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Original Article

Association of single-nucleotide polymorphism on chromosome 9 and ischemic stroke in Heilongjiang province in China

Ming Jin[1*], Hao Zhang[1*], Qi Zhang[1], Xueyan Li[1], Nan Wu[1], Ying Hu[1], Changchun Qiu[1,2], Ningning Wang[1], Keyong Zhang[1]

[1]*Institute of Polygenic Disease, Qiqihar Medical University, Qiqihar, Heilongjiang, China;* [2]*Department of Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China.* [*]*Equal contributors.*

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Abstract: There is a significant correlation between ischemic stroke (IS) and chromosome 9. However, its status was uncertain in China's cold regions. 1920 IS patients, and 1920 healthy individuals were included in the study. Blood samples were collected. The association of SNPs with IS was evaluated by Sequenom, and logistic regression models adjusted for known risk factors of IS were constructed to assess the SNPs' associations in cases and controls. We found rs1333040 and rs2383207 were associated with IS, compared with primitive genotypes. The genotype CT of rs7027526 has a protective role during IS development, while the effect of the genotype TT is still not clear. These results changed after stratification by age and sex. In conclusion, rs1333040 and rs2383207 SNPs in CDKN2BAS are associated with ischemic stroke in the Chinese Han population. This study confirms the association between 9p21.3 and IS.

Keywords: Ischemic stroke, CDKN2B-AS1, FRMD3, Chinese, association

Introduction

Stroke is a well-known medical condition and the second leading cause of cardiovascular death worldwide [1]. It is a complex process influenced by non-genetic and genetic factors [2-6]. Ischemic stroke (IS) is the most common type and occurs in 75%-90% of cases [7]. In previous studies, we used a genome-wide association study (GWAS) to analyze genes associated with IS [8]. We discovered that rs1333040, rs2383207, and rs7027526 SNPs on chromosome 9 were all related to IS. Moreover, these SNPs have been associated with other cardiovascular diseases and diabetic nephropathy [9-15].

In China, the highest prevalence of IS has been reported in Heilongjiang Province (43-47° north latitude), in the northern part of the country. This region is characterized by a long winter and a high salt diet [16], well-known risk factors for stroke. In this study, we further investigated the association between rs1333040, rs2383207, and rs7027526 SNPs in IS cases and healthy volunteers from Heilongjiang province, China.

Materials and methods

Subjects

The present study included 1920 unrelated cases (26-65 years of age) and 1920 unrelated controls (48-86 years of age). All cases were selected from the Second Affiliated Hospital of Qiqihar Medical College. Controls who were volunteers were recruited from the communities following a medical examination. The inclusion criteria and exclusion criteria were as previously described [17, 18].

A complete clinical history, including age, sex, height, weight, blood pressure, triglyceride level, blood oxygen saturation, liver index, blood sugar, and kidney index, were collected from all

Table 1. Information and primers for the SNPs

SNP	Allele	Gene	Chromosome	Position
rs1333040	C>T	CDKN2B-AS1	9p21.3	22083405
rs2383207	A>G	CDKN2B-AS1	9p21.3	22115960
rs7027526	C>T	FRMD3/LOC102723989	9q21.32	83260207

participants by professional and technical personnel. Blood samples were collected at the same time from both groups.

Genetic analysis

Human genomic DNA was extracted from blood samples using the Blood Genomic DNA Midi Kit (produced by Kangwei Shiji Company). Genotype of the investigated SNPs (rs1333040, rs2383207, and rs7027526) was analyzed by a set of reagents on a matrix-assisted laser desorption/ionization-time of flight mass spectrometer (MassArray, Sequenom Inc.) using Sequenom reagents and protocols. Automatic allele calls by the Sequenom software were validated by manual evaluation.

Statistical analysis

Statistical analysis was performed using a SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Measured data (such as age, SBP, DBP) were compared using a t-test. Chi-square test was used to detect Hardy-Weinberg equilibrium and the differences of genotypes and alleles in the two groups. Logistic regression analysis was used to analyze the risk of genotypes and alleles for cerebral infarction. Linkage disequilibrium and haplotype were calculated using the SHEsis program platform (http://analysis.bio-x.cn/SHEsisMain.htm) [19-21]. The lowest frequency threshold (LFT) for haplotype analysis was set to 0.01. The impact of genotypes on stroke was presented by Meta-analysis in different groups. A *P* value of 0.05 or less was considered significant.

Result

Information of the 3 SNPs

rs1333040 and rs2383207 SNPs are located deep in the intron of CDKN2B-AS1 (**Table 1**). Several recent GWAS studies reported an association between these two SNPs, located in the 9p21 region, and CAD [9].

rs7027526 is located in the FRMD3 gene or LOC102723989 gene. The function of the LOC102723989 gene is not clear. In addition, the exact function of the protein encoded by FRMD3 remains unclear. Some studies suggest that the FRMD3 is associated with diabetic nephropathy in type 1 diabetes and may work as a tumor suppressor gene [22].

Characteristics of the study population

In the case group, all three SNPs were detected in 1640 people, and there were 1755 people in the control group. The clinical information of the study participants is summarized in **Table 2**. The mean age of the subjects with IS was 62.66 (±11.75) years, while the mean age of the control subjects was 50.78 (±8.87) years ($P<0.01$). Male patients with IS accounted for 54.72%, 1.21 times of female patients, and this is consistent with other related reports [23, 24].

Significant differences in BMI, SBP, DBP, TG, TC, AST, ALT, γ-GT, Glu, and UA were found between patients and control subjects (all $P< 0.05$), whereas there were no significant differences in SpO_2, Alb, TP, Urea, and Cr (**Table 2**).

Linkage disequilibrium tests and frequency distribution

Linkage disequilibrium test revealed a D' of 0.893 (out-off-balance) between rs1333040 and rs2383207, and the D' of 0.244 between rs2383207 and rs7027526.

The genotype and allele frequency of cases and controls are presented in **Table 3**. The genotype frequencies of rs1333040, rs2383207, and rs7027526 showed a significant difference between IS and controls by logistic regression. A difference existed when comparing the frequency of CC with CT and TT genotypes in rs1333040 (OR (95% CI) = 1.599 (1.242-2.06) and 1.413 (1.102-1.812)). The risk was increased to 1.873 (1.276-2.749) and 1.876 (1.284-2.74) after adjusting for age and sex. The dominant model of rs1333040 showed a reduction in the risk of IS (1.874 (1.297-2.71)).

Table 2. Comparison of general demographic data and clinical characteristics of the study population

	Ischemic Stroke	Control	t/χ^2	*P*
Age, years	62.66±11.75	50.78±8.87	12.840	<0.01**
Sex (M/F, %)	54.72/45.28	33.85/66.15	24.530	<0.01**
Body Mass Index (BMI)	25.12±3.21	23.54±3.21	4.520	<0.01**
Systolic pressure (SBP)	138.4±15.71	118.3±12.96	15.120	<0.01**
Diastolic blood pressure (DBP)	89.67±28.35	77.71±8.86	5.650	<0.01**
Oxygen saturation (SpO_2, %)	94.02±18.48	95.35±15.27	1.259	>0.05
Total Cholesterol (TC)	5.42±1.14	5.04±0.97	5.716	<0.01**
Triglyceride (TG)	2.04±4.3	1.54±2.2	2.309	<0.05*
Aspartate aminotransferase (AST)	33.96±19.83	31.13±16.77	2.476	<0.05*
Alanine aminotransferase (ALT)	21.64±10.8	20.23±6.98	2.435	<0.05*
γ-Glutamyl transpeptidase (γ-GT)	35.57±33.54	27.37±20.58	4.766	<0.01**
Albumin (Alb)	43.16±9.38	44.52±24.2	1.200	>0.05
Total Protein (TP)	69.51±4.2	68.97±5.02	1.871	>0.05
Glucose (Glu)	5.32±3.01	4.96±1.99	2.205	<0.05*
Urea (UR)	4.78±1.72	6.63±24.98	1.628	>0.05
Uric acid (UA)	374.67±129.4	323.71±109.34	6.824	<0.01**
Creatinine (Cr)	64.08±8.55	64.43±9.36	0.627	>0.05

*: P<0.05; **: P<0.01.

Table 3. Genotype and allele frequencies of patients with IS vs. controls

		Ischemic Stroke	Controls	Before adjustment		Adjusted	
		n (%)	N (%)	*P*	OR (95% CI)	*p*	OR (95% CI)
rs1333040	CC	120 (7.3)	185 (10.5)	0.001	1.0	0.004	1.0
	CT	695 (42.4)	670 (38.2)	0.000	1.599 (1.242-2.06)	0.001	1.873 (1.276-2.749)
	TT	825 (50.3)	900 (51.3)	0.006	1.413 (1.102-1.812)	0.001	1.876 (1.284-2.74)
	C	935 (28.51)	1040 (29.63)		1.0		
	T	2345 (71.49)	2470 (70.37)	0.308	1.056 (0.951-1.173)		
Dominant	CC	120 (7.32)	185 (10.54)		1.0		1.0
	CT+TT	1520 (92.68)	1570 (89.46)	0.001	1.493 (1.174-1.898)	0.001	1.874 (1.297-2.71)
Recessive	CC+CT	815 (49.7)	855 (48.72)		1.0		1.0
	TT	825 (50.3)	900 (51.28)	0.569	0.962 (0.841-1.1)	0.302	1.104 (0.915-1.33)
rs2383207	AA	180 (11)	250 (14.2)	0.017	1.0	0.004	1.0
	AG	665 (40.5)	690 (39.3)	0.009	1.339 (1.075-1.667)	0.028	1.412 (1.037-1.921)
	GG	795 (48.5)	815 (46.4)	0.006	1.355 (1.093-1.68)	0.001	1.659 (1.224-2.249)
	A	1025 (31.25)	1190 (33.90)		1.0		
	G	2255 (68.75)	2320 (66.10)	0.020	1.128 (1.019-1.249)		
Dominant	AA	180 (10.98)	250 (14.25)		1.0		1.0
	AG+GG	1460 (89.02)	1505 (85.75)	0.004	1.347 (1.098-1.653)	0.003	1.538 (1.152-2.053)
Recessive	AA+AG	845 (51.52)	940 (53.56)		1.0		1.0
	GG	795 (48.48)	815 (46.44)	0.235	1.085 (0.948-1.242)	0.012	1.274 (1.056-1.537)
rs7027526	CC	1600 (97.6)	1705 (97.2)	0.422	1.0	0.000	1.0
	CT	35 (2.1)	50 (2.8)	0.189	0.746 (0.482-1.155)	0.003	0.225 (0.083-0.611)
	TT	5 (0.3)	0 (0)	-	-	-	-
	C	3235 (98.63)	3460 (98.58)		1.0		
	T	45 (1.37)	50 (1.42)	0.854	0.963 (0.642-1.444)		
Dominant	CC	1600 (97.56)	1705 (97.15)		1.0		1.0
	CT+TT	40 (2.44)	50 (2.85)	0.458	0.853 (0.559-1.299)	0.003	0.225 (0.083-0.611)
Recessive	CC+CT	1635 (99.7)	1755 (100)		-		-
	TT	5 (0.3)	0 (0)	-	-	-	-

OR: Odds Ratio; CI: Confidence Interval; The adjustment factors were age and sex.

The distribution of the variant rs2383207 was significantly different in genotype frequency among cases and controls. After comparing the frequency of AA with AG and GG genotypes, the results were significant (OR (95% CI) = 1.412 (1.037-1.921) and OR (95% CI) = 1.659 (1.224-2.249)). Meanwhile, both dominant and recessive models increased the risk of IS.

A significant association of rs7027526 with IS was shown by heterozygote CT (P = 0.003) and by the dominant model (P = 0.003). Simultaneously, there was a phenomenon accompanying rs7027526. The genotype TT was found only in the control group, which is most likely due to the small sample size. It is also possible that individuals with rs7027526 TT suffered from IS.

Next, we created two independent groups, where the first group included individuals younger than 60 years old, and the other group included those older than 60 years old. The results were more pronounced in those younger than 60 years (**Table 4**). In addition, we further grouped individuals according to gender. The results were more pronounced in females (**Table 5**).

Haplotype analysis among different subgroups

In the control group, the frequency of genotype TT was 0. Consequently, haplotypes were constructed based on the two SNPs (rs1333040 and rs2383207). All 4 possible haplotypes were included in our haplotype analysis (**Table 6** and **Figure 1A**). The T-A haplotype reduced the risk of IS (OR (95% CI) = 0.743 (0.603-0.916)), and the T-G haplotype increased the risk (OR (95% CI) = 1.126 (1.019-1.244)).

In patients younger than 60 years old, the result was not consistent with the overall data (Attached 1, **Figure 1B**). Moreover, the results in the female group were similar to the overall data, while they differed in the male group (Attached 1, **Figure 1C**, **1D**). The individuals with haplotype T-A were not prone to cerebral infarction in males, while those with haplotype T-A were more likely to suffer from it in females.

Discussion

Recently, we discovered an association between three loci on chromosome 9 and cerebral infarction [9]. In the present study, we further examined this association in a large sample population, obtaining results that were consistent with the previous data.

So far, several studies have reported on the CDKN2B-AS1 gene. This region is a significant genetic susceptibility locus for multiple diseases, such as cardiovascular disease, several cancers, and type-2 diabetes. This may be due to the molecular form of RNA affected by transcript variants during transcription [25]. Considering that cardiovascular disease and diabetes are all risk factors for cerebral infarction [26, 27], we assumed that this gene is associated with cerebral infarction.

Several recent studies have reported the association of rs1333040 and rs2383207 with IS. A meta-analysis found an association between those genes and IS in Caucasians (OR = 1.55, P = 0.0007) and African Americans (OR = 0.65, P = 0.023); yet, no correlation with cerebral infarction was found in China [28]. Moreover, Yue *et al* suggested that a recessive model of rs2383207 was a predictor for stroke (OR = 1.28, 95% CI = 1.03-1.59, P = 0.02, Q = 0.03) [29]. Several GWAS have demonstrated that the 9p21 locus was associated with cerebral infarction [30], which was consistent with the results reported by Wang *et al* [31]. Nevertheless, a recent study performed by Cheng *et al* could not replicate these results [32]. These discordant results most likely occurred due to the different genetic backgrounds of these populations.

Our analyses provided novel evidence that 9p21.3 has a key role in cerebral infarction. FRMD3 gene has been considered as a susceptible gene for diabetic nephropathy in type 1 diabetes [33], while an association between FRMD3 genes and stroke has been rarely reported. Considering that diabetes is a traditional risk factor for cerebral infarction [26], we assumed that ischemic stroke shares common risk factors with diabetes. After stratification by sex and age, we found that the results were very different. The effect of haplotype T-A in patients with early-onset cerebral infarction was different from the overall data. At the same time, the role of the haplotypes T-A and T-G in women was opposite to that in men. The above results suggest that patients of different sex and age should be treated differently.

Table 4. Genotype and allele frequencies of patients with IS vs. controls at different ages

		<60						≥60					
		Controls (%)	IS (%)	Before adjustment OR (95% CI)	Before adjustment *P*	Adjusted OR (95% CI)	Adjusted *P*	Controls (%)	IS (%)	Before adjustment OR (95% CI)	Before adjustment *P*	Adjusted OR (95% CI)	Adjusted *P*
rs1333040	CC	11.30	5.20	1.0		1.0		6.80	7.40	1.0		1.0	
	CT	34.90	45.50	2.912 (1.776-4.773)	0.000	2.799 (1.697-4.615)	0.000	54.20	40.70	0.688 (0.387-1.221)	0.201	0.768 (0.429-1.373)	0.373
	TT	53.80	49.40	1.997 (1.223-3.261)	0.006	1.897 (1.155-3.116)	0.011	39.00	51.90	1.217 (0.682-2.172)	0.505	1.314 (0.733-2.358)	0.359
rs2383207	AA	14.00	9.10	1.0		1.0		15.30	13.90	1.0		1.0	
	AG	37.30	44.20	1.881 (1.265-2.797)	0.002	1.72 (1.15-2.574)	0.008	49.20	38.00	0.848 (0.554-1.299)	0.449	0.852 (0.555-1.31)	0.467
	GG	48.60	46.80	1.485 (1.001-2.202)	0.049	1.424 (0.955-2.123)	0.083	35.60	48.10	1.486 (0.963-2.292)	0.073	1.5 (0.969-2.323)	0.069
rs7027526	CC	97.20	97.60	1.0		1.0		98.30	100.00				
	CT	2.80	2.10	0.414 (0.163-1.05)	0.063	0.321 (0.125-0.822)	0.018	1.70	0.00	-	-	-	-
	TT	0.00	0.30	-	-	-	-	0.00	0.00	-	-	-	-

Table 5. Genotype and allele frequencies of patients with IS vs. controls in different sexes

		Male						Female					
		Controls (%)	IS (%)	Before adjustment OR (95% CI)	Before adjustment *P*	Adjusted OR (95% CI)	Adjusted *P*	Controls (%)	IS (%)	Before adjustment OR (95% CI)	Before adjustment *P*	Adjusted OR (95% CI)	Adjusted *P*
rs1333040	CC	8.70	9.00	1.0		1.0		11.40	3.60	1.0		1.0	
	CT	37.40	40.00	1.059 (0.678-1.655)	0.800	1.114 (0.671-1.85)	0.675	38.60	46.40	3.857 (2.205-6.747)	0.000	3.893 (2.014-7.525)	0.000
	TT	53.90	51.00	0.914 (0.591-1.413)	0.686	1.034 (0.629-1.7)	0.894	50.00	50.00	3.203 (1.837-5.587)	0.000	4.016 (2.081-7.747)	0.000
rs2383207	AA	10.40	14.00	1.0		1.0		16.10	9.50	1.0		1.0	
	AG	42.60	42.00	0.752 (0.509-1.111)	0.153	0.865 (0.557-1.342)	0.516	37.70	39.30	1.761 (1.199-2.588)	0.004	2.28 (1.437-3.617)	0.000
	GG	47.00	44.00	0.698 (0.474-1.03)	0.070	0.866 (0.558-1.342)	0.518	46.20	51.20	1.874 (1.287-2.728)	0.001	2.985 (1.895-4.703)	0.000
rs7027526	CC	93.90	99.00					98.70	98.80	1.0		1.0	
	CT	6.10	0.00	-	-	-	-	1.30	1.20	0.936 (0.338-2.591)	0.898	3.276 (1.162-9.238)	0.025
	TT	0.00	1.00	-	-	-	-	0.00	0.00	-	-	-	-

Table 6. Haplotypes and their risk of ischemic stroke

Haplotype	IS (%)	Controls (%)	Before adjustment		Adjusted	
			P	OR (95% CI)	*P*	OR (95% CI)
T G	0.653	0.641	0.373	1.054 (0.937-1.186)	0.373	1.054 (0.937-1.186)
T A	0.067	0.062	0.519	1.077 (0.858-1.352)	0.519	1.077 (0.858-1.352)
C A	0.255	0.276	0.099	0.898 (0.79-1.02)	0.099	0.898 (0.79-1.02)
C G	0.024	0.019	0.305	1.218 (0.834-1.779)	0.305	1.218 (0.834-1.779)

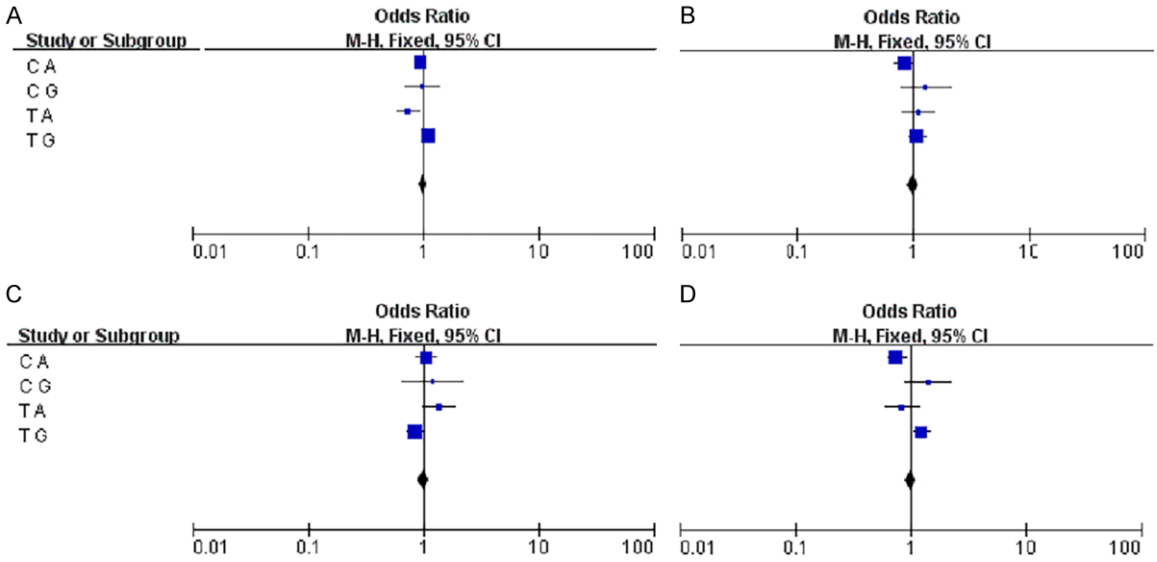

Figure 1. Summary of haplotype analysis. A: In all individuals; B: Early onset cerebral infarction; C: In men; D: In women.

This study has a few limitations. Due to the relatively small sample size, we could not analyze the association of the genotype TT and the haplotype of rs7027526 with IS. Another limitation is the single ethnicity of the study population. Finally, we did not perform analysis in the different subtypes.

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Disclosure of conflict of interest

None.

Address correspondence to: Ningning Wang and Keyong Zhang, Institute of Medical Sciences, Qiqihar Medical University, 333 Bukui North Street, Qiqihar 161006, Heilongjiang, China. E-mail: wnn-891011@sina.com (NNW); kyzhang17@163.com (KYZ)

References

[1] World Health Organization. The global burden of disease: 2004 update. Geneva: WHO 2008; 36-40.

[2] Mostafa MA, El-Nabiel LM, Fahmy NA, Aref H, Shreef E, Abd El-Tawab F and Abdulghany OM. ACE gene in egyptian ischemic stroke patients. J Stroke Cerebrovasc Dis 2016; 25: 2167-2171.

[3] Shi H, Leng S, Liang H, Zheng Y and Chen L. Association study of C-reactive protein associated gene HNF1A with ischemic stroke in Chinese population. BMC Med Genet 2016; 17: 51.

[4] Chon J, Kim HS, Yun DH, Yoo SD, Kim DH, Lee SA, Kim SK, Park HJ, Chung JH, Chung S and Yeo J. Association between a polymorphism (rs2071214) in baculoviral IAP repeat containing 5 gene (BIRC5) and ischemic stroke in Korean opulation. Ann Rehabil Med 2016; 40: 392-400.

[5] Wang X, Zhu L, Wu Y, Sun K, Su M, Yu L, Chen J, Li W, Yang J, Yuan Z and Hui R. Plasma growth differentiation factor 15 predicts first-ever stroke in hypertensive patients. Medicine (Baltimore) 2016; 95: e4342.
[6] Zhang X, Sun Z, Ding C, Tang Y, Jiang X, Xie Y, Li C, Zhang L, Hu D, Li T, Xu G and Sheng L. Metabolic syndrome augments the risk of early neurological deterioration in acute ischemicstroke patients independent of inflammatory mediators: a hospital-based prospective study. Oxid Med Cell Longev 2016; 2016: 8346301.
[7] Huang W. Logistic regression analysis of risk factors for stroke. Medical Information 2011; 22: 3236-3240.
[8] Söderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, Cole JW, Fernandez-Cadenas I, Hankey GJ, Jimenez-Conde J, Jood K, Lee JM, Lemmens R, Levi C, Mitchell BD, Norrving B, Rannikmäe K, Rost NS, Rosand J, Rothwell PM, Scott R, Strbian D, Sturm JW, Sudlow C, Traylor M, Thijs V, Tatlisumak T, Woo D, Worrall BB, Maguire JM, Lindgren A and Jern C; International Stroke Genetics Consortium, the NINDS-SiGN Consortium, and the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) Network. Genome-wide association meta-analysis of functional outcome after ischemic stroke. Neurology 2019; 92: e1271-e1283.
[9] Golabgir Khademi K, Foroughmand AM, Galehdari H, Yazdankhah S, Pourmahdi Borujeni M, Shahbazi Z and Dinarvand P. Association study of rs1333040 and rs1004638 polymorphisms in the 9p21 ocus with coronary artery disease in Southwest of Iran. Iran Biomed J 2016; 20: 122-127.
[10] Lin HF, Tsai PC, Liao YC, Lin TH, Tai CT, Juo SH and Lin RT. Chromosome 9p21 genetic variants are associated with myocardial infarction but not with ischemic stroke in a Taiwanese population. J Investig Med 2011; 59: 926-930.
[11] Beckie TM, Groër MW and Beckstead JW. The relationship between polymorphisms on chromosome 9p21 and age of onset of coronary heart disease in black and white women. Genet Test Mol Biomarkers 2011; 15: 435-442.
[12] El-Menyar AA, Rizk NM, Al-Qahtani A, AlKindi F, Elyas A, Farag F, Bakhsh FD, Ebrahim S, Ahmed E, Al-Khinji M, Al-Thani H and Suwaidi JA. The cardiovascular implication of single nucleotide polymorphisms of hromosome 9p21 locus among Arab population. J Res Med Sci 2015; 20: 346-352.
[13] Shen GQ, Li L, Rao S, Abdullah KG, Ban JM, Lee BS, Park JE and Wang QK. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. Arterioscler Thromb Vasc Biol 2008; 28: 360-365.
[14] Buffon MP, Sortica DA, Gerchman F, Crispim D and Canani LH. FRMD3 gene: its role in diabetic kidney disease. A narrative review. Diabetol Metab Syndr 2015; 7: 118.
[15] Al-waheeb S, Alwohhaib M, Abdelghani A, Al-Sharrah S, Al-Shafey E, Al-Sahow A, Alyatama N, Dashti AA and Hussain N. Evaluation of associations between single nucleotide polymorphisms in the FRMD3 and CARS genes and diabetic nephropathy in a Kuwaiti population. Genet Mol Res 2016; 15.
[16] Sun H, Zou X and Liu L. Epidemiological factors of stroke: a survey of the current status in China. J Stroke 2013; 15: 109-114.
[17] Wang N, Li X, Zhang Q, Zhang H, Zhou L, Wu N, Jin M, Qiu C and Zhang K. Association of angiotensin-converting enzyme gene polymorphism with pulse pressure and its interaction with obesity status in Heilongjiang province. Clin Exp Hypertens 2019; 41: 70-74.
[18] Zhang Q, Cong M, Wang N, Li X, Zhang H, Zhang K, Jin M, Wu N, Qiu C and Li J. Association of angiotensin-converting enzyme 2 gene polymorphism and enzymatic activity with essential hypertension in different gender: a case-control study. Medicine (Baltimore) 2018; 97: e12917.
[19] Shi YY and He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.
[20] Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L and Shi Y. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res 2009; 19: 519-523.
[21] Shen J, Li Z, Chen J, Song Z, Zhou Z and Shi Y. SHEsisPlus, a toolset for genetic studies on polyploid species. Sci Rep 2016; 6: 24095.
[22] Freedman BI, Langefeld CD, Lu L, Divers J, Comeau ME, Kopp JB, Winkler CA, Nelson GW, Johnson RC, Palmer ND, Hicks PJ, Bostrom MA, Cooke JN, McDonough CW and Bowden DW. Differential effects of MYH9 and APOL1 risk variants on FRMD3 association with diabetic ESRD in African Americans. PLoS Genet 2011; 7: e1002150.
[23] Barker-Collo S, Bennett DA, Krishnamurthi RV, Parmar P, Feigin VL, Naghavi M, Forouzanfar MH, Johnson CO, Nguyen G, Mensah GA, Vos T, Murray CJ and Roth GA; GBD 2013 Writing Group; GBD 2013 Stroke Panel Experts Group. Sex differences in stroke incidence, prevalence, mortality and disability-adjusted life years: results from the global burden of dis-

ease study 2013. Neuroepidemiology 2015; 45: 203-214.
[24] Ahnstedt H, McCullough LD and Cipolla MJ. The importance of considering sex differences in translational stroke research. Transl Stroke Res 2016; 7: 261-273.
[25] Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z and Sharpless NE. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. PLoS Genet 2010; 6: e1001233.
[26] Huang T. Modifiable factors for coronary artery disease, myocardial infarction, and stroke: a mendelian randomisation analysis (P15-006-19). Curr Dev Nutr 2019; 3.
[27] Chen R, Ovbiagele B and Feng W. Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcomes. Am J Med Sci 2016; 351: 380-386.
[28] Heckman MG, Soto-Ortolaza AI, Diehl NN, Rayaprolu S, Brott TG, Wszolek ZK, Meschia JF and Ross OA. Genetic variants associated with myocardial infarction in the PSMA6 gene and Chr9p21 are also associated with ischaemic stroke. Eur J Neurol 2013; 20: 300-308.
[29] Yue X, Tian L, Fan X, Xu G, Shi FD and Liu X. Chromosome 9p21.3 variants are associated with cerebral infarction in Chinese population. J Mol Neurosci 2015; 56: 546-552.
[30] Anderson CD, Biffi A, Rost NS, Cortellini L, Furie KL and Rosand J. Chromosome 9p21 in ischemic stroke: population structure and meta-analysis. Stroke 2010; 41: 1123-1131.
[31] Wang C, Li Y, Li H, Sun T, Jin G, Sun Z, Zhou J, Ba L, Huang Z and Bai J. Increased risk of stroke in oral contraceptive users carried replicated genetic variants: a opulation-based case-control study in China. Hum Genet 2012; 131: 1337-1344.
[32] Cheng YC, Anderson CD, Bione S, Keene K, Maguire JM, Nalls M, Rasheed A, Zeginigg M, Attia J, Baker R, Barlera S, Biffi A, Bookman E, Brott TG, Brown RD Jr, Chen F, Chen WM, Ciusani E, Cole JW, Cortellini L, Danesh J, Doheny K, Ferrucci L, Grazia Franzosi M, Frossard P, Furie KL, Golledge J, Hankey GJ, Hernandez D, Holliday EG, Hsu FC, Jannes J, Kamal A, Khan MS, Kittner SJ, Koblar SA, Lewis M, Lincz L, Lisa A, Matarin M, Moscato P, Mychaleckyj JC, Parati EA, Parolo S, Pugh E, Rost NS, Schallert M, Schmidt H, Scott RJ, Sturm JW, Yadav S, Zaidi M, Boncoraglio GB, Levi CR, Meschia JF, Rosand J, Sale M, Saleheen D, Schmidt R, Sharma P, Worrall B and Mitchell BD; GARNET Collaborative Research Group; GENEVA Consortium; International Stroke Genetics Consortium. Are myocardial infarction-associated single-nucleotide polymorphisms associated with ischemic stroke? Stroke 2012; 43: 980-986.
[33] Palmer ND and Freedman BI. Diabetic nephropathy: FRMD3 in diabetic nephropathy--guilt by association. Nat Rev Nephrol 2013; 9: 313-314.