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ORIGINAL ARTICLE

Allelic and genotypic frequencies of platelet glycoprotein polymorphisms in a Portuguese population

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Abstract
Introduction and objectives: We studied genotypic and allelic frequencies of polymorphisms that can affect platelet function, namely the Kozak, VNTR and HPA-2 polymorphisms of glycoprotein Ibα, the Pl^A polymorphism of glycoprotein IIIa and the C807T polymorphism of glycoprotein Ia, in a Portuguese population composed of 227 donors.
Methods: PCR-RFLP was used to assess the Kozak, HPA-2, Pl^A and C807T polymorphisms. The VNTR polymorphism was discriminated by different weight bands on electrophoresis.
Results: All genotypic frequencies were in Hardy-Weinberg equilibrium and do not differ from other Caucasian populations. Genotypic frequencies were 68.3%, 26.9% and 4.8% for $Pl^{A1/A1}$, $Pl^{A1/A2}$ and $Pl^{A2/A2}$ genotypes of the Pl^A polymorphism, 79.3%, 20.3% and 0.4% for TT, TC and CC genotypes of the Kozak polymorphism, 81.1%, 18.9% and 0.0% for aa, ab and bb genotypes of the HPA-2 polymorphism, 15.4%, 0.9%, 70.5%, 11.5%, 1.3% and 0.4% for BC, BD, CC, CD, DD and CE genotypes of the VNTR polymorphism, and 39.7%, 50.2% and 10.1% for CC, CT and TT genotypes of the C807T polymorphism.
Conclusions: The Portuguese population has now been characterized in terms of major platelet glycoprotein polymorphisms, which will be an important tool for further studies to assess the role of platelet glycoproteins in individual predisposition to prothrombotic conditions and response to antithrombotic therapy.

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PALAVRAS-CHAVE
Polimorfismos das glicoproteínas plaquetárias;
Glicoproteína Ib-IX-V;
Glicoproteína Ia-IIa;
Glicoproteína IIb-IIIa

Frequências alélicas e genotípicas de polimorfismos das glicoproteínas plaquetárias numa população portuguesa

Resumo
Introdução e objetivos: Neste estudo determinámos as frequências alélicas e genotípicas de 5 polimorfismos que poderão afetar a funcionalidade plaquetária, nomeadamente os polimorfismos Kozak, VNTR e HPA-2 da glicoproteína Ibα, o polimorfismo Pl^A da glicoproteína IIIa e o polimorfismo C807T da glicoproteína Ia numa população portuguesa constituída por 227 dadores voluntários.
Métodos: A técnica de PCR-RFLP foi usada para determinar os polimorfismos Kozak, HPA-2, Pl^A e C807T. O polimorfismo VNTR foi discriminado através do padrão eletroforético criado por bandas de peso molecular diferente.
Resultados: As frequências genotípicas encontram-se dentro do equilíbrio de Hardy-Weinberg e não diferem estatisticamente de outras populações caucasianas. As frequências genotípicas encontradas foram 68,3, 26,9 e 4,8% para os genótipos $Pl^{A1/A1}$, $Pl^{A1/A2}$ e $Pl^{A2/A2}$ do polimorfismo Pl^A, 79,3, 20,3 e 0,4% para os genótipos TT, TC e CC do polimorfismo Kozak, 81,1, 18,9 e 0,0% para os genótipos aa, ab e bb do polimorfismo HPA-2, 15,4, 0,9, 70,5, 11,5, 1,3 e 0,4% para os genótipos BC, BD, CC, CD, DD e CE do polimorfismo VNTR, e 39,7, 50,2 e 10,1% para os genótipos CC, CT e TT do polimorfismo C807T.
Conclusões: A população portuguesa encontra-se, assim, caracterizada no que respeita aos polimorfismos das principais glicoproteínas plaquetárias, o que poderá servir como ferramenta importante de estudos futuros que avaliem o papel das glicoproteínas plaquetárias na predisposição individual a condições pró-trombóticas e resposta à terapia antitrombótica.

Introduction

The process of hemostasis mediated by blood platelets is complex and involves several receptor-ligand interactions. Most platelet receptors are protein complexes with two or more polypeptide subunits in the platelet membrane. Interindividual differences in platelet responsiveness are usually found, and thus it is reasonable to suggest that in certain circumstances inherited variations in platelet glycoproteins (GP) may contribute to their functional heterogeneity. In fact, three major platelet membrane adhesion receptors – the GPIb-IX-V complex, integrin α2β1 (GPIa-IIa) and integrin α2bβ3 (GPIIb-IIIa) – present genetic polymorphisms that can affect platelet responsiveness.[1–6] Identification of such polymorphisms may thus be useful to assess disease predisposition and response to therapy.

The GPIb-IX-V complex mediates the initial adhesion of platelets to the subendothelial matrix under high shear stress conditions, via von Willebrand factor (vWF) binding.[7] The GPIb subunit is composed of two disulfide-linked polypeptides, GPIbα and GPIbβ. GPIbα, the largest protein of the complex, contains the binding sites for vWF and α-thrombin, both platelet activator ligands.[8] At least three previously described polymorphisms may influence the function and expression of the GPIbα subunit: a molecular weight polymorphism due to a variable number of tandem repeats (VNTR) within the mucin-like macroglycopeptide region of GPIbα,[1] a threonine-to-methionine substitution at amino acid 145 that forms the basis of the HPA-2 platelet alloantigen system,[2] and a polymorphic variation at position -5 from the ATG start codon, where either T or C is present, called the Kozak polymorphism.[3]

Integrin α2β1, also called the GPIa-IIa complex, is a major collagen receptor in platelets and other cell types. GPIa-IIa mediates platelet adhesion to collagen after an initial subendothelial interaction mediated by GPIb-IX-V and vWF.[9] The T allele of the C807T polymorphism within the coding region of the GPIa gene (*ITGA2*) has been associated with high expression of the receptor and with possible platelet hyperreactivity, even though this polymorphism does not alter amino acids.[4,5]

The β3 integrin subunit (GPIIIa) plays a pivotal role in platelet aggregation. The β3 subunit forms a heterodimeric complex with the α2b integrin subunit (GPIIb). The major ligands for this glycoprotein are fibrinogen and vWF, when they are immobilized or in circulation after platelet activation.[10] There are at least nine GPIIIa polymorphisms,[11] incompatibility for the HPA-1 (Pl^A) alloantigens being the most common cause of fetal and neonatal alloimmune thrombocytopenia in Caucasians.[6] Studies on the functional consequences of this polymorphism in thrombotic diseases have yielded conflicting results,[12,13] but an increase in platelet reactivity has been reported in Pl (A2) carriers compared with Pl (A1/A1) individuals.[14]

In view of the importance of genetic variants in platelet function and the controversy surrounding the clinical correlation between them and prothrombotic conditions, further studies appear to be required in specific populations, that will lead to a better understanding of molecular markers with important roles in platelet function. Since there are no data regarding platelet polymorphism frequencies in the Portuguese population, the aim of this study is to assess allelic and genotypic frequencies of polymorphisms that can affect platelet function, namely the Kozak, VNTR