Original Research
Brief Communication

No evidence of association between variant rs2075650 in lipid metabolism-related locus *APOE/TOMM40* and advanced age-related macular degeneration in Han Chinese population

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Abstract

Age-related macular degeneration (AMD) is a late-onset, neurodegenerative disease. Genes related to lipid metabolism are important in AMD pathogenesis. Recently, a variant rs2075650 located in lipid metabolism-related locus *APOE/TOMM40* was identified to be associated with advanced AMD and early AMD, respectively, in two genome-wide association studies with European ancestry, while no association study between rs2075650 and overall advanced AMD in Chinese population has been conducted before. We evaluated the potential effect of this variant on advanced AMD in a Han Chinese cohort with 204 advanced AMD patients and 1536 healthy controls. The results suggested that rs2075650 was neither associated with advanced AMD in allele level ($P = 0.348$) nor in genotype level ($P = 0.890$ under additive model with age and sex adjusted). In conclusion, our study did not confirm the impact of rs2075650 on advanced AMD risk, indicating that rs2075650 is unlikely a superior marker for *APOE/TOMM40* susceptible region with advanced AMD in Han Chinese population.

Keywords: Age-related macular degeneration, *APOE/TOMM40* locus, Han Chinese population, association study, lipid metabolism-related genes

***Experimental Biology and Medicine* 2015; 240: 230–234. DOI: 10.1177/1535370214553770**

Introduction

Age-related macular degeneration (AMD) is the most common cause of blindness in the elderly worldwide and has become a major public health issue.[1] AMD is characterized as the formation of choroidal drusen, yellow deposit in the retinal pigment epithelium, Bruch's membrane or underlying choroid. The advanced stage of AMD will lead to retinal atrophy (geographic atrophy (GA) subtype) or the growth of abnormal choroidal vessels under the retina (choroidal neovascularization (CNV) subtype).[2] One of major age-related changes in the human retina is the accumulation of histochemically detectable lipoprotein particles in normal Bruch's membrane throughout adulthood,[3] which is proposed to lead to the formation of hydrophobic barrier, impairment of nutrient exchange through the choriocapillaris network thus initiating AMD.[3–6] Therefore, genes related to lipid metabolism would play an important role in AMD pathogenesis.

Genetic studies have identified several lipid metabolism-related genes contributing to AMD such as *APOE*,[7–9] *LIPC*,[10] and *TIMP3*.[11] An intronic single nucleotide polymorphism (SNP) rs2075650, within *APOE/TOMM40* region, was found to be associated with advanced AMD in a genome-wide association study (GWAS) ($P = 3.1 \times 10^{-6}$) and combined replication samples ($P = 8.4 \times 10^{-8}$) with European ancestry.[12] Recently, this variant has been identified showing association with early AMD in a GWAS meta-analysis of European population ($P = 1.1 \times 10^{-6}$), and the signal remained in secondary meta-analysis combining two Singapore-based Asian cohorts ($P = 3.2 \times 10^{-6}$).[13] However, Guo *et al.*[14] failed to replicate the association between this variant and neovascular AMD, the CNV subtype of advanced AMD in a northern Chinese cohort ($P = 0.912$), which could be due to the phenotypic discrepancy between AMD subtypes and overall advanced AMD, as well as the genetic discrepancy between different ethnic groups. As no genetic association study of rs2075650 has

been performed for overall advanced AMD in Chinese population before, to shed a light on the source of these contradicting results, it is necessary to study the potential impact of rs2075650 on overall advance AMD risk in Chinese population.

Materials and methods

Subjects

The study cohort with 204 AMD patients and 384 healthy individuals was recruited from the Putuo People's Hospital in Zhoushan, Zhejiang Province, China, which was used in our previous studies.[15,16] To reach sufficient statistical power, another 1152 healthy individuals were selected from a previously studied control set from communities in Pudong District, Shanghai, China.[17–23] All the samples were enrolled concurrently from 2007 to 2008 and represented as southeastern Han Chinese population; therefore, we combined the two sets of controls in the following statistical analyses. AMD clinical diagnosis was defined strictly in accordance with the International Classification of Age-Related Maculopathy and Macular Degeneration.[24] The control subjects are unrelated healthy individuals with no family history of AMD, as well as other metabolic diseases such as type 2 diseases. This study was approved by the Ethics Committee of Shanghai Institutes for Biological Sciences. A standard informed consent was established following the guidelines of the Helsinki Declaration. All the participants obtained and signed the consent. The demographic information of the study subjects was summarized in Table 1.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes of the subjects. The extraction methods were standard phenol-chloroform extraction for 588 subjects from Zhoushan area and QuickGene 610L Automatic DNA/RNA Extraction System (Fujifilm, Tokyo, Japan) for 1152 subjects from Shanghai area, respectively. Genotyping of rs2075650 was determined on ABI ViiA^{TM}7 Thermal Cycler using TaqMan SNP genotyping assay (Life Technologies, Carlsbad, CA, USA) according to the standard TaqMan genotyping protocol. We successfully genotyped more than 95% of the total samples and random 150 samples were repeated to validate the genotyping results with 100% concordance.

Table 1 Characteristics of the participants

Sample	*N*	Age (years)	Female (%)
Control	1536	61.2 ± 10.0	67.9
Control set 1[a]	384	67.6 ± 6.6	55.9
Control set 2[b]	1152	59.1 ± 10.1	71.9
Case	204	74.9 ± 7.0	44.2
GA	77	73.8 ± 5.8	52.7
CNV	54	71.6 ± 7.9	33.3
AMD diagnosis[c]	73	79.9 ± 4.0	43.4

GA: geographic atrophy; CNV: choroidal neovascularization; AMD: age-related macular degeneration.
Data are presented as the mean ± standard deviation.
[a]Control set was recruited from Zhoushan.
[b]Control set was recruited from Shanghai.
[c]These cases were diagnosed with AMD, but we could not acquire specific subtype information.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) for rs2075650 was calculated based on an exact test.[25] To evaluate association between allele and AMD, odds ratios (ORs), 95% confidence intervals (CIs), and corresponding *P* values were calculated based on Fisher's exact test. The association between AMD and the genotype was evaluated using three logistic regression models: additive model, dominant model, and recessive model. As we observed significant difference of age and sex distributions between cases and controls ($P < 0.05$), we further assessed the genotypic associations with adjustment for age and sex. All the statistical tests were carried out by R package (http://www.r-project.org/). Statistical power was *post hoc* calculated using the G*Power program, based on goodness-of-fit test.[26] Statistical significance was defined at $P < 0.05$.

Results

The genotypic distribution of rs2075650 was not deviated from HWE ($P > 0.05$). However, we did not find the association between rs2075650 and overall advanced AMD in our Chinese cohort either in allele level ($P = 0.348$) or in genotype level ($P = 0.326$ under additive model and $P = 0.890$ under additive model with age and sex adjusted) (Table 2). In power calculation, the current sample size had >90% power to detect the modest genetic effect (OR 0.7). Meanwhile, the G allele frequency in our control is 0.111, close to the reported frequency of 0.117 in Han Chinese in Beijing, China (CHB) from the HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), indicating that our result is reliable Therefore, we conclude that this variant is unlikely to contribute to overall advanced AMD risk in Chinese population.

Discussion

We investigated the association between rs2075650 and overall advanced AMD in Chinese population and found no evidence of this variant contributing to advanced AMD susceptibility. Rs2075650 within *APOE/TOMM40* has been studied for its associations with several age-related disorders and diseases in GWAS, such as cognitive decline[27,28] and Alzheimer's disease (AD),[29–31] as well as AMD.[12,13] Apolipoprotein E (ApoE), encoded by the *APOE* gene, was recognized for its crucial role in lipoprotein transport. An endogenous way of retinal lipid removal may rely on the components of reverse cholesterol transport including ApoE.[5,32] In addition, ApoE enhances the degradation of amyloid-beta peptide, as the accumulation of amyloid-beta in patient's specific tissue is a feature common to both AMD and AD.[33] Furthermore, the *TOMM40* gene encodes translocase of outer mitochondrial membrane 40 homolog (TOM40),[34] involved in translocation and sorting

Table 2 *APOE/TOMM40* rs2075650 allele and genotype associations with advanced AMD

	G Allele		GG/AG/AA distributions			Genotypic association[b,c]			Genotypic association[c,d]		
	Count	Frequency	Count	Frequency	Allelic association[a]	Additive	Dominant	Recessive	Additive	Dominant	Recessive
Case	38	0.095	1/36/163	0.005/0.18/0.815	*P* 0.348	0.326	0.408	0.375	0.890	0.762	0.572
Control	324	0.111	18/288/1149	0.012/0.198/0.790	OR 0.84 (95% CI) (0.57–1.20)	0.83 (0.57–1.18)	0.85 (0.57–1.23)	0.40 (0.022–1.96)	1.03 (0.60–1.57)	1.08 (0.67–1.71)	0.54 (0.027–3.19)

GG, AG and AA are the genotypes. [a]ORs, 95% CIs, and *P* values were calculated based on Fisher's exact test.
[b]ORs, 95% CIs, and *P* values were calculated using logistic regression.
[c]Logistic regression was performed under three models. Additive model: GG was coded as 2, while AG was coded as 1, and AA was coded as 0. Dominant model: both GG and AG were coded as 1, while AA was coded as 0. Recessive model: only GG was coded as 1, so both AG and AA were coded as 0.
[d]ORs, 95% CIs, and *P* values were calculated using logistic regression, with adjustment for age and sex.

proteins into mitochondria.[35] Because structural abnormalities and oxidative stress of the mitochondria are known to increase the risk of AD, *TOMM40* may also contribute to AMD in the same manner.[36] Although no evidence was found to support the rs2075650 contributions to advanced AMD risk in Chinese population, the *APOE/TOMM40* region which shows both functional implications in AMD pathogenesis and genetic associations with AMD is still a promising candidate for investigation.

The *TOMM40* gene, located in upstream region to the gene *APOE*, is closely adjacent to *APOE*.[37] One study suggested that the statistical significance of *TOMM40* association with AD is due to linkage disequilibrium (LD),[38] emphasizing a nonsynonymous SNP rs429358 in *APOE*. Cipriani *et al*.[12] found that the association between rs2075650 and advanced AMD turned nonsignificant after adjusted for rs429358, despite of the modest LD ($r^2 = 0.2$). Besides rs2075650, another variant rs6857, near *TOMM40*, was associated with early AMD reported by Holliday *et al*.[13] Rs2075650 and rs6857 occur in high LD ($r^2 = 0.949$, according to CHB and JPT (Japanese in Tokyo, Japan) from the HapMap Project), while both of them are independent of rs429358. We suggest that despite the lack of evidence of association between variants in *TOMM40* block (rs2075650 and rs6857) and advanced AMD in Chinese, other variants in *APOE* locus for advanced AMD susceptibility still need to be studied.

Additionally, in another Chinese study, Guo *et al*.[14] observed no association of rs2075650 with a specific advanced AMD subtype, neovascular AMD, with an OR of 0.979. Although we used the overall advanced AMD, it is unlikely that the negative result was caused by phenotypic heterogeneity of different AMD subtypes in our study, because: (1) The G allele frequencies in different AMD subtypes are close to each other (0.092 for GA type, 0.113 for CNV type, and 0.085 for AMD cases diagnosed with AMD but without further subtype information); and (2) The genotype distributions in total AMD patients as well as in each AMD subgroup were not deviated from HWE ($P > 0.9$). Meanwhile, inconsistent results in Chinese population and European population[12] of advanced AMD might also reflect the genetic heterogeneity in different ethnic groups.

In summary, we did not confirm the association between rs2075650 and advanced AMD in our Chinese cohort, suggesting that rs2075650 is unlikely a superior marker for causal variants in *APOE/TOMM40* susceptible region of advanced AMD in Han Chinese population. Whether other variants in this metabolism-related locus correlate to specific subtypes of AMD (early or advanced AMD) in Chinese population remains to be elucidated in the future study with a larger sample size.

AUTHORS' CONTRIBUTIONS

MK and XW contributed equally to this study. YL and LH conceived the study and helped to draft the manuscript; MK and XW carried out the experiments and performed the statistical analysis and then wrote the manuscript; TW and HZ carried out most part of the experiments; JY, FL, and MX helped

with statistical analysis. DZ provided the sample information for 1152 control set.

ACKNOWLEDGEMENTS

This work was supported by the 973 Program (2010CB529600 and 2011CB504000), the National Key Technology R&D Program (2012BAI01B09), and the National Natural Science Foundation of China (31200954, 81121001, and 81361120389). We gratefully thank Dr Dingguo Qian, who helped with sample collection and AMD diagnosis.

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(Received March 1, 2014, Accepted August 15, 2014)