Interactive Role of CYP2C9 rs4918758 Polymorphism and Sex in Ischemic Stroke Susceptibility

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ABSTRACT
Stroke is a complex health condition caused by several factors. We assessed the possible interaction of the Cytochrome P450 2C9 (CYP2C9) rs4918758 polymorphism with sex in triggering an ischemic stroke. We included 9,197 women and 8,625 men from the Taiwan Biobank (TWB). Data collected between 2008 and 2015 were linked to records in the National Health Insurance Database (NHIRD). We estimated Odds Ratios (OR) for ischemic stroke using logistic regression analysis. We found that ischemic stroke was present in 441 women and 468 men had. Combined TC+CC of rs4918758 did not enhance ischemic stroke risk [OR (95% CI)=1.04 (0.90-1.21]. Compared to women, men did not confer risk for ischemic stroke [OR (95% CI)=1.03 (0.87-1.22). There was an interaction between sex and rs4918758 polymorphism (p for interaction=0.0019). After categorizing by sex, significant odds ratios were found in men with combined TT+CC of rs4918758 but not women [OR, 1.32 (1.07-1.63) vs. 0.83 (0.68-1.02)]. Further stratification by genotypes of rs4918758 polymorphism, TT was protective against ischemic stroke [0.59 (0.44-0.80)] in men compared to women. However, combined TT+CC was causative [1.36 (1.10-1.68)]. Our analyses indicated that TT of rs4918758 was protective whereas combined TT+CC appeared to enhance ischemic stroke risk in Taiwanese men compared to women. This study extends knowledge on the genetic basis of ischemic stroke.

Keywords: Stroke; Genetic variant; Sex

INTRODUCTION
Ischemic stroke is increasingly becoming a serious health issue worldwide especially in people with Coronary Heart Diseases (CHD). It constitutes approximately 74% of all stroke in Taiwan. The disease incidence appears to be increasing significantly among younger adults [1]. Significant risk factors include CHD, atrial fibrillation, diabetes, smoking, hypertension, low high-density lipoprotein cholesterol, and a history of stroke. Several subtypes exist for ischemic stroke, some of which were found to have genetic components among European populations [2]. So far, there is limited evidence on the role of specific genetic variants on the development of ischemic stroke considering that only a few rare variants have been linked to this condition. To our knowledge, a few epidemiological studies have replicated genetic variants linked to ischemic stroke. Polymorphic variants including those of genes encoding the CYP2C subfamily have shown associations with CHD, a potential marker for ischemic stroke [3]. Among them is the rs4918758 polymorphism of the CYP2C9 gene, which was associated with a decreased risk of CHD in Russian and European populations. As far as we know, attempts have been made to investigate this variant mainly in Europe, Japan, and North America. In light of this, we examined associations of rs4918758 with ischemic stroke risk among Taiwanese adults and tested whether they differ by sex [4].

MATERIALS AND METHODS

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Data source
The Institutional Review Board of Chung Shan Medical University approved this study. We included 9,197 women and 8,625 men from the TWB [5]. Diseases were identified using the NHIRD. Data from both sources were collected from 2008-2015. Linkages were made through the Health and Welfare Data Science Center using personal identification numbers [6].

Disease definitions
Disease identification was based on the medical records in National Insurance Research Database. They included 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) coupled with either two outpatient visits or one-time admission [7].

Study population
Initial recruitment included 17985 TWB participants. Exclusion criteria included persons with incomplete questionnaire (n=37) and genotyping information (n=13), and those with hemorrhagic stroke (n=113). After the exclusions, 909 ischemic stroke patients, and 16913 controls were included in the study.

SNP selection and genotyping
The rs4918758 of the CYP2C9 gene was selected based on a literature search and our samples were genotyped for this variant SNP by using the Axiom™ Genome-Wide Array Plate System. For the TWB SNPs array, we followed a standard quality control procedure to exclude SNPs with low call rate (<95%), the p-value for the Hardy-Weinberg Equilibrium (HWE) test of<1.0 × 10^{-3}, and minor allele frequency of<0.05.

STATISTICAL ANALYSIS
We compared differences between continuous and discrete variables using the x2 test and t-test. We also determined the effects of sex and CYP2C9 (rs4918758) on the ischemic stroke using logistic regression analysis and estimated the Odds Ratios (ORs) with their 95% confidence intervals [8]. These analyses were performed using the Statistical Analysis System (SAS) software, version 9.4 (SAS Institute, Inc, Cary, NC) and PLINK software, version 1.09.

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8756</td>
<td>95.2</td>
</tr>
<tr>
<td>Yes</td>
<td>441</td>
<td>4.8</td>
</tr>
<tr>
<td>CYP2C9 (rs4918758)</td>
<td>0.811</td>
<td></td>
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</table>

Table 1: Baseline characteristics for male and female participants.

RESULTS
We observed 441 women and 468 men with ischemic stroke (Table 1). Results of logistic regression is displayed in Table 2. Combined TC+CC of rs4918758 [OR (95% CI)=1.04 (0.90-1.21) did not enhance ischemic stroke risk. Compared to women, men did not confer risk for ischemic stroke [OR (95% CI)=1.03 (0.87-1.22)]. Hyperlipidemia [2.45 (1.45-4.12)], hypertension [2.30 (1.95-2.71)], and diabetes [1.48 (1.26-1.74)] were causative for ischemic stroke. Interestingly, there was an interaction between sex and rs4918758 polymorphism (p for interaction=0.0019). After stratification by genotype of rs4918758 polymorphism, TT was protective against ischemic stroke [0.59 (0.44-0.80)] in men compared to women. In contrast, the combined TT+CC was causative [1.36 (1.10-1.68)]. Further stratification by sex showed substantial association in TT+CC men [1.32 (1.07-1.63)] but not TT+CC women [0.83 (0.68-1.02)].

<table>
<thead>
<tr>
<th>OR</th>
<th>95%CI</th>
</tr>
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<tbody>
<tr>
<td>rs4918758 (ref: TT)</td>
<td></td>
</tr>
<tr>
<td>TC+CC</td>
<td>1.04</td>
</tr>
<tr>
<td>Sex (ref: Female)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.03</td>
</tr>
<tr>
<td>Warfarin use (ref: No)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.72</td>
</tr>
<tr>
<td>Age</td>
<td>1.07</td>
</tr>
<tr>
<td>Yes</td>
<td>1.48</td>
</tr>
<tr>
<td>Hyperlipidemia (ref: No)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Table 2: Association of ischemic stroke with overall variables.
DISCUSSION

Genetic risk factors for ischemic stroke and their possible interactions have not been sufficiently reported. The current study is the first to evaluate relationships of rs4918758 genetic variant and sex with ischemic stroke among TWB participants aged 30 to 70 years [9]. We provide evidence to show that TT of rs4918758 is protective whereas combined TC+CC appears to enhance ischemic stroke risk in Taiwanese men compared to women. This is an indication that rs4918758 polymorphism 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 established. In our primary analysis, we observed no significant differences in ischemic stroke risk among men and women even though a prior study observed male predominance across all age groups. Furthermore, 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 [10].

As stated earlier, polymorphism rs4918758 is a significant marker for heart diseases, specifically in European and Russian populations. However, its specific role in ischemic stroke susceptibility remains to be clarified. Moreover, little is known about this genetic variant in Asia, particularly Taiwan. Despite these, our results represent a great step towards discovering the mechanistic basis for ischemic stroke and genotype associations. Consistent with prior studies, we also found that atrial fibrillation, diabetes, hyperlipidemia, and hypertension were stronger risk factors for ischemic stroke in the general model. Our genotype stratified analysis did not affect these factors, for they remained significantly associated with ischemic stroke risk. They also observed that the overall risk of stroke was higher in women with atrial fibrillation than men. However, in this study, we found men instead of women with atrial fibrillation to be at higher risk of ischemic stroke. Smoking is a preventable risk factor for stroke. In this study, we found no association between ischemic stroke and smoking. However, when we examined the relationships based on genotypes of rs4918758, we found that smoking was causative in TT individuals, but protective in TC+CC. Moreover, we observed that ischemic stroke risk was higher among warfarin users even though the odds were found not significant after we stratified by sex and genotypes. In their study, Tung and colleagues reported higher rates of ischemic stroke in the first 30 days after warfarin initiation [11].

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, the rs4918758 variant has not been specifically linked to ischemic stroke.

CONCLUSION

The choice for this variant was because of its previously established relationship with coronary heart disease, which is a potential marker for ischemic stroke. 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.

REFERENCES