Glycoprotein IIb/IIIa receptor and PlA2 polymorphism

The GPIIb/IIIa receptor represents the final common pathway for platelet activation and several studies pointed out the role of this gene polymorphism on the pathogenesis and development of CAD. GPIIb/IIIa receptor is present on platelet membrane as a non-covalent heterodimeric complex, composed of an alpha subunit (GPIIb, also named αIIb) and a beta unit (GPIIIa, also named β3).

Calcium is necessary to maintain the receptor as an heterodimer and this partially explains the pivotal role of calcium in fibrinogen binding to GPIIb/IIIa receptor. Figure 1 shows a schematic model of the receptor. Its major ligands are fibrinogen and vonWillebrand factor. After platelet adhesion following vascular damage, platelets undergo an activation process that produces a conformational change in the GPIIb/IIIa receptor so that it assumes a high binding affinity for fibrinogen and other proteins involved in the coagulation cascade, such as the vonWillebrand factor.

The GPIIa subunity of such receptor is a high polymorphic protein, with at least seven different allelic isoforms. The most common allelic variant is represented by human platelet antigen-1 (PIA1). In the PIA2 allelic isoform, cytosine is substituted for thymidine in exon 2, which is phenotypically translated in a substitution of proline for leucine at position 33 of the mature GPIIIa.
Previous in vitro studies have shown that the PlA2 variant enhances the binding of the GPllb/llla receptor to fibrinogen and therefore increases the platelet aggregation induced by agonists, leading to an hypercoagulable state. Several studies have shown that subjects harbouring PlA2 allele, both in heterozygosis and in homozygosis, have shortened bleeding time compared with PlA1/PlA1 subjects[13]. Moreover, PlA2-positive platelets displayed a lower threshold for activation when stimulated by various agonists such as adenosine diphosphate or epinephrine[17,19].

In spite of consistent findings reported in molecular studies, clinical studies failed to establish a clear correlation between such polymorphism and ischaemic cardiovascular disease and therefore, up to date, available data are uncertain and still debated. Here we report a review of the current literature regarding the potential association between GpIIa PlA2 polymorphism and cardiovascular disease, focusing on the impact of such polymorphism on CAD and outcome after percutaneous coronary intervention (PCI).

**PlA2 polymorphism and cardiovascular disease**

In 1996, Weiss and co.[18] reported for the first time a significant association between the PlA2 polymorphism and acute coronary thrombosis. In that study, the presence of at least one PlA2 allele was correlated with a 2.8 fold increase in the risk for myocardial infarction (AMI) [19]; the association was stronger in patients who had had coronary events before the age of 60 years, suggesting this polymorphism as an inherited risk factor for ACS[10].

Some years later, the Copenhagen City Heart Study, a prospective study enrolling 9,149 subjects, found a three-fold increased risk of ischaemic cardiovascular disease and a fourfold increased risk of AMI in men <40 years homozygous for PlA2 polymorphism[11]. These findings were further expanded by studies on peripheral atherosclerotic disease. Indeed Mikkelsen et al. reported a significant association between the presence of PlA2 polymorphism and the progression of atherosclerosis in abdominal aorta[12]. Accordingly, our group reported that, in a high risk hypertensive patients population, those harbouring PlA2 allelic variant showed an higher incidence of stroke compared to non-carriers patients[13]. A recent study showed a significant association between PlA2 allele and clinical atherothrombosis in type 2 diabetic patients[14].

Several studies investigating a relation between PlA2 prevalence and traditional cardiovascular risk factor found that the association between PlA2 and MI was strongest in patients with a low cardiovascular risk-profile, suggesting a higher frequency of PlA2 carriers in patients with CAD not explained by traditional risk factors[15]. Accordingly, we recently showed a significant positive association between PlA2 polymorphism and adverse prognosis in two high risk populations represented by patients with severe CAD requiring mechanical myocardial revascularisation and hypertensive patients with previous cerebrovascular events[16].

On the other hand, several studies failed to confirm the association between the presence of PlA2 polymorphism and increased cardiovascular risk. In 1997, Ridker et al. performed a subgroup analysis of the physicians’ health study (PHS)[17] prospectively investigating the association between PlA2 polymorphism and incidence of cardiovascular disease[18]; they studied 1408 patients and found no association between the presence of polymorphism and incidence of myocardial infarction, stroke or venous thrombosis.

Nevertheless, it should be underlined that the PHS was conducted in a very healthy population, with an event rate four-times lower than general population. A meta analysis of 23 independent studies found no evidence supporting a positive relation between MI and PlA2 allele, and this result persisted even after subgroup analysis[19]. A second meta analysis examined 34 studies to explore the correlation between PlA2 and both CAD and MI founding a significant but weak association between such polymorphism and cardiovascular disease[20]. The association appeared to be stronger when less heterogenous groups of patients were considered (i.e., younger patients-age<60 years- or patients who had undergone PCI and experienced stent restenosis)[19]. Since atherosclerosis is a multifactorial disease, the relative impact of PlA2 polymorphism appears to be very hard to assess in this clinical setting: in fact, it may vary notably according to multiple factors, such as genetic heterogeneity, gene-gene and gene-environment interaction[21].

Therefore, it is not possible to explain interindividual variations of cardiovascular disease based on genetic inheritance alone. Mikkelsen et al. investigated the association of PlA2 polymorphism with the development of coronary atherosclerosis by studying 300 middle-aged Finnish men suffering sudden out-of-hospital death. They found that the prevalence of PlA2 allele was higher in sudden death victims with severe coronary narrowing and with coronary thrombosis compared to those with severe coronary stenosis in the absence of thrombosis. Moreover, PlA2 carriers showed a larger area of fissured and ulcerated complicated lesions in their coronary tree, suggesting that PlA2 carriers may harbour more vulnerable coronary plaques and, as a consequence, a higher risk of coronary thrombosis and ACS[22,23].

Therefore, the impact of PlA2 polymorphism on cardiovascular disease could be higher in patients who are at high risk for cardiovascular events, such as patients that already had a clinical manifestation of cardiovascular disease[16].

**PlA2 and outcome after percutaneous coronary intervention**

PCI has become the preferred therapeutic strategy for patients with ACS and stable CAD. Restenosis after angioplasty and stent implantation remains the most significant problem in coronary interventional treatment[24]. Even if the use of drug-eluting stents (DES) has dramatically reduced the rates of restenosis and target vessel revascularisation (TVR) compared to bare-metal stents (BMS), a low rate of in-stent restenosis still exists, and its prevalence cannot be neglected since the large number of patients treated with DES[25]. It has been shown that there is a dependence of restenosis between coronary lesions in patients who undergo a multi-lesion PCI, with higher risk of restenosis for a lesion occurring when the patient experienced a restenosis of another lesion, independently of common cardiovascular risk factors[26].

These data suggest that there are additional patient-specific factors that could play a role in the process of restenosis, and should be considered in the assessment of risk of restenosis. In this regard, it has been shown that coronary stent insertion elicits a major platelet activation, with increased binding of von Willenbrand factor and surface expression of P-selectin on circulating platelets for days after PCI[27] and hyperplastic response[28,29], compared with balloon angioplasty. Stent-thrombosis (ST) occurs rarely (0.5% to 1% per year) but is associated with a very high incidences of death (20% to 40%), MI (50% to 70%) and repeat revascularisation[30-32]. Stent deployment on coronary lesions exposes thrombogenic subendothelium to circulating blood, representing a major stimulus for platelet activation[27].
Therefore, polymorphisms influencing platelet activation and function are likely to be involved in mechanisms of restenosis and thrombosis after coronary stenting. Several studies have investigated the potential role of PLA2 polymorphism in development of stent thrombosis and restenosis. Walter et al. studied 318 patients undergoing coronary stent implantation and they found a strong association between PLA2 and coronary stent thrombosis at 1-month follow-up. The presence of PLA2 polymorphism was the only significant independent predictor of stent-vessel occlusion on multivariate analysis. Interestingly, PLA2 carriers showed a significantly lower classic risk-factor profiles than PLA1/PLA1 patients.\(^{33}\)

Abbate et al. found a trend toward higher incidence of stent restenosis in an Italian population of patients who underwent PCI and were followed-up for six months after stent placement.\(^{15}\) Kastrati et al. studied 1150 consecutive patients who underwent successful coronary stent implantation and investigated the relation between PLA2 polymorphism and stent restenosis at six-month angiographic follow-up. They found a significant association between the PLA2 polymorphism and restenosis after coronary stenting. The association was stronger for patients homozygous for PLA2 allele and in women,\(^{34}\) consistent with previous report of a more easily inducible platelet GPIIb/IIIa receptor activation and lesser epifibatide antithrombotic effect in women compared with men.\(^{35,36}\)

We previously reported that in patients with severe CAD undergoing PCI the presence of PLA2 allelic variant was associated with a significant higher incidence of death, re-MI and overall major adverse cardiac events (MACE) at long term follow-up, and with a non-significant higher incidence of TVR and re- percutaneous transluminal coronary angioplasty (PTCA).\(^{16}\) Moreover, our preliminary data show that in DES-treated patients, presence of PLA2 allele was associated with a significant higher incidence of re-MI, re-PTCA, TVR and overall MACE compared with BMS-treated group.\(^{37}\)

Although our findings were carried out in a small study group and we used clinical surrogate endpoints of stent thrombosis, these results could be consistent with hypothesis of an increased thrombogenic milieu in PLA2 carriers, that can play a pivotal role in the development of coronary thrombosis in context of delaying endoluminal healing at the angioplasty site occurring after DES placement. However, if these findings will be corroborate by further investigation on larger number of patients, PLA2 allele might be also considered as an useful predictive marker in the clinical decision making between BMS or DES during PCI. On this regard, it is valuable that several studies have reported diminished sensitivity to common antiplatelet therapy in PLA2 carriers.\(^{5,38-40}\)

Laule et al. studied 280 consecutive patients with angiographically confirmed coronary-artery disease, treated with coronary stenting and found no significant difference in the composite end-point of TVR, AMI and death between PLA2 carriers and PLA1/PLA1 subjects.\(^{41}\) Moreover, a study including 1759 consecutive patients with stable and unstable angina underwent successful PCI, failed to find a significant association between the presence of PLA2 allele and adverse clinical outcome after stenting at 30-day follow-up, whereas a significant association was shown for homozygous carriers of the PLA2 allele.\(^{42}\) However, since the low frequency of PLA2 homozygosis in the general population, these results should be confirmed in a much larger patients population.

Taken together, these findings could support the relevance of PLA2 polymorphism not only for stratification of cardiovascular risk, but also for planning the most tailored stent type placement and antiplatelet therapy to suppress risk for thrombosis after stent placement in these patients.

**CONCLUSIONS**

Over the past years, several studies have attempted to demonstrate a correlation between the PLA2 polymorphism of GPIIa platelet receptor and increased cardiovascular risk, but at the present time their results appear to be inconclusive. This could be mostly attributed to differences in the design and in the choice of control group and endpoints of the studies. Moreover, ethnicity, environmental factors and selection of patients and controls differ considerably among the studies. Another important cause of irreproducible findings could be variation in linkage disequilibrium.\(^{21}\) Given the multiple possible presentation of CAD, accurate phenotyping is particularly important. Although considerable overlap exists among various CAD phenotypes, the underlying pathophysiological mechanism could vary considerably.

Therefore, every effort has to be done to carefully characterise patients and control phenotype, by using rigorous and uniform criteria for cardiovascular events and for important cardiovascular risk factors such as CAD positive family history or smoking habitus. Therapy provided to patients can also be a source of bias. Notably, studies focusing on investigation of impact of PLA2 on outcome of patients after PCI often differ in terms of antithrombotic therapy provided to patients. An analysis of three of these studies has suggested that the impact of PLA2 on outcome of patients who underwent PCI could be smaller when a more aggressive antithrombotic regimen is provided.\(^{43}\) In summary, PLA2 genetic variant of GPIIa platelet receptor might identify a group of CAD patients with particularly high risk of atherothrombotic complications and, as a consequence, could be considered an useful marker in the clinical and therapeutic decision making.

However, further studies on high-selected patients, focusing on interactions between environment, common cardiovascular risk factors and platelet associated genetic determinants are warranted in order to clarify the populations in which this polymorphism may be more determinant.

**REFERENCES**