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ORIGINAL RESEARCH

Independent and Interactive Effects of Sex and CYP2C9 Variant rs4918758 on Ischemic Stroke Risk in Taiwan Biobank

Jui-Wen Peng[1], Oswald Ndi Nfor[2], Chien-Chang Ho[3,4], Shu-Yi Hsu[2], Ming-Chih Chou[1], Yung-Po Liaw[2,5]

[1]Institute of Medicine, Chung Shan Medical University, Taichung, 40201, Taiwan; [2]Department of Public Health and Institute of Public Health, Chung Shan Medical University, Taichung, 40201, Taiwan; [3]Department of Physical Education, Fu-Jen Catholic University, New Taipei City, 24205, Taiwan; [4]Research and Development Center for Physical Education, Health, and Information Technology, Fu Jen Catholic University, New Taipei, 24205, Taiwan; [5]Department of Medical Imaging, Chung Shan Medical University Hospital, Taichung City, 40201, Taiwan

Correspondence: Yung-Po Liaw, No. 110 Sec. 1 Jianguo N. Road, Department of Public Health and Institute of Public Health, Chung Shan Medical University, Taichung, 40201, Taiwan, Tel +886436097501, Fax +886423248179, Email Liawyp@csmu.edu.tw; Ming-Chih Chou, No. 110 Sec. 1 Jianguo N. Road, Institute of Medicine, Chung Shan Medical University, Taichung, 40201, Taiwan, Tel +886424730022 ext. 11191, Fax +886423248130, Email juhan0223@yahoo.com.tw

Purpose: Stroke is a complex health condition caused by multiple risk factors. We investigated whether the *Cytochrome P450 2C9* (*CYP2C9*) rs4918758 polymorphism and sex were independently and interactively associated with ischemic stroke risk among Taiwan Biobank (TWB) participants.

Material and Methods: We analyzed TWB data pertaining to 9197 female and 8625 male individuals. Data collected between 2008 and 2015 were linked to medical records in the National Health Insurance Database (NHIRD). Based on multiple logistic regression analyses, we estimated odds ratios (OR) and 95% confidence intervals (CI) for ischemic stroke.

Results: We found that 441 women and 468 men had ischemic stroke. There were no differences in the risk of ischemic stroke between individuals with the TC/CC genotype and those with the TT genotype [OR (95% CI) = 1.04 (0.90–1.21)]. When compared to women, men had an OR of 1.03 (95% CI = 0.87–1.22) for ischemic stroke. Based on further analysis, sex was found to interact with polymorphism rs4918758 (p for interaction = 0.0019). After categorizing by sex, men with TC/CC genotype showed significant ORs but not women [OR (95% CI) = 1.32 (1.07–16.33) vs 0.83 (0.68–1.00)]. Further stratification by genotype showed that in comparison with their female counterparts, men with the TT and TC/CC genotypes had ORs of 0.59 (95% CI = 0.44–0.80) and 1.36 (95% CI = 1.10–1.68), respectively.

Conclusion: According to our study, the TT genotype of rs4918758 was associated with a reduced risk of ischemic stroke in Taiwanese men when compared to women, whereas the TC/CC genotype was associated with a greater risk.

Keywords: stroke, genetic variant, sex, ischemic heart, gender

Introduction

Ischemic stroke is increasingly becoming a serious health issue worldwide, especially in people with coronary heart diseases (CHD).[1] It constitutes approximately 74% of all stroke in Taiwan.[2] The incidence of the disease appears to be increasing significantly among younger adults (ie, those under 50 years of age).[3] Significant risk factors include CHD, atrial fibrillation, diabetes, smoking, hypertension, low high-density lipoprotein cholesterol, and a history of stroke.[3]

Several subtypes exist for ischemic stroke, some of which were found to have genetic components among European populations.[4,5] At this point, there are limited data on the impact of genetic variants on the development of ischemic stroke, since only a few rare variants have been linked to this condition.[6] To our knowledge, only a few epidemiological studies have replicated genetic variants that are linked to ischemic stroke.

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Polymorphic variants including those of genes encoding the *CYP2C* subfamily have shown associations with CHD, a potential marker for ischemic stroke.[7] A previous study reported a low risk of ischemic stroke for CYP2C but the underlying mechanisms were not discussed.[8] Additionally, there is evidence that polymorphisms in the *CYP2CP* gene are associated with stroke occurrence and recurrence in Spanish and Chinese populations.[9,10]

The *CYP2C9* protein is involved in the metabolism of several antihypertensive agents.[11] Polymorphisms in this gene have also been associated with warfarin, a potent anticoagulant that has been associated with a 64% decrease in stroke risk.[12] As stated earlier, there are a few polymorphisms in *CYP2C9* that have been investigated for ischemic stroke. However, there is still no conclusive evidence linking certain genetic variants with this condition. CHD is known to increase the risk of ischemic stroke. One of the *CYP2C9* polymorphisms associated with CHD is rs4918758.[7,13] It is evident that this variant has a substantial allele frequency in Asians, and has shown a better similarity in Taiwanese and Japanese populations (39.2% and 40%, respectively).[14] It is noteworthy that variant rs4918758 is associated with CHD, which is potentially a marker for ischemic stroke. However, there here have been attempts to investigate this variant in Europe, Japan, and North America,[15] but not Taiwan. In light of this, we examined the association of rs4918758 with ischemic stroke risk among Taiwanese adults and tested whether they differed by sex.

Materials and Methods

Data Source

Based on TWB data, this study included 9197 females and 8625 males. TWB participants provided written content upon enrollment. The disease information was gathered from the NHIRD, while genetic, demographic, socioeconomic, and lifestyle information was gathered from the TWB dataset. Data from both sources were collected between 2008 and 2015. We linked the data sources using personal identification numbers at the Health and Welfare Data Science Center. The Institutional Review Board (IRB) of Chung Shan Medical University approved this study.

Disease Definitions

Identification of diseases was based on medical records contained in the NHIRD. The diseases were ischemic stroke (ICD-9-CM: 433–437), hypertension (ICD-9-CM: 401–405, A260, A269), diabetes mellitus (ICD-9-CM: 250, A181), hyperlipidemia (ICD-9-CM: 272), and atrial fibrillation (ICD-9-CM: 427.3). This was based on either two outpatient visits or inpatient admission.

Study Population

The initial data analysis involved 17,985 TWB participants. The following were excluded: persons with incomplete questionnaires (n = 37), those without missing genotype information (n = 13), and those who had hemorrhagic strokes (n = 113). After the exclusions, a total of 909 ischemic stroke patients, and 16,913 controls were included in the study.

SNP Selection and Genotyping

The rs4918758 of the *CYP2C9* gene was chosen because of its previously reported association with coronary heart disease, a risk factor for ischemic stroke. Our samples were genotyped for this variant by Affymetrix using the Axiom™ Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA). During the quality control test, this variant met the following criteria: call rate was >95%, minor allele frequency was >0.05, and Hardy-Weinberg equilibrium (HWE) p-value was $>1.0 \times 10^{-3}$.

Statistical Analysis

Using the x^2 test and *t*-test, we compared differences between continuous and discrete variables. Furthermore, we used logistic regression to assess the effects of sex and *CYP2C9* rs4918758 on ischemic stroke and estimated ORs along with the 95% CIs, which were also displayed as forest plots. The analyses were conducted using SAS software, version 9.4 (SAS Institute, Inc, Cary, NC), and PLINK software, version 1.09.

Table 1 Baseline Characteristics of Study Participants

	Female		Male		p-value
	N	%	N	%	
Ischemic stroke					0.056
No	8756	95.2	8157	94.57	
Yes	441	4.8	468	5.43	
CYP2C9 (rs4918758)					0.811
TT	3343	36.35	3150	36.52	
TC/CC	5854	63.65	5475	63.48	
Age (yr), mean (±SD)	48.61	±10.93	49.08	±11.07	0.004
Warfarin use					0.330
No	9130	99.27	8551	99.14	
Yes	67	0.73	74	0.86	
Education					<0.001
Elementary School	712	7.74	315	3.65	
Junior & Senior High school	4032	43.84	2916	33.81	
University & above	4453	48.42	5394	62.54	
Smoking					<0.001
No	8710	94.7	4742	54.98	
Yes	487	5.3	3883	45.02	
Alcohol drinking					<0.001
No	8971	97.54	6999	81.15	
Yes	226	2.46	1626	18.85	
Physical activity					<0.001
No	5534	60.17	4931	57.17	
Yes	3663	39.83	3694	42.83	
BMI (kg/m^2)					<0.001
BMI<18.5	409	4.45	119	1.38	
18.5≤BMI<24	5359	58.27	3208	37.19	
24≤BMI<27	2030	22.07	3120	36.17	
BMI≥27	1399	15.21	2178	25.25	
Diabetes					0.000
No	8033	87.34	7373	85.48	
Yes	1164	12.66	1252	14.52	
Hyperlipidemia					<0.001
No	6638	72.18	5817	67.44	
Yes	2559	27.82	2808	32.56	
Hypertension					<0.001
No	7454	81.05	6205	71.94	
Yes	1743	18.95	2420	28.06	
Atrial fibrillation					0.011
No	9158	99.58	8564	99.29	
Yes	39	0.42	61	0.71	

Abbreviations: SD, standard deviation; BMI, body mass index; yr, year.

Results

We observed 441 women and 468 men with ischemic stroke (Table 1). The results of logistic regression are shown in Table 2. There was no difference in the risk of ischemic stroke between TC/CC and TT genotypes [OR, (95% CI) = 1.04 (0.90–1.21)]. Compared to women, men were not at greater risk of ischemic stroke [OR (95% CI) = 1.03 (0.87–1.22)]. Ischemic stroke was associated with atrial fibrillation [OR (95% CI) = 2.45 (1.45–4.12)], hyperlipidemia [OR (95% CI) = 2.45 (1.45–4.12)], hypertension [OR (95% CI) = 2.30 (1.95–2.71)], and type 2 diabetes [OR (95% CI) =

Table 2 Risk of Ischemic Stroke Among Study Participants

Variable	OR	95% CI
rs4918758 (ref: TT)		
TC/CC	1.04	0.90–1.21
Sex (ref: Female)		
Male	1.03	0.87–1.22
Warfarin use (ref: No)		
Yes	1.72	1.05–2.82
Age	1.07	1.06–1.08
Education (ref: Elementary School)		
Junior & Senior High School	1.07	0.85–1.34
University & above	0.89	0.70–1.13
Smoking (ref: No)		
Yes	0.91	0.75–1.10
Alcohol drinking (ref: No)		
Yes	1.20	0.96–1.51
Physical activity (ref: No)		
Yes	1.03	0.89–1.20
BMI (ref:18.5≤BMI<24)		
BMI<18.5	1.30	0.79–2.14
24≤BMI<27	0.97	0.82–1.14
BMI≥27	1.00	0.82–1.20
Diabetes (ref: No)		
Yes	1.48	1.26–1.74
Hyperlipidemia (ref: No)		
Yes	1.65	1.40–1.94
Hypertension (ref: No)		
Yes	2.30	1.95–2.71
Atrial fibrillation (ref: No)		
Yes	2.45	1.45–4.12

Abbreviations: OR, odds ratio; BMI, body mass index; CI, confidence interval.

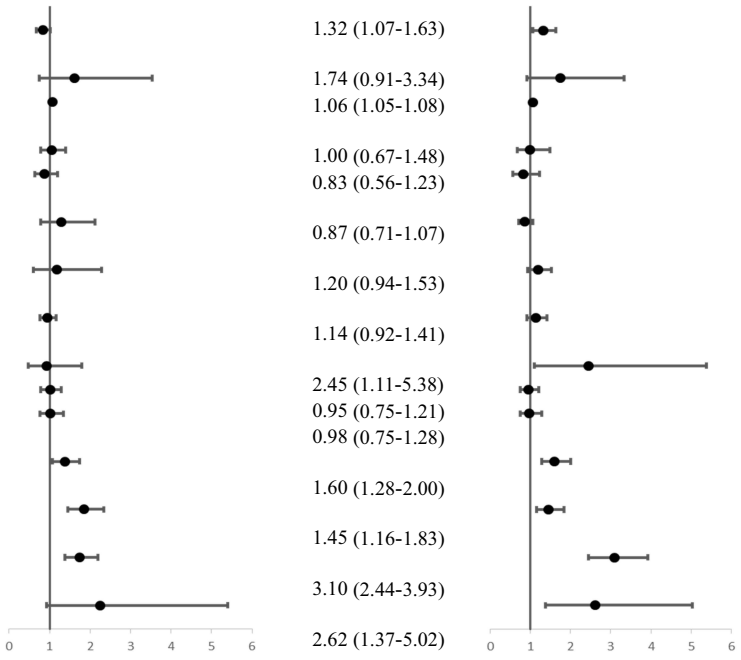
1.48 (1.26–1.74)]. Interestingly, there was an interaction between sex and rs4918758 polymorphism (p for interaction = 0.0019). After stratifying by sex (Figure 1), we found a substantial association for TC/CC men [OR (95% CI) = 1.32 (1.07–1.63), but not for women [OR (95% CI) = 0.83 (0.68–1.02), when compared to the TT genotype. Further stratification by genotypes of rs4918758 (Figure 2) revealed that the OR (95% CI) for ischemic stroke was 0.59 (0.44–0.80) in TT men compared to women, and 1.36 (1.10–1.68) in TC/CC men compared to women, respectively.

Discussion

Genetic risk factors for ischemic stroke and their possible interactions have not been adequately reported. In this study, for the first time, the rs4918758 genetic variant and sex were evaluated in relation to ischemic stroke risk among TWB participants aged 30 to 70 years. We found that the TT genotype of rs4918758 was linked to decreased risk, whereas the TC/CC genotype was associated with increased risk of ischemic stroke in Taiwanese men relative to women. This indicates that rs4918758 and sex may play a role in ischemic stroke risk among adults in Taiwan even though the functional mechanism by which this occurs remains to be determined. While a prior study observed male predominance across all age groups,[16] we did not observe a significant difference in ischemic stroke risk between men and women in our primary analysis. Additionally, we found no association between rs4918758 and ischemic stroke. Interestingly, the test for interaction was significant for sex and polymorphism rs4918758. This prompted us to perform subgroup analyses (based on sex, rs4918758, and ischemic stroke susceptibility), where we observed the most consistent associations for TT and TC/CC genotypes as reported.

As stated earlier, polymorphism rs4918758 has been reported as a significant marker for heart diseases, especially among Europeans and Russians.[7,13,17] However, it is unclear what role it plays in ischemic stroke vulnerability.

Variable	Female OR (95% CI)	Female Forest plot	Male OR (95% CI)	Male Forest plot
rs4918758 (ref: TT)				
TC/CC	0.83 (0.68-1.02)		1.32 (1.07-1.63)	
Warfarin use (ref: No)				
Yes	1.62 (0.75-3.53)		1.74 (0.91-3.34)	
Age	1.08 (1.06-1.09)		1.06 (1.05-1.08)	
Education (ref: Elementary School)				
Junior & Senior High School	1.05 (0.79-1.40)		1.00 (0.67-1.48)	
University & above	0.88 (0.64-1.20)		0.83 (0.56-1.23)	
Smoking (ref: No)				
Yes	1.29 (0.79-2.13)		0.87 (0.71-1.07)	
Alcohol drinking (ref: No)				
Yes	1.18 (0.61-2.28)		1.20 (0.94-1.53)	
Physical activity (ref: No)				
Yes	0.94 (0.76-1.16)		1.14 (0.92-1.41)	
BMI (ref:18.5≤BMI<24)				
BMI<18.5	0.93 (0.48-1.79)		2.45 (1.11-5.38)	
24≤BMI<27	1.01 (0.79-1.28)		0.95 (0.75-1.21)	
BMI≥27	1.02 (0.77-1.35)		0.98 (0.75-1.28)	
Diabetes (ref: No)				
Yes	1.38 (1.08-1.74)		1.60 (1.28-2.00)	
Hyperlipidemia (ref: No)				
Yes	1.85 (1.46-2.34)		1.45 (1.16-1.83)	
Hypertension (ref: No)				
Yes	1.74 (1.38-2.19)		3.10 (2.44-3.93)	
Atrial fibrillation (ref: No)				
Yes	2.24 (0.93-5.40)		2.62 (1.37-5.02)	



OR indicates odds ratio; BMI, body mass index; ref, reference; CI, confidence interval

Figure 1 Risk of ischemic stroke among study participants based on sex.
Abbreviations: OR, odds ratio; BMI, body mass index; ref, reference; CI, confidence interval.

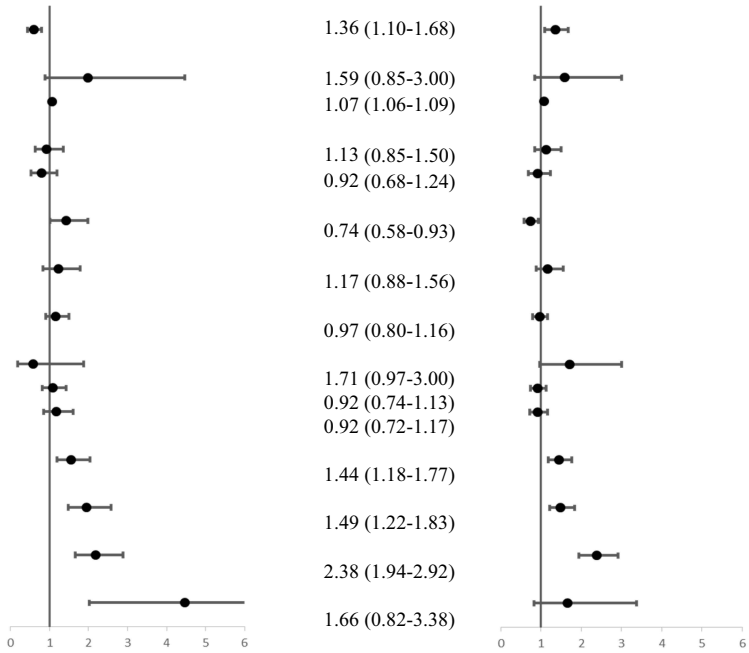
Additionally, little is known about this genetic variant in Asia, particularly Taiwan. These limitations aside, our findings represent a significant step in identifying the mechanistic basis for ischemic stroke and genotype associations.

As in prior studies,[16,18–20] our general model also revealed that atrial fibrillation, diabetes, hyperlipidemia, and hypertension were stronger risk factors for ischemic stroke. Our genotype stratified analysis did not affect these factors, as they remained significantly associated with ischemic stroke risk except for atrial fibrillation and TC/CC genotype combined. Hald et al found that atrial fibrillation is a cause-specific risk factor for ischemic stroke.[18] In addition, they observed that women with atrial fibrillation had a higher overall stroke risk than men. In this study, however, we found that men rather than women with atrial fibrillation were more likely to suffer from ischemic strokes. Smoking is a preventable risk factor for stroke.[21–23] In this study, we did not find an association between ischemic stroke and smoking. The results of our analysis based on genotypes of rs4918758, however, revealed that smoking individuals with the TT genotype were more likely to suffer an ischemic stroke. In contrast, those with TC/CC appeared to be at lower risk. Older age was a risk factor for ischemic stroke no matter the sex or genotype.

Moreover, we found that ischemic stroke risk was higher among warfarin users even though the ORs were not significant after stratification by sex and genotype. In their study, Tung et al reported higher rates of ischemic stroke in the first 30 days following warfarin initiation.[24]

Despite these findings, we discuss our study limitations. First, we might have underestimated cases with ischemic stroke considering that TWB did not enroll participants with a severe type of ischemic stroke. Next, rs4918758 has not been specifically linked to ischemic stroke. Historically, this variant has been associated with CHD, which is a potential marker for ischemic stroke. Our findings, however, are preliminary, so functional studies would help to shed more light on them, including the possible mechanisms involved.

Variable	rs4918758 (TT) OR (95%CI)	Forest plot	rs4918758 (TC/CC) OR (95%CI)	Forest plot
Sex (ref: Female)				
Male	0.59 (0.44-0.80)		1.36 (1.10-1.68)	
Warfarin use (ref: No)				
Yes	1.98 (0.88-4.47)		1.59 (0.85-3.00)	
Age	1.06 (1.04-1.08)		1.07 (1.06-1.09)	
Education (ref: Elementary School)				
Junior & Senior High School	0.93 (0.63-1.36)		1.13 (0.85-1.50)	
University & above	0.79 (0.53-1.19)		0.92 (0.68-1.24)	
Smoking (ref: No)				
Yes	1.43 (1.02-1.99)		0.74 (0.58-0.93)	
Alcohol drinking (ref: No)				
Yes	1.23 (0.84-1.79)		1.17 (0.88-1.56)	
Physical activity (ref: No)				
Yes	1.16 (0.91-1.49)		0.97 (0.80-1.16)	
BMI (ref:18.5≤BMI<24)				
BMI<18.5	0.58 (0.18-1.88)		1.71 (0.97-3.00)	
24≤BMI<27	1.08 (0.81-1.43)		0.92 (0.74-1.13)	
BMI≥27	1.17 (0.85-1.60)		0.92 (0.72-1.17)	
Diabetes (ref: No)				
Yes	1.55 (1.19-2.04)		1.44 (1.18-1.77)	
Hyperlipidemia (ref: No)				
Yes	1.95 (1.48-2.58)		1.49 (1.22-1.83)	
Hypertension (ref: No)				
Yes	2.19 (1.66-2.88)		2.38 (1.94-2.92)	
Atrial fibrillation (ref: No)				
Yes	4.46 (2.02-9.84)		1.66 (0.82-3.38)	



OR indicates odds ratio; BMI, body mass index; ref, reference; CI, confidence interval

Figure 2 Risk of ischemic stroke among study participants based on rs4918758 genotypes.
Abbreviations: OR, odds ratio; BMI, body mass index; ref, reference; CI, confidence interval.

Conclusions

In conclusion, our findings indicate that ischemic stroke risk in Taiwanese male and female adults may be explained by rs4918758 of the *CYP2C9*. Nonetheless, future genome-wide association studies would be required to provide more solid evidence.

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Disclosure

The authors report no conflicts of interest in this work.

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