Platelet GP IIIa Polymorphism HPA-1 (PIA) Protects Against Subarachnoid Hemorrhage

Juan A. Iniesta, PhD; Rocío González-Conejero, PhD; Claudio Piqueras, MD; Vicente Vicente, PhD; Javier Corral, PhD

**Background and Purpose**—Few genetic modifications have been identified to be associated with subarachnoid hemorrhage (SAH), most of them playing a role in the formation or size of aneurysms.

**Methods**—We evaluated the role of common and functional polymorphisms affecting the main platelet adhesive glycoproteins (GP) (GPIIIa: HPA-1; GPIa: HPA-5 and C807T; GPIbα: HPA-2 and VNTR) in the risk of development of the disease and in the severity of the onset. The study was performed in 103 patients with SAH, 103 matched controls, and 473 subjects from the general population.

**Results**—The HPA-1b (PIA2) allele significantly protected against SAH (OR, 0.48; 95% CI, 0.24 to 0.96; *P* = 0.037). Interestingly, patients carrying this allele displayed larger aneurysms, but the extension of their hemorrhage and the clinical grade at presentation was significantly lower when compared with patients HPA-1 a/a (11.9 ± 2.8 mm versus 8.8 ± 2.2 mm, *P* = 0.0001; Fisher grade ≤ 2: 68.4% versus 20%; *P* = 0.0001; Hunt and Hess score < IV: 84.2% versus 53.8%; *P* = 0.0187, respectively). The protection of the HPA-1b allele seemed to be exacerbated by the simultaneous presence of the HPA-2b allele. Thus, no patient carried this combination, which was present in 7.8% of controls (*P* = 0.007).

**Conclusions**—We present the first evidence suggesting a protective role for the platelet GPIIIa HPA-1b allele in SAH. The suggested platelet hyper-reactivity determined by this allele could reduce the risk to suffer SAH, specially if the aneurysm is small, attenuate the severity of the hemorrhage, and reduce the clinical grade at presentation. This effect might be amplified by the simultaneous combination with the GPIbα HPA-2b allele. *(Stroke. 2004;35:2282-2286.)*

**Key Words:** aneurysm ■ genetics ■ hemostasis ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) accounts for 10% of cerebrovascular disease and has an annual incidence of 10 to 15 per 100,000. SAH continues to have a substantial impact on mortality and has long-lasting effects on the functional status and quality of life of patients who survive. The high frequency of SAH with family history suggests that a genetic component might be involved in this disease. Previous reports have shown contradictory results about the role of polymorphisms affecting the α1-antitrypsin and matrix metalloproteinases in the development of intracranial aneurysms. The endothelial nitric oxide synthase T-786C single nucleotide polymorphism has been suggested to play a significant role in the size of ruptured aneurysm, although it seems to have a minor effect in the risk for SAH.

Platelets play a key role in generating the protective hemostatic plug that prevents blood loss at sites of vascular injury. Therefore, modifications of platelet reactivity might favor thrombotic or hemorrhagic disorders. The identification of functional prothrombotic polymorphisms during the past decade encouraged the study of common polymorphisms affecting platelet glycoproteins (GP) in arterial thrombotic disorders. The most frequent platelet polymorphisms evaluated in these studies are those affecting the structure or levels of adhesive receptors. First, GPIIIa HPA-1 (from human platelet alloantigen), also named PIA, is responsible for a structural change (Leu33Pro) in this key platelet protein. From the report of Weiss et al in 1996 suggesting a prothrombotic role in myocardial infarction, this is the platelet polymorphism more studied in arterial thrombosis. Second, GPIa C807T and HPA-5 (Glu505Lys) polymorphisms associate with the levels of the high-affinity collagen receptor. Third, GPIb HPA-2 (Thr145Met) are genetically linked to a VNTR (variable number of tandem repeats) polymorphism. These polymorphisms cause a significant structural change in the Ibα subunit, affecting the binding of von Willebrand factor.

The role of these polymorphisms in arterial thrombosis is conflicting, but all have been suggested to be prothrombotic. We recently observed that prothrombotic polymorphisms such as the factor V Leiden and prothrombin G20210A might protect against cerebral hemorrhage, supporting that prothrombotic polymorphisms could protect...
against hemorrhage. However, only 2 reports analyzed the effect of platelet GP polymorphisms in small groups of patients with intracranial hemorrhage, finding no significant effect.12,13

The aim of this study was to evaluate the role of common functional polymorphisms affecting relevant platelet GP that have been involved in arterial thrombosis in the development and severity of SAH.

Subjects and Methods
A prospective study was performed on all patients admitted to the Arrixaca Hospital and University General Hospital (Murcia, Spain) with aneurysmal and spontaneous nonaneurysmal SAH during a 3-year period. The study was approved by the hospital institutional review board and conducted in accordance with the Helsinki Declaration. Blood samples were obtained via venipuncture for other routine blood tests. Informed consent from patients or their next of kin was obtained before blood samples were taken. Patients are eligible if they are older than age 18 years. The diagnosis of SAH was established by the admission computed tomography (CT) scan or by xanthochromia of the cerebrospinal fluid if the CT was not diagnostic.

The selection of patients and controls was designed to avoid interference of other factors and to determine the role of common polymorphisms that predisposed to or protected against SAH. First, patients at high risk for SAH (patients with SAH caused by trauma, brain tumor, arteriovenous malformation rupture, vasculitis, and other structural lesions, as well as patients in antithrombotic treatment) were excluded. One hundred three patients were finally recruited in our study. All patients underwent standard hematologic screening tests (platelet count, activated partial thromboplastin time and prothrombin time). We recorded baseline demographic data (age, sex, and ethnicity). Moreover, the presence of selected risk factors for SAH (hypertension, smoking history, and alcohol consumption) and the personal history of arterial ischemic events were obtained from clinical data and direct interview of patients. Confounding risk factors of those patients unable to give a meaningful clinical history were obtained from family members. The frequency of deaths within 30 days of the first episode was also recorded (Table 1). The presence and size of the aneurysm was determined on the basis of radiological findings (including cerebral angiography in each case). Severity of SAH on admission CT scan was classified according to the grading system of Fisher (Fisher grades 1 to 4).14 Neurological status on admission was assessed with the Hunt and Hess Scale.15

Second, to avoid overrepresentation of classic hemorrhagic risk factors among patients, their respective controls were selected to match for age, race, sex, and selected risk factors for SAH. Thus, we selected 103 matched controls during the same period of time by reviewing the charts of traumatology and ophthalmology (Table 1). Controls had neither documented history of vascular disease nor personal history of thromboembolic or hemorrhagic disease and were not undergoing antithrombotic therapy.

Finally, the frequency of these polymorphisms in the general population from our region was analyzed in 473 additional healthy unrelated white subjects, mainly blood donors (Table 1).

DNA Studies
Genomic DNA was extracted from whole blood (Wizard genomic DNA purification system; Promega). Genotyping of the studied polymorphisms was performed as described elsewhere.7,8,16

Statistical Analysis
Results are expressed as mean±SD for continuous variables and as percentages for categorical variables. Comparisons between 2 groups were performed by the unpaired t test. Categorical data were compared using the χ² test, and a Fisher exact test was performed. The differences with a 2-tailed P<0.05 were considered significant.

| TABLE 1. Clinical Features, Prevalence of Selected Risk Factors, Coagulation Parameters, and Platelet Counts in Patients, Controls, and General Population |
|---------------------------------|-----------------|----------------|---------|
|                                 | SAH (n=103)     | Control (n=103) | GP (n=473) |
| Age (y), mean±SD                | 59±13           | 60±12           | 0.464    |
| Male, %                         | 38.8            | 38.8            | 1        |
| Hypertension, %                 | 44.7            | 49.5            | 0.485    |
| Current/former smoker, %        | 36.9            | 37.9            | 0.886    |
| Alcohol consumption, %          | 11.7            | 7.8             | 0.347    |
| APTT (seconds), mean±SD         | 26.0±3.2        | 26.8±2.3        | 0.985    |
| PT, %                           | 93.5±13.9       | 95.2±5.2        | 0.328    |
| Platelets ×10⁶/L, mean±SD       | 211.3±37.8      | 203.9±21.3      | 0.148    |
| 30-day survival, %              | 68.0            | —               | —        |
| Aneurysm, %                     | 67.0            | —               | —        |

ND indicates not determined; GP, general population.

The strength of the association of major risk factors and the polymorphism with the occurrence of disease was estimated by calculation of the odds ratio with the EpilInfo software and the Cornfield method for the calculation of 95% CI. Gene–gene interactions were determined by comparing the prevalence of combined carrier for 2 gene variants in patients and controls by analysis of the distribution of 1 chosen gene variant in subgrouped patients and controls who carry another gene variant as genetic background.

Results
Characteristics of the Study Population
The general characteristics of patients and controls are shown in Table 1. We did not detect significant differences in coagulation tests or in number of platelets between patients and controls (Table 1).

Table 1 shows the survival percentage 30 days after admission to the hospital, which is slightly higher than that described in previous reports.17

Prevalence of Polymorphisms in the Case Control Study
The genotypic frequencies for the analyzed polymorphisms in the case control study are shown in Table 2. The frequencies of these polymorphisms in the control group were similar to those identified in the general population from our region (Table 2) and did not differ from those previously reported in other white populations.18 There was no statistical deviation from Hardy–Weinberg equilibrium for all polymorphisms in all groups of subjects.

We observed no significant differences in the distribution of HPA-2, VNTR, C807T, and HPA-5 genotypes between cases and controls (Table 2). However, the prevalence of the GPIIIa HPA-1 polymorphism differed significantly in these 2 groups. The percentage of subjects carrying the HPA-1b allele (PIA2) was significantly lower in patients than in
controls (18.4% versus 32.0%, respectively; *P*=0.037). According to these results, carriers of the HPA-1b allele (a/b + b/b) showed almost a 2-fold decreased risk for SAH than those lacking the genetic variant under the same environmental risk factors (OR, 0.48; 95% CI, 0.24 to 0.96). The protection observed in heterozygous was significantly higher (a/b versus a/a: OR, 0.43; 95% CI, 0.22 to 0.85; *P*=0.0191), whereas the b/b genotype did not protect against SAH (b/b versus a/a: OR, 1.25; 95% CI, 0.21 to 7.70).

**Association of Polymorphisms With Clinical Features of SAH**

We observed no significant association between HPA-2, VNTR, HPA-5, and C807T polymorphisms and clinical features of SAH (data not shown). However, we identified interesting associations concerning the HPA-1 genotype. As indicated in Table 3, we did not find significant differences in age, sex, platelet counts, activated partial thromboplastin time, prothrombin time, presence of aneurysm, smoking, alcohol consumption, and hypertension status according to the HPA-1 genotype. Patients carrying the HPA-1b allele had more previous arterial ischemic events than those with HPA-1 a/a genotype (31.6% versus 7.1%, respectively; *P* = 0.0001). Interestingly, the HPA-1b allele also associated with a milder clinical grade at presentation according to the Hunt and Hess score (Hunt–Hess I to III: 84.2% in carriers of the HPA-1b allele versus 53.8% in patients with HPA-1 a/a genotype; *P*=0.0187).

Finally, this polymorphism did not seem to affect significantly the incidence of early mortality, at least in those patients reaching the hospital. However, there is a trend toward an increase in the survival 30 days after admission to the hospital in those patients carrying the HPA-1b allele (79.0% versus 65.5%; Table 3).

**Synergism Between Polymorphisms**

The studied polymorphisms are common in the white population. Therefore, it was possible to test any association between polymorphisms despite the relative small size of our sample. Only the association between HPA-1 and HPA-2 deserved our attention. No patient simultaneously carried these 2 polymorphisms but 8 controls did (7.8%; a similar percentage was found in the general population [7.5%]). Accordingly, the combination of these 2 polymorphisms strongly increase the protection against the development of SAH (*P*=0.007).

### TABLE 3. Selected Risk Factors, Clinical Severity, and Hemorrhage Extension in Patients With SAH According to the HPA-1 Genotype

<table>
<thead>
<tr>
<th>N</th>
<th>Age, %±SD</th>
<th>Male, %</th>
<th>Plat, %±SD</th>
<th>APTT, %±SD</th>
<th>PT, %±SD</th>
<th>AIE, %</th>
<th>Aneu, %</th>
<th>HT, %</th>
<th>Smok, %</th>
<th>Aic, %</th>
<th>Size, %±SD</th>
<th>Fisher &lt;1%</th>
<th>H&amp;H &lt;IV</th>
<th>Survival, %</th>
</tr>
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<tbody>
<tr>
<td>a/a</td>
<td>84</td>
<td>58±13</td>
<td>39.3</td>
<td>213±40</td>
<td>25.8±3.1</td>
<td>93.0±15.1</td>
<td>7.1</td>
<td>64.3</td>
<td>46.4</td>
<td>36.9</td>
<td>10.7</td>
<td>8.8±2.22</td>
<td>20.3</td>
<td>53.8</td>
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<tr>
<td>a/b</td>
<td>16</td>
<td>60±16</td>
<td>31.3</td>
<td>207±26</td>
<td>25.9±3.4</td>
<td>95.3±6.2</td>
<td>31.3</td>
<td>81.3</td>
<td>37.5</td>
<td>37.5</td>
<td>12.5</td>
<td>11.6±2.9</td>
<td>62.5</td>
<td>87.5</td>
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<tr>
<td>P*</td>
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<tr>
<td>b/b</td>
<td>3</td>
<td>71±21</td>
<td>66.8</td>
<td>190±34</td>
<td>27.2±6</td>
<td>96.7±5.8</td>
<td>33.3</td>
<td>66.7</td>
<td>33.3</td>
<td>33.3</td>
<td>14±1.4</td>
<td>14±1.4</td>
<td>100</td>
<td>66.7</td>
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<tr>
<td>P*</td>
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<tr>
<td>a/b+b/b</td>
<td>19</td>
<td>62±16</td>
<td>36.8</td>
<td>205±27</td>
<td>26.1±3.3</td>
<td>95.5±6.0</td>
<td>31.6</td>
<td>78.9</td>
<td>36.8</td>
<td>36.8</td>
<td>15.8</td>
<td>11.9±2.82</td>
<td>68.4</td>
<td>84.2</td>
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<tr>
<td>P*</td>
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Discussion

Exquisitely regulated hemostatic mechanisms have evolved within the animal kingdom to protect against the ever-present danger of fatal hemorrhage. Platelets and coagulation factors interact to generate the protective hemostatic plug that prevents blood loss at sites of vascular injury. During the past decade, numerous studies have investigated the role of polymorphisms affecting platelet GP in arterial thrombosis with conflicting results.\(^{10,18}\) However, few reports have analyzed the significance of these polymorphisms in hemorrhage. Our report is the first one showing a protective role of a platelet polymorphism in spontaneous SAH. We found that the HPA-1b allele (PlA2) of platelet GPIIIa significantly reduces the risk of SAH. This result is consistent with the suggested platelet hyper-reactivity and high thrombotic risk associated with this variant.\(^{10,18}\)

The association we found between the size of the aneurysm and the HPA-1 genotype probably does not indicate that this polymorphism is related to the cause and pathogenesis of intracranial aneurysm (although the HPA-1 is also expressed on vascular endothelial cells),\(^{19}\) but could be an additional result supporting the mild protective effect of the HPA-1b allele in hemorrhage. Thus, patients carrying the HPA-1b allele were protected for bleeding if the size of the aneurysm is small or medium (<9 mm), but the influence of a mild and common polymorphism cannot avoid hemorrhage in patients with large aneurysms (>9 mm). Because size is the most important factor influencing the possibility of aneurysmal rupture,\(^{20}\) the HPA-1b variant could protect only subjects with small aneurysms.

The role of the platelet HPA-1 polymorphism in SAH is not restricted to the risk of disease; it is also involved in the severity of the onset. In agreement with the protective role identified in the case control study, the HPA-1b allele also seems to reduce the severity of the disease. Thus, the extension of the hemorrhage (measured by the grading system of Fisher) and the clinical grade at presentation (determined by the Hunt and Hess score) are significantly lower in carriers of the HPA-1b allele than in patients with HPA-1 a/a genotype. This is especially relevant considering that patients carrying the HPA-1b allele presented large aneurysms, which have been consistently identified as predictors of mortality and poor functional outcome after SAH.\(^{21}\) Accordingly, the HPA-1b allele seems to be a potent factor reducing the severity of SAH. These data are supported by the high 30-day survival observed in patients carrying the HPA-1b allele (79%).

Our data support previous studies reporting the protective role of prothrombotic polymorphisms (factor V Leiden, prothrombin G20210A, or GPIa C807T) in congenital or acquired bleeding diseases (von Willebrand disease, hemophilia, partum, menstruation, or cerebral hemorrhage).\(^{11}\) Remarkably, the HPA-1b allele also seems to reduce the severity of bleeding in patients with Glanzmann thrombasthenia.\(^{22}\) These results support that a polymorphism may play mild but opposite roles in the pathogenesis of thrombotic and hemorrhagic disorders, suggesting an explanation for the high frequency of these polymorphisms in the general population. Interestingly, the HPA-1b allele is almost absent in the Japanese population,\(^{23}\) which is a population with extremely high incidence of SAH (22.1 per 100,000).\(^{24}\)

Finally, because SAH is a polygenic and multifactorial disorder, the genetic components in this disease may be a combined effect of a number of genes, with each playing only a small role. A recent article suggests a role for certain endothelial nitric oxide synthase polymorphisms in predicting susceptibility toward SAH and post-SAH vasospasm.\(^{25}\) The predisposition imparted by individual genes may act independently or interact with other genes to result in an additive effect or a synergistic co-effect. Synergism between polymorphisms has been identified in other diseases, including hemostatic disorders,\(^{26,27}\) and also involves the HPA-1 polymorphism.\(^{28}\) Our data support that the combination of 2 polymorphisms affecting the most important platelet adhesive receptors, GPIIIa (HPA-1) and GPIbα (HPA-2), strongly increases the protection of the HPA-1b allele in SAH.

In conclusion, we presented the first evidence to our knowledge suggesting a protective role for the platelet GPIIIa HPA-1b allele in SAH. The suggested platelet hyper-reactivity determined by the GPIIIa Pro33 variant could reduce the risk of SAH, especially if the aneurysm is small, and attenuate the severity of the hemorrhage. This effect might be amplified by the simultaneous combination with the GPIbα HPA-2b allele. The sample size is relatively small, but the frequency of the polymorphism is high. Thus, our matched case control sample has a power of 58% to detect a relative risk of 0.48 for the HPA-1 polymorphism with a statistical significance of 5%. The power becomes higher (67%) considering the general population, which shows similar distribution of genotypes that selected controls. Moreover, the conflictive functional and thrombotic role of the GPIIIa HPA-1 polymorphism encourage the performance of further studies including more patients from other populations in association with other polymorphism to confirm whether the HPA-1b allele or the HPA-1 a/b genotype provides protection against SAH or other hemorrhagic disorders.

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