Association Between Platelet Glycoprotein Ibα Genotype and Ischemic Cerebrovascular Disease

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Background and Purpose—Platelets play pivotal roles in the development of ischemic cerebrovascular disease (CVD). The platelet glycoprotein (GP) Ib/IX/V complex is a receptor for von Willebrand factor, which plays a major role in the initial phase of platelet activation under high shear stress conditions. This study was designed to investigate the association between a genetic variation of this receptor and the prevalence of CVD.

Methods—Two hundred patients with ischemic CVD, as confirmed by brain CT and/or MRI, and 317 age- and sex-matched control subjects without clinical evidence of CVD or cardiovascular disease were analyzed for their genotype frequencies of the 145Thr/Met dimorphism of the α-chain of GPIb (GPIbα).

Results—Genotypes with 145Met (T/M and M/M) were more frequently found in the CVD patients (26.5%) than in control subjects (14.2%, \( P = 0.0005 \)). The genotype effect was more obvious in those <60 years of age or without acquired cardiovascular risk factors. The odds ratio for nonsmoking women <60 years of age was 10.6 (95% confidence intervals, 2.2 to 51.7). Although the number of patients studied was small (n=24), transient ischemic attack showed the highest odds ratio (4.3, \( P = 0.0004 \)), followed by lacunar infarction (OR=2.2, \( P = 0.0024 \)) and atherothrombotic infarction (OR=1.5, \( P = 0.3143 \)). Logistic regression analysis revealed that the presence of Met-allele was independently associated with CVD.

Conclusions—Our study suggests that the platelet GPIbα genotype is a genetic risk factor for ischemic CVD. (Stroke. 2000;31:493-497.)

Key Words: cerebrovascular disorders ■ genetics ■ platelets ■ polymorphism ■ thrombosis

Platelets play key roles in cerebrovascular diseases (CVD) and acute coronary syndromes, as demonstrated by histopathologic findings and clinical observations showing the efficacies of antiplatelet therapies for these disorders.1-3 Platelet membrane glycoproteins (GPs) are particularly important to form platelet thrombi in that they mediate platelet adhesion and aggregation. GPs are now targets of antiplatelet therapies. Several lines of evidence have suggested that different GPs are involved in platelet activation processes according to different rheological conditions.4-10 Platelets in flowing blood can be activated and aggregated by high shear stress without chemical agonists. Moreover, shear-induced platelet aggregation has been shown to be enhanced in patients with atherosclerotic disorders including coronary artery diseases and ischemic stroke.11,12 These observations suggest the relevance in ischemic stroke of platelet responsiveness to high shear stress. The molecular mechanisms of shear-induced platelet aggregation have been investigated extensively, and it is now believed that aggregation is initiated by the binding of an adhesive ligand von Willebrand factor (vWF) to its platelet receptor, the GPIb/IX/V complex.10 Binding of vWF to GPIb/IX/V causes intraplatelet signaling, resulting in the activation of the other platelet receptor for vWF, the GPIIb/IIIa complex, to aggregate platelets.10

GPIb/IX/V complex is composed of 4 subunits; GPIbα (CD42b), GPIbβ (CD42c), which is disulfide-bonded to GPIbα, GPIX (CD42a), and GPV (CD42d).13 They are synthesized from different genes. The genomic and cDNA sequences of each component have been identified.14 Two genetic polymorphisms have been reported in the coding sequence of GPIbα. The first polymorphism, a C/T transition at nucleotide 1018 (numbers according to Wenger et al15), results in an amino acid dimorphism (Thr/Met) at residue 145 of GPIbα, which is located within the vWF-binding domain of the receptor. This polymorphism is a known molecular basis of a platelet alloantigen system, HPA 2a/2b, and is involved in the development of platelet transfusion refractoriness.16,17 The second polymorphism is the variable number (1 to 4) of a 13–amino acid sequence repeats. This “size-
polymorphism” is known to be strongly associated with the first polymorphism, alleles with 1 or 2 repeats being linked to 145Met, whereas alleles with 3 or 4 repeats are linked to 145Thr. Moreover, another polymorphism, the Kozak sequence polymorphism, was recently identified and it was suggested that this polymorphism affected the receptor density on the platelet surface.19 The effect of these polymorphisms on platelet function, however, is still not well understood.

The association between GPIbα genotype and the risk of coronary artery disease was first reported in 1997.20 Subsequently, several studies were reported, but only a few studies focused on the association of this genotype with stroke, and the results were not consistent.21–23 Therefore we performed an allelic association study to compare genotype frequencies between CVD patients and age- and sex-matched control subjects.

Subjects and Methods

Patients and Control Subjects

We analyzed 200 unrelated Japanese patients with a history of ischemic cerebrovascular disease (mean age 58.2±7.7 years), and 317 age- and sex-matched unrelated control subjects (mean age 59.0±3.6 years, Table 1). Patients were those who visited the outpatient clinic of Keio University Hospital in Tokyo for regular follow-up. Diagnosis of CVD was made by neurologists at the onset of the disease on the basis of the classification of cerebrovascular diseases III established by the National Institute of Neurological Disorders and Stroke.24 CVD was classified as transient ischemic attack (TIA; n=24), atherothrombotic infarction (n=51), and lacunar infarction (n=125). The distribution of patient number for each subtype is compatible with the reported incidence of CVD in Japan,25 where lacunar infarction is predominant. Patients with cardioembolic cerebral infarction and cerebral hemorrhage were not included in this study. Brain CT and/or MRI were performed for each patient. Magnetic resonance angiography and/or extracranial duplex ultrasonography were available in >80% of cases. For TIA, all patients were evaluated by echocardiography, ECG, magnetic resonance angiography, and extracranial duplex ultrasonography. Control subjects were personnel working in Keio University Hospital. For the recruitment of control subjects, we first registered all subjects between the ages of 50 and 65 years who visited for a regular check-up and gave informed consent. We next excluded those with histories or physical signs of CVD or any cardiovascular disorders. Informed consent was obtained from every patient and control subject after a full explanation of the study. Cardiovascular risk factors were evaluated for both patients and control subjects on the basis of the criteria shown below. Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic pressure >90 mm Hg or current treatment with antihypertensive drugs. Diabetes mellitus was defined by World Health Organization criteria.26 Hypercholesterolemia was defined as a cholesterol level >220 mg/dL or any cholesterol level under cholesterol-lowering treatment.

Polymerase Chain Reaction and Restriction Enzyme Analysis

Identification of GPIbα genotype was performed by the polymerase chain reaction (PCR)–restriction fragment length polymorphism method with the use of Ampdirect (Shimadzu Co), which could eliminate the DNA extraction process and amplify the genomic DNA directly from whole blood. PCR was performed with the use of a Gene Amp PCR System 9600-R (Perkin Elmer). Previously described oligonucleotide primers, 5'-GGAGCTCTCTCCAACCGGC and 5'-GCTTTGCTGGGAACCTTGAC, were used in this study.20 Reaction mixture contained 10 µL Ampdirect, 10 µL Amplification, 250 µmol/L dNTP, 0.5 µmol/L each specific primer, 1 U AmpliTaq DNA polymerase, 0.5 to 1 µL whole blood, with a final volume of 50 µL. After the initial denaturation at 80°C for 15 minutes and at 94°C for 4.5 minutes, 40 amplification cycles were carried out, each consisting of denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, followed by a final extension step at 72°C for 7 minutes. A 588-bp PCR product was digested with 2 U of restriction end-nuclease Hin1I, and genotypes were determined as described.20

Statistical Analysis

The unpaired Student’s t test was used to compare the continuous variables between CVD patients and control subjects. Differences in proportion were analyzed by the χ2 test. Calculation of odds ratio (OR) and 95% confidence intervals (95% CI) estimated the association strength between GPIbα genotype and CVD. OR (95% CI) >1 was considered to be significant. A logistic regression analysis was performed to evaluate the interaction between the GPIbα T/M genotype and other variables in relation to the prevalence of CVD. Independent variables included in this analysis were age (quantitative), sex (male or female), hypertension (yes or no), hypercholesterolemia (yes or no), diabetes (yes or no) and GPIbα genotypes (T/T vs T/M+M/M). A probability value <0.05 was considered to be statistically significant. All statistical analyses were performed with the use of Statview (version 5.0, for Macintosh, SAS Institute, Japan).

Results

Characteristics of Control Subjects and CVD Patients

Table 1 shows the number, mean age, sex distribution, and the prevalences of selected risk factors for CVD in the patients and control subjects. No statistically significant differences were observed for age and sex distribution between the patients and control subjects. As expected, there were significant differences in the frequencies of current smoking, hypertension, and diabetes mellitus between the 2 groups.

Genotype Frequencies Were Different Between Control Subjects and CVD Patients

Genotype and allele frequencies of the CVD patients and control subjects are shown in Table 2. Genotypes with 145Met (T/M or M/M) were 14.2% in control subjects. On the other hand, CVD patients showed a significantly higher percentage of these genotypes (26.5%, P=0.0005). Statistically significant differences were also observed when separate analyses were performed for men (26.1%, P=0.0153) and women (27.9%, P=0.0068).
Effect of GPIbα Genotype Was More Obvious in Those <60 Years of Age or Without Acquired Cardiovascular Risk Factors

Table 3 summarizes the subpopulation analyses for the relation between GPIbα and CVD. In this table, crude ORs (T/T vs T/M + M/M) are described. In all subpopulations analyzed, the ORs were universally higher in those groups without the selected cardiovascular risk factors. The ORs were 3.58 and 1.06 for nonsmokers and smokers, respectively. The OR for nonsmokers <60 years of age was 4.48, for women <60 it was 4.89, and for nonsmoking women <60 it was 10.60 (not shown in this table).

GPIbα Genotype Was Associated With Lacunar Infarction and TIA

We next analyzed genotype frequencies for subtypes of CVD; that is, TIA, atherothrombotic stroke, and lacunar infarction (Table 4). TIA patients showed significantly higher prevalence of T/M or M/M genotypes as compared with control subjects (41.7%, P=0.0004). A similar association was recognized for lacunar infarction (26.4%, P=0.0024). By contrast, the frequency for T/M or M/M genotypes in atherothrombotic stroke patients did not differ from the control subjects (19.6%, P=0.3143). When patients were divided into 2 groups on the basis of the number of cerebral lesions (single or multiple), frequencies of T/M + M/M genotypes were higher in both groups (27.0% for multiple cerebral lesions, P=0.0121, and 23.0% for single cerebral lesion, P=0.0303, Table 4) as compared with control subjects. Thus GPIbα genotype was associated with both single and multiple cerebral lesions.

No Differences Were Seen Between GPIbα Genotypes in Terms of Prevalences of Acquired Vascular Risk Factors

We compared the prevalences of acquired cardiovascular risk factors between different genotypes of GPIbα. In control subjects, there were no statistically significant differences in the distribution of sex, age, frequencies of smoking, hypertension, hypercholesterolemia, and diabetes mellitus between the 2 GPIbα genotype groups (T/T vs T/M + M/M). Also, in CVD patients there were no statistically significant differences in these parameters except that the prevalence of

### TABLE 2. Genotype Distribution and Allele Frequencies of Platelet GPIbα in Control Subjects and CVD Patients

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>T/T, %</th>
<th>T/M + M/M, %</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=317)</td>
<td>272 (85.8)</td>
<td>43 + 2 (14.2)</td>
<td>2.18</td>
<td>0.0005</td>
</tr>
<tr>
<td>CVD (n=200)</td>
<td>147 (73.5)</td>
<td>51 + 2 (26.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=225)</td>
<td>189 (84.0)</td>
<td>34 + 2 (16.0)</td>
<td>1.86</td>
<td>0.0153</td>
</tr>
<tr>
<td>CVD (n=157)</td>
<td>116 (73.9)</td>
<td>40 + 1 (26.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=92)</td>
<td>83 (90.2)</td>
<td>9 + 0 (9.8)</td>
<td>3.57</td>
<td>0.0068</td>
</tr>
<tr>
<td>CVD (n=43)</td>
<td>31 (72.1)</td>
<td>11 + 1 (27.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3. Subpopulation Analyses for Association of GPIbα Genotype With Ischemic CVD

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>OR (95% CI)*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
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<tr>
<td>&lt;60</td>
<td>2.46 (1.37–4.42)</td>
<td>0.0026</td>
</tr>
<tr>
<td>≥60</td>
<td>1.89 (0.97–3.67)</td>
<td>0.0606</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3.58 (2.02–6.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Yes</td>
<td>1.06 (0.52–2.15)</td>
<td>0.8721</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2.19 (1.19–4.03)</td>
<td>0.0117</td>
</tr>
<tr>
<td>Yes</td>
<td>1.82 (0.89–3.74)</td>
<td>0.1030</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2.25 (1.27–4.00)</td>
<td>0.0057</td>
</tr>
<tr>
<td>Yes</td>
<td>1.74 (0.83–3.64)</td>
<td>0.1421</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2.30 (1.41–3.76)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Yes</td>
<td>1.14 (0.36–3.59)</td>
<td>0.8231</td>
</tr>
</tbody>
</table>

* T/T vs T/M + M/M compared between control subjects and CVD patients by χ² test.

### TABLE 4. Genotype Distribution of Platelet GPIbα in Relation to Subtypes of CVD and Number of Cerebral Lesions

<table>
<thead>
<tr>
<th>Subtypes of CVD</th>
<th>T/T, %</th>
<th>T/M + M/M, %</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>272 (85.8)</td>
<td>43 + 2 (14.2)</td>
<td></td>
</tr>
<tr>
<td>TIA</td>
<td>14 (58.3)</td>
<td>10 + 0 (41.7)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Lacunar infarction</td>
<td>92 (73.6)</td>
<td>32 + 1 (26.4)</td>
<td>0.0024</td>
</tr>
<tr>
<td>Atherothrombotic infarction</td>
<td>41 (80.4)</td>
<td>9 + 1 (19.6)</td>
<td>0.3143</td>
</tr>
<tr>
<td>Cerebral lesions†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>46 (73.0)</td>
<td>16 + 1 (27.0)</td>
<td>0.0121</td>
</tr>
<tr>
<td>Single</td>
<td>87 (77.0)</td>
<td>25 + 1 (23.0)</td>
<td>0.0303</td>
</tr>
</tbody>
</table>

* Versus control (χ² test).
† Patients with TIA were excluded from this analysis.
smoking was slightly higher in the T/T genotype, which is, however, the “non-risk” genotype (not shown in tables).

Logistic Regression Analysis Revealed That Presence of the Met-Allele Was an Independent Risk Factor for CVD

In the logistic regression analysis, age, sex, hypertension, hypercholesterolemia, diabetes, and GPIbα genotypes (T/T vs T/M + M/M) were included as independent variables. This analysis revealed that the presence of the Met-allele (T/M or M/M) was one of the independent risk factors for CVD (OR = 1.94, P = 0.0176). Current smoking (OR = 3.28, P < 0.0001), hypertension (OR = 4.63, P < 0.0001), and diabetes mellitus (OR = 5.41, P < 0.0001) were also shown to be independent risk factors (not shown in tables).

Discussion

In the present study, we clearly demonstrated that the genotypes with Met-allele (T/M and M/M genotypes) were associated with CVD in the Japanese population and that it might be a new genetic risk factor for CVD. Our results agree with those obtained by Gonzalez-Conejero et al.21 but contradict the results reported by Carlsson et al.,22 who failed to show any association between the GPIbα genotype and stroke. We performed a careful association study with age- and sex-matched control subjects living in the same area in Japan and carried out detailed analyses for the association. We have found that the effect of GPIbα genotype varies significantly among subpopulations studied. As shown in Table 3, the genotype effect was stronger in those populations who have less acquired vascular risk factors; that is, the ORs were apparently higher in women, those <60 years of age, and nonsmokers. When nonsmoking women <60 years of age were compared, the OR of the Met-allele for CVD was 10.60. This finding is compatible with a published study on the association of GPIbα genotype with coronary artery disease,20 in which the effect of GPIbα genotype was significant only in those ≈60 years of age, and with the 2 previous studies on the relation between CVD and another platelet receptor polymorphism, the PIA1/A2 polymorphism of platelet GPIIb/IIIa.27 In one of these studies, the genotype effect of the PIA1/A2 polymorphism was more obvious among women,28 and in the other study, the genotype effect was stronger in nonsmokers.23 Thus the relative contribution of genetic factors might differ significantly, depending on the populations analyzed. This might in part explain the conflicting results of the published studies.

Although our results suggest that the 145T/M polymorphism is associated with the risk of CVD, we did not find the gene-dose effect; that is, frequencies of heterozygote T/M differed significantly between control subjects and patients (14.2% and 26.5%, respectively), but frequencies of homozygote M/M were not significantly different (0.6% and 1.0%, respectively). This might suggest that there could be a deleterious interaction between 145Thr and Met. However, because the M/M genotype is so rare, further studies with larger cohorts are necessary to test this hypothesis.

In the subtype analysis, the GPIbα genotype was associated with the risks for TIA and lacunar infarction but not for atherothrombotic stroke. We initially assumed that platelet polymorphisms would be more closely related to atherothrombotic stroke and TIA because these two types of ischemic CVD are believed to have a common pathogenesis, and lacunar infarction is mostly dependent on the presence of hypertension. However, this assumption was in part based on the clinical observation that lacunar infarction was relatively resistant to antiplatelet drugs such as aspirin. If one considers that shear-induced, GPIb/IX/V-mediated platelet activation is insensitive to aspirin,13 our results are not surprising, and it could be speculated that the relative contribution of GPIb/IX/V complex for the pathogenesis of CVD might be different among subtypes of CVD.

The roles of vWF-GPIb/IX/V interaction in the development of atherosclerosis and arterial thrombosis have been implicated in several reports.29–31 Agents that block either vWF or GPIb/IX/V inhibited and delayed coronary occlusion in animal models.31 Elevated plasma levels of vWF is a poor prognostic factor of coronary heart disease as well as an independent risk factor for subsequent acute coronary events in patients with angina pectoris.32,33 Participation of vWF-GPIb/IX/V in CVD, however, is not well understood, although shear-induced platelet aggregation was enhanced in ischemic CVD.12

To date, there is no direct evidence showing a relation between the GPIbα genotype and the functional difference of platelets. It is possible that replacement of threonine by methionine at residue 145, which is located within the vWF and thrombin-binding domain of this receptor, might affect the structure and function of this receptor. It is also possible that the effect of 145T/M genotype is merely a reflection of the functional differences caused by the other polymorphism on the coding sequence, the “repeat polymorphism,” located in the macroglycopeptide portion, which is in linkage disequilibrium with 145T/M. Moreover, participation of the third polymorphism of GPIbα, the Kozak sequence polymorphism that is reportedly associated with the receptor density on platelets,19 also should be considered.

Polymorphisms of platelet integrins have also been studied for the association with CVD. These are the PIA1/A2 polymorphism of GPIib/IIIa (αIIbβ3) and a collagen receptor GPIIaIa (α2β1).28,34 GPIIb/IIIa is the final common pathway of activation signals generated by various stimuli and is a key molecule for platelet aggregation, thus being a recent target of antiplatelet therapy. However, because the PIA2 allele (the less frequent allele) is very rare in Japan15 (our unpublished results indicated that PIA2 type is <1% in the normal Japanese), we did not include this polymorphism in our analysis.

Although antiplatelet therapies are widely used for the treatment and prevention of stroke, the efficacy is not always consistent and sufficient, and the participation of platelets in the pathogenesis of different subtypes of CVD still remains to be elucidated. Our present study suggested that vWF and GPIb/IX/V receptor might be involved in lacunar infarction and TIA and would further direct a new strategy for antiplatelet therapy targeting GPIb/IX/V receptor and/or vWF.
References


30. Sonody et al. GPIbalpha Genotype and CVD 497 
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