The Genetic Variation of SORCS1 Is Associated with Late-Onset Alzheimer’s Disease in Chinese Han Population

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Abstract
The variations of SORCS1 gene may play potential key roles in late-onset Alzheimer’s disease (LOAD). To evaluate the relationship between the polymorphism of SORCS1 gene and LOAD in the ethnic Han Chinese, we conducted a case–control study to investigate the association between the single-nucleotide polymorphisms (SNPs) in intron 1 of SORCS1 and LOAD in Chinese Han population. Six reported SNPs in intron 1 of SORCS1 were analyzed by Snapshot, genotyping and haplotyping in 236 Chinese LOAD cases and 233 matched controls. The significant differences in frequencies of two SNPs (rs10884402, rs950809) were found between the two groups. In addition, haplotype analyses revealed that, in the LOAD group, the frequency of haplotypes C-C-G-T-C (alleles in order of rs17277986, rs6584777, rs10884402, rs7078098, rs950809 polymorphisms) were significantly higher (Psim < 0.0001) while haplotype C-C-A-T-C, C-C-A-C-C, T-T-A-C-C were significantly lower (Psim < 0.0001). Our data suggested that the genetic variation of the rs10884402 and rs950809 in intron 1 of SORCS1 was associated with the late-onset AD in the Chinese Han population.

Introduction
Alzheimer’s disease is the most common form of dementia, which is characterized by senile plaques, neurofibrillary tangles and neuron loss [1,2]. In the early-onset family AD (EOFAD), three genes, amyloid precursor protein (APP), presenilin 1 (PS1), or presenilin 2 (PS2) [3,4] were demonstrated to directly influence Aβ metabolism. In contrast to EOFAD, several risk genes such as apolipoprotein E (APOE), EPHA1, CD33 and MS 4A 6A [5,6,7] are involved in the pathogenesis and development of late-onset Alzheimer’s disease (LOAD), in which APOE is the most notable. APOE ε4 allele may account for nearly 50% of the genetic risk in LOAD [8,9].

Although the pathogenetic mechanisms of AD are undetermined, the APP processing and Aβ generation have been proven to be crucial in the pathogenesis of AD [10,11]. Extracellular accumulation of the amyloid-β (Aβ) peptides leads to senile plaques formation. Sorting mechanisms that lead to the colocalization of APP, β-secretases and γ-secretases in the same intracellular compartment may play an important part in Aβ generation in AD [12,13,14]. Sortilin-related VPS10 domain containing receptor 1 (SORCS1), which maps to chromosome 10q23–25, belongs to Vps10p-domain sorting receptor family [15,16,17]. SORCS1 is prominently expressed in the nervous system and may be important for neuronal activities [13,14]. SORCS1 was reported that it could influence APP processing and modulate Aβ metabolism [19,20]. Overexpression of SORCS1 might lead to the reduction of γ-secretase activity and Aβ levels. Oppositely, suppression of SorCS1 increased γ-secretase processing of APP and the levels of Aβ [20]. In addition, one genome-wide association study (GWAS) in French has identified SORCS1 as a candidate gene for AD [21]. All these suggested that SORCS1 were associated with the prevalence of AD.

Based on the result reported by Reitz and his colleagues which indicated several SNPs in intron 1 of SORCS1 were genetic associated with LOAD and memory retention [20,22], we conducted a case–control study (n = 469) to determine the prevalence of six reported SNPs (rs17277986, rs6584777, rs12251340, rs10884402, rs7078098 and rs950809) in patients with LOAD in Chinese Han population of mainland, trying to explore the genetic association between the polymorphism of SORCS1 and LOAD. As APOEε4 allele and the history of type-2 diabetes mellitus were confirmed worldwide as important factors to LOAD, we further analyzed the relationship between these factors and SNPs in LOAD.

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Methods

Subjects
Our study included 236 sporadic LOAD and 233 healthy controls of Chinese Han ethnicity. All patients, which were enrolled from the outpatient clinic at the Department of Neurology, Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine and were evaluated by an experienced neurologist and a psychiatrist, had a clinical diagnosis of possible or probable AD according to the Alzheimer’s Criteria of National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) [23]. All control subjects were recruited from the epidemiological investigation of Shanghai, which were matched for age, gender, and ethnic background. All subjects were unrelated Chinese Han and had no family history of AD. The average age of AD group was 72.20±7.87 years old (Mean age at onset) with the average MMSE score 15.96±3.99, 50.8% were male AD. And the average age of control was 72.88±7.30 years old (Mean age at examination) with the average MMSE score 28.35±1.42, male accounted for 50.3%. The study was approved and authorized by the Research Ethics Committee, Rui Jin Hospital affiliated to School of Medicine, Shanghai Jiao Tong University, Shanghai, China. All participants were fully informed, and had signed a formally written consent.

Genotyping

DNA was isolated from peripheral blood through standardized phenol/chlorine extraction method. Genotyping analysis of APOE was performed as previously described [24]. The SORCS1 SNPs (rs17277986, rs6584777, rs12251340, rs10884402, rs7078098 and rs950809) were genotyped using the method of SNaPshot, which was based on the dideoxy single-base extension of an unlabeled oligonucleotide primer (or primers), with technical support from the Shanghai Southgene Technology Co. LTD.

Table 1. Primer design.

| SNP ID     | Primer Name          | Sequence                        | TM(°C) |
|------------|----------------------|---------------------------------|--------|
| rs17277986 | The PCR Primer       | 5’-TCAGTTCTCCCATTGTGCT-3’       | 59.73  |
| [C/T]      | rs17277986-R         | 5’-AGGCTCTGGAAAGCATTGTTTTTT-3’  | 60.21  |
|           | The anchor probe     | rs17277986-SNP2                 | 57.14  |
| rs6584777  | The PCR Primer       | 5’-CAGAGTGTGATCCCTCTCA-3’       | 58.70  |
| [A/G] and  | rs6584777-R          | 5’-CCTCACCATGGAAACGTG-3’        | 60.00  |
| rs12251340 | [G/T] The anchor probe| 5’-ttttttttcTACCTGATCCAAATTTATCAGC-3’ | 57.03  |
| rs10884402 | The PCR Primer       | 5’-GCCGATGGCAAGGCTCACT-3’       | 60.06  |
| [A/G]      | rs10884402-R         | 5’-AAAAAAAAAAAAGAGAAGATG-3’     | 60.11  |
| rs7078098  | The PCR Primer       | 5’-ACTCCGTAGCTCTGGGAGA-3’       | 59.94  |
| [C/T]      | rs7078098-R          | 5’-AGGGTGCTCCAGATGTG-3’         | 60.12  |
| rs950809   | The PCR Primer       | 5’-ttttttttttttttttttttttTTGTAGGTGATGTTTGCAATCAGT-3’ | 58.97  |
| [C/T]      | rs950809-R           | 5’-CATTTGATCTTCTTCTGAGG-3’      | 60.20  |
|           | The anchor probe     | rs950809-SNP2                   | 59.41  |
| rs950809   | rs950809-SNP2        | 5’-ttttttttttttttttttttttttttGATGAGGCGATAGGGGTCAC-3’ | 57.74  |

All amplification primers were synthesized by standard phosphoramidite chemistry (Sangon Biotech). The primers and probe sequences which were used are summarized in Table 1. The amplification of the target fragment was carried out on a PCR Amplifier (MJ Research PT-100) in a total volume of 10 μl containing ~20 ng of DNA, 0.4 μM each of the primers, 0.3 mM dNTP (Generay Biotech), 0.25 U HotStarTaq DNA Polymerase (QIAGEN). The final concentration of Mg²⁺ in the reaction mixture was adjusted to 3.5 mM. The cycle conditions were as following: denaturation of the template DNA for 1 cycle of 95°C for 5 mins; amplification of the target fragment for 45 cycles of 95°C for 30 s, 60°C for 60 s, and 72°C for 180 s. The PCR products were electrophoresed on 2% agarose gel and visualized under UV light. 2 U Shrimp Alkaline Phosphatase (USB) and 2 U Exonuclease I (Epigenet) was used to purify the target fragment. The mixture was incubated at 37°C for 1 hour and then was incubated at 75°C for 15 mins to inactivate the enzymes. All the primers to be used for SNaPshot reaction should be premixed to reach a final concentration of 0.2 μM. The total volume of SNaPshot PCR mixture was 5 μl containing 1 μl SNaPshot Multiplex Ready Reaction Mix (ABI), 2 μl Pooled PCR products, 1 μl Pooled SNaPshot primers and 1 μl deionized water. The cycle conditions for SNaPshot were as following: denaturation of the template for 1 cycle of 95°C for 10 s; amplification of the target fragment for 25 cycles of 95°C for 10 s, 50°C for 5 s, and 66°C for 30 s. Add 0.5 Unit of Shrimp Alkaline Phosphatase (USB) to the reaction mixture, mix thoroughly, and incubated at 37°C for 1 hour. The enzyme was deactivated by incubating the mixture at 75°C for 15 mins. Dilute 0.5 μl of SNaPshot product and 0.25 μl of GeneScan-120 LIZ (ABI) in 9.25 μl of Hi-Di formamide (ABI), vortex briefly and quick spin, then denature the samples by placing them at 95°C for 5 minutes. Electrophoresis was performed on the ABI PRISM 3730 DNA Analyzer according to the manufacturer’s instructions.

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Statistical Analysis

Statistical calculations were done using SAS v.9.1.3 (Institute Inc., Cary, NC). Means of continuous variables were compared by unpaired t-test. The χ² test or Fisher’s exact test was used to assess the goodness-of-fit between the observed allele frequencies and the expected counterparts by Hardy–Weinberg equilibrium and to evaluate the differences in genotype and allele distributions between cases and controls. Each genotype was assessed by logistic regression analysis assuming additive, dominant and recessive modes of inheritance, respectively. A two-tailed P < 0.05 was accepted as statistically significant.

The linkage disequilibrium patterns were identified in all samples by Haplovie v.4.0 available at www.sourceforge.net. The linkage disequilibrium coefficients were shown as D’ on the basis of 4 gamete color scheme. Traditionally, a haplotype was defined as a combination of multiple alleles in a chromosome. This was because alleles on the same chromosome were in the close proximity and might interact with each other. The haplo.em program was used to estimate the haplotype frequencies for the polymorphisms. This program estimated maximum likelihood of haplotype probability using the progressive insertion algorithm that progressively inserts batches of loci into haplotypes of growing length. The haplo.cc and haplo.glm were employed to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for each haplotype, respectively. These two approaches were based on a generalized linear model, and computed the regression of a trait on haplotypes and other covariates [25,26]. Furthermore, the haplo.score was used to model an individual phenotype as a function of each inferred haplotype, weighed by their estimated probability, to account for haplotype ambiguity. It was based on score statistics, which provided both global tests and haplotype specific tests [27]. Simulated P (Psim) values were obtained from 1000 replicates. The haplo.em, haplo.glm and haplo.score were implemented in the R language (http://www.r-project.org).

Results

The baseline characteristics between patients with Alzheimer’s disease and healthy controls were compared. No statistical differences were observed for age and gender between patients and controls (P > 0.05). But significantly lower MMSE score was found in LOAD patients compared to the controls (P < 0.001). Distributions of the APOE polymorphisms in both AD patients and controls were as expected.

Single-point Association Analysis

There were no deviations from Hardy–Weinberg equilibrium for all studied polymorphisms in LOAD and controls (P > 0.05). Three polymorphisms in SORCS1 did not reach significant differences in the genotype or allele frequencies in the total sample (genotype: rs17277986 P = 0.923, rs6584777 P = 0.982, rs7078098 P = 0.325; Allele: rs17277986 P = 0.719, rs6584777 P = 0.863, rs7078098 P = 0.207). rs10884402 polymorphism was demonstrated to have significant differences in both genotype and allele frequencies between the two groups in the total sample (genotype P = 0.0001; allele P = 0.0004). rs950809 polymorphism showed an edge difference in the genotype frequencies (P = 0.036) but no differences in the allele frequencies (P = 0.79). The results of three genetic modes of inheritance for the six studied genotypic polymorphisms in SORCS1, which were assessed by logistic

Table 2. Genotype and allele frequencies for rs17277986, rs6584777, rs12251340, rs10884402, rs7078098 and rs950809 SNPs and three genetic modes of inheritance for the five studied polymorphisms in SORCS1 gene.

| SNP ID   | Group | n   | Genotype frequency (%) | P-value | MAF     | P-value | Models | OR; 95% CI; P* |
|----------|-------|-----|------------------------|---------|---------|---------|--------|----------------|
|          |       |     | CC                     | CT      | TT      |         |        |                |
| rs17277986 | AD    | 236 | 173(73.3)              | 60(25.4)| 3(1.3)  | 0.922   | 0.14   | 0.719         | 0.92(0.61,1.38),0.69 |
|          | Control | 233 | 167(71.7)              | 63(27)  | 3(1.3)  | 0.15    |         |               | 0.97(0.66,1.41),0.86 |
| rs6584777 | AD    | 236 | 173(73.3)              | 60(25.4)| 3(1.3)  | 0.974   | 0.14   | 0.863         | 0.96(0.64,1.45),0.85 |
|          | Control | 233 | 169(72.5)              | 61(26.2)| 3(1.3)  | 0.14    |         |               | 0.99(0.2,4.94),0.99 |
| rs12251340| AD    | 236 | 236(100)               | 0(0)    | 0(0)    | NA      |         |               | NA               |
|          | Control | 233 | 233(100)               | 0(0)    | 0(0)    | 0       |         |               | NA               |
| rs10884402| AD    | 236 | 102(43.2)              | 112(47.5)| 22(9.3)| 0.0001  | 0.33   | 0.0004        | 0.7(0.48,1.02),0.06 |
|          | Control | 233 | 81(34.8)               | 97(41.6)| 55(23.6)| 0.44    |         |               | 0.33(0.2,0.57),<0.001 |
| rs7078098 | AD    | 236 | 117(49.6)              | 101(42.9)| 18(7.6)| 0.325   | 0.29   | 0.207         | 1.16(0.81,1.66),0.428 |
|          | Control | 233 | 107(45.9)              | 99(42.5)| 27(11.6)| 0.33    |         |               | 1.59(0.85,2.97),0.148 |
| rs950809  | AD    | 236 | 87(36.9)               | 126(53.4)| 23(9.7)| 0.036   | 0.36   | 0.79          | 1.31(0.9,1.9),0.15  |
|          | Control | 233 | 101(43.3)              | 98(42.1)| 34(14.6)| 0.36    |         |               | 0.63(0.36,1.11),0.11 |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MAF, minor allele frequency; NA, not available.

AAdjusting for age and gender.

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regression analysis, were also shown in Table 2. rs10884402 showed significant differences in additive mode (OR = 0.63, 95% CI (0.49, 0.82), P<0.001) and recessive mode (OR = 0.33, 95% CI (0.20, 0.57), P<0.001) (Table 2). Since the genotype and allele frequencies of rs12251340 were completely all the same between the patients and the controls, we did not conduct the further analysis and discussion. When data were stratified by the history of type-2 diabetes mellitus or the severity of AD, no significant difference was observed (data not show). When data were stratified by ApoE e4, the significant differences of rs10884402 polymorphism were observed in both genotype and allele frequencies between the patients and controls in the ApoE e4(-) population (genotype P = 0.008; allele P = 0.003) (Table 3). When data were stratified by gender, the significant differences for rs17277986 and rs6584777 were shown between male and female AD patients (Table 4). These results suggested there were no gender association for all five SNPs.

| SNP ID       | Group          | n   | Genotype frequency (%) | P-value | MAF  | P-value |
|--------------|----------------|-----|------------------------|---------|------|---------|
| rs17277986   | AD             | 130 | 94(72.3)               | 0.899   | 0.15 | 0.94    |
|              | control        | 189 | 136(72)                | 0.15    |      |         |
| rs10884402   | AD             | 130 | 94(72.3)               | 0.988   | 0.15 | 0.91    |
|              | control        | 189 | 138(73)                | 0.14    |      |         |
| rs12251340   | AD             | 130 | 57(43.9)               | 0.0008  | 0.34 | 0.003   |
|              | control        | 189 | 65(34.4)               | 0.46    |      |         |
| rs7078098    | AD             | 130 | 68(52.3)               | 0.515   | 0.28 | 0.24    |
|              | control        | 189 | 87(46)                 | 0.33    |      |         |
| rs950809     | AD             | 130 | 47(36.2)               | 0.202   | 0.38 | 0.76    |
|              | control        | 189 | 80(42.3)               | 0.37    |      |         |

Abbreviations: MAF, minor allele frequency. Genotypes and alleles are expressed as number (percentage). P values were calculated by x 2 test for genotype distribution and 2 x 2 contingency table for allele distribution.

Haplotype Analysis

Since the studied polymorphisms are assigned on the same chromosome, we accordingly performed the linkage analysis (Fig. 1), strong linkage patterns were observed between rs17277986 and rs6584777 in all samples (D’ = 0.96), as well as among rs10884402, rs7078098 and rs950809 (D≥0.97). Therefore, rs17277986 and rs6584777 constitute a block (block1) of two SNPs that are 5 kb apart, and rs10884402, rs7078098 and rs950809 constitute a block (block2) of three adjacent SNPs that are 2 kb apart and are in linkage disequilibrium (LD) in all samples. To facilitate identification of combinational effects of these five polymorphisms on AD risk, we employed haplotype analysis, which studies the frequency of the combination of multiple genetic variants. This is a more powerful statistical method than single-locus analysis. We focused on the haplotypes, which had a frequency of equal to or greater than 1% in all cases. The frequency of haplotypes composed of G-T-C (alleles in order of rs10884402, rs7078098, rs950809 polymorphisms, similarly hereinafter) was 34% in LOAD, which was significantly higher (Psim = 0.002) than that in control, whereas the frequencies of
haplotypes composed of A-C-C and A-T-C was significantly lower (Psim = 0.0001) in LOAD. Frequency of haplotype C-C-G-T-C (alleles in order of rs17277986, rs6584777, rs10884402, rs7078098, rs950809 polymorphisms, similarly hereinafter) was significantly higher (Psim = 0.0031), yet the frequencies of haplotype C-C-A-T-C, C-C-A-C-C and T-T-A-C-C were significantly

| Table 4. Genotype and allele frequencies for rs17277986, rs6584777, rs10884402, rs7078098 and rs950809 stratified by gender in Alzheimer’s patients. |
| SNP ID | Group | n | Genotype frequency (%) | P-value | MAF | P-value |
|--------|-------|---|------------------------|---------|-----|---------|
|        |       | CC | CT | TT | T |        |
| rs17277986 | AD(Male) | 120 | 98 (81.7) | 20 (16.7) | 2 (1.6) | 0.007 | 0.1 | 0.01 |
|        | AD(Female) | 116 | 75 (64.7) | 40 (34.5) | 1 (0.8) | 0.18 |        |
| rs6584777 | AD(Male) | 120 | 98 (81.7) | 20 (16.7) | 2 (1.6) | 0.007 | 0.1 | 0.01 |
|        | AD(Female) | 116 | 75 (64.7) | 40 (34.5) | 1 (0.8) | 0.18 |        |

| Table 5. Genotype and allele frequencies for rs17277986, rs6584777 stratified by gender in overall samples, Alzheimer’s patients and controls. |
| SNP ID | Group | n | Genotype frequency (%) | P-value | MAF | P-value |
|--------|-------|---|------------------------|---------|-----|---------|
|        |       | CC | CT | TT | T |        |
| rs17277986 | male | 256 | 202 (78.9) | 52 (20.3) | 2 (0.8) | 0.003 | 0.09 |
| female | 213 | 138 (64.8) | 71 (33.3) | 4 (1.9) | 0.19 |        |
| rs6584777 | male | 256 | 203 (79.3) | 51 (19.9) | 2 (0.8) | 0.003 | 0.09 |
| female | 213 | 139 (65.2) | 70 (32.9) | 4 (1.9) | 0.18 |        |

Abbreviations: MAF, minor allele frequency.
Genotypes and alleles are expressed as number (percentage). P values were calculated by χ² test 3×2 contingency table for genotype distribution and 2×2 contingency table for allele distribution.
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The Genetic Variation of SORCS1 and LOAD
lower (Psim<0.0001), in LOAD than in controls even after the statistical simulation (Table 6).

**Discussion**

APP processing and Aβ generation were considered to be the most important factors in pathogenesis of AD. Specifically, Small et al had suggested that APP processing might be modulated by Vps10-containing proteins [28], which could mediate the interaction between the retromer complex and APP. APP, β-secretases and γ-secretases were thus colocalized in the same intracellular compartment, where APP processing occurred. The genetic variations of SORCS1, the most recent member of the Vps10 family proteins [18], was also found to be associated with AD [20,22,29,30] in Caribbean Hispanics, Caucasian Hispanics and et al. In our current study, rs10884402 and rs950809 in intron 1 of SORCS1 were found to be associated with LOAD in Chinese Han population. The most noteworthy finding of this study was that rs10884402 showed significant difference between LOAD and the healthy controls in both genotype and haplotype analyses. The further stratified analysis also revealed the significant difference for rs10884402 polymorphism in both genotype and allele frequencies between the patients and controls in the ApoE ε4(2) population. The rs10884402 A allele displayed a significant protective effect against the risk of LOAD compared with the G allele in additive mode. The largerer number of A/A or A/G genotype in the ApoE ε4(2) normal control group reinforces our speculation that rs10884402 A allele is protective against AD. Moreover, we found this protective effect was irrelevant with the severity of AD. rs10884402 AA genotype vs G allele carriers conferred a 67% decreased risk for LOAD in recessive mode.

Table 6. Haplotype frequencies (>1%) of the five SNPs rs17277986, rs6584777, rs10884402, rs7078098 and rs950809 in SorCS1 gene and their relative risks for Alzheimer’s disease.

| Allele | All | LOAD | Control | Psim | OR (95% CI); P | OR (95% CI);P |
|--------|-----|------|---------|------|----------------|----------------|
| Combination | (n = 469) | (n = 236) | (n = 233) | (after ajustment) |
| 1:C-C-A-T-C | 0.03195 | 0 | 0.06212 | <0.0001 | NA | NA |
| 2:C-C-A-C-C | 0.01804 | 0 | 0.03647 | <0.0001 | NA | NA |
| 3:T-T-A-C-C | 0.01081 | 0 | 0.02143 | <0.0001 | NA | NA |
| Totalb | 0.31295 | 0.31208 | 0.32093 | 0.971 | Reference | Reference |
| 4:C-C-A-T-T | 0.10894 | 0.11189 | 0.10636 | 0.732 | 0.98(0.60,1.58);0.925 | 0.91(0.56,1.49);0.716 |
| 5:T-T-G-C-C | 0.0268 | 0.02873 | 0.02403 | 0.489 | 1.04(0.45,2.41);0.925 | 1(0.42,2.35);0.998 |
| 6:C-C-G-T-T | 0.16205 | 0.17556 | 0.14852 | 0.324 | 1.10(0.72,1.69);0.642 | 1.12(0.93,1.9);0.608 |
| 7:C-C-G-C-C | 0.29709 | 0.34099 | 0.2486 | 0.003 | 1.32(0.93,1.9);0.121 | 1.35(0.95,1.94);0.098 |
| Block 1b | 1:C-C | 0.85608 | 0.86017 | 0.85193 | 0.708 | Reference | Reference |
| 2:T-T | 0.14179 | 0.13983 | 0.14378 | 0.916 | 0.96(0.65,1.4);0.815 | 0.91(0.62,1.33);0.617 |
| 1:A-T-C | 0.03203 | NA | 0.06496 | <0.0001 | NA | NA |
| 2:A-C-C | 0.0289 | NA | 0.05759 | <0.0001 | NA | NA |
| Block 2c | 3:A-T-T | 0.32511 | 0.33051 | 0.32091 | 0.889 | Reference | Reference |
| 4:C-C-G-C-C | 0.2729 | 0.28724 | 0.25941 | 0.399 | 0.98(0.71,1.39);0.936 | 0.97(0.68,1.37);0.85 |
| 5:T-G-T | 0.02787 | 0.03088 | 0.02399 | 0.402 | 1.06(0.46,2.44);0.886 | 1.04(0.45,2.44);0.918 |
| 6:T-G-C | 0.30582 | 0.34836 | 0.26181 | 0.002 | 1.23(0.86,1.74);0.254 | 1.26(0.89,1.8);0.195 |

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; Psim, Simulated P values; NA, not available.

bAlleles in total haplotype were arrayed in order of rs17277986, rs6584777, rs10884402, rs7078098 and rs950809.
cAlleles in block 1 haplotype were arrayed in order of rs17277986 and rs6584777.
dAlleles in block 2 haplotype were arrayed in order of rs10884402, rs7078098 and rs950809.
eAdjusting for age and gender.

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Chinese Han population was inconsistent with Reitz et al’s report that A allele of this SNP was associated with lower MMSE scores in Caribbean Hispanics [22]. Haplotype is composed of different alleles, thus haplotype analysis provides more information about the effect of genetic interaction on phenotype than single polymorphism analysis. Haplotype analyses in our study showed that haplotypes A-T-C and A-C-C (alleles in order of rs10884402, rs7078098, rs950809) were observed in controls with total frequencies reaching 12%, whereas frequency of haplotype G-T-C was significantly higher in LOAD group than that in the controls, in agreement with the results of our single-locus analyses. However, this is also different from Reitz et al’s report that A-T-T haplotype for SNPs (alleles in order of rs10884402, rs7078098 and rs950809) were associated with LOAD in both NIA-LOAD dataset and Caribbean Hispanics datasets, and the complementary G-C-C haplotype was associated with higher MMSE scores in the NIA-LOAD dataset [22]. All above results indicated A allele in rs10884402 and C allele in rs950809 seemed to have a synergistic action because their combination was shown a protective effect against the risk of dementia, and A allele in rs10884402 might take a dominant place according to the results.

Our data showed negative association between rs17277986 and AD either in overall samples or in both gender subsets. However, when we reviewed all the case-control studies in the Alzgene data base and recent related studies, we found our result was different from that of Liang’s study [30], in which rs17277986 showed significant association with AD in the overall datasets (p = 0.0025) and in the female subset (allele p = 0.00002). However Liang et al could not confirm the association in their follow-up validation analyses in the validation datasets (CAP, the Collaborative Alzheimer Project; NCRAD, the NCRAD repository at Indiana University; NIMH, the National Institute of Mental Health repository) [30]. Our result was different from Reitz’s report either [30].

All above results indicated that several inconsistencies were presented in the different reports. However, we thought our data was reliable because of the following reasons. All SNPs in our research had been genotyped using the method of SNaPshot, which was an advanced and accurate gene analysis technique. All genotype distributions of SNPs in our research were conformed to the expected Hardy–Weinberg proportions. Moreover, the distributions of the APOE polymorphisms in AD patients and controls showed a significant difference, being the same as what was expected. And interestingly, our data about rs17277906 and rs6504777 are almost coincide with the results of the study in a Northern Han Chinese population conducted by Tan’s group [29], although the samples and experimental procedures were different. All these make our data trustworthy. One major and important reason for the inconsistency between our results and those of former studies is the different genetic background in different ethnic populations. The different environment and lifestyle may also need to be taken into consideration for the inconsistency because of the complexity of the interactions between genes and above factors. In addition, the size of sample group might be the third reason for the discrepancy. The larger sample size would be helpful for the further study.

In summary, our results implicated variations in intron 1 of SORCS1 gene were associated with LOAD in Han Chinese. However, additional studies seeking to provide strong biological or clinical evidence for the association between SORCS1 SNPs and LOAD, as well as longitudinal studies attempting to pursue gene–gene or gene–environment interactions of SORCS1, will be needed in the future.

Author Contributions
Conceived and designed the experiments: JD SC JZ. Performed the experiments: WX JX Y. Analyzed the data: WN. Contributed reagents/materials/analysis tools: WX Y. Wang HT YD RR GW JM Y. Wu. Wrote the paper: WX JD.
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