A2 Allele of GpIIIa Gene Is a Risk Factor for Stroke Caused by Large-Vessel Disease in Males

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Background and Purpose—Glycoprotein IIIa (GpIIIa) is a platelet membrane receptor for fibrinogen and von Willebrand factor. It plays a key role in platelet aggregation. Previous studies in stroke patients, without analysis based on specific subtypes of stroke cause, have not shown any link between GpIIIa A1/A2 polymorphism and stroke risk. We studied the significance of the GpIIIa gene A1/A2 polymorphism in stroke patients with different stroke causes.

Methods—We genotyped 92 patients with stroke caused by large-vessel disease (LVD stroke) and 184 matched controls; 103 patients with stroke caused by small-vessel disease (SVD stroke) and 206 controls; and 182 patients with cardioembolic stroke (CE stroke) and 182 controls (TOAST criteria). The GpIIIa A1/A2 polymorphism was analyzed by polymerase chain reaction followed by restriction enzyme digestion and electrophoresis.

Results—The genotype distribution of the GpIIIa gene in patients with LVD stroke (A1/A1, 63%; A1/A2, 34.8%; A2/A2, 2.2%) differed significantly from their controls (A1A1, 79.3%; A1/A2, 20.1%; A2/A2, 0.6%). The distribution of the GpIIIa A1/A2 polymorphism in patients with SVD stroke and CE stroke was similar to that of their controls. In contrast to females with LVD stroke, we found that males with LVD stroke presented with an overrepresentation of at least 1 A2 allele of the GpIIIa gene when compared with their controls (39.7% versus 23.0%; P=0.003). Conditional logistic regression analysis showed that possession of at least 1 A2 allele of the GpIIIa gene was an independent risk factor for LVD stroke in males (OR, 2.51; 95% CI, 1.21 to 5.20).

Conclusion—A2 allele of the GpIIIa gene is an independent risk factor for LVD stroke in males. (Stroke. 2004;35:1589-1593.)

Key Words: etiology ■ stroke ■ platelet glycoprotein GPIIb/IIIa complex ■ gene expression ■ polymorphism

Glycoprotein IIb/IIIa is a platelet receptor involved in a final step of platelet-mediated thrombus formation on the injured vessel wall. It binds fibrinogen and von Willebrand factor, causing platelet aggregation. The glycoprotein IIb/IIIa receptor consists of a 2-chain glycoprotein IIb subunit noncovalently associated with a single-chain glycoprotein IIIa subunit. The genes encoding glycoprotein IIb and IIIa are located on chromosome 17q21. The A1/A2 polymorphism of glycoprotein IIIa (GpIIIa) gene caused by a T-to-C nucleotide substitution at position 1565 that is associated with the occurrence of the amino acid Leu → Pro variant at residue 33 of the mature protein has been widely studied in cardiovascular diseases. These studies have shown that possession of an A2 allele increases the risk for myocardial infarction, coronary artery disease, and restenosis after stent placement. Most of the studies on stroke patients have yielded negative results, but they did not analyze different stroke causes.

Two studies on subgroups of stroke patients have demonstrated a relation between GpIIIa gene polymorphism and stroke risk. They revealed that possession of at least 1 A2 allele of GpIIIa gene was a risk factor for atherothrombotic stroke in young patients and a risk factor for symptomatic carotid stenosis. Based on data from studies of patients with cardiovascular diseases and studies of stroke patients with atherothrombotic stroke, we hypothesized that the GpIIIa gene polymorphism might increase the risk of stroke caused by large-vessel disease (LVD stroke) only. Unlike other causes of stroke, shares pathophysiological features with acute coronary syndromes. Both diseases result from the formation of a platelet-rich thrombus at the site of the ruptured atherosclerotic plaque.

We studied the significance of the GpIIIa gene polymorphism as a risk factor for stroke caused by LVD, small-vessel disease (SVD), or cardioembolism.
Subjects and Methods

The study population consisted of 632 unrelated patients with ischemic stroke diagnosed, established according to World Health Organization (WHO) criteria, prospectively recruited at the Stroke Unit, University Hospital, Krakow, Poland between 1998 and 2002. This unit serves as a stroke emergency center for 1 district of Krakow and as a referral center for South East Poland (10% to 15% of patients).

All stroke patients underwent cranial computed tomography. Magnetic resonance imaging was performed in 133 cases (21.0%), when indicated. Five hundred twenty (82.3%) patients underwent extracranial arterial ultrasonus examination and 431 (68.2%) patients had echocardiography performed. In patients younger than age 45 years (n=61), we performed transesophageal echocardiography and screening for hypercoagulable state. According to the clinical features and the results of diagnostic workup, the following causes of stroke were diagnosed based on TOAST criteria: LVD stroke, SVD stroke, cardioembolic (CE) stroke, and stroke of unknown or rare etiology. Only patients with established stroke etiology were included for further analysis.

We also studied unrelated control individuals, free of clinically detectable cerebrovascular disease and without any stroke history. They were recruited from consecutive spouses of the patients (30%), or from the community, to maintain the control to case ratio 2:1 for LVD and SVD strokes and control-to-case ratio 1:1 for CE stroke. Controls were randomly matched from the beginning of the study for age (±1 year) and sex with the patients. All stroke patients and controls were whites.

This study was performed according to the Helsinki Declaration with approval of the Ethical Committee of the Jagiellonian University. All individuals included in this study gave informed consent before their inclusion in the study.

Demographic data were collected from stroke patients and their controls. An individual was classified as having arterial hypertension if he/she met 1 of the following criteria: (1) the diagnosis of hypertension in the medical history; (2) antihypertensive treatment before entry into the study; or (3) systolic or diastolic blood pressure ≥140 mm Hg or ≥90 mm Hg, respectively, on at least 2 different occasions (the first 3 days of hospitalization were not considered for the stroke patients). The history of ischemic heart disease and myocardial infarction was established using medical history, examination of previous and current electrocardiograms, and laboratory data. The diagnosis of atrial fibrillation was established on electrocardiogram findings during a hospitalization or on data from medical documentation with electrocardiogram-documented atrial fibrillation. Diabetes mellitus was defined by WHO criteria. Family history of stroke was defined as the occurrence of stroke in first-degree relatives. Smoking habits were defined as current smokers of ≥1 cigarette per day, former smokers, or nonsmokers. For statistical analysis, “current” and “former” smokers were pooled together.

The history was taken from patients or their proxies. To determine patients’ ability to be interviewed, we asked questions about their name, date, and orientation. Irrespective of the information we were able to obtain from the patients, we also questioned all the proxies and studied medical documentation to assemble the most detailed medical history.

For the control subjects, data were collected according to a detailed clinical questionnaire, including information on vascular risk factors, current medication, and physical examination at the time of drawing the blood sample for DNA analysis. Laboratory personnel, blinded for sample identity, performed the genetic analyses. Uncuffed venous blood samples for extraction of DNA were drawn from each subject within 2 days of stroke onset. Leukocyte DNA was extracted using a commercially available kit (High Pure PCR template Preparation Kit; Boehringer Mannheim). The A1/A2 genotyping for the GpIIa polymorphism was established using polymerase chain reaction and restriction enzyme digestion, modified from Weiss et al.

Data on quantitative characteristics are expressed as means±SD. Data on qualitative characteristics are expressed as percent values or absolute numbers, as indicated. Comparisons between the groups were made with χ² test (nominal data) or Student t test (interval data). A value of P<0.05 was considered statistically significant.

Hardy–Weinberg equilibrium was tested by the χ² method. The sample size for LVD stroke was calculated to have a power of 80% to detect the difference between the frequencies of alleles at the 0.05 significance level. The expected proportion of carriers of at least 1 A2 allele of GpIIa among controls for LVD stroke was 23%.

The association of the GpIIa genotype with risk for LVD stroke was tested using conditional logistic regression analysis, considering potential confounding risk variables including age, sex, and other conventional risk factors. A backward elimination procedure was used for multivariable analysis. For multivariate risk predictors, the adjusted odds ratios are given with the 95% CIs.

The calculations were performed using the data analysis software program SPSS 10 for Windows.

Results

Stroke cause was established in 398 of 632 (63%) consecutive patients. SVD stroke was diagnosed in 108 patients, LVD stroke in 99 patients, and CE stroke in 191 patients. Three hundred seventy-seven of 398 (94.7%) patients agreed to participate in the study: 103 patients with SVD stroke, 92 with LVD stroke, and 182 patients with CE stroke. Demographic data and risk factor profiles of patients and their controls are presented in Table 1.

A1/A2 genotypes of the GpIIa gene, determined in 949 individuals, were in Hardy–Weinberg equilibrium for the total group and each group separately (P for each group >0.1).

GpIIa genotype distribution was similar in patients with SVD stroke when compared with their controls, and in CE stroke when compared with their controls. The only significant difference in the GpIIa genotype distribution was found between patients with LVD stroke and their controls. The possession of at least 1 A2 allele was found in 37% of patients with LVD compared with 20.7% of the controls (P=0.003) (Table 2).

Possession of at least 1 A2 allele of GpIIa gene was found significantly more often in males with LVD stroke than in their controls. No such difference was found between females with LVD stroke and their controls. Risk factor profiles of male and female patients with LVD stroke and their controls are presented in Table 3.

Conditional logistic regression analysis showed that the presence of at least 1 A2 allele of GpIIa gene, hypertension, and current or previous smoking history were independent risk factors for LVD stroke in males (Table 4).

Presence of at least 1 A2 allele of GpIIa gene was not an independent risk factor for LVD stroke in females (OR adjusted for all studied risk factors, 1.51; 95% CI, 0.42 to 5.49).

Discussion

This study shows that possession of at least 1 A2 allele of GpIIa gene is an independent risk factor for LVD stroke in males, but not for SVD stroke or CE stroke.

Analysis based on specific stroke cause is crucial in genetic studies. There is a growing body of evidence that stroke causes differ in the age of onset, male/female ratio, and risk factor profile. Jerrard-Dunne et al revealed that a family history of stroke was a risk factor for LVD and SVD strokes, but not for CE stroke. We used TOAST criteria to determine stroke etiology, because they are thought to be the best available clinical criteria to
separate different stroke etiologies, although they present an imperfect relationship to the underlying inherited disease mechanism.22

To prove that GpIIIa polymorphism is involved as a risk for LVD stroke and not for other stroke etiologies, we compared patients with LVD stroke, SVD stroke, and CE stroke with each group's matched controls. We did not use the same control group for different stroke causes, as did the author of a previously published article on this topic.23 The issue of precise matching of patients' populations with their controls is important particularly for the genes having possible deleterious, time-dependent impact on overall health and longevity in humans, such as the GpIIIa gene.24 Thus, even a few years of difference in age of controls may influence the frequency of the alleles in this group and may influence the final results when comparing patient populations with controls.

Several reports have established that LVD stroke affects mostly males, whereas the sex distribution in SVD stroke patients and CE stroke patients did not differ significantly.21 We confirmed this observation showing the male-to-female ratio of 2:1 in patients with LVD.

We did not find any correlation between the possession of at least 1 A2 allele of GpIIIa gene and the age of stroke onset. This correlation has been reported in patients with myocardial infarction26 and once in stroke patients.27 Carter et al, who studied 37 young patients with probable atherothrombotic stroke, showed that at least 1 A2 allele of GpIIIa gene was present twice as often as compared with 74 controls matched by age and sex.14 Studies of young stroke females yielded negative results;23,26 this may be related to the protective role of estrogens in female GpIIIa A2 carriers.27 In our study, the small number of patients with LVD stroke younger than age 50 years (n=8) does not allow us to draw conclusions concerning the relation between the age of stroke onset and GpIIIa polymorphism.

A number of potential biases may have influenced our final results. The most important is the fact that we were not able to establish stroke cause in 37% of consecutive stroke patients fulfilling the entry criteria. This value is similar to the percentage of patients with undetermined stroke causes reported by other authors.20 Nevertheless, this could have influenced the distribution of GpIIIa genotype within the studied groups. We were able to genotype 74% of patients with unknown or rare stroke etiology, and we found that the distribution of the GpIIIa genotype was similar to that of the whole control group (data not shown).

Another weakness of our study may have been the lack of carotid ultrasound examination in the control subjects. We are aware that up to 10% of asymptomatic individuals may have carotid stenosis.28 However, in light of data published by Streifler et al15 showing that the possession of at least 1 A2 allele of GpIIIa gene in asymptomatic patients with significant carotid stenosis was 20.3%, which is similar to our controls, we believe that the lack of this examination in controls does not influence the final results.

| TABLE 1. Demographics and Risk Factors Profile in Patients With Different Stroke Etiologies and Their Matched Controls |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Risk Factor     | SVD Stroke (n=103) | Controls for SVD Stroke (n=206) | P*  | LVD Stroke (n=92) | Controls for LVD Stroke (n=184) | P*  | CE Stroke (n=182) | Controls for CE Stroke (n=182) | P*  |
| Age, y (±SD)    | 62.5±11.2            | 63.2±11.5              | 0.57 | 66.4±12.4          | 65.9±11.3              | 0.75 | 69.0±13.6          | 69.1±13.4              | 0.97 |
| Male/female ratio | 56/47                | 112/94                 | 1.00 | 63/29              | 126/58                 | 1.00 | 82/100             | 82/100                 | 1.00 |
| Hypertension, n (%) | 74 (71.8)           | 108 (52.4)             | 0.0001 | 74 (80.4)         | 98 (53.3)             | <0.00001 | 153 (84.1)       | 101 (55.5)            | <0.00001 |
| Male/female ratio | 56/47                | 112/94                 | 1.00 | 63/29              | 126/58                 | 1.00 | 82/100             | 82/100                 | 1.00 |
| Male/female ratio | 56/47                | 112/94                 | 1.00 | 63/29              | 126/58                 | 1.00 | 82/100             | 82/100                 | 1.00 |
| Male/female ratio | 56/47                | 112/94                 | 1.00 | 63/29              | 126/58                 | 1.00 | 82/100             | 82/100                 | 1.00 |
| Smoking (current/previous), n (%) | 37 (35.9)           | 68 (33.0)              | 0.61 | 36 (39.1)         | 32 (17.4)              | 0.001 | 50 (27.5)          | 51 (28.0)              | 0.90 |
| *Between-group comparison between studied stroke subgroup and their controls with Student t test (age) and χ² test. |
| †Atrial fibrillation was not considered to be a risk factor for SVD stroke and LVD stroke. |

SVD indicates small-vessel disease; LVD, large-vessel disease; CE, cardioembolic.

| TABLE 2. GpIIIa Genotype Distribution in Patients With Different Stroke Etiologies and Their Matched Controls |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Genotype        | SVD Stroke (n=103) | SVD Controls (n=206)  | LVD Stroke (n=92) | LVD Controls (n=184)  | CE Stroke (n=182) | CE Controls (n=182)  |
| A1/A1, n (%)    | 71 (68.9)           | 151 (73.3)         | 58 (63)          | 146 (79.3)        | 142 (78.1)       | 131 (72)         |
| A1/A2, n (%)    | 29 (28.2)           | 53 (25.7)          | 32 (34.8)        | 37 (20.1)         | 35 (19.2)        | 50 (27.5)        |
| A2/A2, n (%)    | 3 (2.9)             | 2 (1.0)            | 2 (2.2)          | 1 (0.6)           | 5 (2.7)          | 1 (0.5)          |
| Statistical calculation based on the comparison of A1/A1 vs A1/A2 and A2/A2 genotypes. |
| *P=NS. |
| †P=0.003 (χ² test). |
Finally, the lack of association of the presence of A2 allele of GpIIa gene in female patients with LVD should be discussed. Women could be protected from the atherothrombotic effect of A2 allele by the action of estrogens. Data exist demonstrating that physiological concentrations of estrogens inhibit the A2-bearing platelets more than the A1-bearing platelets. However, taking into account that our study may lack the power to detect some, possibly minor, role of the A2 allele in females, it should be pointed out that this problem warrants further study.

Identification of a subgroup of stroke patients, i.e., male patients with LVD stroke, having an overrepresentation of A2 allele of GpIIa gene may have implications for planning their future treatment strategies. Studies show that there is a relationship between the effectiveness of aspirin treatment and GpIIa polymorphism. Further studies are needed to elucidate the significance of aspirin in acute treatment and secondary stroke prevention in A1 and A2 carriers with LVD stroke, separately.

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TABLE 3. Demographics and Risk Factor Profile in Males and Females With LVD Stroke and Their Matched Controls

<table>
<thead>
<tr>
<th></th>
<th>Males With LVD Stroke (n=63)</th>
<th>Male Controls for LVD Stroke (n=126)</th>
<th>P*</th>
<th>Females With LVD Stroke (n=29)</th>
<th>Female Controls for LVD Stroke (n=58)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (±SD)</td>
<td>65.7±11.2</td>
<td>65.0±10.8</td>
<td>0.99</td>
<td>67.9±12.2</td>
<td>67.9±12.1</td>
<td>0.99</td>
</tr>
<tr>
<td>A1/A2 or A2/A2 genotype, n (%)</td>
<td>25 (39.7)</td>
<td>29 (23.0)</td>
<td>0.017</td>
<td>9 (31.0)</td>
<td>9 (15.5)</td>
<td>0.097</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>48 (76.2)</td>
<td>68 (54.0)</td>
<td>0.003</td>
<td>27 (93.1)</td>
<td>30 (51.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>25 (39.7)</td>
<td>41 (32.5)</td>
<td>0.33</td>
<td>14 (48.3)</td>
<td>24 (41.4)</td>
<td>0.63</td>
</tr>
<tr>
<td>History of myocardial infarction, n (%)</td>
<td>3 (4.8)</td>
<td>16 (12.7)</td>
<td>0.1</td>
<td>0</td>
<td>2 (3.4)</td>
<td>0.43</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>10 (15.9)</td>
<td>25 (19.8)</td>
<td>0.51</td>
<td>8 (27.6)</td>
<td>4 (6.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Family history of stroke, n (%)</td>
<td>10 (15.9)</td>
<td>18 (14.3)</td>
<td>0.54</td>
<td>3 (10.3)</td>
<td>11 (19.0)</td>
<td>0.28</td>
</tr>
<tr>
<td>Smoking (current/previous), n (%)</td>
<td>30 (47.6)</td>
<td>28 (22.2)</td>
<td>0.0001</td>
<td>5 (17.2)</td>
<td>3 (5.2)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Between-group comparison between studied stroke subgroup and their controls with Student t test (age) and χ² test.

TABLE 4. Independent Predictors of LVD Stroke in Male Patients (Conditional Logistic Regression Model)

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.004</td>
<td>0.97–1.04</td>
<td>NS</td>
</tr>
<tr>
<td>A1/A2 or A2/A2 genotype</td>
<td>2.51</td>
<td>1.21–5.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.77</td>
<td>1.31–5.81</td>
<td>0.007</td>
</tr>
<tr>
<td>Smoking (current/previous)</td>
<td>3.81</td>
<td>1.88–7.73</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Results are presented as ORs (adjusted for age, the presence of at least 1 A2 allele of GpIIa gene, hypertension, smoking, history of myocardial infarction, ischemic heart disease, diabetes mellitus, and family history of stroke). OR indicates odds ratio; NS, nonsignificant.

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14. Carter AM, Catto AJ, Bamford J, Grant PJ. Platelet GP IIIa PIA and GP Ib variable number tandem repeat polymorphisms and markers of platelet


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