Role of Kozak Sequence Polymorphism of Platelet Glycoprotein Ibα as a Risk Factor for Coronary Artery Disease and Catheter Interventions

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OBJECTIVES We sought to determine the role of the −5T/C polymorphism of the platelet glycoprotein (GP) Ibα as a potential risk factor for coronary artery disease (CAD) and adverse events complicating a coronary catheter intervention.

BACKGROUND The platelet GP Ib-IX-V receptor complex plays a crucial role in arterial thrombus formation. The −5T/C polymorphism of GP Ibα is associated with increased receptor density.

METHODS We genotyped 1,000 patients with angiographically confirmed CAD, as well as 1,000 age- and gender-matched control subjects, for this polymorphism by polymerase chain reaction/ restriction fragment length polymorphism. Among the patients with CAD, 269 underwent percutaneous transluminal coronary angioplasty (PTCA), 103 underwent directional coronary atherectomy and 278 underwent stenting. This intervention group was followed for a 30-day composite end point of target vessel revascularization, myocardial infarction or death.

RESULTS Carriers of the −5C allele were significantly over-represented in the group of patients developing acute coronary syndromes (relative risk [RR] 1.43, 95% confidence interval [CI] 1.05 to 1.95, p = 0.02). The −5C allele furthermore predicted an increased risk for developing complications after PTCA (RR 3.75, 95% CI 1.15 to 12.27, p = 0.029).

CONCLUSIONS The −5C allele of the GP Ibα Kozak polymorphism may represent a risk factor in clinical conditions in which thrombosis plays an important role, such as in acute coronary syndromes and in complications after PTCA. (J Am Coll Cardiol 2001;38:1023–7) © 2001 by the American College of Cardiology

Thrombosis at the site of atherosclerotic plaque rupture is a prominent feature in acute coronary syndromes and in vessel wall injury after coronary interventions. The platelet glyco-protein (GP) Ib-IX-V receptor complex, comprising four polypeptides, plays a crucial role in this process by mediating platelet adhesion by binding von Willebrand factor (vWF) at the site of the vessel wall lesion (1,2).

We have previously identified a T/C polymorphism in the Kozak sequence of GP Ibα (the vWF-binding subunit of the complex) at position −5 from the initiator ATG and demonstrated increased GP Ib-IX-V complex density in carriers of the −5C allele. A possible explanation for this

observation could be more efficient messenger ribonucleic acid translation of the −5C allele, due to closer approxima-tion of the consensus nucleotide sequence derived by Kozak (3,4). We hypothesized that a higher GP Ibα receptor density may predispose to thrombotic events.

Very recently, the first published report on the clinical importance of this polymorphism failed to show a positive association between this polymorphism and the risk of coronary artery disease (CAD) in a study of 101 survivors of acute coronary syndromes (5). However, due to the small sample size, a possible association might have been missed (6). We therefore investigated, in a study of 2,000 patients, the role of the GP Ibα −5C allele as a putative risk factor for CAD, as well as for coronary interventions, including percutaneous transluminal coronary angioplasty (PTCA), directional atherectomy (DCA) and stenting.

METHODS

Study group and operational definitions. This study en-rolled 1,000 consecutive patients with angiographically proven CAD admitted to the Charité University Medical Center for elective or emergency angiography between October 1995 and January 1997. An additional 1,000 patients also admitted to this hospital served as the control group. They were matched by age, gender and time of hospital admittance. The disease spectrum of the control group reflects that of a large university hospital that has all internal and surgical disciplines. Exclusion criteria included evidence of coronary or peripheral artery disease, or any kind of vasculitis, according to history, physical examination, electrocardiography, radiography and echocardiography. The design of the study has been described in detail

Abbreviations and Acronyms

CAD	=	coronary artery disease
CI	=	confidence interval
CRP	=	C-reactive protein
DCA	=	directional coronary atherectomy
GP	=	glycoprotein
PAI-1	=	plasminogen activator inhibitor-1
PTCA	=	percutaneous transluminal coronary angioplasty
RR	=	relative risk
vWF	=	von Willebrand factor

elsewhere (7). All participants provided written, informed consent in accordance with a study protocol approved by the local Ethics Committee.

The definition of CAD was based on angiographic criteria; the coronary angiograms were reviewed by experienced cardiologists who had no knowledge of the patients' identity and outcome. Coronary artery disease was defined as ≥50% stenosis in a major coronary artery or in a major branch. The severity of CAD was classified according to the number of affected arteries (i.e., one-, two- or three-vessel disease). A diagnosis of myocardial infarction was established on the basis of World Health Organization criteria (8), as well as on angiographic findings. Operational techniques and indications for coronary interventions (e.g., PTCA, DCA, stenting) have been described in detail (7). Adverse events were assessed by the 30-day composite clinical end point, including the need for target vessel revascularization (repeat PTCA and coronary artery bypass graft surgery), myocardial infarction and death. All patients in the intervention group received aspirin and 10,000 U heparin before the intervention and, as required, repeated doses of 2,500 U heparin to maintain an activated clotting time >250 s. Patients who underwent the stenting procedure also received 500 mg ticlopidine orally after the procedure and for the following four weeks. Acute coronary syndromes comprised unstable angina and acute myocardial infarction. Unstable angina was further stratified according to the Braunwald classification (9).

Genotyping and biochemical determinations. Genotyping by polymerase chain reaction/restriction fragment length polymorphism employed primers 5′-TAGTTTTA-AGTTCTGCAGGCAAGG and 5′-AAGGTGTACA-GGAGGTTCTCACTC (TIB Molbiol, Berlin, Germany). The 353-base pair (bp) polymerase chain reaction product was digested by *Ppu*MI (New England Biolabs, Schwalbach, Germany) into 191-bp and 162-bp fragments when the −5T allele was present. Fibrinogen, C-reactive protein (CRP), plasminogen activator inhibitor (PAI-1) and vWF were determined by standard laboratory methods.

Statistical analysis. Data are presented as the median values (25th to 75th percentiles); groups were compared by the Mann-Whitney U test. Odds ratios are given with 95% confidence intervals (CIs), and p values were calculated by using the chi-square or Fisher exact test. Logistic regression analysis, adjusted for atherogenic risk factors and coagulation variables, was applied to test for the impact of GP Ibα genotypes on the risk of CAD.

For patients who underwent coronary interventions, in a first step, univariate comparison of baseline characteristics, common risk factors and procedural risk factors was done. In a second step, we developed a logistic regression model with the composite end point of adverse events included as the dependent variable. Independent variables were as follows: GP Ibα–Kozak sequence genotype, gender, diabetes, hypercholesterolemia, hypertension, smoking status, procedural determinants (unstable angina, acute myocardial infarction, number of diseased vessels, lesion type [A, B1, B2, C] and recanalization), as well as indicators of procoagulant activation and endothelial function (fibrinogen, CRP, PAI-1 and vWF). All statistical tests were calculated using SPSS, version 9.0.

RESULTS

The baseline demographic and clinical characteristics of the patients are depicted in Table 1. Glycoprotein Ibα genotyping was successful in 971 patients and 984 control subjects. The −5C allele frequencies were 18.2% in patients and 13.8% in control subjects (odds ratio 1.12, 95% CI 0.94 to 1.34, p = 0.22).

Glycoprotein Ibα genotypes and risk of CAD. Genotype frequencies for patients versus control subjects did not differ significantly: 71.7% versus 74.7% for the T/T genotype, 26.3% versus 23.1% for the C/T genotype and 2.1% versus 2.2% for the C/C genotype—comparable to values published previously (3,5).

Glycoprotein Ibα genotypes and acute coronary syndromes. A total of 235 patients with CAD suffered from acute coronary syndromes (144 with unstable angina and 91 with acute myocardial infarction). Further stratification of the patients presenting with unstable angina revealed that 39.6% were in Braunwald class I, 34% were in class II and 26.4% were in class III. Logistic regression analysis, adjusted for age, gender, diabetes, hypertension, hypercholesterolemia, smoking status, vWF, CRP, PAI-1 and fibrinogen, revealed that heterozygous and homozygous carriers of the −5C allele (−5C/T + −5C/C) were at increased risk for development of acute coronary syndromes, as compared with those carriers of the wild-type allele (−5T/T) (relative risk [RR] 1.43, 95% CI 1.05 to 1.95, p = 0.02).

Glycoprotein Ibα genotypes and outcome of coronary interventions. We performed coronary interventions on 673 patients; genotyping was successful in 650 (269 with PTCA, 103 with DCA and 278 with stenting). Genotype frequencies did not differ between the entire population and the intervention group. Table 2 depicts the patients' characteristics stratified according to the type of coronary intervention. In the PTCA group, carriers of the T/T genotype had a significantly lower prevalence of diabetes

Table 1. Demographic and Clinical Data of Patients With CAD (n = 1,000) and Control Subjects (n = 1,000)

	Patients With CAD	Control Subjects	OR	95% CI	p Value
Age (years)	60.6 (55.1, 67.1)	60.5 (54.5, 66.5)			
Males	75.9%	75.9%	—	—	—
History					
Hypertension	55.2%	35.9%	2.20	1.8–2.6	< 0.001
Hypercholesterolemia	52.7%	30.3%	2.56	2.1–3.1	< 0.001
Diabetes	22.8%	11.4%	2.30	1.8–2.9	< 0.001
Smokers	44.0%	35.2%	1.45	1.2–1.7	< 0.001
Age at manifestation <40 years	6.8%				
Acute myocardial infarction	9.1%				
Unstable angina	14.4%				
Three-vessel CAD	33.8%				

Data are presented as the median value (25th, 75th percentile) or percentage of patients or subjects.
CAD = coronary artery disease; CI = confidence interval; OR = odds ratio.

($p < 0.05$) and type A lesions ($p < 0.05$), but a higher proportion of type C lesions ($p < 0.05$), compared with carriers of the −5C allele. A comparison of the intervention groups showed that vessel diameters were smaller in the PTCA group (2.6 mm [range 2.0 to 3.1]) than in the DCA group (3.3 mm [range 2.0 to 3.9]; $p < 0.001$) and stent group (3.1 mm [range 2.0 to 3.5], $p < 0.001$). In the stent group, the number of stents per attempted lesion did not differ significantly between the T/T and T/C + C/C genotypes (1.7 ± 0.9 vs. 1.7 ± 0.8).

The 30-day composite end point was reached by 42 patients (6.5%) (Table 3), a complication rate comparable to that of large registries (10). Of these patients, two were −5C/C homozygotes, 15 were −5C/T heterozygotes and 25 were T/T homozygotes. Logistic regression analysis disclosed that the −5C allele was associated with increased procedural risk in patients undergoing PTCA (Fig. 1), with RR 3.75, adjusted for common risk factors, procedural determinants and indicators of procoagulant activation and endothelial function (95% CI 1.15 to 12.27, $p = 0.029$).

Table 2. Baseline Clinical and Angiographic Characteristics by Interventional Procedure and Kozak Sequence Glycoprotein Ibα Genotype

	PTCA		DCA		Stenting	
	T/T (n = 189)	T/C + C/C (n = 80)	T/T (n = 82)	T/C + C/C (n = 21)	T/T (n = 179)	T/C + C/C (n = 99)
Demographic data						
Age (years)	60.4 (54.1, 67.8)	60.7 (57.2, 67.1)	58.5 (51.9, 63.4)	60.6 (54.7, 65.6)	61.1 (55.7, 66.4)	60.3 (55.0, 67.1)
Male	147 (77.4%)	59 (74.7%)	66 (80.5%)	19 (90.5%)	138 (77.1%)	67 (67.7%)
Risk factors						
Smoking	98 (51.6%)	38 (48.1%)	40 (48.8%)	11 (52.4%)	85 (47.5%)	39 (39.4%)
Diabetes	34 (17.9%)*	25 (31.6%)	19 (23.2%)	4 (19.0%)	39 (21.8%)	22 (22.2%)
Hypercholesterolemia	101 (53.2%)	38 (48.1%)	48 (58.5%)	7 (33.3%)	104 (58.1%)	58 (58.6%)
Hypertension	101 (53.2%)	50 (63.3%)	35 (42.7%)	12 (57.1%)	96 (53.6%)	52 (52.5%)
History						
Acute myocardial infarction	31 (16.3%)	16 (20.3%)	2 (2.4%)	2 (9.5%)	16 (8.9%)	10 (9.9%)
Unstable angina	24 (12.6%)	13 (16.5%)	18 (22.0%)	7 (33.3%)	28 (15.6%)	19 (19.2%)
Recanalization	28 (14.7%)	12 (15.2%)	2 (2.4%)	1 (4.8%)	23 (12.8%)	17 (17.2%)
No. of affected vessels						
One	47 (24.7%)	16 (20.3%)	36 (43.9%)	6 (28.6%)	59 (33.0%)	23 (23.2%)
Two	85 (44.7%)	39 (49.4%)	30 (36.6%)	10 (47.6%)	58 (32.4%)	48 (48.5%)
Three	58 (30.6%)	24 (30.3%)	16 (19.5%)	5 (23.8%)	62 (34.6%)	28 (28.3%)
Lesion type						
A	45 (23.8%)*	28 (35.0%)	34 (41.4%)	4 (19.0%)	1 (0.6%)	4 (4.0%)
B1	18 (9.5%)	7 (8.7%)	4 (4.8%)	1 (4.8%)	20 (11.1%)	5 (5.1%)
B2	89 (47.1%)	37 (46.3%)	26 (31.8%)	10 (47.6%)	111 (62.0%)	59 (59.6%)
C	37 (19.6%)*	8 (10.0%)	18 (22.0%)	6 (28.6%)	47 (26.3%)	31 (31.3%)
Vessel diameter (mm)	2.6 (2.3, 3.1)	2.6 (2.4, 3.1)	3.3 (2.8, 3.5)	3.6 (2.9, 3.9)	3.1 (2.8, 3.5)	3.1 (2.7, 3.5)
Preprocedural TIMI flow grade <3	1 (0.5%)	1 (1.3%)	0	0	1 (0.6%)	1 (1.0%)
MLD						
Before intervention (mm)	1.0 (0.8, 2.1)	1.0 (0.8, 1.3)	1.1 (0.8, 1.2)	1.0 (0.9, 1.2)	0.5 (0.1, 1.0)	0.4 (0.1, 1.2)
After intervention (mm)	2.0 (1.9, 2.8)	1.9 (1.7, 2.5)	2.5 (1.5, 3.0)	2.7 (2.0, 3.0)	3.0 (2.8, 3.4)	3.1 (2.5, 3.6)
Dissection type ≥D	3 (1.6%)	3 (3.8%)	1 (1.2%)	0	12 (6.7%)	4 (4.0%)

*$p < 0.05$ vs. T/C + C/C genotype. Data are presented as the median value (25th, 75th percentiles) or number (%) of patients.
DCA = directional coronary atherectomy; MLD = minimal lumen diameter; PTCA = percutaneous transluminal coronary angioplasty; TIMI = Thrombolysis In Myocardial Infarction trial.

Table 3. Glycoprotein Ibα Genotype Distribution by 30-Day Composite End Point and Type of Intervention in Patients Who Underwent Coronary Interventions

| | Genotype | | p Value* |
	CT + CC	TT	
PTCA (n = 269)			
No complications	72	183	
Composite end point	8	6	0.03
DCA (n = 103)			
No complications	20	77	
Composite end point	1	5	1.00
Stenting (n = 278)			
No complication	91	165	
Composite end point	8	14	0.94

*Calculated by using the chi-square or Fisher exact test.
Abbreviations as in Table 1.

However, the −5C allele did not predict an adverse outcome during DCA and stenting: the RR for C-allele carriers undergoing DCA was 3.42 (95% CI 0.22 to 53.59, p = 0.38) and for those undergoing stenting, it was 1.05 (95% CI 0.31 to 3.59, p = 0.93).

DISCUSSION

The crucial findings of this study were that carriers of the −5C allele have a greater risk of developing acute coronary syndromes and complications after PTCA.

We have recently demonstrated that the presence of C instead of T at position −5 of the GP Ibα–Kozak sequence leads to increased density of the GP Ib-IX-V receptor complex on platelets (3). We therefore hypothesized that this mutation could also possess clinical importance. Thrombus formation at the site of plaque rupture is a dominant feature of acute coronary syndromes and during coronary interventions in which the protective endothelial lining of the arterial wall is disrupted, rendering arteries susceptible to thrombosis (11). Platelet adhesion, aggregation and thrombosis may lead to unstable angina and progress to myocardial infarction (12). The contact of GP Ib-IX-V complex with vWF is a pivotal step in these processes (1). Therefore, it was not unexpected that we observed an increased risk of acute coronary syndromes and a higher complication rate for PTCA in carriers of the −5C allele. However, this did not apply for DCA and stenting. A possible explanation for this discrepancy could be that the stent group received additional ticlopidine, which inhibits platelet aggregation and activation of the fibrinogen receptor, GP IIb-IIIa (12). The reason for the lack of an increased procedural risk in the DCA group might reside in significantly greater vessel diameters. Moreover, the wide confidence interval by no means excludes an increased odds ratio. Therefore, it cannot, of course, be completely excluded that this result is a chance finding.

Although our working hypothesis was initially centered on acute thrombotic events, we also investigated whether the −5C allele predisposes to the development of atherosclerosis, particularly in light of our recent findings that GP Ibα also represents a counter-receptor for endothelial P-selectin and leukocyte Mac-1 (13,14). However, in this large study, no significant association was evident with CAD, which is in accordance with the first report on this
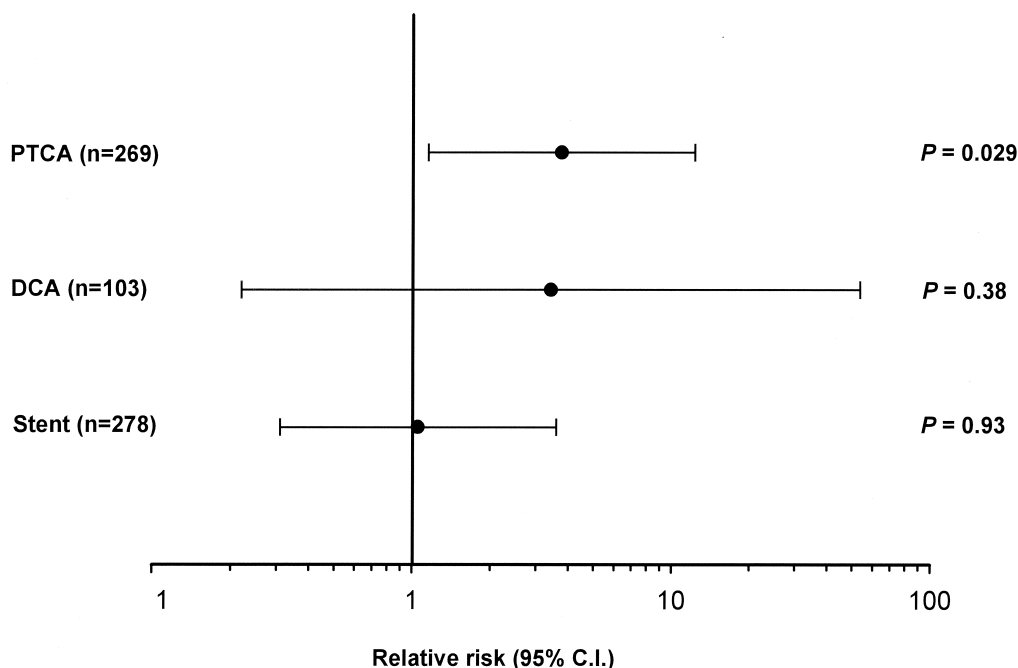
Figure 1. Glycoprotein Ibα–Kozak sequence genotype predicts increased relative risk for the 30-day composite end point after percutaneous transluminal coronary angioplasty (PTCA), but not after directional coronary atherectomy (DCA) or stenting. The figure shows relative risks for the GP Ibα–Kozak sequence (C/C + C/T) genotype, with 95% confidence intervals (CI) adjusted for common risk factors, procedural determinants and indicators of procoagulant activation and endothelial function (see text).

issue by Corral et al. (5), who failed to show an association of the −5C allele with CAD in 101 survivors of acute coronary syndromes.

Potential limitations. In the interpretation of the present results, it has to be kept in mind that the study was of case-control design. Hence, a possible survival bias cannot be excluded for the group of patients with acute coronary syndromes. Furthermore, the patients with CAD in our study must be considered to represent a selected patient group. Therefore, they may not be representative of all patient groups, because these patients were referred to a highly specialized tertiary referral center for a coronary diagnosis and intervention. Despite the large sample size, due to the number of patients in certain subgroups, the confidence limits are broad. Therefore, confirmation of these results in large, independent and prospective studies is required.

Clinical implications. It may be possible to reduce the incidence of early procedure-related complications by identification of subgroups of patients at higher risk. The 28.4% prevalence of genotypes containing one or two −5C alleles among patients with CAD is clinically relevant. Confirmation of our data in further studies would support that genotyping for GP Ibα could prove useful and that intensified antiplatelet therapy should be considered for carriers of the −5C allele. In addition, GP Ibα could also prove to be an interesting target for antiplatelet therapy.

Conclusions. The Kozak sequence mutation of GP Ibα apparently represents a risk factor in clinical conditions in which thrombus formation plays an important role, such as in acute coronary syndromes and adverse events complicating PTCA. These findings—if supported by further studies—suggest that genotyping of GP Ibα could prove important for risk stratification and tailoring of antiplatelet therapy.

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