Amyloid-β-Related Genes *SORL1* and *ACE* are Genetically Associated With Risk for Late-onset Alzheimer Disease in the Chinese Population

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Abstract: Late-onset Alzheimer Disease (LOAD) is a common neurodegenerative disease, and one of its major pathologic characteristics is senile plaques. Proteins encoded by *SORL1* and *ACE* have been shown to be related to the processing, trafficking, and degradation of Amyloid-β, the principal component of senile plaques. In this paper, we investigated whether *SORL1* and *ACE* are associated with LOAD. We recruited 144 LOAD patients and 476 controls from Shanghai, China and conducted a case–control study on 9 single-nucleotide polymorphisms (SNPs): 6 in *SORL1* (rs2070045, rs661057, rs668387, rs689021, rs3824968, rs2282649) and 3 in *ACE* (rs1800764, rs4343, rs1799752). Despite the small case sample size (144), we observed that rs1800764, rs4343, rs1799752 in *ACE*, and rs2070045, rs3824968, rs2282649 in *SORL1* showed significantly different allele frequencies between patients and controls ($P = 4.57 \times 10^{-2}$, 5.24×10^{-3}, 1.95×10^{-4}, 1.77×10^{-4}, $6.44 \times 10^{-3,}$ and $3.11 \times 10^{-3,}$ respectively). Moreover, haplotypes on *ACE* and on *SORL1* were significantly associated with LOAD (all *P*-value < 0.009 in *ACE* and all *P*-value < 0.003 in *SORL1*). In *ACE*, we found the most significant protective haplotype encompasses SNPs rs2070045, rs3824968, and rs2282649 (C-G-D: OR = 0.20, $P = 8.96 \times 10^{-14}$). In *SORL1*, we detected a "complementary" haplotype (G-A-T: OR = 1.54, $P = 2.67 \times 10^{-3}$; T-T-C: OR = 0.63, $P = 2.36 \times 10^{-3}$) composed of SNPs rs2070045, rs3824968, and rs2282649. In addition, we carried out meta-analysis with 3 other Asian populations on 3 SNPs in *SORL1* (rs2070045, rs3824968, and rs2282649). Results supported our initial finding that these 3 SNPs were associated with LOAD. Our data suggested that *SORL1* and *ACE* might play a role in LOAD susceptibility among Han Chinese.

Key Words: *ACE*, Asian, Chinese, haplotype, late-onset Alzheimer's disease, *SORL1*

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Alzheimer Disease (AD), a neurodegenerative disease characterized by progressive memory loss, is the world's most common cause of dementia in the elderly. AD is usually divided into 2 types: early-onset and late-onset, with the later being more widespread. However, owing to the complex etiology of late-onset AD (LOAD), *APOE* is so far the only genetic factor showing clear association with the disease.[1–7] Moreover, the contribution of *APOE* to LOAD has recently been shown to be smaller than what have been earlier reported.[8]

Senile plaques, composed mainly of Aβ, are particularly important in the pathology of AD.[9–11] Among the Aβ-related genes, *ACE, and SORL1* are closely related to the production and degradation of Aβ. *ACE* encodes angiotensin I converting enzyme 1, which affects blood pressure, modulates the effects of cerebrovascular events on Aβ production,[12] and proteolytically degrades Aβ.[13] *SORL1* encodes sortilin-related receptor, a member of the low-density lipoprotein receptor family. Sortilin-related receptor can influence the amount of Aβ by interacting with β-amyloid precursor and regulating its trafficking and localization.[14] We summarized the functions of the 3 genes (*SORL1*, *ACE,* and *APOE*) and indicated their potential role in LOAD pathogenesis in Figure 1.

Rogaeva et al[15] first found the *SORL1* was associated with LOAD in European populations, and Kehoe et al,[16] Meng et al[17,18] reported that SNPs on *ACE* showed association with LOAD. On the basis of these and on the biologic functions of these genes, we conducted a case-control association study on 9 selected SNPs in *ACE* and *SORL1* to investigate whether these SNPs are associated with risk of LOAD in the Chinese population.

MATERIALS AND METHODS

Study Population

We recruited, for our case-control investigation, 144 unrelated LOAD patients (60 males and 84 females) from Shanghai Mental health Center and 476 controls (173 males and 303 females) from homes for older people in Shanghai (Table 1). Patients were diagnosed according to criteria of

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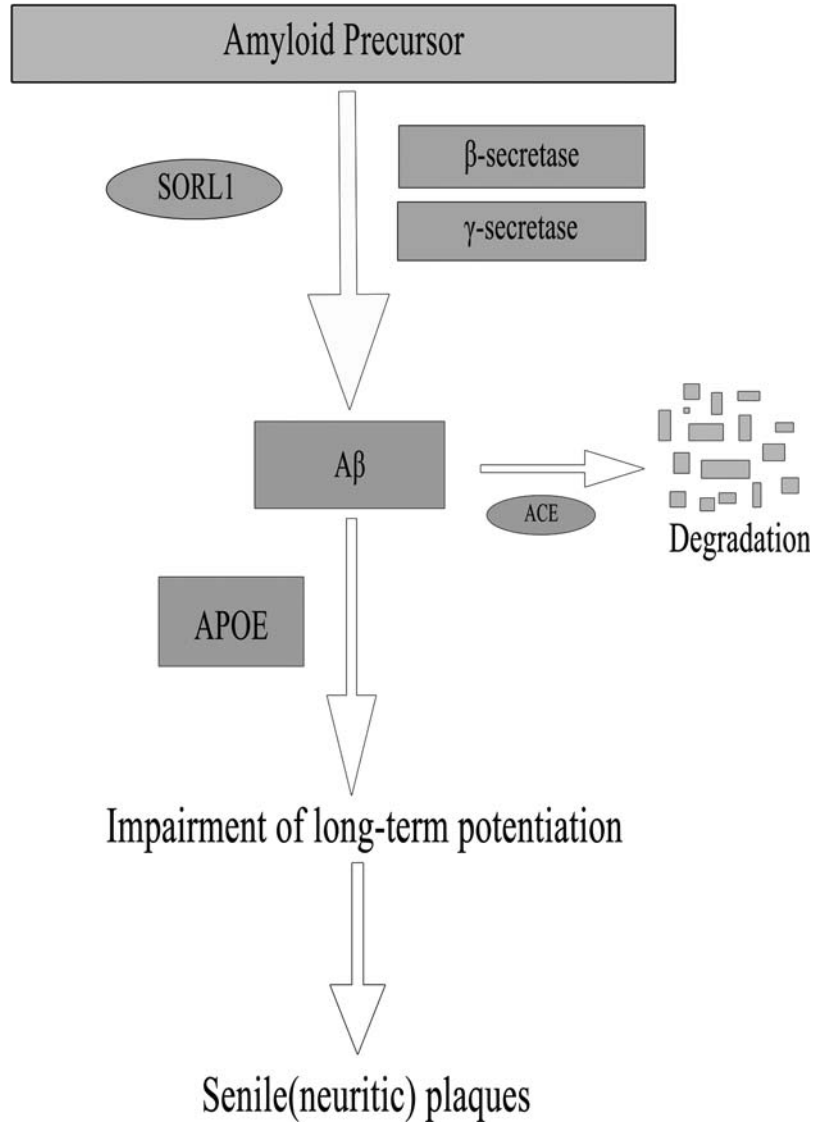

FIGURE 1. Possible pathogenetic roles for *ACE* and *SORL1* in AD. Amyloid precursor produces Aβ through serial cleavage by β-secretase and γ-secretase, or precludes Aβ production by α-secretase. *SORL1* influence the production of Aβ by affecting traffic of Amyloid precursor. *ACE*1 modulates the effects of cerebrovascular events and degradate Aβ. The established late-onset AD gene apolipoprotein E (*APOE*) influences Aβ aggregation. Established AD genes are depicted in square; AD candidate genes are depicted in ellipse.

the Diagnostic and Statistical Manual, fourth edition (DSM-IV) and the National Institute of Neurological Disorders and Stroke–Alzheimer's disease and Related Disorders (NINCDS–ADRDA). Healthy controls took physical examination in Northeast Hospital. All of these healthy controls met the Mini-Mental State Examination (MMSE) cutoff of >25, meaning no dementia. Furthermore, controls were required to be stable residents in the Shanghai areas and free of these conditions: history of stroke, severe psychologic disorders, physical disabilities, cancer, diabetes mellitus, cardiovascular diseases within 6 months; current diagnosis of tuberculosis, AIDs, or other communicable diseases. Recruited patients and controls were all from Shanghai, China. The purpose and procedures of this study were fully explained to all participants before they signed a standard informed consent, which was reviewed and approved by the Shanghai Ethical Committee of Human Genetic Resources. Genomic DNA was prepared from venous blood using the classical phenol-chloroform method.

TABLE 1. Clinical Characteristics of the Participants

		Sex*		
	Age†	Male	Female	Total
LOAD	71.6 ± 7.3	60	84	144
Control	69.3 ± 8.7	173	303	476

*The gender distribution in case and control were compared by χ^2-test and showed no significant difference between case and control.

†The age distribution in case and control were compared by z-test and showed differences (P-value < 0.01), so these analyses were all adjusted by age distribution.

Single-nucleotide Polymorphism Genotyping

We genotyped 7 SNPs (rs2070045, rs661057, rs668387, rs689021, rs3824968, and rs2282649 in SORL1, rs1800764 in ACE) using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Allele-specific probes for these 7 SNPs were labeled with the fluorescent dyes VIC and FAM, respectively. After PCR, all samples were run on ABI PRIM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA), and screened on SDS 2.2 software for allelic discrimination (Applied Biosystems).

rs1799752, an insertion (I)/deletion (D) polymorphism with approximately 250 bp DNA fragment, was genotyped using PCR and agarose gel methods.[19] We amplified region encompassing SNP rs1799752 in DNA Thermal Cycler ABI GeneAmp9700 (Applied Biosystems, Foster City, CA) with primer: 5′-CTGGAGACCACTCCCATCCTTTCT-3′ (forward) and 5′-GATGTGGCCATCACATTCGTCAGAT-3' (reverse) The cycling conditions were: 95°C 10 minutes, 35 cycles of 3 steps (95°C 30 s, 58°C 30 s, and 72°C 30 s), then 72°C 2 minutes and 4°C forever. We genotyped PCR products directly using Ultraviolet illumination and fragment size was determined by 100-bp ladder marker. To exclude agarose gel experimental error, we repeated 32 random samples with 100% concordance in genotyping.

SNP rs4343 was genotyped through direct sequencing. Primers were designed to amplify part of the *ACE* sequence covering the SNP (forward primer: 5′-GCATTCAAA CCCCTACCAGA-3′ and reverse primer: 5′-CACCACAT TACCTGCCTCCT-3′). The cycling conditions were: 95°C 10 minutes, 35 cycles of 3 steps (95°C 30 s, 56°C 30 s, and 72°C 30 s), then 72°C 2 minutes and 4°C forever. Purified PCR products were sequenced using BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 3730XL DNA sequencer (Applied Biosystems, Foster City, CA). Nucleotide changes were detected by visual inspection of chromatograms. Genotype completeness is over 95% for all 9 SNPs analyzed.

Statistical Analysis

Statistical Significance

We used SHEsis[20] to carry out the Hardy-Weinberg Equilibrium (HWE) test and allele/genotype/haplotype analysis. Allele and genotype frequencies of patients and control participants were compared using χ^2-test or Fisher exact test. Statistical significance was defined at $\alpha = 0.05$ level.

Sex and age distribution between case and controls were compared using χ^2-test and z-test, respectively in Microsoft Office Excel 2003. Gender distribution showed no significant difference between case and control whereas age did (P-value < 0.01).

We used R-software[21] to fit models with SNP genotypes. For the additive model, homozygotes of the risk allele (1/1), heterozygotes (1/0), and homozygotes for the nonrisk allele (0/0) were coded into an ordered categoric variable (values 2, 1, and 0, respectively). For the dominant model, 1/1 and 1/0 were both recoded as 1 and 0/0 as 0. Similarly, for the recessive model, 1/1 was coded as 1 and 1/0 and 0/0 were both coded as 1. We then fit these 3 models adjusting for age in R-software.[21] We included age as a covariate and carried out logistic regression. The statistical significance of parameter coefficient, which can denote whether the association is still significant or not after adjusting by age.

TABLE 2. Allele-wise Association Analysis Between LOAS Cases and Controls

Gene	SNP	Alleles†	P‡	Bonferroni Method§
ACE	rs1800764	C/T	$\mathbf{4.57 \times 10^{-2}}$	0.41
	rs1799752	D/I	$\mathbf{1.95 \times 10 - 4}$	1.75×10^{-3}*
	rs4343	G/A	$\mathbf{5.24 \times 10 - 3}$	4.72×10^{-3}*
SORL1	rs2070045	T/G	$\mathbf{1.77 \times 10 - 4}$	1.59×10^{-3}*
	rs661057	T/C	0.09	0.81
	rs668387	C/T	0.18	1.62
	rs689021	G/A	0.11	0.99
	rs3824968	T/A	$\mathbf{6.44 \times 10 - 3}$	5.80×10^{-2}
	rs2282649	C/T	$\mathbf{3.11 \times 10 - 3}$	2.80×10^{-2}*

†The second allele is risk allele (only meaningful for SNPs whose P-value < 0.05).

‡P-values calculated by Fisher exact test, and significant P-values (< 0.05) are in bold.

§P-values after Bonferroni method correction and significant P-values (< 0.05) marked by *.

Multiple Testing Adjustments

To control type I errors we adjusted all the allele-wise association resulting P-values using Bonferroni method (results summarized in Table 2).

Power Analyses

We estimated the statistical power of this study using the Genetic Power Calculator.[22] For a disease SNP with minor allele frequency of 0.2 and OR of 1.5, the statistical power using the sample size in this study to detect its association with disease exceeds 80%.

Haplotype Analyses

We carried out haplotype analysis using SHEsis,[20,23] in which haplotype frequency difference between case and control groups was assessed using Fisher exact test. Haplotypes with frequency < 0.03 were ignored in analysis.

Meta Analyses

Using keywords "*SORL1,*" "Alzheimer," "sortilin-related receptor," we found 17 studies through PubMed. Among the 17 studies, we found 3 meeting all of these criteria: (1) presented sufficient data to calculate OR with CI and P-value; (2) were studies in Asian populations; (3) were association studies written in English. The 3 studies, were conducted in Japanese[24,25] or Chinese[26] populations. Two had data for all of rs2070045, rs3824968, rs2282649, whereas 1 study only contains rs3824968, rs2282649 data. The analyzed data cover all English-language publications up to February 2010.

Data from the case-control studies were summarized using 2-by-2 tables. For meta-analysis of rs2070045, 3 studies (including ours, but without Nobuto Shibata because they did not investigate the association between rs2070045 and LOAD) were included, comprising of 804 cases and 1190 controls; for rs3824968 and rs2282649, all 4 studies were included for a total of 984 cases and 1320 controls.

For each site, we used Cochran χ^2-based Q statistic test to assess heterogeneity, and I^2 statistic to measure the extent of inconsistency among studies. Egger test was used to assess publication bias. The significance of the overall OR was determined by z-test. Each study was removed in turn and the remaining was reanalyzed to ensure that no

individual study was entirely responsible for the positive result. We used Review Manager 4.2 to conduct the above analysis.[27]

RESULTS

Single Marker Analysis

We genotyped 144 LOAD cases and 476 controls with 3 SNPs in *ACE* and 6 SNPs in *SORL1*. All samples were from Shanghai China, and gender distribution showed no difference between case and control, whereas age distribution did (Table 1). The allele frequencies of 3 *ACE* SNPs (rs1800764, rs4343, and rs1799752) and 6 *SORL1* SNPs (rs2070045, rs661057, rs668387, rs689021, rs3824968, and rs2282649) in LOAD patients and controls were summarized in Table 2. All SNPs except rs4343 and rs1799752 were in HWE in both case and control groups at $\alpha = 0.05$ level. These 2 SNPs were in HWE in controls but not in cases.

We first carried out χ^2-test for allele frequency difference. We found that among these SNPs, 3 SNPs rs1800764, rs4343, and rs1799752 in *ACE* and 3 SNPs rs2070045, rs3824968, and rs2282649 in *SORL1* were significantly associated with LOAD in our sample (*P*-values were 4.57×10^{-2}, 5.24×10^{-3}, 1.95×10^{-4}, 1.77×10^{-4}, 6.44×10^{-3}, and 3.11×10^{-3}, respectively) and, except for rs1800764 in *ACE* and rs3824968 in *SORL1*, association remained significant after conservative Bonferroni correction (Table 2).

We then tested the addictive, dominant, and recessive models using likelihood-ratio test, and found the positive SNPs in our study were in accordance with addictive models except for rs4343 and rs1799752, for both recessive model fits best. We found age distribution showed significant difference between case and control groups whereas gender distribution did not (Table 1). We therefore, adjusted for age in the analysis of all 6 positive SNPs. Genotype-wise association analyses of the 6 SNPs were summarized in Table 3. ORs for these positive SNPs were above 1.5 (with the exception of rs1800764 in *ACE*), which represented moderate risk for the disease.

LD Structure and Haplotype Analysis

Linkage disequilibrium (LD) among the 6 SNPs in *SORL1* and 3 SNPs in *ACE* were presented in Figure 2. The LD data of *SORL1* were calculated from HapMap and *ACE* data were based on our own data because HapMap did not have LD information about rs1799752 and rs4343. We also analyzed the frequencies of 2 haplotypes: 1 consisting of 3 positive *SORL1* SNPs rs2070045, rs3824968, and rs2282649, and the other consisting of 3 positive *ACE* SNPs rs1800764, rs4343, and rs1799752 (at least 3% frequency in either case or control groups). For the *ACE* haplotype, we found the most significant protective haplotype C-G-D (OR = 0.20, $P = 8.96 \times 10^{-14}$). For the *SORL1* haplotype, we detected a "complementary" haplotype (G-A-T: OR = 1.54, $P = 2.67 \times 10^{-3}$; T-T-C: OR = 0.63, $P = 2.36 \times 10^{-3}$) (Table 4). Further analysis showed no gene–gene interaction between *ACE* and *SORL1* (data not shown).

We also conducted a meta-analysis which showed that the 3 SNPs in *SORL1* were significantly associated with LOAD across 4 Asian populations (Table 5 and in Discussion Section).

DISCUSSION

With the development of genome-wide association studies and data mining, a number of genes have been identified as playing a potential role in Late-Onset Alzheimer Disease. Among these genes, *SORL1* and *ACE* are closely related to the processing, trafficking, and degradation of Amyloid-β, which is the principal component of senile plaques, a major characteristic in Alzheimer disease. In this study, we genotyped 9 SNPs within the *SORL1* and *ACE* genes in 144 Chinese LOAD patients and

TABLE 3. Genotype-wise Association Analysis Between LOAD Cases and Controls

Gene	SNP	Risk Allele	Genotype Distribution	Control n (%)	LOAD n (%)	Adjusted Addictive Model* OR (95% CI)
ACE	rs1800764	T	CC	93 (0.21)	23 (0.16)	1.38 (1.04-1.84)
			CT	215 (0.48)	59 (0.42)	
			TT	145 (0.32)	28 (0.41)	
	rs4343	A	GG	58 (0.12)	17 (0.12)	2.14 (1.43-3.20)†
			AG	226 (0.49)	44 (0.31)	
			AA	179 (0.39)	79 (0.56)	
	rs1799752‡	I	DD	67 (0.14)	17 (0.12)	2.38 (1.58-3.59)†
			ID	229 (0.49)	39 (0.28)	
			II	173 (0.37)	82 (0.59)	
SORL1	rs2070045	G	GG	158 (0.35)	72 (0.51)	1.68 (1.24-2.28)
			GT	222 (0.49)	58 (0.41)	
			TT	77 (0.17)	12 (0.09)	
	rs3824968	A	AA	176 (0.38)	69 (0.49)	1.54 (1.13-2.08)
			AT	214 (0.47)	62 (0.44)	
			TT	69 (0.15)	11 (0.08)	
	rs2282649	T	CC	80 (0.17)	13 (0.09)	1.57 (1.17-2.12)
			CT	208 (0.45)	61 (0.43)	
			TT	172 (0.37)	69 (0.48)	

*Additive model were assessed by logistic regression after adjusting for age.
†Recessive model were assessed by logistic regression after adjusting for age.
‡rs1799752 is an insertion/deletion polymorphism, and D represented deletion whereas I represented insertion.

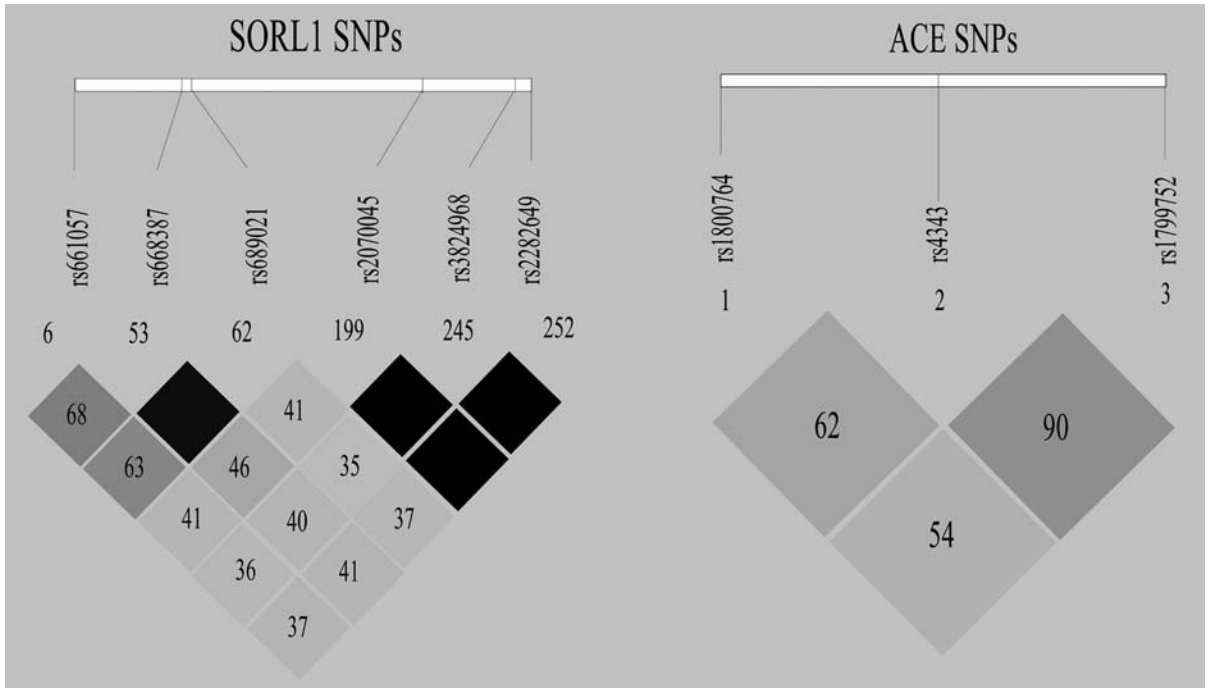

FIGURE 2. Linkage disequilibrium of *SORL1* SNPs and *ACE* SNPs. A, The number in each box stands for the pairwise LD as assessed by D′; in which there is no number D′=1. B, LD for *SORL1* SNPs is on the left of figure and the data from HapMap, whereas LD for *ACE* SNPs is on the right of figure and data from our own results.

476 healthy controls. Despite the modest case sample size, we observed positive association between SNPs in *ACE*, *SORL1,* and LOAD, and significant frequency difference in haplotypes on *SORL1*/*ACE* between LOAD cases and controls in our Chinese population. The significant results were verified using a variety of additional statistical analyses.

Earlier, 2 Japanese groups[24,25] reported results in opposite directions (1 report is negative and the other is positive) and our study confirmed the positive results of rs3824968, rs2282649 as presented in a larger Japanese population. Besides, another Chinese study produced results similar to our own for the risk allele in rs3824968, rs2282649.[26]

Compared with studies in northern European populations,[15] our results for SNPs in *SORL1* showed some differences: the risk alleles of rs2070045 and rs2282649 were identical but not for rs3824968. In fact, the results of SNPs in *SORL1* were not consistent across different populations. This discrepancy, as Rogaeva et al[15] reported, may be owing to ethnic difference, suggesting the existence of population-specific locus. In addition, considering other publications, different populations reported different effect sizes for these 3 SNPs (rs2070045, rs2282649, rs3824968). For example, within populations in Belgium,[28] Europe, and the United States,[29] and in a mixed population of African Americans (34%), Caribbean Hispanics (51%), and non-Hispanic whites (15%)[30]; these 3 SNPs (rs2070045, rs2282649, rs3824968) showed no significant difference between cases and controls.

These evidences indicate that in Asian populations, *SORL1* is a possible candidate gene associated with LOAD. We therefore, conducted a meta-analysis in 4 Asian populations of SNPs rs2070045, rs3824968, rs2282649 (Table 5). Tests showed no heterogeneity among the 4 populations for rs3824968 and rs2282649 (P-value = 0.70, $I^2 = 0.000$; and P-value = 0.74, $I^2 = 0.000$, respectively), and

TABLE 4. Estimated Haplotypes in the Case-control Samples in *ACE* and *SORL1*

	Haplotype†	LOAD n (%)	Control n (%)	Fisher's P*	Odds Ratio (95% CI)
ACE	C A I‡	67.34 (0.26)	84.66 (0.10)	$\mathbf{2.60 \times 10-11}$	3.25 (2.27-4.65)
	C G D	22.95 (0.09)	277.37 (0.32)	$\mathbf{8.96 \times 10-14}$	0.20 (0.13-0.32)
	T A I	115.46 (0.44)	440.88 (0.507)	0.06	0.76 (0.58-1.01)
	T G D	37.85 (0.15)	26.17 (0.03)	$\mathbf{2.08 \times 10-12}$	5.49 (3.26-9.24)
	T G I	7.83 (0.03)	7.57 (0.009)	$\mathbf{9.33 \times 10-3}$	3.52 (1.28-9.65)
SORL1	G A T	185.56 (0.66)	485.92 (0.55)	$\mathbf{2.67 \times 10-3}$	1.54 (1.16-2.04)
	G T C	11.44 (0.04)	26.52 (0.03)	0.41	1.35 (0.67-2.73)
	T A T	10.44 (0.04)	43.94 (0.05)	0.36	0.72 (0.36-1.44)
	T T C	71.56 (0.25)	306.34 (0.35)	$\mathbf{2.36 \times 10-3}$	0.63 (0.46-0.85)

*P-values (< 0.05) are in bold.
†The *ACE* haplotype composed of the rs1800764-rs4343-rs1799752; the *SORL1* haplotype composed of rs2070045- rs3824968- rs2282649.
‡rs1799752 is an insertion/deletion polymorphism, and D represented deletion whereas I represented insertion.

TABLE 5. Meta-analysis of Case-control Studies Between *SORL1* Polymorphisms and LOAD

SNP	First Researcher	Racial Descent	Number (LOAD/Control)	Allele Distribution				Odds Ratio (95% CI)	*P*
				G-LOAD	T-LOAD	G-Control	T-Control		
rs2070045	M.Ning*	China	144/476	202	82	538	378	1.73 (1.30-2.31)	
	E.K.Tan	China	223/263	277	169	301	225	1.23 (0.95-1.59)	
	R.Kimura	Japan	437/451	428	446	393	507	1.24 (1.03-1.49)	
	Fixed model		804/1190					1.33 (1.16-1.52)	$< 10^{-4}$
	Random model		804/1190					1.35 (1.11-1.65)	3×10^{-3}
rs3824968	M.Ning*	China	144/476	200	84	566	354	1.49 (1.12-1.98)	
	E.K.Tan	China	223/263	298	148	319	207	1.31 (1.00-1.70)	
	Nobuto Shibata	Japan	180/130	171	189	113	147	1.18 (0.85-1.62)	
	R.Kimura	Japan	437/451	439	433	405	497	1.24 (1.03-1.50)	
	Fixed model		984/1320					1.29 (1.14-1.46)	$< 10^{-4}$
rs2282649	M.Ning*	China	144/476	199	87	552	370	1.53 (1.15-2.04)	
	E.K.Tan	China	223/263	293	153	316	210	1.27 (0.98-1.65)	
	Nobuto Shibata	Japan	180/130	171	163	112	148	1.39 (1.00-1.92)	
	R.Kimura	Japan	437/451	439	425	402	500	1.28 (1.07-1.55)	
	Fixed model		984/1320					1.34 (1.19-1.52)	$< 10^{-5}$

*Ning is regarded as our association study of the Chinese population.

therefore, fixed effect model was used. For rs2070045, heterogeneity test *P*-value was 0.12 and $I^2 = 0.519$. We therefore, used both random effect model and fixed effect model. In these Asian populations, rs2070045, rs3824968, and rs2282649 were all significantly associated with LOAD (*P*-value for the 3 SNPs are all < 0.0001 in fixed effect model, Table 5).

As for SNPs in the *ACE* gene, our case-control study confirmed that rs1800764 T-allele, rs4343 A-allele, rs1799752 insertion-allele (I-allele) were associated with risk of LOAD in the Chinese population. rs1800764 locates in the promoter region of *ACE*, fits the addictive model rather than the recessive model[6] in our study. Our study is the first that showed evidence of association between rs1800764 and LOAD in an Asian population. Many investigations of association between *ACE* and AD have examined SNPs rs1799752 and rs4343. rs1799752 is an insertion/deletion polymorphism, the results of association studies about it were conflicted,[17,31–34] so meta-analysis have been done and suggested that I-allele increases the risk of AD[16,35,36] similar to our results. Rs4343 encodes a silent mutation in exon 16, and studies about it are also conflicted. Meng et al[17] reported that the G-allele of rs4343 increased the risk of AD in Israeli Arab Community whereas Kehoe et al[37] suggested A-allele was the risk allele, and other studies also showed different risk allele. Considering rs4343 A-allele is in almost perfect LD with rs1799752 I-allele,[37] our results which contains both A and I allele (risk alleles in our study) seemed more convincing.

Furthermore, we also found the most significant protective haplotype C-G-D (OR = 0.20, $P = 8.96 \times 10$ to 14). The human genome has been hypothesized, after empirical analyses, to be constitutive of blocks that present little internal recombination.[38] As the strong LD between rs4343 and rs1799752 ($D' = 0.90$, Fig. 2) and relatively weak LD between rs1800764 and rs4343 ($D' = 0.62$, Fig. 2), we suggested there might be an unidentified polymorphism for protective effect on disease between these 3 markers.

As we mentioned before, Aβ composed senile plaques which is an important pathology of AD,[9–11] whereas *ACE* modulate Aβ production[12] and degration.[13] In fact, rs4343 was reported that influence Aβ42 level in cerebrospinal fluid (CSF),[16] and rs1799752 insertion allele associated with reduce levels of circulation and cellular activity of *ACE*.[39] However, we do think more functional studies are needed to verify these results.

We used the Bonferroni method to control Type I errors, and after correction, the p-values of rs1800764, rs3824968 were larger than 0.05, whereas rs2070045, rs2282649, rs4343, and rs1799752 were still significant. Actually, the Bonferroni method is partially appropriate for our analysis as these SNPs were not picked randomly but were specifically chosen based on the earlier studies, which means that the H_0 assumption is for a positive result rather than a negative one. Besides, the 3 positive SNPs on *SORL1* were not independent of each other but were in a linkage disequilibrium region, which the same situation also exists in *ACE* (Fig. 2). So rs1800764, rs3824968 were quite possibly a true-positive rather than a false-positive site. In addition, after the correction, the remaining 3 SNPs were quite definitely associated with LOAD in the Chinese population.

In summary, our results provide evidence that the *ACE* and *SORL1* genes are associated with LOAD in the Chinese population. Our meta-analysis also showed that *SORL1* was associated with LOAD in Asian populations. We think studies of more SNPs in large populations and different ethnic are needed, and the functional studies.

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