Protein Kinase Cη Polymorphism and the Susceptibility to Ischemic Stroke in the Taiwan Population

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Background: Prior studies suggested that protein kinase Cη (*PRKCH*) 1425G/A polymorphism was associated with lacunar infarction. This study examined whether the association was independent of traditional risk factors in each of the stroke subtypes.

Methods: This study included 206 ischemic stroke patients and 337 controls. Multivariable logistic regression was used for analyses. Co-variables of age, sex, hypertension, diabetes mellitus (DM), and smoking were included to delineate independency of associations.

Result: *PRKCH* 1425G/A was associated with ischemic stroke [odds ratio (OR) =1.5, 95% confidence interval (CI): 1.1–2.2, $p = 0.024$] when adjusted for age and sex. However, the significance of association became borderline when adjusted for co-variables (OR = 1.5, 95% CI: 1.004–2.3, $p = 0.048$). Of the infarction subtypes, *PRKCH* 1425G/A was associated with lacunar infarction (OR = 1.8, 95% CI: 1.1–2.9, $p = 0.025$), which remained significant when adjusted for co-variables (OR = 2.0, 95% CI: 1.1–3.5, $p = 0.015$). No association was found between the polymorphism and the other infarction subtypes. When stratified by age group, the magnitude of significance became stronger in patients >65 years old. Specifically, *PRKCH* 1425G/A was significantly associated with ischemic stroke in patients older than 65 years, when adjusted for all co-variables (OR = 2.0, 95% CI: 1.05–3.8, $p = 0.036$). Still, in patients older than 65 years, the association was only observed in lacunar infarction when adjusted for all co-variables (OR = 4.2, 95% CI: 1.7–10, $p = 0.001$). No association of *PRKCH* 1425G/A with stroke and any of the subtypes was identified in patients >65 years old.

Conclusion: The association between *PRKCH* 1425G/A and lacunar infarction was independent of traditional stroke risk factors. *PRKCH* 1425G/A in stroke susceptibility differed between infarction subtypes and age groups. *(Biomed J 2015;38:433-438)*

At a Glance Commentary

Scientific background of the subject

PKC-η was expressed mainly in vascular endothelial cells and was involved in the development and progression of atherosclerosis. Previous Asian studies have shown contradictory results regarding the associations between stroke and PRKCH 1425G/A. We examined if PRKCH 1425G/A was independently associated with stroke risk factors in the Taiwan population.

What this study adds to the field

The association between *PRKCH* 1425G/A and lacunar infarction was independent of traditional stroke risk factors. *PRKCH* 1425G/A in stroke susceptibility differed between infarction subtypes and age groups.

Key words: disease association, epidemiology, ischemic stroke, lacunar infarction, *PRKCH*, single-nucleotide polymorphism

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Stroke is the second leading cause of death worldwide and a major cause of disability and mortality in the elderly.[1] The risk factors of stroke traditionally consist of hypertension, diabetes mellitus (DM), smoking, and cardiac diseases.[2,3] Currently, with the progress in genotyping technologies, genome-wide searches for "stroke gene" have become imminent.[4] Recently, protein kinase Cη (PKC-η), encoded by the *PRKCH* gene, was identified as a new risk locus for ischemic stroke.[5] Kubo *et al*. found that two single-nucleotide polymorphisms (SNPs) in exon 9 of *PRKCH*, 1425G/A (rs2230500) and 1427A/C (rs2230501), had an absolute linkage disequilibrium between each other. The variant of 1425G/A was focused on because it is non-synonymous.[5]

PKC-η is a member of serine–threonine specific protein kinase C (PKC) family which regulates important cellular functions including proliferation, differentiation, and apoptosis.[6] PKC-η was found to be expressed mainly in vascular endothelial cells and foamy macrophages, and was involved in the development and progression of atherosclerosis.[5] The 1425G/A SNP (rs2230500) within the ATP-binding site of the *PRKCH* gene leads to V374I that enhances the activity of protein kinase.[7] The *PRKCH* 1425G/A is a common variant in Asians, but not in other ethnicities. The HapMap database showed that the minor allele frequency (MAF) of rs2230500 was less than 0.001 in CEU (Caucasian) and YRI (Yoruba in Ibadan, Black ethnicity), but was 0.018 in Japanese and 0.037 in Chinese Han ancestry (in Beijing, China). In our previous report, the MAF of rs2230500 was 0.023 in the Taiwan population.[8] Previous Asian studies have shown contradictory results regarding the associations between stroke and *PRKCH* 1425G/A.[5,9-11] While this polymorphism was shown to increase the risk of stroke in two Japanese and one Chinese study,[5,10,11] it was not replicated in another Chinese study.[9] Meta-analysis of the above studies suggested that 1425G/A in *PRKCH* was associated with ischemic stroke, particularly lacunar infarction.[12]

Because cerebral infarction is a common complex disease, evaluation of an independent role of a stroke risk factor is essential. Therefore, we conducted a case–control study to examine if *PRKCH* 1425G/A was associated with stroke risk and if the association was independent of the environmental factors. We also examined whether there were disparities in the associations of *PRKCH* 1425G/A between stroke subtypes in the Taiwan population.

METHODS

Subjects

This is a retrospective case–control study. Subjects were recruited from the Department of Neurology, Chang Gung Memorial Hospital (CGMH), Linkou Medical Center, which is a tertiary referral center serving residents, mainly in northern Taiwan. Patients with stroke were diagnosed based on both clinical presentations and computed tomography (CT) of brain to separate hemorrhagic stroke from ischemic stroke.[13] The inclusion criteria for the study group were: (1) patients with stroke and (2) a patient or her/his legally acceptable representative willing to provide written informed consent to participate. Patients with a traumatic stroke, brain tumor, or vascular anomaly were excluded. The control group was recruited randomly from the community of northern Taiwan, which has been described in detail elsewhere.[14,15] Briefly, the inclusion criteria were as follows: (1) subjects providing informed consent; (2) subjects who came to CGMH for a health examination or for treatment for diseases other than neurodegenerative diseases, inflammatory diseases, or cerebrovascular diseases; (3) those with no medical history of neurodegenerative diseases, inflammatory diseases, and cerebrovascular diseases including previous stroke or transient ischemic attack (TIA); and (4) those with all the medical records that had been reviewed by at least two ne urologists. This study was approved by the Institutional Review Board of CGMH.

Clinical information

Anthropometric data and 12-h fasting blood were collected from all participants. Hypertension was defined as an average of three independent measures of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive drugs. For stroke patients without prior diagnosis of hypertension, patients were considered to have hypertension when the blood pressure measured after the acute phase of stroke (14 days of onset) repeatedly exceeded 140 mm Hg (systolic) and/or 90 mm Hg (diastolic). DM was defined based on the World Health Organization (WHO) criteria.[16] Smoking was defined as former (adults who have smoked at least 100 cigarettes in their lifetime, but say they currently do not smoke) or current smoking [adults who have smoked 100 cigarettes in their lifetime and currently smoke cigarettes every day (daily) or some days (not daily)].[17] Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Alcohol use was defined as drinking ≥210 g per week.

Ischemic stroke was classified into the following subtypes: lacunar infarction, atherothrombotic infarction, cardioembolic infarction, other determined cause, and undetermined subtypes. The diagnoses of stroke and its subtypes were confirmed by two neurologists (C. M. Chen and Y. C. Chen) based on the clinical presentation and the available brain imaging, including cerebral angiography [including CT angiography (CTA), magnetic resonance angiogra-

phy (MRA), and digital subtraction angiography (DSA)], echocardiography, and carotid duplex imaging. Subtypes of cerebral infarction were determined on the basis of the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria with modifications as follows: (1) lacunar infarction was diagnosed when the brainstem or subcortical hemispheric ischemia was <1.5 cm in diameter on brain imaging, and there was no clinical evidence of cerebral cortical or cerebellar dysfunction; (2) atherothrombotic infarction was diagnosed when the ischemic lesion was >1.5 cm on brain imaging and arterial stenosis was >50% of any of the carotid/cerebral artery; (3) cardioembolic infarction was diagnosed by the presence of high-risk sources of cardioembolism, including atrial fibrillation, recent myocardial infarction, mechanical valve, rheumatic mitral stenosis, cardiac thrombus, and endocarditis;[18] (4) other determined cause of stroke was made when a secondary cause contributing to stroke was identified, such as arterial dissection; and (5) undetermined subtype of stroke included all cerebral infarction patients for whom the subtype could not be determined because of insufficient clinical or morphological information.

Genotyping

DNA was extracted from the leukocytes using the standard protocols. Genotypes for *PRKCH* 1425G/A (rs2230500) were determined by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) assays. For RFLP detection, DNA was amplified with mismatch forward 5′-CATAGGTGATGCTTGCAACA-3′ and reverse 5′-TCTGAAAGCAGCAGAACAA-3′ primers. The polymorphism results in loss of an *Hpy*CH4III endonuclease site. For SSCP detection, hex-labeled forward primer 5′-CATAGGTGATGCTTGCAAG-3′ was used. The amplified 174-bp products were denatured and subjected to electrophoresis by using GeneGel Excel gels (Pharmacia Biotech, Uppsala, Sweden). Alleles G and A were confirmed by DNA sequencing. *PRKCH* 1425G/A was in Hardy–Weinberg equilibrium in each of the stroke groups and controls (χ^2 test, significance level of 0.005).

Statistical analysis and power estimation

The Pearson's χ^2 test or Student's *t*-test was utilized to compare demographic data and the distribution of genotypes of *PRKCH* between controls and patients. Two-tailed *p* values were derived from the χ^2 test or Fisher's exact test. Multivariable logistic regression was used to analyze the phenotype–genotype associations of stroke and stroke subtypes, with *PRKCH* genotypes under dominant genetic models. Co-variables of age and sex in the first model and additional adjustment of hypertension, DM, and smoking in the second model were included to delineate the independent role of *PRKCH* genotypes in stroke susceptibility.

In the present case–control study, at 5% significance level, we had power greater than 0.8 to identify an association under a dominant genetic model when the disease allele frequency was greater than 0.2 and the per-allele genetic effect was greater than an odds ratio (OR) of 1.6 for ischemic stroke. All the data analyses were performed using SAS software version 9.1.3 (SAS Institute, Cary, NC, USA).

RESULTS

The characteristics of the study groups are presented in Table 1. There were 206 stroke patients and 337 controls in this study. The proportions of hypertension, DM, and smoking were significantly higher in stroke patients than in controls (χ^2 test, $p < 0.0001$). Age, gender, BMI, level of cholesterol, and proportion of alcohol use were similar between the stroke and control groups.

In our stroke patients, 89.9% had carotid duplex imaging examinations, 8.7% had CTA, 22.8% had MRA, and one patient had DSA. Echocardiography was performed as an ancillary examination for clinical confirmation in 59.2% of all the stroke patients. Undetermined type included patients (7%) with two or more identified causes and patients (4%) with incomplete study. Etiology of patients with other determined causes of stroke included arterial dissection, Moyamoya disease, and venous thrombosis.

Distribution of *PRKCH* 1425G/A differed between stroke patients and controls ($p = 0.012$) [Table 1]. The associations of *PRKCH* 1425G/A with stroke risks showed disparities in subgroups on using logistic regression analyses [Table 2]. Specifically, *PRKCH* 1425G/A was significantly associated with ischemic stroke [OR = 1.5, 95% confidence interval (CI): 1.1–2.2, $p = 0.024$] even after age and sex adjustment (model 1). The association became borderline when adjusted for co-variables (OR = 1.5, 95% CI: 1.004–2.3, $p = 0.048$) (model 2). When further stratified

Table 1: Demographic data in patients with ischemic stroke and in controls

	Stroke (*n*=206)	Controls (*n*=337)	*p*
Age (years)	64.6±12.1	63.0±11.3	0.12
Range of age (years)	27-88	41-88	
Male	59.7%	54.3%	0.22
BMI	24.9±3.9	25.3±3.5	0.26
Hypertension	81.5%	49.5%	<0.0001
Diabetes mellitus	42.4%	16.7%	<0.0001
Smoking	43%	23.5%	<0.0001
Alcohol use	16.4%	11.3%	0.11
Total cholesterol (mmol/l)	4.87±1.06	5.02±0.99	0.15
PRKCH 1425 GG/GA/AA	108/92/6	208/110/19	0.012

Data are expressed as percentage or mean±SD. Comparisons between controls and stroke cases were analyzed by χ^2 test or Student's *t*-test where appropriate

Table 2: Frequencies and associations of genotype in patients with ischemic stroke and in controls

Genotypes	GG	GA+AA	Model 1 OR (95% CI), *p*	Model 2 OR (95% CI), *p*
Total controls	208	110+19		
Total stroke patients	108 (100)	92+6 (100)	1.5 (1.1-2.2), 0.024	1.5 (1.004-2.3), 0.048
Lacunar	38 (35.2)	38+3 (41.8)	1.8 (1.1-2.9), 0.025	2.0 (1.1-3.5), 0.015
Atherothrombotic	43 (39.8)	31+1 (32.7)	1.3 (0.8-2.1), 0.37	1.2 (0.6-2.2), 0.57
Cardioembolic	15 (13.9)	8+0 (8.2)	0.9 (0.4-2.3), 0.90	1.1 (0.4-2.9), 0.83
Others	1 (0.01)	4+1 (5.0)	6.4 (0.7-56), 0.10	6.1 (0.7-56), 0.11
Undetermined	11 (10.2)	11+1 (12.2)	1.8 (0.8-4.3), 0.17	1.9 (0.7-4.8), 0.18
Controls >65 years old	97	36+7		
Stroke patients >65 years old	59 (100)	45+1 (100)	1.7 (1.01-2.9), 0.045	2.0 (1.05-3.8), 0.036
Lacunar	16 (27.1)	22+1 (50.0)	3.2 (1.5-6.6), 0.002	4.2 (1.7-10), 0.001
Atherothrombotic	25 (42.4)	13+0 (28.3)	1.1 (0.5-2.3), 0.85	1.2 (0.5-3.0), 0.71
Cardioembolic	10 (16.9)	3+0 (6.5)	1.1 (0.5-2.3), 0.59	1.03 (0.2-4.5), 0.97
Others	1 (1.7)	0	0.95	0.93
Undetermined	7 (11.9)	7+0 (15.2)	2.3 (0.8-7.2), 0.14	3.0 (0.8-11), 0.10

Abbreviations: OR: Odds ratio, CI: Confidence interval. Values are *n* (%). Logistic regression analyses, adjusting for age and sex in a dominant model (model 1), and additional adjustment for hypertension, diabetes mellitus, and smoking (model 2)

by stroke subtypes, the association was only observed in lacunar infarction (OR = 1.8, 95% CI: 1.1–2.9, $p = 0.025$). This association remained significant when adjusted for co-variables (OR = 2.0, 95% CI: 1.1–3.5, $p = 0.015$). There was no association between *PRKCH* 1425G/A and the other stroke subgroups.

When further stratified by age of 65 years, we found that the magnitude of significance became stronger in patients >65 years old when compared to controls of age >65 years [Table 2]. Specifically, *PRKCH* 1425G/A was significantly associated with ischemic stroke in patients older than 65 years (OR = 1.7, 95% CI: 1.01–2.9, $p = 0.045$) in model 1. The association remained when adjusted for co-variables (OR = 2.0, 95% CI: 1.05–3.8, $p = 0.036$) (model 2). When further stratified by stroke subtypes, the association was still only observed in lacunar infarction in patients older than 65 years (OR = 3.2, 95% CI: 1.5–6.6, $p = 0.002$). This association remained significant when adjusted for co-variables (OR = 4.2, 95% CI: 1.7–10, $p = 0.001$). Still, there was no association between *PRKCH* 1425G/A and the other stroke subgroups in patients older than 65 years. In addition, there was no association of *PRKCH* 1425G/A with stroke and any of the subtypes in patients ≦65 years old.

DISCUSSION

PKC is classified into three subfamilies based on their molecular structure and cofactor requirements. Classical PKC isoforms (α, βI, βII, and γ) are regulated by calcium, diacylglycerol, and phospholipids. Novel PKC isoforms (δ, ε, η, and θ) are regulated by diacylglycerol and phospholipids, but are insensitive to calcium. Atypical PKC isoforms (ζ and λ or ι) are insensitive to either calcium or diacylglycerol.[6]

Despite their high degree of sequence homology, different PKC isoforms mediate different cellular functions and phosphorylate unique protein substrates.[19]

The *PRKCH* gene is located in chromosome 14q22-q23 in humans,[20] and is known to be expressed predominantly in the epithelial tissues in mouse, but its expression pattern in humans was unknown.[21] Although the association between PKC-η and the development of atherosclerosis has been reported,[5,6] the pathophysiology remains unclear. Kubo *et al*. suggested the involvement of PKC-η in the inflammation pathway, which has been demonstrated by abundant expression of PKC-η in foamy macrophages and vascular endothelial cells during all phases of coronary atherogenesis.[5,7]

The other possible mechanism of the effect of PKC-η on stroke was through oxidative stress and immune mediators.[22] PKC-η is necessary for the development of the inducible NO synthase (iNOS) in monocytes, and contributes to the development of inflammatory conditions and atherosclerotic diseases.[5,6,22] A study in Chinese population also showed a remarkable association of an increased risk of coronary artery disease with the minor allele of SNP1425G/A in *PRKCH* gene.[23] In contrast, this SNP was shown to be associated with decrease in carotid intima–media thickness.[24] , possibly mediated through a decrease in C3 complement. C3 is a major component of the inflammatory pathway and is known to be a major predictor of atherosclerosis; a decrease in C3 complement decreases the progression of atherosclerotic disease.[25] Due to the conflicting results of the association of *PRKCH* 1425G/A with atherosclerosis, further studies should be undertaken for confirmation.

Previous studies which focused on Chinese and Japanese populations showed contradictory results about the

association between 1425G/A SNP in *PRKCH* and ischemic stroke.[5,9-11,26] *PRKCH* 1425G/A was shown to increase the risk of lacunar infarction in two independent Japanese reports and one meta-analysis.[5,10,12] It was found to have positive association with ischemic stroke in one Chinese report,[11] but was insignificant in another small Chinese study.[9] A recent study showed that *PRKCH* 1427A/C (rs2230501) had borderline significance to susceptibility of ischemic stroke ($p = 0.039$).[27] Because 1427A/C and 1425G/A are absolute linked, in the present case–control study, we selected 1425G/A to examine its associations with different subgroups of ischemic stroke in the Taiwan population. The study supported the finding that the common genetic variation of *PRKCH* 1425G/A increased the risk of lacunar infarction. The association was independent of the effects of age, sex, hypertension, DM, and smoking. In addition, the study herein was the first to demonstrate age difference in the association of *PRKCH* 1425G/A with lacunar stroke.

The reason of *PRKCH* 1425G/A being a risk of lacunar infarction only[5,10,11] and, particularly, in patients >65 years old is unknown at this time. However, because *PRKCH* is involved in oxidative stress and inflammation pathway, future functional study in this area may help illuminate different pathophysiology underlying stroke subtypes[18,28] and age groups. In addition, discrepancy of association between *PRKCH* 1425G/A and susceptibility to intracerebral hemorrhage (ICH) subtypes has been previously demonstrated by our group.[8] *PRKCH* 1425G/A was shown to be a risk of lobar ICH, but not deep ICH. Although deep ICH and lacunar infarction have been considered to attribute to common pathophysiology involving lipohyalinotic small-vessel disease,[29] our prior report and the finding herein suggest that *PRKCH* 1425G/A might not be the common pathway of the two types of small-vessel disease. Furthermore, we found a unique distribution of *PRKCH* 1425G/A in the other determined stroke group, when compared to the other stroke subgroups and controls. As an association study, this study is not able to provide the exact answer of pathophysiology. However, future study focusing on atypical stroke subgroups will be necessary. Therefore, appropriate stratification of stroke subtypes instead of mixing them together will be necessary for the future studies in PKC-η and other genetic association studies to elucidate the possible mechanism of *PRKCH* 1425G/A with lacunar infarction.

This is the first study that included the traditional risk factors when analyzing the associations between *PRKCH* 1425G/A and infarction subtypes, which allowed us to delineate the independent role of *PRKCH* in stroke susceptibility. It is worth mentioning that the sample size of stroke patients in this study is just adequate to identify an association greater than 0.8 when the disease allele frequency was greater than 0.2 and the per-allele genetic effect was greater than 1.6 for stroke. In addition, there was a high ratio of atherothrombotic subtype in our study. As the patients were enrolled by our research team, instead of enrolled from the whole hospital, selection bias could exist. Also, including both extracranial and intracranial stenosis in the definition of atherothrombotic subtype may lead to increased ratio of atherothrombotic subtype. Furthermore, replication in large cohorts is needed before *PRKCH* 1425G/A can be viewed as an independent risk factor of lacunar infarction. Contradiction among previous studies may be due to differences in definition of stroke classification, age group, and sample numbers. In addition, *PRKCH* 1425G/A may not pose a specific risk factor only for stroke, given that this polymorphism could be a risk factor for numerous conditions.[7,8,23,30] The study will need to be replicated before the polymorphism can be viewed as an independent risk factor of lacunar infarction. The other limitation of this study is that we did not measure PKC-η level, which might have otherwise provided additional functional information to support our hypothesis. Also, the control group was defined as no medical history of neurodegenerative diseases, inflammatory diseases, and cerebrovascular diseases including previous stroke or TIA. Lack of neuroimaging in the control group may also be the study limitation. A further study examining the correlation between *PRKCH* and PKC-η level will be useful.

Conclusions

In the Taiwan population, the genetic variation of *PRKCH* 1425G/A was independently associated with susceptibility to lacunar stroke in patients >65 years old.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet 2008;371:1612-23.
2. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. Stroke 2003;34:2518-32.
3. Folsom AR, Rosamond WD, Shahar E, Cooper LS, Aleksic N,

Nieto FJ, *et al*. Prospective study of markers of hemostatic function with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation 1999;100:736-42.

4. Nilsson-Ardnor S, Janunger T, Wiklund PG, Lackovic K, Nilsson AK, Lindgren P, *et al*. Genome-wide linkage scan of common stroke in families from northern Sweden. Stroke 2007;38:34-40.

5. Kubo M, Hata J, Ninomiya T, Matsuda K, Yonemoto K, Nakano T, *et al*. A nonsynonymous SNP in PRKCH (protein kinase C eta) increases the risk of cerebral infarction. Nat Genet 2007;39:212-7.

6. Spitaler M, Cantrell DA. Protein kinase C and beyond. Nat Immunol 2004;5:785-90.

7. Takata Y, Hamada D, Miyatake K, Nakano S, Shinomiya F, Scafe CR, *et al*. Genetic association between the PRKCH gene encoding protein kinase Ceta isozyme and rheumatoid arthritis in the Japanese population. Arthritis Rheum 2007;56:30-42.

8. Chen YC, Chen P, Wu YR, Shie SS, Chen ST, Lee-Chen GJ, *et al*. Protein kinase Ceta polymorphism and the susceptibilities to intracerebral hemorrhage in the Taiwan population. Neurosci Lett 2012;528:170-3.

9. Cheng H, Wang F, Ding X, Ding H, Song X. Association of PRKCH gene with lacunar infarction in a local Chinese Han population. Neurosci Lett 2009;464:146-9.

10. Serizawa M, Nabika T, Ochiai Y, Takahashi K, Yamaguchi S, Makaya M, *et al*. Association between PRKCH gene polymorphisms and subcortical silent brain infarction. Atherosclerosis 2008;199:340-5.

11. Wu L, Shen Y, Liu X, Ma X, Xi B, Mi J, *et al*. The 1425G/A SNP in PRKCH is associated with ischemic stroke and cerebral hemorrhage in a Chinese population. Stroke 2009;40:2973-6.

12. Li J, Luo M, Xu X, Sheng W. Association between 1425G/A SNP in PRKCH and ischemic stroke among Chinese and Japanese populations: A meta-analysis including 3686 cases and 4589 controls. Neurosci Lett 2012;506:55-8.

13. Morgenstern LB, Hemphill JC III, Anderson C, Becker K, Broderick JP, Connolly ES Jr, *et al*. Guidelines for the management of spontaneous intracerebral hemorrhage: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2010;41:2108-29.

14. Chen CM, Chen YC, Wu YR, Hu FJ, Lyu RK, Chang HS, *et al*. Angiotensin-converting enzyme polymorphisms and risk of spontaneous deep intracranial hemorrhage in Taiwan. Eur J Neurol 2008;15:1206-11.

15. Chen YC, Hu FJ, Chen P, Wu YR, Wu HC, Chen ST, *et al*. Association of TNF-α gene with spontaneous deep intracerebral hemorrhage in the Taiwan population: A case control study. BMC Neurol 2010;10:41.

16. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.

17. US Centers for Disease Control and Prevention. Health behaviors of adults: United States, 2005-2007. Vital and Health Statistics, Series 10, Number 245, Appendix II. 2010:80.

18. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, *et al*. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.

19. Poole AW, Pula G, Hers I, Crosby D, Jones ML. PKC-interacting proteins: From function to pharmacology. Trends Pharmacol Sci 2004;25:528-35.

20. Osada S, Mizuno K, Saido TC, Akita Y, Suzuki K, Kuroki T, *et al*. A phorbol ester receptor/protein kinase, nPKC eta, a new member of the protein kinase C family predominantly expressed in lung and skin. J Biol Chem 1990;265:22434-40.

21. Osada S, Hashimoto Y, Nomura S, Kohno Y, Chida K, Tajima O, *et al*. Predominant expression of nPKC eta, a Ca (2+)-independent isoform of protein kinase C in epithelial tissues, in association with epithelial differentiation. Cell Growth Differ 1993;4:167-75.

22. Pham T. Protein kinase C-eta (PKC-η) is required for the development of inducible nitric oxide synthase (iNOS) positive phenotype in human monocytic cells. Nitric Oxide 2003;9:123-34.

23. Zhu J, Yan JJ, Kuai ZP, Gao W, Tang JJ, Jia EZ, *et al*. The role of PRKCH gene variants in coronary artery disease in a Chinese population. Mol Biol Rep 2012;39:1777-82.

24. Wu L, Xi B, Hou D, Zhao X, Liu J, Cheng H, *et al*. The SNP (rs2230500) in PRKCH decreases the risk of carotid intima-media thickness in a Chinese young adult population. PLoS One 2012;7:e40606.

25. Szeplaki G, Prohaszka Z, Duba J, Rugonfalvi-Kiss S, Karadi I, Kokai M, *et al*. Association of high serum concentration of the third component of complement (C3) with pre-existing severe coronary artery disease and new vascular events in women. Atherosclerosis 2004;177:383-9.

26. Li JX, Luo M, Xu XW, Sheng WL. Association between 1425G/A SNP in PRKCH and ischemic stroke among Chinese and Japanese populations: A meta-analysis including 3686 cases and 4589 controls. Neurosci Lett 2012;506:55-8.

27. Zhu Y, Liu K, Tang X, Wang J, Yu Z, Wu Y, *et al*. Association between NINJ2 gene polymorphisms and ischemic stroke: A family-based case-control study. J Thromb Thrombolysis 2014;38:470-6.

28. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. New approach to stroke subtyping: The A-S-C-O (phenotypic) classification of stroke. Cerebrovasc Dis 2009;27:502-8.

29. Lammie GA. Pathology of small vessel stroke. Br Med Bull 2000;56:296-306.

30. Goto Y, Hishida A, Matsuo K, Tajima K, Morita E, Naito M, *et al*. PRKCH gene polymorphism is associated with the risk of severe gastric atrophy. Gastric Cancer 2010;13:90-4.