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Interaction between an ATP-Binding Cassette A1 (*ABCA1*) Variant and Egg Consumption for the Risk of Ischemic Stroke and Carotid Atherosclerosis: a Family-Based Study in the Chinese Population

Jing Song[1], Xia Jiang[2, 3], Yaying Cao[1], Juan Juan[4], Tao Wu[1] and Yonghua Hu[1]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China
[2]Program in Genetic Epidemiology and Statistical Genetics, Harvard T.H. Chan School of Public Health. Boston, USA
[3]Unit of Cardiovascular Epidemiology, Institute of Environmental Health, Karolinska Institute, Stockholm, Sweden
[4]Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing, China

Aim: ATP-binding cassette A1 (*ABCA1*) plays an important role in reducing the risk of stroke. Egg is the major source of dietary cholesterol and is known to be associated with the risk of stroke and atherosclerosis. We aimed to assess the effects of interaction between an *ABCA1* variant (rs2066715) and egg consumption on the risk of ischemic stroke (IS), carotid plaque, and carotid-intima media thickness (CIMT) in the Chinese population.

Methods: In total, 5869 subjects (including 1213 IS cases) across 1128 families were enrolled and divided into two groups based on the median egg consumption (4 eggs per week). In the analyses for the presence of carotid plaque and CIMT, 3171 out of 4656 IS-free controls without self-reported history of coronary heart disease and lipid-lowering medications were included. Multilevel logistic regression models were used to model the genetic association of rs2066715 with the risk of IS, and mixed-effect linear regression for the genetic association of rs2066715 with carotid plaque, and CIMT. The gene-by-egg cross-product term was included in the regression model for interaction analysis.

Results: We found that rs2066715 was associated with the increased risk of carotid plaque among those who consumed <4 eggs per week after adjustment (odds ratio [95% confidence interval]: 1.61 [1.08, 2.39], P=0.019). A significant effect of interaction between rs2066715 and egg consumption on the risk of carotid plaque was identified (P=0.011).

Conclusion: rs2066715 was found to interact with egg consumption in modifying the risk of carotid plaque in the Chinese population.

Key words: ATP-binding cassette A1, Egg consumption, Ischemic stroke, Carotid plaque, Interaction

Introduction

Stroke has increasingly become a burden for global public health in the past two decades, particularly in developing countries[1]. In China, stroke has become the leading cause of death and adult disability[2]. Ischemic stroke (IS) is the most common type of stroke, accounting for 43%−79% of all stroke cases[2]. It is widely acknowledged that IS is a multifactorial disease; however, its mechanisms remain unclear. Twins and family studies have indicated that genetic factors might play a role in the risk of stroke[3-5], where the heritability estimate was approximately 37.9% for all IS cases and approximately 16.1%−40.3% for the cases of its subtypes[6]. ATP-binding cassette transporter A1 (ABCA1) is a transmembrane protein involved in the cellular cholesterol efflux, and the mutation of *ABCA1* leads to a deficiency or absence of high-density lipoprotein cholesterol (HDLC)[7, 8] and may cause atherosclerotic cardiovascular diseases (e.g. coronary artery disease [CHD]

Address for correspondence: Yonghua Hu, Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China
E-mail: yhhu@bjmu.edu.cn

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and IS)[9-12]. Rs2066715, a nonsynonymous single nucleotide polymorphism (SNP) in *ABCA1*, is reported to be associated with the increased risk of IS solely in Chinese individuals[13-15], though evidences were conflicting. A small Chinese Kazakh population's sample study ($N=118$) reported that the frequency of A allele at rs2066715 was significantly higher in patients with cerebral infarction than controls (0.300 versus 0.168, $\chi^2=6.12$, $P<0.05$)[13]. However, in another two studies involving Chinese Han population ($N=476/1100$), the allele frequencies at rs2066715 did not significantly differ between the atherothrombotic cerebral infarction or IS group and control group[14, 15]. In addition, rs2066715 possibly interacts with other risk factors for IS. One study observed that both AG (odds ratio [OR] [95% confidence interval, CI] 3.91 [1.93, 7.93]) and GG genotypes (OR [95% CI] 2.42 [1.04, 5.62]) exerted synergetic effects jointly with hypertension on the risk of IS in another Chinese Han population[14]. These results suggest that rs2066715 likely exerts a modest effect on the risk of IS, which could be amplified by other risk factors (e.g., hypertension), and is difficult to be identified in underpowered studies with a small sample size. Therefore, the role of rs2066715 in the risk of IS in the Chinese population remains unclear, and to validate it, further studies with adequate statistical power are needed.

Dietary cholesterol, mostly studied as egg consumption, has been suggested as a risk factor of stroke, although the evidence was inclusive[16-23]. The underlying mechanisms were probably through accelerating the oxidation of low-density lipoprotein cholesterol (LDLC), prompting the adverse role of dietary saturated fat, and increasing the postprandial lipid levels[22]. ABCA1 functions as the transporter in the uptake of dietary cholesterol, and in-vitro evidence has shown that egg yolk affects the mRNA expression of *ABCA1* in a dose-dependent manner[24]. A randomized controlled trial (RCT) has found the intake of whole eggs with restriction on carbohydrate intake to be associated with higher *ABCA1* expression and that it affected cholesterol homeostasis in 37 adults with metabolic syndrome (MetS)[25]. However, whether there is an interactive effect between *ABCA1* polymorphisms and egg intake on the risk of IS or its subtypes remains unknown. In this study, we aimed to investigate the interaction between whole egg consumption and rs2066715 for the risk of IS and its subtypes in the Chinese population. We also assessed the genetic association of rs2066715 with carotid-intima media thickness (CIMT) and carotid plaque (especially ruptured plaque), which are considered as subclinical atherosclerotic measures and strong risk predictors of stroke[26-32].

Materials and Methods

(1) Study Population

The present study was nested within the Fangshan/Family-based Ischemic Stroke Study in China (FISSIC) program, a region located on the stroke belt in China[33]. The details of FISSIC have been described elsewhere[34]. In brief, this ongoing family-based, epidemiological study has enrolled 5869 individuals (including 1213 confirmed IS cases and 4656 controls) across 1128 families using the proband-initiated contact method[35]. An IS case was included if all of the following criteria were met: (1) the diagnosis of IS should be confirmed by a neurologist, from at least a secondary hospital based on the medical records, and head imaging by computed tomography (CT) or magnetic resonance imaging (MRI); (2) ≥ 18 years old at the time of survey; (3) at least one full sibling alive by the time of recruitment; (4) long-term inhabitants of the Fangshan District (>5 years by the time of investigation); and (5) written informed consent provided. Patients with self-reported IS but without the availability of CT or MRI images and those diagnosed with IS associated with autoimmune conditions, endocarditis, surgical procedures (e.g. coronary artery pass endarterectomy), or Mendelian diseases (e.g. sickle cell anemia or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]) were excluded from the study population. The IS cases included in our study were diagnosed based on the monitoring trends and determinants of cardiovascular disease (MONICA) diagnostic criteria[36], which includes brain infarction due to occlusion of precerebral arteries (international classification of diseases [ICD]-9 433), brain infarction due to cerebral thrombosis (ICD-9 434), and embolic brain infarction (ICD-9 434). Based on the Trial of Org 10172 in acute stroke treatment (TOAST)[37], IS was classified into 5 etiological subtypes: large-artery atherosclerosis (LAA), small-vessel occlusion (SAO), cardioembolism (CE), stroke of other determined etiology, and stroke of undetermined etiology. All patients with IS were ascertained and subtyped by two qualified neurologists from the department of neurology at Peking University Third Hospital based on patients' medical records, CT and MRI images, and results from other auxiliary tests (e.g. transcranial Doppler ultrasonography [TCD], carotid artery ultrasonography, radiography of cervical vertebra, electrocardiograph [ECG], and Doppler cardiogram [DCG]). When their diagnoses disagreed, the diagnosis by a third neurologist was considered to determine the status of IS and its subtypes. In this study, 835 IS cases were classified into the LAA subtype, 217 into the SAO subtype, and 138

into the other subtypes (including CE and stroke of other determined and undetermined etiologies). 23 IS cases could not be classified into any specific subtype due to incomplete medical records; therefore, they were removed from the analysis for IS subtypes. Subjects were included in this study as controls if all of the following parameters were satisfied: (1) ≥18 years old at the time of survey; (2) at least one sibling alive confirmed as a patient with IS; (3) without a self-reported history of stroke, which was verified by the provision of negative answers to all the questions in the questionnaire for verifying stroke-free status; and (4) written informed consent provided. Among the 4656 controls, 3731 subjects were free of history of self-reported coronary heart disease and claimed that no lipid-lowering medication had been taken by them by the time of investigation. Thus, we selected these 3171 individuals as study subjects for the analysis of subclinical carotid atherosclerosis. This study has been approved by the Peking University Institutional Review Board.

(2) Assessment of Diet

The data on consumption of eggs, red meat, and green vegetables were collected by our trained interviewers using a semi-quantitative food frequency questionnaire (FFQ) designed for Chinese population[38]. The questions regarding the consumption of each type of food included: (1) usual consumption frequency (measured as times per week) and (2) portion size during each consumption (measured as grams for consumption of red meat and vegetables and counts for eggs). Based on the median egg consumption in all individuals (4 per week), we further stratified the whole study population into two groups.

(3) Assessment of Carotid Atherosclerosis

The images of CIMT and carotid plaque were collected by qualified physicians from Fangshan District Center for Disease Control and Prevention using GE Vivid I ultrasound machine (GE Healthcare, Tokyo, Japan). The details have been described previously[39]. Briefly, the high-solution dynamic images of CIMT at 3 segments (proximal end, distal end, and bifurcation) were recorded for the far wall of carotid artery on each side, lasting for at least 6 cardiac cycles. In addition, the carotid plaques were scanned from the proximal end to bifurcation on both sides, with dynamic images in both coronal and sagittal plane recorded for each plaque.

The measurement of CIMT was performed by trained researchers using the Vascular Research Tools 6 DEMO software (Medical Imaging Applications LLC, Coralville, Iowa, USA). As described before[39], the CIMT was measured as the interval between two parallel bright lines on the image of the far wall of each segment. The upper line represented the borderline between intima and lumen, while the lower line represented the borderline between media and adventitia. All the CIMTs were measured at a position free of atherosclerotic plaque. For each of the images, CIMT was measured twice at both systole and diastole and then averaged. The mean of overall CIMT was calculated as the average of CIMT at all 3 segments of carotid artery on both sides (6 values). To control the quality of CIMT measurement, the interobserver and intraobserver intraclass correlation coefficients were >0.90.

(4) Assessment of Other Covariates

The demographic information (age and sex), self-reported medical history (including diabetes and hypertension), drug-taking history (including lipid-lowering medication, anti-hypertensive medication, hypoglycemic drugs, or insulin use), smoking and alcohol drinking status (ever or never), and physical activity at leisure time (type of physical activity, frequency, and duration) were collected through the questionnaire administered by trained researchers. The ever-smokers were defined as individuals who smoked at least one cigarette per day for at least one year in their life. The ever-drinkers were defined as those who consumed ≥50 ml of liquor or any alcoholic beverage containing equivalent alcohol units.

Physical examinations, including height, weight, and blood pressure, were performed by trained researchers. Laboratory tests of biochemical indexes, such as HDLC, LDLC, triglyceride (TG), fasting blood glucose (FBG), and hemoglobin A1c (HbA1c), were performed by qualified technicians from the Laboratory of Molecular Epidemiology in the Department of Epidemiology at the Peking University. The standard protocol of physical examinations and laboratory tests was described elsewhere[34]. In our study, patients with diabetes included self-reported patients with diabetes and those without a self-reported history of diabetes but having abnormal glycemic markers (FBG ≥7.0 mmol/L or HbA1C >6.5%) based on our laboratory tests. Patients with hypertension included self-reported patients with hypertension and/or abnormal high levels of blood pressure (systolic blood pressure [SBP] ≥140 mmHg,] and/or diastolic blood pressure [DBP] ≥90 mmHg) in the blood pressure screening. Patients with dyslipidemia included those with following criteria: (1) TG ≥1.04 mmol/L; (2) HDLC <1.03 mmol/L for men and <1.29 mmol/L for female; (3) LDLC ≥4.14 mmol/L; or (4) regular intake of lipid-lowering medications during the past 2 weeks. The body mass index (BMI) was calculated as the ratio of weight (kg) and squared height (m^2).

Table 1. Characteristics of the study population and distribution of rs2066715 genotype frequencies for ischemic stroke ($N=5869$).

	stroke-free control ($N=4656$)	IS cases ($N=1213$)	p[§]
age (years)	58.39 (8.96)	59.81 (8.54)	<0.001
sex (%female)	54.07	35.20	<0.001
smoking (%ever-smoker)	43.59	59.60	<0.001
alcohol drinking (%ever-drinker)	33.81	44.02	<0.001
body mass index (kg/m^2)	25.88 (3.61)	26.43 (3.53)	<0.001
hypertension (%)	66.25	82.52	<0.001
diabetes (%)	5.93	3.63	0.002
dyslipidemia (%)	16.77	26.55	<0.001
moderate-high intensity physical activity (%)	46.99	35.81	<0.001
egg consumption (count/week)	4.73 (3.89)	3.54 (2.94)	<0.001
red meat consumption (kg/week)	0.29 (0.39)	0.24 (0.37)	<0.001
green vegetables consumption (kg/week)	4.69 (5.76)	4.47 (6.47)	0.287
mean CIMT (mm)	0.74 (0.15)	0.77 (0.15)	<0.001
carotid plaque (%)	55.92	68.1	<0.001
rs2066715_A (%)			0.393
AA	14.78	15.50	
AG	47.00	49.38	
GG	37.22	35.12	

Continuous variables are present as mean and standard deviation, and categoric variables are shown as percentage.
[§] P-value of chi-square test for categoric variables, and t test for continuous variables; Abbreviation: CIMT, carotid-intima media thickness.

(5) Genotyping

The genomic DNA was extracted and purified from venous blood sample using LabTurbo 496-Standard System (TAIGEN Bioscience Corporation, Taiwan). The purity and concentration of genomic DNA were measured using ultraviolet spectrophotometry. The DNA sample qualified for the next step of genotyping if the ratio of optical density at 260 nm and 290 nm ranged from 1.8 to 2.0. Subsequently, the genomic DNA sample was sent to be genotyped with the time of flight mass spectrum using MassARRAY® System (Agena Bioscience, San Diego, CA). To control the quality of genotype, two negative (blanks) and three positive (known genotypes of rs2066715: AA, AG, and GG) controls were used, and the call rate of rs2066715 was examined (>95%) to check the accuracy of genotyping. The SNP Hardy−Weinberg test was performed in randomly selected individuals, one from each family, and those who were free of IS ($P=0.468$).

(6) Statistical Methods

Categorical variables, such as sex, smoking status, alcohol consumption, moderate-high intensity physical activity, hypertension, diabetes, dyslipidemia, and the presence of carotid plaque were described as percentages in the case and control groups. Continuous variables, including age, BMI, LDLC, HDLC, TG, egg consumption per week, green vegetables' consumption, and mean CIMT were presented as mean (standard deviation [SD]). For the genotype rs2066715, an additive genetic model was employed to describe the distribution of risk allele.

To model the association between the count of A allele at rs2066715 and IS or its subtypes, multilevel logistic regressions were used to accommodate family-based design and ORs were derived to represent the genetic effect for an additional risk allele (rs2066715_A). To assess the effect of modification of egg consumption on the genetic association of rs2066715 with IS, we further divided the whole study population into two subgroups based on the median egg consumption per week (4 per week), and stratification analysis was implemented using the above-mentioned model. In addition, another multilevel logistic regression model was used to test the interaction between rs2066715 and egg consumption for the risk of IS or its subtypes by incorporating a cross-product term of rs2066715_A*egg consumption, as well as the main effect of rs2066715_A and egg consumption.

After analyzing the risk of IS or its subtypes, we investigated the association of rs2066715 with two measures of subclinical atherosclerosis (CIMT and pres-

Table 2. Association between rs2066715_A and ischemic stroke in all individuals and stratified by egg consumption category.

model	ischemic stroke		LAA subtype		SAO subtype		other subtypes	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
all individuals (*N* = 3731)	all individuals (*N* = 5869)		all individuals (*N* = 5491)		all individuals (*N* = 4873)		all individuals (*N* = 4794)	
model 1	1.06 (0.97, 1.16)	0.201	1.06 (0.95, 1.18)	0.294	1.01 (0.83, 1.23)	0.910	0.99 (0.77, 1.26)	0.909
model 2	1.07 (0.97, 1.17)	0.154	1.07 (0.96, 1.20)	0.191	1.03 (0.84, 1.25)	0.790	0.99 (0.77, 1.27)	0.941
model 3	1.07 (0.94, 1.21)	0.306	1.04 (0.91, 1.19)	0.524	1.04 (0.85, 1.27)	0.725	0.99 (0.77, 1.26)	0.922
stratification 1	egg consumption < 4/week (*N* = 3055)		egg consumption < 4/week (*N* = 2838)		egg consumption < 4/week (*N* = 2485)		egg consumption < 4/week (*N* = 2366)	
model 1	1.05 (0.93, 1.18)	0.433	1.00 (0.87, 1.14)	0.959	0.90 (0.70, 1.16)	0.406	0.95 (0.72, 1.24)	0.694
model 2	1.05 (0.93, 1.19)	0.391	1.02 (0.88, 1.17)	0.820	0.92 (0.72, 1.19)	0.541	0.97 (0.73, 1.27)	0.811
model 3	1.11 (0.94, 1.31)	0.224	0.95 (0.79, 1.13)	0.552	0.87 (0.67, 1.13)	0.304	0.97 (0.73, 1.28)	0.813
stratification 2	egg consumption ≥ 4/week (*N* = 2814)		egg consumption ≥ 4/week (*N* = 2653)		egg consumption ≥ 4/week (*N* = 2388)		egg consumption ≥ 4/week (*N* = 2428)	
model 1	1.08 (0.93, 1.26)	0.297	1.18 (0.99, 1.40)	0.061	1.23 (0.89, 1.71)	0.209	1.09 (0.62, 1.91)	0.759
model 2	1.10 (0.94, 1.28)	0.230	1.19 (1.00, 1.42)	0.047	1.24 (0.89, 1.72)	0.196	1.08 (0.61, 1.89)	0.796
model 3	1.01 (0.83, 1.23)	0.941	1.22 (0.99, 1.51)	0.066	1.31 (0.94, 1.83)	0.106	1.05 (0.59, 1.87)	0.865

Model 1 is unadjusted. Model 2 is adjusted for age, sex, and body mass index. Model 3 is further adjusted for smoking and alcohol drinking status, hypertension, diabetes, dyslipidemia, green vegetable consumption, red meat consumption, and moderate-high intensity physical activity. The cut-off of categorical egg consumption was defined based on the median of egg consumption per week in all individuals. Abbreviation: LAA, large artery atherosclerosis; SAO, small artery occlusion; OR, odds ratio; CI, confidence interval.

ence of carotid plaque). A total of 3731 subjects without confirmed stroke, self-reported coronary heart disease, and a history of taking lipid-lowering medications were included in the analyses. A mixed-effect linear regression model was used, and the coefficients (β) along with standard error (SE) were used to quantify the genetic effect on CIM, and a multilevel logistic regression model was used to assess the genetic effect on the risk of carotid plaque. Likewise, stratification analyses and interaction analyses were performed for carotid atherosclerosis.

To control the confounders, we established three models for each of the analysis mentioned above. Model 1 was unadjusted (no covariates) and model 2 was only adjusted for age, sex, and BMI. In model 3, we further adjusted for other covariates, including smoking and alcohol drinking status, hypertension, dyslipidemia (for the association of rs2066715 or rs2066715-egg interaction with IS or its subtypes) and levels of HDLC, LDLC, and TG (for the association of rs2066715 or rs2066715-egg interaction with carotid atherosclerosis), green vegetables' consumption, red meat consumption, and moderate-high intensity physical activity. Only those associations that remained significant in all the three models were considered robust and reliable. A *P*-value of < 0.05 (2-sided) was considered statistically significant. All the statistical analyses were conducted using R version 3.4.3.

Results

The characteristics of the study population and distribution of rs2066715 genotype frequencies for the risk of IS are presented in **Table 1**. Compared to

Table 3. Interaction between rs2066715_A and egg consumption and their associations with ischemic stroke.

model	ischemic stroke (N=5869)			LAA subtype (N=5491)			SAO subtype (N=4873)			other subtype (N=4794)		
	OR (95% CI)	P	$P_{interaction}$	OR (95% CI)	P	$P_{interaction}$	OR (95% CI)	P	$P_{interaction}$	OR (95% CI)	P	$P_{interaction}$
model 1												
rs2066715_A	1.10 (0.95, 1.28)	0.182	0.519	1.05 (0.89, 1.24)	0.572	0.862	0.89 (0.66, 1.2)	0.450	0.261	0.89 (0.62, 1.27)	0.508	0.461
egg	0.91 (0.88, 0.94)	<0.001		0.91 (0.87, 0.94)	<0.001		0.86 (0.80, 0.93)	<0.001		0.80 (0.73, 0.88)	<0.001	
model 2												
rs2066715_A	1.12 (0.96, 1.30)	0.141	0.494	1.08 (0.91, 1.28)	0.389	0.979	0.93 (0.69, 1.26)	0.659	0.360	0.92 (0.64, 1.33)	0.659	0.587
egg	0.90 (0.87, 0.93)	<0.001		0.90 (0.87, 0.93)	<0.001		0.86 (0.80, 0.92)	<0.001		0.79 (0.72, 0.88)	<0.001	
model 3												
rs2066715_A	1.22 (1.00, 1.50)	0.053	0.131	1.01 (0.81, 1.27)	0.897	0.732	0.87 (0.64, 1.19)	0.390	0.168	0.92 (0.63, 1.32)	0.637	0.600
egg	0.92 (0.88, 0.96)	<0.001		0.88 (0.84, 0.92)	<0.001		0.86 (0.80, 0.93)	<0.001		0.80 (0.72, 0.88)	<0.001	

Model is unadjusted. Model 2 is adjusted for age, sex, and body mass index. Model 3 is further adjusted for smoking and alcohol drinking status, hypertension, diabetes, dyslipidemia, green vegetable consumption, red meat consumption, and moderate-high intensity physical activity. Egg consumption is used as continuous variable (counts/week) here. Abbreviation: LAA, large artery atherosclerosis; SAO, small artery occlusion; OR, odds ratio; CI, confidence interval.

IS-free controls, the cases of IS were characterized by a higher BMI, larger numbers of smokers and drinkers, higher prevalence of hypertension and dyslipidemia, presence of carotid plaques, and thicker CIMT (P< 0.001).

After adjusting for the known covariates (age, sex, BMI, smoking and alcohol drinking status, hypertension and diabetes comorbidity, dyslipidemia, consumption of green vegetables and red meat, and moderate-high intensity physical activity), no significant association between the count of A allele at rs2066715 and IS (OR [95% CI]: 1.07 [0.94, 1.21], P=0.306) or any of its subtypes (LAA: 1.04 [0.91, 1.19], P=0.524; SAO: 1.04 [0.85, 1.27], P=0.725; and other: 0.99 [0.77, 1.26], P=0.922) was observed (**Table 2**). Subsequently, we investigated these associations stratified by egg consumption and did not observed any significant findings for IS or any of its subtypes after adjustment for all the covariates (**Table 2**). In the analysis of gene-egg interaction with the risk of IS (**Table 3**), no significant interaction between the count of A allele at rs2066715 and egg consumption was found associated with the risk of IS or any of its subtypes (IS: $P_{interacation}$=0.131; LAA: $P_{interacation}$=0.732; SAO: $P_{interacation}$=0.168; and other: $P_{interacation}$=0.600).

To further study the genetic association of rs2066715 with subclinical carotid atherosclerosis, we performed a series of similar analyses in individuals without a history of diagnosed stroke and self-reported coronary heart disease and not taking any lipid-lowering medications in the past six months (N=3731). Similar to the results observed for IS, we did not find any significant genetic association of rs2066715 with CIMT (β [SE], 0.01 [0.01], P=0.073) or with the risk of carotid plaque (OR [95% CI]: 1.06 [0.82, 1.36], P= 0.675) after adjustment for multiple covariates in all individuals (**Table 4**). However, after stratification for egg consumption (**Table 4**), we observed a significant and positive association of the count of A allele at rs2066715 with the risk of carotid plaque in subjects consuming <4 eggs per week (1.61 [1.08, 2.39], P=0.019). In individuals consuming ≥4 eggs per week (**Table 4**), the genetic association was not statistically significant (0.75 [0.53, 1.07], P=0.111). In the interaction analysis for subclinical carotid atherosclerosis (**Table 5**), an interaction between the count of A allele at rs2066715 and egg consumption was detected for the risk of carotid plaque ($P_{interacation}$=0.011). The risk of carotid plaque increased as egg consumption for the GG genotype at rs2066715 increased (1.12 [1.02, 1.22], P=0.013), but such trend was not observed for the AG or AA genotype (AG: 1.01 [0.94, 1.07], P=0.855; AA: 0.95 [0.82, 1.11], P=0.538)

Discussion

In this study, we found an interaction between an *ABCA1* variant (rs2066715) and egg consumption

Table 4. Association between rs2066715_A and carotid atherosclerosis in all individuals and stratified by egg consumption category.

model	mean CIMT (mm)		carotid plaque	
	β (SE)	*P*	OR (95% CI)	*P*
all individuals (*N* = 3731)				
model 1	0.01 (0.01)	0.183	0.99 (0.83, 1.18)	0.892
model 2	0.01 (0.01)	0.302	1.04 (0.85, 1.27)	0.727
model 3	0.01 (0.01)	0.073	1.06 (0.82, 1.36)	0.675
egg consumption < 4/week (*N* = 1655)				
model 1	0.01 (0.01)	0.468	1.41 (1.02, 1.95)	0.040
model 2	0.01 (0.01)	0.569	1.57 (1.09, 2.28)	0.016
model 3	0 (0.01)	0.849	1.61 (1.08, 2.39)	0.019
egg consumption ≥ 4/week (*N* = 2076)				
model 1	0.01 (0.01)	0.533	0.70 (0.52, 0.96)	0.026
model 2	0.01 (0.01)	0.291	0.70 (0.51, 0.98)	0.038
model 3	0.01 (0.01)	0.177	0.75 (0.53, 1.07)	0.111

Model 1 is unadjusted. Model 2 is adjusted for age, sex, and body mass index. Model 3 is further adjusted for smoking and alcohol drinking status, hypertension, diabetes, levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride, green vegetable consumption, red meat consumption, and moderate-high intensity physical activity. The cut-off of categorical egg consumption was defined based on the median of egg consumption per week in all individuals. Abbreviation: CIMT, carotid-intima media thickness; SE, standard error.

for the risk of carotid plaque after adjustment for multicovariates. In individuals who consume < 4 eggs per week, the A allele at rs2066715 increases the risk of carotid plaque. For those who carry the GG genotype on rs2066715, the risk of carotid plaque increases with the increase in egg intake per week. We did not detect any interactions for the risk of IS or CIMT.

Animal experiments have shown that the deficiency of ABCA1 was associated with the risk of atherosclerotic plaque[40, 41]. Epidemiological studies have also indicated that a decreased *ABCA1* expression, or mutation on *ABCA1*, was significantly associated with carotid plaques, especially unstable plaques[42-46]. The atherogenic property of abnormal *ABCA1* expression might be mediated by both impaired reverse cholesterol transportation from macrophage foam cell lipids to HDL and enhanced inflammation through peripheral lymphocyte proliferation and lipid raft formation[47-50] and modulated by the binding to several types of microRNAs[51-53]. Although rs2066715 has not been observed to be directly associated with metrics of carotid atherosclerosis, the function analysis of *ABCA1* supported our findings by revealing that rs2066715 substitution with A allele has a lower activity in mediating cholesterol efflux[54], which is further associated with increased CIMT, carotid plaque morphology, and atherosclerotic cardiovascular diseases[55, 56]. Eggs are cost-effective and nutrition-rich food containing a variety of bioactive components (e.g. phospholipids, lutein, zeaxanthin, egg proteins, and cholesterol) that influence pro- and anti- inflammation as well as pro- and anti- atherosclerosis processes[24, 57]. An in-vitro study suggested that high levels of egg yolk phosphatidylcholine promoted the ABCA1 activity and increased cholesterol absorption[24]. An RCT partly supported this relationship between egg intake and ABCA1 level by showing that whole egg intake increased the expression of *ABCA1* and improved endothelial inflammatory markers in patients with MetS under carbohydrate restriction diet compared with those consuming yolk-free eggs[25]. In the present study, we found a significant genetic association between the A allele at rs2066715 and the presence of carotid plaque only in those with low consumption of eggs, which might be explained by the synergistic effect of genetic mutation on *ABCA1* variant and reduced intake of bioactive ingredients in egg yolk (e.g. phosphatidylcholine).

Several strengths of this study are worth mentioning. First, our study is nested in a family-based study and multilevel regressions were used to model the genetic associations, which is robust against population stratification or admixture[58]. Second, most of the existing studies on the association between egg consumption and atherosclerotic diseases did not consider the diet components (such as red meat[59]) and physical activity parameters that might compete for the risk of IS[60-62]. In this study, we included diet (red meat and green vegetables' intake) and physical activity in addition to commonly used covariates (BMI, diabetes, hypertension, dyslipidemia, smoking and alcohol drinking status) as adjustment in the interaction analysis for the risk of IS and carotid atherosclerosis.

The first limitation of our study is that it is a cross-sectional study and that the information regard-

Table 5. Interaction between rs2066715_A and egg consumption and their associations with carotid atherosclerosis.

model	mean CIMT (mm, $N=3731$)			carotid plaque ($N=3731$)		
	β (SE)	P	$P_{interaction}$	OR (95% CI)	P	$P_{interaction}$
model 1						
rs2066715_A	0.01 (0.01)	0.215	0.402	1.50 (1.02, 2.22)	0.042	0.008
egg	0 (0)	0.601		1.10 (1.03, 1.18)	0.004	
model 2						
rs2066715_A	0.01 (0.01)	0.219	0.497	1.67 (1.09, 2.56)	0.019	0.006
egg	0 (0)	0.708		1.11 (1.03, 1.19)	0.007	
model 3						
rs2066715_A	0.01 (0.01)	0.440	0.823	1.70 (1.09, 2.68)	0.021	0.011
egg	0 (0)	0.828		1.11 (1.03, 1.20)	0.006	

Model 1 is unadjusted. Model 2 is adjusted for age, sex, and body mass index. Model 3 is further adjusted for smoking and alcohol drinking status, hypertension, diabetes, levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride, green vegetable consumption, red meat consumption, and moderate-high intensity physical activity. Egg consumption is used as continuous variable (counts/week) here. Abbreviation: CIMT, carotid-intima media thickness; SE, standard error.

ing diet, physical activity, and smoking and alcohol drinking status was not collected prior to IS diagnosis; thus, the results might be influenced by recall bias and misclassification due to the changes in dietary pattern (e.g. quit smoking and alcohol drinking, reduce red meat and egg intake, and increase vegetable consumption) as an intervention for IS patients, leading to a more conservative result. Secondly, we collected the whole egg intake through a semi-quantitative FFQ, which could not distinguish between those who consumed whole eggs and those who consumed egg yolks only. As the bioactive components are localized to different parts of eggs (e.g. cholesterol, phospholipid, lutein, and zeaxanthin were mainly found in egg yolks, but egg proteins are localized to egg whites), future studies should investigate the interaction with different fractions of eggs, in addition to different nutrients[57].

Conclusions

In conclusion, our study indicated that A allele at rs2066715 interacts with egg consumption for the risk of carotid plaque at a statistically significant level in the Northern Chinese population. Our results suggest that there is a synergistic effect of healthful egg-yolk components and the protective genotype of a variant on *ABCA1* gene on carotid atherosclerosis. However, due to the limitations of our study, the conclusion must be draw with caution and further longitudinal studies are needed to confirm the interaction between A allele at rs2066715 and egg consumption.

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Conflicts of Interest

The authors declare no conflicts of interest.

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