Interactive Association between CYP2C9 rs2860905 Polymorphism and Atrial Fibrillation on Ischemic Stroke in Taiwan Biobank Participants

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ABSTRACT
Objective: Ischemic stroke accounts for approximately 85% of all strokes. Risk factors include atrial fibrillation, metabolic disorders, and genetic and lifestyle factors. There is limited evidence to support the association between atrial fibrillation and the risk of ischemic stroke based on genetic variants. We assessed the relationship between ischemic stroke and atrial fibrillation among Taiwan biobank (TWB) participants based on the rs2860905 variant of the cytochrome P450 Family 2 Subfamily C Member 9 (CYP2C9) gene.

Methods: Using logistic regression analysis, we estimated the odds ratios (OR) and 95% confidence intervals (CI) for ischemic stroke among 17,726 biobank adults recruited from 2008 through 2015.

Results: Of the eligible individuals (n=17,726), 906 were identified with ischemic stroke. Atrial fibrillation was positively associated with ischemic stroke (OR=3.70; 95% CI, 2.21-6.20), whereas the rs2860905 variant was not. The OR for ischemic stroke among those with GA/AA genotype was 1.00 (95% CI, 0.82-1.22) compared to those with GG genotype. Based on the genotype-stratified analysis, the OR of atrial fibrillation for ischemic stroke was 4.68 (95% CI, 2.70-8.09) in those with GG genotype.

Conclusions: These results demonstrate that GG genotype of CYP2C9 rs2860905 variant may enhance ischemic stroke risk in Taiwanese individuals.

Keywords: Genetic variation; Risk prediction; Stroke

INTRODUCTION
Stroke remains one of the significant health threats in Taiwan. Globally, about 13.7 million people over the age of 25 develop stroke each year, and the incidence is higher in men than women. There are three main types, but generally, ischemic stroke is the most frequent type, especially in people under 70. Ischemic stroke accounts for almost 85% of all stroke. According to a previous report in Taiwan, ischemic stroke is predominant among adults aged 65 and 69. Several metabolic and lifestyle factors play a role in the development of ischemic stroke. Atrial fibrillation is among the independent factors previously associated with ischemic stroke in Taiwan and other populations. Dysrhythmia, which affects millions of people worldwide, has been linked to an interplay between genetic predisposition and several other factors.

Stroke that develops due to AF is associated with a higher fatality because of large thrombi in the atria. Warfarin, a plasma-bound anticoagulant, is commonly used to decrease stroke risk. According to a previous meta-analysis, warfarin use was associated with a 64% decrease in stroke risk. Despite these benefits, it has been suggested that sensitivity to this anticoagulant is affected by genetic variation. The Cytochrome P450 2C9 (CYP2C9) gene is among the genes whose variants have previously shown associations with warfarin dose. It is responsible for the metabolic clearance of warfarin. Rs 2860905
is among the CYP2C9 variants that remained significantly associated with warfarin dose after experiment-wise adjustments for multiple testing. Compared to other CYP2C9 variants combined, rs2860905 has been associated with a better prediction of warfarin dose among Caribbean Hispanics of Puerto Rico. Assessing the pharmacogenomic influence on warfarin across different ethnicity is essential in clinical pharmacology. In this study, we evaluated the relationship between ischemic stroke and atrial fibrillation among Taiwan biobank participants based on the rs2860905 variant of the warfarin sensitivity (CYP2C9) gene.

**METHODS AND MATERIALS**

**Data source**

Genetic data from 2008 through 2015 were obtained from Taiwan Biobank (TWB) database. They were linked to population data from 1998 through 2015 that were available in the National Health Insurance (NHI) databases provided by the Health and Welfare Data Science Center. We used the NHI data were used to identify ischemic stroke, atrial fibrillation, and warfarin prescription. The TWB data and the NHI data were linked using personal identification numbers. The Institutional Review Board of Chung Shan Medical University approved this study.

**Study participants**

We enrolled a total of 17,985 participants in the Taiwan Biobank database and excluded persons with an incomplete questionnaire (n=37) and genotyping information (n=109). Also excluded were 113 persons with hemorrhagic stroke. The final enrollment included 906 participants with ischemic stroke and 16,820 control individuals.

**Definition of the variables**

We identified ischemic stroke based on the International Classification of Diseases Clinical Modification, 9th Revision (ICD-9-CM) 433-437, and atrial fibrillation based on either two outpatient visits or one admission with reported ICD-9-CM 427.3 code. The selected lifestyle variables of interest have been previously defined. Prior to the diagnosis of ischemic stroke, we identified persons who had received a prescription for warfarin.

**Variant selection and genotyping**

Based on the literature search, we selected the rs2860905 variant in the CYP2C9 gene. We chose this variant based on its previously established associations with warfarin. Our samples were genotyped for this variant SNP by using the Axiom™ Genome-Wide Array Plate System. We performed quality control by excluding SNPs.

**STATISTICAL ANALYSIS**

We compared the differences between discrete and continuous variables using the Chi-square and t-test. Then, we performed logistic regression analysis to investigate the association between atrial fibrillation and CYP2C9 in relation to ischemic stroke. Our regression model variables included were age, sex, alcohol drinking, educational level, smoking, physical activity, body mass index, diabetes, hypertension, hyperlipidemia, and warfarin use. We estimated corresponding Odds Ratios (ORs) with their 95% confidence intervals. Statistical analyses were performed using the Statistical Analysis System (SAS) software (version 9.4) and PLINK.

**RESULTS**

Baseline characteristics are provided in Table 1. Of the 17,726 eligible individuals, we identified 906 with ischemic stroke. Among them, 773 were those with the GG genotype and 133 carried the GA/AA genotype. About 1.21% of patients with ischemic stroke and 0.65% of control individuals had a history of warfarin use. In our overall analysis (Table 2), atrial fibrillation was associated with increased ischemic stroke risk (OR=3.70; 95% CI, 2.21–6.20). We found no significant association between the rs2860905 variant and ischemic stroke risk. The OR for ischemic stroke among those with GA/AA genotype was 1.00 (95% CI, 0.82–1.22). We also found strong associations between ischemic stroke risk and diabetes (OR=1.49; 95% CI, 1.26–1.75), hypertension (OR=2.28; 95% CI, 1.94–2.69), and hyperlipidemia (OR=1.66; 95% CI, 1.41-1.96). We also found that the ischemic stroke risk associated with warfarin use was lower but not significant (OR=0.54; 95% CI, 0.26-1.11).
In this study, we assessed the relationship between ischemic stroke and atrial fibrillation among Taiwan Biobank participants based on the CYP2C9 rs2860905 polymorphism. To our knowledge, such associations have not been reported in Taiwan. In the primary analysis, we observed that atrial fibrillation but not rs2860905 variant was a significant risk factor for ischemic stroke. However, when stratified by CYP2C9 genotypes, atrial fibrillation remained a risk factor only among GG genotype individuals with OR of 4.68 (95% CI: 2.7-8.09). On the contrary, GA+AA predicted a decreased but non-significant risk for ischemic stroke. Strong associations have been observed between ischemic stroke and genom-wide measures of atrial fibrillation. The genetic variants analyzed were particularly those that have been previously associated with atrial fibrillation. We firmly believe that our findings expand knowledge on associations between ischemic stroke risk and atrial fibrillation by relating a genetic variant that has been previously associated with warfarin use, as stated in the introduction. Of note, warfarin predicts a decrease in the risk of ischemic stroke, but its dose is affected by genetic variants including the rs2860905 variant. Considering that this variant has not been replicated in Taiwan and that little is known about it in Asia, we thought it wise to determine whether it contributes to the association between ischemic stroke and AF.

We observed that other well-established risk factors for ischemic stroke like diabetes, hypertension, and hyperlipidemia remained strongly associated with ischemic stroke risk. We also observed that the odds ratio associated with warfarin use was lower but not statistically significant. Moreover, we found that the risk of ischemic stroke occurrence did not differ significantly between men and women.

Thus far, efforts have been made to determine the relationship between ischemic stroke and genetic variants even though results have been inconsistent. Among these studies, those that were conducted in Taiwan included variants of other genes. Of note, they did not include atrial fibrillation in their model as we have done. Having presented these findings, we also acknowledge a few limitations. First, patients with a severe type of ischemic stroke were not enrolled in the TWB. Therefore, we may have underestimated the number of people with ischemic stroke. Next, we could not determine the actual dose of warfarin taken by those who had an ischemic stroke from the NHIRD and TWB database.

**CONCLUSION**

Taken together, our findings indicate that the association between ischemic stroke and atrial fibrillation may be stronger among adults with the CYP2C9 rs2860905 GG genotype.
Further studies would be required to see whether the results reported here apply to other populations.

REFERENCES


