Platelet GP IIIa Pl^A and GP Ib Variable Number Tandem Repeat Polymorphisms and Markers of Platelet Activation in Acute Stroke

A.M. Carter, A.J. Catto, J.M. Bamford, P.J. Grant

Abstract—A number of polymorphisms of the platelet glycoprotein (GP) Ib-V-IX and IIb/IIIa complexes have been described, and the Pl^A polymorphism of GP IIIa has been associated with coronary thrombosis. We determined the levels of β-thromboglobulin (β-TG) and platelet factor 4 (PF4) and the genotype distributions of Pl^A and a variable number tandem repeat (VNTR) polymorphism of GP 1b in subjects with acute stroke (n=609) and healthy control subjects (n=435). Levels of β-TG were higher in patients both initially (47.4 [44.7 to 50.2] ng/mL, P<0.0001) and after 3 months (42.9 [40.3 to 45.7] ng/mL, P=0.03) compared with control subjects (39.4 [37.7 to 41.2] ng/mL). Initial levels of β-TG were significantly higher in those who subsequently died (58.7 [52.3 to 65.8] ng/mL) compared with those still alive (42.7 [40.1 to 45.5] ng/mL, P<0.0001). In a logistic regression model, β-TG remained an independent predictor of poststroke mortality, with an odds ratio for an increase in 10 ng/mL of 1.12 (1.03 to 1.21, P=0.006). In subjects who had never smoked, there was a significant difference in the genotype distributions of patients with atherothrombotic stroke (A1/A1=147, A1/A2=70, and A2/A2=2) compared with controls (A1/A1=165, A1/A2=47, and A2/A2=5, P=0.03). The Pl^A distribution of subjects with atherothrombotic stroke before the age of 50 years (A1/A1=19 and A1/A2+A2/A2=18) was also significantly different from age- and sex-matched controls (A1/A1=54 and A1/A2+A2/A2=20, P=0.02). We found no association of VNTR with stroke or poststroke mortality. These data indicate that there is a persistent state of enhanced platelet activation in subjects with acute stroke, which is associated with poststroke mortality. The increased frequency of the Pl^A allele in young subjects with atherothrombotic stroke lends further support for a role of the Pl^A polymorphism in acute thrombosis. (Arterioscler Thromb Vasc Biol. 1998;18:1124-1131.)

Key Words: platelets ■ polymorphisms ■ β-thromboglobulin ■ platelet factor 4

Two receptor complexes mediate the integral role of platelets in hemostasis: GP Ib-V-IX and GP IIb/IIIa, which are involved in the processes of platelet activation and aggregation.1,2 Under conditions of high shear stress associated with both MI and stroke.7–11 Increased platelet activation has been associated with both MI and stroke.

A number of polymorphisms of GP Ia and GP IIb/IIIa have been identified.12 The Pl^A polymorphism of platelet GP IIIa has been reported to be independently associated with coronary thrombosis in some studies,13,14 with the strongest associations observed in young subjects.15 A 39-bp VNTR polymorphism has been identified in the macroglycopeptide region of the GP 1bα gene, resulting in 1 to 4 repeats of a 13–amino acid sequence in the mature protein.6 It has been postulated that this might result in an increase in the length of the extracellular portion of GP 1b with increasing repeat motifs; this would lead to extension of the vWF binding site, which is located at the amino terminal of GP 1b, further into the circulation.16 This raises the possibility that this VNTR may be associated with an increased risk of thrombosis.

The aims of this study were to (1) determine the association of the Pl^A and VNTR polymorphisms and levels of β-TG and PF4 with stroke in subjects with acute stroke and healthy control subjects free of vascular disease; (2) relate these factors to pathological stroke type, subtypes of cerebral infarction, and poststroke mortality; (3) determine whether there is any association of these polymorphisms with circu-
lating levels of β-TG, PF4, vWF, and fibrinogen; and (4) determine the genotypic distributions of PI^a and VNTR in subjects with atherothrombotic stroke before the age of 50 years.

Methods

Subjects
White European subjects (n=609) with a clinical diagnosis of acute stroke whose pathological type was confirmed by noncontrast cranial CT scan were recruited from 4 hospitals in Leeds. Healthy white European control subjects (n=435) free from vascular disease were recruited from Family Health Services Authority general practice registers. Patients and controls gave informed consent according to a protocol approved by the United Leeds Teaching Hospitals Research Ethics Committee. Patients were “flagged” with the Office of Population Censuses and Surveys for notification of death. On the basis of the CT scan results, stroke was classified as either ICI or ICH. We further subclassified ICI according to the Oxfordshire Community Stroke Project classification17 as either probable small-vessel disease (lacunar infarction) or probable large-vessel infarction (total anterior circulation infarction or partial anterior circulation infarction), as described previously.18 Those subjects with posterior circulation infarcts, which are considered to be of mixed vascular pathology, were excluded from the relevant subgroup analyses.

Patients who survived the acute event for 2 years) and sex with 2 healthy control subjects for separate analysis.

Patients and controls were classified as smokers if they had ever smoked >1 cigarette per day for at least 1 year. Current therapy, including antihypertensives, aspirin, warfarin, and in-hospital heparin administration, was recorded. The presence of hypertension was defined as two preadmission blood pressures of ≥160/95 mm Hg or current antihypertensive therapy. BMI was calculated as the weight in kilograms divided by the square of height in meters.

Fibrinogen and vWF levels were determined as previously described.19,20 PF4 and β-TG levels were determined by ELISA (Diagnostica Stago), with intra-assay and interassay coefficients of variation, respectively, of 5.3% and 11.4% for β-TG and of 8.0% and 31% for PI^a.

DNA Analysis
DNA samples were not available for 21 patients and 33 controls. The PI^a polymorphism was determined as previously described.21 Primers, designed to flank the VNTR region, were modified from those described by Simsek et al22; forward primer was 5’CAC TAC TGA ACC AAC CCC AAG 3’ and the reverse primer 5’TGG TGG CAG ACA CCA GGA TGG 3’ to give total fragment lengths of 197 to 314 bp, depending on the number of repeats. Polymerase chain reaction conditions of 25 pmol of each primer, 100 ng DNA, 200 μmol/L of each dNTP, 10 mmol/L Tris HCl (pH 8.8), 1.5 mmol/L MgCl2, 50 mmol/L KCl, 0.1% Triton X-100, and 0.75 U Dynazyme II DNA polymerase (Flowgen) were used, involving 32 cycles of 93°C for 1 minute denaturing, 1 minute annealing at 67°C, and 1 minute at 72°C for extension, followed by a final 5-minute extension at 72°C. Genotype was determined by 2% agarose gel electrophoresis containing ethidium bromide, visualized by UV light, and sized with reference to a DNA ladder. VNTR polymorphism was classified according to the scheme of Moroi et al23 as D (single copy), C (2 copies), B (3 copies), and A (4 copies).

Statistics
The distributions of β-TG, PF4, fibrinogen, vWF, cholesterol, triglycerides, and BMI levels were positively skewed and were therefore logarithmically transformed to normalize the distributions and allow analysis by parametric tests. Initial and 3-month levels in patients were compared by paired t test. Patient levels at both visits were compared with those of controls by unpaired t test. For results that were logarithmically transformed, they were expressed as geometric means and the antilogs of the 95% confidence intervals. All other values were expressed as the mean (95% confidence intervals). Ages were compared by Mann-Whitney U tests and expressed as medians and interquartile range. One-way ANOVA was used to investigate the relationship of circulating factor levels to stroke subtypes and genotypes with Scheffé’s post hoc analysis. Multiple stepwise linear regression analysis was used to identify the determinants of β-TG and PF4 levels in each group. Genotype distributions were compared by gene counting and χ^2 analysis. Logistic regression analysis was used to determine significant determinants of stroke and poststroke mortality. All statistical analyses were performed with the SPSS for Windows statistical package (SPSS Inc).

Results

Subject Characteristics
The characteristics of the patients and control subjects are presented in Table 1. There was no significant difference in the ages of patients and controls. Female patients were significantly older than male patients, and female controls were significantly older than male controls. There was a greater proportion of women in the control group compared with the patient group. More men than women and more male patients than male controls were smokers. There were more hypertensives and more subjects with atrial fibrillation in the patient group compared with controls. Control subjects had significantly higher cholesterol levels than did patients. Of the patients, 76 had a history of MI and 166 a history of previous stroke.

Selected Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>GP</td>
<td>glycoprotein</td>
</tr>
<tr>
<td>ICH</td>
<td>intracerebral hemorrhage</td>
</tr>
<tr>
<td>ICI</td>
<td>intracerebral infarction</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>PF4</td>
<td>platelet factor 4</td>
</tr>
<tr>
<td>β-TG</td>
<td>β-thromboglobulin</td>
</tr>
<tr>
<td>VNTR</td>
<td>variable number tandem repeat</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
</tr>
</tbody>
</table>
Markers of Platelet Activation in Relation to Stroke, Stroke Subtype, and Poststroke Mortality

**Patient β-TG and PF4 Levels Compared With Controls**
Both initial and follow-up determinations of β-TG and PF4 were available for 219 patients, with an additional 113 patients having only an initial and 27 patients having only a follow-up determination. Levels of β-TG and PF4 were available for 330 control subjects. Subjects for whom samples for the determination of β-TG and PF4 levels were available were slightly younger than the overall group (although there remained no significant difference in the ages of patients and controls), with a slightly lower proportion of patients with atrial fibrillation and previous stroke; all other variables presented in Table 1 were not significantly different in those with and without β-TG and PF4 determinations (data not shown).

In all patients, levels of β-TG were significantly higher in patients both initially (n = 332, 47.4 [44.7 to 50.2] ng/mL, \( P = 0.0001 \)) and after 3 months (n = 246, 42.9 [40.3 to 45.7] ng/mL, \( P = 0.03 \)) compared with controls (n = 330, 39.4 [37.7 to 41.2] ng/mL). Initial levels of PF4 were higher in patients (7.7 [7.1 to 8.4] ng/mL) than controls (6.4 [6.0 to 6.8] ng/mL, \( P < 0.0001 \)), but there was no significant difference in these levels after 3 months (6.1 [5.5 to 6.8] ng/mL, \( P = 0.5 \)). In the 219 patients with both initial and follow-up determinations of β-TG and PF4, there was no significant difference in levels of β-TG between these two visits (initial, 44.6 [41.5 to 48.0]; follow-up, 43.1 [40.3 to 46.1], \( P = 0.3 \)). Levels of PF4 were, however, significantly lower in these subjects at follow-up (initial, 7.1 [6.4 to 7.8]; follow-up, 5.9 [5.3 to 6.6], \( P = 0.002 \)).

**Determinants of β-TG and PF4 Levels**
Bivariate correlation coefficients are presented in Table 2. As expected, levels of β-TG and PF4 were strongly correlated in patients both initially and at follow-up and in control subjects.

β-TG levels were significantly positively correlated with age, fibrinogen, vWF, and platelet count and negatively with BMI in all groups. PF4 was associated with fibrinogen, vWF, age, and BMI in patients initially, with age alone at follow-up, and with platelet count alone in control subjects.

There was no association of β-TG or PF4 with aspirin or warfarin use, smoking, diabetes mellitus, or atrial fibrillation in any group (data not shown). Initial levels of PF4 were significantly lower in patients receiving heparin during hospitalization (n = 18, 5.2 [3.9 to 6.9] ng/mL) than in those who

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**TABLE 1. Characteristics of Patients and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 301)</td>
<td>Female (n = 308)</td>
</tr>
<tr>
<td>Age, y</td>
<td>70.0 (61.0–77.0)</td>
<td>75.0 (68.0–82.0)‡</td>
</tr>
<tr>
<td>BM, kg/m²</td>
<td>25.6 (25.1–26.2)</td>
<td>24.7 (24.0–25.3)‡</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.2 (5.0–5.4)*</td>
<td>5.5 (5.3–5.7)‡</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.57 (1.46–1.69)</td>
<td>1.55 (1.45–1.65)</td>
</tr>
<tr>
<td>Platelet count, × 10⁹/L</td>
<td>238 (228–248)</td>
<td>265 (256–275)‡</td>
</tr>
<tr>
<td>Current smokers</td>
<td>No</td>
<td>235 (0.78)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>66 (0.22)</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>No</td>
<td>82 (0.27)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>219 (0.73)*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>No</td>
<td>177 (0.59)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>124 (0.41)*</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>No</td>
<td>258 (0.86)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>43 (0.14)*</td>
</tr>
<tr>
<td>Previous CVA</td>
<td>No</td>
<td>221 (0.73)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>80 (0.27)</td>
</tr>
<tr>
<td>Previous MI</td>
<td>No</td>
<td>257 (0.85)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>44 (0.15)</td>
</tr>
</tbody>
</table>

CVA indicates cerebrovascular accident. Values are mean or geometric mean and 95% CI.

* \( P < 0.05 \) for male patients vs male controls.

† \( P < 0.05 \) for female patients vs female controls.

‡ \( P < 0.05 \) for male patients vs female patients.

§ \( P < 0.05 \) for male controls vs female controls.
did not (n = 314, 7.9 [7.2 to 8.6] ng/mL, P = 0.03). There was no difference in levels of β-TG or PF4 in patients with a history of MI or previous stroke compared with those without (data not shown).

**Independent Predictors of β-TG Levels in Patients and Controls**

Factors significantly associated with levels of β-TG and PF4 in univariate analyses were entered into stepwise multiple linear regression models to identify independent predictors of these levels. In patients, initial levels were independently associated with fibrinogen, accounting for 20.3% of the variation, while age, platelet count, and vWF together accounted for an additional 8.3% of the variation in levels. At follow-up, levels were associated with fibrinogen, age, and platelet count, accounting for 9.2%, 4.5%, and 3.9%, respectively, of the variation in these levels. In controls, levels were independently associated only with vWF (27.3%) and fibrinogen (5.0%).

**Independent Predictors of PF4 Levels in Patients and Controls**

In patients, initial levels were independently associated only with fibrinogen, accounting for 5.7% of the variation. No factors were identified as being independently associated with levels at follow-up in patients or in control subjects.

**Levels of β-TG and PF4 According to Stroke Type**

There was no significant difference in levels of β-TG or PF4 in patients with CT-confirmed ICH compared with all those with ICI, in those with either small- or large-vessel infarction, or in controls, as shown in Table 3. Subjects with large-vessel infarction had higher levels of β-TG than did controls at both the initial (P < 0.0001) and the follow-up (P = 0.04) visits.

**Association of β-TG and PF4 Levels With Poststroke Mortality**

One hundred eight patients with initial determinations of β-TG and PF4 died between the initial visit and April 1997, representing a median (interquartile range) follow-up period of 3.1 (2.6 to 3.4) years. Of these, 45 survived until after the 3-month follow-up. Initial levels of β-TG were significantly higher in those who died (58.7 [52.3 to 65.8] ng/mL) compared with those still alive (n = 224, 42.7 [40.1 to 45.5] ng/mL, P < 0.0001). Levels at 3 months were also significantly higher in those subjects who subsequently died (n = 45, 52.3 [44.9 to 60.9] ng/mL compared with those still alive after follow-up (n = 201, 41.1 [38.4 to 43.9] ng/mL, P = 0.003). Initial but not follow-up levels of PF4 were also significantly higher in those who died (9.0 [7.7 to 10.5] ng/mL) compared with those still alive (7.2 [6.5 to 7.9] ng/mL, P = 0.01). In a stepwise logistic regression model including age, previous stroke, sex, atrial fibrillation, smoking, stroke type, and initial levels of β-TG and PF4, β-TG remained an independent predictor of poststroke mortality, with an odds ratio for an increase in 10 ng/mL of 1.12 (1.03 to 1.21, P = 0.006).

**Platelet Glycoprotein Polymorphisms in Relation to Stroke, Stroke Subtype, and Poststroke Mortality**

**Association of VNTR With Stroke and Poststroke Mortality**

The VNTR genotype distributions in patients and controls were in Hardy-Weinberg equilibrium. There was no difference in the genotype distribution of VNTR in patients and controls, nor in those with ICH or ICI compared with controls (Table 4). In addition there was no difference in VNTR by subtypes of ICI or by prior treatment with aspirin or warfarin in relation to age, and there was no interaction of VNTR with any of the variables in Table 1. In addition, there was no difference in levels of β-TG, PF4, vWF, or fibrinogen by VNTR genotype (data not shown) and no difference in VNTR

### Table 2. Correlation Coefficients in Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (Initial)</th>
<th>Patients (Follow-up)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-TG PF4</td>
<td>β-TG PF4</td>
<td>β-TG PF4</td>
</tr>
<tr>
<td>β-TG</td>
<td>...</td>
<td>0.71‡</td>
<td>0.71‡</td>
</tr>
<tr>
<td>PF4</td>
<td>0.71‡</td>
<td>...</td>
<td>0.71‡</td>
</tr>
<tr>
<td>Age</td>
<td>0.27‡</td>
<td>0.13*</td>
<td>0.26‡</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.25‡</td>
<td>0.18†</td>
<td>0.22†</td>
</tr>
<tr>
<td>vWF</td>
<td>0.28§</td>
<td>0.16†</td>
<td>0.26‡</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.24‡</td>
<td>0.10</td>
<td>0.20†</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.12*</td>
<td>−0.12*</td>
<td>−0.11</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>−0.10</td>
<td>−0.08</td>
<td>−0.04</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.03</td>
<td>−0.13</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*0.01 < P < 0.05, †0.001 < P < 0.01, and ‡P < 0.001.

### Table 3. Levels of β-TG and PF4 by Stroke Subtype

<table>
<thead>
<tr>
<th></th>
<th>ICH (n = 29)</th>
<th>Small Vessel (n = 96)</th>
<th>Large Vessel (n = 163)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial β-TG, ng/mL</td>
<td>42.9 (35.0–52.5)</td>
<td>44.4 (39.9–49.4)</td>
<td>51.2 (47.0–55.7)*</td>
</tr>
<tr>
<td>Follow-up β-TG, ng/mL</td>
<td>37.8 (32.4–44.0)</td>
<td>41.8 (37.3–46.9)</td>
<td>44.7 (40.5–49.3)*</td>
</tr>
<tr>
<td>Initial PF4, ng/mL</td>
<td>6.7 (5.1–8.9)</td>
<td>7.3 (6.2–8.5)</td>
<td>8.2 (7.2–9.2)*</td>
</tr>
<tr>
<td>Follow-up PF4, ng/mL</td>
<td>7.7 (7.0–8.4)</td>
<td>6.3 (5.2–7.5)</td>
<td>6.1 (5.2–7.2)</td>
</tr>
</tbody>
</table>

Values are mean or geometric mean and 95% CI.

*P < 0.05 for patients with large-vessel infarction compared with controls.
distribution in subjects who died compared with those still alive (data not shown).

**Association of PlA Genotype With Cerebral Infarction and Subsequent Mortality in All Patients**
Because PlA has been associated with thrombosis, we restricted the analysis of PlA to those patients with CT-confirmed ICI (n=505). The PlA genotype distributions in patients and controls were in Hardy-Weinberg equilibrium. In the patients with ICI as a whole, there was no difference in the genotype distributions between patients and controls, as shown in Table 5. There was no difference in levels of β-TG or PF4 by PlA genotype; there was no difference in genotype distributions by aspirin or warfarin therapy prior to the acute event, nor was there any association of PlA with other risk factors presented in Table 1. There was, however, a significant difference in the genotype distributions of smokers and nonsmokers. As with the group as a whole, there was no association of PlA with stroke in smokers (data not shown). In the young subjects, there was a significant difference in the genotype distributions of patients and controls, as shown in Table 6. Significantly more of these patients had diabetes, were hypertensive, and smoked compared with controls. In these young subjects, there was a significant difference in the genotype distributions of patients (A1/A1=19, A1/A2+A2/A2=18) and controls (A1/A1=54, A1/A2+A2/A2=20, P=0.02), as shown in Table 6. There was no evidence for an interaction of PlA genotype with any other risk factors for stroke in these subjects (data not shown). In a logistic regression model including PlA, smoking, hypertension, and diabetes, the odds ratio (95% confidence interval) for stroke in those possessing the A2 allele was 1.68 (1.00 to 2.82, P=0.05). Hypertension and smoking were also independent stroke predictors in these subjects (data not shown).

## Discussion

**Markers of Platelet Activation in Relation to Stroke, Stroke Subtype, and Poststroke Mortality**
Although a number of studies have investigated the association of platelet activation with MI and stroke, the majority have been relatively small. Elevated levels of β-TG and PF4 have been found in subjects with thromboembolic and cardioembolic stroke compared with those with small-vessel infarction and healthy control subjects; similarly Konstantopoulos et al found increased shear-induced platelet aggregation and a persistent increase in the percent of circulating activated platelets in subjects with large-vessel infarction compared with those with small-vessel infarction or control subjects. Increased ADP-induced platelet aggregation and increased spontaneous platelet aggregation have also been found in subjects with transient ischemic attack or reversible ischemic neurologic deficit and cerebral infarction compared with healthy controls. In the Caerphilly Collaborative Heart Disease Study, hypersensitivity to ADP was found in subjects with a past history of MI or ECG evidence of ischemic heart disease.

**β-TG and PF4 Levels in Relation to Acute Stroke and Poststroke Mortality**
We found considerable variation in the performance of the PF4 ELISA compared with that for β-TG, which is in keeping with other studies. As a result of this variation, we found

### Table 4. VNTR Genotype Distributions in Controls and in Patients As a Whole and by Pathological Stroke Type

<table>
<thead>
<tr>
<th></th>
<th>Patients (All)</th>
<th>Patients (ICH)</th>
<th>Patients (IC)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>3 (&lt;0.01)</td>
<td>...</td>
<td>3 (&lt;0.01)</td>
<td>...</td>
</tr>
<tr>
<td>BC</td>
<td>20 (0.03)</td>
<td>3 (0.05)</td>
<td>16 (0.03)</td>
<td>7 (0.02)</td>
</tr>
<tr>
<td>BD</td>
<td>1 (&lt;0.01)</td>
<td>...</td>
<td>1 (&lt;0.01)</td>
<td>...</td>
</tr>
<tr>
<td>CC</td>
<td>510 (0.87)</td>
<td>51 (0.89)</td>
<td>438 (0.87)</td>
<td>370 (0.88)</td>
</tr>
<tr>
<td>CD</td>
<td>44 (0.07)</td>
<td>2 (0.04)</td>
<td>39 (0.08)</td>
<td>35 (0.08)</td>
</tr>
<tr>
<td>DD</td>
<td>10 (0.02)</td>
<td>1 (0.02)</td>
<td>8 (0.02)</td>
<td>10 (0.02)</td>
</tr>
</tbody>
</table>

Values are numbers of individuals in each group and (percentage).

### Table 5. PlA Genotype Distributions of Patients With ICI and Controls As a Whole and by Smoking Status

<table>
<thead>
<tr>
<th></th>
<th>All Subjects</th>
<th>Nonsmokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
</tr>
<tr>
<td>A1/A1</td>
<td>353 (0.70)</td>
<td>296 (0.73)</td>
<td>146 (0.67)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>141 (0.28)</td>
<td>96 (0.24)</td>
<td>70 (0.32)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>11 (0.02)</td>
<td>10 (0.02)</td>
<td>2 (0.01)</td>
</tr>
</tbody>
</table>

Values are numbers of individuals in each group and (percentage).
TABLE 6. Characteristics of Patients With ICI Before the Age of 50 Years and Healthy Age- and Sex-Matched Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=37)</th>
<th>Controls (n=74)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>43.0 (33.0–47.0)</td>
<td>43.8 (35.7–47.0)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.7 (23.0–29.9)</td>
<td>25.7 (23.3–27.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.5 (4.6–6.6)</td>
<td>5.4 (4.8–6.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.5 (1.2–2.8)</td>
<td>1.4 (0.9–2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/L</td>
<td>266 (236–305)</td>
<td>277 (230–310)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>23:14</td>
<td>46:28</td>
<td>NS</td>
</tr>
<tr>
<td>P1a genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1/A1</td>
<td>19 (0.51)</td>
<td>54 (0.73)</td>
<td>P=0.02</td>
</tr>
<tr>
<td>A1/A2+A2/A2</td>
<td>18 (0.49)</td>
<td>20 (0.27)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>72</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>74</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>61</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median and (interquartile range). Groups were compared by Mann Whitney U test.

the β-TG levels to be more informative than those of PF4. Levels of β-TG were significantly higher in patients both at the time of acute stroke and after 3 months when compared with healthy control subjects. On analysis by stroke type, only subjects classified as suffering from large-vessel infarction had levels of β-TG significantly higher than those of control subjects. These results are in keeping with previous studies demonstrating increased levels of β-TG or hypersensitivity to ADP in subjects with large-vessel infarction compared with healthy control subjects but no difference in these parameters in subjects with small vessel infarction compared with controls. It has been suggested that platelet hypofunction may predispose to ICH. Our finding that there was no significant difference in the levels of β-TG or PF4 in these subjects compared with control subjects does not support this idea, although the number of subjects with ICH and β-TG determinations was small.

Whether the observed elevation in levels of β-TG and PF4 reflect platelet activation and α-granule release as a result of the acute ischemic event or whether they predated and played a causative role in the acute event is unclear. It has been reported that platelet hyperaggregability observed at the time of acute stroke normalizes within 6 weeks. In contrast to this result, we found a persistent elevation in the levels of β-TG after 3 months compared with those in control subjects; also, in patients with both initial and follow-up determinations of β-TG, there was no significant difference between these two visits, suggesting that increased levels predated the acute event. In support of a causative role for platelet activation in the pathogenesis of both atherosclerosis and thrombosis, Numano et al have demonstrated that incubation of endothelial cells with activated platelets resulted in endothelial cell disruption. In this way subjects with hypersensitive platelets may be predisposed to endothelial cell disruption leading to further platelet activation, release of mitogenic and chemotactic factors, progression of atherosclerosis, and subsequent thrombus formation.

We previously described elevated levels of fibrinogen and vWF in these subjects. In patients at both visits and in control subjects, β-TG levels were significantly correlated with both of these circulating hemostatic factors. vWF is the major ligand involved in the adhesion of platelets at sites of endothelial damage, especially at areas of atherosclerotic narrowing of vessels, and fibrinogen is involved in platelet aggregation. It is possible that elevated levels of both vWF and fibrinogen lead to an increase in platelet adhesion, activation, and aggregation, as indicated by elevated levels of circulating β-TG, to support a causative role for platelet activation in the pathogenesis of acute thrombotic stroke.

In a prospective study of healthy men, Thaulow et al found that increased sensitivity to ADP-induced platelet aggregation was associated with total and cardiovascular mortality during 13.5 years of follow-up. We found a strong association of levels of β-TG and PF4 with poststroke mortality. Initial levels of both β-TG and PF4 were significantly higher in subjects who died, and this association remained after adjustment for age, sex, stroke type, and other confounding factors, indicating that this association is not merely a reflection of age or the severity of stroke. Levels of β-TG at follow-up were also significantly higher in subjects who subsequently died compared with those still alive, suggesting that there is persistent platelet activation that is unlikely to be due to the acute event itself. These data suggest that in subjects with acute stroke, an ongoing state of heightened platelet activation exists, in part due to elevated levels of fibrinogen and vWF, which may result in poor resolution of the existing thrombus and also predispose to further fatal thrombotic events.

Platelet GP Polymorphisms in Relation to Stroke, Stroke Subtype, and Poststroke Mortality

Association of VNTR With Stroke and Poststroke Mortality

In keeping with other studies in white subjects, we found no individuals who possessed the larger A allele, despite its documented prevalence in Oriental subjects. There was no association of this polymorphism with stroke, subtypes of stroke, or poststroke mortality, and we found no evidence of an interaction with other conventional risk factors for thrombosis. Therefore, these data do not support the hypothesis that the postulated extension of the vWF binding site from the platelet surface leads to an increased risk of platelet activation and ultimately thrombus formation in relation to cerebrovascular disease in this European population.

Association of P1α With Acute Ischemic Stroke and Poststroke Mortality

Weiss et al reported the P1α polymorphism of GP IIIa to be associated with coronary thrombosis. We also found an association of P1α with MI, particularly in young subjects.
Others have not supported these findings; in particular, the prospective Physicians’ Health Study found no association of PI^A2 with MI, stroke, or venous thrombosis.\(^30\) In the present study, we found an association of PI^A2 with ICI in nonsmokers and subjects under the age of 50 years; possession of PI^A2 remained an independent predictor of stroke in logistic regression models including the classic risk factors in these subjects. There remained no association of PI^A with ICI in smokers, either in univariate or multivariate analyses. As expected, atrial fibrillation, hypertension, and diabetes were independent predictors of ICI in both smokers and nonsmokers, suggesting that apart from smoking status, these two groups are comparable in terms of classic risk factor profile. Smoking is a well-documented risk factor for vascular disease, and the present data suggest that in subjects who smoke, any potential influence of PI^A is masked by the detrimental effect of smoking. In keeping with the results of Weiss et al\(^31\) and our previous findings in young subjects with MI,\(^15\) we found a 49% incidence of the A2 allele in patients under the age of 50 years compared with 27% in age- and sex-matched control subjects. The reason for the lack of association of PI^A with stroke in the Physicians’ study\(^30\) is unclear. However, it has been previously noted\(^13\) that this group is not representative of the population as a whole. In keeping with the results of the Physicians’ study, we found no difference in the genotype distributions by prior treatment with aspirin; therefore, this is unlikely to account for the differences observed. Further large population-based studies, both case-control and prospective, are required to clarify these findings.

The mechanisms whereby this polymorphism leads to an increased risk of thrombosis remain unclear. We did not find any association of PI^A with levels of fibrinogen, vWF, PF4, or β-TG. This may indicate that this polymorphism plays a role in postactivation events, possibly leading to increased binding of fibrinogen. This hypothesis appears to be negated by the finding of Weiss et al\(^32\) of decreased fibrinogen binding to platelets. However, the system used to quantify the number of bound fibrinogen molecules involves the use of exogenously labeled fibrinogen in a washed platelet system.\(^33\) This leads to the possibility that PI^A platelets in this system have increased amounts of bound endogenous fibrinogen compared with PI^A1, which would then be reflected as a decreased binding of exogenous fibrinogen. Further in vitro studies are required to clarify this observation.

MI and atherothrombotic stroke are multifactorial diseases involving a complex interplay of environmental and genetic factors, many of which are common to both disorders.\(^34\) The importance of platelet activation is reinforced by studies demonstrating the efficacy of aspirin in the secondary prevention of stroke and MI and the use of antagonists to GP IIb/IIIa in the prevention of restenosis after coronary angioplasty.\(^35\) Data from the present study suggest that there are strong relationships between circulating levels of platelet activation markers (in particular β-TG) and the platelet receptor ligands fibrinogen and vWF. Activation of platelets and of platelet-fibrinogen and platelet-vWF binding appears to play a central role in the pathogenesis of acute stroke, and it is likely that the genetic and environmental factors affecting these relationships will become important therapeutic targets.

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