Genetic Variants of Platelet Glycoprotein Receptors and Risk of Stroke in Young Women

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Background and Purpose—A number of studies have examined the relationship between genetic platelet glycoprotein variants and early-onset atherothrombotic disease, particularly acute myocardial infarction. Data on the association of these genetic susceptibility markers with ischemic stroke are more limited, and their role in hemorrhagic stroke has not been previously examined.

Methods—We performed genotype analysis for 5 common diallelic platelet glycoprotein polymorphisms in a population-based study of 78 white women aged <45 years with arterial stroke (36 ischemic cases and 42 hemorrhagic cases) and 346 demographically similar control subjects.

Results—The 807T variant of glycoprotein Ia was associated with a 2-fold increased risk of ischemic stroke (age-adjusted odds ratio [OR] = 2.24; 95% CI = 0.99 to 5.06). The Met145 allele of glycoprotein Ibα was associated with a trend toward an increased risk of ischemic stroke that was more pronounced in the homozygous state (OR = 10.36), but the CI is extremely wide because of the small numbers of subjects (95% CI = 1.43 to 79.34). Homozygosity for the Ser843 allele of the glycoprotein IIb was associated with an ~5-fold increased risk of ischemic stroke among subgroups of women who carried a diagnosis of hypertension or diabetes (OR = 4.51; 95% CI = 1.01 to 20.13) or had elevated plasma homocysteine levels (OR = 5.94; 95% CI = 1.53 to 23.05). The genotype distributions for all 5 platelet glycoprotein polymorphisms were similar among hemorrhagic stroke cases and controls.

Conclusions—Several inherited platelet glycoprotein variants may be associated with an increased risk of ischemic stroke in young women. These associations seemed to be confined to women with other cardiovascular risk factors. Additional studies involving larger numbers of subjects are needed to confirm these preliminary findings. (Stroke. 2000;31:1628-1633.)

Key Words: platelets ■ polymorphism ■ stroke, hemorrhagic ■ stroke, ischemic

Platelet thrombosis is mediated by several platelet membrane receptor complexes, including glycoprotein Ib/IX, glycoprotein Ia/IIa, and glycoprotein IIb/IIIa (Table 1). Several genetic platelet glycoprotein variants have been associated with an increased risk of early-onset atherothrombotic disease, particularly acute myocardial infarction. The potential contributions of these genetic platelet glycoprotein variants to the occurrence of ischemic stroke have been examined in a smaller number of studies. The Pro39 variant of platelet glycoprotein IIIa and the Met145/VNTR-B allele of glycoprotein Ibb have been associated with ischemic stroke in some studies, but not in others. Two other common platelet glycoprotein dimorphisms, glycoprotein IIb IIb/Ser403 and glycoprotein Ia Gln/Lys305, were not associated with ischemic stroke in a single study, but the Ile403 variant of glycoprotein IIb was recently associated with increased mortality following ischemic stroke in an elderly patient population. A nucleotide 807T variant of glycoprotein Ia that correlates with increased platelet surface levels of glycoprotein Ia/IIa (the platelet collagen receptor) was recently associated with an increased risk of ischemic stroke with onset at a young age. The inconsistent results of studies examining the risk of stroke associated with genetic platelet glycoprotein variants may be partly due to differences in demographic characteristics between study populations. For example, genetic prothrombotic factors may be more important in younger individuals or premenopausal women who are less likely to have advanced atherosclerotic disease. Furthermore, the effect of single genetic factors on the risk of stroke may be weak when analyzed individually but may be more pronounced in the presence of other common genetic or envi-
TABLE 1. Platelet Glycoprotein Receptor Polymorphisms Implicated in Atherothrombotic Disease

<table>
<thead>
<tr>
<th>Platelet Receptor</th>
<th>Major Ligand(s)</th>
<th>Function</th>
<th>Polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein Ib/IX</td>
<td>vWF</td>
<td>Initial platelet adhesion</td>
<td>Glycoprotein Ib Thr/Met&lt;sup&gt;145&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycoprotein Ib VNTR</td>
</tr>
<tr>
<td>Glycoprotein Ia/IIa</td>
<td>Collagen</td>
<td>Stable platelet adhesion</td>
<td>Glycoprotein Ia C807T</td>
</tr>
<tr>
<td>Glycoprotein IIb/IIIa</td>
<td>Fibrinogen, vWF</td>
<td>Stable platelet adhesion; platelet cohesion</td>
<td>Glycoprotein IIb Ile/Ser&lt;sup&gt;43&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycoprotein Ila Leu/Pro&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

vWF indicates von Willebrand factor; VNTR, variable number of tandem repeats.

In a previous analysis of the overall study sample, we determined that there was a significantly greater proportion of black women among the hemorrhagic stroke cases (11.8%) and ischemic stroke cases (11.7%) than among the control subjects (2.5%). Since the distribution of platelet glycoprotein alleles differs according to race, white and black subjects must be analyzed separately. However, because of the inadequate number of black women in the control group, we restricted the comparison of platelet glycoprotein genotypes to the subset of study subjects with DNA samples who were white (36 ischemic stroke cases, 42 hemorrhagic stroke cases, and 346 controls).

Platelet glycoprotein allele frequencies were calculated by gene counting. The association of platelet glycoprotein genotypes with stroke was examined by unconditional logistic regression adjusted for age and was expressed as odds ratios (ORs) and 95% CIs. Unless otherwise noted, ORs for the 5 biallelic platelet glycoprotein polymorphisms were calculated comparing individuals who were either heterozygous or homozygous for the less common allele with individuals who were homozygous for the more common allele. We also assessed the extent to which associations with platelet glycoprotein polymorphisms were modified by other cardiovascular risk factors, including smoking, obesity, hypertension, diabetes, and hyperhomocysteinemia, through analyses stratified on these cardiovascular risk factors.

**Subjects and Methods**

This analysis is based on data and genetic material collected in a population-based case-control study of incident nonfatal stroke in young women. 14,15 The study population consisted of women aged 18 to 44 years residing in 3 contiguous counties in western Washington State between July 1, 1991, and February 28, 1995. All subjects gave informed consent according to a protocol approved by the University of Washington and participating local hospitals. Stroke was defined as evidence of new focal neurological deficit(s) lasting >24 hours. On the basis of review of hospital records, brain imaging studies, and lumbar puncture results, strokes were further classified by the study neurologist (W.T.L.) as ischemic, hemorrhagic, venous, or “other” (including arterial dissections). Random-digit telephone dialing was used to identify female control subjects, who were frequency matched to the age distribution of the cases.

One hundred forty-nine of 198 eligible patients with nonfatal stroke and 525 of 684 eligible control subjects were willing to participate in an in-person interview. The interview involved ascertainment of demographic characteristics and histories of traditional cardiovascular risk factors, including hypertension, diabetes, cigarette smoking, hypercholesterolemia, height, weight, contraceptive practices, menstrual status, and frequency of vigorous exercise. All interview questions elicited information about the time period before each subject’s reference date, which was the date of stroke for cases and a date assigned at random from among the potential stroke occurrence dates for controls. A woman was classified as hypertensive, diabetic, or hypercholesterolemic if she reported ever receiving the diagnosis by a physician. Smokers were defined as subjects who reported smoking both currently (within a month of the reference date) and regularly (≥5 cigarettes per week for ≥6 consecutive months). Obesity was defined as a body mass index ≥27.3 kg/m². Physical inactivity was defined as exercising vigorously <1 time per week. Hyperhomocysteinemia was defined as a serum homocysteine level >12.6 μmol/L.

At the time of interview, venous blood samples were collected into EDTA-treated evacuated tubes from 106 participating stroke cases (54 hemorrhagic, 41 ischemic, 2 venous, and 9 “other”) and from 391 participating control subjects. Plasma and genomic DNA samples were prepared as described previously. 14,15 Genotyping for 5 platelet glycoprotein polymorphisms (glycoprotein Ila Leu/Pro<sup>33</sup>, glycoprotein IIb Ile/Ser<sup>43</sup>, glycoprotein Ib Thr/Met<sup>145</sup>, glycoprotein Ia Glu/Lys<sup>149</sup>, and glycoprotein Ia C807T) was performed by polymerase chain reaction amplification of genomic DNA followed by restriction enzyme digestion (polymerase chain reaction–restriction fragment length polymorphism), according to previously published methods. 16–18

Environmental cardiovascular risk factors (eg, hypertension, smoking, obesity). We therefore assessed the association of 5 common platelet glycoprotein polymorphisms with stroke in women aged <45 years and examined potential interactions between these genetic variants and other traditional cardiovascular risk factors.

**Results**

A comparison of the characteristics of stroke cases and control subjects among the subset of white women with DNA samples is presented in Table 2. The frequencies of various cardiovascular risk factors among ischemic stroke cases, hemorrhagic stroke cases, and control subjects were similar to those previously reported in an analysis based on a larger sample size from this study population. 14 Current cigarette smoking, hypertension, and physical inactivity were more common in both ischemic stroke cases and hemorrhagic stroke cases than control subjects. Higher plasma levels of total homocysteine were also noted in both the ischemic and hemorrhagic stroke patients compared with control subjects. In contrast, higher frequencies of obesity and diabetes were confined to the ischemic stroke cases.

The genotype distributions of the 5 platelet glycoprotein polymorphisms among the ischemic stroke cases and control subjects are compared in Table 3. A trend toward an association with risk of ischemic stroke was observed for the glycoprotein Ia C807T, glycoprotein Ib Thr/Met<sup>145</sup>, and glycoprotein IIb Ile/Ser<sup>43</sup> polymorphisms, but none of these reached the level of statistical significance in the overall study sample. The age-adjusted OR for women carrying ≥1 copy of the 807T allele was 2.24, and the lower confidence limit was just <1.0 (95% CI=0.99 to 5.06). When analyzed by subgroups defined according to the presence or absence of other cardiovascular risk factors, the risk of ischemic stroke...
associated with the 807T allele was highest in the subgroup of women (16 cases and 91 controls) who were obese (OR = 5.324; 95% CI = 0.86 to 12.18) and in the subgroup of women (13 cases and 86 controls) who had elevated plasma homocysteine levels (OR = 4.03; 95% CI = 0.83 to 19.67).

The presence of 1 copy of the Met145 allele of glycoprotein Iba was associated with a more modestly increased risk of ischemic stroke (OR = 1.48; 95% CI = 0.64 to 3.41). The risk associated with the Met145 allele of glycoprotein Iba was highest in the subgroup of women (15 cases and 40 controls) who carried a diagnosis of either hypertension or diabetes (OR = 3.86; 95% CI = 0.98 to 15.17). In the overall study population, the increased risk associated with the Met145 variant was primarily due to an overrepresentation of the Met/Met145 genotype among the ischemic stroke cases (5.6%) compared with the controls (0.6%). This results in a large point estimate for the risk of ischemic stroke associated with Met145 homozygosity (age-adjusted OR = 10.63), but the CI is extremely wide (95% CI = 1.43 to 79.34) because of the relatively low frequency of this genotype.

Homozygosity for the Ser843 allele of glycoprotein IIb was also more prevalent in ischemic stroke cases (22.2%) than controls (14.4%) (OR = 1.69; 95% CI = 0.73 to 3.92). The risk of ischemic stroke associated with carrying 2 copies of the Ser843 allele was particularly high in the subgroup of women with hypertension or diabetes (OR = 4.51; 95% CI = 1.01 to 20.13) and in the subgroup of women with elevated plasma homocysteine levels (OR = 5.94; 95% CI = 1.53 to 23.05). By

### TABLE 2. Characteristics of Stroke Patients and Control Subjects

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allele Frequency</th>
<th>Genotype Frequency</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein IIb</td>
<td>Ile843 0.569 0.630</td>
<td>Ile843/Ile843 13 (36.1) 140 (40.5)</td>
<td>1.20 (0.59–2.45)</td>
</tr>
<tr>
<td>Glycoprotein Ila</td>
<td>Leu33 0.833 0.838</td>
<td>Leu33/Leu33 25 (69.4) 241 (69.7)</td>
<td>1.01 (0.48–2.13)</td>
</tr>
<tr>
<td>Glycoprotein Ia</td>
<td>Glu505 0.917 0.909</td>
<td>Glu505/Glu505 30 (83.3) 286 (82.7)</td>
<td>0.96 (0.38–2.41)</td>
</tr>
<tr>
<td>Glycoprotein Ib</td>
<td>C807T 0.514 0.627</td>
<td>C807T/C807T 8 (22.2) 135 (39.0)</td>
<td>2.24 (0.99–5.06)</td>
</tr>
<tr>
<td>Glycoprotein Iba</td>
<td>Thr145 0.861 0.916</td>
<td>Thr145/Thr145 28 (77.8) 290 (83.8)</td>
<td>1.48 (0.64–3.41)</td>
</tr>
</tbody>
</table>

*ORs are adjusted for age and calculated comparing carriers of 1 or 2 copies of the less common allele (eg, carriers of glycoprotein IIb Ser843) to individuals who are homozygous for the more common allele (eg, carriers of glycoprotein IIb Ile843/Ile843 genotype).
contrast, the risk associated with homozygosity for the glycoprotein Iib Ser43 allele was not increased in women without hypertension or diabetes (OR = 0.94; 95% CI = 0.27 to 3.33) or in women with normal plasma homocysteine levels (OR = 0.83; 95% CI = 0.23 to 2.94).

The genotype distributions of the 5 platelet glycoprotein polymorphisms in hemorrhagic stroke cases and control subjects are compared in Table 4. The genotype distributions were similar between hemorrhagic stroke cases and controls, either overall or in the presence or absence of other cardiovascular risk factors (data not shown).

### Discussion

Our results suggest possible associations between 3 platelet glycoprotein polymorphisms, glycoprotein Ia C807T, glycoprotein Iib Ile/Ser43, and glycoprotein Ibα Thr/Met415, and risk of ischemic stroke in young women. Although the observed associations were modest, and the study was limited by small numbers of stroke cases, 2 features of the study suggest that these preliminary results may represent authentic associations. First, there was evidence that the risk of ischemic stroke associated with these genetic platelet glycoprotein variants was highest in subgroups of women with other known cardiovascular risk factors. These results are consistent with previous studies that indicate the importance of genetic prothrombotic factors in combination with other cardiovascular risk factors in the occurrence of atherothrombotic diseases such as myocardial infarction in young adults. Second, we did not observe an association between any of the platelet glycoprotein polymorphisms and risk of hemorrhagic stroke, a disorder that does not involve platelet-dependent thromboembolism.

Glycoprotein Ia/Iiα is the major platelet collagen receptor and is responsible for platelet adherence to exposed vascular subendothelium. The 807T allele of glycoprotein Ia was recently associated with a statistically significant 3-fold increased risk of stroke in men and women aged <50 years but was not associated with stroke in patients aged ≥50 years. Our results in young women indicate an ~2-fold increased risk of ischemic stroke associated with the 807T variant that was highest in women who were either obese or had elevated plasma homocysteine levels. An increased risk of myocardial infarction associated with the glycoprotein Ia 807T allele similarly has been reported in young obese men.

The platelet glycoprotein Iib/IX complex is the major platelet receptor for von Willebrand factor and is responsible for initial platelet adhesion and activation following exposure to vascular subendothelium under high shear rates. Gonzalez-Conejero et al and Sonoda et al each reported a 2- to 3-fold increased risk of ischemic stroke or transient ischemic attack in men and women who possessed ≥1 copy of the Met415 allele. Our results indicate a trend toward an increased risk of ischemic stroke associated with the presence of ≥1 copy of the Met415 allele in young women, particularly in the subgroup of women who were hypertensive or diabetic. Moreover, women who possessed 2 copies of the glycoprotein Iba Met415 allele had an ~10-fold increased risk of ischemic stroke, although this estimate of effect was statistically unstable because of the small sample size and relatively low frequency of the Met415 variant.

The bridging of adjacent platelets through the glycoprotein IIIb/IIa receptor is important for thrombus formation, and glycoprotein IIIb/IIa inhibitors are used increasingly in the treatment of atherothrombotic disease. Carter et al reported an association between the Pro13 variant of platelet glycopro-

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### Table 4. Platelet Glycoprotein Allele Frequencies and Genotypes in Hemorrhagic Stroke Cases and Controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allele</th>
<th>Cases</th>
<th>Controls</th>
<th>Genotype</th>
<th>No. of Cases (%)</th>
<th>No. of Controls (%)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein Iib</td>
<td>Ile43</td>
<td>0.631</td>
<td>0.630</td>
<td>Ile43/Ile43</td>
<td>16 (38.1)</td>
<td>140 (40.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ser43</td>
<td>0.369</td>
<td>0.370</td>
<td>Ser43/Ser43</td>
<td>21 (50.0)</td>
<td>156 (45.1)</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein Illa</td>
<td>Leu33</td>
<td>0.833</td>
<td>0.838</td>
<td>Leu33/Leu33</td>
<td>28 (66.7)</td>
<td>241 (69.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu/Pro33</td>
<td>0.167</td>
<td>0.162</td>
<td>Leu33/Pro33</td>
<td>14 (33.3)</td>
<td>98 (28.3)</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein Ia</td>
<td>Glu505</td>
<td>0.905</td>
<td>0.909</td>
<td>Glu505/Glu505</td>
<td>34 (81.0)</td>
<td>286 (82.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lys505</td>
<td>0.095</td>
<td>0.091</td>
<td>Lys505/Lys505</td>
<td>8 (19.0)</td>
<td>57 (16.4)</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein Ia</td>
<td>C807T</td>
<td>0.619</td>
<td>0.627</td>
<td>C807T/C807T</td>
<td>15 (35.7)</td>
<td>135 (39.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>807T</td>
<td>0.381</td>
<td>0.373</td>
<td>807T/807T</td>
<td>22 (52.4)</td>
<td>164 (47.4)</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein Iba</td>
<td>Thr415</td>
<td>0.905</td>
<td>0.916</td>
<td>Thr415/Thr415</td>
<td>34 (81.0)</td>
<td>290 (83.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Met415</td>
<td>0.095</td>
<td>0.084</td>
<td>Met415/Met415</td>
<td>8 (19.1)</td>
<td>54 (15.6)</td>
<td></td>
</tr>
</tbody>
</table>

*ORs are adjusted for age and calculated comparing carriers of 1 or 2 copies of the less common allele (eg, carriers of glycoprotein Iib Ser43) to individuals who are homozygous for the more common allele (eg, carriers of glycoprotein Iib Ile43/Ile43 genotype).
tein IIIa and risk of atherothrombotic stroke only in the subgroup of patients aged <50 years. Wagner et al\(^9\) reported a trend toward an association between the Pro\(^3\) variant of platelet glycoprotein IIIa and cerebral infarction in women aged <45 years when the analysis was restricted to whites. In contrast, we found no association between the glycoprotein IIIa Pro\(^3\) variant and risk of ischemic stroke in our population of young white women. Our negative findings are consistent with the results of 2 other case-control studies,\(^6,7\) as well as a prospective study of male physicians.\(^8\)

The platelet glycoprotein Ib/IIa receptor contains another prevalent polymorphism, glycoprotein Ib Ile/Ser\(^{843}\). Two studies have not observed an association between the Ile/Ser\(^{843}\) polymorphism of glycoprotein Ib and risk of cerebrovascular disease in older patient populations.\(^7,9\) Our results suggest a trend toward an association with increased risk of ischemic stroke in young women who are homozygous for the Ser\(^{843}\) variant. The risk associated with the glycoprotein Ib Ser/Ser\(^{843}\) genotype was most pronounced in women with hypertension or diabetes (OR=4.51; 95% CI=1.01 to 20.13) and in women with elevated plasma homocysteine levels (OR=5.94; 95% CI=1.53 to 23.05). Although the CIs were wide around these point estimates, they did not overlap 1.0. A recent preliminary report indicated that platelets containing the glycoprotein Ib Ser\(^{843}\) allele demonstrate increased in vitro platelet aggregation and clot retraction compared with those lacking the Ser\(^{843}\) allele\(^10\) and thus provides a possible explanation for the association between the Ser\(^{843}\) variant and risk of atherothrombosis.

Our findings are in contrast with another recent study that noted an association between the more common Ile\(^{843}\) variant of glycoprotein Ib and increased risk of poststroke mortality in an older patient population.\(^9\) Since our study analyzed only women who survived their acute event, it is possible that the women who died acutely had an overrepresentation of the Ile\(^{843}\) variant. Given the possible increased in vitro thrombogenicity of the Ser\(^{843}\) variant,\(^23\) one may speculate that the less adhesive Ile\(^{843}\) allele is associated with a decreased tendency toward embolization of a preexisting platelet thrombus; this might account for increased mortality following the acute atherothrombotic event.\(^9\)

Several limitations of our study should be noted. First, the relatively small number of cases and the performance of multiple subgroup analyses may increase the likelihood of a spurious association (type I statistical error). In this regard, some polymorphisms that have been associated with stroke or myocardial infarction in smaller studies have not been verified in larger, prospective studies.\(^1,8\) Second, the small number of cases decreases the likelihood of detecting weak associations (type II error). Third, blood samples for DNA analysis were collected at the time of subject interview; thus, as noted above, our study includes only women who survived an acute stroke. Therefore, if a particular platelet glycoprotein allele was associated with increased morbidity or early mortality, the effect could be underestimated because of exclusion of women who died acutely or were severely disabled. Fourth, our results apply to white, premenopausal women with acute stroke and may not be generalized to other demographic and ethnic groups.

Thus, confirmation of the role of genetic platelet glycoprotein variants as risk factors for early-onset ischemic stroke requires further study involving larger numbers of subjects and other populations.

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References
15. Longstreth WT Jr, Rosendaal FR, Siscovick DS, Vos HL, Schwartz SM, Psaty BM, Ragnhunathan TE, Koepsell TD, Reitsma PH. Risk of stroke in...


