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Association of Genetic Polymorphisms in the Fibrinogen and Platelet Glycoprotein Genes with Unstable Angina in Chinese Patients

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Summary

Background: Inherited predisposition has been associated with coronary artery disease (CAD) in the white population.

Hypothesis: The objective of this study was to investigate the association between the risk of unstable angina (UA) and genetic factors believed to be associated with an increased tendency toward thrombosis (the variable number of tandem repeats [VNTR] polymorphism of the platelet glycoprotein [GP] Ibα gene, $PI^{A1/A2}$ of the platelet GP IIIa gene, 448G/A of the Bβ fibrinogen gene and Thr312Ala of the Aα fibrinogen gene) in Chinese patients with UA.

Methods: We performed a case/control study evaluating 69 Chinese patients (43 men, 26 women) with UA and 69 control subjects without CAD, individually matched for age and gender. The restriction fragment length polymorphism (RFLP) method was used to determine the genetic polymorphisms.

Results: The frequencies of GP Ibα C/B genotype and Bβ fibrinogen 448A allele were higher in patients with UA (46.4 vs. 30.4%, odds ratio [OR] 1.977, 95% confidence interval [CI] 0.98–3.97, p = 0.054, and 49.3 vs. 20.3%, OR 3.816, 95% CI 1.797–8.103, p = 0.000, respectively). Only four subjects (two cases, two controls) with GP IIIa PI^{A2} allele were found, and there was no association between Aα fibrinogen Thr312Ala polymorphism and UA.

Conclusions: Chinese patients with UA had increased frequencies of GP Ibα C/B genotype and Bβ fibrinogen 448A allele. These data suggest that some genetic factors may influence the development of UA.

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Introduction

Coronary artery disease (CAD) is an important cause of death and disability in the developed western world and in China. Unstable angina (UA) is caused by the total or subtotal, transient or typical obstruction of a coronary artery, which results from rupture or erosion of atheromatous plaque with consequent platelet adhesion and aggregation and cleavage of fibrinogen by thrombin to form fibrin. Therefore, any genetic difference that might alter function or production of the glycoprotein (GP) receptors and fibrinogen could influence risk for adverse outcomes as a result of the hemostatic process.

Two receptor complexes mediate the integral role of platelets in hemostasis: GP Ib-IX-V and GP IIb/IIIa. Under conditions of high shear stress associated with atherosclerotic narrowing of vessels, the binding of GP Ib-IX-V complex and von Willebrand factor (vWF) leads to platelet adhesion and activation and a conformational change of GP IIb/IIIa complexes ($\alpha_{IIb}\beta_3$ integrin) that facilitates fibrinogen binding and platelet aggregation.[1] Many GP polymorphisms have been found. The C/B genotype of GP Ibα VNTR polymorphism has been reported to be associated with CAD and cerebral vascular disease,[2] and most recently Afshar-Kharghan *et al.* have found an association between the CC genotype and a lower risk of coronary heart disease in blacks.[3] However, many studies have failed to confirm any link between VNTR polymorphism and cardiovascular disease.[4] A meta-analysis of 12 epidemiologic studies, spanning over 3,400 patients and 3,400 controls, mostly white persons, has provided the evidence that $PI^{A1/A2}$ polymorphism is associated with an increased risk of coronary heart disease.[5]

An elevated plasma fibrinogen level has been established to be an independent predictor of CAD.[6] There is evidence that up to 51% of the variation in fibrinogen levels may be due to genetic factors, and a relation between the Bβ 448G/A and β-455G/A polymorphisms and fibrinogen level has been reported both in white[7] and Chinese persons.[8] Furthermore, an increased frequency of Bβ fibrinogen 448A allele has been

found in patients without flow-limiting stenosis after myocardial infarction (MI) compared with patients with ≥ 1 flow-limiting stenosis.[9] A recent report that Ala312 of the Aα fibrinogen Thr312Ala polymorphism can influence clot structure and properties may provide a mechanism by which Ala312 fibrinogen could predispose to clot embolization.[10]

The aim of this study was to determine whether the VNTR polymorphism of the GP Ibα gene, the $Pl^{A1/A2}$ polymorphism of GP IIIa, the 448G/A polymorphism of the Bβ fibrinogen, and the Thr312Ala polymorphism of the Aα fibrinogen gene are associated with UA in Chinese patients.

Materials and Methods

Patient Population

After informed consent was obtained, we enrolled 138 consecutive patients (mean age 59.0 ± 10.1 years) who were referred to the Department of Cardiology of Peking University People's Hospital for diagnostic or therapeutic cardiac intervention. All patients had undergone coronary angiography during hospitalization. Case patients were defined as subjects with at least one coronary stenosis ≥ 50%, whereas the age- and gender-matched control patients had no luminal narrowing or stenosis < 50%. All case patients were diagnosed with UA according to American College of Cardiology/American Heart Association (ACC/AHA) guidelines for UA and non-ST-segment elevation MI.[11] Risk factors and patient history were assessed by questionnaire. Fasting blood samples for measurement of routine clinical chemistry profile, as well as isolation of DNA were obtained in the early morning. Detection of the genotypes was performed in a blinded fashion with regard to clinical data and DNA analysis.

Determination of DNA Genotypes

Genomic DNA was isolated from white blood cells using digestion with proteinase K and sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by phenol/chloroform extraction and dialysis. Three restriction fragment length polymorphisms (RFLP) were detected by polymerase chain reaction (PCR) and the restriction enzymes RsaI for Aα fibrinogen, MnlI for Bβ fibrinogen, and MspI for glycoprotein IIIa. Glycoprotein Ibα VNTR polymorphism was determined only by PCR. Genomic DNA (100 ng) was amplified using slightly modified standard PCR conditions.[12] The first three primer sequences were identical to those previously used for these sites,[8, 13, 14] and we designed and synthesized the VNTR primers (Table I). Amplified products were digested either with RsaI and DdeI, MnlI, or MspI (New England BioLabs, Beverly, Mass., USA) and, together with VNTR PCR products, electrophoresed with 10% polyacrylamide gels and visualized by silver staining.

Statistical Analysis

The Student's *t*-test was used to compare age between case patients and control subjects. All other variables were analyzed by chi-square test or continuity-corrected chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the presence of C/B genotype compared with all other kinds of genotypes of GP Ibα VNTR polymorphism, and for the presence of at least one 448A allele compared with homozygous carriers of the 448G allele of Bβ fibrinogen 448G/A polymorphism. A p value of ≤ 0.05 was considered to indicate statistical significance. All p values were two tailed. Statistical analyses were performed by the Statistical Package for Social Sciences for windows 10.0 (SPSS, Inc., Chicago, Ill., USA).

Results

Characteristics of the Study Population

Table II shows the age and gender of the study subjects. No significant differences were found in the prevalence of select-

TABLE I Primers and allelic fragments

			Alleles (bp)	
Gene	RFLP	Primers (5'−3')	1	2
FGA	RsaI	GGAAGGCATTAACAGACATG	Thr (ACT)	Ala (GCT)
		GAGCTCTTTATCTCCTTTAG	78, 39	117
FGB	MnlI	AACATCAGATCCCAGAAAACAG	Arg (AGG)	Lys (AAG)
		GGTGAGCAAGAGAAATGAAGAA	183, 132	315
GP IIIa	MspI	GCTCCAATGTACGGGGTAAAC	Pl^{A1}	Pl^{A2}
		GGGGACTGACTTGAGTGACCT	282	157, 125
			A B	C D
GP Ibα VNTR		TTCCCCACCAAAGCCCATACAAC	692 653	614 575
		AGGGGGAGGAGGCAGCAAAAGTC		

Abbreviations: RFLP = restriction fragment length polymorphism, FGA = Aα fibrinogen, FGB = Bβ fibrinogen, GP = glycoprotein.

TABLE II Age and gender of study subjects and prevalence of risk factors for acute coronary syndrome

	Patients (n = 69)	Controls (n = 69)	p Value [a]
Age (years)	60.6 ± 9.0	57.4 ± 11.0	
Mean ± SD (range)	(34–77)	(33–79)	NS
Male gender (%)	62.3	62.3	NS
Risk factors (%)			
Hypertension [b]	43.5	36.2	NS
Type I or II diabetes [c]	17.4	17.4	NS
Dyslipidemia [d]	18.8	15.9	NS
Smoking	34.8	31.9	NS
Family history [e]	5.8	8.7	NS

[a] Student's *t*-test (for age) and chi-square test (for all other variables) were used to compare the values for case patients and controls.
[b] Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg at the time of admission to the hospital.
[c] Diabetes was defined as a fasting blood glucose > 7.8 mmol/l at the time of admission to the hospital.
[d] Dyslipidemia was defined as a total serum cholesterol level ≥ 5.72 mmol/l, or low-density lipoprotein cholesterol level ≥ 3.64 mmol/l, or triglyceride level ≥ 1.7 mmol/l at the time of admission to the hospital.
[e] Family history was defined as at least one first-degree relative who had suffered from coronary artery disease.

ed risk factors for CAD (hypertension, diabetes, dyslipidemia, smoking, or family history) among patients and controls in this case/control study.

Glycoprotein Polymorphisms and Unstable Angina

The genotypes of the VNTR polymorphism in the case/control study are summarized in Table III. No allele D carriers were found. Genotype analysis showed an association of the C/B genotype and UA (p = 0.054; OR, 1.977; 95% CI, 0.98–3.97). There were no frequency differences of the A, B, and C alleles between the case patients and controls.

The Pl^{A2} allele existed in only 4 (2 cases and 2 controls) of 138 Chinese subjects (2.9%) in this study. The low prevalence of this polymorphism compared with that in white persons,[5, 14] is similar to that in Japanese[15] and suggests an ethnic difference.

Fibrinogen Polymorphisms and Unstable Angina

Table III summarizes the genotyping data of the Bβ fibrinogen 448G/A polymorphism. We found a significant association between 448A allele and UA. Thus, of 69 such patients, 49.3% carried at least one 448A allele, compared with 20.3% of the controls (p = 0.000, OR, 3.816; 95% CI, 1.797–8.103). The prevalence of the 448A allele was significantly higher in the group of patients with UA than in the respective control group (54.4 vs. 30%; p = 0.002; OR, 2.786; 95% CI, 1.441–5.386).

There was no association between Aα fibrinogen Thr312Ala polymorphism and UA (data not shown).

Discussion

To assess the association between these four polymorphisms and UA, we compared the risk of CAD among case and control subjects matched for age and gender. Considering that genetic and environmental factors act additively or synergistically to determine an individual's risk of CAD, our strategy for identification of polymorphisms predisposing to the disease was to avoid overrepresentation of classic risk factors in the UA group compared with controls. Thus, we tried to approximate risk factors between every case patient and a single age- and gender-matched control.

In this study, we found increased frequencies of GP Ibα C/B genotype and Bβ fibrinogen 448A allele (p = 0.054, OR 1.977, 95% CI 0.98–3.97; p = 0.000, OR 3.816, 95% CI 1.797–8.103, respectively) in Chinese patients with UA compared with subjects without CAD.

Glycoprotein Ibα plays an essential role in platelet activation and aggregation. Some previous studies have shown an association between GP Ibα C/B genotype and cardiovascular disease,[2] although the results are controversial.[4] The GP Ibα VNTR polymorphism is a molecular weight polymorphism within the mucin-like macroglycopeptide region of GP Ibα, resulting in the duplication of a 13-amino acid sequence once (VNTR D), twice (VNTR C), three times (VNTR B), or four times (VNTR A). One possible hypothesis is that this could extend the GP Iba binding sites for vWF and thrombin further above the plane of the plasma membrane, increasing the avidity for these ligands and accounting for the observed increased

TABLE III Frequency of genotypes of the GP Ibα VNTR and Bβ fibrinogen 448G/A polymorphisms in case patients and controls

Genotype	No. of patients (%)	No. of controls (%)	p Value (OR) [a]
GP Ibα VNTR	p = 0.290 [b]		
AB	2 (2.9)	4 (5.8)	0.676
AC	1 (1.4)	1 (1.4)	—
BB	22 (31.9)	23 (33.3)	0.856
BC	32 (46.4)	21 (30.4)	0.054 (1.977)
CC	12 (17.4)	20 (29.0)	0.107
FGB 448G/A	p = 0.000 (3.816) [b]		
1,1	35 (50.7)	55 (79.7)	
1,2 + 2,2	33 + 1 (49.3)	13 + 1 (20.3)	

[a] Chi-square test was used to compare the value of a particular GP Ibα VNTR genotype among case patients and controls.
[b] Chi-square test was used to compare the values of the distribution of GP Ibα VNTR and Bβ fibrinogen 448G/A genotypes among case patients and controls.
Abbreviations: GP = glycoprotein, FGB = Bβ fibrinogen, OR = odds ratio.

risk for acute CAD associated with the longer variants,[2] but this could not explain why C/B genotype rather than B/B or A/A genotype is associated with cardiovascular disease. Recently, Afshar-Kharghan *et al.* have proposed another mechanism by which the VNTR polymorphism may influence CAD susceptibility.[3] They believe that it was the disparity in length between the two allele products in a heterozygous individual that influences the interaction of GP Ibα with the receptor, and it is partly proved by the shorter collagen-adrenaline-induced closure time in carriers of C/D versus C/C genotype;[16] however, because of the small sample size, we could not prove this hypothesis in this case/control study.

The conversion of fibrinogen to fibrin is the ultimate event in the activated coagulation cascade. It is widely accepted that plasma fibrinogen levels are strongly associated with and an independent predictor of CAD.[6] Some studies reported a relation between the Bβ 448G/A and β-455G/A polymorphisms and fibrinogen level both in white[7] and Chinese persons,[8] and β-455G/A polymorphism, which is in linkage disequilibrium with 448G/A polymorphism, has been shown to be associated with MI[17] and CAD.[18] As far as we know, there is only one study demonstrating the association between 448G/A polymorphism and MI. French *et al.*[9] reported that patients without flow-limiting stenosis after MI have increased frequencies of Bβ fibrinogen 448A allele, compared with patients with ≥1 flow-limiting stenosis (42 vs. 27%, OR 2.0, 95% CI 1.1–3.5, p = 0.018), while in this study we found that the frequency of 448A allele was higher in patients with UA and at least one coronary stenosis ≥50%. The subjects enrolled in the former study were all survivors of MI, and angiography was performed 1 month after MI. This might exclude patients who died and were at high genetic risk and patients with coronary repatency after therapy, which may cause a selection bias.

Several limitations should be taken into account when interpreting results. First, the lumenogram obtained by coronary angiography that cannot elucidate the true morphology of the lumen and the relatively small number of patients could lead to a selection bias. Second, our study refers to the association between these four polymorphisms and UA specifically in the Chinese population. The relevance of these polymorphisms should be investigated in other populations and with prospective and family studies.

Conclusions

Chinese patients with UA have increased frequencies of GP Ibα C/B genotype and Bβ fibrinogen 448A allele. Large and prospective studies are needed to clarify the role of these and other platelet receptor and fibrinogen polymorphisms. Additional in vitro studies of the functional relevance underlying these polymorphisms are needed to provide a sound biological explanation for the results of clinical correlations.

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