BIN1* gene locus was identified as having a possible association with AD using GWAS in the GERAD1 study (Harold et al., 2009) but it was not until the study by Seshadri et al., 2010 that this locus for AD reached genome-wide statistical significance, a finding that was confirmed in other large GWAS (Kamboh et al., 2012; Naj et al., 2011) and the largest family-based GWAS (Wijsman et al., 2011). After these original observations, several independent candidate gene studies examined the association between *BIN1* polymorphisms and LOAD risk in independent cohorts (Carrasquillo et al., 2011; Lambert et al., 2011; Lee et al., 2011; Logue et al., 2011), but the results have been conflicting in ethnically distinct populations (Chung et al., 2013; Tan et al., 2013a). Based on large, multicenter data sets, meta-analysis results also strongly indicate that *BIN1* is an AD susceptibility locus (Carrasquillo et al., 2011; Hollingworth et al., 2011; Hu et al., 2011; Kamboh et al., 2012; Lambert et al., 2013). Genetic variants of *BIN1* associated with susceptibility to LOAD are known for individuals of European ancestry, but whether the same or different variants within *BIN1* gene account for the genetic risk of AD in Han Chinese individuals is still unknown. In this study, to investigate the involvement of the *BIN1* gene in LOAD in the Han Chinese individuals, a 2-step design study was carried out. We first sequenced in the promoter, exons, the 5′ and 3′ untranslated regions, and exon–intron boundaries of the *BIN1* gene for mutations in a total of 50 LOAD patients and 50 controls. The identified variants were then partially selected to be analyzed in a much larger case–control sample (n = 2292), which was well matched for age, gender, and ethnic background in the Han Chinese individuals.

2. Methods

2.1. Subjects

Our study comprised 1133 LOAD patients (age at onset ≥65 years) and 1159 healthy controls matched for gender and age. All LOAD patients and control subjects were unrelated Han Chinese residents from Qingdao, which is located in the North of China. The AD patients were recruited from the Department of Neurology at Qingdao Municipal Hospital, and several other hospitals in Qingdao. A consensus clinical diagnosis of probable AD was established by at least 2 neurologists according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and/or Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) (McKhann et al., 1984). All patients were defined as sporadic because none of their first-degree relatives had dementia in their family history. Age at onset and family history were determined from caregivers. The age- and gender-matched healthy controls were collected from the Health Examination Center of each collaborating hospital and were confirmed healthy and neurologically normal by medical history, general examinations, laboratory examinations, and Mini-Mental State Examination score by physicians and neurologists. Demographic details of the sample set are shown in Table 1. Informed consent was acquired from all subjects or from their caregivers, and the protocol of this study was approved by the Institutional Ethics Committees.

2.2. DNA sequencing

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard genomic DNA purification kit (Cat. #A1125, Promega, USA) following the manufacturer's protocol. We performed sequencing of the *BIN1* (GeneID: 274; chromosome 2, NC_000002.11 [127805599.127864903, NCBI build 37.5]; isoform 1: NM_139343.1, which is the longest transcript of the gene) by using genomic DNA of 50 AD patients and 50 control subjects matched for age and gender, who were selected randomly from the main study population. The *BIN1* gene sequences within the promoter (~2kb upstream of *BIN1*), exons, the 5′ and 3′ untranslated regions, and the exon–intron boundaries were obtained from the UCSC Genome Browser Database (http://genome.UCSC.edu) and referred to Human Genome Resources. Primer pairs were designed by primer 3 software. The purified polymerase chain reaction products were then sequenced using the BigDye Terminator v3.1 sequencing chemistry from Applied Biosystems and run on ABI3130XL genetic analyzer according to the manufacturer's instructions. The sequences were analyzed with the PolyPhred analysis software (Table 2).

Table 1
The characteristics of the study population

	AD (n = 1133)	HC (n = 1159)	*p*
Age at examination, y; mean ± SD	79.94 ± 8.12	74.48 ± 6.29	0.078[a]
Age at onset, y; mean ± SD	75.01 ± 8.00		
Gender, n (%)			
Male	464 (41.0)	518 (44.7)	0.070
Female	669 (59.0)	641 (55.3)	
MMSE score, mean ± SD	10.06 ± 3.82	28.26 ± 1.08	<0.001
ApoE-ε4 status, n (%)			
ApoE-ε4 carrier	315 (27.8)	158 (13.6)	<0.001
ApoE-ε4 noncarrier	818 (72.2)	1001 (86.4)	

Key: AD, Alzheimer's disease; *ApoE*, apolipoprotein E; HC, healthy controls; MMSE, Mini-Mental State Examination; SD, standard deviation.
[a] *p*-value was calculated with the age of onset for late-onset AD and age at examination for HC. Differences in the characteristics of age and MMSE score between the 2 groups were examined using the Student *t* test. Differences in gender and *ApoE*-ε4 status between AD patients and HC were assessed using the Pearson χ^2 test.

2.3. Genotyping

The single-nucleotide polymorphisms (SNPs) of *BIN1* gene were selected based on the following criteria as described previously (Jiang et al., 2014; Ma et al., 2014; Yu et al., 2013). First, SNPs with minor allele frequency >0.05 in our data validated by sequencing analysis will be taken forward to genotyping in the full cohort. Second, for SNPs which were located in 1 haplotype block and were in complete linkage disequilibrium (LD) (determined with the criterion of $r^2 > 0.8$), one of them will be randomly selected for the subsequent genotyping. Under these criteria, 13 tagSNPs (shown in Table 3) were selected for genotyping in our full cohort. In addition, another 2 rare missense mutations in the exon region (p.Pro318Leu and p.Pro431Leu) in the direct sequencing were also taken forward to genotyping in our full cohort. The 15 selected polymorphisms in *BIN1* gene were genotyped on the remaining cases and control subjects and analyzed using all 2292 individuals who had been taken as the main study population by polymerase chain reaction-ligase detection reaction using TaqMan genotyping assays on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA) (Favis et al., 2000; Xiao et al., 2006), with technical support from the Shanghai Genesky Biotechnology Company. Data analysis was achieved using GeneMapper Software v4.0 (Applied Biosystems). Randomly selected DNA samples from each genotype were analyzed in duplicate using ligation detection reaction and sequence analysis method. Consistent results were obtained by these 2 methods.

2.4. Statistical analysis

Goodness-of-fit to Hardy–Weinberg equilibrium in the controls; and genotype and allele distributions between AD patients and controls were calculated using the χ^2 test or Fisher exact test. The strength of association between SNPs and AD was estimated with the odds ratios (ORs) with 95% confidence intervals (CIs) by logistic regression, adjusting for age, gender, and *ApoE* ε4 status (presence