Platelet receptors for adhesion and activation. Variability as a factor in susceptibility to cardiovascular diseases

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Key words:
Myocardial infarction; Platelets; Polymorphisms; Receptors; Stroke.

Polymorphisms in coagulation factors leading to altered susceptibility to cardiovascular diseases have been known for some time and some are now well-established risk factors. More recently, an increasing number of polymorphisms have been identified in platelet receptors and a series of studies indicate that these too may play a role as individual risk factors for stroke and myocardial infarction. The effect of these platelet polymorphisms appears less clear-cut than some of the coagulation factor effects and other, associated, risk factors may be important in defining their role. In this review platelet receptor polymorphisms and their role as risk factors are surveyed and their possible relevance discussed.

(Ital Heart J 2001; 2 (11): 816-818)

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Introduction

Platelets are major players in preventing bleeding – hemostasis – and in its pathological variant, thrombosis. As such they have been the subject of a great deal of interest over the past couple of decades in the search for new antithrombotics. Cardiovascular diseases remain the major cause of death and disability in countries with a Western lifestyle and are rapidly becoming a serious health problem in developing countries that adopt similar habits. A better knowledge of platelet function could help to elucidate their possible role in the development of these disorders. For many years platelets were regarded as innocent bystanders in the development of cardiovascular diseases. The idea that variability in platelets could affect susceptibility to cardiovascular disorders was not considered. With the demonstration that sequence differences in coagulation factors can have a decided influence on thrombosis rates, some enhancing and others protective, together with the discovery of diversity in platelet receptor sequences, the whole area has been reconsidered, and a large number of papers have appeared over the past 5 or so years touching on various aspects of this problem. These studies have already been the subject of several reviews[1,2].

Platelet receptor polymorphisms

It has been known for some time that there are amino acid differences in the principal platelet integrin glycoprotein (GP) IIb/IIIa ($\alpha_{IIb}\beta_3$)[3] because this was a main cause of antiplatelet antibodies following transfusion. The first indication that such differences might have other consequences came from later studies reporting that myocardial infarction was commoner in people with the L33 version of the 33L/P (HPA-1) polymorphism in GPIIIa[4]. It was then reported that GPIIb/IIIa containing this polymorphism binds immobilized fibrinogen more avidly as well as demonstrating enhanced outside-in signaling and therefore platelets with this type might form large aggregates more easily[5]. On the other hand defective responses to arachidonic acid and thromboxane A_2 were reported in the Pl(A2) polymorphism of β_3[6]. No hypersensitivity *ex vivo* was observed using plasma levels of β-thromboglobulin determined by flow cytometry but increased sensitivity to antiaggregatory effects of aspirin was observed in this study with a relatively small number of subjects. No increased risk was found in heterozygous carriers of PlA2[7].

Homozygosity for Ser843 in GPIIb (HPA-3) has recently been reported to give a 5 times higher risk for stroke in high risk groups of young women such as subgroups with hypertension or diabetes[8]. However,

other studies found no effect on cardiovascular disease or myocardial infarction[9].

The HPA-5 immunogen is caused by a 505 E/K (A/G 1648) polymorphism on α_2 of $\alpha_2\beta_1$[10,11]. In one study, coronary heart disease and acute myocardial infarction rates were higher in subjects expressing the 1648GG homozygous polymorphism[12]. Another variant caused by silent polymorphisms (807T/C; 873A/G) in the coding region of the α_2 gene affects levels of $\alpha_2\beta_1$ expression and the higher level of expression was associated with higher levels of stroke in younger patients. Higher $\alpha_2\beta_1$ expression might be supposed to lead to easier activation following exposure to collagen after vessel damage or plaque rupture. Several groups[13-15] reported a higher incidence of myocardial infarction or stroke associated with the T807 variant, with the association being particularly marked in patients younger than 50. However, other studies found no effect after stenting[16] and no association with malignant arrhythmia[17].

In a study on stroke in young women the risk was found to be 2-fold higher in subjects expressing the 807T polymorphism[18]. An even stronger association was found in a study of a large group but was concentrated among high-risk subgroups. Thus, there was a 14.1X risk factor for 807TT homozygous carriers versus homozygous CC for cardiovascular mortality in high-risk women (≥ 2 risk factors, smoking, diabetes or microalbuminuria) in a large prospective study with 12 239 women in breast cancer screening program[19]. In this total population the risk factor of 807TT versus CC was only 1.2X.

Frequencies of myocardial infarction in 210 Japanese myocardial infarction patients and control groups were not related to C807T polymorphism[20].

A further major receptor with polymorphisms that might be implicated in differences in cardiovascular disease rates is GPIb-V-IX. Variants detected so far fall in three categories. The first is single amino acid polymorphisms within the leucine-rich repeat domain of GPIbα, such as 145T/M[21], which might affect von Willebrand factor binding to platelets by changing the conformation or flexibility of the leucine-rich repeat domain. One study reported that the M145 polymorphism showed a trend to higher risk of stroke in young women[18]. The second category is variations in the number of copies of a 13 amino acid segment in the *O*-glycosylated stalk-like domain holding the binding sites out from the platelet surface[22]. Here it is not clear what might be the dominant factor affecting platelet activation. On one hand, it could be argued that platelets with longer GPIb molecules might be more easily activated at lower shear stress than those with shorter GPIb molecules. On the other hand, incompatibility in the length of GPIb molecules derived from the two different alleles might also affect the platelet response by reducing receptor accessibility and, therefore, the effective density, and indeed some studies can be interpreted in this direction. The third category involves a polymorphism that was described in the Kozak sequence of the promoter of GPIbα (-5T/C), which affects levels of expression. Several studies have suggested that this might or might not affect susceptibility to myocardial infarction with the majority finding of no association. In a study with young women, 78 of whom had non-fatal myocardial infarction and 106 non-fatal stroke, the -5C polymorphism might even be associated with a reduced risk of myocardial infarction[23]. However, a recent report found a strong association between a combination of the two, T145M and -5T/C, polymorphisms, with the incidence of stroke[24].

Polymorphisms in other receptors that have so far been poorly investigated could also contribute to differences in platelet reactivity. These receptors include the seven transmembrane, G-protein associated group where in some cases polymorphisms have been reported. A T102C polymorphism in the serotonin (5-HT) 2A receptor has been associated with non-fatal acute myocardial infarction[25]. Other tyrosine kinase-linked receptors might also be implicated. Some cases of thrombocytopenia were shown to be due to a defect in the thrombopoietin receptor gene[26] and might be expected to reduce susceptibility to cardiovascular disease.

The FcγRIIa H/R131 polymorphism[27] might also affect platelet reactivity if this receptor really has a role in GPIb-V-IX signaling.

Overall the studies which have been made to try to relate the differences in sequence of platelet receptors with increased incidence of cardiovascular disease remain controversial. Probably this is because any effects are fairly small, compared, for example, to certain polymorphisms in coagulation factors. This does not mean that these effects are negligible but that they may only show up against a certain background of overall platelet/coagulation polymorphisms or when several polymorphisms occur in the same individual. An example of a difference that could be detected when both a platelet receptor and a plasma factor were affected is the increased bleeding noted when a polymorphism affecting low levels of expression of $\alpha_2\beta_1$ was combined with mild von Willebrand factor involving decreased amounts of von Willebrand factor[28].

It is important to notice that in several, more recent, studies an effect of a platelet polymorphism was quite strong but only if subgroups of subjects with multiple risk factors possibly affecting vascular integrity were considered. This raises again the interesting question, at which stage does variation in platelet reactivity, in this case determined by receptor structure, affect the incidence of cardiovascular disease? Which might bring us back, more or less, to the “innocent bystander” idea where platelets are involved in life threatening events only at the last stage when plaque rupture occurs. More reactive platelets would then tend to form larger, more stable thrombi leading to an increased risk of major events. On the other hand more reactive platelets could also contribute to plaque growth over a longer period, or even modify the nature of the plaque by contributing

more growth factors/cytokines to minor injury sites. If such a mechanism really occurs, long-term treatment with aspirin or other drugs that affect platelet reactivity might also affect vascular condition.

In this review only known receptor polymorphisms have been considered but other critical receptors exist such as GPVI, where little is known about variability, and which could also affect cardiovascular disease susceptibility. We also know very little about polymorphisms in signaling molecules downstream of platelet receptors. The future clearly lies in larger scale prospective studies incorporating data on as wide a range of molecular polymorphisms as possible linked to detailed multivariant analysis.

Acknowledgment

I thank the Swiss National Science Foundation for research support (grant 31-52396.97).

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