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Research Report

Relationship Between *FERMT2*, *CELF1*, *COPI*, *CHRNA2*, and *ABCA7* Genetic Polymorphisms and Alzheimer's Disease Risk in the Southern Chinese Population

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Abstract.

Background: Alzheimer's disease (AD) is a multi-gene inherited disease, and apolipoprotein E (*APOE*) ε4 is a strong risk factor. Other genetic factors are important but limited.

Objective: This study aimed to investigate the relationship between 17 single-nucleotide polymorphisms (SNPs) and AD in the Southern Chinese populations.

Methods: We recruited 242 AD patients and 208 controls. The SNaPshot technique was used to detect the SNPs.

Results: Adjusted for sex and age, we found rs6572869 (*FERMT2*), rs11604680 (*CELF1*), and rs1317149 (*CELF1*) were associated with AD risk in the dominant (rs6572869: $p = 0.022$, OR = 1.55; rs11604680: $p = 0.007$, OR = 1.68; rs1317149: $p = 0.033$, OR = 1.50) and overdominant models (rs6572869: $p = 0.001$, OR = 1.96; rs11604680: $p = 0.002$, OR = 1.82; rs1317149: $p = 0.003$, OR = 1.80). rs9898218 (*COPI*) was associated with AD risk in the overdominant model ($p = 0.004$, OR = 1.81). Further, rs2741342 (*CHRNA2*) was associated with AD protection in the dominant ($p = 0.002$, OR = 0.5) and additive models ($p = 0.002$, OR = 0.64). Mutations in rs10742814 (*CELF1*), rs11039280 (*CELF1*), and rs3752242 (*ABCA7*) contributed to AD protection. Among them, rs10742814 (*CELF1*), rs3752242 (*ABCA7*), and rs11039280 (*CELF1*) were more significantly associated with AD carrying *APOE* ε4, whereas rs1317149 (*CELF1*) showed an opposite trend. Interestingly, rs4147912 (*ABCA7*) and rs2516049 (*HLA-DRB1*) were identified to be relevant with AD carrying *APOE* ε4. Using expression quantitative trait locus analysis, we found polymorphisms in *CELF1* (rs10742814 and rs11039280), *ABCA7* (rs4147912), *HLA-DRB1* (rs2516049), and *ADGRF4* (rs1109581) correlated with their corresponding gene expression in the brain.

Conclusions: We identified four risk and four protective SNPs associated with AD in the Southern Chinese population, with different correlations between *APOE* ε4 carriers and non-carriers. rs4147912 (*ABCA7*) and rs2516049 (*HLA-DRB1*) were associated with AD carrying *APOE* ε4.

Keywords: Alzheimer's disease, Apolipoprotein E, neurodegenerative disease, single nucleotide polymorphisms

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INTRODUCTION

Alzheimer's disease (AD) is a common age-related neurodegenerative disease associated with genetic factors and accounts for most dementia in the elderly population worldwide [1]. Genetic factors explain 58–79% of AD heritability [2]. Therefore, the identification of AD-related loci can provide a reliable basis for a timely and reliable diagnosis. Apolipoprotein E (*APOE*) ε4 is a major risk factor for sporadic AD [3]. A meta-analysis in Caucasians showed that individuals with the ε4 allele were at increased risk of AD (ε2/ε4: odds ratio [OR] = 2.6; ε3/ε4: OR = 3.2, ε4/ε4: OR = 14.9) [4]. However, *APOE* accounts for only 10–20% of the risk of late-onset AD, suggesting that other genetic risk factors are also important in AD pathogenesis [5].

More than 30 AD-related susceptibility genes have been identified by genome-wide association study (GWAS) meta-analyses, including *FERMT2, ABCA7, CR1, HLA-DRB*, and *CELF1* [6–9]. Furthermore, AD-related variants may affect the expression of nearby genes that influence the pathological processes of brain amyloidosis, tauopathy, and neurodegeneration, which provides valuable insights into the molecular mechanisms of AD pathogenesis [10]. A GWAS analysis of 1,126,563 individuals from European populations recently identified 3,915 variants across 38 independent loci associated with AD and proposed five new susceptibility loci (*TNIP1, NTN5, AGRN, LILRB2, HAVCR2*) [9]. The association between SNPs within *ABCA7*, *EPHA1*, and *CD2AP* and AD was not observed in an East Asian study, which was identified in Caucasians previously [11].

The genetic basis of race-specific AD susceptibility may be due to differences in minor allele frequency and the complexity of the underlying genetic structure inherent [11, 12]. Most GWAS and subsequent replication studies have been conducted almost exclusively in Caucasian populations, with relatively limited but significant AD inheritance in other populations. Replication of GWAS results in different ethnic populations may help identify SNPs associated with AD [13]. Therefore, in this study, we selected 17 SNP loci in 12 genes that have never been reported in Asian populations (*FERMT2* rs6572869, *ABCA7* rs4147912 and rs3752242, *CELF1* rs10742814, rs1317149, rs11604680 and rs11039280, *COPI* rs9898218, *CHRNA2* rs2741342, *ADGRF4* rs1109581, *HLA-DRB1* rs2516049, *MS4A* rs7116190 and rs667897, *NRXN1* rs698842, *CR1* rs4266886, *CSMD1* rs7813641, and *OR51I1* rs77336780), to investigate whether these SNPs are independently or in combination with *APOE* ε4 concerning AD risk in southern Chinese populations. In addition, we used expression quantitative trait loci (eQTL) datasets to assess the potential effects of these loci on gene expression, contributing to a better understanding of disease-related mechanisms and the discovery of new therapeutic pathways.

MATERIALS AND METHODS

Subjects

All enrolled subjects were evaluated by at least two experienced neurologists and underwent a standard series of examinations, including medical history, physical examination, systematic neuropsychological, such as standard Mini-Mental State Examination (MMSE) and clock drawing test (CDT) and neuroimaging tests such as magnetic resonance. Among them, 36 healthy controls and 52 AD patients received ^{18}F-florbetapir positron emission tomogram examination. The results of AD patients met the criteria for probable AD defined by National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA criteria) [14]. The physicians assessed the healthy controls recruited to confirm that they had no cognitive impairment [15, 16]. All participants were Han Chinese living in southern China. We excluded participants with a history of other neurological disorders that could lead to dementia, such as Parkinson's disease, stroke, multiple sclerosis, brain tumors, and major depressive disorder [17].

This study conformed with the Declaration of Helsinki and was approved by the Ethics Committee of Ruijin Hospital, affiliated with the Shanghai Jiao Tong University School of Medicine (2018-No.6). All participants and their caregivers were fully informed about the study and signed a written consent form.

Genotype analysis

Genomic DNA was obtained from the peripheral blood leukocytes of the participants using the phenol-chloroform-isopropyl alcohol method. We designed the extension primers and polymerase chain reaction (PCR) using Primer5 software (version 5.00) and used SNaPshot technology (Applied Biosystems,

Foster City, CA, United States) to genotype SNPs. SNPs of *APOE* (rs429358 and rs7412) were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[3]. The primer sequences are listed in Supplementary Table 1.

Gene expression analysis

Two databases, Braineac and Genotype-Tissue Expression (GTEx), were used to investigate the relationship between genotype and gene expression. The Braineac dataset included eQTL data for ten brain regions from 134 individuals of European descent without neurodegenerative disease. The GTEx database contained genotype and gene expression data of about 1000 individuals, mostly from Caucasian populations, covering 54 non-diseased tissues.

Statistical analyses

Statistical analyses were performed using SPSS software (version 26.0). A *t*-test and chi-square analysis were used to compare the demographic characteristics of the two groups. We performed a chi-square analysis to assess the distribution of allele and genotype and Hardy-Weinberg equilibrium (HWE) for each SNP. Four genetic models were applied to evaluate the risk of AD for each SNP after adjusting for age and sex. With M defined as the major allele and m as the minor allele, the models are: Dominant: 1 (mm + Mm) versus 0 (MM); Recessive: 1 (mm) versus 0 (MM + Mm); Additive: 0 (MM) versus 1 (Mm) versus 2 (mm); Overdominant: 1 (Mm) versus 0 (MM + mm). Statistical significance was set at $p < 0.05$. Bonferroni correction was performed.

RESULTS

Demographic characteristics of the study population

A total of 242 patients with AD and 208 healthy controls were recruited in this study. Table 1 shows the demographic and clinical information of the participants. There was no significant difference between the two groups in age and sex. Patients with AD had lower educational attainment than controls, which is consistent with previous studies [18].

Analysis of the association between SNPs and AD

All genotypes followed the HWE. The allele and genotype frequencies of the SNPs are listed in Supplementary Table 2. rs2741342 of *CHRNA* was associated with AD in allele and genotype frequencies (allele: $p = 0.003$, OR = 0.67, 95% confidence interval [CI] = 0.52–0.88; genotype: $p = 0.005$) (Table 2). Differences persisted after Bonferroni corrections for allele frequencies. The genotype or allele frequencies of rs6572869 in *FERMT2*, rs11604680, rs1317149, and rs11039280 in *CELF1*, rs9898218 in *COPI*, and rs3752242 in *ABCA7* were also associated with AD (Table 2). However, these associations vanished after the Bonferroni correction.

Four risk SNPs and four protective SNPs associated with AD were identified. Adjusted for sex and age, rs6572869 of *FERMT2* and rs11604680 and rs1317149 of *CELF1* were associated with the risk of AD in the dominant (rs6572869: $p = 0.022$, OR = 1.55, 95 % CI = 1.07–2.26; rs11604680: $p = 0.007$, OR = 1.68, 95% CI = 1.15–2.44; rs1317149: $p = 0.033$, OR = 1.50, 95% CI = 1.03–2.19) and overdominant models (rs6572869: $p = 0.001$, OR = 1.96, 95% CI = 1.32–2.92; rs11604680: $p = 0.002$, OR = 1.82, 95% CI = 1.24–2.67; rs1317149: $p = 0.003$, OR = 1.80, 95% CI = 1.23–2.64) (Table 2). After the Bonferroni correction, these differences persisted in the overdominant model. In addition, rs9898218 of *COPI* is associated with AD risk in the overdominant model ($p = 0.004$, OR = 1.81, 95% CI = 1.21–2.71) (Table 2).

Further, rs2741342 of *CHRNA2* was associated with AD protection in the dominant ($p = 0.002$, OR = 0.5, 95% CI = 0.33–0.77) and additive models ($p = 0.002$, OR = 0.64, 95% CI = 0.48–0.85), even after Bonferroni correction (Table 2). The recessive model results of rs10742814 and rs11039280 of *CELF1* showed that they are protective SNPs associated with AD (rs10742814: $p = 0.039$, OR = 0.55, 95% CI = 0.31–0.97; rs11039280: $p = 0.016$, OR = 0.49, 95% CI = 0.28–0.88) (Table 2). These associations vanished after the Bonferroni correction. The results also showed that rs3752242 (*ABCA7*) was marginally associated with AD protection in the dominant and additive models with AD protection (Table 2).

Association analysis of subgroups stratified by APOE ε4 status

As expected, *APOE* ε4 increased susceptibility to AD (Table 1). Next, we stratified the participants according to their *APOE* ε4 carrier status. Other discrepancies were observed in the allele and genotype frequencies of the above risk and protective SNPs

combined with *APOE* ε4 (Supplementary Tables 4 and 6).

Adjusted for sex and age, the association of risk SNPs (rs6572869 of *FERMT2*, rs11604680 of *CELF1*, rs9898218 of *COPI*) and protective SNPs (rs2741342 of *CHRNA2*) persisted in AD with and without *APOE* ε4, consistent with the results when unstratified (Table 3). Protective SNPs (rs10742814 of *CELF1*, rs3752242 of *ABCA7*, and rs11039280 of *CELF1*) were still associated with AD in APOE ε4 carriers, and the differences were more pronounced, but not in *APOE* ε4 non-carriers (Table 3). Nonetheless, the risk SNP rs1317149 of *CELF1* was significantly associated with AD in *APOE* ε4 non-carriers but not in *APOE* ε4 carriers (Table 3).

Table 1
Characteristics of the study population

	Patients ($N = 242$)	Controls ($N = 208$)	p
Female, *n* (%)	157 (64.9%)	127 (61.1%)	0.40
Male, *n* (%)	85 (35.1%)	81 (38.9%)	
Age at examination (y, mean ± SD)	72.81 ± 9.56	71.19 ± 9.76	0.076
Education level (y, mean ± SD)	8.32 ± 4.97	11.95 ± 3.23	**<0.001**
MMSE score (mean ± SD)	12.08 ± 5.46	29.08 ± 0.76	**<0.001**
CDT score (mean ± SD)	1.83 ± 1.57	3.90 ± 0.14	**<0.001**
APOE genotype (%)			
ε2/ε2	2 (0.8%)	1 (0.5%)	
ε2/ε3	13 (5.4%)	25 (12.0%)	
ε2/ε4	7 (2.9%)	5 (2.4%)	
ε3/ε3	96 (39.7%)	121 (58.2%)	
ε3/ε4	102 (42.1%)	50 (24.0%)	
ε4/ε4	22 (9.1%)	6 (2.9%)	
APOE ε4 carriers	131 (54.1%)	61 (29.3%)	**<0.001**

MMSE, Mini-Mental State Examination; *APOE*, apolipoprotein E, *APOE* ε4 carriers have at least one copy of the ε4 allele; SD, standard deviation; Bolding indicates statistically significant values; CDT, clock drawing test.

Table 2
Association of SNP with AD risk

SNP	Gene	minor allele	P allele OR (95%CI)	P genotype	Dominant p OR (95%CI)	Recessive p OR (95%CI)	Additive p OR (95%CI)	Overdominant p OR (95%CI)
rs6572869	*FERMT2*	A	0.35	**0.005**	**0.022**	0.13	0.33	**0.001**
			1.15		1.55	0.63	1.15	1.96
			0.87–1.53		1.07–2.26	0.35–1.14	0.87–1.51	1.32–2.92
rs11604680	*CELF1*	G	0.078	**0.007**	**0.007**	0.53	0.065	**0.002**
			1.31		1.68	0.81	1.32	1.82
			0.98–1.75		1.15–2.44	0.41–1.56	0.98–1.78	1.24–2.67
rs1317149	*CELF1*	A	0.35	**0.009**	**0.033**	0.13	0.33	**0.003**
			1.15		1.50	0.6	1.16	1.80
			0.87–1.53		1.03–2.19	0.31–1.16	0.87–1.55	1.23–2.64
rs9898218	*COPI*	T	0.53	**0.005**	0.065	0.073	0.48	**0.004**
			1.11		1.43	0.51	1.11	1.81
			0.82–1.51		0.98–2.10	0.24–1.07	0.83–1.50	1.21–2.71
rs2741342	*CHRNA2*	A	**0.003**	**0.005**	**0.002**	0.068	**0.002**	0.16
			0.67		0.5	0.64	0.64	0.77
			0.52–0.88		0.33–0.77	0.4–1.03	0.48–0.85	0.53–1.11
rs10742814	*CELF1*	A	0.23	0.17	0.61	**0.039**	0.17	0.39
			0.84		0.91	0.55	0.83	1.18
			0.63–1.10		0.62–1.32	0.31–0.97	0.63–1.09	0.81–1.72
rs11039280	*CELF1*	A	**0.046**	0.064	0.26	**0.016**	**0.048**	0.63
			0.75		0.81	0.49	0.76	1.1
			0.57–0.99		0.56–1.17	0.28–0.88	0.58–0.99	0.75–1.61
rs3752242	*ABCA7*	A	**0.037**	0.10	**0.047**	0.26	**0.049**	0.23
			0.74		0.68	0.73	0.76	0.80
			0.57–0.98		0.47–0.99	0.43–1.26	0.58–1.00	0.55–1.16

The P allele and P genotype were examined using the Chi-squared test. The p value was adjusted for sex and age using binary logistic regression. Bold indicates statistically significant values.

Table 3
Association of SNPs of candidate genes with AD risk stratified by *APOE* ε4 status

SNP	Gene	MAF	*APOE* ε4	Dominant model (adjusted)			Recessive model (adjusted)		
				p	OR	95%CI	*p*	OR	95%CI
rs6572869	*FERMT2*	A	+	**0.009**	2.33	1.23–4.40	0.2	2.32	0.64–8.39
			–	0.43	1.22	0.74–2.01	**0.014**	0.33	0.14–0.80
rs11604680	*CELF1*	G	+	**0.016**	2.20	1.16–4.17	0.95	1.03	0.34–3.15
			–	**0.028**	1.76	1.06–2.92	0.38	0.65	0.25–1.68
rs9898218	*COPI*	T	+	0.45	1.28	0.68–2.40	**0.023**	0.23	0.065–0.81
			–	0.066	1.61	0.97–2.66	0.82	0.90	0.35–2.29
rs1317149	*CELF1*	A	+	0.058	1.85	0.98–3.47	0.95	1.03	0.34–3.15
			–	**0.038**	1.72	1.03–2.86	0.062	0.4	0.15–1.05
rs2741342	*CHRNA2*	A	+	**0.028**	0.43	0.20–0.91	0.15	0.56	0.25–1.24
			–	**0.017**	0.51	0.29–0.89	0.41	0.77	0.42–1.43
rs10742814	*CELF1*	A	+	0.39	0.76	0.40–1.43	**0.004**	0.30	0.13–0.68
			–	0.77	0.93	0.57–1.52	0.35	0.66	0.28–1.58
rs3752242	*ABCA7*	A	+	**0.042**	0.52	0.28–0.98	**0.035**	0.40	0.17–0.94
			–	0.69	0.90	0.54–1.50	0.81	1.09	0.53–2.25
rs11039280	*CELF1*	A	+	0.24	0.68	0.36–1.29	**0.004**	0.30	0.13–0.68
			–	0.31	0.77	0.47–1.27	0.16	0.53	0.22–1.28
rs4147912	*ABCA7*	A	+	**0.034**	2.28	1.06–4.87	0.68	1.60	0.17–14.74
			–	0.94	1.02	0.61–1.72	0.45	2.53	0.22–28.51
rs2516049	*HLA-DRB1*	G	+	0.69	1.14	0.60–2.16	**0.033**	0.30	0.10–0.91
			–	0.61	0.87	0.53–1.45	0.69	1.30	0.36–4.67
rs1109581	*ADGRF4*	A	+	0.33	0.72	0.37–1.40	0.81	0.83	0.19–3.66
			–	**0.04**	1.85	1.03–3.32	0.40	0.49	0.092–2.58
SNP	Gene	MAF	*APOE* ε4	Additive model (adjusted)			Overdominant model (adjusted)		
				p	OR	95%CI	*p*	OR	95%CI
rs6572869	*FERMT2*	A	+	**0.01**	1.99	1.17–3.36	0.058	1.88	0.98–3.61
			–	0.53	0.89	0.62–1.28	**0.011**	1.97	1.17–3.33
rs11604680	*CELF1*	G	+	0.058	1.64	0.98–2.74	**0.015**	2.32	1.18–4.56
			–	0.18	1.31	0.88–1.94	**0.007**	1.99	1.20–3.30
rs9898218	*COPI*	T	+	0.72	0.91	0.56–1.50	**0.044**	2.02	1.02–4.02
			–	0.18	1.30	0.88–1.92	**0.04**	1.74	1.03–2.94
rs1317149	*CELF1*	A	+	0.14	1.47	0.89–2.43	0.056	1.90	0.98–3.69
			–	0.46	1.16	0.79–1.70	**0.002**	2.29	1.37–3.82
rs2741342	*CHRNA2*	A	+	**0.021**	0.57	0.35–0.92	0.33	0.73	0.39–1.38
			–	**0.044**	0.69	0.48–0.99	0.14	0.69	0.42–1.14
rs10742814	*CELF1*	A	+	**0.036**	0.63	0.41–0.97	0.19	1.55	0.81–2.95
			–	0.52	0.88	0.60–1.29	0.80	1.07	0.65–1.76
rs3752242	*ABCA7*	A	+	**0.015**	0.59	0.38–0.90	0.57	0.83	0.43–1.59
			–	0.87	0.97	0.67–1.4	0.59	0.87	0.53–1.43
rs11039280	*CELF1*	A	+	**0.022**	0.60	0.39–0.93	0.32	1.39	0.73–2.64
			–	0.16	0.76	0.52–1.11	0.87	0.96	0.58–1.59
rs4147912	*ABCA7*	A	+	**0.046**	2.03	1.01–4.07	**0.043**	2.24	1.02–4.91
			–	0.81	1.06	0.65–1.74	0.92	0.97	0.58–1.65
rs2516049	*HLA-DRB1*	G	+	0.52	0.86	0.53–1.38	0.084	1.89	0.92–3.88
			–	0.76	0.93	0.60–1.45	0.49	0.83	0.49–1.40
rs1109581	*ADGRF4*	A	+	0.38	0.78	0.46–1.35	0.36	0.72	0.36–1.46
			–	0.14	1.45	0.88–2.39	**0.013**	2.18	1.18–4.04

p-values were adjusted for sex and age using binary logistic regression. Bold indicates statistically significant values.

Interestingly, rs4147912 of *ABCA7* and rs2516049 of *HLA-DRB1* were identified to be relevant with AD in *APOE* ε4 carriers (dominant model of rs4147912: $p = 0.034$, OR = 2.28, 95% CI = 1.06–4.87; additive model of rs4147912: $p = 0.046$, OR = 2.03, 95% CI = 1.01–4.07; overdominant model of rs4147912: $p = 0.043$, OR = 2.24, 95% CI = 1.02–4.91; recessive model of rs2516049: $p = 0.033$, OR = 0.30, 95% CI = 0.10–0.91) (Table 3). Moreover, in non-*APOE* ε4 carriers, rs1109581 of *ADGRF4* in the dominant and overdominant models was associated with AD (dominant model: $p = 0.04$, OR = 1.85, 95% CI = 1.03–3.32; overdominant model: $p = 0.013$, OR = 2.18, 95% CI = 1.18–4.04) (Table 3). However, these correlations were not available after Bonferroni correction (Table 3).

Table 4
Effects of rs2516049 and rs1109581 on *HLA-DRB1* and *ADGRF4* gene expression in different regions of the normal human brain in GTEx

SNP	Variant ID	Tissue	Samples	Genotype			*p*	NES
				TT	TC	CC		
rs2516049	chr6_32602623_T_C_b38	Brain - Anterior cingulate cortex (BA24)	147	72	58	17	1.00E-16	–0.59
		Brain - Cortex	205	115	69	21	8.40E-13	–0.46
		Brain - Frontal Cortex (BA9)	175	92	66	17	2.30E-11	–0.44
		Brain - Putamen (basal ganglia)	170	89	69	12	3.80E-11	–0.51
		Brain - Amygdala	129	58	59	12	2.40E-10	–0.34
		Brain - Cerebellum	209	105	85	19	1.10E-09	–0.44
		Brain - Caudate (basal ganglia)	193	103	73	17	1.20E-09	–0.34
		Brain - Nucleus accumbens (basal ganglia)	202	102	81	19	2.30E-09	–0.37
		Brain - Cerebellar Hemisphere	175	88	68	19	2.80E-09	–0.44
		Brain - Substantia nigra	114	58	47	9	7.90E-08	–0.35
		Brain - Hypothalamus	170	90	66	14	4.00E-07	–0.28
		Brain - Hippocampus	165	81	68	16	0.0000015	–0.25
SNP	Variant ID	Tissue	Samples	Genotype			*p*	NES
				CC	CT	TT		
rs1109581	chr6_47710446_C_T_b38	Brain - Cerebellum	209	114	83	12	0.000082	0.35

Samples: Number of RNA-seq samples with genotype, *p*-value: from a *t*-test that compares observed NES from single-tissue eQTL analysis to a null NES of 0, NES: normalized effect size, the slope of the linear regression of normalized expression data versus the three genotype categories using single-tissue eQTL analysis representing the eQTL effect size.

Analysis of the association between SNPs and related gene expression

We applied eQTL analysis to investigate the correlation between genotype and gene expression of the above 11 candidate loci to understand further the relationship between the loci and the pathogenesis of AD. According to GTEx, we found that the TT genotype at rs2516049 was associated with the high expression level of *HLA-DRB1* in multiple brain regions, such as the anterior cingulate cortex (BA24), cortex, frontal cortex (BA9), putamen (basal ganglia), amygdala, cerebellum, caudate (basal ganglia), nucleus accumbens (basal ganglia), cerebellar hemisphere, substantia nigra, hypothalamus, and hippocampus (Table 4). In addition, the TT genotype at rs1109581 was associated with a higher level of *ADGRF4* in the cerebellum (Table 4). According to the Braineac database, the A allele of rs4147912 was positively correlated with *ABCA7* expression in the frontal cortex ($p = 0.00012$, probe set 3815488). Both A alleles of rs10742814 and rs11039280 increased *CELF1* expression in the medulla oblongata (rs10742814: $p = 0.00034$, probe set 3372267; rs11039280: $p = 0.00032$, probe set 3372267) (Fig. 1). In the GTEx and Braineac databases, no other genotypes of SNPs were found to be significantly correlated with the expression levels of related genes in the brain.

DISCUSSION

In this study, the associations between 17 SNPs in 12 genes and the risk of AD were validated. To our knowledge, these SNPs have been investigated for the first time in the Southern Chinese population. Four risk SNPs and four protective SNPs were identified. Mutations in rs6572869 (*FERMT2*), rs11604680 (*CELF1*), rs1317149 (*CELF1*), and rs9898218 (*COPI*) were associated with a higher susceptibility to AD, whereas rs2741342 (*CHRNA2*), rs10742814 (*CELF1*), rs11039280 (*CELF1*), and rs3752242 (*ABCA7*) were protective against AD pathogenesis. In addition, the correlation between the above-mentioned related SNPs and AD is different when participants are divided into two groups according to their *APOE* ε4 carrier status. Furthermore, individuals with the A allele at rs4147912 had a higher AD susceptibility in *APOE* ε4 carriers, while homozygous GG at rs2516049 reduced AD morbidity. Heterozygote AG at rs1109581 of *ADGRF4* in *APOE* ε4 non-carriers increased the risk of AD. The associations of the remaining six SNPs with AD were not replicated in the Southern Chinese group (Supplementary Tables 3, 5, and 7). Similarly, various populations may carry different AD-risk genetic variants.

CELF1 is an essential gene associated with AD genetic factors [19]. Aret, the homologue of *CELF1*

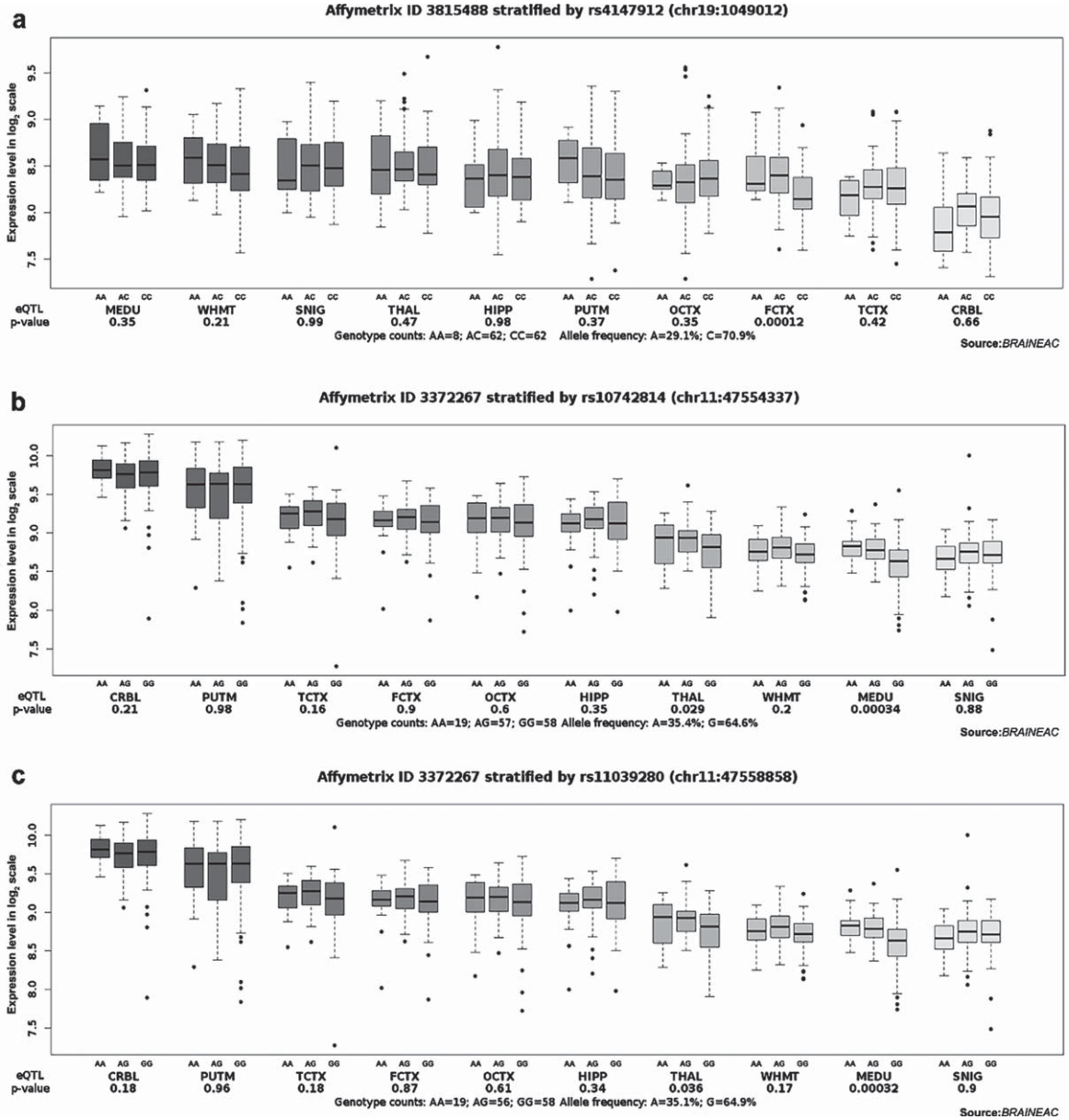

Fig. 1. Effect of rs4147912, rs10742814, and rs11039280 on *ABCA7* and *CELF1* expression in normal human brain regions in Braineac. a) The A allele of rs4147912 increased *ABCA7* expression in the frontal cortex with probe set 3815488 ($p = 0.00012$). b) The A allele of rs10742814 increased *CELF1* expression in the medulla oblongata with probe set 3372267 ($p = 0.00034$). c) The A allele of rs11039280 increased *CELF1* expression in the medulla oblongata with probe set 3372267 ($p = 0.00032$).

in fly, was associated with AD by regulating tau protein aggregation [20]. Furthermore, previous studies have found a significant correlation between *CELF1* and the establishment of hippocampal white matter integrity [21]. Moreover, the *CELF1* variants may correlate with higher levels of $A\beta_{42}$ in cerebrospinal fluid, thus associated with the AD pathology. In the Caucasian population, when rs11604680, rs1317149, rs10742814, and rs11039280 were aggregated with other SNPs, they were associated with cognition, but the association between a single SNP and AD has not been explored [22]. No studies have investigated the association between rs11604680, rs1317149, rs10742814, and rs11039280 of the *CELF1* gene and AD risk in Asian populations. We provide evidence that heterozygous AG at rs11604680 and rs1317149

increased AD susceptibility, while homozygous AA at rs10742814 and rs11039280 decreased AD risk. Moreover, the correlations between these SNPs in *APOE* ε4 carriers and non-carriers were significantly inconsistent. For the first time, we hypothesized that they might interact with *APOE* and participate in the progression of AD, which needs to be confirmed by future studies.

ABCA7 encodes an ATP-binding cassette transporter that facilitates lipid efflux across membranes [23, 24]. *ABCA7* is second only to *APOE* ε4 concerning amyloid deposition, and its loss-of-function increases β-secretase cleavage of the amyloid-β protein precursor (AβPP) [25]. *ABCA7* knockout in mice carrying mutant human AβPPs increased insoluble Aβ deposition and amyloid plaques [26]. Two variants of *ABCA7* (rs4147912 and rs3752242) were identified to be associated with cerebral amyloidosis and susceptibility to AD, but not with brain atrophy [25]. In this study, we discovered that the A allele at rs3752242 might be protective against AD, particularly in *APOE* ε4 carriers. Moreover, the A allele at rs4147912 is a risk factor for AD susceptibility in *APOE* ε4 carriers. We speculated that *ABCA7* might interact with *APOE* ε4 and be associated with AD. *APOE* ε4 may promote the transport of oxidized lipids by ABCA7 through the production of reactive oxygen species (ROS). Studies have shown that *APOE* ε4 in astrocytes and neurons induce greater cPLA2 activation through p38 MAPK pathway, leading to more LTB4, iNOS, and ROS production, increased oxidative stress and neuroinflammation [27]. ROS induces neuronal lipid synthesis, leading to lipid peroxidation isolation in glial lipid droplets to delay neurotoxicity, and ABCA7 in neurons is involved in this process [28]. In addition, studies have shown that *APOE* ε4 is impaired in lipid transport and promotes neurodegeneration [29], which may have a synergistic effect with ABCA7 in this process.

FERMT2 is expressed in the brain, and its corresponding protein targets cell-matrix adhesion structures [30]. Reduction of *FERMT2* in human neurons leads to reduced extracellular $A\beta_{40}$ and $A\beta_{42}$ levels and reduced total and phosphorylated tau, demonstrating the link between *FERMT2* and AD pathology [31]. A recent study revealed that *FERMT2* directly regulates AβPP metabolism and that inadequate expression of *FERMT2* affects synaptic connectivity and axonal growth in an AβPP-dependent way [32]. GWAS results based on International Genomics of Alzheimer's Project showed that rs6572869 of *FERMT2* were significantly correlated with the incidence of AD [13, 33]. Our study confirmed that heterozygous AG of rs6572869 might be an influential genetic risk factor in AD pathophysiology.

Cell trafficking and recycling are risk mechanisms for AD [34]. *COPI*-dependent trafficking alters subcellular localization and cell surface expression of AβPP. *COPI*-dependent trafficking is significantly associated with the reduction of amyloid plaques in the neurocortex and hippocampus of the AD mouse [34]. A large meta-analysis examining transcriptional changes in ageing and AD found that *COPI* subunit zeta 2 (COPZ2) was upregulated in AD [35]. Analysis of an AD GWAS dataset of European ancestry demonstrated that rs9898218 of *COPI* is significantly associated with AD risk [36]. Consistent with these results, we provided the first supporting evidence for the association between rs9898218 and AD risk in the Southern Chinese population.

It remains unknown whether rs2741342 of *CHRNA2* is relevant to AD risk in the Chinese population. *CHRNA2* encodes the alpha-2 subunit of neuronal acetylcholine receptor (nAChR), which is expressed in the hippocampal CA1 region and influences hippocampal-dependent memory and the plasticity of CA1 hippocampal synapses [37, 38]. *CHRNA2* is currently one of the targets of AD clinical drug research [39]. rs2741342 has been confirmed to be associated with AD risk in people with Caucasian ancestry [13]. However, our study observed that the A allele of rs2741342 reduces susceptibility to AD, which may be due to race specificity and requires further validation.

HLA-DRB1, a genetic factor in AD pathogenesis, has shown a nominal association with the diagnosis of pathological AD, and methylation in this locus is associated with Aβ loading correlated with tau entanglement density [6, 40]. Analysis of GWAS from North America and Europe found that rs2516049 was significantly associated with neurofibrillary tangle pathology and correlated with AD progression [41]. rs1109581 is in an intron of *ADGRF4*, which is an adhesion G-protein-coupled receptor [42]. And GWAS results based on IGAP showed that rs1109581 of *ADGRF4* were significantly correlated with the incidence of AD [13, 33]. Our results showed that, in the Southern Chinese population, homozygosity for the G allele of rs2516049 (*HLA-DRB1*) protects *APOE* ε4 carriers from AD susceptibility, whereas mutation of rs1109581 (*ADGRF4*) may increase the risk of AD in *APOE* ε4 non-carriers. The association of

rs2516049 and rs1109581 with *HLA-DRB1* and *ADGRF4* expression in the brain suggested that mutations in these two loci might be involved in AD pathogenesis by affecting gene expression and interacting with *APOE*, which requires confirmation in future studies.

The associations between the other six SNPs and AD risk could not be replicated in this study, which may be due to heterogeneity in ethnic origin and/or small sample size [12, 43]. eQTL analysis can establish a link between genotype and gene expression patterns and help identify disease-causing genes. Many eQTLs affect the expression of local transcripts and distal genes [43]. Based on eQTL analysis, we further found that polymorphisms in *HLA-DRB1* (rs2516049), *ADGRF4* (rs1109581), *CELF1* (rs10742814 and rs11039280), and *ABCA7* (rs4147912) correlated with their corresponding gene expression in the brain, which demonstrated that polymorphisms in susceptibility loci might affect gene expression in different regions of the brain and thus affect AD pathogenesis. Meanwhile, positive eQTL association analysis could support the validity of this study and contribute to understanding the role of genetic variation in AD-related mechanisms.

In conclusion, our study identified several risk SNPs and protective SNPs associated with AD that have never been reported in Asian populations. Different SNP variants are either a risk factor or protective factor for AD and are associated with *APOE* ε4 carrier status, suggesting that the polymorphism may interact with *APOE*. Genetic polymorphisms remain insufficiently understood in the development of AD. AD pathogenesis is highly correlated with genetic factors, and the identification of AD-associated loci could provide a reliable basis for timely and reliable diagnosis. Our findings provide new insights into the relationship between risk loci and AD development in a southern Chinese population and contribute to the general understanding of the genetic basis of AD risk. Given the association between these loci and AD risk, future studies must investigate the role of risk genes in the pathogenesis of AD, as demonstrated here, to help further inform future AD-targeted therapies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/ADR-230072.

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