Associations for *BCO2*, *PCSK9*, and *TR1B1* Polymorphism and Lifestyle Factors with Ischemic Stroke: A Nested Case-Control Study

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Purpose: To investigate associations for polymorphisms in β-carotene 9',10'-oxygenase (*BCO2*, rs10431036 and rs11214109), proprotein convertase subtilisin kexin type 9 (*PCSK9*, rs11583680), and tribbles pseudokinase 1 (*TRIB1*, rs17321515 and rs2954029), as well as lifestyle factors, with ischemic stroke (IS).

Materials and Methods: This nested case-control study included 161 patients with IS and 483 matched control individuals. We collected medical reports, lifestyle details, and blood samples from individuals and used the PCR-ligase detection reaction method to genotype single nucleotide polymorphisms (SNPs).

Results: The GA+AA genotype of rs10431036 (*p*<0.001) and rs17321515 (*p*=0.003), the CT+TT genotype of rs11214109 (*p*=0.005), and the TA+AA genotype of rs2954029 (*p*=0.006) in dominant models increased the risk of IS. In additive models, the GG genotype of rs17321515 (*p*=0.005) and the TT genotype of rs2954029 (*p*=0.008) increased the risk of IS. Adequate intake of fruits/vegetables reduced the risk of IS (*p*=0.005). Although there was no interaction between genes and fruits/vegetables, people with inadequate intake of fruits/vegetables who carried a risk genotype had a higher risk of IS than those only having inadequate fruits/vegetables intake or those only carrying a risk genotype. Also, the haplotypes AC, AT, and GT (comprising rs10431036 and rs11214109) and GT (comprising rs2954029 and rs17321515) were found to be associated with an increased risk of IS (*p*<0.05).

Conclusion: Polymorphisms in *BCO2* and *TRIB1* and fruits/vegetables intake were associated with IS. These results provide the theoretical basis for gene screening to prevent chronic cerebrovascular diseases.

Key Words: *BCO2*, *PCSK9*, *TR1B1*, polymorphism, ischemic stroke

INTRODUCTION

Stroke, as a heterogeneous syndrome, is one of the leading causes of mortality and morbidity, with astronomical financial repercussions for health systems worldwide. According to the World Health Organization, 15 million people suffer strokes

worldwide each year. Of these, more than 6 million die and another 5 million are permanently disabled.[1] Epidemiological studies have indicated that about 70% of cases worldwide are ischemic strokes (IS), which are featured by disruption of cerebral blood flow and a hypoxia in the affected area.[2]

IS is caused by many factors, such as diabetes mellitus, obesity, dyslipidemia, and atherosclerosis.[3-5] It has a strong genetic basis, demonstrated by family associations and degrees of genetic influence as high as 40−50%. Genetic studies have shown that a few specific gene variants of lipid metabolism affect the pathogenesis of IS.[6-10] Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) plays a major part in type 2 diabetes and atherogenesis development, which ultimately leads to IS.[11,12] β-carotene 9',10'-oxygenase (*BCO2*) and tribbles pseudokinase 1 (*TRIB1*) are important to maintaining normal lipid and cholesterol homeostasis,[13,14] reducing the occurrence of cardiovascular and

cerebrovascular diseases. In addition, lifestyle factors, such as fruits and vegetables intake, have been suggested as influencing factors for IS.[15-17]

Therefore, we sought to investigate associations for five single nucleotide polymorphisms (SNPs) in *BCO2*, *PCSK9*, and *TRIB1* and for lifestyle factors (fruits/vegetables intake) with IS in southern China by a community-based nested case-control study.

MATERIALS AND METHODS

Subjects

A total of 2323 people who underwent physical examinations at the community health service center and had dyslipidemia from April 2013 to July 2013 were randomly selected from four townships under the jurisdiction of a certain district of Ningbo, Zhejiang Province as a survey point using cluster random sampling. All subjects were unrelated and >40 years of age. Tumors, aneurysms, patients after surgery or trauma, infections, subarachnoid hemorrhage, and women during pregnancy and baby nursing period were excluded. We gather all the subjects' life behavior information and blood samples. In order to understand the incidence of IS among the subject, we paid a visit in August 2016. The case group comprised 161 patients diagnosed with IS between April 2013 and August 2016. A total of 483 subjects were matched 1:3 as controls according to age and gender. All participants joined this study with informed consents, which got the permission by the Medical Ethics Committee of Hangzhou Normal University (No. 2013020).

Diagnostic criteria

Dyslipidemia was defined according to the Guidelines for the Prevention and Treatment of Diabetes in Chinese Adults prepared by the Joint Committee of the Chinese Association for the Prevention and Treatment of Dyslipidemia. Total cholesterol (TC) >5.18 mmol/L, triglycerides (TG) >1.70 mmol/L, high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L, and low-density lipoprotein cholesterol (LDL-C) >3.37 mmol/L represent abnormal values. One or more of the four blood lipid indicators above were deemed indicative of a diagnosis of dyslipidemia. Subjects diagnosed with dyslipidemia and taking hypolipidemic drugs were also considered under the dyslipidemia category. Diagnosis of IS was based on the diagnostic criteria issued by the Society of Neurology, Chinese Medical Association in 2014.[18] Patients were diagnosed as having IS with clinical manifestation and brain MRI and/or head CT.

Epidemiological investigation

The field epidemiological survey mainly consisted of fundamental demographic criteria such as age, sex, occupation, and education level, as well as information on lifestyle such as smoking, drinking, the intake of fruits/vegetables. The intake of fruits/vegetables and fruits survey employed a semi-quantita-

tive questionnaire: average daily intake of fruits/vegetables less than 300 g was defined as "inadequate fruits/vegetables intake," and average daily fruits/vegetables intake more than 300 g was defined as "adequate fruits/vegetables intake." Anthropometric data, including body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), and HDL-C and LDL-C levels, were evaluated by professional medical examination according to standard protocols.

SNP selection and genotyping

Five SNPs in *BCO2*, *TRIB1*, and *PCSK9* were selected using the HapMap website (http://www.hapmap.org) and the Haploview 4.2 software (Broad Institute, Cambridge, MA, USA). The SNPs included rs10431036 and rs11214109 in *BCO2*, and rs17321515 and rs2954029 in *TRIB1*, rs11583680 in *PCSK9*. The minor allele frequencies (MAFs) of these five SNPs were greater than 5%, and the linkage disequilibrium (LD) was $r^2>0.8$.

A refrigerator at -80°C was selected to store 5 mL of whole blood from each fasted individual, which was anticoagulated with EDTA. DNA was extracted using Tiangen Blood Genomic DNA extraction kits (Tiangen Biotech, Beijing, China) and sent to Shanghai Jierui Biological Engineering Co., Ltd. (Shanghai, China), for genotyping analysis using the PCR-ligase detection reaction method (Generay Biotech Company, Shanghai, China). For quality control, we randomly chose 10% of samples for re-genotyping, and the concordance rates were 96.8% (rs10431036), 97.7% (rs11214109), 98.9% (rs17321515), and 99.2% (rs2954029).

Statistical analysis

Statistical analysis was completed using SPSS 24.0 software (IBM Corp., Armonk, NY, USA). Demographic characteristics and the SNP genotypes of genes were evaluated using Student's t-test, χ^2 test, Fisher's exact test (for categorical variables), and Wilcoxon's rank sum test (for continuous variables). The χ^2 test was used to test for Hardy-Weinberg equilibrium (HWE). Allelic frequencies were analyzed using Fisher's exact test to generate the reported *p* values. Logistic regression analysis was used to determine the odds ratio (OR) of the associations between genetic model and lifestyle factors and IS risk. Using Microsoft Excel according to Knol, et al.,[19] we identified relative excess risk due to interaction (RERI), ORs, and 95% confidence intervals (CIs). The associations between gene haplotypes and the risk of IS were calculated using the R language package "haplo.stats" (R Foundation for Statistical Computing, Vienna, Austria). In all analyses, differences were deemed significant if *p*<0.05.

RESULTS

Basic characteristics, lifestyle factors, and genotype distribution in case and control groups

The subjects included 161 cases and 483 controls: 45.3% of the

subjects were female, and 54.7% were male. The medians of LDL-C in the case group were significantly higher than those in the control group ($p<0.05$) (Table 1). The χ² test revealed significant differences in fruits/vegetables intake and the genotypes of rs10431036, rs11214109, rs11583680, rs17321515, and rs2954029 between the case group and the control group ($p<0.05$). All of the studied SNPs in the control subjects were in HWE ($p>0.05$) (Supplementary Table 1, only online).

Associations of genetic models and lifestyle with the risk of IS

The associations among *BCO2* and *TRIB1* and lifestyle factors with IS were examined under each gene model. With or without adjustment for confounding factors of age, sex, waist circumference, smoking, drinking, LDL-C, the dominant models of four SNPs (rs10431036, rs11214109, rs17321515, and rs2954029), the additive models of two SNPs (rs17321515 and rs2954029), and fruits/vegetables intake were found to be significantly as-

Table 1. General Characteristics, Lifestyle Factors, and Genotype Distribution between Case and Control Groups

Characteristics	Case (n=161)	Control (n=483)	t/z/χ²	*p* value*
Age, median (IQR), yr	66 (19)	66.5 (16)	-0.236	0.813
Sex, n (%)			0.000	0.999
Male	88 (55.1)	264 (55.1)		
Female	73 (44.9)	219 (44.9)		
Waist circumference, median (IQR), cm	81 (11)	80 (11)	-0.329	0.732
SBP, median (IQR), mm Hg	139 (30)	140 (26)	-1.538	0.124
DBP, median (IQR), mm Hg	82 (14)	83 (13)	-0.895	0.371
BMI, median (IQR), kg/m²	22.98 (3.85)	23.18 (4.07)	-1.056	0.291
TC, median (IQR), mmol/L	4.93 (1.54)	4.95 (1.26)	-0.164	0.870
TG, median (IQR), mmol/L	1.34 (0.87)	1.33 (0.89)	-0.109	0.913
HDL-C, median (IQR), mmol/L	1.21 (0.35)	1.21 (0.37)	-0.728	0.466
LDL-C, median (IQR), mmol/L	3.165 (1.43)	2.94 (1.15)	-2.477	0.013
Current smoking, n (%)	35 (20.2)	106 (21.2)	0.073	0.787
Current alcohol intake, n (%)	43 (24.9)	148 (29.6)	1.423	0.233
Fruits/vegetables intake, n (%)			9.284	0.002
Inadequate	24 (14.9)	129 (26.7)		
Adequate	137 (85.1)	354 (73.3)		
rs10431036			18.957	<0.001
GG	45 (28.3)	217 (45.02)		
GA	95 (59.7)	203 (42.12)		
AA	19 (12)	62 (12.86)		
rs11214109			11.582	0.009
CC	49 (2.3)	212 (3.8)		
CT	88 (27.2)	210 (34.4)		
TT	22 (70.5)	60 (61.8)		
rs11583680			7.42	0.06
CC	114 (2.9)	384 (79.84)		
CT	41 (29.5)	93 (19.33)		
TT	4 (67.3)	4 (0.83)		
rs17321515			14.642	0.002
GG	31 (19.3)	162 (33.60)		
GA	90 (60)	236 (48.97)		
AA	38 (23.6)	84 (17.43)		
rs2954029			13.071	0.004
TT	33 (20.75)	163 (33.82)		
TA	88 (55.35)	235 (48.76)		
AA	38 (23.9)	84 (17.42)		

IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
p value <0.05 was considered statistically significant and maintained significance using the Benjamini-Hochberg procedure with the false discovery rate at 0.09.
*Student's t-test, χ² test and Wilcoxon's rank sum test.

Table 2. Associations of Genetic Models and Lifestyles with Risk of Ischemic Stroke

SNP/lifestyle	Genotype/Classification	Unadjusted OR (95% CI)	Unadjusted *p* value	Adjusted OR (95% CI)	Adjusted *p* value
rs10431036					
Additive	GA/GG	2.26 (1.51–3.38)	<0.001	2.36 (1.56–3.57)	<0.001
	AA/GG	1.48 (0.81–2.71)	0.207	1.61 (0.87–2.99)	0.131
Dominant	GA+AA/GG	2.0 (1.36–2.93)	<0.001	2.08 (1.41–20.08)	<0.001
Recessive	AA/GA+GG	1 (0.59–1.70)	0.998	1.07 (0.62–1.82)	0.828
rs11214109					
Additive	CT/CC	1.81 (1.22–2.70)	0.003	1.86 (1.24–2.79)	0.003
	TT/CC	1.59 (0.89–2.83)	0.118	1.59 (0.88–2.87)	0.124
Dominant	CT+TT/CC	1.70 (1.17–2.48)	0.006	1.73 (1.18–2.54)	0.005
Recessive	TT/CT+CC	1.21 (0.73–2.02)	0.460	1.20 (0.72–2.02)	0.484
rs17321515					
Additive	GA/AA	2.0 (1.27–3.14)	0.003	1.93 (1.22–3.06)	0.005
	GG/AA	2.36 (1.37–4.06)	0.002	2.23 (1.28–3.87)	0.005
Dominant	GA+AA/GG	1.98 (1.29–3.03)	0.002	1.94 (1.26–2.99)	0.003
Recessive	AA/GA+AA	1.55 (1.01–2.37)	0.045	1.51 (0.98–2.32)	0.064
rs2954029					
Additive	TA/AA	1.85 (1.18–2.89)	0.007	1.79 (1.14–2.82)	0.012
	TT/AA	2.23 (1.31–3.81)	0.003	2.10 (1.20–3.63)	0.008
Dominant	TA+AA/TT	1.85 (1.22–2.82)	0.040	1.82 (1.19–2.77)	0.006
Recessive	AA/TA+TT	1.55 (1.01–2.37)	0.045	1.51 (0.98–2.33)	0.064
rs11583680					
Additive	CT/CC	1.49 (0.97–2.273	0.067	1.42 (0.90–2.22)	0.132
	TT/CC	3.37 (0.83–13.68)	0.089	3.84 (0.30–16.87)	0.075
Dominant	(CT+TT) / CC	1.54 (1.02–2.33)	0.038	1.49 (0.96–2.31)	0.074
Recessive	TT/CT+CC	3.07 (0.98–9.68)	0.055	2.79 (0.83–9.42)	0.097
Fruits/vegetables	Inadequate/adequate	0.48 (0.30–0.78)	0.003	0.49 (0.30–0.80)	0.005

OR, odds ratio; CI, confidence interval.
Adjusted for age, sex, body mass index, waist, low-density lipoprotein cholesterol, smoking, drinking in logistic regression model. *p* value <0.05 was considered statistically significant and maintained significance using the Benjamini-Hochberg procedure with the false discovery rate at 0.09.

sociated with IS (Table 2). In dominant models, the GA+AA genotype of rs10431036, the CT+TT genotype of rs11214109, the GA+AA genotype of rs17321515, and the TA+AA genotype of rs2954029 increased the risk of IS (adjusted OR=2.08, 95% CI = 1.41–20.08, *p*<0,001; adjusted OR=1.73, 95% CI=1.18–2.54, *p*= 0.005; adjusted OR=1.94, 95% CI=1.26-2.99, *p*=0.003; adjusted OR=1.82, 95% CI=1.19–2.77, *p*=0.006). In additive models, the GG genotype of rs17321515 and the TT genotype of rs2954029 increased the risk of IS (adjusted OR=2.23, 95% CI=1.28–3.87, *p*=0.005; adjusted OR=2.10, 95% CI=1.20–3.63, *p*=0.008). Adequate intake of fruits/vegetables posed lower susceptibility to IS than inadequate fruits/vegetables intake (adjusted OR=0.49, 95% CI=0.30–0.80, *p*=0.005).

Interactions among *BCO2*, *TRIB1*, lifestyle factors, and IS

Table 3 show the effects of the interactions among four SNPs (rs10431036, rs11214109, rs17321515, and rs2954029) in two genes (*BCO2* and *TRIB1*) and lifestyle factors on IS. The following results were adjusted for age, sex, waist circumference, BMI, smoking, drinking, and LDL-C. In rs10431036, compared

with people who carried the GG genotype and had adequate intake of fruits/vegetables, those with the GA+AA genotype who had adequate/inadequate intake of fruits/vegetables and with the GG genotype who had inadequate fruits/vegetables were at a higher risk of IS (OR=5.84, 95% CI=1.63-20.86, *p*= 0.007; OR=9.33, 95% CI=2.82-30.86, *p*<0.001; OR=5.12, 95% CI= 1.51-17.28, *p*=0.009). In rs11214109, compared with people who carried the GG genotype and had adequate intake of fruits/vegetables, those with the CT+TT genotype who had adequate/ inadequate intake of fruits/vegetables and with the CC genotype who had inadequate fruits/vegetables were at a higher risk of IS (OR=5.66, 95% CI=1.58-20.25, *p*=0.008; OR=8.53, 95% CI=2.57-28.29, *p*<0.001; OR=5.74, 95% CI=1.70-19.35, *p*= 0.005). Compared with people who carried the GG genotype of rs17321515 and had adequate intake of fruits/vegetables, those with the GA+AA genotype who had adequate or inadequate intake of fruits/vegetables and those with the GG genotype who had inadequate fruits/vegetables were at a higher risk of IS (OR=4.67, 95% CI=1.03-21.22, *p*=0.046; OR=8.77, 95% CI= 2.04-37.80, *p*=0.004; OR=4.81, 95% CI=1.08-21.40, *p*= 0.039). In rs2954029, compared with people who carried the

Table 3. Interaction between Gene Polymorphism and Fruits/Vegetables Intake for the Risk of Ischemic Stroke

Genotypes	Adequate fruits/ vegetables		Inadequate fruits/ vegetables		OR (95% CI) for inadequate fruits/vegetables within strata of genotype	RERI* (95%CI)	p value
	Case/ control (n)	OR (95% CI)	Case/ control (n)	OR (95% CI)			
rs10431036						-0.62 (-5.19–3.94)	0.789
Non-risk allele carriers (GG)	3/56		44/162				
		1		5.12 (1.51–17.28) p=0.009	5.12 (1.51–17.28) p=0.009		
Risk allele carriers (GA+AA)	21/73		93/192				
		5.84 (1.63–20.86) p=0.007		9.33 (2.82–30.86) p<0.001	1.52 (0.86–2.70) p=0.151		
OR (95% CI) for risk allele carriers within strata of fruits/vegetables intake		5.84 (1.63–20.86) p=0.007		1.84 (1.20-2.82) p=0.005			
rs11214109						-1.82 (-7.05–3.39)	0.493
Non-risk allele carriers (CC)	3/55		48/158				
		1		5.74 (1.70–19.35) p=0.005	5.74 (1.70–19.35) p=0.005		
Risk allele carriers (CT+TT)	21/74		89/196				
		5.66 (1.58–20.25) p=0.008		8.53 (2.57–28.29) p<0.001	1.51 (0.99–2.29) p=0.055		
OR (95% CI) for risk allele carriers within strata of fruits/vegetables intake		5.66 (1.58–20.25) p=0.008		1.46 (0.82–2.60) p=0.200			
rs17321515						0.287 (-3.49–4.06)	0.881
Non-risk allele carriers (GG)	2/38		31/125				
		1		4.81 (1.08–21.40) p=0.039	4.81 (1.08–21.40) p=0.039		
Risk allele carriers (GA+AA)	22/91		106/229				
		4.67 (1.03–21.22) p=0.046		8.77 (2.04–37.80) p=0.004	1.79 (1.03–3.09) p=0.036		
OR (95% CI) for risk allele carriers within strata of fruits/vegetables intake		4.67 (1.03–21.22) p=0.046		1.19 (1.17–2.98) p=0.009			
rs2954029						-0.13 (-4.10–3.82)	0.950
Non-risk allele carriers (TT)	2/38		33/126				
		1		5.07 (1.14–22.53) p=0.033	5.07 (1.14–22.53) p=0.033		
Risk allele carriers (TA+AA)	22/91		104/228				
		4.66 (1.03–21.17) p=0.046		8.61 (1.99–37.11) p=0.004	1.78 (1.03–3.07) p=0.04		
OR (95% CI) for risk allele carriers within strata of fruits/vegetables intake		4.66 (1.03–21.17) p=0.046		1.74 (1.10–2.75) p=0.018			

OR, odds ratio; CI, confidence interval; RERI, relative excess risk due to interaction.
Adjusted for age, sex, waist circumference, body mass index, smoking, drinking, low-density lipoprotein cholesterol. p value <0.05 was considered statistically significant and maintained significance using the Benjamini-Hochberg procedure with the false discovery rate at 0.09.
*RERIs were calculated according Knol, et al.[19]

TT genotype and had adequate intake of fruits/vegetables, those with the TA+AA genotype who had adequate/ inadequate intake of fruits/vegetables and those with the CC genotype who had inadequate fruits/vegetables were at a higher risk of IS (OR=4.66, 95% CI=1.03–21.17, p=0.046; OR=8.61, 95% CI= 1.99–37.11, p<0.004; OR=5.07, 95% CI=1.14–22.53, p=0.033).

The interactions between gene polymorphism and fruits/ vegetables intake were not found in the additive model (p values of RERI >0.05).

Associations for *BCO2* and *TRIB1* haplotypes with IS risk

LD analysis for the SNPs showed obvious LD between two pairs of SNPs (Supplementary Table 2, only online). The hap-

Table 4. Frequencies of Haplotypes among Cases and Controls and Association with Risk of Ischemic Stroke Haplotypes

Haplotypes	Frequency	Case, n (%)	Controls, n (%)	OR (95% CI)	p value
rs10431036–rs11214109					
GC	0.6241	174.55 (54.9)	624.89 (64.8)	1	
AC	0.0148	11.45 (3.6)	9.11 (0.9)	6.30 (2.34–16.95)	<0.001
AT	0.3432	121.55 (38.2)	317.89 (33.0)	1.42 (1.07–1.88)	0.015
GT	0.0180	10.45 (3.3)	12.11 (1.3)	3.11 (1.28–7.56)	0.012
rs2954029–rs17321515					
AA	0.5530	152.00 (47.8)	560.00 (58.1)	1	
GT	0.4446	164.00 (51.6)	403.00 (41.8)	1.52 (1.16–1.99)	0.002
Rare group*	0.0024	1.93 (0.6)	1.00 (0.1)	10.36 (0.90–119.47)	0.061

OR, odds ratio; CI, confidence interval.
Adjusted for age, sex, waist circumference, body mass index, smoking, drinking, low-density lipoprotein cholesterol. p value <0.05 was considered statistically significant and maintained significance using the Benjamini-Hochberg procedure with the false discovery rate at 0.09.
*Haplotypes with frequency <0.01 were pooled into the rare group.

lotype frequencies of the four SNPs were compared between IS cases and control subjects (Table 4). The haplotypes AC, AT, and GT (comprising rs10431036 and rs11214109) were found to be associated with an increased risk of IS (OR=6.30, 95% CI= 2.34–16.95, *p*<0.001; OR=1.42, 95% CI=1.07–1.88, *p*=0.015; OR= 3.11, 95% CI=1.28–7.56, *p*=0.012). The haplotype GT (comprising rs2954029 and rs17321515) was also found to be associated with an increased risk of IS (OR=1.52, 95% CI=1.16–1.99, *p*= 0.002).

DISCUSSION

Here, we investigated associations for *BCO2*, *TR1B1*, and *PCSK9* polymorphisms with IS in Chinese Han people in southern China. The results showed that different genetic backgrounds and lifestyles would lead to different risks of IS.

TRIB1 is a gene that plays a structural role in the cholesterol metabolism and the atherosclerosis process. Our study showed that the GG genotype of rs17321515 and the TT genotype of rs2954029 in *TRIB1* in dominant and addictive models posed a higher risk of IS. The association remained the same after adjustment for confounding factors. These results indicated that the G mutation site of rs17321515 and the T mutation site of rs2954029 were risk factors for IS, which was consistent with the study by Járomi, et al.[20] rs2954029 is located on chromosome 8q24 and encodes the protein tribbles homolog 1, which has been shown to be associated with LDL-C, HDL-C, and TG concentrations, as well as the risk of CAD.[21] Also, rs17321515 was discovered as a very compelling SNP effecting lipoprotein metabolism.[14] The overexpression of *TRIB1* significantly reduced the plasma levels of very low-density lipoprotein, LDL-C, HDL-C, and TG, which may be a possible mechanism underlying the association between *TRIB1* and IS.

BCO2 is a potential candidate gene that plays a substantial role in the inner membrane of mitochondria through regulation of electron transport chain complexes. Our study showed that the GG genotype of rs10431036 and the CC genotype of

rs11214109 of *BCO2* in a dominant model posed a higher risk of IS. The association remained after adjustment for confounding factors. These were newly identified SNPs for susceptibility to IS. No studies on *BCO2* and IS have been reported. Nevertheless, genetic studies have linked *BCO2* mutations to obesity in humans,[22,23] and obese people are thought to be more susceptible to IS, suggesting that *BCO2* might influence IS in obese individuals.

This study also analyzed associations between fruits/vegetables intake and IS. We found that adequate fruits/vegetables intake could reduce the risk of IS, which was consistent with most previous studies.[15-17] Fruits/vegetables can effectively protect the cardio-cerebrovascular system from damage, while lacking fruits/vegetables was found to result in up to twice the incidence of cardio-cerebrovascular events.[15,16] This association can be explained by several biological mechanisms. An adequate intake of fruits/vegetables lowers blood pressure and improves microvascular function.[24] It also has favorable effects on other cardiovascular risk factors, such as plasma levels of TC and LDL-C, BMI, waist circumference, and inflammation, thereby reducing the risk of IS. In addition, adequate intake of fruits and vegetables increases micronutrients, carbohydrates, and fiber levels and potentially reduces fat intake and the risk of IS.[17]

In case-control studies, RERI is generally considered the standard measure of additive model interactions. Our study investigated the interactions of gene-lifestyle factors with the risk of IS. People who ate inadequate fruits/vegetables or carried risk genotypes had a higher risk of IS. The risk of IS increased when two risk factors were present together, compared to either of them alone, though the interaction index of four SNPs and fruits/vegetables intake was not statistically significant. In addition, we found that the haplotypes AC, AT, and GT, composed of rs10431036 and rs11214109, and the haplotype GT, composed of rs2954029 and rs17321515, increased the risk of IS.

This study had some limitations. First, the study might lead to unstable research results, owing to the small sample size, which makes our observations highly susceptible to type 2 er-

rors. Second, community-based cohorts involving self-reported data might suffer from an uncertainty concerning the validity of the data. Third, a change of exposure to environmental risk factors during the follow-up period were difficult to assess. Finally, the research conclusions might only be applicable to people in southern China because of geographical restrictions. To confirm our present findings, randomized, large-scale, long-term studies from different regions and ethnic or social background will be needed.

In conclusion, polymorphisms of two SNPs (rs10431036 and rs11214109) in *BCO2*, two SNPs (rs17321515 and rs2954029) in *TRIB1*, and fruits/vegetables intake were found to be associated with IS in people in southern China. These results provide the theoretical basis for gene screening to prevent cerebrovascular diseases.

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AUTHOR CONTRIBUTIONS

Tian-Yu Zhao, Zheng Li, and Lei Yang had the original idea for the study and, with all co-authors, carried out the design. Song Lei was responsible for recruitment and follow-up of study participants. Tian-Yu Zhao drafted the manuscript, which was revised by all authors. Liu Huang and Song Lei provided advanced statistical methods. All authors read and approved the final manuscript.

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