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**Review**

# Genetic factors underlying differential blood platelet sensitivity to inhibitors

Marcin Rozalski, Magdalena Boncler, Bogusława Luzak, Cezary Watala

Department of Hemostasis and Hemostatic Disorders, Medical University of Łódź, Żeromskiego 113,  
PL 90-549 Łódź, Poland

**Correspondence:** Cezary Watala, e-mail: cwatala@csk.am.lodz.pl

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**Abstract:**

Blood platelets are not only the primary defence mechanism involved in physiological hemostasis, but also their disorders constitute a crucial risk factor in arterial thrombosis. As arterial thrombi are composed of predominantly platelets formed under conditions of elevated shear stress at sites of atherosclerotic vascular injury and disturbed blood flow, the prevention of arterial thrombosis has been for years the main target for antiplatelet therapy. Individual differences in the rate of platelet activation and reactivity markedly influence normal hemostasis and the pathological outcome of thrombosis. Such an individual variability is largely determined by environmental and genetic factors. These are known to either hamper platelets' response to agonists, and thereby mimic the pharmacological modulation of platelet function or mask therapy effect and sensitize platelets. Some clinical studies have indicated that platelet glycoprotein polymorphisms are genetic factors contributing to arterial thrombosis. In spite of some discrepancies between different studies, there is substantial evidence that the integrin  $\beta_3$   $PI^{A2}$  allele, the variants  $GPIIb\alpha$  Met<sup>145</sup> and  $GPIIb\alpha$  <sup>-5</sup>C haplotype or the integrin  $\alpha_2$  haplotype 1 (<sup>807</sup>T) each contribute to the risk for and morbidity of thrombotic disease. In this article, we reviewed a role of the aforementioned polymorphisms in modulating platelet function and platelet response to inhibitors. The paper focuses on the association between  $PI^{A1/A2}$  polymorphism and sensitivity (or resistance) to aspirin and the inhibitory efficacy of  $GPIIb$ -IIIa antagonists. Additionally, a potential role of <sup>807</sup>C/T polymorphism ( $GPIa$ ), polymorphisms of  $GPIb$  and platelet purinoreceptor  $P2Y_{12}$  in affecting platelet sensitivity to blocking agents is discussed.

**Key words:**

thrombosis, blood platelets, polymorphism,  $GPIIb$ -IIIa antagonists, aspirin

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## A role of blood platelets in hemostasis and thrombosis

Blood platelets are small anuclear cells involved in blood coagulation. Upon vessel injury, platelets become activated, release a content of granules, undergo a shape change, aggregation and adhesion, which finally leads to clot formation and prevention of bleeding. In addition to these types of early platelet response, altogether called a primary hemostasis, platelets also promote the activation of coagulation factors

on the surface of plasma membrane. The normal platelet response is critical to the maintenance of physiological hemostasis, notwithstanding, platelet hyperactivity leads to undesirable thrombosis [4]. Arterial thrombi are composed of predominantly platelets formed under conditions of elevated shear stress at sites of atherosclerotic vascular injury and disturbed blood flow. Hence, the prevention of arterial thrombosis has been for years the main target for antiplatelet therapy. For about 50 years, acetylsalicylic acid (Aspirin®, ASA) has been employed as the major

antiplatelet agent [6, 10, 32]. Despite its clinical effectiveness and safety, it is a relatively weak antiplatelet agent having some limitations [6, 10]. This prompted a search for other antiplatelet strategies and recently there has been a considerable progress in this field. Amongst several new-generation inhibitors of platelet-mediated thrombosis, including inhibitors of platelet adhesion, inhibitors of specific platelet agonist-receptor interactions (antithrombins, fibrinogen receptor antagonists, thromboxane A<sub>2</sub> receptor antagonists, ADP receptor blockers) or inhibitors of thromboxane synthase and arachidonic acid metabolism, only the antagonists of platelet receptors have been demonstrated to allow for more global interruption of both the initial and final steps of platelet activation, adhesion and aggregation [12, 126, 137, 144].

#### **Genetic and environmental factors governing platelet reactivity**

Individual differences in the rate of platelet reactivity markedly influence normal hemostasis and the pathological outcome of thrombosis. Such an individual variability is largely determined by environmental and genetic factors. These are known to either hamper platelets' response to agonists, and thereby mimic the pharmacological modulation of platelet function or mask therapy effect and sensitize platelets. Apart from some common environmental factors, like age or sex, smoking, exercise, hormonal effects, depression or vitamin supplementation [37], also diet and hyperlipidemia largely contribute to an overall picture of platelet reactivity. Whereas the former is relevant to the regulation of prostanoid metabolism, the latter seems critical in modulating of platelet membrane lipid fluidity and intracellular signaling [41, 44, 64, 113].

Some clinical studies indicate that also genetic polymorphisms of platelet receptors are factors having an impact on platelet reactivity, and, hence, could contribute to arterial thrombosis. Discrepancies between different studies in the degree to which platelet glycoprotein polymorphisms contribute to overall risk for clinical thrombosis largely originate from the fact that most of the clinical studies differ by patient population size, ethnicity, bias in the selection of patients and controls, plurality in clinical endpoints and variation of environmental factors. In spite of these differences, there is a substantial evidence that the following allelic variants: the integrin  $\beta_3$  P1<sup>A2</sup>, GPIIb  $\alpha$  Met<sup>145</sup> and GPIIb  $\alpha$  -<sup>5</sup>C haplotype, and the integrin  $\alpha_2$  <sup>807</sup>T

each contribute to the risk for and morbidity of thrombotic disease, although they may remain disputable as to the extent of their contribution [2, 112, 115].

#### **P1<sup>A1/A2</sup> as a major genetic polymorphism of glycoprotein IIb-IIIa**

The platelet heterodimer GPIIb-IIIa ( $\alpha_{IIb}\beta_3$ ), a member of integrins, is a fibrinogen receptor, which also exhibits an ability for binding the von Willebrand factor, fibronectin and thrombospondin. The GPIIb-IIIa complex is expressed exclusively on blood platelets and megakaryocytes and is the most abundantly represented glycoprotein on platelet surface (40,000–50,000 copies per platelet on resting cells). The binding of fibrinogen to the active form of GPIIb-IIIa is the final agonist-independent step leading to platelet aggregation and formation of thrombus [20, 49]. The subunit  $\beta_3$  (GPIIIa) of this platelet membrane integrin appears to be dimorphic in the position of Leu  $\rightarrow$  <sup>33</sup>Pro, which results from a single nucleotide C<sup>1565</sup>  $\rightarrow$  T transition in the GPIIIa gene and is commonly known as platelet-specific antigen (P1<sup>A1/A2</sup>) polymorphism [105].

In 1996, based on the observation of the higher frequency of the P1<sup>A2</sup> allele in patients with unstable angina and myocardial infarction in comparison with healthy donors, Weiss et al. suggested that the carriers of the P1<sup>A2</sup> are at potentially increased risk of arterial thrombosis [151]. Since then, however, conflicting results on the role of this polymorphism have been reported in a number of case-controlled clinical studies. Many investigators found no differences in P1<sup>A1/A2</sup> variant frequency between healthy donors and patients with myocardial infarction [17, 18, 50, 54, 61, 62, 87, 116, 122], ischemic heart disease (IHD) [36, 50] or stroke [21, 36, 116]. In contrast, in other reports, a higher prevalence of the P1<sup>A2</sup> allele was demonstrated in stroke [23, 24, 139, 147], IHD and myocardial infarction [24, 50, 56]. Meta-analysis of 12 epidemiological studies showed that there was an association between P1<sup>A2</sup> variant and an increased risk of coronary heart disease [18]. Interestingly, some reports suggest that P1<sup>A1/A1</sup> homozygotes may be prone to early atherosclerosis and more rapid progression of stable coronary artery disease [60, 98], whereas carriers of the P1<sup>A2</sup> allele are more prone to thrombotic complications [98].

Noteworthy, a controversy also exists about influence of the  $PI^{A1/A2}$  polymorphism on functional platelet parameters, such as reactivity and ligand binding. There are reports pointing to either hyperreactivity [51, 97, 150] or hyporeactivity [9, 88] of  $PI^{A2}$ -positive platelets. Some authors found moderately but significantly enhanced binding of fibrinogen to  $PI^{A2}$ -positive cells [58], while others failed to observe any association [36, 96, 97]. The impact of  $PI^A$  on adhesive properties of GPIIb-IIIa was studied using Chinese hamster ovary cells and human kidney embryonal 293 cells overexpressing the  $PI^{A1}$  or  $PI^{A2}$  polymorphic forms of the GPIIb-IIIa [145]. In this elegantly designed model, it was demonstrated that although the  $\alpha_{IIb}\beta_3$ -mediated binding of soluble fibrinogen was not different between  $PI^{A1}$  and  $PI^{A2}$  forms, the  $PI^{A2}$ -positive cells bound more easily to immobilized fibrinogen. The fibrinogen binding was also enhanced by shear stress [146]. The increased binding of the  $PI^{A2}$ -positive cells to immobilized fibrinogen is related to the increased actin polymerization, greater cell spreading, enhanced tyrosine phosphorylation of pp125<sup>FAK</sup> and greater fibrin clot retraction [145].  $PI^{A2}$  variant was also associated with augmented activation of ER kinase (ERK2).

As regards the association between  $PI^A$  genotype and platelet sensitivity to antiplatelet drugs, two aspects should be discussed: i)  $PI^{A1/A2}$  and ASA resistance and ii)  $PI^{A1/A2}$  and sensitivity to GPIIb-IIIa blockers.

#### $PI^{A1/A2}$ polymorphism and “resistance to aspirin”

ASA is a non-steroidal anti-inflammatory drug, which shows antiplatelet effect at lower concentrations, and, therefore, is widely used in prevention and treatment for thromboembolic complications. Antithrombotic action of ASA is caused by irreversible acetylation-dependent inhibition of platelet cyclooxygenase (at the functionally important amino acid Ser<sup>530</sup>), an enzyme responsible for biosynthesis of prostanoids such as PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , PGI<sub>2</sub> and also thromboxane A<sub>2</sub> (TxA<sub>2</sub>) that is described to be involved in developing of thrombosis [90, 107, 133].

Several controlled studies demonstrated a remarkable inter-individual variability regarding the inhibition of platelet function by ASA, especially at low drug doses [27, 65]. Along with such a variability, there is also a considerable heterogeneity in the yield of a protection against thromboembolic complications

by ASA, reported particularly in some clinical states, including patients with unstable angina or those undergoing cardiac surgery [19]. This phenomenon is known as reduced blood platelet sensitivity to ASA and often referred to as the so-called “ASA-resistance”. Nowadays, there is no unambiguous evidence pointing to the cause of the reduced blood platelet response to ASA, nevertheless, multiple mechanisms of the resistance have been proposed, including the increased reactivity to platelet aggregating factors, genetic polymorphisms, alternate pathways for Tx synthesis, hypertension or ASA competition with short-lived, alternative non-steroidal anti-inflammatory drugs (e.g. ibuprofen) [39, 65, 107, 108, 127, 150].

Noteworthy,  $PI^{A1/A2}$  polymorphism has been postulated to modulate an anti-platelet effect of ASA but, again, results are inconsistent. Cooke et al. found that ASA inhibited *in vitro* platelet aggregation stronger in  $PI^{A2/A2}$  homozygotes as compared to  $PI^{A1/A1}$  variant [34]. Interestingly, Michelson et al. [97], using epinephrine-induced platelet aggregation, showed that  $PI^{A1/A2}$  heterozygotes were the most sensitive to ASA *in vitro* (2.5 and 5  $\mu$ mol/l), whereas  $PI^{A2/A2}$  homozygotes were characterized by the highest IC<sub>50</sub> value. Macchi et al., based on *ex vivo* studies with the use of platelet function analyser (PFA-100), found that  $PI^{A1/A1}$  homozygosity was associated with depressed sensitivity to low-dose ASA (160 mg/d for a month) [93]. In contrast, Undas et al. using *ex vivo* studies, provided evidence that in healthy donors taking 75 mg of ASA daily for 7 days, ASA depressed thrombin generation (measured as an increase in F1 + 2 fragment plasma concentration) significantly higher in  $PI^{A1/A1}$  homozygotes, whereas  $PI^{A2}$  carriers were resistant to the drug [142]. The same research group investigated the relationship between the  $PI^{A1/A2}$  polymorphism and bleeding time in young healthy men treated with ASA [140]. Before ASA ingestion, bleeding time was found to be shorter in carriers of the  $PI^{A2}$  than in carriers of the  $PI^{A1/A1}$  allele. At 4 h after ingestion of 300 mg of ASA, bleeding time was prolonged, and the inter-group difference was enhanced [140]. The same authors in their next work examined more thoroughly effects of  $PI^{A1/A2}$  polymorphism on blood coagulation and efficiency of ASA [141]. In normal subjects, the authors assessed an effect of ASA ingestion (75 mg/d for 7 days) on a plethora of hemostatic parameters, including the activation of prothrombin and factor V, factor XIII and fibrinogen removal, thrombin-antithrombin complex generation, and levels of fibri-

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nopeptides A and B, in blood collected every 30 s at the sites of standardized microvascular injury. In general, the results suggested that carriers of the P1<sup>A2</sup> allele were more resistant to the antithrombotic action of ASA [141].

### **P1<sup>A1/A2</sup> polymorphism and platelet sensitivity to GPIIb-IIIa antagonists**

Since fibrinogen binding to its platelet receptor is the final agonist-independent step leading to platelet aggregation and formation of thrombus [20, 49], GPIIb-IIIa has become an attractive target for a blockade with various antagonists. GPIIb-IIIa blockers can be classified into several groups according to their basic chemical structure. The classes of GPIIb-IIIa antagonists include monoclonal antibodies, disintegrins (peptides isolated from snake venoms), synthetic peptides and non-peptide agents [120]. Despite development of a number of GPIIb-IIIa antagonists, only a few of them underwent all the steps of clinical trials. To date, abciximab (monoclonal antibody c7E3-Fab), eptifibatide (synthetic cyclic heptapeptide) and tirofiban (non-peptide agent) found clinical applications.

In our laboratory, we evaluated the inhibition of ADP- and collagen-induced platelet aggregation by disintegrins: kistrin and echistatin, as well as the low molecular-weight blockers: GR144053F (4-(4-(4-(aminoiminomethyl)phenyl)-1-piperazinyl)-1-piperidineacetic acid, hydrochloride trihydrate) and eptifibatide (N<sup>6</sup>-(aminoiminomethyl)-N<sup>2</sup>-3-mercapto-1-oxopropyl-L-lysylglycyl-L- $\alpha$ -aspartyl-L-tryptophyl-L-prolyl-L-cysteinamid, cyclic (1–6)-disulfide). We found that kistrin (10–30 nM) inhibited ADP- and collagen-induced platelet aggregation stronger in P1<sup>A2</sup>-negative donors and the difference between IC<sub>50</sub> values was significant [121]. The same tendency occurred at moderate concentrations of eptifibatide (40–100 nM), and also at low concentrations of GR144053F (5–10 nM), and high concentrations of echistatin (80–150 nM), although in the case of the two latter inhibitors, the estimated IC<sub>50</sub> values were not significant [121]. In general, our results suggest that GPIIb-IIIa blockers representing various classes are less effective inhibitors of platelet aggregation in the P1<sup>A2</sup>-positive carriers, however, the effect of the genotype is both agonist- and antagonist-dependent [121]. On the other hand, Michelson et al. found that P1<sup>A1/A2</sup> heterozygotes were more sensitive to abciximab as compared to other genotypes [97]. Weber et al. failed to find any

association between P1<sup>A1/A2</sup> genotype and the inhibition of fibrinogen binding by abciximab or eptifibatide.

Overall, the presently available data still do not allow for the conclusion that the GPIIIa polymorphism alone represents a cardiovascular risk factor in the general population. The observed differences in platelet function and sensitivity to inhibitors between P1<sup>A1</sup> and P1<sup>A2</sup> carriers are hardly modest or even controversial. From the evolutionary point of view, this seems not surprising, simply because more profound alterations would have certainly resulted in the elimination of P1<sup>A2</sup>-positive phenotypes by natural selection [121, 146]. However, some model experiments and a series of clinical studies suggest that it may be a risk factor in certain subgroups of patients under certain clinical conditions.

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### **Polymorphisms of platelet collagen receptors and platelet sensitivity to antagonists**

Collagen is a major adhesive protein exposed to platelets after a blood vessel injury [31]. Interaction between collagen and blood platelets results in their adhesion, activation and aggregation, that in consequence leads to formation of hemostatic plug [132]. Platelets interact with collagen *via* several surface receptors, and the major role is attributed to  $\alpha_2\beta_1$  integrin (GPIIb/IIIa) [128, 131] as well as glycoprotein VI (GPVI) [99, 106, 125, 143]. Recently, the concept of a ‘multisite multistep’ model of the platelet-collagen interaction has been considered [148]. In this model, the primary receptor for platelet-collagen adhesion  $\alpha_2\beta_1$  is engaged in the initial direct contact with collagen and is responsible for the arrest of platelets on the subendothelial collagen surface [45, 66, 129]. This allows another collagen receptor molecule, GPVI, to further activate blood platelets and transmit the signal through the GPVI-FcR $\gamma$ -chain pathway. It is commonly believed that GPVI is responsible for collagen-induced platelet activation resulting in protein phosphorylation (FcR $\gamma$ , tyrosine kinase *syk*, PLC $\gamma$ 2) [13, 55] and platelet aggregation [110]. Other receptors, like GPIV, TIIICBP (Type III Collagen Binding Protein) or p65, may be involved in very early platelet-collagen interactions, but their roles are minor.

### Genetic polymorphisms of integrin $\alpha_2\beta_1$

Integrin  $\alpha_2\beta_1$  is involved in early steps of platelet adhesion to collagen under high shear stress [5, 132]. It has been demonstrated that  $\alpha_2\beta_1$  is activated by various agonists, including thrombin, ADP or Txs (inside-out signaling) and it leads to a different affinity of collagen binding to the receptor [70–73]. The transduction of signal originated from the interactions of platelets with collagen by GPVI can evoke the high-affinity state of  $\alpha_2\beta_1$  [72]. Nowadays, it is accepted that GPVI and  $\alpha_2\beta_1$  play complementary roles in collagen-induced thrombus formation under flow conditions [82] and both the receptors are required for a full collagen-induced platelet activation [29].

Several single-nucleotide polymorphisms have been described in cDNA coding for GPIa subunit. The most important polymorphism seems to be  $^{807}C/T$  polymorphism that remains in a linkage disequilibrium with other GPIa polymorphisms ( $^{837}C/T$ ,  $^{873}A/G$ ,  $Br^{a/b}$ ) and gives rise to three alleles [80, 114]. The polymorphism  $^{807}C/T$  (codon Phe<sup>224</sup>),  $^{837}C/T$  (codon Ala<sup>234</sup>) and  $^{873}A/G$  (codon Thr<sup>246</sup>) are conservative and do not alter the deduced amino acid sequence of the translated protein [80]. However, the polymorphism  $Br^{a/b}$  ( $^{1648}G/A$ ) which determines HPA-5 a/b alloantigen system is related to an amino acid change of Glu<sup>505</sup> to Lys<sup>505</sup> [124]. A significant association between the expression levels of integrin  $\alpha_2\beta_1$  on platelets and a presence of  $^{807}C/T$  allelic variants has been established [80, 85, 86]. The highest receptor density was found to be attributed to  $^{807}T/T$  homozygotes (allele 1), while lower density was related to  $^{807}C/C$  variant (alleles 2 and 3) [80, 85]. Differences in the receptor density directly correlate with a rate of platelet attachment to type I collagen in whole blood under high shear rates (1500/s) [80]. The occurrence of the GPIa  $^{807}T$  allele causes a higher GPIaIIa expression, thus enhancing platelet binding to collagen [84]. Indeed, variation in GPIaIIa receptor density, associated with the above-mentioned dimorphisms, has been shown to correlate with functional differences in platelet adhesiveness to collagens, as well as differentiated platelet sensitivity to ADP, when the  $^{807}T/T$  genotype is related to the increased platelet adhesion to type I monomeric collagen induced by ADP [92]. Moreover, the variations in plasma vWF and  $\alpha_2\beta_1$  expression affected platelet adhesion to collagen under flow conditions [118]. The occurrence of allele 1 ( $^{807}T/T$ ) has been regarded as a risk factor of throm-

boembolic complications in patients with diabetic retinopathy [94] or myocardial infarction and of stroke in young patients [22, 81, 123]. As deduced from clinical trials and epidemiological studies, the discussed polymorphisms of GPIa seems to be rather mild risk factor that is particularly important in synergism with other known risk factors, such as smoking, hypertension, or diabetes, which may enhance its contribution to the overall risk of cardiovascular and atherothrombotic events [117].

### Antagonists of collagen receptors and a potential modulating effect of genetic polymorphisms

A number of agents inhibiting platelet adhesion to collagen *via* integrin  $\alpha_2\beta_1$  have been identified. They include snake venom proteins (crovidisin, catrocolastatin, jaracetin, jararhagin) [30, 42, 75], monoclonal antibodies (Gi9, 6F1) [101, 104] and synthetic peptides (DGEA, GFOGER) [79, 136]. Undoubtedly, depending on their specificity, the antagonists of platelet receptors are not only useful tools in model studies on the molecular mechanisms of platelet function, but also antiplatelet drugs are of a potential use in antithrombotic therapy. Of the variety of  $\alpha_2\beta_1$  antagonists, anti- $\alpha_2\beta_1$  monoclonal antibodies are the most frequently used for model studies on  $\alpha_2\beta_1$  function. The promising group of blockers involves peptides having sequences similar to those of collagen molecule, which are recognized by  $\alpha_2\beta_1$ . Staatz et al. [136] were the first who described a series of peptides derived from the  $\alpha 1(I)$ -CB3 fragment of type I collagen, which are recognized by  $\alpha_2\beta_1$  and have the ability to inhibit cell adhesion to collagen. In these studies, the minimal active recognition sequence Asp-Gly-Glu-Ala (DGEA), corresponding to residues 435–438 of type I collagen sequence, was identified. Since DGEA-containing peptides effectively inhibited the  $\alpha_2\beta_1$ -mediated  $Mg^{2+}$ -dependent platelet adhesion to collagen, this tetrapeptide was considered as a specific blocker of  $\alpha_2\beta_1$ -dependent functions [136]. In the next years, the inhibiting properties of DGEA were questioned by some other investigators [15]. Recently, we have shown that DGEA peptide is a strong antagonist interfering with a variety of collagen-platelet interactions and it can be recognized not only by  $\alpha_2\beta_1$  but also by other collagen receptors [91].

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In the literature, there are only scarce papers reporting a role of GPIa polymorphisms in platelet function and platelet sensitivity to inhibitors. In our laboratory, we studied an effect of <sup>807</sup>C/T polymorphism on platelet sensitivity to an adhesion inhibitor, anti-GPIaIIa–Gi9 monoclonal antibody. We observed that in <sup>807</sup>T/T subjects, both spontaneous and either thrombin- or ADP-induced platelet adhesion to type I fibrillar collagen was significantly blocked by Gi9 (10 µg/ml). Moreover, <sup>807</sup>T/T platelets were significantly more sensitive to Gi9 in case of both spontaneous and thrombin-evoked adhesion, as compared to <sup>807</sup>C/C platelets. In <sup>807</sup>C/C subjects, Gi9 only slightly inhibited platelet adhesion. Irrespectively of <sup>807</sup>C/T genotype Gi9 antibodies significantly inhibited platelet adhesion to monomeric collagen with stronger effect for both ADP- and U46619-induced adhesion compared to the adhesion evoked with thrombin. There was a tendency, although below statistical significance, that platelet adhesion to monomeric collagen was inhibited by Gi9 much stronger in <sup>807</sup>T/T donors vs. <sup>807</sup>C/C genotype, which suggests that <sup>807</sup>C/C platelets are less sensitive to Gi9. It could be speculated that the strength of inside-out signaling per every single copy of the  $\alpha_2\beta_1$  receptor is crucial for its affinity to collagen. If so, <sup>807</sup>C/C platelets characterized by low receptor density could have higher affinity to collagen and be less sensitive to blockers in comparison to <sup>807</sup>T/T platelets stimulated under the same conditions [91].

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## Polymorphisms of glycoprotein Ib and platelet pharmacology

The GPIb-V-IX receptor is a key mediator of platelet adhesion under high shear conditions [119]. As within the extracellular domain of GPIb  $\alpha$  there are a few distinct binding sites for its physiological protein targets, von Willebrand factor (vWF) and thrombin, subunit  $\alpha$  of GPIb is considered to be the most important one in the receptor complex [25, 43, 59, 77]. Moreover, GPIb  $\alpha$  is highly polymorphic. The molecular weight polymorphism located in the heavily glycosylated macroglycopeptide region of GPIb  $\alpha$  results from a variable number of tandem repeats (VNTR) of 39-bp sequence and comprises four variants of GPIb  $\alpha$  (D, C, B and A ranging from the short-

est to the longest) determining the external projection of GPIb molecule [100]. In such a situation, the D and C isoforms contain one and two 13-amino acid repeats, whereas B and A isoforms are characterized by as many as three and four tandem repeats. Close to vWF- and thrombin-binding sites, the Thr/Met<sup>145</sup> dimorphism has been found, which determines the antigenicity of GPIb and is referred to as Human Platelet Antigen 2 (HPA-2) [102]. A third polymorphism in the GPIb  $\alpha$  gene, a single nucleotide substitution (T/C) in the noncoding region, 5 base pairs upstream of the initiation codon was first reported by Kaski et al. [76]. The sequence surrounding the translation start site is crucial for effective translation of eucariotic mRNA and it has been shown that <sup>-5</sup>T/C polymorphism predicts the levels of GPIb  $\alpha$  on the cell membrane due to diverse protein expression determined by the <sup>-5</sup>T/C variants [1, 83]. In contrast to VNTR and HPA-2 polymorphisms that are in nearly complete linkage disequilibrium, Kozak sequence polymorphism seems to be unlinked to another sequence [11, 35, 67, 130]. Hypothetically, VNTR polymorphism might be a genetic determinant of the accessibility of GPIb-V-IX receptor for natural ligands, HPA-2 dimorphism could have an impact on the binding affinity to GPIb ligands, while Kozak polymorphism might affect receptor density. Consequently, at unfavorable combination of haplotype, polymorphism(s) could promote a prothrombotic state. According to this, all the above polymorphisms are believed to play an essential role in etiopathology of cardiovascular diseases as potential inherited risk factors of thromboembolic complications but, to date, the case-controlled studies did not revealed a clear standpoint in this matter [3, 23, 38, 57, 68, 98, 103, 134, 135]. None of GPIb polymorphisms has been profoundly tested for its effects on GPIb  $\alpha$  function. A functional significance of these polymorphisms in determining platelet response to activating agents has been reported only for VNTR and HPA-2 polymorphism. Whereas the influence of VNTR on platelet reactivity in the presence of collagen under high shear stress has been postulated [48], no such positive evidence was presented regarding the impact of the Thr/Met<sup>145</sup> dimorphism on the binding of vWF to GPIb. [89, 95]. However, an effect of the latter on GPIb-vWF interaction has not been examined under conditions of shear forces that regulate GPIb  $\alpha$  function *in vivo*.

### Blockade of von Willebrand factor-GPIb-V-IX interactions and a potential modulating effect of genetic polymorphisms

Since platelet adhesion is an important component of platelet response, it seems obvious that subunit of GPIb became a target for a design of antiplatelet drugs. The creation of new antithrombotic strategy by the inhibition of vWF-GPIb-V-IX interactions is expected to be used in combined therapy together with  $\alpha_{IIb}\beta_3$  blockade or to be alternative one for ASA-resistant patients. A plethora of antagonists of GPIb-vWF interaction have been tested, nevertheless, these drugs are still at the level of preclinical testing [14, 28, 40, 69, 78, 109, 111, 152]. For example, one of the last described GPIb-vWF blockers, humanized anti-vWF monoclonal antibody, AJW200 [74], which was tested in *in vitro* and *ex vivo* study in monkeys revealed its strong antiplatelet effect, probably due to a shear-stress-dependent inhibitory action. In addition, the authors suggested that it might be preferable in clinical practice owing to low bleeding profile.

The contribution of the GPIb gene polymorphisms to the discrimination of blood platelet reactivity in the presence of some GPIb-vWF antagonists has been recently reported by Boncler et al. [16]. This report demonstrates the possible functional role of VNTR and Thr/Met<sup>145</sup> polymorphisms under conditions where the vWF-dependent platelet agglutination was inhibited by aurintricarboxylic acid (ATA), the antagonist of vWF. In the suspensions of isolated platelets, ATA appeared to be a more efficient antagonist of VNTR B/Met<sup>145</sup>-positive platelets, as reflected by the significantly higher IC<sub>50</sub> values in VNTR B/Met<sup>145</sup>-negative individuals. Therefore, under certain experimental conditions, corresponding to a low shear stress, the VNTR-B and Met<sup>145</sup> have been demonstrated to enhance platelet sensitivity to the action of these antagonists. As far as VNTR-B/Met<sup>145</sup>-positive platelets might be more susceptible to such an inhibition, then their natural propensity to aggregate and perpetuate thrombosis would be lower, indicating that such the array of genotypes might offer a protective effect. The observed elevated efficacy of ATA-mediated inhibition of vWF-GPIb interaction(s) and reduced platelet agglutination may have two reasons: either the vWF-mediated platelet agglutination in the presence of ATA could be less efficient in VNTR-B/Met<sup>145</sup>-positive platelets, or the formation of vWF-ATA complexes could be more efficient for longer variants of GPIb  $\alpha$  [14].

### Polymorphisms of purinoreceptors and their potential influence on platelet pharmacology

Although ADP is regarded as a weak agonist of blood platelets, it remains an important mediator of platelet activation evoked by other agonists, which induce ADP release from dense granules where ADP concentration is in a range of moles [7, 8]. Of three types of platelet receptors for ADP and/or ATP, receptor P2Y<sub>12</sub>, which has been cloned recently [63], is of special importance. The receptor is responsible for amplification of platelet response and is essential for a full activation of GPIIb-IIIa by ADP, stabilization of platelet aggregates. It enhances also the release reaction [7, 8, 26, 138].

Since P2Y<sub>12</sub> receptor occurs mainly on blood platelets (at a low number of copies it is present also in the brain tissue), it has become an attractive target for antagonists, being potential anti-platelet drugs. It is known that thienopyridine derivatives irreversibly block P2Y<sub>12</sub> *via* covalent modification of cysteine residues located in extracellular domain of the receptor [33, 46, 47]. Recently, the extensive search has been made for new, competitive blockers of P2Y<sub>12</sub>, such as AR-C69931MX, the analogue of ATP described by AstraZeneca [138]. Due to the fact that P2Y<sub>12</sub> receptor was cloned in 2001 [63], a little is known about genetic variation of the receptor. In the work published recently [52], five genetic polymorphisms of P2Y<sub>12</sub> receptor have been described in a cohort of healthy donors. It was found that 4 of these polymorphisms are in complete linkage disequilibrium giving rise to two haplotypes: H1 and H2. Further, it was demonstrated that a maximal aggregation induced by low ADP concentrations was increased in H1/H2 heterozygotes, and the highest values were recorded in H2/H2 homozygotes. Besides, the ADP-induced drop in cytosolic cAMP level was more enhanced in carriers of H2 haplotype [52]. The subsequent work (case-control study) [53] proved that H2 haplotype is a risk factor of peripheral artery disease. So far, there is a lack of information whether H1/H2 haplotype of P2Y<sub>12</sub> receptor affects platelet sensitivity to P2Y<sub>12</sub> inhibitors. However, taking into consideration a recent huge interest in P2Y<sub>12</sub> inhibitors and recalling the fact that H1/H2 appears to affect the functioning of the receptor, this research area seems to be promising in the very near future.

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## Concluding remarks

The hemostatic response of platelets of an individual is influenced by the total population of receptors expressed on the platelet surface. Overall receptors' functionality largely underlies platelet reactivity and is governed by: (a) the density of each receptor, originating from the rate at which genes are transcribed and receptors produced and (b) receptor accessibility and affinity for potential ligands, relevant to both the presence of structural receptor polymorphisms and genetic profile of a whole population of platelet surface receptors, and variations in physicochemical nature of physicochemical membrane lipid bilayer. It has been estimated that up to 30% of natural variation in platelet reactivity is related to genetic inheritance. The implication of such a genetic variability is that atherosclerosis and its platelet-derived complications are the result of complex interactions between the environment and genetic factors. The influence of genetic make-up on natural platelet reactivity seems essential also in the context of effective antiplatelet therapy [1, 83, 112]. We have to keep in mind, however, that the reliable estimation which of these genetic factors should be considered 'true', i.e. the unconfounded, risk factor, may appear extremely difficult in clinical practice. On the other hand, we are now capable of identifying a large number of polymorphic sites with potential clinical relevance. Due to a technical simplicity of basic genetic analysis and digital nature of analyses' outcomes, studying of platelet receptor polymorphisms as risk factors has grown in popularity. The continuous search for new polymorphic sites in platelet receptor glycoprotein of potential pharmacogenomics interest becomes even more attractive since the influence of genetic make-up on natural platelet reactivity seems essential also in the context of effective antiplatelet therapy.

Once established as a risk factor, a genetic polymorphism of a given platelet receptor GP has the potential to aid selective prophylaxis and therapy of disease.

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