Interaction effects between variants in *TOMM40* and *PVRL2* with plasma amyloid- β and Alzheimer's disease among Chinese older adults: a population-based study

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Keywords: Alzheimer's disease, TOMM40, PVRL2, gene-gene interaction, plasma biomarkers, population-based study

Posted Date: August 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-832373/v1>

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Abstract

Background

Emerging evidence has linked *TOMM40* and *PVRL2* polymorphisms with Alzheimer's disease (AD). We examined their associations with AD and plasma AD biomarkers and interaction on AD risk among Chinese older adults.

Methods

This population-based study included 4876 participants (age ≥ 65 years, 57.2% women) from MIND-China. *TOMM40*(rs2075650) and *PVRL2*(rs6859) polymorphisms were detected using multiple-polymerase chain reaction amplification. Plasma A β 40, A β 42, and t-tau were measured using SIMOA in a subsample (n = 1257). Data was analyzed using multiple logistic and general linear regression models.

Results

AD was diagnosed in 182 participants. The multi-adjusted odds ratio of AD was 6.24(95%CI 1.73–22.48) for *TOMM40*GG (vs. AA), 1.47(0.89–2.42) for *PVRL2*AA (vs. GG), and 12.87(3.97–41.73) for having both risk alleles ($P_{\text{interaction}}=0.0003$). In the plasma biomarker subsample, *TOMM40*GG was significantly associated with lower plasma A β 42 and the A β 42-to-A β 40 ratio ($p < 0.05$).

Conclusions

TOMM40 and *PVRL2* genes could interact to substantially increase the likelihood of AD, possibly through influencing A β metabolism.

1. Background

Alzheimer's disease (AD) is the most common neurodegenerative disease among elderly people[1]. The etiology and pathogenesis of AD remain poorly understood. The heritability of late-onset AD was estimated to be approximately 56%-79%, suggesting that genetic components play an important role in the pathogenesis[2]. Thus, exploring genetic factors may help increase our understanding of the etiopathogenesis of AD. Indeed, the genome-wide association studies (GWAS) have identified a large number of susceptibility loci, of which, the chromosome 19q13 region has been the strongest genetic loci for AD[3]. In addition to the well-known *APOE* gene within this region, translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) gene and poliovirus receptor-related 2 (*PVRL2*) gene have been identified to be associated with AD[4, 5].

TOMM40, an *APOE* nearby gene[6], encodes a subunit of the translocase of the outer mitochondrial membrane complex, which is involved in AD-related pathology[7]. Evidence has shown that multiple single nucleotide polymorphisms (SNPs) within *TOMM40* are associated with AD risk, of which, the SNP rs2075650 has been frequently reported in various ethnic populations[8–11]. *PVRL2*, which locates near the *TOMM40*, also serves as a risk variant that is involved in AD pathology[12]. In addition, *TOMM40* and *PVRL2* genes have been implicated in common biological pathways, such as lipid metabolism[13] and immunity[14, 15]. Thus, it is biologically plausible to hypothesize that *TOMM40* and *PVRL2* genes may confer an interaction effect in the pathogenesis of AD. However, this hypothesis has yet to be explored.

Although GWAS studies have identified the associations of *TOMM40* and *PVRL2* with AD risk, the neuropathological pathways underlying their associations remain unclear. Aggregation of the β -amyloid (A β) peptide and neurofibrillary tangles in the brain are the neuropathological hallmarks of AD[16]. Data from the Framingham Heart Study showed that lower plasma A β and higher plasma total tau (t-tau) proteins were associated with an increased risk of AD[17, 18]. However, whether *TOMM40* and *PVRL2* are associated with AD via influencing plasma A β and tau proteins remains largely unknown.

Therefore, in this population-based study of rural-dwelling Chinese older adults, we aimed to investigate the associations of *TOMM40* rs2075650 and *PVRL2* rs6859 as well as their interactions with AD risk, and to further explore the associations of these two genes with plasma AD biomarkers (A β and t-tau proteins).

2. Methods

2.1 Study population

This population-based study involved the participants from the ongoing Multimodal Interventions to Delay Dementia and Disability in Rural China (MIND-China)[19–21], a participating project in the World-Wide FINGERS Network[22]. Briefly, all registered residents who were aged ≥ 60 years and living in 52 villages of Yanlou Town, Yanggu County, western Shandong Province, China, were eligible for the MIND-China study. From March to September 2018, 5765 participants were enrolled. Of these, 519 participants aged 60–64 years were excluded, because we aimed to investigate the late-onset sporadic AD. Of the 5246 participants who were aged ≥ 65 years, 370 were excluded due to missing genotyping information ($n = 230$), insufficient information for dementia diagnosis ($n = 46$), and other types of dementia than AD ($n = 94$). Thus, the analytical sample included 4876 participants. Compared with the excluded participants ($n = 370$), individuals included in the analytical sample ($n = 4876$) were younger (mean age, 71.6 vs. 73.6 years, $p < 0.001$) and more educated (mean years of education, 3.14 vs. 2.60, $p < 0.001$). However, the two groups did not differ significantly in sex distribution (female, 57.2% vs. 56.2%, $p = 0.701$).

In addition, data on plasma AD biomarkers were available in a subsample of 1257 individuals that were derived from the participants of MIND-China. This subsample was used for the analysis involving plasma AD biomarkers. Figure 1 shows a flowchart of the study participants.

The MIND-China project was approved by the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong. All participants provided written informed consent for both study enrollment and blood sample collection, or in case of persons with severe cognitive impairment, their informants provided informed consent. Research within MIND-China has been conducted in accordance with the ethical principles expressed in the Declaration of Helsinki as well as relevant national guidelines and regulations. MIND-China was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

2.2 Data collection and assessments

The procedure of baseline data collection was described elsewhere[19, 20]. Briefly, all data were collected by trained staff via face-to-face interviews, clinical examinations, cognitive testing, and laboratory tests. The in-person interview was performed following a structured questionnaire covering demographic data (e.g., age, sex, and education), lifestyle factors (e.g., smoking and alcohol drinking), health conditions (e.g., hypertension, diabetes, and hyperlipidemia), medications, and cognitive function. Alcohol consumption and smoking status were categorized as current, former, and never drinking or smoking, respectively. After a 5-minute rest, seated arterial blood pressure was measured on the right upper arm using an electronic sphygmomanometer (HEM-7127J, Omron Corporation, Kyoto, Japan). After an overnight fast, venous blood samples were collected and blood glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an Automatic Biochemical Analyzer (CS-600B, DIRUI Corporation, Changchun, China). Hypertension was defined as arterial blood pressure $\geq 140/90$ mmHg or taking antihypertensive agents. Diabetes was defined as the fasting blood glucose ≥ 7.0 mmol/L or current use of antidiabetic agents. Hyperlipidemia was defined as TC ≥ 6.2 mmol/L or TG ≥ 2.3 mmol/L or LDL-C ≥ 4.1 mmol/L or HDL-C < 1.0 mmol/L or taking hypolipidemic agents.

2.3 Genomic DNA extraction and genotyping

Genomic DNA was extracted from venous blood leukocytes using the TIANamp blood DNA kit (Tiangen, Beijing, China) according to manufacturer's instructions. Then, DNA was quantified using Nanodrop 3300 spectrometry and a total amount of 100 ng genomic DNA per sample was used for the DNA sample preparation. Subsequently, sequencing libraries were generated using MultipSeqCustom Panel (iGeneTech, Beijing, China) following manufacturer's recommendations and index codes were added to each sample. Finally, qualified libraries were subjected to next-generation sequencing (NGS) on a Novaseq system (Illumina), and raw data were filtered to remove low-quality reads using FastQC. Genotyping was conducted by an operator who was blinded to the clinical data.

In addition to the *APOE* genotype, two SNPs of interest, rs2075650 (*TOMM40*) and rs6859 (*PVRL2*), were detected using multiple-polymerase chain reaction amplification (iGeneTech Bioscience Co., Ltd., Beijing, China).

2.4 Measurements of Plasma A β 42, A β 40 and Tau

Blood samples were collected in EDTA-coated vacutainers, followed by centrifugation to obtain plasma. The samples were stored at -80°C and thawed immediately before component quantification. Levels of plasma Aβ42, Aβ40, and t-tau were measured using a Human Neurology 3-Plex A assay (N3PA) Kit run on the fully automated single molecule array (SIMOA) instrument (Quanterix Corp, MA, USA) according to the manufacturer's protocol. Two quality control samples were run in duplicates on each plate for each analyte. The upper and lower detection limits for quality sample of each index were within the range indicated in the manual, and the inter-assay coefficient of variability (CV) and inter-plate inter-assay CV were controlled within 13%.

2.5 Diagnosis of Alzheimer's disease

We employed a 3-step procedure for the clinical diagnosis of dementia. In brief, clinicians and trained junior interviewers conducted comprehensive assessments for each participant following the structured questionnaires. The assessments included health-related factors, medical history, a neurocognitive assessment battery, the Chinese version of activities of daily living (ADLs), and the Clinical Dementia Rating Scale (CDR). Then, the neurologists specialized in dementia diagnosis and care reviewed all the data collected from clinical examination, in-person interviews, and neurocognitive testing, and made a preliminary judgement for participants who were suspected to have dementia. In addition, ~ 9.5% had incomplete data for the assessment of dementia status. Finally, all these persons were contacted by neurologists to conduct the second interview and reassess their medical history, cognitive status, ADLs, and whenever available, neuroimaging data. If the participants were not able to take the interview due to severe cognitive impairment or not available for the face-to-face interviews (~ 13%), the neurologists interviewed their family members, neighbors, or village doctors (primary care providers to local residents). Based on all the assessments, a diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, criteria[23]. In case of uncertainty, a senior neurologist was consulted and discussed, and a consensus diagnosis of dementia was reached. The diagnosis of AD was made according to the National Institute on Aging-Alzheimer's Association criteria for probable AD[24].

2.6 Statistical Analysis

Characteristics of study participants were present and compared using the non-parametric test for continuous variables or chi-square test for categorical variables. We used chi-square test to assess whether the allele frequencies agreed with expectations in Hardy-Weinberg equilibrium (HEW). The Haploview software was used to explore the stratified linkage disequilibrium (LD) patterns of SNPs[25]. Multivariable logistic regression models were used to calculate the odds ratio and 95% confidence interval (95% CI) of AD associated with *TOMM40* and *PVRL2* genotypes. Furthermore, we evaluated the interaction and joint exposures of *TOMM40* and *PVRL2* genotypes associated with AD risk. We reported the main results from two models: Model 1 was controlled for age, sex, and education, and Model 2 was additionally controlled for current smoking, current drinking, diabetes, hyperlipidemia, hypertension, and *APOE* genotype. In the plasma biomarker subsample, we used the multiple general linear models to examine the associations of *TOMM40* and *PVRL2* genotypes with plasma AD biomarkers. The statistical

significance was set to be two-tailed $p < 0.05$. All analyses were performed using IBM SPSS Statistics version 26.0 (IBM SPSS Inc., Chicago, Illinois, USA) and Haploview version 4.2 (Cambridge, MA02141, USA).

3. Results

3.1 Characteristics of study participants (n = 4876)

Of the 4876 participants, the mean age was 71.6 (SD, 5.4) years, 57.2% were women, and the average years of formal schooling was 3.1 (SD, 3.4; 39.9% were illiterate) years. Among all the participants, 182 (3.7%) were ascertained to have AD. Compared with people without dementia, participants with AD were older, more likely to be women, and less educated ($p < 0.001$). The distribution of the *TOMM40* and *PVRL2* genotypes conformed to Hardy-Weinberg equilibrium (HWE). In addition, there was a significant difference in the distributions of current smoking, alcohol drinking, and *TOMM40* and *PVRL2* genotypes between the two groups. However, the two groups did not significantly differ in the distributions of diabetes, hyperlipoidemia, hypertension, and carrying *APOE* $\epsilon 4$ allele (Table 1).

Table 1
Characteristics of the study participants

Characteristics	Total sample (n = 4876)	Non-dementia (n = 4694)	AD (n = 182)	P-value
Age, y, mean (SD)	71.6(5.4)	71.4(5.1)	77.5 (7.2)	< 0.001
Female, n (%)	2791(57.2)	2653(56.5)	138(75.8)	< 0.001
Education, y, mean (SD)	3.1(3.4)	3.2(3.4)	1.2(2.3)	< 0.001
Current smoking, n (%)	729(15.0)	711(15.2)	18(9.9)	< 0.001
Current drinking, n (%)	1416(29.0)	1392(29.7)	23(12.6)	< 0.001
Diabetes, n (%)	678(13.9)	649(13.8)	29(15.9)	0.420
Hyperlipoidemia, n (%)	1133(23.2)	1085(23.1)	48(26.4)	0.307
Hypertension, n (%)	3251(67.7)	3134(66.8)	117(64.3)	0.700
<i>APOE</i> ε4 allele, n (%)	781(16.0)	747(15.9)	34(18.7)	0.318
<i>TOMM40</i> (rs2075650), n (%)				
AA	4076(83.6)	3930(83.7)	146(80.2)	0.006
AG	763(15.6)	732(15.6)	31(17.0)	
GG	37(0.8)	32(0.7)	5(2.8)	
P(HWE)	0.98			
<i>PVRL2</i> (rs6859), n (%)				
GG	2379(48.8)	2287(48.7)	92(50.6)	0.024
AG	2070(42.4)	2005(42.7)	65(35.7)	
AA	427(8.8)	402(8.6)	25(13.7)	
P(HWE)	0.31			
The number of participants with missing values was 39 for hypertension. As a covariate in the subsequence analysis, a dummy variable was created for hypertension to represent these with missing values.				
Abbreviations: AD, Alzheimer's disease; SD, standard deviation; <i>APOE</i> , apolipoprotein E gene; HWE, Hardy-Weinberg equilibrium; <i>TOMM40</i> , Translocase of outer mitochondrial membrane 40 homolog gene; <i>PVRL2</i> , poliovirus receptor-related 2 gene.				

3.2 Associations of *TOMM40* and *PVRL2* SNPs with AD risk (n = 4876)

To dissect AD-associated genetic structure among *TOMM40* rs2075650 and *PVRL2* rs6859, we used Haploview software to investigate stratified LD patterns of the two SNPs among AD cases and non-demented subjects respectively (Figure S1). People with AD manifested a diverse genomic structure relative to people who were free of dementia, represented by a stronger D' value among rs2075650 and rs6859 variants (0.91 vs. 0.79).

Controlling for age, sex, and education, *TOMM40* GG genotype was significantly associated with an increased likelihood of AD (Table 2, Model 1). When additionally adjusting for smoking, drinking, diabetes, hyperlipidemia, hypertension, and *APOE* genotype, the association between *TOMM40* GG and AD remained significant (Table 2, Model 2). There was no significant association of *PVRL2* genotype with AD, although the *PVRL2* AA genotype (vs. GG genotype) was associated with a non-significantly increased likelihood of AD (odds ratio = 1.56) (Table 2).

Table 2
Associations of *TOMM40* and *PVRL2* SNPs and their interaction with Alzheimer's disease risk

Genotype		No. of subjects	No. of cases	Odds ratio (95% confidence interval)	
				Model 1*	Model 2*
TOMM40 rs2075650					
AA		4076	146	1.00 (Reference)	1.00 (Reference)
AG		763	31	1.25(0.83–1.89)	1.34(0.60–3.01)
GG		37	5	5.77(2.03–16.44) ^a	6.24(1.73–22.48) ^a
PVRL2 rs6859					
GG		2379	92	1.00 (Reference)	1.00 (Reference)
AG		2070	65	0.83(0.60–1.17)	0.79(0.56–1.12)
AA		427	25	1.56(0.96–2.51)	1.47(0.89–2.42)
TOMM40 rs2075650	PVRL2 rs6859				
AA + AG	GG + AG	4433	157	1.00 (Reference)	1.00 (Reference)
GG	GG + AG	16	0	-	-
AA + AG	AA	406	20	1.36(0.83–2.24)	1.37(0.83–2.28)
GG	AA	21	5	14.01(4.56–42.99) ^a	12.87(3.97–41.73) ^a
*Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for smoking, alcohol consumption, hypertension, diabetes, hyperlipoidemia, and APOE genotype.					
^a p<0.05.					
Abbreviations: TOMM40, Translocase of outer mitochondrial membrane 40 homolog gene; PVRL2, poliovirus receptor-related 2 gene.					

We detected a statistical interaction of *TOMM40* and *PVRL2* genotypes on the likelihood of AD (p for interactions = 0.0003). Further analysis stratified by both genotypes showed that *TOMM40* GG genotype in combination with *PVRL2* AA genotype was significantly associated with a substantially increased likelihood of AD compared with *TOMM40* AA + AG genotype in combination with *PVRL2* GG + AG genotype (Table 2, Model 1). Further adjusting for additional confounders, including *APOE* genotype, slightly decreased the association with AD (Table 2, Model 2).

3.3 Associations of *TOMM40* and *PVRL2* SNPs with plasma AD biomarkers (n = 1257)

Multiple general linear regression analysis showed that *TOMM40* AG and GG genotypes (vs. AA genotype) were significantly associated with a lower level of plasma A β 42 after controlling for age, sex, and education (Table 3, Model 1). When additionally adjusting for smoking, drinking, diabetes, hyperlipoidemia, hypertension, and *APOE* genotype, the association between *TOMM40* GG genotype and lower plasma A β 42 was still significant (Table 3, Model 2). The associations of *TOMM40* genotype with the A β 42/A β 40 ratio were similar to those with plasma A β 42 (Table 3). There were no significant associations of the *PVRL2* genotype with either plasma A β 42 or the A β 42/A β 40 ratio.

Table 3

Associations of *TOMM40* and *PVRL2* polymorphisms with plasma Alzheimer's disease biomarkers

Genotype	No. of participants	β-coefficient (95% confidence interval)	
		Model 1*	Model 2*
Plasma Aβ42 (pg/ml)			
TOMM40 rs2075650			
AA	1054	0.000(Reference)	0.000(Reference)
AG	194	-0.553(-1.004–0.102) ^a	-0.060(-0.897-0.776)
GG	9	-2.733(-4.666–0.801) ^a	-2.196(-4.264–0.128) ^a
PVRL2 rs6859			
GG	633	0.000(Reference)	0.000(Reference)
AG	514	-0.013(-0.356-0.331)	0.159(-0.197-0.515)
AA	110	0.266(-0.333-0.865)	0.565(-0.053-1.183)
Plasma Aβ40 (pg/ml)			
TOMM40 rs2075650			
AA	1054	0.000(Reference)	0.000(Reference)
AG	194	1.378(-5.924-8.680)	-3.070(-16.595-10.455)
GG	9	6.000(-25.274-37.274)	2.997(-30.438-36.431)
PVRL2 rs6859			
GG	633	0.000(Reference)	0.000(Reference)
AG	514	-0.735(-6.267-4.796)	-1.235(-6.981-4.510)
AA	110	8.895(-0.744-18.534)	8.357(-1.618-18.331)
Plasma t-tau (pg/ml)			
TOMM40 rs2075650			
AA	1054	0.000(Reference)	0.000(Reference)

*Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for smoking, drinking, hypertension, diabetes, hyperlipoidemia, and *APOE* genotype.

^ap<0.05.

Abbreviations: A β , amyloid- β ; t-tau, total tau protein; *TOMM40*, Translocase of outer mitochondrial membrane 40 homolog gene; *PVRL2*, poliovirus receptor-related 2 gene.

Genotype	No. of participants	β -coefficient (95% confidence interval)	
		Model 1*	Model 2*
AG	194	-0.013(-0.162-0.135)	-0.008(-0.283-0.267)
GG	9	0.016(-0.620-0.652)	0.075(-0.605-0.755)
<i>PVRL2</i> rs6859			
GG	633	0.000(Reference)	0.000(Reference)
AG	514	-0.013(-0.126-0.099)	-0.010(-0.126-0.107)
AA	110	-0.126(-0.322-0.070)	-0.136(-0.339-0.067)
Plasma Aβ42-to-Aβ40 ratio			
<i>TOMM40</i> rs2075650			
AA	1054	0.000(Reference)	0.000(Reference)
AG	194	-0.004(-0.0061–0.001) ^a	0.001(-0.004-0.005)
GG	9	-0.017(-0.028–0.006) ^a	-0.013(-0.024–0.001) ^a
<i>PVRL2</i> rs6859			
GG	633	0.000(Reference)	0.000(Reference)
AG	514	0.000(-0.002-0.002)	0.001(-0.001-0.003)
AA	110	-0.002(-0.005-0.002)	0.000(-0.003-0.004)
*Model 1 was adjusted for age, sex, and education; Model 2 was additionally adjusted for smoking, drinking, hypertension, diabetes, hyperlipoidemia, and <i>APOE</i> genotype.			
^a p<0.05.			
Abbreviations: A β , amyloid- β ; t-tau, total tau protein; <i>TOMM40</i> , Translocase of outer mitochondrial membrane 40 homolog gene; <i>PVRL2</i> , poliovirus receptor-related 2 gene.			

4. Discussion

In this population-based study of rural-dwelling Chinese older adults, we found that *TOMM40* GG genotype was associated with an increased likelihood of AD. Whereas, there was a marginal association of *PVRL2* AA genotype with AD. An interaction between *TOMM40* and *PVRL2* on the likelihood of AD was detected such that carrying both *TOMM40* GG and *PVRL2* AA genotypes was associated with an over 12-fold increased likelihood of AD. In our subsample, the *TOMM40* GG genotype, but not *PVRL2* AA genotype, was associated with a reduced level of plasma A β 42. Taken together, these results suggest that

TOMM40 and *PVRL2* risk alleles may act interactively to increase the likelihood of AD, possibly through influencing A β metabolism.

The GWAS analyses suggested that *TOMM40* SNPs may play some role in AD[14, 26]. In addition, case-control studies from Italy[9], Colombia[10], and China[11] also revealed that the *TOMM40* G allele serves as a risk variant for AD. In line with previous studies, our community-based study confirmed that the *TOMM40* G allele could confer a substantial risk to AD. However, the mechanism underlying the association of *TOMM40* with AD is not well established. The neuroimaging and neuropathological data showed that the *TOMM40* was associated with amyloid burden in the brain parenchyma and vessels[27]. Coincidentally, reports from ADNI database demonstrated that *TOMM40* was a susceptible putative locus associated with cerebrospinal fluid AD biomarkers (e.g., A β 42, p-tau181p/A β 42 ratio, and t-tau/A β 42 ratio)[28, 29]. In the current population-based study, we found that *TOMM40* GG genotype was associated with a lower level of plasma A β 42 and the A β 42/A β 40 ratio. Taken together, these results suggested that *TOMM40* might be involved in the A β metabolism, which in turn may be linked with AD.

The GWAS analyses among Chinese or European populations showed that *PVRL2* rs6859 was associated with AD, with OR ranging from 1.46 to 1.61[4, 5, 26]. In line with these GWAS analyses, our community-based study showed that *PVRL2* AA genotype was associated with a 56% increased likelihood of AD after controlling for demographic factors (OR = 1.56; 95% CI 0.96–2.51), although the association was not statistically evident, possibly owing to limited statistical power. Thus, large-scale population-based studies are warranted to replicate the association of *PVRL2* genotype with AD risk.

Our study revealed a synergistic interaction between *TOMM40* and *PVRL2* variants on AD, such that having both *TOMM40* GG and *PVRL2* AA genotypes was associated with an over 12-fold likelihood of AD. To the best of our knowledge, this is the first population-based study that identified the interaction between *TOMM40* and *PVRL2* genes on AD risk. The neurobiological mechanisms underlying their interaction effect on AD are not clear. The expression quantitative trait loci (eQTL) analysis indicated that the *TOMM40* rs2075650 G allele could increase the binding affinity of transcription factors (TFs), thus, increasing *PVRL2* gene expression[15]. In this context, the synergistic interaction of *TOMM40* GG and *PVRL2* AA genotypes with AD may partly attribute to the mechanism that *TOMM40* G allele might enhance the effect of *PVRL2* risk allele. Alternatively, the synergistic effects of *TOMM40* and *PVRL2* risk variants on AD might be resulted from their shared molecular pathways involved in AD pathological mechanisms such as A β metabolism[30, 31] and inflammation[14, 15].

Previous studies reported that *TOMM40* and *PVRL2* might be indirectly involved in the accumulation of A β [32, 33]. Our results showed that *TOMM40* GG carriers had the lower level of plasma A β 42 than carriers of other genotypes, supporting a possible link of *TOMM40* with metabolism of A β 42, but not A β 40. However, we did not find associations between *PVRL2* genotype with any of the three plasma AD biomarkers (A β 40, A β 42, and t-tau), which is in line with the lack of evident association between *PVRL2* genotype and AD risk. In addition, the lack of associations between plasma t-tau and *TOMM40* or *PVRL2*

genotype may be due partly to the facts that plasma t-tau is not associated with dementia and AD[34]. Alternatively, *TOMM40* and *PVRL2* genes may be not involved in the process of tau pathology.

Our study was based on a large-scale sample of rural-dwelling Chinese older adults, where epidemiological, clinical, neuropsychological, and genetic data were integrated with plasma AD biomarkers. Thus, we are able to explore the associations and interaction effects of *TOMM40* and *PVRL2* genes with AD risk as well as the possible neuropathological mechanisms.

5. Limitation

Our study also has limitations. First, due to the cross-sectional nature of the study design, the observed associations might be subject to selective survival bias, which usually leads to an underestimation of the true association. Furthermore, the study participants were recruited from a single rural area in northern China, which should be kept in mind when generalizing our findings to other populations.

6. Conclusions

In conclusion, our study confirms the association of *TOMM40* GG genotype with AD among rural-dwelling Chinese older adults and further reveals a potential genetic interaction between *TOMM40* and *PVRL2* risk alleles on AD risk and the association of *TOMM40* GG genotype with low plasma A β 42. These results increase our understanding of the roles of *TOMM40* and *PVRL2* genes in AD and the potential neuropathological mechanisms.

Abbreviations

AD, Alzheimer's disease; SNPs, single nucleotide polymorphisms; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog gene; *PVRL2*, poliovirus receptor-related 2 gene; *APOE*, apolipoprotein E; A β , amyloid- β ; t-tau, total tau protein; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; MIND-China, Multimodal Intervention to Delay Dementia and Disability in Rural China; OR, odds ratio; SD, standard deviation.

Declarations

Ethics approval and consent to participate

The MIND-China project was approved by the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong. All participants provided written informed consent for both study enrollment and blood sample collection, or in case of persons with severe cognitive impairment, their informants provided informed consent. Research within MIND-China has been conducted in accordance with the ethical principles expressed in the Declaration of Helsinki as well as relevant guidelines and regulations. MIND-China was registered in the Chinese Clinical Trial Registry (registration no.: ChiCTR1800017758).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used for the analysis are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

MIND-China was supported by the grant from the National Key R&D Program of China (grant no.: 2017YFC1310100) and by additional grants from the National Nature Science Foundation of China (grants no.: 81861138008 and 82011530139), the Academic Promotion Program of Shandong First Medical University (2019QL020), and the Taishan Scholar Program of Shandong Province, China. C Qiu received grants from the Swedish Research Council (grants no.: 2017-00740, 2017-05819, and 2020-01574), the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) (grant no.: CH2019-8320) for the Joint China-Sweden Mobility program, and the Karolinska Institutet, Stockholm, Sweden. The funding agency had no role in the study design, data collection and analysis, the writing of this manuscript, and in the decision to submit the work for publication.

Author's contributions

X.L., T.H., Y.D., and C.Q. designed the study. X.L., T.H., K.L., C.L., Y.W., R.L., W.F., N.T., Y.C., and N.W. contributed to data collection and assessment. X.L., T.H., and K.L. analyzed the data. X.L. drafted the manuscript. Y.D. and C.Q. supervised the study. All authors made critical comments on the manuscript and approved the final manuscript.

Acknowledgements

We would like to thank all participants of the MIND-China project as well as our research staff for their collaboration in data collection and management.

References

1. Dubois B, Feldman HH, Jacova C, Cummings JL, DeKosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol.* 2010;9(11):1118–27.
2. Sims R, Hill M, Williams J. The multiplex model of the genetics of Alzheimer's disease. *Nat Neurosci.* 2020;23(3):311–22.

3. Andrews SJ, Fulton-Howard B, Goate A. Interpretation of risk loci from genome-wide association studies of Alzheimer's disease. *Lancet Neurol*. 2020;19(4):326–35.
4. Jia L, Li F, Wei C, Zhu M, Qu Q, Qin W, et al. Prediction of Alzheimer's disease using multi-variants from a Chinese genome-wide association study. *Brain*. 2021;144(3):924–37.
5. Zhou X, Chen Y, Mok KY, Kwok TCY, Mok VCT, Guo Q, et al. Non-coding variability at the APOE locus contributes to the Alzheimer's risk. *Nat Commun*. 2019;10(1):3310.
6. Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, et al. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. *Pharmacogenomics J*. 2010;10(5):375–84.
7. Caselli RJ, Dueck AC, Huentelman MJ, Lutz MW, Saunders AM, Reiman EM, et al. Longitudinal modeling of cognitive aging and the TOMM40 effect. *Alzheimers Dement*. 2012;8(6):490–5.
8. Seripa D, Bizzarro A, Pilotto A, Palmieri O, Panza F, D'Onofrio G, et al. TOMM40, APOE, and APOC1 in primary progressive aphasia and frontotemporal dementia. *J Alzheimers Dis*. 2012;31(4):731–40.
9. Bagnoli S, Piaceri I, Tedde A, Bessi V, Bracco L, Sorbi S, et al. TOMM40 polymorphisms in Italian Alzheimer's disease and frontotemporal dementia patients. *Neurol Sci*. 2013;34(6):995–8.
10. Ortega RJ, Morales L, Guerrero E. Association Analysis of Polymorphisms in TOMM40 CR1 PVRL2 SORL1 PICALM and 14q3213 Regions in Colombian Alzheimer Disease Patients. *Alzheimer Dis Assoc Disord*. 2016;30(4):305–9.
11. Ma YX, Yu TJ, Wang W. Association of TOMM40 Polymorphisms with Late Onset Alzheimers Disease in a Northern Han Chinese Population. *Neuromolecular Med*. 2013;15(2):279–87.
12. Wingo AP, Liu Y, Gerasimov ES, Gockley J, Logsdon BA, Duong DM, et al. Integrating human brain proteomes with genome-wide association data implicates new proteins in Alzheimer's disease pathogenesis. *Nat Genet*. 2021;53(2):143–6.
13. Miao L, Xingyin R, LingPan S. BCL3 PVRL2 TOMM40 SNPs gene-gene and gene-environment interactions on dyslipidemia. *Sci Rep*. 2018;18(1):6189.
14. Zhou X, Chen Y, Mok KY, Zhao Q, Chen K, Chen Y, et al. Identification of genetic risk factors in the Chinese population implicates a role of immune system in Alzheimer's disease pathogenesis. *Proc Natl Acad Sci U S A*. 2018;115(8):1697–706.
15. Liu C, Chyr J, Zhao W, Xu Y, Ji Z, Tan H, et al. Genome-Wide Association and Mechanistic Studies Indicate That Immune Response Contributes to Alzheimer's Disease Development. *Front Genet*. 2018;9:410.
16. Watts JC, Prusiner SB. beta-Amyloid Prions and the Pathobiology of Alzheimer's Disease. *Cold Spring Harb Perspect Med*. 2018;8(5):a023507.
17. Pase MP, Beiser AS, Himali JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of Plasma Total Tau Level as a Predictive Biomarker for Dementia and Related Endophenotypes. *JAMA Neurol*. 2019;76(5):598–606.

18. Chouraki V, Beiser A, Yountkin L, Preis SR, Weinstein G, Hansson O, et al. Plasma amyloid-beta and risk of Alzheimer's disease in the Framingham Heart Study. *Alzheimers Dement*. 2015;11(3):249–57.e1.
19. Han X, Jiang Z, Li Y, Wang Y, Liang Y, Dong Y, et al. Sex disparities in cardiovascular health metrics among rural-dwelling older adults in China: a population-based study. *BMC Geriatr*. 2021;21(1):158.
20. Dong Y, Wang Y, Liu K, Liu R, Tang S, Zhang Q, et al. Olfactory Impairment Among Rural-Dwelling Chinese Older Adults: Prevalence and Associations With Demographic, Lifestyle, and Clinical Factors. *Front Aging Neurosci*. 2021;13:621619.
21. Cong L, Ren Y, Hou T, Han X, Dong Y, Wang Y, et al. Use of Cardiovascular Drugs for Primary and Secondary Prevention of Cardiovascular Disease Among Rural-Dwelling Older Chinese Adults. *Front Pharmacol*. 2020;11:608136.
22. Kivipelto M, Mangialasche F, Snyder HM, Allegri R, Andrieu S, Arai H, et al. World-Wide FINGERS Network: A global approach to risk reduction and prevention of dementia. *Alzheimers Dement*. 2020;16(7):1078–94.
23. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. fourth ed. Washington, DC: American Psychiatric Association; 1994.
24. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement*. 2011;7(3):263–9.
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21(2):263–5.
26. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet*. 2009;41(10):1088–93.
27. Valant V, Keenan BT, Anderson CD, Shulman JM, Devan WJ, Ayres AM, et al. TOMM40 in Cerebral Amyloid Angiopathy Related Intracerebral Hemorrhage: Comparative Genetic Analysis with Alzheimer's Disease. *Transl Stroke Res*. 2012;3(Suppl 1):102–12.
28. Souza MB, Araujo GS, Costa IG, Oliveira JR. Alzheimer's Disease Neuroimaging I. Combined Genome-Wide CSF Aβ₄₂'s Associations and Simple Network Properties Highlight New Risk Factors for Alzheimer's Disease. *J Mol Neurosci*. 2016;58(1):120–8.
29. Kim S, Swaminathan S, Shen L. Genome wide association study of CSF biomarkers Aβ_{1–42} t-tau and p tau_{181p} in the ADNI cohort. *Neurology*. 2011;76(1):69–79.
30. Hu W, Wang Z, Zheng H. Mitochondrial accumulation of amyloid β (Aβ) peptides requires TOMM22 as a main Aβ receptor in yeast. *J Biol Chem*. 2018;293(33):12681–9.
31. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. *J Pathol*. 2009;217(1):131–8.

32. Itzhaki RF. Herpes simplex virus type 1 and Alzheimer's disease: increasing evidence for a major role of the virus. *Front Aging Neurosci.* 2014;6:202.

33. Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci.* 2006;26(35):9057–68.

34. de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ, et al. Plasma tau, neurofilament light chain and amyloid-beta levels and risk of dementia; a population-based cohort study. *Brain.* 2020;143(4):1220–32.

Figures

Figure 1

The flowchart of the study participants. Abbreviations: MIND-China, Multimodal Interventions to Delay Dementia and Disability in Rural China; AD, Alzheimer’s disease.

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