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Full Length Article

Genetic determinants of Platelet Large-Cell Ratio, Immature Platelet Fraction, and other platelet-related phenotypes

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ABSTRACT

Introduction: Platelets play a significant role in arterial thrombosis and are involved also in venous thrombosis. The genetic determinants of several platelet-related phenotypes have been studied previously. However, to the best of our knowledge, the genetic determinants of other platelet phenotypes have not been reported such as platelet-large-cell ratio (P-LCR) index, immature platelet fraction (IPF) parameters and overall platelet function measured through the PFA-100 system.

Materials and Methods: As part of the GAIT-2 (Genetic Analysis of Idiopathic Thrombophilia 2) Project, 935 individuals from 35 large Spanish families, ascertained through a proband with thrombophilia, were studied. Using variance component methods, implemented in the SOLAR package, the heritability of the following sets of platelet-related phenotypes was determined: platelet count and indices, IPF, and platelet function.

Results and Conclusions: High heritabilities of the platelet count and index phenotypes (from 0.41 to 0.64) were found, especially for those related to platelet volume. The heritabilities of the IPF phenotypes, as a measure of platelet turnover, were the highest (from 0.65 to 0.69). The heritabilities of the platelet function phenotypes were high also (0.45 and 0.62). The covariate age influenced all of the platelet phenotypes. Smoking influenced the platelet indices related to platelet volume and all the IPF phenotypes. Venous thrombosis showed a heritability of 0.67. We did not find a genetic correlation between any of the platelet-related phenotypes and venous thrombosis. The high heritabilities found for all of the platelet phenotypes provid promising data for the identification of new genes that underly these phenotypes.

1. Introduction

The development of thrombosis depends on a complex and variable interaction between genetic and environmental factors. Several genetic abnormalities that determine susceptibility to thrombosis have been described. However, in more than 50 % of the families with thrombosis none of these abnormalities is found [1]. Despite this fact, and by using a family-based approach, we demonstrated that the heritability of the liability to thromboembolic disease in the Spanish general population is higher than 60 % [2]. Ulterior studies, using different strategies, gave similar results [3]. The high heritability of the risk of thrombosis indicates the importance to search for susceptibility genes. The identification of these

Abbreviations: c^2, household effect; Col-ADP, cartridge of the PFA-100 device containing a membrane coated with both collagen and ADP; Col-Epi, cartridge of the PFA-100 device coated containing a membrane coated with both collagen and epinephrine; GAIT Project, Genetic Analysis of Idiopathic Thrombophilia Project; GAIT-1 Project, First phase of the GAIT Project; GAIT-2 Project, Second phase of the GAIT Project; h^2, heritability; HIPF%, percentage of the most immature platelets, included in IPF%; IPF, immature platelet fraction = fraction of platelets containing RNA; IPF%, percentage of immature platelets with respect to the platelet count; IPF#, absolute number of immature platelets; IPF-X, mean RNA content per platelet; MPV, mean platelet volume; P-LCR, platelet-large cell ratio = percentage of platelets measuring more tha 12 fl; PCT, plateletcrit; PDW, platelet distribution width; SOLAR, computer package Sequential Oligogenic Linkage Analysis Routines.

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genes would be of great interest for subsequent screening in the general population to identify people at risk. It may help also to understand the mechanism underlying the increased risk of thrombosis, thereby suggesting preventive strategies. This would lead ultimately to reduce the thrombotic-related morbidity and mortality in the population.

The classical case–control studies have evaluated by association whether a gene has any effect on the risk of thrombosis [1]. However, this approach is not useful for identifying unknown genes with effects on the disease. Unlike case–control studies, the GAIT (Genetic Analysis of Idiopathic Thrombophilia) Project is a family-based genetic study that is able to localize directly the potential effect of a locus through pedigree-based variance component analyses [4]. The first step of this type of study is to select intermediate phenotypes that could play a role in the susceptibility to thrombosis, based on their biological activities and on previously published evidence. Then, using the variance component method, the correlation in a given phenotype between relatives leads to the partition of the variance into components attributable to genes (heritability) and to environment [2,4]. The first phase of the GAIT Project (GAIT-1) was focused mainly on coagulation phenotypes and gave interesting results [5–7].

The second phase of the GAIT Project (GAIT-2) has investigated a larger number of individuals and families and has included a larger number and types of phenotypes, among them numerous platelet-related phenotypes.

Platelets play a key role in primary hemostasis and are involved in the development of arterial thrombosis [8]. Moreover, there is growing evidence that platelets are involved also in venous thrombosis through several mechanisms such as the release of polyphosphates which activate the coagulation intrinsic pathway and increase the clot resistance to fibrinolysis [9,10], the production of procoagulant-antifibrinolytic microparticles [11] or the involvement in inflammatory cellular interactions [12,13].

Several studies have focused on whether platelet number or platelet volume (which are both determinants of platelet mass) are risk factors for thrombosis. Elevated values of mean platelet volume have been related to risk factors for cardiovascular disease, with arterial and venous thrombosis and with impaired antiplatelet therapy responses [14–19]. In contrast, increased platelet counts have failed to demonstrate a consistent association with thrombosis in the majority of studies [14–16]. Other studies have related the elevated levels of different platelet indices -plateletcrit, platelet distribution and width, platelet-large cell ratio- to risk factors for cardiovascular disease, coronary syndromes, venous thromboembolism or poor response to aspirin [19–23].

Taking into account that immature platelets have a higher functional capacity than mature platelets, it has been investigated if an increased proportion of circulating immature platelets (the immature platelet fraction or IPF) is a risk factor for thrombosis. Similar studies have been performed on the platelet hyperreactivity that leads to increased platelet activation and aggregation. Increased levels of IPF or of platelet reactivity have been related to cardiovascular risk factors, coronary diseases or poor responses to antiaggregant drugs [24–31].

Using different approaches it has been established that there is a genetic contribution to the platelet count, to some platelet index phenotypes and to some phenotypes involving platelet function [32–36]. The aim of the present study was to analyze the genetic determinants of platelet-related phenotypes, including platelet count and indices, IPF, and platelet function, as part of the GAIT-2 Project.

2. Methods

2.1. Enrollment of Individuals and Families

As part of the GAIT-2 Project, 935 individuals from 35 large Spanish families were studied at the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain. The families were recruited following the same criteria as in the GAIT-1 Project [2], namely they were ascertained through a proband with thrombophilia and the condition of having at least of 10 members and at least 3 generations. Thrombophilia was defined as multiple thrombotic events (at least one of which was spontaneous), a single spontaneous event with a first-degree relative affected also, or onset of thrombosis before age of 45 years. The thrombotic events were diagnosed by objective methods and they were considered spontaneous when all of the known biological causes of thrombosis were excluded. These exclusion criteria were: deficiencies of antithrombin, Protein S, Protein C, heparin cofactor II, or plasminogen, activated protein C resistance, Factor V Leiden, dysfibrinogenemia, lupus anticoagulant and antiphospholipid antibodies. To recruit the individuals following the same criteria as in GAIT-1 Project [2], initiated in 1995, the prothrombin A20210G mutation was not considered an exclusion criterion. The unification of the recruitment crietaria would have some advantages such as to have a larger statistical sample for future analyses.

The families had between 10 and 68 individuals and all the families had three generations including 14 families with more than three generations. In total, these families gave 8649 pairs of relatives. Among the individuals studied, 465 were males and 470 were females. The mean age of the individuals was 39.5 (minimum 2.6, maximum 101, SD 21.4), and 197 of them were children up to 18-years. A total of 187 thrombotic events (121 venous and 66 arterial) were registered, affecting a total of 120 individuals: 86 with venous thrombosis, 47 with arterial thrombosis, and 13 with both.

The study was performed according to the Declaration of Helsinki. Written informed consent was obtained from all adult patients and for parents of children. All procedures were approved by the Institutional Review Board at the Hospital de la Santa Creu i Sant Pau.

The subjects were interviewed by a physician to collect demographic data and medical history including episodes of venous or arterial thrombosis and the age and circumstances at which these episodes occurred. Only documented events were considered. The subjects were questioned about their current medication to confirm that they had not taken antiplatelet drugs (such as aspirin or clopidogrel) in the last two weeks or they had not taken other drugs with slight effect on platelet function (such as nonsteroidal antiinflammatory drugs or serotonin reuptake inhibitor drugs) in the last week. Also, we collected data on the smoking habit and, in women, on the use of oral contraceptives or hormonal replacement therapy.

2.2. Blood Collection and Platelet Phenotype Analyses

Whole blood samples were obtained by venipuncture, under basal conditions, after a 12-hour overnight fast, and between 9:00 and 9:30 am. to minimize the circadian fluctuation. A 5 ml sample was obtained in EDTA-K_3 for determining platelet count and indices and for IPF phenotypes. Another 5 ml sample was obtained in sodium citrate for determining platelet function. Both samples were processed within 2 and 4 hours of collection. Using the impedance channel of the automated hematology analyzer Sysmex XE-2100 (Roche Diagnostics, Kobe, Japan), the following platelet phenotypes were determined: platelet count, mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) and platelet-large cell ratio (P-LCR). In the functional description of the Sysmex XE-2100, PDW is defined as the platelet volume distribution width at the 20 % frequency level, assuming that the peak height of platelet volume histogram is 100 % (Fig. 1), and the P-LCR is defined as the percentage of platelets with more than 12 fl of volume (Fig. 1). Staining the cells with fluorescent dyes for RNA and using its optical channel, the Sysmex XE-2100 determined the IPF parameters: percentage of platelets containing RNA with respect to the platelet count (IPF%), absolute number of platelets containing RNA (IPF#), percentage of platelets with the highest RNA content (HIPF%), included in IPF%, and mean RNA content per platelet (IPF-X).

The overall platelet function was measured by means of a routine laboratory test in daily clinics, the PFA-100 system (Siemens Healthcare Diagnostics, Marburg, Germany) which measures the primary hemostasis