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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_{unadj} = 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) [4-6]. 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

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documented by delayed recall in the auditory-verbal learning test (AVLT); (3) preserved general cognitive function, measured using a Mini-Mental State Examination (MMSE) with a score of 24 or higher; (4) a clinical dementia rating (CDR) score of 0.5; (5) intact daily living activities; and (6) not demented, or insufficient to meet the NINCDS-ADRDA criteria for AD. Participants with epilepsy, major depression, alcoholism, head injury, Parkinson disease, other neurological or psychiatric illnesses or other with dementia were excluded. The controls had no memory complaint or cognition dysfunctions, and were confirmed to be healthy and neurologically normal. All aMCI patients and healthy controls were selected from the Wuxi Mental Health Center and underwent a comprehensive evaluation through a neuropsychological test battery, including the AVLT, Rey-Osterrieth complex figure test, Activities of daily living, Trail Making Tests A and B, and Clock Drawing Test. Subject characteristics are summarized in Table 1.

The clinical study was in compliance with the World Medical Association (WMA) Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The Ethics Committees of the Wuxi Mental Health Center approved this study. In addition, all patients and controls were not blood relations. Before enrollment in this study, all participants provided written consent forms.

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.

Table 1: Demographic characteristics of the study subjects

	Control n (%)	aMCI n (%)
Total	179	63
Female	96 (53.6)	35 (55.6)
Male	83 (46.4)	28 (44.4)
Age (years)	78.2 ± 8.7	77.4 ± 9.3
Education (years)	13.2 ± 3.6	12.8 ± 5.1
Clinical dementia rating (CDR)	0	0.5
Mini mental state exam (MMSE)	28.2 ± 2.5	25.6 ± 1.4*
Activities of daily living (ADL)	15.1 ± 0.5	15.3 ± 0.8
Auditory verbal learning test delayed recall	8.01 ± 1.42	3.14 ± 1.85*
Rey-Osterrieth complex figure test delayed recall	17.9 ± 6.1	10.4 ± 7.8*
Trail making test A (seconds)	70.6 ± 25.7	90.8 ± 29.4*
Trail making test B (seconds)	140.2 ± 40.1	181.6 ± 63.7*
Clock drawing test	9.6 ± 0.9	8.1 ± 1.5*

Note: data are presented as mean ± SD. *indicates had statistical difference between aMCI and control group, *P* value < 0.05, Mann-Whitney *U* test.

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/haploview/), 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

Table 2: Association study of three SNPs in *SORL1* under different models

	Control		aMCI			
SNP(A1/A2)†/Test Model	n	Freq. §	n	Freq. §	P_{unadj}*	P_{adj}*
rs11218304(C;T)						
Trend: C/T	47/311	13.1%	15/111	11.9%	0.7188	1.000
Allelic: C/T	47/311	13.1%	15/111	11.9%	0.7237	1.000
Dominant: (CC+CT)/TT	45/134	1.12%	14/49	22.2%	NA	NA
Recessive: CC/(CT+TT)	2/177		1/62	1.59%	NA	NA
rs689021 (G;A)						
Trend: G/A	145/213	40.5%	58/68	46.0%	0.2852	0.856
Allelic: G/A	145/213	40.5%	58/68	46.0%	0.2794	0.838
Dominant: (GG+GA)/AA	114/65	63.7%	45/18	71.4%	0.2656	0.797
Recessive: GG/(GA+AA)	31/148	17.3%	13/50	20.6%	0.5572	1.000
rs11218343 (C;T)						
Trend: C/T	82/276	22.9%	45/81	35.7%	0.004209	0.013
Allelic: C/T	82/276	22.9%	45/81	35.7%	0.004941	0.015
Dominant: (CC+CT)/TT	76/103	42.5%	36/27	57.1%	0.04438	0.133
Recessive: CC/(CT+TT)	6/173	3.35%	9/54	14.3%	0.001966	0.006

†A1/A2, indicates minor allele/major allele

§The minor allele frequency for allelic and trend model, "DD+Dd" frequency for dominant model, and "DD" for recessive model, where "D" indicates minor allele, "d" indicates the major allele

P_{adj} is adjusted by Bonferroni correction.

*Cochran-Armitage trend test p-value for the Trend model; for other models, asymptotic p-value was calculated by Chi-Squared test

Table 3: Association of SORL1 SNP rs11218343 with aMCI in ApoE ε4 stratified samples

	N	Genotype n (%)			*P*	Allele n (%)		*P*	OR (95% CI)
		CC	CT	TT		C	T		
Total									
aMCI	63	9 (14.3)	27 (42.9)	27 (42.9)	0.004	45 (34.3)	81(65.7)	0.005	1.870 (1.204-2.903)
Control	179	6 (3.35)	70 (39.1)	103 (57.5)		82 (22.9)	276 (77.1)		
ApoE ε4 (+)									
aMCI	12	3 (25.0)	3 (25.0)	6 (50.0)	0.093	9 (35.7)	15 (64.3)	0.011	5.400 (1.438-20.273)
Control	20	1 (5.00)	2 (10.0)	17 (85.0)		4 (10.0)	36 (90.0)		
ApoE ε4 (-)									
aMCI	51	6 (11.8)	23 (45.1)	22 (43.1)	0.040	35 (34.0)	67 (66.0)	0.052	1.607 (0.993-2.603)
Control	159	5 (3.14)	68 (42.8)	86 (54.1)		78 (24.5)	240 (75.5)		

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

Haplotypes	aMCI (freq)	Controls (freq)	Fisher's P_{unadj} value	OR (95% CI)
A-C-C	24.43 (0.194)	35.49 (0.099)	0.005062	2.206 (1.257-3.873)
A-C-T	24.51 (0.195)	66.69 (0.186)	0.819670	1.062 (0.633-1.780)
A-T-C	17.41 (0.138)	39.35 (0.110)	0.381657	1.308 (0.713-2.396)
A-T-T	44.65 (0.354)	169.47 (0.473)	0.021905	0.611 (0.400-0.933)
G-C-T	5.91 (0.047)	35.65 (0.100)	0.071315	0.447 (0.182-1.095)
G-T-T	5.93 (0.047)	4.18 (0.012)	0.016384	4.210 (1.183-14.978)

allele 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 $P_{adj} = 0.013$, allelic test $P_{adj} = 0.015$, the χ^2 test under recessive model: $P_{adj} = 0.006$ for aMCI) between aMCI and the minor allele ('C') of the SORL1 gene SNP rs11218343 (Table 2). In addition, due to the ε4 allele of the apolipoprotein E gene (ApoE ε4) is the most important susceptibility gene for AD, a stratified analysis by ApoE ε4 status was performed. Higher frequencies of the minor allele ('C') of SORL1 were observed in aMCI patients with ApoE ε4 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 ε4 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, $P_{unadj} = 0.0051$ for A-C-C) (Table 4).

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 a stronger positive signal between the "C" allele and aMCI was observed in the stratified analysis by ApoE ε4 status, the high OR may be caused by the relatively small sample size, which is a major limitation in this study that may affect the interpretation of the results. Thus, follow-up studies with larger sample sizes are warranted in the future.

Recently, converging lines of evidence demonstrated that SORL1 genetic variants may be significantly associated with the risk of AD and aMCI in the Han Chinese population [16-19]. Especially, the association of SORL1 gene with the high risk of conversion from MCI to AD has been reported [20]. Thus, elucidating the effects of SORL1 SNPs on the pathogenesis of AD is crucial. It is well known that functional genetic variants may affect normal neurodevelopment, clinical symptoms, brain structure and neurocognitive functioning. The SNP rs11218343 is located in the intronic region of the SORL1 gene and therefore does not affect the amino acid sequences or protein structure. However, this genetic variant may indirectly affect protein function through LD or gene expression through influencing alternative splicing of RNA. An LD analysis from HapMap database indicated that rs11218343 and a functional disease-associated SNP rs2070045 belong to a complete LD region (D' = 1.0, $r^2 = 0.567$) and that the SNP rs11218343 is predicted to play a role in transcriptional regulation by rSNPBase (data not shown). These observations suggest that the SORL1 gene SNP rs11218343 may be a risk locus with potential functions. Notably, more than 20 loci have been found to be associated with AD in the past years [21-23], while AD is a multifactorial disease affected by both inherited and environmental factors. Therefore, the interaction of gene-gene and/or SNP-SNP should not be ignored.

In summary, despite some limitations, the present case-control study of Han Chinese suggested that the SORL1 gene intronic variant rs11218343 may affect aMCI risk. However, the current findings need to be further replicated and validated.

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