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The PI^{A2} allele and cardiovascular disease: the pro^{33} and con

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Commentary

Cardiovascular diseases, the leading cause of death in Western nations, carry enormous socioeconomic costs and have been a focus of clinical and basic research throughout the past century. Genetic analyses have become a staple of such research, and several single nucleotide polymorphisms (SNPs) are now known that correlate well with an increased risk for thrombotic events. Most of these SNPs, including the Leiden variant of factor V and the 2021 A allele of prothrombin, are associated with enhanced fibrin deposition. Individuals who carry these alleles are at increased risk of venous thrombosis, because fibrin is the dominant component of thrombi in the veins. In arteries, however, platelets play a more prominent role in thrombus formation, and these SNPs have little prognostic value. Until 1996, none of the SNPs present in genes determining platelet function had been suggested to influence the risk of arterial thrombosis. The seminal but still controversial publication of Weiss et al. (1), identified the PIA2 allele as a significant risk factor for acute myocardial infarction and unstable angina, particularly in younger Caucasian males. As discussed below, a massive amount of clinical data regarding this association has accumulated since that time, but the literature is equivocal in the extreme. Skeptics demand a mechanism by which this SNP could give rise to important differences in platelet function. The article […]

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See related article, pages 793–802.

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Cardiovascular diseases, the leading cause of death in Western nations, carry enormous socioeconomic costs and have been a focus of clinical and basic research throughout the past century. Genetic analyses have become a staple of such research, and several single nucleotide polymorphisms (SNPs) are now known that correlate well with an increased risk for thrombotic events. Most of these SNPs, including the Leiden variant of factor V and the 2021 A allele of prothrombin, are associated with enhanced fibrin deposition. Individuals who carry these alleles are at increased risk of venous thrombosis, because fibrin is the dominant component of thrombi in the veins. In arteries, however, platelets play a more prominent role in thrombus formation, and these SNPs have little prognostic value. Until 1996, none of the SNPs present in genes determining platelet function had been suggested to influence the risk of arterial thrombosis.

The seminal but still controversial publication of Weiss et al. (1), identified the Pl^{A2} allele as a significant risk factor for acute myocardial infarction and unstable angina, particularly in younger Caucasian males. As discussed below, a massive amount of clinical data regarding this association has accumulated since that time, but the literature is equivocal in the extreme. Skeptics demand a mechanism by which this SNP could give rise to important differences in platelet function. The article by Vijayan et al. (2) in this issue of the *JCI* begins to address their concern. In essence, these authors have established that the Pl^{A1} and Pl^{A2} alleles create multiple differences in the function of the platelet integrin $\alpha_{IIb}\beta_3$, strengthening the case for an association between the polymorphism and cardiovascular disease.

The Pl^{A1}/Pl^{A2} polymorphism lies within the gene for the β_3 (GPIIIa) subunit of $\alpha_{IIb}\beta_3$ (GPIIb-IIIa). This integrin serves as a receptor for adhesive proteins fibrinogen and von Willebrand factor and is critical for the cohesive properties of platelets. Recognition of these proteins as soluble ligands requires $\alpha_{IIb}\beta_3$ to be activated by exposing platelets to one of several agonists. The transition of the receptor from a resting to an activated state depends on inside-out signals, which are transmitted across the cell membrane to render the extracellular face of $\alpha_{IIb}\beta_3$ competent to bind soluble ligands. Occupancy of $\alpha_{IIb}\beta_3$ also induces a series of outside-in signals culminating in the activation of numerous kinases and phosphatases and in rearrangement of the platelet cytoskeleton, allowing platelets to spread on an adhesive surface. Recognition of fibrin or fibrinogen as an immobilized substrate does not require inside-out signaling, but still induces outside-in signaling.

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Pl^{A1}/Pl^{A2}, one of several allelic systems on $\alpha_{IIb}\beta_3$, was originally recognized in transfusion medicine as a cause of neonatal alloimmune thrombocytopenia and posttransfusion purpura. The structural basis of the Pl^{A1}/Pl^{A2} polymorphism arises from a single base difference, a thymidine or a cytosine, which leads to an amino acid difference at position 33 in the mature β_3 subunit: a leucine in Pl^{A1} or a proline in Pl^{A2} (3). Because this simple substitution introduces multiple neoepitopes within β_3 (4), it may exert substantial and long-range effects on the conformation and, consequently, on the function of $\alpha_{IIb}\beta_3$.

Since the initial report that the Pl^{A2} polymorphism is a cardiovascular risk factor, no fewer than 17 studies have presented findings on this topic. Of these, 9 support a role for the Pl^{A2} allele as a thrombotic risk factor, and 8 do not. By far the largest of these studies was the analysis by Ridker et al. (5) who found no evidence of an association between the Pl^{A2} allele and myocardial infarction, stroke, or venous thrombosis among 14,916 men. However, several reports within the last 2 years (6, 7) identified a significant association of Pl^{A2} with the severity of coronary artery disease or stenosis. Some of these studies (7) suggested that clinical consequences could even be seen with expression of the heterozygous $Pl^{A1/2}$ genotype. The search for a mechanism by which the Pl^{A1}/Pl^{A2} polymorphism might affect platelet function has only added to this confusion. For example, although Goodall et al. (8) noted a difference in binding of soluble fibrinogen to platelets with Pl^{A1} or Pl^{A2}, other investigators (9, 10) found no such evidence. In addition, Feng et al. (11) reported that Pl^{A2} platelets activated at lower doses of one platelet agonist (adenosine diphosphate), whereas Lasne et al. (12) reported that higher doses of another platelet agonist (thrombin receptor activating peptide) were required to activate Pl^{A2} platelets. As summarized in Figure 1, other functional differences between

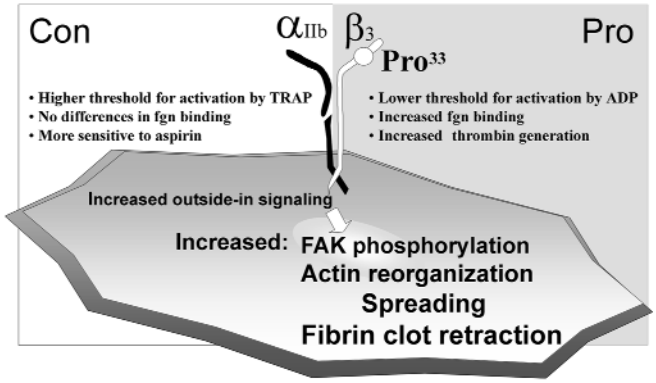

Figure 1
Functional consequences of the Pl^{A2} allele within $\alpha_{IIb}\beta_3$. Previous reports have used platelets and have yielded conflicting results, whereas the study by Vijayan et al. (2) identifies differences in outside-in signaling using a cell line expressing Pl^{A1} or Pl^{A2}. Fgn, fibrinogen.

Pl^{A1} and Pl^{A2} platelets have been reported (Figure 1), but no consistent pattern has emerged (13, 14).

A fundamental problem confronting investigators in such studies is the notorious variability of platelet function assays. To circumvent this problem, Vijayan et al. (2) established cell lines expressing either the Pl^{A1} or Pl^{A2} forms of $\alpha_{IIb}\beta_3$. Working with these cells, the authors found no differences in soluble fibrinogen binding to the Pl^{A1} and Pl^{A2} cells. However, significantly more Pl^{A2} than Pl^{A1} cells adhered to immobilized fibrinogen. The greater adhesion of the Pl^{A2} cells was associated with greater spreading and more extensive actin polymerization on the fibrinogen substrate. Such changes are likely to reflect differences between Pl^{A2} and Pl^{A1}, in the nature or the efficiency of outside-in signals that are generated when $\alpha_{IIb}\beta_3$ encounters a thrombogenic substrate. Consistent with this interpretation, tyrosine phosphorylation of $pp125^{FAK}$ — a kinase that is frequently activated during integrin signaling — was enhanced in the Pl^{A2} cells. Also, clot retraction, another response that depends on outside-in signaling, was enhanced in the Pl^{A2} cells. The latter observation is particularly notable because retracted clots are more resistant to thrombolysis, so clots containing Pl^{A2} platelets would be expected to persist longer and pose greater danger than clots that form with only Pl^{A1}. To be sure, the differences in $\alpha_{IIb}\beta_3$ function between the Pl^{A1} and Pl^{A2} cells were quite subtle, but marked differences would not be expected: the Pl^{A2} allele is proposed as a risk factor, and not a cause of thrombosis.

The challenge now lies in verifying whether the differences identified by Vijayan et al. are indeed responsible for the thrombotic risks associated with the Pl^{A2} polymorphism. Additional epidemiological studies and data analyses are needed to determine whether the Pl^{A2} genotype is a risk of cardiovascular disease. At a more general level, the possibility remains that still other platelet SNPs of equal or even greater prognostic value can be identified.

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