Renin–angiotensin system gene polymorphisms predict the risk of stroke in patients with atrial fibrillation: A 10-year prospective follow-up study

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BACKGROUND Little evidence is available regarding the impact of genetic polymorphisms on the risk of stroke in patients with atrial fibrillation (AF). Angiotensin II plays a pathophysiologic role in prothrombotic atrial endocardial remodeling.

OBJECTIVE The purpose of this study was to investigate the effect of polymorphisms of renin–angiotensin system genes on the incidence of stroke in a prospective cohort of patients with AF.

METHODS A total of 712 AF patients were longitudinally followed-up for 10.3 ± 2.7 years. Eight polymorphisms of renin–angiotensin system genes were genotyped.

RESULTS Patients carrying the G-6 allele in the promoter of the angiotensinogen gene, which was associated with higher promoter activity, were more likely to develop stroke than were noncarriers (hazard ratio 2.54, 95% confidence interval [1.26–5.12], $P = .009$ after adjustment for $CHADS_2$ score). G-6A polymorphism provides information additional to $CHADS_2$ on stroke risk prediction (*C*-statistic 0.672 vs 0.724, $P = .039$). In haplotype analysis, angiotensinogen gene promoter haplotypes containing –217G/–6G, which was associated with the highest promoter activity, were associated with an increased risk of stroke ($P = .004$). G-217/G-6 haplotype carriers were even more likely to develop stroke than were noncarriers (hazard ratio 2.78, 95% confidence interval 1.37–5.64, $P = .003$ after multivariable adjustment). In pharmacogenetic analysis, the increased risk of stroke in subjects carrying G-6 was eliminated by concomitant treatment with an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker ($P = .012$ for interaction).

CONCLUSION In addition to the $CHADS_2$ score, angiotensinogen gene polymorphisms may be considered an additional genetic predictor of stroke in patients with AF. Genotyping of the angiotensinogen gene is helpful to determine which AF patients may benefit from treatment with an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker.

KEYWORDS Atrial fibrillation; Stroke; Prospective; Polymorphisms; Renin–angiotensin system; Pharmacogenetics

ABBREVIATIONS **AF** = atrial fibrillation; **ACE** = angiotensin-converting enzyme; **ACEI** = angiotensin-converting enzyme inhibitor; **AUC** = area under the curve; **CI** = confidence interval; **HR** = hazard ratio; **ICAM** = intercellular adhesion molecule; **ROC** = receiver operating characteristic; **VCAM** = vascular cell adhesion molecule

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Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. In addition, AF is associated with an increased risk of stroke and thromboembolism[1] because it is associated with a prothrombotic or hypercoagulable state.[2,3] AF was associated with increased fibrin D-dimer and von Willebrand factor levels, a marker for intravascular thrombogenesis and an index of endothelial dysfunction, respectively.[4,5] The presence of enhanced platelet activation with a higher level

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of soluble P-selectin has been recognized in chronic AF patients.[6] Another potential mechanism may be related to the association of AF with an inflammatory state.[7,8] Increased atrial endocardial expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM), could be an important link between the initiation of inflammatory and thrombogenic mechanisms responsible for thrombus development at the atrial endocardium.[9,10]

Although clinical factors that predict the risk of stroke in patients with AF (e.g., $CHADS_2$ or $CHADS_2$-Vasc score) have been identified, these clinical factors do not explain the entire susceptibility to stroke among patients with AF. Furthermore, limited data are available on regarding the impact of genetic polymorphisms on the risk of stroke in AF patients. Most of these studies focused on the genes related to thromboembolisms and were cross-sectional, and the results still are controversial.[11,12]

We previously showed that polymorphisms of the renin–angiotensin system genes were associated with an increased risk for AF.[13] It was previously demonstrated that AF or rapid atrial pacing increases endocardial VCAM expression, which may be related to endocardial thrombus formation and can be attenuated by angiotensin II receptor blockade (ARB).[14] This finding provides evidence that angiotensin II plays a pathophysiologic role in prothrombotic endocardial remodeling and mural thrombus formation. Therefore, we hypothesized that polymorphisms of the renin–angiotensin system genes also may influence the risk of stroke in patients with AF and provide additional information other than the $CHADS_2$ or $CHADS_2$-Vasc score for predicting the risk of stroke among AF patients. We conducted the first long-term longitudinal observational study to prove this hypothesis.

We genotyped a total of eight polymorphisms within the genes related to the renin–angiotensin system, which included G-217A, G-152A, A-20C, G-6A, M235T, and T174M polymorphisms of the angiotensinogen (AGT) gene, insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene, and A1166C polymorphism of the angiotensin II type 1 receptor (AT1R) gene. For the polymorphisms in the AGT gene, there were two polymorphisms (M235T and T174M) in exon 2 and four polymorphisms in the promoter (G-217A, G-152A, A-20C, G-6A), which have been shown to modulate AGT gene expression.[15,16] The polymorphism in the ACE gene is a 287-bp insertion/deletion polymorphism in intron 16 of the ACE gene, which has also been shown to determine serum and tissue ACE levels.[17] The A1166C polymorphism of the AT1R gene localizes in the 3′ untranslated region of the AT1R,and has also been shown to affect AT1R response to angiotensin II stimulation.[18]

Methods

Patient population and follow-up

The study population consisted of 712 consecutive adult patients who were diagnosed as having AF after January 1998 at National Taiwan University Hospital and National Taiwan University Hospital Yun-Lin Branch and were followed-up in the Cardiovascular Clinic through February 2012. The enrollment was staggered, and details of the selection of AF patients were described previously.[13] Sixty-nine percent of the enrolled AF patients had persistent AF. Median follow-up time was 10.3 years (range 3.2–12.2 years). The study protocols were reviewed and approved by the Institutional Review Committee, and all patients agreed to participate in the study.

Clinical and outcome assessments

Stroke was defined as a sudden onset of neurologic deficit that was not explained by other origins, with supporting evidence from the imaging study. Hemorrhagic stroke was not included in this study. All patients underwent complete transthoracic echocardiography to measure left atrial and left ventricular dimensions and left ventricular ejection fraction (LVEF) and to detect significant valvular disease (define as at least moderate valvular regurgitation or stenosis). All baseline characteristics were collected as previously described.[13,19]

We focused on whether treatment with angiotensin-converting enzyme inhibitor (ACEI) or ARB might influence the impact of genetic polymorphisms on the risk of stroke in patients with AF. Patients receiving ACEI or ARB treatment were defined as those who continued to take standard doses of ACEI or ARB throughout at least half of the follow-up years and were still taking ACEI or ARB at the end of follow-up or when the outcome (stroke) occurred.[20]

Identification of diallelic polymorphisms

Genomic DNA was extracted, and DNA fragments were amplified by polymerase chain reaction. Genotyping of the ACE gene I/D, AT1R gene A1166C, and AGT gene polymorphisms was performed using our previously reported methods.[13,21–23]

Statistical analysis

Data are presented as mean ± SD. For comparisons of baseline patient characteristics, the between-group data were compared using the Student unpaired t test for continuous data and the χ^2 or Fisher exact test for categorical data. Methods for evaluation of Hardy-Weinberg equilibrium and single-locus analyses were described previously.[13,22]

The six polymorphisms in the AGT gene are in tight linkage dysequilibrium[22] and do not segregate independently. Therefore, we used haplotype analysis to determine the overall effect of these six polymorphisms and their associations with stroke risk. We used a regression-based approach for AGT gene haplotype analysis as previously reported[19,24] because the risk of stroke was also substantially influenced by environmental factors or nongenetic covariates. We used the Haplo.stats program (haplo.score and haplo.glm), which was specially designed to analyze haplotype data by the regression method, as previously described.[25]

Survival analysis was performed for the significant polymorphism(s) detected in single-locus analysis or haplotype analysis. The times to first attack of stroke were depicted