The 1425G/A SNP in *PRKCH* Is Associated With Ischemic Stroke and Cerebral Hemorrhage in a Chinese Population

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Background and Purpose—*PRKCH* (the gene encoding protein kinase C η) has a role in the pathogenesis of ischemic stroke. The 1425G/A SNP in *PRKCH* (rs2230500) is significantly associated with ischemic stroke in Japanese. The aim of the present study is to investigate the associations in ischemic stroke and other types of stroke in the Chinese population.

Methods—A total of 1209 patients with stroke and 1174 controls were examined using a case–control methodology. The 1425G/A SNP in *PRKCH* was genotyped by allele-specific real-time PCR assay.

Results—The 1425G/A SNP in *PRKCH* was significantly associated with both ischemic stroke (odds ratio [OR]=1.31; 95% confidence interval [CI], 1.08 to 1.60; P=0.0058) and cerebral hemorrhage (OR=1.94; 95% CI, 1.21 to 3.10; P=0.0054) under a dominant model. Even after age- and sex-adjustment, the significant associations remained (in ischemic stroke, for AA+AG versus GG, OR=1.37, 95% CI, 1.12 to 1.67, P=0.0019; in cerebral hemorrhage, for AA+AG versus GG, OR=1.96, 95% CI, 1.21 to 3.19, P=0.0064).

Conclusions—The 1425G/A SNP in *PRKCH* increases the risk of both ischemic stroke and cerebral hemorrhage in the Chinese population. **(*Stroke*. 2009;40:2973-2976.)**

Key Words: ischemic stroke ■ cerebral hemorrhage ■ *PRKCH*

Stroke is the second leading cause of death worldwide and a major burden on health care.[1,2] In China, stroke is the dominant type of cardiovascular disease.[3] Stroke is generally classified into ischemic stroke and hemorrhagic stroke. The proportion of ischemic stroke was about 43.7 to 78.9% of all strokes in Chinese population.[4] Different from Western countries, the incidence of stroke is higher than that of coronary heart disease, and the proportion of hemorrhagic stroke is also high in China.[5]

The risk factors of stroke conventionally consist of hypertension, diabetes mellitus, smoking, and cardiac diseases.[6,7] In recent years, accumulating evidence suggests that inflammation and atherosclerosis play important roles in the development of stroke.[8,9] However, the molecular mechanisms are not fully understood.

Protein kinase C η (PKCη), a serine-threonine kinase, is involved in the development and progression of atherosclerosis in human.[10] It has been reported that a 1425G/A SNP in *PRKCH* (the gene encoding PKCη) increases the risk of ischemic stroke in the Japanese population,[10,11] yet the replicated studies have not been carried out in other populations. In addition, whether the 1425G/A SNP in *PRKCH* is associated with cerebral hemorrhage has not been studied.

The minor allele frequencies (MAF) of the 1425G/A SNP in *PRKCH* were reported in the HapMap database as 0.008 in CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), 0.00 in YRI (Yoruba in Ibadan, Nigeria), 0.239 in JPT (Japanese in Tokyo, Japan), and 0.178 in CHB (Han Chinese in Beijing, China).[10] These data indicate that this SNP is specific to Asian populations, and it is important to investigate the association between the SNP and stroke in another Asian population.

We have previously conducted a genetic study of stroke in Chinese patients. The types of stroke recruited for the study include ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage, transient ischemic attacks (TIA), and stroke with undetermined cause. Therefore, we investigated the association between the 1425G/A SNP in *PRKCH* and ischemic stroke, also potential associations between this SNP and other types of stroke in the cohort.

Received March 11, 2009; final revision received May 3, 2009; accepted May 8, 2009.

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Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.109.551747**

Materials and Methods

Subjects

The Stroke Hypertension Investigation in Genetics (SHINING) study was conducted by the Beijing Hypertension League Institute. Between 1997 and 2000, patients and control subjects from 6 geographical regions within China were recruited for the case–control study (70% subjects came from in and near Beijing).[12] SHINING study comprised subjects exclusive to Han ethnicity. Cases were recruited from the community of those who were discharged from hospitals. Any stroke patients who suffered a stroke within the past 5 years were eligible to participate in the study.[12] All patients have medical records with diagnosis of brain CT/MRI. Patients with ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage, or transient ischemic attacks were included. Control subjects were selected according to the case–control study criteria during the same period (control subjects matched to cases by sex, age within 3 years, geographic location, and blood pressure category (<140/90, ≥140/90 and ≤180/105, >180/105 mm Hg)).[12] Data collected included age, sex, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension. Hypertensives were defined as having current or past antihypertensive medication, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg.[12]

We obtained written informed consent from all study participants, and the study was approved by ethics committees of the Beijing Hypertension League Institute.

Genotyping

A total of 3119 participants (1559 stroke cases and 1560 controls) were recruited for the SHINING study, and of these, 2383 participants (1209 stroke cases and 1174 controls) had DNA samples available. The 1425G/A SNP in *PRKCH* (rs2230500) was genotyped by allele-specific real-time polymerase chain reaction[13] using GeneAmp 5700 Sequence Detector (Applied Biosystem).

The PCR amplifications were performed using the following primers:

Common primer, 5′-GCAGAATCACGTCCTTCTTCAG-3′;
Allele-specific primer (A), 5′-CATAGGTGATGCTTGCAAGAA-3′;
Allele-specific primer (G), 5′-CATAGGTGATGCTTGCAAGAG-3′.

Individual DNA sample was genotyped for single SNP by using an equal aliquot of samples with 2 allele-specific PCR reactions, each containing 1 of the allele-specific (A-S) primers and a common primer. PCR reaction with the A-S primer that matched the allele in the template DNA amplified normally, whereas PCR reaction with the other A-S primer that mismatched the allele in the template was prevented or delayed when PCR reaction was monitored in real-time (by including SYBR Green I in the PCR and following fluorescence cycle-by-cycle). For each amplification, a fluorescence threshold near the baseline fluorescence was used to calculate a cycle threshold value, which was then used to call the genotype of the sample. PCR was carried out on the GeneAmp 5700 Sequence Detector with procedure of 12 minutes at 95°C, followed by 45 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and finished by 20 minutes dissociation at 60°C. Genotype was directly obtained with the GeneAmp 5700 SDS software. The genotyping call rate was 98%. For validating the accuracy of genotyping, we sent 94 samples to direct sequencing and observed 100% concordance between 2 genotyping methods.

Statistical Analysis

Clinical data about continuous variables expressed as mean±SD, and differences between groups were assessed by Mann–Whiteney *U* test.[14] Categorical variables were represented as percentage and were tested by χ^2 analysis. Hardy–Weinberg equilibrium was also assessed by χ^2 analysis. Our analyses concerned the whole study group and were subsequently stratified by 2 major types of stroke, ischemic stroke and cerebral hemorrhage. In each stratum, cases were compared with the corresponding control groups. Odds ratios in dominant model with corresponding 95% confidence intervals were computed by the Woolf's method to test the effect of genotype on stroke risk. Adjusted odds ratios for age and sex were performed by multiple logistic regression with genotypes, age and sex as the independent variables.[15] Data were analyzed using SAS statistical software (version 9.1, SAS Institute Inc). $P<0.05$ was used to indicate statistically significant differences.

Table 1. Characteristics of Study Participants

	Stroke Patients	Controls	*P* Value
No. of subjects	1209	1174	
Age,* y	60.0±10.3	61.0±10.5	0.024
Sex, % male	58.6	60.0	0.511
BMI,* kg/m^2	24.3±3.0	25.0±3.3	<0.0001
SBP,* mm Hg	144.1±23.1	143.8±23.0	0.544
DBP,* mm Hg	86.7±12.5	86.6±12.8	0.616
Hypertension, % yes	79.1	86.4	<0.0001

*Continuous variables were expressed as mean±SD.

Results

The characteristics of the study participants are shown in Table 1. The means of age and BMI and the frequency of presence of hypertension were lower in cases than those of in controls. SBP and DBP in cases were not different from those in controls. There was a greater proportion of hypertension in controls because the original goal of SHINING study was to identify SNPs that predispose to stroke independent of blood pressure.[12]

Supplemental Table I, available online at http://stroke.ahajournals.org, shows the types of stroke in patients. The percentage of patients with ischemic stroke and cerebral hemorrhage is 72.6% and 12.7% of overall patients, respectively. We genotyped the 1425G/A SNP in *PRKCH* (rs2230500) in the cohort (Table 2). The SNP was tested to be in Hardy–Weinberg equilibrium ($P>0.05$) in controls except the controls matching to the cases with cerebral hemorrhage ($P=0.043$). This could be attributable to the small sample size (144 controls) in our study for these controls. Associations between the 1425G/A SNP in *PRKCH* and different types of stroke are shown in Table 2. The A allele of 1425G/A SNP not only increased the risk of ischemic stroke but also increased the risk of cerebral hemorrhage.

Odds ratios for the incidence of ischemic stroke and cerebral hemorrhage are shown in Table 3. The 1425G/A SNP in *PRKCH* increased the risk of both ischemic stroke (age- and sex-adjusted odds ratio=1.37; 95% CI, 1.12 to 1.67; $P=0.0019$) and cerebral hemorrhage (age- and sex-adjusted odds ratio=1.96; 95% CI, 1.21 to 3.19; $P=0.0064$) under a dominant model. Supplemental Table II shows the results in stroke (including all subtypes of stroke in the study). For 1425G→A, age- and sex-adjusted odds ratio of AA and AG genotype combined was 1.46 (95% CI, 1.23 to 1.73; $P=0.000012$) compared with GG genotype.

Discussion

In the present study, we examined the 1425G/A SNP in *PRKCH* with different types of stroke in the Chinese population. This SNP was previously shown to increase the risk of ischemic stroke in the Japanese population.[10,11] Our results indicate that the 1425G/A SNP in *PRKCH* increases the risk

Table 2. Case-Control Study Showing Association Between the 1425G/A SNP in *PRKCH* and Stroke

	Case					Control					MAF		Unadjusted (AA+AG vs GG)	
Samples	AA	AG	GG	Sum	H-W*	AA	AG	GG	Sum	H-W*	Case	Control	Odds ratio (95% CI)	*P* Value†
Screening														
Stroke	66	478	659	1203	0.084	50	371	726	1147	0.766	0.254	0.205	1.42 (1.21–1.68)	0.000027
Ischemic stroke	35	349	489	873	0.005	28	281	517	826	0.172	0.240	0.204	1.31 (1.08–1.60)	0.0058
IH stroke‡	0	18	24	42	0.077	2	7	27	36	0.135	0.214	0.153	2.25 (0.85–5.94)	0.098
Cerebral hemorrhage	17	59	76	152	0.289	10	39	95	144	0.043	0.306	0.205	1.94 (1.21–3.10)	0.0054
Subarachnoid hemorrhage	2	7	3	12	0.545	2	2	8	12	0.054	0.458	0.25	6.00 (1.02–35.37)	0.041
TIA	8	34	47	89	0.608	6	30	56	92	0.475	0.281	0.228	1.39 (0.77–2.51)	0.274
Undetermined	4	11	20	35	0.224	2	12	23	37	0.793	0.271	0.216	1.23 (0.48–3.16)	0.664

*H-W indicates Hardy–Weinberg equilibrium.

†*P* values were assessed with χ^2 test.

‡IH stroke indicates patients with both ischemic stroke and cerebral hemorrhage.

of ischemic stroke (Tables 2 and 3). It also increases the risk of cerebral hemorrhage (Table 2), and this association remains significant under a dominant model after age- and sex-adjustment (Table 3).

Patients with IH stroke (patients with both ischemic stroke and cerebral hemorrhage), TIA, and stroke with undetermined cause were also included in the analysis (Table 2). The results showed the 1425G/A SNP in *PRKCH* increased the risk of all types of stroke listed above, but did not reach the statistical significance. This could be because of the small sample size in our study for the above types of stroke. The association between the SNP and subarachnoid hemorrhage reached statistical significant (shown in Table 2). We are cautious about this positive result because we have only 12 cases versus 12 controls. Studies with greater sample size are needed to examine the SNP with other types of stroke to clarify the associations.

Protein kinase C (PKC) mediates a wide variety of signaling pathways and regulates multiple crucial cellular functions including proliferation, differentiation, and apoptosis.[10,16] The PKC family consists of 10 different isoforms including PKCη.[17] Unlike classical PKC isoforms, PKCη is insensitive to calcium and regulated by diacylglycerol and phospholipids.[18] It has been reported that PKCη is involved in the induction of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) release.[19] NO plays important roles in many physiological and pathological processes.[20] PKCη has been proved to relate to rheumatoid arthritis (RA).[21] Kubo et al reported that PKCη was expressed mainly in vascular endothelial cells and it has a role in the development of atherosclerotic diseases.[10]

PRKCH is located in chromosome 14q22-q23 in human. A 1425G/A SNP (leading to V374I) which lies in exon 9 and within the ATP-binding site of PKCη enhances the kinase activity.[10] Previous studies showed that the nonsynonymous SNP in *PRKCH* increases the risk of ischemic stroke in the Japanese population.[10,11] The minor allele frequency in the HapMap database indicates that this SNP is specific to Asian populations.[10] It is important to replicate the result in other Asian populations, in addition, to investigate the association between the 1425G/A SNP in *PRKCH* and cerebral hemorrhage.

Atherosclerosis is a common risk factor for ischemic stroke and cerebral hemorrhage.[8,22] It has been reported that PKCη is involved in the development of atherosclerotic diseases.[10] Previous studies tested whether the 1425G/A SNP in *PRKCH* is associated with ischemic stroke in the Japanese population.[10,11] Based on clinical and neuroimaging data, ischemic stroke (cerebral infarction) is further classified into the following subtypes: lacunar infarction, atherothrombotic infarction, cardioembolic infarction.[10,23] It was reported by Kubo et al that this SNP was significantly associated with

Table 3. Odds Ratios for the Incidence of Ischemic Stroke and Cerebral Hemorrhage

				Age- and Sex-Adjusted*	
Samples	Genotype of the 1425G/A SNP	No. of Cases	Total No. of Subjects	Odds Ratio (95% CI)	*P* Value
Ischemic stroke	GG	489	1006	1.00	
	AG	349	630	1.37 (1.12–1.68)	0.0023
	AA	35	63	1.33 (0.79–2.25)	0.286
	AA+AG	384	693	1.37 (1.12–1.67)	0.0019
Cerebral hemorrhage	GG	76	171	1.00	
	AG	59	98	1.82 (1.08–3.06)	0.025
	AA	17	27	2.61 (1.09–6.22)	0.031
	AA+AG	76	125	1.96 (1.21–3.19)	0.0064

*Multiple logistic regression was performed with adjustment for age and sex.

lacunar infarction (a subtype of ischemic stroke) in 2 independent Japanese samples (crude odds ratio=1.40; $P=5.1\times10^{-7}$), and a 14-year follow-up cohort study in Hisayama (Fukuoka, Japan) supported the involvement of the SNP in the development of ischemic stroke (age- and sex-adjusted hazard ratio=2.83; $P=0.03$).[10] Serizawa et al reported that this SNP was associated with silent lacunar infarction (SLI, a subtype of lacunar infarction) under a dominant model after adjustment for confounding factors (adjusted odds ratio=1.27; 95% CI, 1.09 to 1.48; $P=0.0026$ for AA+AG versus GG).[11]

The current study supports the findings that *PRKCH* is involved in ischemic stroke, also our results suggested that *PRKCH* is involved in all types of stroke, whether those associations were mediated through atherosclerosis is yet to be determined.

Summary

Our study showed that the 1425G/A SNP in *PRKCH* were significantly associated with both ischemic stroke and cerebral hemorrhage in the Chinese population. The future studies are needed in other Asian populations as well as in a Chinese population with a greater sample size. The physiological function of PKCη and the molecular mechanisms of its involvement in stroke need to be investigated to clarify the associations.

Acknowledgments

We thank Guiyou Liu for statistical analysis support, and Jian Li, Wei Zhang, and Jinli Xing for technical assistance.

Sources of Funding

This study was supported by the Beijing Hypertension League Institute, in part through an unrestricted educational grant from F. Hoffmann-La Roche and the National Infrastructure Program of Chinese Genetic Resources (2005DKA21300) and China Postdoctoral Science Foundation (No. 20080430852).

Disclosures

K.L. is an employee of F. Hoffmann-La Roche Ltd, which provided an unrestricted educational grant to the Beijing Hypertension League Institute.

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