PRKCH 1425G/A polymorphism predicts recurrence of ischemic stroke in a Chinese population

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Abstract

A recent genome-wide association study (GWAS) identified a nonsynonymous SNP (1425G/A) in *PRKCH* which was associated with increased risk of ischemic stroke. The purpose of this study was to examine whether this functional polymorphism is associated with stroke onset and prognosis in a Chinese population. We genotyped *PRKCH* 1425G/A using Improved Multiple Ligase Detection Reaction in 919 patients with ischemic stroke. Analyses of genotype association with onset and prognosis outcomes were assessed by the Kaplan-Meier method, the log-rank test, and the Cox proportional hazards models. *PRKCH* 1425G/A was not associated with age of stroke onset ($P = 0.323$). However, this functional polymorphism was significantly associated with risk of stroke recurrence in recessive models (hazard ratio [HR] = 2.23; 95% confidential interval [CI], 1.06 to 4.68; $P = 0.014$), and this effect was more predominant among smokers (HR = 3.67; 95% CI, 1.47-9.18; $P = 0.005$). Moreover, the variant genotypes of *PRKCH* 1425G/A are an independent prognostic factor for ischemic stroke in the final multivariate Cox regression model. Our findings show that *PRKCH* 1425G/A may be a useful biomarker for predicting the recurrence of ischemic stroke.

Keywords: *PRKCH*; Polymorphism; Stroke; Recurrence

Abbreviations

HR: hazard ratio.

CI: confidence interval.

GWAS: genome-wide association study.

Introduction

Stroke is a major cause of death and long-term disability worldwide, leading to serious public health problems [1]. Primary prevention of stroke is therefore an important medical and social issue. Reports involving twin and family studies have shown that genetics play crucial roles in the development of ischemic stroke, and there has been substantial research evaluating specific genetic risk factors for ischemic stroke [2, 3].

To date, genome-wide association study (GWAS) has emerged as a new promising tool to identify potential susceptibility variants with moderate genetic risk on many complex diseases [4]. In 2007, *PRKCH* was identified as a novel candidate gene for ischemic stroke using genome-wide SNP analysis, and the nonsynonymous SNP (1425G/A) in *PRKCH* was reported to be associated with the risk of ischemic stroke in a Japanese population-based sample [5]. This SNP is located in the linkage disequilibrium block in the *PRKCH* gene (Fig. 1) that encodes PKC η , a molecule that plays important roles in the process of atherosclerosis [5]. Subsequently, the study was followed by many studies in an attempt to replicate this finding in other populations [6-8]. Nevertheless, most of these studies focused on stroke susceptibility. The roles of *PRKCH* 1425G/A polymorphism in stroke onset and prognosis are still largely unknown. Therefore, we hypothesized that the *PRKCH* 1425G/A polymorphism was associated with onset and prognosis of ischemic stroke and conducted a cohort study to test this hypothesis in a Chinese population.

Methods

Study subjects

Our study was approved by the Institutional Review Board of Jinling Hospital (Nanjing,

China). A total of 919 ischemic stroke patients were prospectively recruited between December 2009 and May 2011 from the Nanjing Stroke Registry Program (NSRP) [9]. All ischemic stroke patients suffered a focal neurologic deficit lasting > 24 hours and were confirmed by computed tomography (CT) or magnetic resonance imaging (MRI). The detailed diagnosis of ischemic stroke patients was described previously [10]. Those who smoked daily for >1 year were defined as smokers. Patients were prospectively followed up every 3 months after enrollment via telephone interview or clinical visit until the study endpoint (recurrent stroke) or the latest follow-up time period (November 2013). The median follow-up time was 25.8 months. During the follow-up period, 64 patients were lost to follow-up, and 75 had recurrent stroke. Those lost to follow-up were considered as censored data.

Genotyping

Genomic DNA was extracted according to standard procedures. Genotyping was conducted by the Improved Multiple Ligase Detection Reaction (iMLDR) [11], with technical support from the Center for Human Genetics Research, Shanghai Genesky Biotechnology Company. About 5% of the samples were randomly selected for confirmation, and the results were 100% concordant.

Statistical analysis

One-way ANOVA was adopted to compare the average age at stroke onset. Survival time was calculated from the date of stroke diagnosis to the date of study endpoint or the time of last follow-up. Log-rank test was used to compare the different survival times according to demographic, clinical information, and genotypes. Univariate and multivariate Cox

regression models were performed to estimate the crude hazard ratios (HRs) or adjusted HRs and their 95% confidential intervals (CIs). All tests were two-sided by using the SAS (version 9.1.3) and STATA (version 12.0).

Results

Genotyping results and their associations with stroke onset

We first evaluated if *PRKCH* 1425G/A affected the age of disease onset. The mean age of onset for the GG, GA and AA groups were 61.33 ± 12.65 , 60.28 ± 13.16 and 62.89 ± 11.70 , respectively ($P = 0.323$) (Fig. 2). The result indicated that the *PRKCH* 1425G/A SNP was not associated with age of stroke onset.

Genotyping results and their association with recurrence

The final population for prognosis of this study consisted of 855 ischemic stroke patients. The demographic and clinical information is shown in Table 1. The median age was 62 years, including 602 males (70.4%) and 253 females (29.6%). Age and hypertension were significantly associated with recurrence time (log-rank $P < 0.05$). As shown in Table 2, Cox regression analyses were used to assess the association of *PRKCH* 1425G/A with ischemic stroke recurrence in different genetic models. As a result, *PRKCH* 1425G/A was significantly associated with risk of recurrence in a recessive model (log-rank $P = 0.014$, Fig. 3). Cox regression analyses indicated that the AA variant genotype had a 123% significantly increased recurrence risk (HR = 2.23; 95% CI, 1.06-4.68), compared to the GG/GA genotypes. In the subgroup analyses by stroke subtype, the variant AA genotype was associated with a higher risk of recurrence for both large-artery atherosclerosis (LAA) and small-vessel disease (SVD), which was not statistically significant ($P = 0.218$ and 0.095 ,

respectively).

Stratification analyses

Cox proportional hazard regression analysis showed that *PRKCH* 1425 AA was a significantly unfavorable prognostic factor for ischemic stroke (adjusted HR, 2.23, 95% CI, 1.06-4.68; $P = 0.014$; Table 2). In Table 3, age, sex, hypertension and smoking all seem to be associated with an increased risk for recurrence of ischemic stroke in the AA genotype.

Further stratification analysis indicated that this increased risk was more pronounced among smokers (HR = 3.67; 95% CI, 1.47-9.18; $P = 0.005$; Table 3). Moreover, multivariate cox proportional hazard analysis also showed that the *PRKCH* 1425G/A is an independent prognostic marker for recurrence of ischemic stroke ($P = 0.033$; Table 4). Next, we evaluated whether there exist potential interaction between the *PRKCH* 1425G/A polymorphism and tobacco smoking on recurrence risk of ischemic stroke. As shown in Supplementary Table 1, compared with nonsmokers who carried the GG/GA genotype, smokers with the GG/GA genotype had a 1.002-fold (95% CI, 0.613-1.638) increased recurrence risk of ischemic stroke and non-smokers with the AA had a 1.308-fold (95% CI, 0.316-5.410) increased recurrence risk of ischemic stroke, whereas smokers with AA genotype had the highest risk, with the HR being 3.436 (95% CI, 1.457-8.102), which is 3-fold greater than the product of the HR for smokers with the GG/GA genotype and the HR for non-smokers with AA genotype (Supplementary Table 1).

Discussion

In this cohort study, we investigated whether there is a correlation between the *PRKCH* 1425G/A polymorphism and onset and prognosis of ischemic stroke in a Chinese population.

Our results indicated that this functional SNP was not associated with age of stroke onset. However, we found that *PRKCH* 1425G/A was significantly associated with risk of stroke recurrence, and this effect was more prominent among smokers. Moreover, the variant genotypes of *PRKCH* 1425G/A was an independent prognostic factor for ischemic stroke in the final multivariate Cox regression model. These findings showed that *PRKCH* 1425G/A may be a useful biomarker for predicting the recurrence of ischemic stroke.

PKC family mediates various signaling pathways and regulates multiple important cellular functions such as proliferation, differentiation, and apoptosis [5, 12]. PKC η , encoded by *PRKCH*, is a serine-threonine kinase and is involved in the development and progression of atherosclerosis [5]. Recently, Kubo et al reported that PKC η was mainly expressed in vascular endothelial cells and it plays crucial roles in the development of atherosclerotic diseases such as stroke [5]. The nonsynonymous SNP (1425G/A), which lies in exon 9 and within the ATP-binding site of PKC η , causes enhancement of PKC activity, which may increase stroke risk.

In our study, smokers with the *PRKCH* 1425 AA genotype had the highest recurrent risk of ischemic stroke, suggesting that smoking may have a joint effect with *PRKCH* 1425G/A on recurrence of ischemic stroke. Smoking is known to be an important risk factor for ischemic stroke [13]. Tobacco smoke contains thousands of potentially harmful chemicals, some of which are known to promote atherosclerosis [14]. In addition, tobacco smoke causes vascular endothelial dysfunction with related alteration in hemostatic and inflammatory markers [15, 16]. Of note, it has been reported that smoking could also increase the concentration of fibrinogen and aggregability of platelet, reduce fibrinolytic activity, and

cause polycythemia [17-20]. Thus, it is biologically plausible that smokers with the AA genotype had the highest recurrent risk for ischemic stroke.

The limitation of our study is its hospital-based design, leading to the possibility of selection bias. However, the genotype distributions in our study population were similar to that reported in published data for Chinese populations. For instance, the frequencies of the GG, GA, and AA genotypes among our southern Chinese subjects were 58.7%, 36.1%, and 5.2%, respectively, compared with 56.0%, 40.0%, and 4.0% in northern Chinese populations in the study by Wu et al. [8]. However, the MAF of this SNP is relatively low in European and African populations. Thus, more evidence was needed from other populations to further investigate the association between the *PRKCH* 1425 G/A and recurrence of ischemic stroke.

In addition, the number of patients is relatively small in some stratification analyses, which has insufficient statistical power to detect a slight effect or may have generated a fluctuated risk estimate. Moreover, the function and the signaling pathway of *PRKCH* are still largely unknown. Thus, more molecular and cellular experiments should be designed to further illuminate the mechanism involved.

In conclusion, our study showed that the *PRKCH* 1425G/A polymorphism was an independent predictor of ischemic stroke recurrence in a Chinese population. Large well-designed studies with diverse populations and functional evaluations of PKC η are warranted to confirm and extend our findings.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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Figure legend

Fig. 1 Linkage disequilibrium (LD) pattern of SNPs on chromosome 14q22-q23 based on the HapMap reference sample (Han Chinese in Beijing, China) and NCBI36/hg18 genome build. The LD structure indicates the pairwise calculation of D' for each possible combination of SNPs.

Fig. 2 Correlation between the *PRKCH* 1425G/A and age at onset. (A) A box plot of age at onset between 3 groups of patients with the wild type (GG), heterozygote (GA), or mutant homozygote (AA). • indicates outliers. (B) Cumulative incidence curve of the 3 groups of patients with the wild type (GG), heterozygote (GA), or mutant homozygote (AA).

Fig. 3 Kaplan-Meier survival curves for recurrence among ischemic stroke cases.

Table 1 Patient characteristics and clinical features

Variables	Patients N = 855 (%)	Recurrence N = 75	Log-rank <i>P</i>
Age (years)			0.026
≤ 60	381 (44.6)	25	
> 60	474 (55.4)	50	
Sex			0.122
Male	602 (70.4)	59	
Female	253 (29.6)	16	
Hypertension			0.026
No	307 (35.9)	18	
Yes	548 (64.1)	57	
Diabetes			0.275
No	677 (79.2)	56	
Yes	178 (20.8)	19	
Smoking			0.567
No	530 (62.0)	43	
Yes	325 (38.0)	32	
TOAST			0.076
LAA	515 (60.2)	41	
SVD	250 (29.3)	26	
CE	30 (3.5)	5	
UND	60 (7.0)	3	

LAA, large-artery atherosclerosis; SVD, small-vessel disease; CE, cardiac embolism; UND, other determined and undetermined causes.

Table 2 Association between *PRKCH* 1425G/A and recurrence of ischemic stroke

Genetic models	Genotypes	All cases (n = 855)			
		Patients (n)	Recurrence (n)	Log-rank <i>P</i>	HR (95%CI) ^a
Codominant model	GG	502	49	0.008	1.00
	GA	309	18		0.60 (0.35-1.03)
	AA	44	8		1.91 (0.90-4.05)
Dominant model	GG	502	49	0.252	1.00
	GA/AA	353	26		0.76 (0.47-1.23)
Recessive model	GG/GA	811	67	0.014	1.00
	AA	44	8		2.23 (1.06-4.68)
LAA (n = 515)					
		Patients (n)	Recurrence (n)	Log-rank <i>P</i>	HR (95%CI) ^a
Codominant model	GG	297	29	0.041	1.00
	GA	187	8		0.39 (0.18-0.85)
	AA	31	4		1.43 (0.49-4.12)
Dominant model	GG	297	29	0.075	1.00
	GA /AA	218	12		0.51 (0.26-1.01)
Recessive model	GG/GA	484	37	0.218	1.00
	AA	31	4		1.87 (0.66-5.36)
SVD (n = 250)					
		Patients (n)	Recurrence (n)	Log-rank <i>P</i>	HR (95%CI) ^a
Codominant model	GG	147	15	0.241	1.00
	GA	91	8		1.17 (0.49-2.80)
	AA	12	3		2.10 (0.60-7.31)
Dominant model	GG	147	15	0.819	1.00
	GA /AA	103	11		1.34 (0.61-2.94)
Recessive model	GG/GA	238	23	0.095	1.00
	AA	12	3		2.00 (0.59-6.73)

^a Adjusted for age, sex, hypertension, diabetes and smoking.

Table 3 Stratified analysis of *PRKCH* 1425G/A genotypes associated with recurrence of ischemic stroke

Variable	Genotype (recurrence/patients)		HR (95%CI) ^a
	GG/GA	AA	
Total	67/811	8/44	2.23 (1.06-4.68)
Age (years)			
≤ 60	22/363	3/18	2.81 (0.82-9.60)
> 60	45/448	5/26	2.00 (0.79-5.10)
Sex			
Male	51/565	8/37	2.62 (1.24-5.57)
Female	16/246	0/7	—
Hypertension			
No	17/294	1/13	1.24 (0.16-9.63)
Yes	50/517	7/31	2.48 (1.12-5.51)
Diabetes			
No	49/639	7/38	2.31 (1.04-5.14)
Yes	18/172	1/6	1.94 (0.25-15.04)
Smoking			
No	41/510	2/20	1.14 (0.27-4.73)
Yes	26/301	6/24	3.67 (1.47-9.18)

^a Adjusted for age, sex, hypertension, diabetes and smoking.

Table 4 Multivariate cox regression analysis for recurrence

Variables	β	SE	HR	95% CI	<i>P</i>
Age	0.47	0.25	1.61	0.99-2.62	0.057
Sex	-0.46	0.30	0.63	0.35-1.14	0.127
Hypertension	0.47	0.28	1.60	0.93-2.74	0.091
Diabetes	0.30	0.27	1.35	0.79-2.31	0.267
Smoking	0.05	0.25	1.05	0.64-1.72	0.838
<i>PRKCH</i> 1425 (GG/GA vs. AA)	0.80	0.38	2.23	1.06-4.68	0.033

β : regression coefficient.

Figure 1

Figure 2

Figure 3