PVRL2 rs6859 Modifies the Effect of Triglyceride on Progression of Mild Cognitive Impairment: The Shanghai Aging Study

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Abstract

**Background** Mild cognitive impairment (MCI) is an intermediate stage between normal cognition and Alzheimer’s disease (AD). Genome-wide association studies (GWAS) have identified many AD-risk variants and indicated the important role of lipid metabolism pathway in AD progression. This study aimed to investigate the effects of triglyceride (TG) and genetic risk factors on progression from MCI to AD (MCI-AD progression).

**Methods** The current study sample comprised of 305 MCI subjects aged 50 and over who were prospectively followed up for average 4.5 years in a sub-cohort of the Shanghai Aging Study. A consensus diagnosis of incident AD was conducted according to Diagnostic and Statistical Manual of Mental Disorders-IV and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria. Fasting blood samples were obtained at baseline for analyzing serum TG. Single nucleotide polymorphisms (SNPs) genotyping was performed using a MassARRAY system. The effect of TG, genetic variants and their interaction on MCI-AD progression were analyzed using Cox proportional hazards regression model.

**Results** During a mean (±SD) follow-up period of 4.5±1.3 y, 58 subjects developed incident AD. The SNP, rs6859 in the Poliovirus Receptor–Related 2 (PVRL2) gene, was significantly associated with incident AD (false discovery rate (FDR)-adjusted \( P = 0.018 \)). In multivariate cox model, the PVRL2 rs6859 AG, AA and AG+AA genotypes were associated with significantly increased incident AD, compared with the GG genotype (hazard ratio [HR] = 2.29, \( P = 0.029 \), and HR = 2.92, \( P = 0.013 \), and HR = 2.47, \( P =0.012 \), respectively). In PVRL2 rs6859 AG/AA carriers, higher ln TG was significantly associated with increased risk of incident AD (adjusted HR =2.64, \( P = 0.034 \)). Ln TG and PVRL2 rs6859 had interactive effect on the MCI-AD progression (\( P_{\text{Ln TG × rs6859}} = 0.001 \)).

**Conclusion** The present study indicated that PVRL2 rs6859 modified the effect of TG on MCI-AD progression. Precision prevention in MCI population based on genetic information should be considered to avoid progression to AD.

Introduction

Mild cognitive impairment (MCI) is a transitional clinical status between normal and Alzheimer's disease (AD)-type dementia [1], and its incidence in elderly population ranges from 21.5 to 71.3 per 1,000 person-years and prevalence ranges from 3–42% worldwide [2]. Our previous findings suggested that 20% of Chinese elderly (> 60 years old) were affected by MCI [3], and approximately 6% of elderly with MCI progressed to dementia annually in the Shanghai Aging Study in China [4]. Although MCI has been associated with greater risk of AD occurrence, many individuals revert to normal state or do not progress [5]. Therefore, understanding the predictive factors and potential targets for progression from MCI to AD (MCI-AD progression) is fundamental to seek effective and timely intervention.

About a third of AD cases is attributable to potentially modifiable risk factors, especially vascular risk factors [6]. As lipids levels represent easily modifiable potential targets for prevention, exploring their relationship with AD risk is of major interest. Unlike cholesterol measurements, the effect of TG on cognitive function in the elderly has not garnered enough attention in recent years [7]. Additionally, previous epidemiological studies regarding the impact of TG on cognitive function in elderly populations have not reached a consensus, including associations of adverse TG with an increased AD risk [8–10], absence of an association [11–15], or even inverse associations [7, 16]. Gina et al. found that two AD-risk SNPs had interaction with midlife TG level on AD risk (rs11218343 and APOE e4) in the Framingham Heart Study [17], which indicated that genetic factor might be one of the reasons for inconsistent results of TG-AD relationship. APOE e4 is the strongest genetic risk factor for sporadic AD [18], however, 40–50% people with sporadic AD do not carry the APOE e4 allele [19, 20], meaning that additional genetic variants likely exist. Genome-wide association studies (GWAS) have identified a number of independent AD risk-associated single nucleotide polymorphisms (SNPs) [21–26]. Tosto et al. reviewed these AD-risk SNPs identified from GWAS, and indicated that several AD-related genes clustered lipid metabolism pathway, such as APOE, Clusterin (CLU), ATP-binding cassette transporter A7 (ABCA7) and poliovirus receptor–related 2 (PVRL2) [27]. All these findings compel us to study whether the relationship between TG and risk of AD is modified by genetic risk variants of AD, which has rarely been reported.
In the present study, we aimed to investigate the effect of TG, genetic variants and their interaction on MCI-AD progression in a prospective community-based cohort in order to verify the hypothesis that the genetic-risk variants could modify the effect of TG on cognitive decline.

**Methods**

**Recruitment of study subjects**

All subjects came from the Shanghai Aging Study (SAS), a longitudinal, community-based cohort study launched at January 1, 2010 in central downtown, Shanghai [28]. At baseline, we conducted face-to-face interviews and clinical examinations with 3836 registered residents aged 50 years or older and diagnosed 696 individuals with MCI. Subsequently, we established the MCI sub-cohort. The detailed procedures of the recruitment have been published elsewhere [3].

The present study was approved by the Medical Ethics Committee of Huashan Hospital, Fudan University (No.2009-195) and Ethics Committee of Department of Public Health in Fudan University, Shanghai, China (No.2018-01-0662). Written informed consent was obtained from all subjects or their legally acceptable representative.

**Baseline demographic characteristics and medical history**

At baseline, demographic information, lifestyle characteristics, and medical histories were collected via an in-person questionnaire survey (either at Huashan Hospital, or at their homes). Data included age, gender, educational background, dementia family history, cigarette smoking and alcohol consumption. History of hypertension, diabetes, stroke, and coronary artery disease (including valvular heart disease, cardiomyopathy, heart failure, and arrhythmias, etc.) were collected and confirmed with subjects’ medical records. Body mass index (BMI) was calculated by dividing weight in kilograms (kg) by height in meters (m) squared. Mini-Mental State Examination (MMSE) score was used for screening for global cognition and the Center for Epidemiologic Studies Depression Scale (CESD) was used to determine mood for each individual [29].

**Neuropsychological assessments**

We translated, adapted and standardized neuropsychological tests from western countries harmonized with Chinese culture, covering the domains of global cognition, executive function, spatial construction function, memory, language, and attention. Tests used in the study were: (1) MMSE; (2) Conflicting Instructions Task (Go/No Go Task); (3) Stick Test; (4) Modified Common Objects Sorting Test; (5) Auditory Verbal Learning Test; (6) Modified Fuld Object Memory Evaluation; (7) Trail-making test A&B; (8) Renminbi (official currency of China) Test, translated from the EURO test. Tests (1) to (5) and (7) were used for individuals with more than 6 years of education, while tests (1) to (4) and (6) and (8) were used for those with less than 6 years of education. Detailed clinical and neuropsychological evaluation and diagnosis procedures were described in our previous report of MCI prevalence [3]. All tests were conducted in the Chinese language in 90 minutes or less.

**Interview at the follow-up**

From April 1, 2014 to December 31, 2016, neurologists conducted a follow-up study for this MCI cohort and those who had been traced and agreed to participate were scheduled to clinical interview. Those who could not be traced, refused to participate, or deceased were defined as “lost-to-follow-up”.

At the in-person interview, subjects (or the proxy) were firstly rated by the Clinical Dementia Rating scale for cognitive complaints [30]. Also, the time and hospital name were recorded if the subject was diagnosed as dementia by neurologists at other hospitals. Subjects were measured the Lawton and Brody Activity of Daily Living (ADL) scale, and functionally intact of physical self-maintenance and instrumental activities of daily living were considered if ADL score > 16 [31]. Cognitive function of subjects was evaluated by using the same neuropsychological batteries which were used at baseline survey. For those deceased individuals, the cause and date of death were provided by their family members via the telephone call and confirmed by the death certificates from the Center of Disease Control.

**Consensus diagnosis**
Neurologists and neuropsychologists in our study group reviewed the medical, neuropsychological data and reached a consensus diagnosis of incident AD using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) and the National Institute of Neurological and Communicative Diseases and Stroke /Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [32, 33]. Those who were not diagnosed with dementia were considered for a diagnosis of MCI, which was defined according to the Petersen's criteria [34]: (1) cognitive concern or complaint by the subject, informant, nurse, or physician, with CDR = 0.5; (2) objective impairment in at least 1 cognitive domain based on performance 1.5 SD below the mean using the norms obtained in the pilot study; (3) essentially normal functional activities (determined from the CDR and the ADL evaluation); (4) absence of dementia (by DSM-IV). Diagnostic procedures and criteria were as same as those at baseline.

Serum TG Determination

Baseline blood samples were collected from each subject by research nurses in the morning after overnight fasting. Serum TG profiles were measured by Hitachi 7600 fully automatic biochemical analyzer in central laboratory of Huashan hospital.

SNP selection, genotyping and quality control

A total of 35 sporadic AD risk-associated SNPs were selected from AD GWAS in European population and Asia population. All these SNPs were reported with AD-risk association exceeded the threshold of a genome-wide significance level (P < 5 × 10^{-8}) and were mutually independent, defined by pairwise r^2 < 0.2 estimated from the HapMap CHB (Han Chinese in Beijing, China) population. The information of all SNPs that be chosen was listed in Table S1.

Genomic DNA was extracted from peripheral blood samples at the baseline using the QIAamp DNA Blood Mini kit (QIAGEN GmbH, Hilden, Germany). Genotyping of selected SNPs was performed on the MassARRAY system (iPLEX; Sequenom, Inc. San Diego, CA) by using the Agena Biosciences iPLEX Gold Genotyping reagent. Four duplicate test samples and four water samples (PCR negative controls) that were blinded to the technician were included in each 384-well plate to monitor genotyping accuracy. The average concordance rate was 100% among these duplicate samples. All assays were conducted blinded to subjects status. SNPs with genotyping rate of < 95%, minor allele frequency (MAF) < 0.01 or P < 0.001 in a Hardy–Weinberg Equilibrium (HWE) test were further removed. Additionally, samples with a missing rate of > 10% were also removed from the study.

Apolipoprotein E (APOE) genotyping was conducted by the TaqMan SNP method [35]. The presence of at least one ε4 allele was treated as being APOE ε4 positive.

Statistical analysis

Continuous variables were expressed as the mean and standard deviation (SD), and categorical variables were expressed as number and frequencies (%). The Student t-test, one-way ANOVA test, Wilcoxon rank-sum test and Pearson Chi-square test were used to compare continuous and categorical variables. Subjects with MCI who had not converted to AD at the last follow-up visit or those lost-to-follow-up were regarded as censored. The effect of individual SNP genotypes on MCI-AD progression was assessed by log-rank test and Cox proportional hazards regression model adjusting for age, gender, APOE ε4 and included SNPs. The false discovery rate (FDR) as proposed by Benjamini and Hochberg was calculated using R software for multiple comparison [36]. Ln-transformed TG (Ln TG) were used in statistical tests because the distribution of TG was skewed [15, 17]. The effects of baseline ln TG on MCI-AD progression in total samples and in subpopulation stratified by genotypes were analyzed using the Cox proportional regression hazards model adjusting for age, gender, education years, APOE ε4 in model 1 and additionally adjusting for coronary heart disease, diabetes mellitus, hypertension, BMI, stroke, smoking, drinking in model 2. The weighted Schoenfeld residual-based test was used to check the proportional hazards (PH) assumption. P values of the all variables were all greater than 0.05, indicating that each variable satisfied the PH assumption. TG was also analyzed as categorical variable according to the Guidelines for Prevention and Treatment of Adult Dyslipidemia (2016 revised edition) in China [38]: low: TG < 1.7 mmol/L, high: TG ≥ 1.7 mmol/L. Hazard ratios (HRs) and 95% confidence intervals (CIs) were used to assess the risk effect.

Statistical analyses were performed with the PLINK 1.07 software and Stata 14.0 software. All tests were two-sided and P < 0.05 was considered as statistically significant.
Results

Baseline characteristics of study subjects

Among 696 MCI subjects at baseline, 391 subjects were excluded due to lost to follow-up (n = 311), lack of baseline blood sample (n = 39), failure of genome DNA extraction (n = 27), SNPs genotyping missing rate > 0.1 (n = 3) (Fig. 1). After the average 4.5 years (SD = 1.3) of follow-up, 58 incident AD, 11 other types of dementia, and 247 non-demented were diagnosed. We finally included 305 subjects with incident AD and non-dementia for following analysis. These excluded MCI subjects were older (74.18 versus 72.07, \( P = 0.003 \)), with higher MMSE score (26.84 versus 26.13, \( P = 0.001 \)), higher education years (10.53 versus 9.27, \( P = 0.001 \)), lower BMI level (23.98 versus 24.52, \( P = 0.048 \)), less CHD sufferers (11.31% versus 22.70%, \( P < 0.001 \)) and more smokers (14.91% versus 8.85%, \( P = 0.016 \)) than the 305 included MCI subjects (Table 1). There was no significant difference between these two groups in gender, CES-D score, \( APOE \) \( \varepsilon \)4 positive ratio, drinking status, medical history of diabetes mellitus, hypertension and stroke, and TG concentrations at baseline.

| Characteristics                  | All MCI subjects (n = 696) | MCI subjects included (n = 305) | \( P \) Value | AD cases (n = 58) | Control (n = 247) | \( P \) Value |
|----------------------------------|---------------------------|---------------------------------|--------------|------------------|------------------|--------------|
| age, mean ± SD, (y)              | 74.18 ± 9.91              | 72.07 ± 8.07                    | 0.003        | 78.48 ± 5.03     | 71.26 ± 8.02    | < 0.001      |
| Female, n (%)                    | 224 (57.29)               | 170 (55.74)                     | 0.682        | 33 (56.90)       | 137 (55.47)     | 0.843        |
| MMSE score, mean ± SD           | 26.84 ± 2.66              | 26.13 ± 3.01                    | 0.001        | 25.31 ± 3.29     | 27.47 ± 2.07    | < 0.001      |
| Education year, mean ± SD, (y)  | 10.53 ± 4.53              | 9.27 ± 4.86                     | 0.001        | 8.72 ± 5.09      | 10.89 ± 4.22    | 0.001        |
| Follow up months                 | -                         | 52.88 ± 15.48                   | -            | 45.38 ± 17.72    | 54.64 ± 14.39   | < 0.001      |
| \( APOE \) \( \varepsilon \)4 positive, n (%) | 55 (16.82)                | 49 (17.07)                      | 0.933        | 15 (28.85)       | 45 (19.15)      | 0.120        |
| CES-D, mean ± SD                | 10.09 ± 8.81              | 10.01 ± 9.05                    | 0.913        | 11.60 ± 10.59    | 9.64 ± 8.63     | 0.137        |
| BMI, mean ± SD, (kg/m2)          | 23.98 ± 3.51              | 24.52 ± 3.65                    | 0.048        | 23.58 ± 3.41     | 24.64 ± 3.99    | 0.063        |
| Diabetes mellitus, n (%)         | 57 (14.69)                | 61 (20.07)                      | 0.062        | 10 (17.24)       | 43 (17.48)      | 0.966        |
| Hypertension, n (%)              | 218 (56.19)               | 190 (62.50)                     | 0.094        | 34 (58.62)       | 134 (54.25)     | 0.547        |
| Stroke, n (%)                    | 62 (15.86)                | 59 (19.34)                      | 0.228        | 15 (25.86)       | 32 (13.01)      | 0.015        |
| Coronary heart disease, n (%)    | 44 (11.31)                | 69 (22.70)                      | < 0.001      | 9(15.52)         | 32(13.01)       | 0.615        |
| Smoking, n (%)                   | 58 (14.91)                | 27 (8.85)                       | 0.016        | 6(10.34)         | 28(11.38)       | 0.822        |
| Drinking, n (%)                  | 40 (10.28)                | 19 (6.29)                       | 0.063        | 3(5.17)          | 26(10.61)       | 0.205        |
| TG, mean ± SD (mmol/L)           | 1.57 ± 0.91               | 1.73 ± 1.21                     | 0.079        | 1.61 ± 1.29      | 1.64 ± 0.94     | 0.461        |

Abbreviations: MCI, mild cognitive impairment; MMSE, Mini-Mental State Exam; \( APOE \) Apolipoprotein E; CES-D, Center for epidemiological Survey, Depression Scale; BMI, body mass index; TG, triglycerides.

Continuous variables were expressed as the mean and standard deviation (SD), and categorical variables were expressed as number and frequencies (%). The Student \( t \) test, Wilcoxon rank-sum test and Pearson Chi-square test were used to compare continuous and categorical variables.
The baseline characteristics of 305 individuals with MCI showed that AD cases were more likely to be older (78.48 versus 71.26, $P < 0.001$) and had a lower MMSE score (25.31 versus 27.47, $P < 0.001$), lower education year (8.72 versus 10.89, $P = 0.001$), shorter follow up duration (45.38 versus 54.64, $P < 0.001$) and more stroke (25.86% versus 13.01%) compared with controls (Table 1). We did not find a difference in gender, $APOE \varepsilon 4$ positive ratio, CES-D score, BMI, smoking, drinking and TG levels, prevalence of diabetes mellitus, hypertension and coronary heart disease between AD cases and controls.

**Effect of genotypes on MCI-AD progression**

Among the 35 selected SNPs analyzed in the study, two SNPs were excluded due to MAF < 0.01 (rs7274581) and deviation from HWE at $P < 0.001$ (rs4663105). Each of the remaining 33 SNPs was tested for its association with MCI-AD progression by the log-rank test to choose the significant SNPs for constructing the next step analysis. The $PVRL2$ rs6859 was found to have significantly association with incident AD (FDR-adjusted $P = 0.018$, Table 2). Considering that $PVRL2$ rs6859 located at the 19 chromosome, we analyzed its LD with $APOE$ genotype (treating $\varepsilon 3$ and $\varepsilon 2$ as the same allele and $\varepsilon 4$ as another allele), and found no evidence of strong LD between $PVRL2$ rs6859 and $APOE$ ($r^2 = 0.28$).
| Chromosome | SNPs       | Position  | The nearest gene | Allele (Major/Minor) | MAF (CHB) | MAF (our study) | P (HWE) | P (Log-rank) | P(FDR) |
|------------|------------|-----------|------------------|----------------------|-----------|-----------------|---------|-------------|--------|
| 1q32.2     | rs3818361  | 207611623 | CR1              | G/A                  | 0.3350    | 0.3864          | 0.2013  | 0.469       | 0.675  |
| 2q12.3     | rs4676049  | 109018801 | RANBP2           | C/T                  | 0.1650    | 0.1372          | 0.6433  | 0.789       | 0.894  |
| 2q14.3     | rs744373   | 127137039 | BIN1             | A/G                  | 0.3301    | 0.3474          | 0.6284  | 0.233       | 0.561  |
| 2q14.3     | rs7561528  | 127132061 | BIN1             | G/A                  | 0.1214    | 0.1254          | 0.6195  | 0.203       | 0.561  |
| 5q14.3     | rs190982   | 88927603  | MEF2C            | A/G                  | 0.1553    | 0.1360          | 1       | **0.017**   | 0.153  |
| 6q25.1     | rs11754661 | 151207078 | MTHFD1L          | G/A                  | 0.0875    | 0.0380          | 1       | 0.295       | 0.561  |
| 6p21.32    | rs9271192  | 32610753  | HLA-DRB1         | A/C                  | 0.1796    | 0.1526          | 0.8332  | 0.584       | 0.751  |
| 6p12.3     | rs9349407  | 47485642  | CD2AP            | G/C                  | 0.1262    | 0.1273          | 0.4528  | 0.869       | 0.894  |
| 7q35       | rs11767557 | 143412046 | EPHA1            | T/C                  | 0.1408    | 0.1333          | 0.1492  | 0.858       | 0.894  |
| 7q35       | rs11771145 | 143413669 | EPHA1            | A/G                  | 0.4369    | 0.4758          | 0.0366  | 0.975       | 0.975  |
| 7q22.1     | rs1476679  | 100406823 | ZCWPW1           | T/C                  | 0.3252    | 0.3202          | 0.3132  | **0.010**   | 0.120  |
| 7p14.1     | rs2718058  | 37801932  | NME8             | A/G                  | 0.2573    | 0.1964          | 0.0223  | 0.296       | 0.561  |
| 8p21.1     | rs11136000 | 27607002  | CLU              | C/T                  | 0.1990    | 0.2221          | 0.7520  | 0.279       | 0.561  |
| 8p21.1     | rs28834970 | 27337604  | PTK2B            | T/C                  | 0.2282    | 0.2864          | 0.8928  | 0.509       | 0.705  |
| 8p21.1     | rs569214   | 27630273  | CLU              | T/G                  | 0.4563    | 0.4755          | 0.4388  | 0.067       | 0.345  |
| 11q11.2    | rs10838725 | 47536319  | CELF1            | T/C                  | 0.3155    | 0.2915          | 0.5968  | 0.342       | 0.584  |
| 11q24.1    | rs11218343 | 121564878 | SORL1            | T/C                  | 0.3107    | 0.3015          | 0.1924  | 0.179       | 0.561  |
| 11q14.2    | rs17817600 | 85677471  | PICALM            | A/G                  | 0.1083    | 0.0426          | 0.1006  | 0.540       | 0.720  |
| 11q14.1    | rs2373115  | 78380104  | GAB2             | C/A                  | 0.3883    | 0.3894          | 0.4888  | 0.357       | 0.584  |
| 11q14.2    | rs3851179  | 86157598  | PICALM            | C/T                  | 0.4563    | 0.4009          | 0.2500  | 0.052       | 0.312  |
| 11q12.2    | rs4938933  | 60266956  | MS4A4A           | T/C                  | 0.2621    | 0.2674          | 0.0912  | 0.166       | 0.561  |
| 11q12.2    | rs610932   | 60171834  | MS4A6A           | G/T                  | 0.3350    | 0.3278          | 0.5346  | **0.006**   | 0.108  |
| 11q12.2    | rs983392   | 59923508  | MS4A6A           | A/G                  | 0.0243    | 0.0301          | 1       | 0.129       | 0.561  |
| 14q32.12   | rs10498633 | 92460608  | SLC24A4          | G/T                  | 0.1165    | 0.1224          | 0.1968  | 0.454       | 0.675  |
| 14q22.2    | rs17125944 | 52933911  | FERMT2           | T/C                  | 0.2524    | 0.2356          | 0.2851  | 0.757       | 0.894  |
| 19q13.32   | rs157580   | 44892009  | TOMM40           | G/A                  | 0.4515    | 0.4260          | 0.3705  | 0.190       | 0.561  |
| 19q13.32   | rs2075650  | 44892362  | TOMM40           | A/G                  | 0.1117    | 0.1239          | 0.6121  | 0.052       | 0.312  |
| 19p13.3    | rs3764650  | 1046521  | ABCA7            | T/G                  | 0.2670    | 0.2812          | 0.7856  | 0.312       | 0.562  |
| 19q13.41   | rs3865444  | 51224706  | CD33             | C/A                  | 0.1796    | 0.1873          | 0.0471  | 0.817       | 0.894  |
| 19p13.3    | rs4147929  | 1063444  | ABCA7            | G/A                  | 0.2816    | 0.3015          | 0.1177  | 0.239       | 0.561  |

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; CHB, Han Chinese in Beijing, China; HWE, Hardy-Weinberg equilibrium; FDR, false discovery rate; NA, not available.
### Table 1

| Chromosome | SNPs | Position | The nearest gene | Allele (Major/Minor) | MAF (CHB) | MAF (our study) | MAF (HWE) | P (Log-rank) | P (HWE) | P (FDR) |
|------------|------|----------|------------------|----------------------|-----------|-----------------|-----------|-------------|----------|--------|
| 19q13.32   | rs6859 | 44878777 | PVRL2            | G/A                  | 0.3204    | 0.3529          | 0.1797    | >0.001      | >0.001   | 0.018   |
| 2q14.3     | rs4663105 | 127133851 | BIN1             | A/C                  | NA        | 0.3910          | 6.472E-30 | NA          | NA       | NA      |
| 20q13.31   | rs7274581 | 55018510 | CASS4            | T/C                  | NA        | 0.0015          | 1         | NA          | NA       | NA      |

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; CHB, Han Chinese in Beijing, China; HWE, Hardy-Weinberg equilibrium; FDR, false discovery rate; NA, not available.

### Multivariate analysis of association of PVRL2 rs6859 genotype with incident AD

Multivariate analysis of association of PVRL2 rs6859 genotype with incident AD was conducted by using Cox regression model, adjusted for age, gender, APOE genotype. As shown in Table 3, the AG, AA and AG/AA genotypes of PVRL2 rs6859 were significantly associated with increased incident AD, compared with GG genotype (HR = 2.29, P = 0.029, and HR = 2.92, P = 0.013, and HR = 2.47, P = 0.012 in model 2, respectively). Additionally, in APOE ε4+ subjects, PVRL2 rs6859 still had a significant association with MCI-AD progression (AG/AA vs. GG, HR = 3.09, P = 0.005, Table S2). Hence, we defined the AG/AA genotypes of PVRL2 rs6859 as risk genotypes for MCI-AD progression. Meanwhile, APOE ε4 was significantly associated with increased risk of incident AD in univariate model (HR = 1.85, P = 0.045), while this significant association was not surviving in multivariate model (P = 0.154, Table 3).

### Table 3

Univariate and multivariate Cox regression analysis of genotypes in MCI subjects.

| Genotype | No. of subjects | No. of events | Univariate model |  |  |  | Multivariate model a |  |  |  |
|----------|-----------------|---------------|------------------|---|---|---|----------------------|---|---|---|
|          |                 |               |                  | HR| 95% CI| p Value| HR| 95% CI| p Value|
| APOE     |                 |               |                  |   |       |       |   |       |       |
| Non-ε4   | 227             | 37            | 1(Ref.) -        |   |       |       | 1(Ref.) -        |   |       |       |
| At least one ε4 b | 60 | 15 | 1.85 | 1.01–3.39 | 0.045 | 1.61 | 0.84–3.10 | 0.154 |
| rs6859   |                 |               |                  |   |       |       |   |       |       |
| GG       | 131             | 13            | 1(Ref.) -        |   |       |       | 1(Ref.) -        |   |       |       |
| AG       | 119             | 32            | 3.31 | 1.73–6.31 | <0.001 | 2.29 | 1.09–4.81 | 0.029 |
| AA       | 47              | 12            | 2.89 | 1.32–6.34 | 0.008 | 2.92 | 1.26–6.78 | 0.013 |
| AG/AA b  | 166             | 44            | 3.18 | 1.71–5.92 | <0.001 | 2.47 | 1.21–5.00 | 0.012 |

a Adjusted for age, gender, APOE genotype and rs6859 genotype.

b There are 57 subjects with APOE ε4 heterozygote and only 3 subjects with APOE ε4 homozygote.

### Effect of TG-genetic interaction on MCI-AD progression

The distribution of ln TG and TG did not significantly differ in PVRL2 rs6859 genotypes and APOE genotypes in our study (Tables S3, S4 and S5). We found an interactive relationship between ln TG and PVRL2 rs6859 genotypes on MCI-AD progression (P Ln TG×rs6859 = 0.001, Table 4). Stratified analyses showed that among MCI subjects with non-risk genotype of PVRL2 rs6859 (GG), higher ln TG was significantly associated with decreased risk of MCI-AD progression in model 1 (HR = 0.19, P = 0.018), and this association was marginally significant in model 2 (HR = 0.23, P = 0.066). However, in MCI subjects with risk genotype of PVRL2 rs6859 (AG/AA), higher ln TG possessed significant association with increased risk of MCI-AD progression in model 2 (HR = 2.64, P = 0.034). For total samples, no significant association between ln TG and MCI-AD progression were found (Table 4). The similar
results were also found in subgroups stratified by *APOE* ε4 status (Table S6). Ln TG and *APOE* ε4 also have significant interactive effect on MCI-AD progression ($P_{\text{Ln TG}*\text{APOE}} < 0.001$). In *APOE* ε4- subjects, ln TG showed a protective effect on risk of incident AD in model 1 (HR = 0.44, $P = 0.029$), while this significant association was not surviving in model 2 (HR = 0.49, $P = 0.091$). In *APOE* ε4+ subjects, ln TG showed a promotive effect on risk of incident AD (model 1: HR = 4.19, $P = 0.006$; model 2: HR = 5.14, $P = 0.030$). All these results suggested the potential opposite role of ln TG in different genetic risk subgroups.

Table 4

**PVRL2 rs6859 stratified analysis of TG effect on progression of MCI to AD.**

| Ln (TG) | Model 1a | Model 2b |
|---------|----------|----------|
|         | HR       | 95% CI   | $P$   | HR       | 95% CI   | $P$   |
| Total sample | 0.81     | 0.43–1.52 | 0.506 | 0.92     | 0.45–1.86 | 0.811 |
| Ln (TG)*rs6859 | 9.97     | 2.44–40.67 | **0.001** | 13.56    | 2.97–61.94 | **0.001** |
| Rs6859 GG | 0.19     | 0.05–0.75 | **0.018** | 0.23     | 0.05–1.10 | 0.066 |
| Rs6859 AG/AA | 1.76     | 0.79–3.93 | 0.165 | 2.64     | 1.07–6.48 | **0.034** |

a model 1: adjusted factors are age, gender, education year, *APOE* genotype.

b model 2: adjusted factors are age, gender, education year, *APOE* genotype, BMI, coronary heart disease, diabetes mellitus, hypertension, stroke, smoking, drinking.

For clinical application consideration, we furtherly divided TG concentration into two categorical scales by 1.7 mmol/L referring to the Guidelines for Prevention and Treatment of Adult Dyslipidemia (2016) in China. An analysis of the joint effects of TG concentration and *PVRL2* rs6859 on the MCI-AD progression identified that, taking the subjects with non-risk genotype at *PVRL2* rs6859 (GG) and TG < 1.7 mmol/L as the reference, subjects with non-risk genotype at *PVRL2* rs6859 (GG) and TG $\geq$ 1.7 mmol/L had approximately 5-fold lower risk of MCI-AD progression (HR = 0.20, 95% CI 0.04–0.93, $P = 0.040$), but subjects with risk genotype at *PVRL2* rs6859 (AG/AA) and TG $\geq$ 1.7 mmol/L was 2.6-fold higher risk of MCI-AD progression (HR = 2.61, 95% CI 1.04–6.52, $P = 0.040$) (Fig. 2 and Table 5). Similar results were also found for analysis of the joint effects of TG concentration and *APOE* (Figure S1).

Table 5

**Joint effect of rs6859 genotypes and TG categories on MCI-AD progression**

| Rs6859 | TG level | No. of patients | No. of events | Model 1a | Model 2b |
|--------|----------|----------------|---------------|----------|----------|
|        | HR(95% CI) | $P$ | HR(95% CI) | $P$ |
| GG     | low      | 75  | 10  | 1(Ref.) | 1(Ref.) |
| GG     | high     | 56  | 3   | 0.18(0.04–0.81) | **0.025** | 0.20(0.04–0.93) | **0.040** |
| AG/AA  | low      | 116 | 28  | 1.19(0.54–2.59) | 0.669 | 1.39(0.62–3.12) | 0.427 |
| AG/AA  | high     | 49  | 15  | 1.77(0.74–4.24) | 0.198 | 2.61(1.04–6.52) | **0.040** |

Note: low TG level: $\leq$ 1.7 mmol/L, high TG level: $\geq$ 1.7 mmol/L.

a model 1: adjusted for age, gender, education year, *APOE* genotype.

b model 2: adjusted for age, gender, education year, *APOE* genotype, BMI, coronary heart disease, diabetes mellitus, hypertension, stroke, smoking, drinking.

**Discussion**

The present study indicated that *PVRL2* rs6859 could modify the TG effect on the MCI-AD progression. High TG level showed a protective role on the MCI-AD progression in MCI subjects carrying non-risk genotype of *PVRL2* rs6859 GG, while a promotive role
in those carrying risk genotype of \textit{PVRL2} rs6859 AG/AA.

To our knowledge, this is the first prospective cohort study to report the interactive effect of TG and \textit{PVRL2} rs6859 on MCI-AD progression in Chinese elderly population. Previous studies largely focused on the relationship between TG and AD risk irrespective of AD risk-related SNPs, and there was no unified conclusion [7–16]. For example, Power, M.C found that elevated TG at midlife were associated with cognitive decline over the follow-up of 20 years in non-dementia subjects of the Atherosclerosis Risk in American population [10]. To the contrary, Lv Y.B et al. found that each 1-mmol/L increase of TG was associated with a nearly 20% lower risk of cognitive decline during the 5 follow-up years in 930 Chinese oldest (mean age: 94.0 years) [7]. Moreover, Sabrina found that higher baseline TG concentrations were associated with mixed dementia while it disappeared after adjusting for vascular risk factors in the Three-City (3C) study of European population (mean age = 76.3 years) with up to 13 years of follow-up [8]. We think these discrepancies might derive from differences in effect based on age at lipid assessment or duration of follow-up [37], selection bias in older cohorts [38], ethnicity of study population, sample size and so on. For genetic factors, the Framingham Heart Study (FHS) indicated the interrelationships between genetic risk score (GRS) of AD susceptibility loci and TG on AD risk [17], however, this study did not find the significant results regarding to the TG effect on AD risk in stratification analysis by genetic markers.

Importantly, we found that \textit{PVRL2} rs6859 was significantly associated with MCI-AD progression and that \textit{PVRL2} rs6859 and \textit{APOE} ε4 had similar role of modifying the TG effect on MCI-AD progression. Consistent with our finding, \textit{PVRL2} rs6859 was previously confirmed as the AD risk factor in a cross-sectional study in Chinese Han [39]. Although the \textit{APOE} ε4 allele is the strongest genetic risk factor for sporadic AD, 40–50% people with sporadic AD do not carry the \textit{APOE} ε4 allele [19, 20]. In our study, only 20.8% of MCI subjects carried \textit{APOE} ε4, but 55.2% of MCI subjects carried the risk genotype of \textit{PVRL2} rs6859 AG/AA; even 46.2% of \textit{APOE} ε4- subjects carried the \textit{PVRL2} rs6859 AG/AA genotype (Table S7). Additionally, in \textit{APOE} ε4- subjects, \textit{PVRL2} rs6859 still had a significant association with MCI-AD progression (AG/AA vs. GG, HR = 3.09, P = 0.005, Table S2). Therefore, our study suggested that \textit{PVRL2} rs6859 could be used to supplement the \textit{APOE} ε4 to better predict the MCI-AD progression in Chinese population. Furthermore, \textit{PVRL2} rs6859 and \textit{APOE} ε4 similarly modified the TG effect on MCI-AD progression. TG possessed a protective role of MCI-AD progression in subjects with \textit{PVRL2} rs6859 GG or \textit{APOE} ε4-, however a promotive role in subjects with \textit{PVRL2} rs6859 AG/AA or in \textit{APOE} ε4+. Hence, \textit{PVRL2} rs6859 may influence the pathological process of AD through a similar mechanism with \textit{APOE} ε4, at least in part. All these results suggested that \textit{PVRL2} rs6859 should be considered for a complementary risk assessment of AD to implement targeted prevention by controlling the TG level in Chinese population.

There were some possible mechanisms of TG effect on nervous system for explaining and understanding the opposite effect of TG in different genetic risk group. On the one hand, TG was reported to have protective role for neurons. Medium-chain triglyceride (6–12 carbons), the main constituent of coconut and palm kernel oils, is currently prescribed for mild to moderate Alzheimer's disease (AD) patients [40], because its metabolite (ketone body) serves as an alternative source of energy to the brain and has neuroprotective effects [41, 42]. In mouse model of AD, ketone body was also reported to show a cognition-sparing property and to reduce Aβ deposition and tau pathology [43, 44]. Alzheimer's disease (AD) patients have been shown to exhibit decreases in brain glucose metabolism and glycolytic enzymes, however, brain ketone uptake is still normal in MCI and in early AD, which could help explain why ketogenic interventions improve cognitive function in MCI and AD subjects [45]. However, on the other hand, previous preclinical studies suggested that high serum levels of TG might play a promotive role in cerebral amyloidosis [46, 47]. Katarina found that increased levels of TG at midlife could predict brain Aβ and tau pathology 20 years later in cognitively healthy individuals independent of age, gender, \textit{APOE} ε4 and vascular risk factors [48]. Mamo et al found that Aβ was associated with plasma lipoproteins, especially those enriched with TG, and that TG-rich lipoprotein particles in blood may serve as Aβ carriers [49]. The circulating TG-rich lipoprotein-Aβ complex may compromise blood–brain barrier (BBB) integrity and ultimately increased cerebral amyloid deposition [50]. Therefore, TG has both protective and promotive roles. Based on our findings that \textit{PVRL2} rs6859 and \textit{APOE} modified the TG effect on MCI-AD progression, we suspected that genetic factor was one of the factors influencing TG function.

\textit{APOE} ε4 is the strongest genetic risk factor for sporadic AD [18], it is critical in the transport of lipid, and activation of lipolytic enzymes [51]. Consistent with our findings that TG possessed a protective role of MCI-AD progression in subjects with non-genetic risk genotype (\textit{PVRL2} rs6859 GG or \textit{APOE} ε4–), while promotive role in subjects with genetic risk genotype (\textit{PVRL2} rs6859 AG/AA or \textit{APOE} ε4+). Reger et al. found that single intake of medium-chain triglyceride drink significantly facilitated performance on the
Alzheimer’s Disease Assessment Scale-Cognitive Subscale (ADAS-cog) for APOE ε4 – subjects, but not for APOE ε4 + subjects in American population [52]. The consistent results were also found in Japanese population [53] and Chinese population [54]. It is possible that APOE ε4 – individuals were better able to utilize ketones (produced by TG) than were the APOE ε4 + subjects. PVRL2, also known as cell adhesion molecule 2 (NECTIN2) [55], is important for maintaining proper cell junction and controlling BBB permeability, and may protect brain from spreading the viral infection which was also suggested for APOE [56]. Liu M found that PVRL2 rs6859 AG + AA genotypes were significantly associated with higher TG level and increased risk of dyslipidemia compared with GG genotype in the Chinese Maonan population [57], but this relation was not found in our study with Chinese Han population. In addition, the high confidence (determined by a methylation individual-nucleotide-resolution crosslinking and immunoprecipitation experiment) m6A-SNP rs6859 in the 3'-untranslated region of PVRL2 was found to be associated with TG levels, as well as PVRL2 mRNA expression in artery tibial and whole blood [58]. Based on evidence above, we made an assumption that person with different genotypes of AD risk related-SNPs had different pathway of TG metabolism and subsequent Aβ formation, thereby causing different TG effect on AD progression. In individuals with APOE ε4 – or rs6859 GG, TG could be normally metabolized to ketone bodies which had neuroprotective effects and meanwhile the circulating TG-rich lipoprotein-Aβ complex maintained a low level. Hence, TG showed a protective role on MCI-AD progression. However, APOE ε4 + or rs6859 AG/AA may cause the dysfunction of TG metabolism and destroy the equilibrium of TG-rich lipoprotein-Aβ complex in blood. Therefore, high TG may compromise blood–brain barrier integrity and increase cerebral amyloid deposition and virus infection, thus shows the promotive role for risk of MCI-AD. Further researches are justified to formally test the hypothesis.

There are some limitations in this study as well. First, this study was composed of individuals of Chinese descents aged 72.07 ± 8.07 years and therefore these results had a limited generalizability to populations of different age-ranges and other ethics. Secondly, we had a relatively small sample size for statistical analysis because nearly half of the subjects were lost to follow-up. The small sample size may skew our results to be statistically non-significant. For example, APOE was not observed as significant risk factor on MCI-AD progression in our study. In addition, the subjects who were lost to follow-up were older than those who were followed-up, and may be more likely to develop AD thus AD incidence and effect of the association between TG and AD incidence might be underestimated. Thirdly, the study had a short follow-up time. However, this average 4.5-year follow-up study was able to achieve a sufficient statistical power in analysis because MCI is a transitional clinical state between normal and AD-type dementia thus has a relative higher conversion rate to AD than cognitive normal status (18% in our study) within a short follow-up time. Fourthly, the effect of TG and TG-genetic interaction on MCI-AD progression were not adjusted for TG-lowing therapy because only < 10% subjects in our study have lipid-lowering medication intake.

**Conclusion**

In summary, results from this preliminary study provided evidence that the relationship of TG level and the progression of MCI to AD may be mediated by PVRL2 rs6859. PVRL2 rs6859 can be used to supplement APOE to better define the individual’s risk for AD. The observed relation emphasizes the need for concomitant use of TG and AD risk-related SNPs in older individuals in order to identify susceptible subgroups and implement precision prevention strategy. Further studies are justified to test the hypothesis and explore its mechanism furtherly.

**Supplementary information**

**Additional file 1: Table S1.** The information of selected SNPs from GWAS. **Table S2.** Effects of PVRL2 rs6859 genotypes on the MCI-AD progression among APOE ε4- subjects. **Table S3.** Association between PVRL2 rs6859 genotypes and TG levels. **Table S4.** Association between APOE ε4 genotypes and TG levels. **Table S5.** Association between APOE ε2/ε3 genotypes and TG levels. **Table S6.** APOE ε4 stratified analysis of triglyceride’s effect on progression of MCI to AD. **Table S7.** Correlation between APOE genotypes and PVRL2 rs6859. **Figure S1.** The association between TG and MCI-AD progression in subgroups stratified by APOE ε4.

**Abbreviations**

MCI, mild cognitive impairment; APOE, Apolipoprotein E; MMSE, Mini-Mental State Exam; CES-D, Center for epidemiological Survey, Depression Scale; BMI, body mass index; TG, triglycerides; SNP, single nucleotide polymorphism; GWAS, genome-wide association
studies; HWE, Hardy-Weinberg equilibrium; Chr, chromosome; OR, odd ratio; CI, confidence interval; CHB, Han Chinese in Beijing, China; MAF, minor allele frequency; Ref., reference; NA, not available. PVRL2, Poliovirus Receptor Related 2; Aβ, Amyloid deposition.

Declarations

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Author's contributions

Study conception and design: QX and DD. Data collection: DD, JX, QZ, QG, XL, and LZ. Acquisition, analysis, or interpretation of data: RW, AA, JX, QX, DD. Statistical analysis: RW, AA and JX. Manuscript drafting: RW and QX. Review and comment to manuscript: DD, HF and HZ. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Huashan Hospital, Fudan University (No.2009-195) and Ethics Committee of Department of Public Health in Fudan University, Shanghai, China (No.2018-01-0662). Written informed consent was obtained from all participants or their legally acceptable representative.

Consent for publication

All the patients included in this study provided written informed consent for publication of this article and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Competing interests

None of the authors has declared a conflict of interest.

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Figures
Figure 1

Flowchart of subjects in the MCI sub-cohort of the Shanghai Aging Study.

Figure 2

The association between TG and MCI-AD progression in subgroups stratified by PVRL2 rs6859 genotypes. AG/AA in PVRL2 rs6859 was defined as risk genotype and GG was non-risk genotype. Model was adjusted for age, gender and education years, APOE ε4, BMI, coronary heart disease, diabetes mellitus, hypertension, stroke, smoking, drinking. TG concentration was classified
two categories according to the Guidelines for Prevention and Treatment of Adult Dyslipidemia in China: low: TG < 1.7 mmol/L, high: TG ≥ 1.7 mmol/L.

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