Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 36, No. 1, 2000
ISSN 0735-1097/00/$20.00
PII S0735-1097(00)00710-5

## EDITORIAL COMMENT

# $Pl^{A2}$, a Variant of GPIIIa Implicated in Coronary Thromboembolic Complications*

Pascal J. Goldschmidt-Clermont, MD, FACC, Glen E. Cooke, MD, Gregory M. Eaton, MD, Philip F. Binkley, MD, FACC
*Columbus, Ohio*

## THE $Pl^{A2}$ POLYMORPHISM OF GPIIb-IIIa

Ischemic heart disease (IHD) and adverse events after coronary stent placement represent polygenic disorders. They correspond to complex traits induced not only by multiple genes but also by the interaction of these genes with the environment. Multiple loci likely to be implicated in IHD are polymorphic, such that there are two or more variant forms of the gene in the population. These variants have been created by mutations that can change the coding sequence of the gene in question. A missense mutation, for example, would produce a different form of the encoded protein, whose function might be slightly altered compared with the ancestor protein. Alternatively, the mutation, might fall within the regulatory domains of a gene, thereby changing the level of expression of the gene product and/or when and where the gene is being activated. The specific impact of such mutations is further dependent on a variety of factors, both genetic and environmental.

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The platelet antigen 2 ($Pl^{A2}$) polymorphism represents one such variant that has been implicated in arterial thrombosis (1–3). Due to the substitution of a cytosine for a thymidine at position 1565 in exon 2 of the glycoprotein IIIa (GPIIIa or integrin $Beta_3$) gene, the $Pl^{A2}$ variant displays a proline instead of a leucine at amino acid 33 (4). Hence, the ancestor gene is called $Pl^{A1}$ and the variant gene $Pl^{A2}$, such that individuals can be $Pl^{A1/A1}$ homozygous, $Pl^{A1/A2}$ heterozygous or $Pl^{A2/A2}$ homozygous. Glycoprotein IIIa, together with GPIIb, constitutes the fibrinogen receptor (GPIIb-IIIa), whose engagement represents the final common pathway for platelet activation (5). Therefore, GPIIIa with its $Pl^{A2}$ polymorphism is positioned as a candidate gene that contributes to arterial thromboembolic complications. The $Pl^{A2}$ polymorphism affects the structure of GPIIb-IIIa, an assumption that was supported by the discovery of the $Pl^{A2}$ variant as the cause for a severe form of neonatal alloimmune thrombocytopenia.

We initially observed a high prevalence of $Pl^{A2}$ positivity in a population of siblings of patients with a history of premature IHD (IHD manifestations before age 60) (6) and in patients with severe unstable coronary syndromes (7). In complex traits, it is unlikely that individual genes have an effect that would account for more than a few percent of the variance. Consistent with this concept, in a meta-analysis, we have found that the relative risk of myocardial infarction (MI) associated with $Pl^{A2}$ is small (1.2) but significant ($p = 0.034$) (8). Several studies have not confirmed the association between $Pl^{A2}$ and MI, in particular, the Physicians' Health Study (9). Hence, as a consequence of such discrepancy among well conducted epidemiological studies, the link between $Pl^{A2}$ and spontaneous MI is perceived as controversial (2). In contrast with epidemiological data, most studies on the molecular effect of the $Pl^{A2}$ polymorphism on GPIIb-IIIa function have been consistent. These studies have shown that the L33P mutation results in increased platelet responsiveness (10,11).

Genetic factors can influence illnesses in very specific ways. Considering that a clinical diagnosis of MI encompasses a complex phenotype, the relative impact of the $Pl^{A2}$ polymorphism on MI may vary according to multiple factors, including the presence or absence of ST elevation, associated diseases like diabetes, gender, age, environment and medications. Studies of patients who have died suddenly and at a young age can be highly instructive, as the culprit lesion can be identified and characterized, and the number of contributing modifiers is often limited. Indeed, sudden death is a prominent first manifestation of coronary artery disease in younger individuals, such that sudden death victims do not have the opportunity to benefit from drugs that could interfere with the effect of a gene variant. Mikkelsson et al. (12) have reported that the prevalence of $Pl^{A2}$ is elevated in sudden death victims whose coronary vessels contain a thrombus. They also reported that $Pl^{A1/A1}$ homozygous individuals have a heightened atherosclerotic plaque burden compared with their $Pl^{A2}$ counterparts. These results suggest that $Pl^{A2}$, in conjunction with other genetic and environmental risk factors, might contribute to driving the course of atherosclerosis toward a path of coronary plaque rupture and thrombus formation.

## ENDOVASCULAR CORONARY PROCEDURES AND $Pl^{A2}$

Catheter-based intervention with stent placement has become a preferred management for patients with symptomatic coronary disease (both acute coronary ischemic syndromes and acute MI). Although associated with a risk that is not negligible, the procedure has become safer because of technological advances and the availability of powerful

*Editorials published in *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Heart and Lung Institute and Division of Cardiology, Department of Internal Medicine, College of Medicine and Public Health, The Ohio State University, Columbus, Ohio.

antithrombotic drugs (13). The use of intracoronary stents has improved the immediate and short-term efficacy of endovascular procedures by maximizing acute procedural luminal gain and reducing the unfavorable geometric vascular remodeling of stenotic coronary vessels. We have hypothesized that, to be efficient, an antithrombotic strategy must target the impact of specific susceptibility genes implicated in coronary thrombosis under various clinical circumstances (14). This principle is pertinent to strategies directed towards minimizing thromboembolic complications after stent revascularization. Platelet antigen 2 was the first platelet polymorphism implicated in thromboembolic complications associated with stent placement (15).

Several classes of drugs are clinically utilized to reduce platelet activity during coronary stenting: 1) acetylsalicylate (ASA), 2) thrombin antagonists (unfractionated heparin, mainly), 3) thienopyridine derivatives (clopidogrel and ticlopidine), and 4) GPIIb-IIIa antagonists (ReoPro or abciximab, Tirofiban or aggrastat, and Integrilin or eptafibitide). The role of dipyridamole for stent patients has not yet been established but could be of interest for drug combination strategies. Two combinations of drugs have been highly successful in reducing adverse events associated with coronary stent placement: a) ASA plus an antithrombin plus a thienopyridine derivative or b) aspirin plus an antithrombin plus a GPII-IIIa blocker. It is likely that the multidrug approach has the potential of covering a larger number of "thrombotic susceptibility genes" compared with single agent therapy. Experiments performed in vitro have consistently shown an increased responsiveness to agonists of platelets displaying the $Pl^{A2}$ polymorphism (10,11). In this context, the study of Kastrati et al. (17) published in this issue can be viewed as investigating whether standard antithrombotic strategies have reached a sufficient level of control for the thrombotic diathesis conferred by the $Pl^{A2}$ polymorphism.

## $Pl^{A2}$ AND ADVERSE EVENTS AFTER STENT PLACEMENT

There have been three studies published on the topic of $Pl^{A2}$ and adverse events after stent revascularization. Walter et al. (15) studied 318 consecutive patients who received a coronary stent for indications of dissection, acute occlusion or suboptimal angioplasty. The primary end points of death, MI, stent thrombosis and coronary bypass surgery were followed over a period of 30 days after stent placement. All patients received aspirin and heparin during the procedure. Ticlopidine, 250 mg per day, was started and given for the next four weeks, in combination with aspirin (100 mg per day), which was continued indefinitely. Occlusion of the stent vessel was more frequent in $Pl^{A2}$ positive individuals (9.5% in $Pl^{A2}$ positive patients vs. 1.9% in $Pl^{A1/A1}$ homozygous, odds ratio: 5.26, $p = 0.01$). There was also an excess of MI in the $Pl^{A2}$ positive patient group.

Laule et al. (16) studied 1,000 consecutive patients with angiographically confirmed coronary artery disease, 653 of whom received intervention (280 with stent placements and 102 with directional coronary atherectomy). A composite end point of death, MI and target vessel revascularization (TVR) was followed for 30 days after intervention. All patients received aspirin and heparin during the procedure and after the procedure aspirin (100 mg indefinitely). Stented patients also received ticlopidine 500 mg daily for four weeks. A total of 41 patients reached the 30-day composite end point (6.3%). For the 653 who received intervention, the odds ratio for the composite end point ($Pl^{A2}$ positive vs. $Pl^{A1/A1}$) was 1.36, 1.46 for TVR and 1.31 for MI (nonsignificant). For the stented patients, the odds ratio for the composite end point ($Pl^{A2}$ positive vs. $Pl^{A1/A1}$) was 1.42, 1.78 for TVR and 1.24 for MI (nonsignificant). Due to the small number of homozygous $Pl^{A2/A2}$, the analysis pooled $Pl^{A2/A2}$ with $Pl^{A1/A2}$ patients.

The study by Kastrati et al. (17) included 1,759 consecutive patients with stable or unstable angina and successful stent placement. The composite end point of death, MI and urgent revascularization was followed for 30 days after stent placement. All patients received aspirin (100 mg twice a day) indefinitely and ticlopidine (250 mg twice a day) for four weeks. Patients with residual thrombi or flow limiting dissection received abciximab (bolus plus 12 h infusion). Angiographic stent thrombosis occurred in 2.1% of $Pl^{A2}$ positive, versus 1.7% of the $Pl^{A1/A1}$ homozygous patients (nonsignificant). Death and Q-wave MI were significantly more frequent in $Pl^{A2}$ carriers. Moreover, $Pl^{A2/A2}$ homozygous patients ($n = 46$) had either a clear trend or a significantly higher event rate compared with heterozygous and $Pl^{A1/A1}$ homozygous patients. The primary end point was reached in 5.4% for $Pl^{A1/A1}$ patients, $Pl^{A1/A2}$ 4.8% and $Pl^{A2/A2}$ 13.0% ($p = 0.06$); the incidence of death or MI was 4.3% for $Pl^{A1/A1}$ patients, $Pl^{A1/A2}$ 4.2% and $Pl^{A2/A2}$ 13.0% ($p = 0.02$), and the incidence of angiographic stent thrombosis was 1.7% for $Pl^{A1/A1}$ patients, $Pl^{A1/A2}$ 1.5% and $Pl^{A2/A2}$ 8.7% ($p = 0.002$). $Pl^{A2}$ positive patients with stent thrombosis tended to be younger than $Pl^{A1/A1}$ homozygous patients with stent thrombosis. In a multivariate model, the $Pl^{A2/A2}$ genotype was associated with an adverse outcome after stenting (odds ratio: 2.6), compared with the $Pl^{A1/A1}$ genotype. Only 12.1% of the patients received abciximab.

From the analysis of these three studies that surveyed 2,357 patients for a period of 30 days, one may conclude that the $Pl^{A2}$ polymorphism of GPIIIa represents a risk factor for adverse events in patients undergoing coronary stenting. Although all three studies were conducted by expert investigators, they differ in their complication rates and in their conclusion relative to the strength of the impact of $Pl^{A2}$ on adverse events. Although some unrecognized factor(s) may have contributed to such a discrepancy, it is notable that the studies were not uniform in terms of the antithrombotic regimen provided to the patients (Table 1). For example, in the study by Walter et al. (15), whose

**Table 1.** $Pl^{A2}$ and Stent Thrombosis

| | n | ASA | ACT > 250 | Ticlid | GPIIb-IIIa-B | OR |
|---|---|---|---|---|---|---|
| Walter (15) | 318 | 100 | + | 250 | − | 5.3* |
| Laule (16) | 280 | 100 | + | 500 | − | 1.5 (NS) |
| Kastrati (17) | 1,759 | 200 | + | 500 | 12% | 1.2† |

In the report of Laule et al. (16), stent thrombosis rate was calculated from target vessel revascularization and myocardial infarction rates. Odds ratio (OR) are used to estimate risk associated with $Pl^{A2}$.

*$p < 0.05$ for $Pl^{A2}$ positive patients (at least one $Pl^{A2}$ allele: $Pl^{A1/A2}$ + $Pl^{A2/A2}$); †$p > 0.05$ for $Pl^{A2}$ positive patients, whereas OR for stent thrombosis in $Pl^{A2/A2}$ homozygous patients was 5.1 ($p < 0.05$). ASA or acetylsalicylic acid was provided at the indicated daily dose in mg. Ticlid 250 or 500 indicates the daily dose in mg. of ticlopidine used for each study. ACT > 250 indicates that patients were receiving a dose of heparin sufficient to maintain an activated clotting time (ACT) beyond 250 s. The GPIIb-IIIa-B column refers to patients receiving a GPIIb-IIIa blocker (abciximab).

findings support that $Pl^{A2}$ has a powerful effect on adverse events (OR:5.3), the patients received only 100 mg of ASA and 250 mg of ticlopidine daily for four weeks. In the report of Laule et al. (16), patients received 100 mg of ASA and 500 mg of ticlopidine (but no GPIIb-IIIa blocker), and the effects of $Pl^{A2}$ was less pronounced (OR:1.5). Finally, Kastrati and colleagues in this issue (17) have reported an even smaller effect of $Pl^{A2}$ (OR:1.2), and their patients received 200 mg of ASA, 500 mg of ticlopidine, and a small minority of patients received abciximab.

It is tempting to speculate that differences in the $Pl^{A2}$ effect among studies was due to the strength of the antithrombotic regimen. As indicated earlier, we have hypothesized that, to be efficient, an antithrombotic strategy must target a specific susceptibility gene implicated in coronary thrombosis, including thrombotic events that follow stent placement. According to this theory, most $Pl^{A1/A1}$ patients may find enough protection against adverse events after stent placement from an antithrombotic strategy that includes heparin, ASA (≥325 mg daily) and a thienopyridine derivative (full dose) for four to six weeks. The $Pl^{A1/A2}$ heterozygous patients would need the same triple therapy, but a dose of ASA of 160 mg daily might suffice (14), while the duration of the administration of thienopyridine derivative (clopidogrel, 75 mg daily) might need to be extended for a period of six to 12 months. Whereas the "toughest nuts to crack", the $Pl^{A2/A2}$ homozygous platelets, may need a GPIIb-IIIa blocker to lower their risk to the level of $Pl^{A1/A1}$ or $Pl^{A1/A2}$ patients, in addition to receiving ASA (≥325 mg daily), clopidogrel (75 mg daily for 6 to 12 months) and heparin. Additional large scale studies will be needed to test the usefulness of stratifying procedural risk with knowledge of $Pl^{A}$ status in patients undergoing catheter based interventions. Considering important issues of safety and increasing cost, it might be of interest not only to stratify risk with genomic information but then target the use of increasingly powerful and costly antithrombotic strategies to those patients who will derive the greatest benefits.

## $Pl^{A2}$ AND RESTENOSIS

In a previous study of 1,150 consecutive patients with successful coronary stent placements and followed for an average of 163 days, Kastrati et al. (18) showed that $Pl^{A2}$ positive patients have a significantly higher rate of restenosis (defined angiographically as ≥50% diameter stenosis at six months after procedure): 53.1% in $Pl^{A2/A2}$, 46.1% in $Pl^{A1/A2}$ and 38.4% in $Pl^{A1/A1}$ patients. The effect of $Pl^{A2}$ on restenosis rate was particularly pronounced for women: 52% restenosis for $Pl^{A2}$ positive versus 33% for $Pl^{A1/A1}$ homozygous women compared with 45% restenosis for $Pl^{A2}$ positive versus 40% for $Pl^{A1/A1}$ homozygous men.

The use of stents for coronary interventions has expanded our understanding of the pathophysiology of restenosis, as it has nearly eliminated the component of "recoil" of the vessel wall from the restenotic process. In the presence of a stent, the contribution of neointimal hyperplasia (smooth muscle cell proliferation) to restenosis is more important than after conventional balloon angioplasty. Platelet adhesion and aggregation does contribute substantially to restenosis by promoting the migration and growth of smooth muscle cells (19). Even when acute stent thrombosis is efficiently prevented in $Pl^{A2}$ carriers using combination antiplatelet therapy with ASA, heparin and ticlopidine, the residual platelet accumulation might be sufficient to promote restenosis. It may, therefore, be reasonable to extend the duration of the administration of a thienopyridine such as clopidogrel, which causes fewer chronic hematological side effects than ticlopidine, to a full six-month course for patients with the $Pl^{A2}$ polymorphism. Such genomic-based therapeutic decisions are currently being tested and could have a substantial effect on the efficacy and the cost of antiplatelet therapy in the context of stent placement and restenosis.

Other polymorphisms have been studied in the context of restenosis. The blood level of angiotensin-converting enzyme (ACE) has been linked to an insertion/deletion (I/D) polymorphism of the ACE gene, such that DD genotype carriers have a higher level of ACE than either ID or II. Although, the I/D polymorphism is not associated with restenosis after balloon angioplasty, the D allele is a risk factor for restenosis after coronary stent implantation (20). In contrast, the HPA-3 polymorphism of platelet GPIIb (Bak) is not associated with an increase in the risk of thrombosis and restenosis over one year after coronary stent placement (21).

In summary, over the past few years, we have seen an exponential rise of genetic information that may turn out to be of great relevance not only for assessment of risk for individual patients but also for the design of tailored

therapeutic strategies, the pharmacogenetic approach to therapy. By utilizing a pharmacogenetic approach to therapy in patients undergoing percutaneous coronary intervention with stent placement, we should be able to tailor the combination of antiplatelet drugs that most accurately suppress risk for acute thrombosis and prevent restenosis, thereby enhancing the clinical benefit, without exposing the patient to the unnecessary risk of bleeding and society, in general, to excess health care costs.

**Reprint requests and correspondence:** Dr. Pascal J. Goldschmidt-Clermont, Heart and Lung Institute, 514 Medical Research Facility, 420 W. 12th Street, Columbus, Ohio 43210. E-mail: goldschmidt-1@medctr.osu.edu.

## REFERENCES

1. Nurden AT. Platelet glycoprotein IIIa polymorphism and coronary thrombosis. Lancet 1997;350:1189–91.
2. Goldschmidt-Clermont PJ, Roos CM, Cooke GE. Platelet PlA2 polymorphism and thromboembolic events: from inherited risk to pharmacogenetics. J Thromb 1999;8:89–103.
3. Bray PF. Integrin polymorphisms as risk factors for thrombosis. Thromb Haemost 1999;92:337–44.
4. Newman PJ, Derbes RS, Aster RH. The human platelet alloantigens, PlA1 and PlA2, are associated with a leucine33/proline33 amino acid polymorphism in membrane glycoprotein IIIa and are distinguishable by DNA typing. J Clin Invest 1989;83:1778–81.
5. Coller BS. Blockade of platelet GPIIb/IIIa receptors as an antithrombotic strategy. Circulation 1995;92:2373–80.
6. Goldschmidt-Clermont PJ, Coleman LD, Pham YM, et al. Higher prevalence of GPIIIa PlA2 polymorphism in siblings of patients with premature coronary heart disease. Arch Pathol Lab Med 1999;123: 1223–9.
7. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996;334:1090–4.
8. Burr D, Doss H, Cooke GE, Goldschmidt-Clermont PJ. A meta-analysis of studies on the association of the platelet PlA polymorphism of glycoprotein IIIa and risk of coronary heart disease. Unpublished data.
9. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K. $PI^{A1/A2}$ polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke and venous thrombosis. Lancet 1997; 349:385–8.
10. Feng D, Lindpaintner K, Larson MG, et al. Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 1999; 19:1142–7.
11. Michelson AD, Furman MI, Goldschmidt-Clermont PJ, et al. Platelet GPIIIa $Pl^{A}$ polymorphisms display different sensitivities to agonists. Circulation 2000;101:1013–8.
12. Mikkelsson J, Perola M, Laippala P, et al. Glycoprotein IIIa/Pl(A) polymorphism associates with progression of coronary artery disease and with myocardial infarction in an autopsy series of middle-aged men who died suddenly. Arteroscler Thromb Vasc Biol 1999;19:2573–8.
13. Topol EJ, Byzova TV, Plow EF. Platelet GPIIb-IIIa blockers. Lancet 1999;353:227–31.
14. Cooke GE, Bray PF, Hamlington JD, Pham DM, Goldschmidt-Clermont PJ. $Pl^{A2}$ polymorphism and efficacy of aspirin. Lancet 1998;351:1253.
15. Walter DH, Schachinger V, Elsner M, Dimmeler S, Zeiher AM. Platelet glycoprotein IIIa polymorphisms and risk of coronary stent thrombosis. Lancet 1997;350:1217–9.
16. Laule M, Cascorbi I, Stangl V, et al. A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study. Lancet 1999; 353:708–12.
17. Kastrati A, Koch W, Gawaz M, et al. $Pl^{A}$ polymorphism of glycoprotein IIIa and risk of adverse events after coronary stent placement. J Am Coll Cardiol 2000;36:84–9.
18. Kastrati A, Schomig A, Seyfarth M, et al. $Pl^{A}$ polymorphism of platelet glycoprotein IIIa and risk of restenosis after coronary stent placement. Circulation 1999;99:1005–10.
19. Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 1991;253:1129–32.
20. Amant C, Bauters C, Bodart JC, et al. D allele of the angiotensin I-converting enzyme is a major risk factor for restenosis after coronary stenting. Circulation 1997;96:56–60.
21. Bottiger C, Kastrati A, Koch W, et al. Polymorphism of platelet glycoprotein IIb and risk of thrombosis and restenosis after coronary stent placement. Am J Cardiol 1999;84:987–91.