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An exploratory study on *STX6*, *MOBP*, *MAPT*, and *EIF2AK3* and late-onset Alzheimer's disease

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ABSTRACT

Both Alzheimer's disease (AD) and progressive supranuclear palsy (PSP) are a class of neurodegenerative diseases associated with the pathologic aggregation of tau protein in the human brain. They share some clinical and pathologic characteristics. A recent genome-wide association study reported several single-nucleotide polymorphisms at the *STX6*, *MOBP*, *MAPT*, and *EIF2AK3* in association with PSP. To explore whether these single-nucleotide polymorphisms are associated with AD risk, we conducted a case-control study to investigate the PSP-associated loci in 1592 Han Chinese subjects. Rs242557 at the *MAPT* locus was associated with late-onset AD (LOAD) (odds ratio [OR], 1.175; $p = 0.026$), which appeared to be stronger for LOAD patients with apolipoprotein E (*APOE*) ε4 allele (OR, 1.510), and this positive association was not changed after adjusting for age, sex, and the *APOE* ε4-carrier status (additive model: OR, 1.163; $p = 0.036$; dominant model: OR, 1.315; $p = 0.010$). Rs1768208 in *MOBP* and rs7571971 in *EIF2AK3* showed association only in the *APOE* ε4 positive subjects, and these did not appear to be independent of *APOE*. As for rs1411478 in *STX6*, we did not explore any association with LOAD. Our exploratory analysis mainly suggests an association of *MAPT* with LOAD, especially in *APOE* ε4 carriers. Genotypes at *MOBP* and *EIF2AK3* confer risk predominantly in *APOE* ε4-positive subjects, with indications of an interaction between *APOE* and *MOBP* or *EIF2AK3* on AD risk.

1. Introduction

Both Alzheimer's disease (AD) and progressive supranuclear palsy (PSP) are a class of neurodegenerative diseases associated with the pathologic aggregation of tau protein in the human brain (Crespo-Biel et al., 2012; Höglinger et al., 2011). These 2 conditions share some clinical and pathologic characteristics. On the 1 hand, PSP cases display tau pathology similar to late-onset AD (LOAD) (Abraham et al., 2009). Both of them involve abnormal accumulation of tau protein within neurons as neurofibrillary tangles (NFT). On the other hand, they are clinically progressive and manifest cognitive decline eventually (Waldemar et al., 2007). These connections suggest that AD and PSP might have some shared risk factors and/or common pathogenic mechanisms. However, little effort to date has been made to research potential genetic risk factors that might contribute to both diseases.

Recently, a large genome-wide association study (GWAS) has reported several novel susceptibility genetic loci for PSP, including syntaxin6 (*STX6*), myelin oligodendrocyte-associated basic protein (*MOBP*) and eukaryotic translation initiation factor 2-α kinase 3 (*EIF2AK3*) (Höglinger et al., 2011). Meanwhile, the tau gene (*MAPT*) was also confirmed in this PSP GWAS. The expression levels and functional features of these genes exclusively supported their associations with PSP (Zou et al., 2012). Therefore, we explored the relationship of LOAD with selected single-nucleotide polymorphisms (SNPs) from the PSP GWAS (rs1411478 for *STX6*, rs1768208 for *MOBP*, rs7571971 for *EIF2AK3*, and rs8070723 and rs242557 for *MAPT*) in a large LOAD case-control study.

2. Methods

2.1. Subjects

A total of 796 sporadic LOAD cases (400 male and 396 female; age ≥ 65 years; age at onset = 75.28 ± 6.57 years) and 796 healthy control subjects (408 male and 388 female; mean age = 74.81 ± 6.96 years) matched for sex and age were recruited for this study. All the subjects were Han Chinese originally. The patients were from the Department of Neurology at Qingdao Municipal Hospital,

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and several other hospitals in Shang dong Province. A clinical probable AD was diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (McKhann et al., 1984). None of the AD patients had a family history of dementia. The control subjects were selected from the Health Examination Center of the Qingdao Municipal Hospital, and they were confirmed healthy and neurologically normal by medical history, general examinations, laboratory examinations, and Mini Mental State Examination. Our study was conducted with informed consent of all individuals or legal guardians and with approval from the Institute Ethical Committee.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures (Promega). The selected 5 SNPs in *STX6, MOBP, EIF2AK3*, and *MAPT* were genotyped with the method of polymerase chain reaction (PCR)–ligase detection reaction (LDR) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) (Favis et al., 2000; Xiao et al., 2006), with technical support from the Shanghai Genesky Biotechnology Company. The primer sequences used for the PCR reaction were: rs1411478, forward: CCCTTCAAAGAGCCTGGCACAC, reverse: GGTGT GACCATGGGAGGTTTTG; rs1768208, forward: TCCCAAAACTGACTGGT CTAATTTCTACA, reverse: GAGGGAGATGTTTGGCTGGTTTG; rs7571971, forward: TCTCCCAGAAAAATAGAAGCATCATACC, reverse: GACCCTTTT GGCCCATGGTAAA; rs242557, forward: TTGCCTTTCATCTGATTGTAAA CATAATACA, reverse: GTGACACCCTGGGGACAGAGC; and rs8070723, forward: GTGAATTTGTACGTGGAATC, reverse: GCAATACTCTGCTGG GAG. The PCR reaction mixture (10 μL) contained 1x GC-I buffer (Takara), 3.0 mM Mg^{2+}, 0.3 mM dNTP, 1 U HotStarTaq polymerase (Qiagen Inc), 1 μL of sample DNA and 1 μL of each primer. PCR amplification was performed under the following conditions: 95 °C for 2 minutes and 11 cycles at 94 °C for 20 seconds, 65 °C for 40 seconds, 72 °C for 1.5 minutes, and 24 cycles at 94 °C for 20 seconds, 59 °C for 30 seconds, and 72 °C for 1.5 minutes, and a final extension at 72 °C for 2 minutes. PCR products were purified with 1 U of Shrimp Alkaline Phosphatase and 1 U of Exonuclease I to degrade excess dNTPs and primers.

Two allele-specific probes and 1 a fluorescently-labeled probe was used for LDR (probes sequences are available from the corresponding author). LDR was carried out in 1 uL of 10x binding buffer, 0.25 uL of thermostable Taq DNA ligase, 0.4 uL of 1 μM 5′ ligation primers mixture, 0.4 uL of 2 μM, 3′ ligation primers mixture, 2 uL of multiplex PCR product, 6 uL 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 stored at 4 °C. Half a microliter of the reaction mixtures were denatured at 95 °C for 5 minutes in 9 μL Hi-Di formamide along with 0.5 μL of the LIZ-500 size standard, and run on the ABI3130XL genetic analyzer. Data analysis was achieved using GeneMapper Software v4.0 (AppliedBiosystems). DNA sequencing was used to validate the genotyping by LDR. Results of LDR corresponded with the results of sequencing for the randomly selected DNA samples from each genotype.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. Goodness-of-fit to the Hardy–Weinberg equilibrium and genotype and allele distributions between AD and controls were compared by χ^2 test. Logistic regression analysis was used to investigate the association of tested SNPs with LOAD risk after adjustment for age, sex, and apolipoprotein E (*APOE*) ε4 status. Estimation of the statistical power was performed with the STPLAN 4.3 software. Statistically significant value was defined as $p \leq 0.05$.

3. Results

Demographic and clinical characteristics of AD and control subjects are shown in Table 1. The 796 LOAD cases were well-matched with the 796 control subjects in terms of sex ($p = 0.668$) and age ($p = 0.166$). As expected, *APOE* ε4-carriers had higher risk for LOAD (odds ratio [OR], 1.854; 95% confidence interval [CI], 1.443–2.382; $p < 0.001$).

All SNPs in case and control subjects were in the Hardy–Weinberg equilibrium ($p > 0.05$). In the total sample (Table 2), only the genotype and allele frequencies of rs242557 in *MAPT* were significantly different between LOAD cases and control subjects (genotype: $p = 0.032$; allele: $p = 0.026$). The minor allele G significantly raised the risk of LOAD (OR, 1.175; 95% CI, 1.020–1.353). None of the SNPs rs1411478 for *STX6*, rs1768208 for *MOBP*, and rs7571971 for *EIF2AK3* was related to LOAD risk. Similarly, multivariate logistic regression still revealed that only rs242557 polymorphism was associated with LOAD (additive model: OR, 1.163; 95% CI, 1.010–1.339; $p = 0.036$; dominant model: OR, 1.315; 95% CI, 1.068–1.619; $p = 0.010$) after adjusting for age, sex, and the *APOE* ε4-carrier status (Table 3). The G allele was associated with an increased risk of LOAD.

Furthermore, we divided these data into 2 subgroups according to the *APOE* ε4 status (Table 4). Interestingly, besides rs242557 in *MAPT*, there was a significant difference for rs1768208 in *MOBP* when alleles or genotypes were compared between AD and controls with *APOE* ε4 alleles. The G allele of rs242557 has a 1.51-fold increased risk compared with the A allele (OR, 1.51; 95% CI, 1.089–2.090; $p = 0.013$), and the C allele of rs1768208 has a 37.2% decreased risk compared with the T allele (OR, 0.628; 95% CI, 0.454–0.868; $p = 0.005$). Moreover, in subjects with *APOE* ε4 alleles, the genotype distribution of rs7571971 for *EIF2AK3* was also significantly different between AD and control subjects ($p = 0.009$). No significant differences were found for all SNPs when alleles or genotypes were compared between non-*APOE* ε4 AD and control subjects.

For rs8070723 in *MAPT*, all subjects in this study were identified as AA homozygous.

4. Discussion

STX6, *MOBP*, *MAPT*, and *EIF2AK3* were all implicated by PSP pathology and shown to have genetic and expression effects on tauopathy of the neurodegenerative diseases (Höglinger et al., 2011; Zou et al., 2012). To explore whether these genes cause susceptibility to LOAD, the most common tauopathy, we analyzed *STX6*, *MOBP*, *MAPT*, and *EIF2AK3* loci in a Han Chinese data set of 1592 subjects. In this large case-control study, we found preliminary evidence that rs242557 at the *MAPT* locus might be associated with LOAD (OR, 1.175), and this association appeared to be stronger for

Table 1
The characteristics of the study population

	AD (796)	Control (796)	*p*
Sex, male:female	400:396	408:388	0.668
Age, mean ± SD, y	75.28 ± 6.57[a]	74.81 ± 6.96[b]	0.166
MMSE score	12.12 ± 5.56	28.26 ± 1.09	<0.001
APOE ε4 carrier (*n*)	200	122	<0.001

Key: AD, Alzheimer's disease; MMSE, Mini Mental State Examination.
[a] Mean age at onset.
[b] Mean age at examination.