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<b>Author(s)</b>	<b>Chen, LH; Kao, PYP; Fan, YH; Ho, DTY; Chan, CSY; Yik, PY; Ha, JCT; Chu, LW; Song, YQ</b>
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**Polymorphisms of *CRI*, *CLU* and *PICALM* confer susceptibility of Alzheimer's disease in southern Chinese population**

Lu Hua Chen <sup>a</sup>, Patrick Yu Ping Kao <sup>a</sup>, Yan Hui Fan <sup>a</sup>, Deborah Tip Yin Ho <sup>b</sup>, Cherry Sze Yan Chan <sup>b</sup>,  
Ping Yiu Yik <sup>b</sup>, Joyce Cheuk Tung Ha <sup>b</sup>, Leung Wing Chu <sup>b,c,d,\*</sup> and You-Qiang Song <sup>a,d,e,\*</sup>

*<sup>a</sup>Department of Biochemistry, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong*

*<sup>b</sup>Division of Geriatric Medicine, Department of Medicine, Queen Mary Hospital, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong*

*<sup>c</sup>Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong*

*<sup>d</sup>Alzheimer's Disease Research Network, SRT Healthy Aging, the University of Hong Kong, Hong Kong*

*<sup>e</sup>Centre for Reproduction, Development and Growth, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong*

**Correspondence to:**

\*Dr. You-Qiang Song, Department of Biochemistry, Li Ka Shing Faculty of Medicine, the University of Hong Kong, 102 Pokfulam Road, Hong Kong; Tel: 00852 28199245, Fax: 00852 28151254, Email: [songy@hku.hk](mailto:songy@hku.hk)

\*Prof. Leung Wing Chu, Division of Geriatric Medicine, Department of Medicine, Queen Mary Hospital, the University of Hong Kong, 102 Pokfulam Road, Hong Kong; Tel: 00852 22553315, Fax: 00852 29741171, Email: [lwchu@hku.hk](mailto:lwchu@hku.hk)

## ABSTRACT

In this case-controlled study, we tested susceptible genetic variants for Alzheimer's disease (AD) in *CRI*, *CLU* and *PICALM* from Genome Wide Association studies (GWAS) in southern Chinese population. 812 participants consisting of 462 late-onset Alzheimer's disease (LOAD) patients and 350 non-demented controls were recruited. We found by multivariate logistic regression analysis, that single nucleotide polymorphisms (SNPs) in *CRI* (rs6656401 adjusted allelic  $p=0.035$ , adjusted genotypic  $p=0.043$ ) and *CLU* (rs2279590 adjusted allelic  $p=0.035$ , adjusted genotypic  $p=0.006$ ; rs11136000 adjusted allelic  $p=0.038$ , adjusted genotypic  $p=0.009$ ) were significantly different between LOAD patients and non-demented controls. For *PICALM*, LOAD association was found only in the *APOE*  $\epsilon 4$  (-) subgroup (rs3851179 adjusted allelic  $p=0.028$ , adjusted genotypic  $p=0.013$ ). Our findings showed evidence of *CRI*, *CLU* and *PICALM* and LOAD susceptibility in an independent southern Chinese population, which provides additional evidence for LOAD association apart from prior GWAS in Caucasian population.

**Keywords:** *CRI*, *CLU* and *PICALM*, Alzheimer's disease, genetics, Chinese

## 1. Introduction

Alzheimer's disease (AD), characterized with progressive cognitive impairments, is the most common form of dementia in an aging population. Although the underlying cause is uncertain, it is widely acknowledged that the involvement of heredity genetic risk factors in AD's predisposition and progression (Goate, et al., 1991, Rogaev, et al., 1995, Sherrington, et al., 1995). At present, *APOE* which carries three alleles  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ , is the unique and well established susceptibility gene for LOAD (Saunders, et al., 1993). The consistent result of association between *APOE*  $\epsilon 4$  and an increased disease risk has been confirmed in different ethnic populations based on candidate-gene approaches as well as Genome Wide Association studies (GWAS) (Bertram and Tanzi, 2008). Moreover, researches showed the heterozygous *APOE*  $\epsilon 4$  carriers have two-fold increased risk and the homozygous carriers have eleven-fold increased risk for LOAD development compared to *APOE*  $\epsilon 3$  carriers in Caucasian population (Bickeboller, et al., 1997). However, with 65% sensitivity and 68% specificity, the impact of *APOE*  $\epsilon 4$  allele for LOAD diagnosis is limited and can't benefit clinics (Mayeux, et al., 1998). Thus, further study to identify novel LOAD genetic risk markers is warranted.

To date, a number of genetic variants from different genes have been reported to be related with LOAD susceptibility by using new large scale genotyping technologies according to AlzGene database (<http://www.alzforum.org/res/com/gen/alzgene/largescale.asp>). However, none of those genes exhibited replicable results for disease risk association until recently *CLU* was simultaneously reported by two independent research groups both based on GWAS approach. In one GWAS performed by Lambert and his colleagues among subjects recruited

from France, Finland, Belgium, Spain and Italy, results showed evidence of SNPs in *CLU*, *CRI* and *LOAD* association. Further analysis revealed the interaction between those loci and *APOE*  $\epsilon$ 4 status for disease risk (Lambert, et al., 2009). Findings from the other GWAS conducted by Harold et al on Europeans and Americans demonstrated a novel locus at 5' in *PICALM* associated with *LOAD* in addition to the same susceptible variant in *CLU*. However, contrary to Lambert's results, they didn't detect the interaction between those polymorphisms with *APOE*  $\epsilon$ 4 status on *LOAD* risk (Harold, et al., 2009).

The overlap, as well as the contradiction of these two GWAS' results promoted the present replication genetic study focusing on the Chinese population. Currently, the prevalence of AD in China is 1.6% in elderly (>60 years) which is imposing a tremendous economic burden to family and society (Dong, et al., 2007, Song and Wang, 2010). To better understand the genetic aspect of *LOAD* in Chinese ethnic population, we therefore followed those susceptible polymorphisms of *CLU*, *CRI* and *PICALM* from GWAS in an independent Chinese sample set.

## **2. Methods**

### *2.1 Subjects*

Subjects of this case-controlled study were recruited from the Memory Clinic of Queen Mary Hospital and community elderly social centers in Hong Kong. This is an on-going aging and dementia project in the University of Hong Kong, which has been reported previously (Li, et al., 2006, Li, et al., 2009, Li, et al., 2010). The Institutional Review Board (IRB) of the

University of Hong Kong and the Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB) approved this study. Written consents were obtained from all participants. In addition to detailed physical and neurological examinations, all participants were subjected to laboratory investigations: thyroid function test; serum vitamins B12 and folate levels tests; and red blood cell (RBC) folate level test. CT brain scans were done for patients with LOAD but not for the non-demented controls. All subjects were rated by a single rater (L.W.C) with the Clinical Dementia Rating Scale (CDR) (Hughes, et al., 1982). Cognitively normal controls were given CDR rating of 0. For LOAD patients, the CDR ranged from 0.5 to 3.0, depending on the severity of the dementia. The probable LOAD was diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA criteria) (McKhann, et al., 1984). We excluded subjects with secondary causes of dementia, non-AD types of dementia and familial AD.

## *2.2 DNA extraction*

Genomic DNA was isolated from the whole blood by using QIAamp DNA Blood Mini Kit (Qiagen, Germany) in accordance with protocol. The quality and quantity of DNA were evaluated on a fluorometer.

## *2.3 Genotyping*

Seven previously published GWAS SNPs (rs3818361 and rs6656401 of *CRI*; rs11136000, rs2279590 and rs9331888 of *CLU*; rs3851179 and rs541458 of *PICALM*) were selected for

genotyping. Both amplification and single allele extension primers were designed by Mass ARRAY AssayDesign software. Subsequent genotyping for those selected polymorphisms were investigated on Sequenom<sup>®</sup> San Diego, CA platform following the manufacturer's protocols. *APOE* genotyping was done by the restriction fragment length polymorphism (RFLP) approach according to a modified method (Song, et al.,1998).

#### *2.4 Statistical analysis*

Data analysis was performed by PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink>). The differences of allele frequencies and genotype distributions between groups were calculated by  $\chi^2$  test. Multivariate logistic regression model was used to obtain adjusted association p-values (adjusted for age, sex and *APOE*  $\epsilon 4$  status) to exclude the potential effects of confounding factors. Being a replication study with association evidence supported by GWAS, multiple testing corrections were not applied. We regarded p-value of 0.05 was an acceptable cut-off for statistical significance.

### **3. Results**

A total of 812 subjects participated in the study (462 LOAD and 350 non-demented controls). Consistent with previous studies, there was a higher prevalence of *APOE*  $\epsilon 4$  carriers (presence of one or two  $\epsilon 4$  alleles versus absence of  $\epsilon 4$  allele) in LOAD patients (38%) than non-demented controls (16.6%) ( $p=2.27 \times 10^{-11}$ , OR=3.09) (Supplement table).

All analyzed polymorphisms were satisfied with the Hardy-Weinberg equilibrium distribution

( $p > 0.001$ ). For allele frequency association of *CRI*, both A allele of rs6656401 ( $p=0.034$ , OR=1.69) and T allele of rs3818361 ( $p=0.029$ , OR=1.27) were found to increase LOAD risk (Table 1). Further genotypic analysis of those two SNPs showed significant different distributions between LOAD and non-demented controls by applying a dominant model (Table 2). Neither allele frequency nor genotypic distribution was detected to be significant for *PICALM* by simple association analysis in our whole dataset.

Since age and *APOE*  $\epsilon 4$  status were of significant difference between groups, multivariate logistic regression analysis was employed (Table 3). After adjustment for age, sex and *APOE*  $\epsilon 4$  status, the rs6656401 but not rs3818361 in *CRI* remained of significant different distribution between disease and control groups. In contrast, both allele frequencies and genotypic distributions of rs2279590 and rs11136000 in *CLU* exhibited more significant protective effects after adjustment. Although, after multivariate logistic regression analysis no association was found for SNPs in *PICALM* between cases and controls among the entire dataset, significant LOAD association for rs3851179 of *PICALM* was found in the *APOE*  $\epsilon 4$  (-) but not in the *APOE*  $\epsilon 4$  (+) subgroup. In the *APOE*  $\epsilon 4$  (-) subgroup, compared to G allele, the A allele carrier of this SNP would decrease disease risk by 28% (adjusted  $p=0.028$ ). The (AA+AG) genotype of this SNP carrier would reduce LOAD risk by 42% (adjusted  $p=0.013$ ) compared to (GG) genotype (data not shown).



#### 4. Discussion

In our Hong Kong dataset, allelic association of the *CRI* and *CLU* exhibited significant difference between LOAD cases and non-demented controls which were in agreement with previous studies (Harold, et al., 2009, Lambert, et al., 2009). By multivariate logistic regression analysis, the A allele of rs6656401 (adjusted OR=1.97) was identified as an independent genetic risk factor for LOAD, while the A allele of rs2279590 (adjusted OR=0.72) and T allele of rs11136000 (adjusted OR=0.73) were observed to be independent genetic protective factors for disease development. However, the adjusted OR of *CRI* rs6656401 we presented here was of notable variance from Lambert's original GWAS data (OR=1.21) (Lambert, et al., 2009). For *CLU*, adjusted ORs from our findings were similar to those in initial GWAS (0.86 ORs for both rs2279590 and rs11136000) (Harold, et al., 2009, Lambert, et al., 2009). Up to date, in addition to two published GWAS, another two case-controlled studies (Carrasquillo, et al., 2010, Corneveaux, et al., 2010) and one meta-analysis (Jun, et al., 2010) carried out in parallel on Caucasians successfully demonstrated significant disease associations for *CRI*, *CLU* and *PICALM* with compatible genetic effect sizes (1.28 OR for *CRI* rs6656401; 0.82-0.92 OR for *CLU* rs11136000). A replication study performed by Kamboh et al (Kamboh, et al., 2010) based on 2707 Caucasian Americans failed to detect significant LOAD associations for *CRI*, *CLU* and *PICALM*, however, further meta-analysis combing their own data with Lambert's and/or Harold's GWAS data demonstrated improved significant associations between these three genes and disease risk (1.18 OR for *CRI* rs6656401; 0.87, 0.86 ORs for *CLU* rs2279590 and rs11136000, separately). In contrast to the Caucasian population studies, the study of Zhang et al (Zhang, et al., 2010) which was

performed on Chinese ethnics in Shandong province (the northern part of China), showed significant association of *CRI* and LOAD risk with 2.6 unadjusted OR for rs6656401. The disease allelic effect size was almost double compared to that of the Caucasian population and even 30% larger than that of our sample. Their result exhibited larger genetic effect tendency for polymorphism in *CRI* which was similar with the findings from our southern Chinese population comparison to Caucasian population. On the contrary to obtaining usually decreased OR by replication study following the original GWAS, in Chinese, both the northern and southern populations investigated by independent research centers exhibited a larger genetic effect of polymorphism in *CRI* compared to the Caucasian population. Thus, we hypothesize that *CRI* confers LOAD susceptibility with more remarkable genetic effect in Chinese than in Caucasians. Nevertheless, it is necessary to clarify this association speculation in the future based on a much larger sample size.

Although significant association was successfully replicated in *CRI* and *CLU* based on our dataset, no genotyping difference in *PICALM* was identified between cases and controls among the entire dataset. However, after stratification our dataset according to the *APOE*  $\epsilon 4$  status, significant association for *PICALM* rs3851179 was found in the *APOE*  $\epsilon 4$  (-) but not in the *APOE*  $\epsilon 4$  (+) subgroup. The possible interpretation is due to the powerful *APOE*  $\epsilon 4$  risky allele, which weakens the protective effect of polymorphism in the *APOE*  $\epsilon 4$  (+) subgroup. Therefore, its disease association was exhibited only in the *APOE*  $\epsilon 4$  (-) subgroup. Currently, it is notable that apolipoprotein E modulates amyloid- $\beta$  ( $A\beta$ ) peptide aggregation and clearance (Bell, et al., 2007, Holtzman, et al., 2000). Results from our study suggest that, by

interaction with *APOE*, *PICALM* may indirectly participate in amyloid precursor protein (APP) processing pathway and finally be involved in LOAD pathogenesis.

Compared to the cited GWAS with thousands of subjects, our study was conducted on a much smaller sample size. This may partially explain why no association was observed for *PICALM* with LOAD in our whole dataset. However, we did show the evidences of *CRI*, *CLU* and *PICALM* and LOAD susceptibility based on our southern Chinese population. This successful follow-on study performed in an independent Chinese population provides additional evidence for LOAD association apart from prior GWAS in the Caucasian population. Biologically, complement receptor 1 encoded by *CRI* has been revealed to be involved in clearance peripheral A $\beta$  peptides (Rogers, et al., 2006). Clusterin encoded by *CLU* has been found to bind with A $\beta$  to modulate A $\beta$  metabolism (Bell, et al., 2007, DeMattos, et al., 2002) and related with oxidative stress as well as neuronal apoptosis (Nuutinen, et al., 2009, Trougakos and Gonos, 2006). Phosphatidylinositol-binding clathrin assembly protein encoded by *PICALM* has been reported to participate in APP processing (Carey, et al., 2005, Nordstedt, et al., 1993). Moreover, two new GWAS studies also indicated *CRI*, *CLU* and *PICALM* are associated with LOAD (Naj, et al., 2011, Hollingworth, et al., 2011). Combing above biological information with evidences from GWAS as well as our study, it is warranted to further investigate those genes' function and to clarify their different roles in Alzheimer's disease.

### **Conflicts of interest**

There was no conflict of interest for this study.

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### **Supplement table**

Supplement table is available at

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**Table 1 Allelic association analysis of polymorphisms in *CRI*, *CLU* and *PICALM* with LOAD**

			All participants (AD=462, Control=350)			
		Allele	AD (n, %)	Control (n, %)	<i>P</i>	OR(95% CI)
<i>CRI</i>	rs6656401	A	53(5.79%)	24(3.49%)	<b>0.034*</b>	<b>1.69(1.04-2.77)</b>
		G	863(94.21%)	662(96.51%)		
	rs3818361	T	316(34.73%)	201(29.56%)	<b>0.029*</b>	<b>1.27(1.02-1.57)</b>
		C	594(65.27%)	479(70.44%)		
<i>CLU</i>	rs9331888	C	453(49.45%)	335(48.98%)	0.850	1.02(0.84-1.24)
		G	463(50.55%)	349(51.02%)		
	rs2279590	A	189(21.05%)	159(23.38%)	0.268	0.87(0.69-1.11)
		G	709(78.95%)	521(76.62%)		
	rs11136000	T	192(21.29%)	162(23.96%)	0.207	0.86(0.68-1.09)
		C	710(78.71%)	514(76.04%)		
<i>PICALM</i>	rs541458	C	439(48.89%)	338(49.71%)	0.747	0.97(0.79-1.18)
		T	459(51.11%)	342(50.29%)		
	rs3851179	A	364(39.82%)	275(40.32%)	0.840	0.98(0.80-1.20)
		G	550(60.18%)	407(59.68%)		

\* $P < 0.05$

**Table 2 Genotypic association analysis of polymorphisms in *CRI*, *CLU* and *PICALM* with LOAD**

			All participants (AD=462, Control=350)				
Genotype			AD (n, %)	Control (n, %)	<i>P</i>	OR(95%CI)	
<i>CRI</i>	rs6656401 <sup>a</sup>	AA	2(0.4%)	0(0%)	<b>0.048*</b>	<b>1.67(1.00-2.77)</b>	
		AG	49(10.7%)	24(7%)			
		GG	407(88.9%)	319(93%)			
	rs3818361 <sup>a</sup>	TT	51(11.2%)	27(8.0%)	<b>0.047*</b>	<b>1.33(1.00-1.77)</b>	
		TC	214(47.0%)	147(43.2%)			
		CC	190(41.8%)	166(48.8%)			
<i>CLU</i>	rs9331888 <sup>b</sup>	CC	109(23.8%)	79(23.1%)	0.817	1.04(0.75-1.45)	
		CG	235(51.3%)	177(51.8%)			
		GG	114(24.9%)	86(25.1%)			
	rs2279590 <sup>b</sup>	AA	13(2.9%)	21(6.2%)	<b>0.028*</b>	<b>0.45(0.22-0.92)</b>	
		AG	163(36.3%)	117(34.4%)			
		GG	273(60.8%)	202(59.4%)			
	rs1113600 <sup>b</sup>	TT	15(3.3%)	24(7.1%)	<b>0.018*</b>	<b>0.45(0.23-0.87)</b>	
		TC	162(35.9%)	114(33.7%)			
		CC	274(60.8%)	200(59.2%)			
	<i>PICALM</i>	rs541458 <sup>a</sup>	CC	112(24.9%)	92(27.1%)	0.882	1.02(0.75-1.41)
			CT	215(47.9%)	154(45.3%)		
			TT	122(27.2%)	94(27.6%)		
rs3851179 <sup>a</sup>		AA	77(16.8%)	56(16.4%)	0.679	0.94(0.70-1.26)	
		AG	210(46.0%)	163(47.8%)			
		GG	170(37.2%)	122(35.8%)			

<sup>a</sup> Dominant model for genotypic analysis; <sup>b</sup> Recessive model for genotypic analysis

\**P*<0.05

**Table 3 Multivariate logistic regression analysis for polymorphisms in *CRI*, *CLU* and *PICALM***

		Allele/Genotype	<i>P</i>	OR(95%CI)
<i>CRI</i>	rs6656401	A vs G	<b>0.035*</b>	<b>1.97(1.05-3.71)</b>
		AA+AG vs GG	<b>0.043*</b>	<b>1.95(1.02-3.74)</b>
	rs3818361	T vs C	0.092	1.27(0.96-1.66)
		TT+TC vs CC	0.096	1.35(0.95-1.93)
<i>CLU</i>	rs9331888	C vs G	0.353	1.13(0.87-1.46)
		CC vs CG+GG	0.427	1.18(0.78-1.79)
	rs2279590	A vs G	<b>0.035*</b>	<b>0.72(0.53-0.98)</b>
		AA vs AG+GG	<b>0.006**</b>	<b>0.29(0.12-0.70)</b>
	rs11136000	T vs C	<b>0.038*</b>	<b>0.73(0.54-0.98)</b>
		TT vs TC+CC	<b>0.009**</b>	<b>0.33(0.14-0.77)</b>
<i>PICALM</i>	rs541458	C vs T	0.534	0.93(0.72-1.18)
		CC+CT vs TT	0.670	0.92(0.61-1.37)
	rs3851179	A vs G	0.320	0.88(0.68-1.13)
		AA+AG vs GG	0.150	0.76(0.53-1.10)

Adjustment *P* value with age, sex and APOE  $\epsilon$  4 status as covariates

\*  $P < 0.05$ , \*\*  $P < 0.01$



### Supplement table Baseline characteristics of 812 Chinese participants

	AD (n=462)	Control (n=350)	<i>P</i>
Females (n, %)	330 (71.4%)	248 (70.9%)	0.86
Male (n, %)	132 (28.6%)	102 (29.1%)	
Age, years (mean $\pm$ SD)	79.4 $\pm$ 7.24	70.7 $\pm$ 6.33	< 0.001
<i>ApoE</i> $\epsilon$ 4 (+) carrier (n, %)	175 (38%)	58 (16.6%)	2.27 $\times$ 10 <sup>-11</sup>
$\epsilon$ 3/ $\epsilon$ 4: $\epsilon$ 3/ $\epsilon$ 3	149 (32.3%)	48 (13.7%)	1.24 $\times$ 10 <sup>-9</sup>
$\epsilon$ 4/ $\epsilon$ 4: $\epsilon$ 3/ $\epsilon$ 3	17 (3.7%)	3 (0.9%)	0.002