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Platelet glycoprotein IaC807T polymorphisms and ischemic stroke in young Chinese Han Population

J. ZHANG, D. HUANG, J. YANG, H. AN[1], R. OJHA, C. DU, R. LIU

Department of Neurology, East Hospital, Tongji University School of Medicine, Shanghai, China.
[1]Department of Neurology, Sixth People's Hospital of Shanghai Jiaotong University, Shanghai, China

Abstract. – OBJECTIVES: The objective of this study was to investigate the association between platelet glycoprotein (GP) Ia C807T polymorphisms and ischemic stroke in young Chinese Han Population.

MATERIALS AND METHODS: We conducted a case-control study in 92 consecutive young (</= 50 years) first-ever hospitalized ischemic stroke inpatients and outpatients, 86 elder ischemic stroke control (> 50 years), and 160 age- and sex-matched healthy control. Genotyping of platelet GP Ia C807Tpolymorphisms was performed by polymerase chain reaction followed by sequencing nucleic acid with dideoxy chain-termination method and an ABI PRISM3100 (Perkin-Elmer Co) genetic analyzer. Student's t-test, chi-square test, and logistic regression modeling were used for data significance analyses.

RESULTS: Hypertension and smoking were found to be the independent risk factors for ischemic stroke patients (aged </= 50 years), while hypertension, diabetes and smoking were the independent risk factors for ischemic stroke patients (aged > 50 years). There was no significant difference observed in the T allele frequency of GPIa C807T polymorphisms between young stroke patients and corresponding controls.

CONCLUSIONS: These findings suggest that there is no role of GPIa C807T polymorphisms in the development of young first-ever ischemic stroke in Chinese Han Population.

Key Words:
Ischemic stroke, Risk factors, GPIa C807T polymorphisms, Hypertension.

Introduction

The development of stroke is known to be related to different vascular risk factors such as hypertension, diabetes mellitus, high cholesterol and smoking[1]. Moreover, genetic predispositions may enhance the risk of developing a cerebrovascular thrombotic event, especially in young ischemic stroke patients under the age of 50 years[2,3]. The molecular basis for the genetic risk of ischemic stroke is likely to be multigenic and influenced by environmental factors. Several case-control studies have suggested associations between ischemic stroke and polymorphisms of genes that code for coagulation cascade proteins and platelet receptors[4].

Platelet plays a pivotal role in the pathogenesis of thrombotic cardiovascular diseases. The glycoprotein (GP) Ia/IIa complex, also known as $\alpha_2\beta_1$, is one of the major collagen receptors that are physiologically active on platelets[5]. Functional characterization reveals that platelet via GP Ia/IIa attaches to type I collagen in case of collagen-induced platelet aggregation[6]. Two silent biallelic polymorphisms on GP Ia (α_2) have been identified, $C_{807}T$ in exon7 and $G_{873}A$ in exon8. These two mutations are in complete linkage disequilibrium, having shown to be associated with the density of GP Ia/IIa expression. The GP IaC_{807}/G_{873} allele is associated with low receptor density and GP IaT_{807}/A_{873} allele with high receptor density[7].

The hypothesis that the GP Ia/IIa polymorphisms may affect the risk of developing cerebral vascular disease has prompted a large number of clinical studies. Two reports have indicated that genetic risk factors may be especially relevant to the development of stroke in younger patients[7-9], but this result has not been confirmed in other four studies[10-13]. The frequency of GP IaC807T polymorphisms shows significance among distinct populations. Therefore, the purpose of this study is to evaluate the distribution of GP Ia/IIa polymorphisms and its association with young ischemic stroke among Chinese Han population.

Corresponding Author: Huang Dongya, MD, Ph.D.; e-mail: dongyah@hotmail.com

Materials and Methods

Study Design and Participants

This case-control study was approved by the Local Ethics Committee of Shanghai East Hospital. All patients and control subjects provided informed consent.

From Oct. 2005 to Feb. 2010, a total of 92 consecutive young (≤ 50 years) first-ever hospitalized ischemic stroke inpatients and outpatients, 86 elder ischemic stroke controls (> 50 years), and 160 age- and sex-matched healthy controls were recruited from Neurology Ward and Outpatient Department of Shanghai East Hospital. Patients with focal neurological symptoms due to complete stroke were included, and those with an intracerebral, subarachnoid hemorrhage, vasculitis or myocardial infarction were excluded.

Measurement and Data Collection

All stroke patients underwent computerized tomography (CT)/magnetic resonance imaging (MRI), ultrasonography (USG), electrocardiography (ECG), echocardiography, and lab testing. Trans-esophageal echocardiography and digital subtraction angiography were optional. Each case was diagnosed and confirmed as stroke and matched subtype by a skilled neurologist, based on the data from clinical assessments and neurological images. Information about patients' age, sex, and risk factors of stroke were obtained through history taking during admission. Fasting venous blood of the stroke patients and healthy control was drawn into two 5 ml heparinized tubes for routine lab testing and analysis of length variability of target gene, respectively.

Analysis of Genetic Polymorphisms

Genomic DNA was isolated from 2 ml of peripheral blood leukocytes by using the phenol-chloroform method. Genotyping of platelet glycoprotein GP Ia C807T polymorphisms was performed by polymerase chain reaction (PCR) with the following primers: sense, 5'-TGGTGCTTACCTGTGGAGAG-3', and antisense primer, 5'-ATGAACAGCCAATGGGAAAG-3'. The PCR protocol 35 cycles, as follows: denaturation at 94°C for 20 s, primer annealing at 64°C for 30 s and extension at 72°C for 30 s. The PCR cycling parameters was initial denaturation at 95 °C for 15min; 12 cycles of amplification (denaturation at 94°C for 40 s, primer annealing from 63-58°C for 40 s by 0.5°C decrease every second cycles, 72°C for 1 min); 30 cycles of amplification (94°C for 40 s, 56°C for 40 s, 72°C for 1 min); and 72°C for 10 min. The PCR products were analyzed on 1.5% agarose gels and visualized using ethidium bromide staining. The nucleic acid sequences were detected by dideoxy chain-termination method and an ABI PRISM3100 (Perkin-Elmer Co, Norwalk, CT, USA) genetic analyzer.

Statistical Analysis

Age was expressed as mean ± standard definition (SD) and the significance of differences between patients and controls with respect to continuous variables was tested using Student's *t* test. The allele frequencies and genotypes determined were compared by chi-square test. A statistically significant difference was defined as a *p*-value ≤ 0.05. Odd's ratios (OR) with the corresponding 95% confidence interval[10] were assessed for major stroke risk factors (hypertension, diabetes, high cholesterol, smoking, positive family history of stroke, and the presence of the GPIa T_{807} allele). A logistic regression was applied to estimate the OR controlling for conventional risk factors of stroke. The statistical analyses were performed using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of Patients and Controls

Baseline characteristics of study groups were presented in Table I. A total of 178 stroke patients were included for this study. Among them, 92 stroke patients (70 male and 22 female) were classified into the young groups aged ≤ 50 years (mean, 42.6±6.8 years), and other 86 patients (53 male and 33 female) were included into the elder groups aged > 50 years (mean, 69.2±10.1 years). In addition, the age and sex-matched control groups comprised of 160 healthy individuals, 80 of them were ≤ 50 years of age (mean, 40.3±6.4 years) and 80 were > 50 years of age (mean, 68.2±8.7 years). Representation of conventional risk factors was compared between stroke patients and controls group. In the young group ≤ 50 years of age, hypertension ($p = 0.009$) and smoking ($p = 0.013$) were significantly over represented in stroke patients compared with controls (Table I). However, in the elder group > 50 years of age, hypertension ($p = 0.001$), diabetes mellitus ($p = 0.019$) and smoking ($p = 0.032$) were more common in patients than in controls

(Table I). Hypercholesterolemia, as well as positive family history was found with no significant differences between the two groups (Table I).

Allele Frequencies and Genotype Distributions of GPIa C807T

The genotype distribution and allele frequencies of GPIa C807T were compared between (1) stroke patients (aged ≤ 50 years, n=92) and corresponding age-matched controls (n=80); and (2) stroke patients (aged > 50 years n=86) and its age-matched controls (n=80) (Table II). The findings indicated the distribution of GPIa C807T CC, CT and TT was similar in patients and controls group regardless of the age ≤ 50 years (CC53.2% vs 56.3%, CT37.0% vs 35.0%, TT10.9% vs 8.8%, respectively, $p = 0.593$) or the age > 50 years (CC46.5% vs 50.0%, CT40.7% vs 40.0%, TT12.8% vs 10.0%, respectively, $p = 0.652$). There was also no significant difference in the T allele frequency of GPIa C807T polymorphism between stroke patients and controls in both group ≤ 50 years (29.3% vs 26.3%, $p = 0.624$) and group > 50 years (33.1% vs 30.0%, $p = 0.637$).

Logistic Regression Analysis of Gpia C807T Polymorphism and the Risk of Ischemic Stroke

To determine whether the GPIa C807T polymorphism is associated with stroke or not, a logistic regression analysis was performed (Table III). There was association between GPIa C807T polymorphism and ischemic stroke neither in the young stroke patients, nor in the elder stroke patient. Our results further confirmed, hypertension ($p = 0.004$) and smoking ($p = 0.005$) were independent risk factors for ischemic stroke patients ≤ 50 years, while hypertension ($p < 0.0001$), diabetes mellitus ($p < 0.0001$) and smoking ($p = 0.006$) were independent risk factors for ischemic stroke patients > 50 years of age.

Discussion

In this case-control study, we identified the role of the GPIa C807T polymorphism in ischemic stroke in Chinese Han population. Our results showed that the variant 807CT/807CT was associated neither with ischemic stroke patients ≤ 50 years (OR 1.41; 95% CI 0.74-2.79) nor with ischemic stroke patients > 50 years (OR 0.16; 95% CI 0.59-2.32).

Table I. Baseline characteristics of patients in ischemic stroke and control subjects.

	Subjects ≤ 50 years of age					Subjects > 50 years of age				
	Patients (n=92)	Controls (n=80)	OR	95% CI	*p*-value	Patients (n=86)	Controls (n=80)	OR	95% CI	*p*-value
Mean age (SD)	42.6 (6.8)	40.3 (6.4)	–	–		69.2 (10.1)	68.2 (8.7)	–	–	
Male (%)	70 (76.1)	60 (75.0)	1.06	0.53-2.13	0.869	53 (61.6)	49 (61.3)	1.02	0.54 -1.90	0.960
Hypertension (%)	48 (52.2)	26 (32.5)	2.27	1.22- 4.22	0.009	57 (66.3)	33 (38.4)	2.80	1.49 -5.26	0.001
Hypercholesterolemia (%)	23 (25.0)	17 (21.3)	1.24	0.61-2.52	0.561	24 (27.9)	21 (26.3)	1.03	0.51-2.05	0.943
Diabetes mellitus (%)	16 (17.4)	9 (11.3)	1.66	0.69-3.40	0.254	30 (34.9)	15 (18.8)	2.32	1.14 -4.75	0.019
Smoking (%)	30 (32.6)	13 (16.3)	2.50	1.20-5.21	0.013	35 (40.7)	20 (25.0)	2.06	1.06-4.00	0.032
Positive family history of smoke (%)	17 (18.5)	11 (13.8)	1.42	0.62-3.25	0.402	11 (12.8)	8 (10.0)	1.32	0.50-3.47	0.573

Table II. Genotype distribution and allele frequency of GPIa 807CT in patients and controls.

	Genotype (number of cases, %)			Allele frequency (%)	
	CC	CT	TT	C	T
Patients (≤ 50 years)	48 (53.2%)	34 (37.0%)	10 (10.9%)	70.7%	29.3%
Controls (≤ 50 years)	45 (56.3%)	28 (35.0%)	7 (8.8%)	73.8%	26.3%
Patients (> 50 years)	40 (46.5%)	35 (40.7%)	11 (12.8%)	66.9%	33.1%
Controls (> 50 years)	40 (50.0%)	32 (40.0%)	8 (10.0%)	70.0%	30.0%

It has been demonstrated that differences in platelet GPIa/IIa density correlate with the rate of platelet attachment in whole blood to type I collagen. The fact that integrin a2 1 is considered the major receptor involved in this process leads to the hypothesis that subjects with the 807T allele have an increased potential of platelet adhesion and a tendency to arterial thrombosis, hence an increased risk of cerebrovascular disease[14]. Its thrombotic complications have prompted a large number of clinical studies. Two pilot studies have found an association between the GPIa C807T polymorphism and the incidence of stroke[8,9]. Reiner found an increased risk of ischemic stroke with the C807T variant in 36 women ≤ 45 years of age[8]. Carlsson reported a positive association in 45 stroke patients ≤ 50 years of age, but not in older patients[9]. Several other studies also have evaluated the association between this polymorphism and ischemic stroke. However, the results were contradictory[10-13]. Similarly, our data did not confirm the results found by Carlsson and Reiner: the distribution of GPIa C807T CC, CT and TT was similar in patients and control group no matter the age ≤ 50 years or > 50 years of age ($p > 0.05$). There was no significant difference in the T allele frequency of GPIa C807T polymorphism between stroke patients and controls in group ≤ 50 years of age (29.3% vs 26.3%, $p > 0.05$) and group > 50 years of age (33.1% vs 30.0%, $p > 0.05$). The T allele frequency in our patients and controls ≤50 years of age (29.3% vs 26.3%) is similar to that in the controls (28%) of an acute myocardial infarction study conducted in northern China[14] and to that in the controls (27.1%) of a young stroke study carried out in Taiwan[15], but is much lower than that found in healthy Caucasians (39% and 43.8%)[8,9]. These wide variations among different populations may be related, at least in part, to ethnic heterogeneity and to differences in study design.

In order to clarify the influence caused by ischemic stroke risk factors including GPIa C807T polymorphism, a logistic regression analysis was performed. Our data showed the similar results as in other studies, arterial hypertension and smoking were significantly more common in patients than in controls ≤ 50 years, while arterial

Table III. Logistic regression analysis of GPIa C807T polymorphism and the risk of stroke.

Patient group	Variable	B	S.E.	Wals	Odds Ratio	*p*-value
≤ 50 yr (n = 172)	Gender (male)	-0.12	0.39	0.001	0.98 (0.46-2.11)	0.976
	Hypertension	0.98	0.34	8.13	2.68 (1.36-5.23)	0.004*
	High cholesterol	0.23	0.40	0.32	1.25 (0.58-2.72)	0.570
	Diabetes mellitus	0.26	0.49	0.30	1.30 (0.50-3.38)	0.589
	Smoking	1.17	0.42	7.93	3.25 (1.43-7.37)	0.005*
	Positive family	0.14	0.45	0.10	1.16 (0.48-2.79)	0.749
	GPIa807CT+807TT	0.35	0.33	1.08	1.41 (0.74-2.79)	0.298
> 50 yr (n = 166)	Gender (male)	-0.17	0.36	0.23	0.84 (0.41-1.71)	0.631
	Hypertension	1.61	0.39	16.74	5.00 (2.31-10.81)	0.000*
	High cholesterol	0.73	0.41	3.24	2.07 (0.94-4.58)	0.072
	Diabetes mellitus	1.59	0.44	13.25	4.91 (2.08-11.55)	0.000*
	Smoking	1.07	0.39	7.64	2.91 (1.37-6.21)	0.006*
	Positive family	0.65	0.56	1.37	1.91 (0.64-5.67)	0.243
	GPIa807CT+807TT	0.16	0.35	0.20	0.16 (0.59-2.32)	0.651

*$p < 0.01$ was considered as statistical significance.

hypertension, smoking and diabetes mellitus were significantly more common in patients than in controls > 50 years. Diabetes mellitus has been strongly implicated as a risk factor for ischemic stroke with age > 50 years[16]. Our data did not confirm the results that the GPIa C807T polymorphism is an independent risk factor of cerebral ischemic stroke both in young and elder ischemic stroke patients.

Furthermore, the stratified analysis of different risk factors in group ≤ 50 years of age revealed that, arterial hypertension carrying GPIa 807CT/GPIa 807 TT had 25 fold (data not shown) higher risk of ischemic stroke than those in controls; while subjects with positive family history of stroke and unknown etiology carrying GPIa 807CT/GPIa 807 TT displayed higher risk of stroke than in controls, but there was no significant difference in the death rate. Although various causes of young ischemic strokes were identified, yet a clear underlying cause has not been found in many cases till now. Additional studies will be needed for further assessment.

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