Platelet PlA2 Polymorphism and the Risk for Thrombosis in Heparin-Induced Thrombocytopenia

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Key Words: Heparin-induced thrombocytopenia; HIT; HPA1; PlA2

DOI: 10.1309/BMW4M8NBV07KFRX

Abstract

Platelet glycoprotein (GP) IIb/IIIa has an important role in platelet aggregation. A polymorphism of platelet GPIIIa (PlA2, also called HPA1b) has been associated with a higher risk of thrombosis, but its implication in heparin-induced thrombocytopenia (HIT) is unclear. To investigate the hypothesis that the PlA2 polymorphism influences the prothrombotic effects of HIT, we conducted a prospective study of 66 consecutive patients with a laboratory diagnosis of HIT. The end point of the study was the diagnosis of a thrombus within 30 days of the positive HIT test result. The Diagnostica Stago (Asnières, France) enzyme-linked immunosorbent assay was used to detect HIT antibodies, and a polymerase chain reaction assay was used to detect the PlA2 polymorphism. Of the 66 patients, thrombotic complications developed in 27 (41%). Patients with the PlA2 allele demonstrated a significantly higher thrombosis risk than did patients without (69% vs 32%; P = .0088; odds ratio, 4.68; 95% confidence interval, 1.39-15.72). The risk was stronger for arterial thrombosis and for patients 60 years or older. There was a significant association between the PlA2 polymorphism of GPIIIa and the risk of thrombosis in patients with HIT antibodies.

Heparin-induced thrombocytopenia (HIT) is an immune-mediated, potentially life-threatening, adverse effect of heparin therapy, which is frequently associated with venous and arterial thrombosis.1,2 HIT is caused by antibodies against heparin bound to platelet factor 4 (PF4). The antibodies bind to heparin-PF4 complexes and to platelets, inducing platelet activation and aggregation. The decrease in platelet count typically begins 4 to 12 days after the initiation of heparin. At least 30% of patients with HIT have thrombotic events, with rates up to approximately 75% in some series.2,3

Platelet glycoprotein (GP) IIb/IIIa is a platelet membrane receptor for fibrinogen. GPIIb/IIIa mediates platelet aggregation by linking activated platelets together through fibrinogen. The PlA2 polymorphism is a leucine-to-proline substitution at residue 33 of the β3 subunit of platelet GPIIb/IIIa (also called αIIbβ3) due to a thymidine-to-cytosine substitution at position 1565 in exon 2 of the GPIIIa gene. This polymorphism is present in approximately 15% to 20% of the healthy population in the United States.4,5

Many4,6-11 but not all12-15 studies have shown an association of PlA2 with thrombosis. In 1996, Weiss et al4 reported an association between the PlA2 allele and increased risk of premature coronary thrombosis. A meta-analysis of 34 studies on coronary artery disease and 6 studies for restenosis after revascularization found a significant odds ratio (OR) of 1.10 for coronary artery disease and 1.21 among people younger than 60 years. The OR for restenosis after revascularization procedures was also significant at 1.31.16 Similarly, many but not all in vitro and ex vivo studies have shown an association of PlA2 with thrombosis. In an in vitro study using Chinese hamster ovary and human kidney embryonal cells overexpressing PlA1 or PlA2, significantly more PlA2 cells bound to
immobilized fibrinogen and had greater fibrinogen clot retraction than did PlA1 cells. Epinephrine- and adenosine diphosphate–induced platelet aggregation was increased among patients with PlA1 with elevated fibrinogen levels, whereas patients with PlA2 had increased aggregation regardless of their fibrinogen levels. Another study also found evidence that the PlA2 is associated with increased adenosine diphosphate–induced platelet aggregation. In contrast, another study found no evidence for a PlA2 influence on collagen-induced platelet thrombus formation.

Because the PlA2 polymorphism has been shown to influence platelet aggregation in many studies and because the mechanism for thrombosis in HIT is platelet activation, we speculated that this platelet polymorphism might influence risk for thrombosis in patients with HIT. A previous study showed no influence of the factor V Leiden mutation in the thrombosis risk of HIT, presumably because HIT itself is a strong risk factor for thrombosis, and the thrombosis risk with factor V Leiden does not directly involve platelets. The pathogenesis of thrombosis in HIT involves platelet activation, producing platelet-rich thrombi. In the present study, it was hypothesized that a prothrombotic polymorphism on the platelet itself may have a role in the thrombosis risk of HIT.

Materials and Methods

Patient Selection and Clinical Data

The PlA1/2 genotype was determined for 66 consecutive HIT-positive patients tested in the Coagulation Laboratory, Massachusetts General Hospital, Boston. Specimens had been submitted to the laboratory by the patients’ clinicians based on a clinical suspicion of HIT. This study was approved by the Massachusetts General Hospital Institutional Review Board.

The following demographic and clinical data for each patient were obtained: age, sex, smoking history, fibrinogen level, platelet count, aspirin use, and mortality. Fibrinogen and platelet count data were taken from the day of HIT testing. The medical records were examined to determine the presence or absence of a thrombotic event occurring during heparin exposure or within 30 days after the positive HIT test result. Medical records were examined without knowledge of PlA genotype to avoid bias. Objective documentation of the thrombotic event (eg, confirmation by radiologic studies or microscopic examination of thrombi) was recorded.

Determination of PlA Genotype

The PlA region of the GPIIIa gene was amplified by polymerase chain reaction using genomic DNA as a template. Genomic DNA was isolated from 200 µL of citrated whole blood with the QIAamp DNA blood mini kit (Qiagen, Valencia, CA). The primers used were 5'-TTCTGATTGCTGGACTTTCTTT-3' and 5'-TCTTCTCCCCATGCGCAAAGCT-3'. The resulting polymerase chain reaction products were digested with MspI (New England Biolabs, Beverly, MA). Digestion products were separated by electrophoresis on a 2.5% agarose gel containing ethidium bromide, which distinguishes among PlA1 homozygotes (wild type), PlA1/PlA2 heterozygotes, and PlA2 homozygotes.

Determination of HIT Antibodies

Blood samples were collected into 3.2% sodium citrate (9:1, blood/citrate), and plasma was prepared by centrifugation at 1,500g for 15 minutes and stored at −70°C. The heparin-PF4 enzyme-linked immunosorbent assay (Asserachrom HPIA, Diagnostica Stago, Asnières, France) was performed according to the manufacturer’s instructions. Briefly, the plasma that was suspected to contain heparin-PF4 antibodies was incubated in a plastic microwell that was precoated with heparin-PF4 complexes to capture any anti–heparin-PF4 (HIT) antibodies present. If HIT antibody is present in the patient sample, the antibody binds to the plate, leading to a color-generating reaction that increases the optical density at 492 nm when a second antibody is added.

Statistical Analysis

Continuous data (platelet counts and age in years) were analyzed by using the Student t test. Discrete data (PlA genotype, thrombosis, mortality, smoking, aspirin use, fibrinogen level >400 mg/dL [11.8 µmol/L], and age <60 years) were analyzed by using the Fisher exact test. The strength of the association of PlA2 with thrombosis was estimated by calculation of the OR. The association of PlA2 with thrombosis, adjusted for an age younger than 60 years and sex, was determined by using the multiple logistic-regression method using Stata statistical software (version 4.0, StataCorp, College Station, TX). P values less than .05 were considered statistically significant.

Results

Demographic and Clinical Characteristics

Table I shows the prevalence of demographic and clinical variables that could influence the risk for thrombosis among the patients in the study. Overall, thrombosis developed in 27 (41%) of 66 HIT-positive patients. Age, sex, current or former cigarette smoking, current aspirin use, and elevated fibrinogen level were not significantly different between patients with and without thrombosis.

The mean and median ages of all patients were 67.1 and 70.0 years, respectively. The mean and median ages of patients with thrombosis were 64.0 and 70.0 years, respectively and for patients without thrombosis, 69.3 and 70.0 years, respectively (P = .13). The mean and median ages also did not differ significantly.
between patients heterozygous for PI$^{A2}$ (mean, 63.4 years; median, 67.0 years) and patients with wild-type PI$^{A1}$ (mean, 68.3 years; median, 71.0 years; $P = .23$).

**Prevalence of PI$^{A2}$ and Thrombosis**

**Table 2** shows the genotype data for the patients in this study. Of the 66 patients, 16 (24%) were heterozygous for PI$^{A2}$, and the remaining 50 patients (76%) were PI$^{A1}$/PI$^{A1}$ homozygotes (wild type). No PI$^{A2}$ homozygotes were identified.

As mentioned, thrombosis developed in 27 (41%) of 66 HIT-positive patients. Among PI$^{A2}$ heterozygotes, thrombosis developed in 11 (69%) of 16, whereas it developed in 16 (32%) of 50 PI$^{A1}$ homozygotes ($P = .0088$; OR, 4.68; 95% confidence interval [CI], 1.39-15.72). Stated differently, the prevalence of PI$^{A2}$ among subjects with thrombosis was 41% (11/27), which was significantly higher than the prevalence among subjects without thrombosis (13% [5/39]; $P = .0088$).

To determine if PI$^{A2}$ had a stronger influence in younger or older patients, the prevalence of PI$^{A2}$ and thrombosis was compared using age 60 years as a cutoff. Of 9 patients 60 years or older with thrombosis, 7 (78%) were heterozygous for PI$^{A2}$ vs 10 (25%) of 40 patients 60 years or older without thrombosis ($P = .0047$). This relationship was not seen in younger patients. Of 10 patients younger than 60 years with thrombosis, 4 (40%) were heterozygous for PI$^{A2}$ vs 3 (43%) of 7 patients younger than 60 years without thrombosis ($P = .378$). In addition, the percentage of patients 60 years or older was lower among PI$^{A2}$-positive patients (9/16 [56%]) than among patients with wild-type PI$^{A1}$ (40/50 [80%]), regardless of thrombosis ($P = .0467$).

The types of thrombotic events that occurred are shown in **Table 3**. Of the 16 PI$^{A2}$-positive patients, 6 (38%) experienced an arterial thrombotic event compared with only 4 (8%) of 50 PI$^{A1}$-positive patients ($P = .027$). For PI$^{A2}$ vs 10 (25%) of 40 patients 60 years or older without thrombosis ($P = .00470$). This relationship was not seen in younger patients. Of 10 patients younger than 60 years with thrombosis, 4 (40%) were heterozygous for PI$^{A2}$ vs 3 (43%) of 7 patients younger than 60 years without thrombosis ($P = .378$). In addition, the percentage of patients 60 years or older was lower among PI$^{A2}$-positive patients (9/16 [56%]) than among patients with wild-type PI$^{A1}$ (40/50 [80%]), regardless of thrombosis ($P = .0467$).

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patients with wild-type PI\(^{A1}\) (\(P = .027; \) OR, 6.9; 95% CI, 1.64-29.07). In addition, 1 of the 4 arterial events among patients with wild-type PI\(^{A1}\) was an extension of a preexisting ischemic stroke (all other thrombotic events in this study occurred de novo, except for 1 extension of preexisting pulmonary embolism in a patient with wild-type PI\(^{A1}\)). Of 16 PI\(^{A2}\)-positive patients, 3 (19%) experienced a venous thrombotic event compared with 10 (20%) of 50 patients with wild-type PI\(^{A1}\) (\(P = .28\)).

A multiple logistic-regression model that adjusted for sex and age younger than 60 years provided an OR of 4.39 (95% CI, 1.29-14.94; \(P = .018\)) for the association between thrombosis and PI\(^{A2}\).

Although records were examined for thromboses occurring up to 30 days after the positive HIT test result, the latest thromboses to occur were on day 17 (in a patient with PI\(^{A1/A1}\)) and day 20 (in a patient with PI\(^{A1/A2}\)) after HIT testing. All other thromboses occurred within 5 days of the positive HIT test result. In addition, even though thromboses beyond 30 days would not have been included in the analysis, records of the patients without thrombosis were further examined for 30 to 60 days after the HIT test, and no thromboses were observed during that time.

### Mortality

Among all PI\(^{A2}\)-positive patients, 6 (38%) of 16 died, whereas 14 (28%) of 50 patients with wild-type PI\(^{A1}\) died (\(P = .18; \) OR, 1.54; 95% CI, 0.47-5.05). Mortality was significantly more frequent among patients with thrombosis compared with patients without thrombosis (\(P = .026; \) OR, 3.1; 95% CI, 1.05-9.19). Age above 60 years did not significantly influence mortality (\(P = .198; \) OR, 1.99; 95% CI, 0.696-5.70), nor did fibrinogen level, smoking, or aspirin use (data not shown).

### Platelet Count

The median platelet count at the time of the positive HIT test result was 86 × 10^3/µL (86 × 10^9/L) among the PI\(^{A2}\) heterozygous patients and 103 × 10^3/µL (103 × 10^9/L) among the wild-type PI\(^{A1}\) homozygous patients (\(P = .86\)). Thus, there was no significant difference in platelet count at the time of HIT testing between PI\(^{A2}\) heterozygous and PI\(^{A1}\) homozygous patients.

The median platelet count at the time of the positive HIT test result was 95 × 10^3/µL (95 × 10^9/L) among all subjects with thrombosis and 99 × 10^3/µL (99 × 10^9/L) among all subjects without thrombosis (\(P = .94\)). Thus, there was no significant difference in platelet count at the time of HIT testing between patients with and without thrombosis.

### Discussion

Our results demonstrate a significant association between the PI\(^{A2}\) polymorphism of GPIIb/IIIa and the occurrence of thrombosis in HIT-positive patients. We found a significantly higher prevalence of the PI\(^{A2}\) genotype among HIT-positive patients with thrombosis than among HIT-positive patients without thrombosis. Furthermore, the prevalence of arterial thrombosis was significantly higher among patients with the PI\(^{A2}\) genotype compared with wild type, but the rate of venous thrombosis was similar between the groups. This observation is consistent with the notion that platelets have a more important role in arterial thrombosis than in venous thrombosis. Consequently, if a platelet polymorphism such as PI\(^{A2}\) increases the risk for thrombosis, it would be reasonable to expect it to demonstrate a greater effect on arterial thrombosis than on venous thrombosis.

The presence of PI\(^{A2}\) in patients 60 years or older was associated with a higher risk for thrombosis. This finding could be because older patients are more likely to have underlying atherosclerosis, which promotes arterial thrombosis.

In the Physicians’ Health Study, the prevalence of PI\(^{A2}\) among 704 healthy men in the Massachusetts area was 15%. This value is similar to the prevalence of PI\(^{A2}\) among HIT-positive patients without thrombosis in the present study (13%). This suggests that the genotype data on subjects without thrombosis in the present study accurately reflect the genetics of the population in our geographic area and that this is a valid group to compare with the HIT thrombosis patients.

The results suggest that further study could be indicated to determine if the PI\(^{A2}\) genotype helps predict thrombotic risk in patients with HIT. If this is confirmed, additional studies could determine if PI\(^{A2}\) HIT-positive patients would benefit from additional treatment protocols such as GPIIb/IIIa inhibitors, providing a rationale for testing for the PI\(^{A2}\) polymorphism in patients with HIT.

Another recent study did not find an association between PI\(^{A2}\) and thrombosis in HIT. This could be because the previous study had proportionally fewer patients with arterial thrombosis than did the present study, and the PI\(^{A2}\) effect is seen predominantly with arterial thrombosis. Arterial thrombosis was not analyzed separately from venous events in that study. Another possible explanation for the differing results is a difference in HIT test methods. The present study used the Diagnostica Stago enzyme-linked immunosorbent assay method, which is a very sensitive method of detecting HIT antibody. The previous study used a heparin-induced platelet activation assay, which has the advantage of being a functional assay rather than an immunonasay, but it demonstrated some inconsistent results in an interlaboratory study.

The presence of PI\(^{A2}\) heterozygosity in HIT-positive patients was associated with an increased incidence of thrombosis, in particular with arterial thrombosis and patients 60 years or older.

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References