Association of SORL1 Polymorphisms with the Risk of Amnestic Mild Cognitive Impairment in the Han Chinese Population

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Abstract
Mild cognitive impairment (MCI) is defined as the symptomatic predementia stage, characterized by cognitive impairment that is not severe enough to influence the usual activities of daily living. MCI is suggested to be a transitional state between healthy aging and clinically probable Alzheimer’s disease (AD). Previous studies have identified that single nucleotide polymorphisms (SNPs) in the sortilin-related receptor, L (DLR class) A repeats containing (SORL1) gene are associated with amnestic MCI (aMCI). To further investigate the relationships between SORL1 genetic variants and aMCI, we conducted the present case-control study in 63 aMCI unrelated patients and 179 unrelated healthy controls. The genotypes of three SNPs: rs11218304, rs689021, and rs11218343 in the SORL1 gene were analyzed using the ligase detection reaction-polymerase chain reaction (LDR-PCR) methods. We found significant association (OR = 1.870, 95% CI = 1.204-2.903, P = 0.005) between the ‘C’ allele of the SORL1 SNP rs11218343 and aMCI. The stratified analysis by ApoE ε4 status indicated that the interaction of the rs11218343 ‘C’ allele with ApoE ε4 may increase the effect of rs11218343 on the susceptibility to aMCI. Moreover, we also observed a significant association of SORL1 three-marker haplotypes (rs11218304-rs689021-rs11218343) with the risk of aMCI (for ACC: OR = 2.206, 95% CI = 1.257-3.873, P= 0.005). These findings suggest that the SORL1 SNP rs11248343 may alter the risk for aMCI in the Han Chinese population.

Keywords
SORL1, Mild cognitive impairment, Polymorphism, Han Chinese, Association

Introduction
Alzheimer’s Disease (AD) is an age-related progressive neurodegenerative disorder characterized by impairments in memory and other cognitive functions (Roses 1996), which is a most common polygenic multifactorial disease and suggested to be the result of the interaction of genetic and environmental factors [1]. Mild Cognitive Impairment (MCI) is defined as the symptomatic predementia stage, characterized by cognitive impairment that is not severe enough to influence the usual activities of daily living. The subtypes of MCI mainly consist of non-amnestic MCI and amnestic MCI (aMCI), and most aMCI patients are considered to have a prodromal stage of AD that will progress to AD at a rate of 10% to 15% per year. The translational rate is significantly higher than that in healthy controls [2]. Therefore, an aMCI genetic association study expected to provide important insights into the risks of developing dementia.

In a recent association study, sortilin-related receptor (SORL1, also called LR11 or sorLA) single nucleotide polymorphisms (SNPs) were found to be associated with the risk of AD [3]. SORL1 is a member of the low-density lipoprotein receptor family that reduces amyloid-β (Aβ) production by regulating the intracellular transport and processing of amyloid precursor protein (APP) [-6]. Both genetic and biological evidence indicate that SORL1 could have a role in AD susceptibility. Using meta-analysis methods, we further identified SORL1 SNPs rs11218304 and rs689021 were associated with susceptibility to SAD [7]. In addition, recent studies indicated that SORL1 SNP rs11218343 was associated with an increased risk of AD [8,9]. However, the associations of these SNPs: rs11218304, rs689021, and rs11218343, with the risk of aMCI in the Han Chinese population are still unknown to date. Thus, we conducted the present case-control study.

Material and Methods
Study population
We recruited 63 unrelated aMCI patients (35 women and 28 men aged 77.4 ± 9.3 years at recruitment), and 179 unrelated healthy control subjects (96 women and 83 men aged 78.2 ± 8.7 years at recruitment), who were drawn from a Chinese population of Han descent. All aMCI patients satisfied the following clinical diagnostic criteria [2,10]: (1) subjective memory impairment corroborated by subject and an informant; (2) weak objective memory performance...
DNA extraction

Blood samples were collected from all participants using K2EDTA tubes, and a Blood Genotyping DNA Extraction Kit (Tiangen Biotech, Beijing, China) was used to extract genomic DNA from 150 μl of peripheral blood. DNA samples were then stored at -80 °C for the purpose of genotype analysis.

SNP selection

SORL1 SNPs rs11218304, rs689021, and rs11218343 had been identified to be associated with AD in previous studies [8,9], however, the association of these SNPs with aMCI in the Han Chinese is still unknown. Thus, we selected the three SNPs for genotyping in present study.

SNP genotyping

The genotypes of three SNPs were analyzed by the Shanghai Biowing Applied Biotechnology Co., Ltd (www.biowing.com.cn) using LDR-PCR method [11-13]. Genomic DNA extracted from clinical samples was first subjected to multiplex PCR to obtain a PCR product that included SNPs. This PCR product and the LDR probes were then subjected to a multiplex LDR reaction with a DNA sequencer to detect the products. To validate this procedure, approximately 10% of the samples were randomly selected and retested using the same process. The concordance rate for the blind duplicates is 100%. The results in the retested samples were consistent with those obtained in the larger sample group.

Statistical analysis

Our statistical analyses were performed using the PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) and included association studies, Hardy-Weinberg equilibrium (HWE) tests, and the calculation of genotype and allele frequencies in aMCI and healthy control subjects. Haplotype analysis was conducted using the SHeSis software (http://analysis.bio-x.cn/myAnalysis.php/) [14]. HapMap data were used to assess linkage disequilibrium (LD) using the Haploview software (http://www.broadinstitute.org/haplovew/), and function prediction was conducted using rSNPBase (rsnp.psych.ac.cn/) [15].

Results

In total, our study participants included 63 aMCI patients and 179 healthy subjects. Our HWE tests indicated that the allelic frequency distribution of SORL1 polymorphisms does not deviate significantly from the HWE; P = 0.6119 for rs689021, P = 0.4770 for rs11218304, and P = 0.1512 for rs11218343. Total genotyping rate in all individuals was 100%.

A genetic model refers to a specific mode of inheritance. Specific
lobe or genotype carriers may imply the different risk of disease. Thus, multiple genetic models were used in the present study. In association study, our results suggest that there are significant associations (Trend test $P_{\text{adj}} = 0.013$, allelic test $P_{\text{adj}} = 0.015$, the $\chi^2$ test under recessive model: $P_{\text{adj}} = 0.006$ for aMCI) between aMCI and the minor allele (‘C’) of the SORL1 gene SNP rs11218343 (Table 2). In addition, due to the $e4$ allele of the apolipoprotein E gene (ApoE $e4$) is the most important susceptibility gene for AD, a stratified analysis by ApoE $e4$ status was performed. Higher frequencies of the minor allele (‘C’) of SORL1 were observed in aMCI patients with ApoE $e4$ allele compared with the control subjects (OR = 5.400, 95% CI = 1.438-20.273, $P = 0.011$). We also observed a positive signal in the subjects without ApoE $e4$ allele in genotypic test ($P = 0.040$) (Table 1).

### Discussion

To our knowledge, this is the first study to associate the ‘C’ allele within the SORL1 SNP rs11218343 with an increased risk for aMCI in the Han Chinese population. Interestingly, the ‘C’ allele of rs11218343 was identified to play a protective role for AD in previous studies [8,9], which is in contrast to our findings. The frequency of the ‘C’ allele is 2%, 40%, and 28% in European, Japanese, and Han Chinese populations, respectively (HapMap database), suggesting populations from different geographic regions might exhibit different genetic markers for AD development. Although no significant LD was found between these SNPs (data not shown), we observed an association in the three-marker haplotype analyses at SNP rs11218304-rs689021-rs11218343 (Table 2). In addition, due to the $e4$ allele of the apolipoprotein E gene (ApoE $e4$) is the most important susceptibility gene for AD, a stratified analysis by ApoE $e4$ status was performed. Higher frequencies of the minor allele (‘C’) of SORL1 were observed in aMCI patients with ApoE $e4$ allele compared with the control subjects (OR = 5.400, 95% CI = 1.438-20.273, $P = 0.011$). We also observed a positive signal in the subjects without ApoE $e4$ allele in genotypic test ($P = 0.040$) (Table 3). However, no significant association signals were observed in other loci (all $P > 0.05$).

LD patterns and haplotype structures for candidate gene are instructive for the genetic association analysis of complex disease. Although no significant LD was found between these SNPs (data not shown), we observed an association in the three-marker haplotype analyses at SNP rs11218304-rs689021-rs11218343 (OR = 2.206, 95% CI = 1.257-3.873, $P_{\text{adj}} = 0.0051$ for A-C-C) (Table 4).

### Acknowledgements

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### References


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Table 3: Association of SORL1 SNP rs11218343 with aMCI in ApoE $e4$ stratified samples

<table>
<thead>
<tr>
<th>Genotype n (%)</th>
<th>P</th>
<th>Allele n (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype n (%)</td>
<td>P</td>
<td>Allele n (%)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aMCI</td>
<td>63</td>
<td>9 (14.3)</td>
<td>27 (42.9)</td>
<td>27 (42.9)</td>
</tr>
<tr>
<td>Control</td>
<td>179</td>
<td>6 (3.5)</td>
<td>70 (38.1)</td>
<td>103 (57.5)</td>
</tr>
<tr>
<td>ApoE $e4$ (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aMCI</td>
<td>12</td>
<td>3 (25.0)</td>
<td>3 (25.0)</td>
<td>6 (50.0)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>1 (5.0)</td>
<td>2 (10.0)</td>
<td>17 (85.0)</td>
</tr>
<tr>
<td>ApoE $e4$ (-)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aMCI</td>
<td>51</td>
<td>6 (11.8)</td>
<td>23 (45.1)</td>
<td>22 (43.1)</td>
</tr>
<tr>
<td>Control</td>
<td>159</td>
<td>5 (3.14)</td>
<td>68 (42.8)</td>
<td>86 (54.1)</td>
</tr>
</tbody>
</table>

Table 4: Association of SORL1 three-marker haplotypes (rs11218304-rs689021-rs11218343) with aMCI

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>aMCI (freq)</th>
<th>Controls (freq)</th>
<th>Fisher’s $P_{\text{adj}}$ value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-C-C</td>
<td>24.43 (0.194)</td>
<td>35.49 (0.099)</td>
<td>0.005062</td>
<td>2.206 (1.257-3.873)</td>
</tr>
<tr>
<td>A-C-T</td>
<td>24.51 (0.195)</td>
<td>66.69 (0.186)</td>
<td>0.819670</td>
<td>1.062 (0.633-1.780)</td>
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<tr>
<td>A-T-C</td>
<td>17.41 (0.138)</td>
<td>39.35 (0.110)</td>
<td>0.381657</td>
<td>1.308 (0.713-2.396)</td>
</tr>
<tr>
<td>A-T-T</td>
<td>44.65 (0.354)</td>
<td>169.47 (0.473)</td>
<td>0.021905</td>
<td>0.611 (0.400-0.933)</td>
</tr>
<tr>
<td>G-C-T</td>
<td>5.91 (0.047)</td>
<td>35.65 (0.100)</td>
<td>0.071315</td>
<td>0.447 (0.182-1.095)</td>
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<tr>
<td>G-T-T</td>
<td>5.93 (0.047)</td>
<td>4.18 (0.012)</td>
<td>0.016834</td>
<td>4.210 (1.183-14.978)</td>
</tr>
</tbody>
</table>


