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Associations between *CLU* polymorphisms and memory performance: The role of serum lipids in Alzheimer's disease

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ABSTRACT

CLU encoding clusterin, has been reported to associate with Alzherimer's disease (AD) by genome-wide association studies (GWAS) based on Caucasian populations. Our previous case-control study has independently confirmed the disease association of *CLU* in Chinese population. Since little is known about the underlying mechanism of *CLU* in AD, we have conducted this study to investigate whether the genetic impact of *CLU* polymorphisms on cognitive functioning is via serum lipid's dysfunction. Three GWAS previously published *CLU* polymorphisms including rs2279590, rs11136000 and rs9331888, were genotyped in 689 subjects. Serum levels of triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured and tested as mediators. Delayed Word Recall Test (DWRT) was used to evaluate subjects' memory performance. Multiple mediation analysis, a nonparametric procedure to create confidence interval, was performed according to Preacher and Hayes's Bootstrapping method. Our findings suggested significant correlation between *CLU* polymorphism and DWRT scores for rs11136000 ($p = 0.045$) after adjustment for age, gender, body mass index, and *APOEε4* status, with borderline significant correlation for rs2279590 ($p = 0.058$). Both T allele of rs11136000 and A allele of rs2279590 were negatively correlated with serum TG levels ($p = 0.003$; $p = 0.001$, separately). Moreover, A allele of rs2279590 was positively correlated with serum HDL-C levels ($p = 0.015$). Consistent with our hypotheses, the genetic impact of *CLU* polymorphisms on memory performance were partially mediated through TG (rs11136000 95% CI [-0.099,-0.003] and rs2279590 95% CI [-0.104, −0.004]), but not through HDL-C and LDL-C. Our findings indicate *CLU* polymorphisms may modify AD susceptibility through lipid metabolic pathway.

1. Introduction

Clusterin known as apolipoprotein J (apoJ), is identified to play a critical role in Alzheimer's disease (AD) development. Being the second major apolipoprotein in human brain, clusterin has been revealed to share some similarities with Apolipoprotein E (apoE). Both of them are synthesized and released by astrocytes and neurons (DeMattos et al., 2001); involved in transporting cholesterol by constituting high-density lipoprotein (HDL) particles (Gelissen et al., 1998; Pitas et al., 1987); acting as an Aβ chaperone and therefore regulating Aβ deposition in the brain (DeMattos et al., 2004).

Following *APOE* which encoding apoE and is the well-established genetic risk factor, *CLU* encoding clusterin, has appeared to be the second impactful genetic risk factor for AD in the recent decade. In 2009, Lambert et al. (2009) and Harold et al. (2009), have simultaneously identified significant associations between *CLU* single-nucleotide polymorphisms (SNPs) and AD by two different genome-wide association studies (GWAS) based on Caucasian populations. Subsequently, these

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findings have been replicated by another two GWAS, with one by Seshadri et al., in 2010 (Seshadri et al., 2010) and the other one by Naj et al., in 2011 (Naj et al., 2011). For the Chinese population, a previous case-control study conducted by us has independently confirmed the association of *CLU* and AD susceptibility in Hong Kong (South China), with T allele of rs11136000 and A allele of rs2279590 exhibiting significant protective effects (Chen et al., 2012). Other replication studies conducted in Shanghai (East China) (Wang et al., 2016), Changsha (Central South China) (Jiao et al., 2015), and Qinghai-Tibet plateau (Southwest China) (Huang et al., 2016), all have consistent findings for association of *CLU* in AD. Lin et al. have also revealed significant AD association with *CLU* polymorphism in Taiwan (Lin et al., 2012). Further re-assessing the effect of *CLU* SNPs by meta-analysis has validated these significant findings in populations of Caucasian and Asian (Chinese, Japanese, Indian, and Korean) for rs11136000, with effect size of 0.87 in both populations (Du et al., 2016; Jun et al., 2010; Liu et al., 2014; Wang et al., 2016).

Additionally, accumulating evidence has suggested *CLU* has a critical role in cognitive functioning. In a prospective longitudinal Danish cohort study, significant positive correlation has been detected between dosage of *CLU* rs11136000 T allele (protective allele) and better cognitive performance in elderly using cognitive composite score as measurement, which is computed by combining the scores of five tests, including verbal fluency test, forward and backward digit span tests, immediate and delayed recall tests (Mengel-From et al., 2011; Mengel-From et al., 2013). Moreover, this allele has been reported to correlate with better verbal episodic memory in immediate and delayed recall trials based on a mixture sample of AD cases and controls in Americans (Pedraza et al., 2014). Intriguingly, Thambisetty et al. has reported carriers of the *CLU* rs11136000 C allele (risky allele) demonstrate faster rates of impairments in memory performance when measured by California Verbal Learning Test (immediate and delayed recall trials) in American elderly (Thambisetty et al., 2013). Coincidently, Cai et al. have found that rs11136000 C allele carrier exhibits an increased plasma clusterin level in Chinese, which is negatively correlated with the auditory verbal learning test of delayed recall trial (Cai et al., 2016). In line with the above findings, in Australians, carriers of the risky C allele of rs11136000, have demonstrated a distinct profile of lower white matter integrity in fornix, cingulum, corpus callosum, and longitudinal fasciculi, regions which are known to be degenerated in AD (Braskie et al., 2011). However, so far, the underlying mechanism that links *CLU* polymorphisms to cognitive functioning in AD has been poorly explored.

Since *CLU* polymorphisms are also related with serum lipid levels (Miwa et al., 2005) and epidemiological data has suggested the serum lipid levels are related with risk of AD (Reitz, 2013), we therefore proposed the current study to investigate whether *CLU* polymorphisms have impact on memory performance in Chinese population, and whether this impact is a direct or indirect event which may be mediated via serum lipids. Because impairment of episodic memory is one of the earliest signs and symptoms of AD (Gold and Budson, 2008), our study which uses episodic memory as measurement of cognitive functioning should be a powerful approach to early evaluate the downstream consequence of *CLU* genetic polymorphisms.

2. Materials and methods

2.1. Subjects

In this cross-sectional study performed in Hong Kong, we recruited AD patients from the Memory Clinic of Queen Mary Hospital and controls from the elderly social centers in the community. The study was approved by local ethics committee (Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster), which required written consents to be acquired from each participant.

In the current study, consecutive patients who received a diagnosis of AD were recruited from February, 2004 to May, 2010, if they fulfilled the inclusion and exclusion criteria. The clinical diagnosis of AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984), which has been shown to be valid and reliable (Blacker et al., 1994). Subjects who had secondary causes of dementia, including non-AD types of dementia and familial AD, were excluded from the current study. The controls had normal cognition without cognitive complaint or impairment. The exclusion criteria of controls included cancer within the past five years, active infection, end-stage renal/cardiac/liver/respiratory failure, stroke, Parkinson's disease, depression, deafness, and other communication barriers.

2.2. Memory measuring & laboratory investigations

The Delayed 10-Word Recall Test (DWRT) which reflects impairment in episodic memory, a key deficit feature in AD and mild cognitive impairment (MCI) (Backman et al., 2005; Maruff et al., 2004), was used to assess memory performance. We had reported the use of DWRT in our previous study (Chu et al., 2008), which composed by 10 Chinese words. Each participant was firstly given three immediate registration and immediate recall trials. After 10 min, each participant was given a delayed free recall trial. Higher DWRT scores indicate better memory performance (Altepeter et al., 1990).

Serum lipid levels, including triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, were performed at the Division of Clinical Biochemistry in Queen Mary Hospital. The Hitachi 717 analyzer (Beohringer Mannhein, Germany) was used for cholesterol and triglyceride measurements by the cholesterol oxidase/glycerol kinase methods. The HDL-C level was measured using the cholesterol oxidase method, after precipitation of the apolipoprotein B containing lipoproteins. Calculation of LDL-C level was done by the Friedwald equation (Janus et al., 1997).

2.3. Genotyping

The subject's DNA was extracted from the whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), and quality and quantity were evaluated using the fluorometer. Three GWAS previously published *CLU* SNPs (rs11136000, rs2279590 and rs9331888) were selected for genotyping. We used Mass ARRARY AssayDesign software to design both amplification and single allele extension primers. Following, genotyping for the three selected SNPs were investigated on a high-throughput Sequenom® genotyping platform (Sequenom, San Diego, CA) at Centre for PanorOmic Sciences-Genomics and Bioinformatics Cores, the University of Hong Kong.

2.4. Statistical analysis

Data analysis was conducted using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink) and SPSS 23.0. Genotypes of the three SNPs were coded as 0, 1, 2, which represented homozygotes for the major alleles, heterozygotes for the major and minor alleles, and homozygotes for the minor alleles, separately. Independent sample t-tests were carried out to compare means between cases and controls for clinical continuous variables, including age, body mass index (BMI), lipid levels and cognitive scores. Pearson's Chi-square tests were performed to compare frequencies between groups for clinical categorical variables, including gender and *APOEε4* status. Linear regression was conducted to examine the specific correlations of each variable (SNPs/lipid levels) with cognitive scores (DWRT), separately.

Multiple mediation analyses utilizing Preacher and Hayes's Bootstrapping approach (Preacher and Hayes, 2008) implemented in SPSS via Syntax were performed to test the proposed hypothesis. It is a nonparametric procedure to create an empirical approximation of the

sampling distribution, which in turn will generate a confidence interval (CI) to test the indirect effect related to the mediation model. The advantage of this analyses is that the assumption of normality is not required when applying it. The indirect effect of the independent variable on the dependent variable through multiple mediators, presenting as path coefficient, will be estimated. The multiple mediation analyses can calculate not only the collective indirect effect through all mediators but also the specific indirect effect of each one (Hayes, 2009). The 95% bias corrected CI is determined utilizing bootstrap resamples, which technique involves repeatedly randomly sampling observations with replacement from the existing dataset. For the computed CI which doesn't include 0, it suggests the significant indirect effect of the mediator in the analyzed model. All mediation analyses were adjusted by potential confounding effects of age, gender, BMI and *APOE ε4* status with 10,000 bootstrapping resamples applied. We regarded $p < 0.05$ was statistically significant.

3. Results

3.1. Demographic characteristics of all subjects

A total of 689 subjects (400 LOAD patients and 289 controls) were recruited for the current study. Compared to controls, AD patients were much older with lower BMI and lower DWRT score. There was no significant difference for levels of TG, HDL-C as well as LDL-C between AD patients and controls in our sample. All three genotyped *CLU* SNPs were satisfied with the Hardy-Weinberg equilibrium distribution ($p > 0.001$). In line with our previous findings (Chen et al., 2012), both T allele of rs11136000 and A allele of rs2279590 were associated with decreased AD risk (p value adjusted by age, gender, BMI and *APOE ε4* status), although no significant disease association was detected for rs9331888 C allele (Table 1).

3.2. Multiple mediation analysis

Both T allele of rs11136000 and A allele of rs2279590 were negatively correlated with serum TG levels ($b = -0.139$, $p = 0.003$; $b = -0.153$, $p = 0.001$, separately). Moreover, A allele of rs2279590 was positively correlated with serum HDL-C levels ($b = 0.072$, $p = 0.015$), while the correlation between T allele of rs11136000 and HDL-C levels was borderline significant ($b = 0.056$, $p = 0.051$). No significant correlation was detected between *CLU* polymorphisms and serum LDL-C levels. Of interest, serum TG levels would positively predict DWRT scores ($b = 0.312$, $p = 0.009$) and serum LDL-C levels would negatively predict DWRT scores ($b = -0.354$, $p = 0.006$) in our models (Fig. 1 and Fig. 2).

For rs11136000, significant total effect (direct path + indirect path via lipid mediators) ($b = 0.404$, $p = 0.045$) and direct effect (direct path) ($b = 0.425$, $p = 0.035$) on DWRT scores were found after adjustment for age, gender, BMI, and *APOE ε4* status. Thus, subjects carrying rs11136000 T allele were correlated with higher DWRT scores which indicated better memory performance. When taken as a set, the multiple-mediator model accounted for 36.73% of the variance in DWRT scores (F (5,654) = 89.97, $p < 0.0001$) (Fig. 1). Moreover, the significant effects of rs11136000 on memory performance were partially mediated through serum TG (95% CI [-0.099, −0.003]), but not through HDL-C (95% CI [-0.005, 0.084]) and LDL-C (95% CI [-0.039, 0.041]) (Table 2). For rs2279590, borderline significant effects were found on DWRT scores for total effect ($b = 0.390$, $p = 0.058$) and direct effect ($b = 0.402$, $p = 0.053$) after adjustment for age, gender, BMI, and *APOE ε4* status. The overall multiple-mediator model accounted for 36.66% of the variance in DWRT scores (F (5,654) = 90.28, $p < 0.0001$) (Fig. 2). Similar to rs11136000, the significant effects of rs2279590 on memory performance were partially mediated through serum TG (95% CI [-0.104, −0.004]), but not through HDL-C (95% CI [-0.007, 0.094]) and LDL-C (95% CI [-0.026, 0.055]) (Table 3). With regard to rs9331888, the total effect ($p = 0.405$), direct effect ($p = 0.312$), and specific indirect effect on DWRT scores through specific lipid mediators were all non-significant (Table 4).

Subsequently, investigation of the pairwise contrasts of the specific mediators demonstrated that the mediating effect through TG was significantly larger than that through HDL-C in models of rs11136000 (95% CI [-0.155, −0.008]) (Table 2) and rs2279590 (95% CI [-0.173, −0.010]) (Table 3), but not in model of rs9331888 (95% CI [-0.012, 0.085]) (Table 4).

Table 1
Clinical characteristics of patients and controls.

	All participants (n = 698)		
	AD (n = 400)	Control(n = 289)	*P*
Female (n, %)	281 (70.25%)	207 (71.63%)	0.70
Male (n, %)	119 (29.75%)	82 (28.37%)	
APOEε4 (+) carrier (n, %)	155 (38.60%)	51 (17.64%)	<0.001
APOEε4 (−) carrier (n, %)	245 (61.40%)	238 (82.36%)	
CLU rs11136000			0.04*
T (n, %)	164 (21.08%)	131 (23.48%)	
C (n, %)	614 (78.92%)	427 (76.52%)	
rs2279590			0.03*
A (n, %)	160 (20.62%)	127 (22.68%)	
G (n, %)	616 (79.38%)	433 (77.32%)	
rs9331888			0.44*
C (n, %)	386 (48.74%)	279 (49.47%)	
G (n, %)	406 (51.26%)	285 (50.53%)	
Age, years (mean ± SD)	80.1 ± 6.99	71.0 ± 6.29	<0.001
BMI, kg/m^2 (mean ± SD)	22.75 ± 3.85	23.99 ± 3.78	<0.001
TG, mmol/L (mean ± SD)	1.37 ± 0.61	1.45 ± 1.05	0.17
HDL-C, mmol/L (mean ± SD)	1.50 ± 0.43	1.54 ± 0.44	0.16
LDL-C, mmol/L (mean ± SD)	3.11 ± 0.82	3.03 ± 0.84	0.20
DWRT score (mean ± SD)	0.31 ± 0.91	7.22 ± 1.26	<0.001

APOE, Apolipoprotein E gene; BMI, Body Mass Index; TG, Triglyceride; HDL-C, High-density Lipoprotein Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol; DWRT, Delayed Word Recall Test.
**P* value adjusted by age, gender, BMI and *APOE ε4*.

4. Discussion

In the current study, using a sample of Chinese elderly in Hong Kong, we have found that *CLU* polymorphism can predict DWRT scores. Compared to non-carriers, subjects carrying T allele of rs11136000 (protective allele) are correlated with better memory performance. The *CLU* polymorphisms are also related to serum lipid levels, which in turn are related to DWRT scores. Moreover, TG but not HDL-C and LDL-C in the lipid metabolic pathway, plays as an inconsistent mediator suppressing the protective effects of *CLU* polymorphisms on subjects' episodic memory.

We have identified that *CLU* polymorphisms not only confer AD risk but also influence memory performance, which reinforce their important roles in AD development. Previously, cognitive functioning has been suggested to be a suitable endophenotype in clinical study of early AD (Bennett et al., 2009). Given that so far most studies investigating *CLU* polymorphisms and cognitive endophenotypes are carried out in Caucasian populations (Barral et al., 2012; Mengel-From et al., 2011, 2013; Pedraza et al., 2014), the current study provides additional evidence by way of significant findings based on the Chinese population. Consistently, *CLU* polymorphism, rs11136000 T allele, is positively associated with episodic memory which is implicated in capability of human hippocampus and its interconnections with brain cortex (Gallagher and Koh, 2011). And, carriers of *CLU* polymorphism in healthy individuals have exhibited abnormal coupling between prefrontal cortex and hippocampus in episodic memory retrieval (Erk et al., 2011). Supportively, recent findings from neuroimaging studies based on Chinese cohorts have revealed impacts of *CLU* rs11136000 on grey matter atrophy of parahippocampal gyrus (Qiu et al., 2016) as well as functional

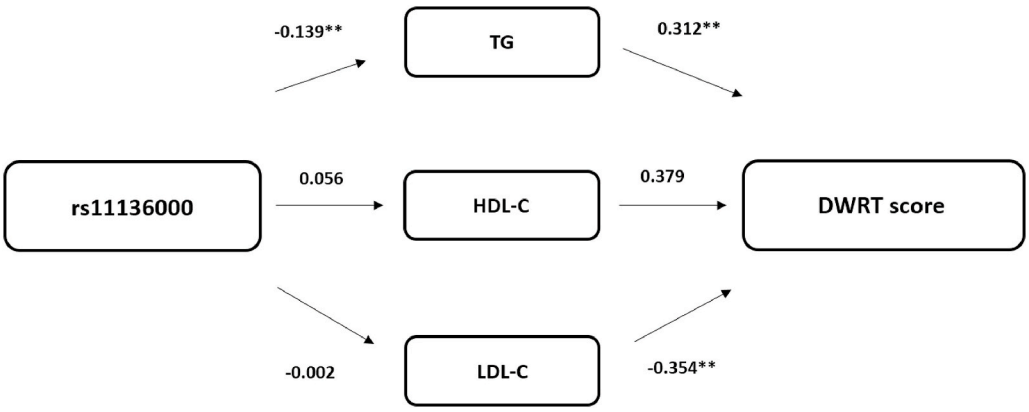

Fig. 1. The multiple mediator model for rs11136000 on memory performance. Unstandardized Coefficient (*b*) presenting the effect of rs11136000 T allele on mediators and DWRT scores. Significant effects were found for the total effect of rs11136000 T allele on DWR scores ($b = 0.404$, $p = 0.045$) and direct effect of rs11136000 T allele on DWRT scores ($b = 0.425$, $p = 0.035$). The multiple mediator model accounting for 36.73% of the variance in DWRT scores ($F(5,654) = 89.97$, $p < 0.0001$).*Significant at $p < 0.05$, **Significant at $p < 0.01$.

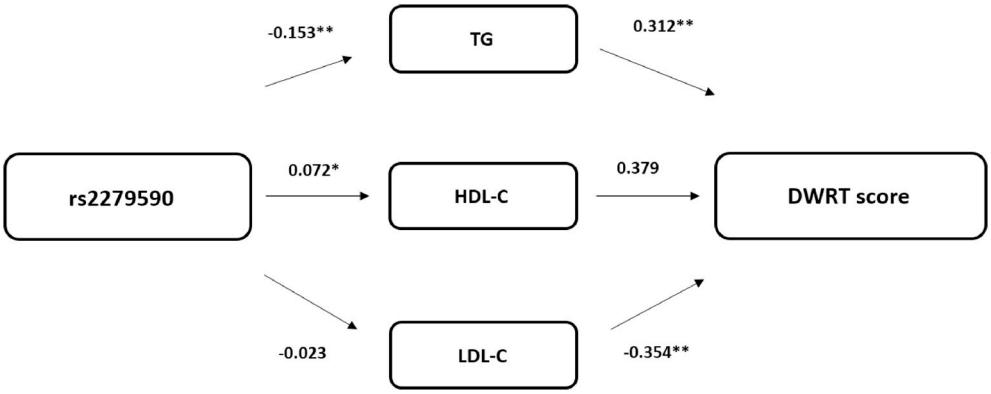

Fig. 2. The multiple mediator model for rs2279590 on memory performance. Unstandardized Coefficient (*b*) presenting the effect of rs2279590 A allele on mediators and DWRT scores. Borderline significant effects were found for the total effect of rs2279590 A allele on DWR scores ($b = 0.390$, $p = 0.058$) and direct effect of rs2279590 A allele on DWRT scores ($b = 0.402$, $p = 0.053$). The multiple mediator model accounting for 36.66% of the variance in DWRT scores ($F(5,654) = 90.28$, $p < 0.0001$). *Significant at $p < 0.05$, **Significant at $p < 0.01$.

Table 2
Indirect effects of multiple mediation model for rs11136000 on DWRT scores through serum lipid levels.

Pathway	*b* (Unstandardized Coefficient)	SE	BC 95% CI	
			Lower	Upper
TG	−0.043	0.024	−0.099	−0.003
HDL-C	0.021	0.021	−0.005	0.084
LDL-C	0.001	0.019	−0.039	0.041
Total	−0.021	0.033	−0.086	0.048
TG vs HDL-C	−0.065	0.036	−0.155	−0.008
TG vs LDL-C	−0.044	0.031	−0.112	0.012
HDL-C vs LDL-C	0.021	0.029	−0.028	0.087

DWRT, Delayed Word Recall Test; SE, Standard Error; BC, Bias Corrected; CI, Confidence Interval; TG, Triglyceride; HDL-C, High-density Lipoprotein Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol.

Table 3
Indirect effects of multiple mediation model for rs2279590 on DWRT scores through serum lipid levels.

Pathway	*b* (Unstandardized Coefficient)	SE	BC 95% CI	
			Lower	Upper
TG	−0.047	0.025	−0.104	−0.004
HDL-C	0.028	0.025	−0.007	0.094
LDL-C	0.008	0.019	−0.026	0.055
Total	−0.011	0.035	−0.079	0.063
TG vs HDL-C	−0.075	0.040	−0.173	−0.010
TG vs LDL-C	−0.056	0.033	−0.129	0.002
HDL-C vs LDL-C	0.019	0.031	−0.037	0.089

DWRT, Delayed Word Recall Test; SE, Standard Error; BC, Bias Corrected; CI, Confidence Interval; TG, Triglyceride; HDL-C, High-density Lipoprotein Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol.

connectivity of hippocampus (Zhang et al., 2015). Congruently, significant correlations identified from the current study combining neuroimaging studies from others, all suggest the considerable role of *CLU* polymorphisms in memory performance, which highlights an underlying mechanism that needs efforts to be further explored.

Moreover, we have observed significant correlations between *CLU* polymorphisms and serum TG as well as HDL-C levels in Chinese elderly. In combined case-control dataset, compared to non-carriers, subjects carrying rs2279590 A allele were found to have lower serum TG levels (Fig. 3 Panel B), but higher HDL-C levels (Fig. 4 Panel B). Similarly, subjects carrying 11136000 T allele were found to have lower serum TG levels (Fig. 3 Panel A), while higher HDL-C levels (borderline significant) (Fig. 4 Panel A). Specifically, such genetic effects of *CLU* polymorphisms on serum TG levels demonstrated in a dose-dependent manner. It has been suggested that an individual's lipoprotein levels can be influenced by *CLU* polymorphisms (Nestlerode et al., 1999), with previous studies mainly focused on cardiovascular diseases (Miwa et al., 2005; Pan et al., 2011). Only recently, such influence on cognitive disorders has been started to be investigated. Aghajanpour-Mir et al. have found that compared to the CC genotype, the (TC + TT) genotype of rs11136000 are significantly associated with increased amount of HDL-C in MCI group (Aghajanpour-Mir et al., 2019), which is in line with tendency of the current study (Fig. 4 Panel A). It has been reported that HDL-C can functionally decrease Aβ accumulation as well as debilitate Aβ induced neuroinflammation in the human vessel model (Button et al., 2019). And findings from clinical studies suggest that higher HDL-C circulating levels are related with lower AD risk (Reitz et al., 2010; Zuliani et al., 2010) and less AD severity (Merched et al.,

Table 4
Indirect effects of multiple mediation model for rs9331888 on DWRT scores through serum lipid levels.

Pathway	*b* (Unstandardized Coefficient)	SE	BC 95% CI	
			Lower	Upper
TG	0.023	0.019	−0.002	0.073
HDL-C	−0.001	0.011	−0.027	0.021
LDL-C	0.005	0.015	−0.020	0.041
Total	0.028	0.025	−0.014	0.085
TG vs HDL-C	0.024	0.024	−0.012	0.085
TG vs LDL-C	0.018	0.023	−0.023	0.070
HDL-C vs LDL-C	−0.006	0.019	−0.049	0.028

DWRT, Delayed Word Recall Test; SE, Standard Error; BC, Bias Corrected; CI, Confidence Interval; TG, Triglyceride; HDL-C, High-density Lipoprotein Cholesterol; LDL-C, Low-density Lipoprotein Cholesterol.

2000). Consistent with above evidence from both basic and clinical human investigations, the positive correlations between *CLU* polymorphisms with higher serum HDL-C levels identified in the current study further support their protective effects in cognitive functioning.

In the following mediation analysis of lipid pathway, the total indirect effect through three lipid mediators (the difference between total and direct effects) is with a point estimate of −0.021 in model of rs11136000 which is different from zero (Table 2). While subsequent examination of the specific indirect effect reveals that TG is a significant mediator, with neither HDL-C nor LDL-C contributing to the indirect effect mentioned above. When the variances accounted by three lipid mediators were controlled for, we have revealed stronger association between rs11136000 T allele and memory performance (the direct effect > the total effect) in our model. The direct effect opposite in sign to the indirect effect which is found in the current study has been referred to be inconsistent mediation (MacKinnon et al., 2007). It is suggested that mediators and suppressors can coexist in the model involved in multiple intervening variables (MacKinnon et al., 2000). The effect of serum TG has a different sign (negative) than serum HDL-C (positive), LDL-C (positive) in our model, indicating an inconsistent mediation model which yields two opposing mediational processes (MacKinnon et al., 2007), although effects of the latter two mediators can't achieve statistical significance. Therefore, serum TG level, being an inconsistent mediator or suppressor (Darlington, 1968; MacKinnon et al., 2000; McFatter, 1979), weakens the positive association between rs11136000 T allele and memory performance in our overall model.

Since the pair-wise r^2 value is 0.96 between rs11136000 and rs2279590 in our Chinese dataset (data not shown) which indicates a strong linkage disequilibrium (LD) relationship, it is not a surprise for us to detect a similar mediation pattern for rs2279590. The total effect of rs2279590 A allele on memory performance is borderline significant, in other words obscured significant, which may be due to the direct effect and indirect effect cancelling each other out. This counterbalanced

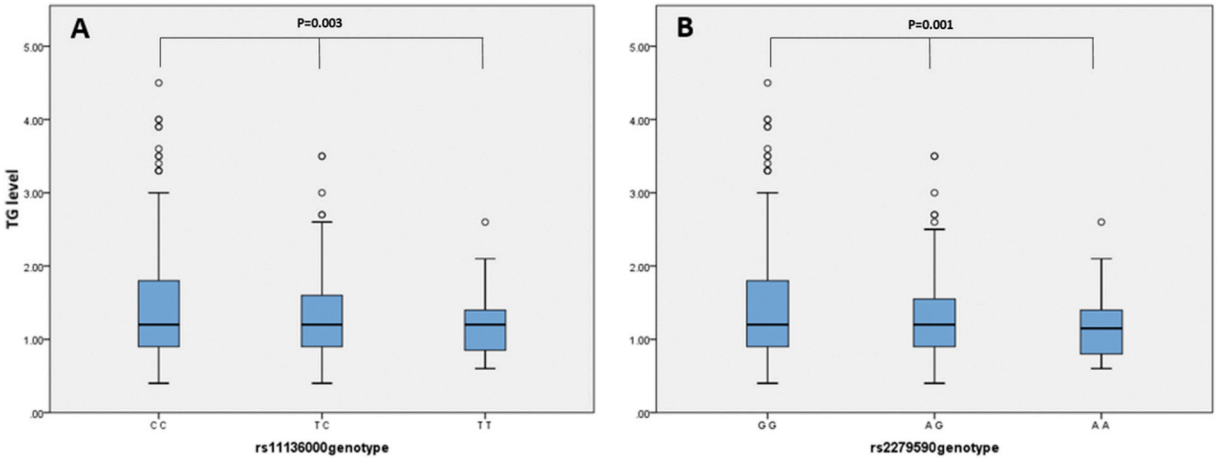

Fig. 3. Boxplots of TG levels stratified by *CLU* three genotypes. Panel A and B represented distributions of serum TG levels across 3 genotypes of rs11136000 and rs2279590, separately. Data was presented as median (hortizontal line through box) with first and third quartile (box boudnaries).

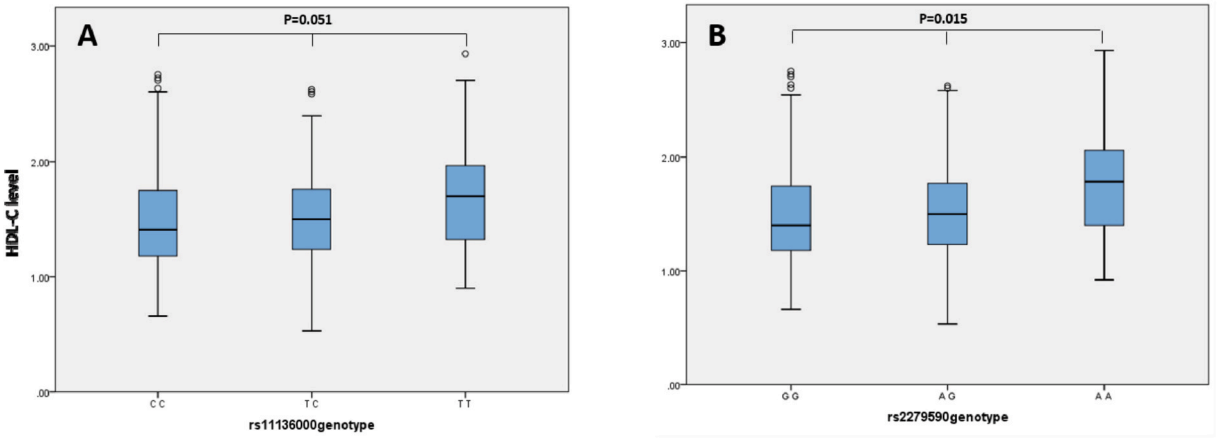

Fig. 4. Boxplots of HDL-C levels stratified by *CLU* three genotypes. Panel A and B represented distributions of serum HDL-C levels across 3 genotypes of rs11136000 and rs2279590, separately. Data was presented as median (hortizontal line through box) with first and third quartile (box boudnaries).

phenomenon is attributed by the suppression impact of serum TG level acting as an inconsistent mediator/suppressor with opposite indirect effect sign compared to HDL-C and LDL-C in the current multiple mediator model. It has been suggested that the function of a suppressor would cause both direct and total effects to appear small or nonsignificant by its omission, while meditation actually exists (Rucker et al., 2011). Thus, our findings of borderline significant total and direct effects of rs2279590 A allele on episodic memory while with significant indirect effect via serum TG level, are consistent with the concealing mediation theory induced by the suppression impact of inconsistent mediator (Rucker et al., 2011).

Recently, TG has been found to cross the human blood–brain barrier and subsequently to be detected in cerebrospinal fluid (CSF) (Banks et al., 2018). By injecting TG into mice's lateral ventricle, Farr et al. have reported impaired cognitive functioning, including both learning and memory (Farr et al., 2008). A longitudinal cohort study has found midlife TG levels can predict abnormal CSF $A\beta_{42}$ levels, CSF $A\beta_{42}$/phosphorylate-tau ratios, and brain Aβ depositions in later life (Nagga et al., 2018). In a more recent study based on MCI and AD patients registered in "Alzheimer's Disease Neuroimaging Initiative (ADNI)" project, Bernath et al. have reported significant associations between TG principal component scores with patients' hippocampus volumes (Bernath et al., 2019). Consistent with above findings, our results based on Chinese elderly highlight TG may represent a risk factor by interfering with the protection effect of *CLU* on cognition and AD. It has been suggested that clusterin participates in lipid transportation and is enriched in TG (Burkey et al., 1992). Since deficiency of clusterin dysregulates TG metabolism (Heo et al., 2018), it is presumed that *CLU* may modulate AD susceptibility via lipid metabolism by involving in TG-rich lipoprotein particles, which is in accordance with TG's role revealed by the current study. Nevertheless, a following study using prospective longitudinal research design to validate this cross-sectional finding is warranted.

Neither AD diagnosis nor memory performance was found to associate with *CLU* rs93331888 in Chinese elderly. Although significant disease association for rs933188 has been well established in Caucasian population by GWAS (Harold et al., 2009; Lambert et al., 2009), the non-significant result for AD diagnosis is consistent with finding from system-review and meta-analysis based on Chinese (Zhu et al., 2017) and Asian populations (Zhang et al., 2016). Further investigation to test the genetic influence of rs93331888 on memory performance also demonstrated non-significant finding, although previously it has been linked with cognitive decline in a Danish cohort study (Mengel-From et al., 2013).The inconsistent finding between Caucasian population and Chinese population may suggest that *CLU* rs93331888 acts as an ethnicity-dependent variant in the pathogenesis of AD.

The human brain is a high lipid content with lipids playing a critical role in developing its structure and maintaining its function. Consistently, genetic alterations on the lipid metabolic pathway have been identified by revealing correlations between *CLU* polymorphisms and memory performance in Chinese elderly. The risky role of lipid TG but not HDL-C and LDL-C, further implicates the potential dysfunction of lipid metabolic pathway in the AD pathogenesis. Clinically, HMG-CoA reductase inhibitor, a lipid-lowering medication known as Statin, has shown a beneficial effect on AD (Wanamaker et al., 2015). Simvastatin, for example, can decrease AD incidence, especially in patients who are *APOEε4* homozygous carriers (Geifman et al., 2017). Given that cardiovascular risk factor is a significant AD predictor, current findings support the lipid-centric therapy as a promising therapeutic target in treating the devastating disease. With advancement in lipid analysis technology, to conquer the limitations of current study, it's possible to use "lipidomics" to identify and specify the subfraction profiles of TG changes in the future which will instruct us on understanding more about the potential causal role of lipid metabolic pathway in AD.

In summary, reduced likelihood of neuroprotective effects of *CLU* rs11136000 T allele and rs2279590 A allele on episodic memory correlated with serum TG levels have been revealed in Hong Kong Chinese elderly. Our findings afford additional evidence of the association between lipid metabolic pathway and cognitive functioning, which may suggest *CLU* polymorphisms modify AD susceptibility through serum lipids' levels. This would offer a new perspective for effective lipid-centric therapy in neurodegenerative disease.

Contributors

Leung Wing Chu and **You-Qiang Song** were responsible for the overall drafting and reviewing the manuscript. **Lu Hua Chen** contributed to conceiving and performing the experiment, statistical analysis, interpretation of data, and writing the manuscript. **Deborah Tip Yin Ho** was performed the experiment and conducted data collection. **Yanhui Fan** was responsible for genetic analysis. **Pak Chung Sham** and **Timothy Shin Heng Mak** were responsible for multiple-mediation statistical analysis.

All authors approved the final submitted version of the current manuscript.

Declaration of competing interest

None of the authors has any financial or any other kind of personal conflicts with this study.

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