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RESEARCH ARTICLE

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Association study of polymorphisms in the *ABO* gene and their gene-gene interactions with ischemic stroke in Chinese population

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Aims: To investigate the impact of 4 single nucleotide polymorphisms (SNPs) within *ABO* gene and their gene-gene interactions on ischemic stroke (IS) susceptibility in Chinese Han population.

Methods: A total of 1993 participants (1375 males, 618 females) were selected, including 991 IS patients and 1002 normal controls. The SNPstats (http://bioinfo.iconcologia.net/SNPstats) was used for Hardy-Weinberg equilibrium (HWE) test. Generalized multifactor dimensionality reduction (GMDR) was used to screen the best interaction combination among 4 SNPs within *ABO* gene. Logistic regression was performed to calculate the ORs (95%CI) for interaction between SNPs.

Results: Both rs579459 and rs505922 within *ABO* gene were associated with IS risk in additive and dominant models. IS risks were higher in those with minor alleles of rs579459 and rs505922 than those with wild-type homozygotes, OR (95%CI) were 1.62 (1.19-2.10) and 1.69 (1.23-2.18), respectively. We did not find any relation of rs651007 and rs529565 with IS risk in both additive and dominant models. GMDR model indicated a significant two-locus model ($P = .0010$) involving rs505922 and rs579459, indicating a potential interaction between rs505922 and rs579459, the cross-validation consistency of the two-locus models was 9/10, and the testing accuracy was 60.72%. We also found that participants with rs505922- TC/CC and rs579459- TC/CC genotype have the highest IS risk, compared to participants with rs505922- TT and rs579459- TT genotype, OR (95%CI) was 2.94 (1.28-4.66).

Conclusions: We found that rs579459 and rs505922 within ABO gene and their interaction were both associated with increased IS risk in Chinese population.

KEYWORDS

ABO gene, interaction, ischemic stroke, single nucleotide polymorphism

1 | INTRODUCTION

Stroke is one of the main causes of death and adult disability around the world.[1,2] In China, there were 2.5 million new stroke cases each year and 7.5 million stroke survivors.[2] In the subtypes of the stroke, the ischemic stroke (IS) is the most type, and about 43%-79% of all strokes are ischemic in China.[2,3] Increasing evidence indicated that IS was a complex clinical syndrome resulting from environmental and genetic factors.[4,5] Many environmental risk factors for IS have been reported, including age, hypertension, type 2 diabetes mellitus (T2DM), obesity, smoking. However, these conventional risk factors could not completely explain all IS risk, family and twin-based studies demonstrated that genetic factors also play a key role in the development of IS.[6,7]

The ABO blood group system is encoded by *ABO* gene, which located around 9q34.2, encodes glycosyltransferases, catalyze the

transfer to different carbohydrate groups onto the H antigen, thus forming A and B antigens of the *ABO* system.[8,9] In recent years, several single nucleotide polymorphisms (SNPs) have been reported, and the genetic variants of *ABO* gene were associated with several diseases, including coronary artery disease (CAD),[10] venous thromboembolism,[11] however, just few studies focused on the association between *ABO* gene SNPs and IS were reported and the results of these studies were inconsistent.[12-14] In addition, no study focused on the impact of interaction among several SNPs within *ABO* gene on IS risk, particularly in Chinese population. So the aim of this study was to investigate the impact of *ABO* gene SNPs, and their gene-gene interactions on IS risk based on Chinese population with a relatively larger sample size.

2 | MATERIALS AND METHODS

2.1 | Subjects

Our study sample recruited 1993 participants (1375 males, 618 females) between July 2011 and March 2015 from the first Affiliated Hospital of Jinan University and the People's Hospital of Maoming, including 991 IS patients and 1002 normal controls. The mean age of all participants was 67.9 ± 15.2 years. The diagnosis of IS the subtype of IS were determined in accordance with World Health Organization criteria[15,16] and the original TOAST20 (Trial of ORG 10172 in Acute Stroke Treatment) criteria. All patients were diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) within 48 hours after the admission. Blood vessels were evaluated with neck vascular ultrasound and brain color Doppler as well as CT angiography or magnetic resonance angiography. Controls were matched by approximate 1:1 matched to patients on the basis of age (±2 years) and sex. Normal controls with family history of IS were excluded. Written informed consent was obtained from all participants. Both doctors and study subjects provided consent to participate in this study. The protocol of this study was approved by the Ethics Committee of Jinan University.

TABLE 1 General characteristics of 1993 study participants in case and control group

Variables	Case group (n = 991)	Control group (n = 1002)	*P*-values
Age (y)	68.3 ± 15.9	67.6 ± 16.3	.332
Males, N (%)	680 (68.6)	695 (69.4)	.720
BMI(kg/m^2)	24.6 ± 9.3	23.7 ± 9.6	.034
FPG (mmol/L)	7.1 ± 3.5	6.1 ± 3.7	<.001
TG (mmol/L)	1.5 ± 0.7	1.3 ± 0.8	<.001
TC (mmol/L)	4.8 ± 1.2	4.4 ± 1.3	<.001
HDL (mmol/L)	1.26 ± 0.8	1.31 ± 0.9	<.001
Smoking, N (%)	473 (47.7)	368 (36.7)	<.001
Alcohol drinking, N (%)	512 (51.7)	428 (42.7)	<.001
T2DM, N (%)	329 (33.2)	192 (19.2)	<.001
Hypertension, N (%)	548 (55.3)	327 (32.6)	<.001

BMI, body mass index; FPG, fast plasma glucose; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglyceride.
Means ± standard deviation for age, BMI, FPG, TC, TG, HDL; Number and percentages for males, smokers, drinkers.

2.2 | Definition

Current cigarette smokers were those who self-reported smoking cigarettes at least once a day for 1 year or more. Alcohol consumption was expressed as the sum of milliliters of alcohol per week from wine, beer, and spirits. Hypertension was defined as systolic blood pressure (SBP) ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥90 mm Hg and/or use of antihypertensive medication. The criteria for the diagnosis of type 2 diabetes mellitus (T2DM) included a fasting glucose ≥126 mg/dL (7.0 mmol/L), or a 2 hours postprandial blood glucose ≥200 mg/dL (11.0 mmol/L), or if hypoglycemic therapy (oral agents or insulin) had been started in the interim.

2.3 | Genomic DNA extraction and genotyping

SNPs within *ABO* gene were evaluated and selected using the HapMap database (https://hapmap.ncbi.nlm.nih.gov/) according to the following criterion: (1) a minor allele frequency (MAF) >5%; (2) which have been reported associations with IS or IS related risk factors in previous studies. At last, a total of 4 SNPs within *ABO* gene were selected for genotyping: rs505922, rs579459, rs651007, and rs529565. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at −20°C until use. Genotyping for 4 SNPs were tested using Sequenom MassARRAY platform (San Diego, U.S) at CapitalBio Corporation (Beijing, China). Genomic DNA was isolated from human peripheral blood samples of each individual through Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA concentration was determined by DNA spectrophotometer (ND-1000, NanoDrop, Wilmington, NC, USA). Specific assays including a locus-specific PCR reaction based on a locus-specific primer extension reaction were designed using the MassARRAY Assay Design software package (v3.1 Sequenom Inc., San Diego, CA, USA). Mass determination was carried out with the MALDI-TOF mass spectrometer and Mass ARRAY Type 4.0 software was used for data acquisition.

Genotyping results were confirmed by randomly assaying 8% of the original specimens for replication to exclude genotyping errors. There were no discrepancies between genotypes determined in duplicate.

2.4 | Statistical analysis

SPSS 22.0 software package (SPSS Inc, Chicago, IL, USA) for Windows 7 (Microsoft Corp, Redmond, WA, USA) was used for statistical analyses in this study. The means and SDs were calculated for normally distributed continuous variables and compared using Student's *t* test, percentages were calculated for categorical variables and analyzed using χ^2 test. The SNPstats (http://bioinfo.iconcologia.net/SNPstats) was used to assess the Hardy-Weinberg equilibrium for genotype

TABLE 2 Description for allele and genotype frequencies and analysis on association between 4 SNPs and IS risk

SNP	Genotypes and alleles	Frequencies N (%)		OR (95%CI)[a]	*P*-values	HWE test for controls
		Cases (n = 991)	Controls (n = 1002)			
rs579459	Additive					
	TT	494 (49.8)	639 (63.8)	1.00		0.257
	TC	396 (40.0)	315 (31.4)	1.59 (1.21-2.06)	<.001	
	CC	101 (10.2)	48 (4.8)	1.73 (1.15-2.33)	<.001	
	Dominant					
	TT	494 (49.8)	639 (63.8)	1.00		
	TC+CC	497 (50.2)	363 (36.2)	1.62 (1.19-2.10)	<.001	
	Allele, C (%)	598 (30.2)	411 (20.5)			
rs651007	Additive					
	CC	565 (57.0)	597 (59.6)	1.00		0.422
	CT	354 (35.7)	347 (34.6)	1.08 (0.72-1.51)	.431	
	TT	72 (7.3)	58 (5.8)	1.23 (0.66-1.92)	.723	
	Dominant					
	CC	565 (57.0)	597 (59.6)	1.00		
	CT+TT	426 (43.0)	405 (40.4)	1.12 (0.70-1.61)	.665	
	Allele, T (%)	498 (25.1)	463 (23.1)			
rs505922	Additive					
	TT	511 (51.6)	657 (65.6)	1.00		0.652
	TC	406 (41.0)	306 (30.5)	1.65 (1.26-2.12)	<.001	
	CC	74 (7.5)	39 (3.9)	1.83 (1.14-2.64)	<.001	
	Dominant					
	TT	511 (51.6)	657 (65.6)	1.00		
	TC+CC	480 (48.4)	345 (34.4)	1.69 (1.23-2.18)	<.001	
	Allele, C (%)	554 (28.0)	384 (19.2)			
rs529565	Additive					
	TT	521 (52.6)	578 (57.7)	1.00		0.195
	TC	383 (38.6)	356 (35.5)	1.33 (0.81-1.94)	.526	
	CC	87 (8.8)	68 (6.8)	1.51 (0.70-2.41)	.742	
	Dominant					
	TT	521 (52.6)	578 (57.7)	1.00		
	TC+CC	470 (47.4)	424 (42.3)	1.40 (0.77-2.00)	.705	
	Allele, C (%)	557 (28.1)	492 (24.6)			

[a]Adjusted for gender, age, smoking, drinking, TC, TG, HDL. Bonferroni correction threshold: $P < .00417$.

TABLE 3 Generalized multifactor dimensionality reduction analysis on the best gene-gene interaction combinations

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	*P*-values[a]
2	rs505922 rs579459	9/10	0.6072	.0010
3	rs505922 rs579459 rs651007	7/10	0.5399	.3770
4	rs505922 rs579459 rs651007 rs529565	6/10	0.4958	.4258

[a]Adjusted for gender, age, smoking, drinking, TC, TG, HDL.

frequencies and association between SNPs and IS. Generalized multifactor dimensionality reduction (GMDR)[17] was used to screen the best interaction combination among 4 SNPs within *ABO* gene. Interaction between SNPs within *ABO* gene in relation to the risk of IS was estimated by multiple logistic regressions, ORs (95%CI) for interaction between SNPs were calculate. All reported *P*-values were two-tailed, and to correct for multiple testing we defined a Bonferroni corrected-threshold in different tables.

3 | RESULTS

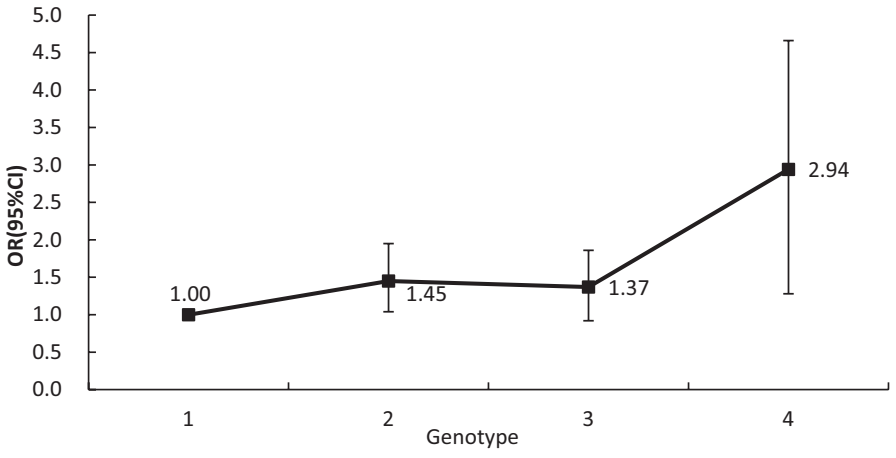
Table 1 shows the general and clinical characteristics of all study participants in case and control group. A total of 1993 participants (1375 males, 618 females) were selected, including 991 IS patients and 1002 normal controls. The mean age of all participants was 67.9 ± 15.2 years. The rate of smoking, alcohol drinking, hypertension, T2DM and the means of body mass index (BMI), fasting plasma glucose (FPG), triglyceride (TG) and total cholesterol (TC) are significantly higher in cases than that in controls, the mean of high-density lipoprotein (HDL) was lower in cases than that in controls. The mean age and rate of males were not different between cases and controls.

Table 2 shows the frequencies of genotypes and alleles for 4 SNPs and results for analysis on association between SNPs within *ABO* gene and IS risk. Logistic regression analysis showed that rs579459 and rs505922 within *ABO* gene were both associated with IS risk in additive and dominant models, after adjustment for gender, age, smoking, drinking, TC, TG, HDL. The carriers of homozygous and heterozygous mutant of rs579459 and rs505922 are associated with increased IS risk than those with wild-type homozygotes, OR (95%CI) were 1.62 (1.19- 2.10) and 1.69 (1.23-2.18), respectively. We did not find any relation of rs651007 and rs529565 with IS risk in both additive and dominant models.

Generalized multifactor dimensionality reduction model was used to screen the best interaction combination among 4 SNPs within *ABO* gene. Table 3 summarized the results obtained from GMDR analysis, which indicated a significant two-locus model (*P* = .0010) involving rs505922 and rs579459, indicating a potential interaction between rs505922 and rs579459, the cross-validation consistency of the two-locus models was 9/10, and the testing accuracy was 60.72%. To obtain the odds ratios and 95% CI for the joint effects, we conducted an interaction analysis using logistic regression (Figure 1). We found that participants with rs505922- TC/CC and rs579459- TC/CC genotype have the highest IS risk, compared to participants with rs505922- TT and rs579459- TT genotype, OR (95%CI) was 2.94 (1.28-4.66).

4 | DISCUSSION

In this study, we found that both rs505922 and rs579459 within ABO gene were associated with increased IS risk in additive and dominant models. We did not find any relation of rs651007 and rs529565 in ABO gene with IS risk in two models. Some studies have focused on the association between ABO SNPs and some others diseases, but to date, few study[11] has yet reported the association between ABO gene polymorphisms and the risk of IS in the Chinese population, and they concluded negative results, which maybe resulted by relatively small sample, and in addition, they did not consider the impact of interaction among several SNPs within ABO gene on IS risk. Hanson et al[14] provided evidence that ABO genotype does not have a major impact in the pathophysiology of IS or any of the subtypes. But Ling et al[18] concluded different results in another Chinese study, they indicated that genetic variations of ABO gene may contribute to susceptibility of large-artery atherosclerosis (LAA) but not IS and small-vessel diseases (SVD) in the Chinese population, but their preliminary results should be further validated in prospective independent studies with expanded sample size. Abino et al[19] suggested that a relationship of non-O blood groups in pathogenesis of thrombosis events and a possible protective effect of O blood group, mainly in young-adults patients with diagnosis of IS. In addition to these studies, several studies have focused on the association between *ABO* gene and others diseases, such as coronary heart disease (CHD), MI and CAD. Williams et al[13]



1: rs505922-TT+rs579459-TT; 2：rs505922-TC/CC+rs579459-TT;
3: rs505922-TT+rs579459-TC/CC; 4: rs505922--TC/CC+rs579459-TC/CC

FIGURE 1 Logistic regression for interaction between rs505922 and rs579459

suggest that *ABO* gene variants are associated with large-vessel and cardioembolic stroke but not small-vessel disease in the EuroCLOT Study. Previous studies found that the non-O phenotypes were more frequent in IS patients than controls.[20-22] Wu et al[23] confirmed the historical impression of linkage between some vascular disorders and non-O blood group status, although the odds ratios are similar to those predicted by the effect of ABO (H) on von Willebrand factor levels. Some subtypes of IS and CAD shared many common risk factors, for example, atherosclerosis plaque were observed in both LAA and CAD as a common pathophysiologic mechanism. Consequently, it was speculated that genetic variants of ABO gene associated with CAD,[10] may be also associated with LAA.

The complex diseases, such as IS, was a result of many gene polymorphism and gene-gene interactions, hence, it was necessary to investigate the impact of gene-gene interactions on susceptibility to IS. In considered of the multidimensional issue, GMDR model was used to screen the best interaction combination among 4 SNPs within *ABO* gene, we found a potential interaction between rs505922 and rs579459 on IS risk, participants with rs505922-TC/CC and rs579459-TC/CC genotype have the highest IS risk, compared to participants with s505922-TT and rs579459- TT genotype. To our knowledge, this study was the first study focused on the impact of *ABO* gene- gene interaction on IS risk. The results of this study suggest that IS risk may be modified by the two SNPs and they influenced with each other in susceptibility to IS. The potential mechanism for this interaction was not very clearly, it maybe that both SNPs were associated with some IS or related-risk factors, such as T2DM, obesity, and hypertension and so on, and this combined or crossover effect could lead to the interaction between the two SNPs on IS risk.

This study also has several limitations. First, limited number of SNPs within *ABO* gene was included in this study, these SNPs merely represented limited genetic variability in *ABO* gene. Second, the sample this study was relatively small, limited size of the cohort might reduce the power to detect association, so prospective independent studies with a comparatively larger sample size are required in the future. Third, interaction between this gene and others gene or environmental risk factors should be investigated in the future studies.

In conclusion, we found that rs579459 and rs505922 within *ABO* gene and their interaction were both associated with increased IS risk in Chinese population.

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