Platelets

A novel Ser123Pro substitution in the MIDAS domain of integrin 3 associated with variant Glanzmann's thrombasthenia in an Indian patient

We report a novel 465T→C (S123P) mutation in exon 3 of the GPIIIa gene in a patient with type III or variant Glanzmann's thrombasthenia (GT). Though this mutation did not affect fibrinogen binding to GPIIb-IIIa in activated platelets, it interfered with the platelet aggregation in a manner similar to GT.

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Glanzmann's thrombasthenia is an autosomal recessive disorder of platelet function caused by deficiency or abnormality of platelet glycoprotein (GP) IIb-IIIa receptor and results in a lifelong bleeding tendency due to defective platelet plug formation.[1,2] GT can be classified as type I (total absence or <5% GPIIb-IIIa), type II (reduced levels) and type III or variant forms (normal levels of dysfunctional GPIIb-IIIa on platelets). The analysis of the molecular defects has led to an improved understanding of the biogenesis, expression and function GPIIb-IIIa subunit.

A 9-year old female child from Mumbai born of a first degree consanguineous marriage presented with a history of excessive bleeding from gums, recurrent episodes of epistaxis and prolonged bleeding from cuts. There was no family history of bleeding. Platelet aggregation tests were performed using commercial reagents (Chronolog Corp., Havertown, PA, USA): ristocetin (1.25 mg/mL), ADP (6 μM) and collagen (4 μg/mL). Flow cytometry and Western blot analysis were performed as described elsewhere[3,4] using FITC-tagged antibodies (Dako, Glostrup City, Denmark) to detect the abnormal glycoproteins. Denaturating gradient gel electrophoresis (DGGE) analysis and DNA sequencing were done to detect molecular abnormalities.[5,6] Flow cytometric analysis showed the presence of normal levels of GPIIb, GPIIIa, GPIIb-IIIa and GPIb-IX on the patient's platelet surface. The fibrinogen binding study on activated platelets showed normal levels of fibrinogen binding[3,4] (Figure 1). Western blot analysis showed the presence of GPIIb and GPIIIa bands with normal migration patterns in the patient's platelets. DGGE analysis of all the exons of the GPIIIa gene in the patient's sample showed an abnormality in exon 3. All the exons of the GPIIb gene showed normal migration patterns when compared to those of a normal control. DNA sequencing of exon 3 of the GPIIIa gene identified a 465T→C mutation, which corresponds to an amino acid substitution of serine by proline at residue 123 of the GPIIIa molecule (Figure 2).

According to the hypothesis of Loftus *et al.*,[7] β-subunit mutations affecting the Asp residue at 119 in integrin 3 should inhibit ligand binding function in other integrins as would mutations affecting four other oxygenated residues. Ser123 is also conserved among the β-subunits and the substitution of serine by proline, as in our case, may give the same effect. Serine contains aliphatic hydroxyl groups or side chains, which make them hydrophilic and reactive. Though proline also has aliphatic side chains, its side chains are bound to both nitrogen and α carbon atoms. Proline is a heterocyclic amino acid known to give structural rigidity

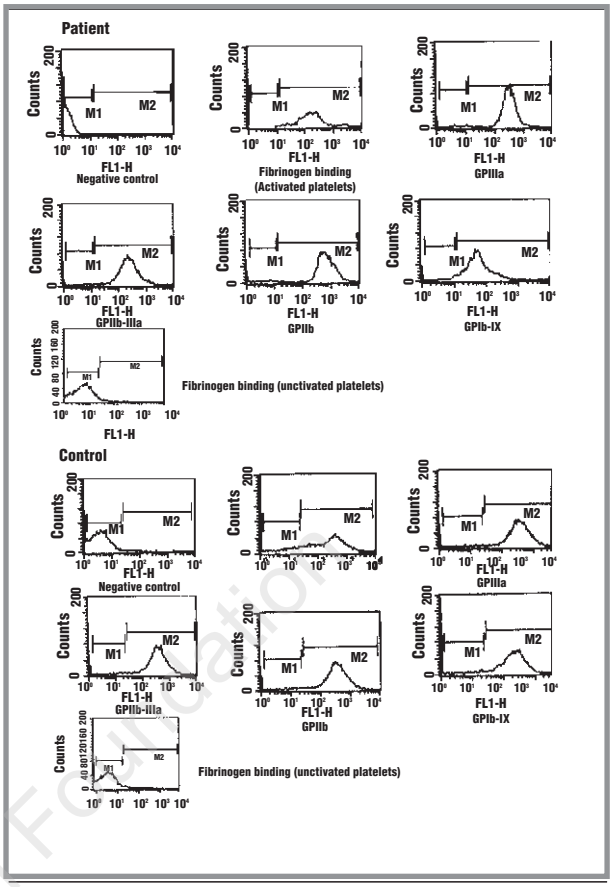

Figure 1. Flow cytometric analysis of the patient and a control. Washed platelets were used. Activated and unactivated platelets were used to study fibrinogen binding both in the patient and in a control. Platelets were activated using 0.02 mM ADP. Fibrinogen binding occurred on activated platelets whereas anti-GP binding did not.The patient showed normal fibrinogen binding and levels of GPIIb, GPIIIa, GPIIb-IIIa and GPIb-IX receptors when compared to those of a normal healthy control.

to the protein molecule thus markedly influencing the protein architecture. Hence replacement of serine with proline may hinder the folding of the GPIIIa molecule and its proper association with the GPIIb molecule on activation; furthermore, the hydroxyl group of the serine residue is known to be involved in many enzymatic reactions (serine protease).[8,9] However, our patient showed normal levels of GPIb-IIIa complex, GPIIb and GPIIIa on the platelet surface, as detected by specific FITC-tagged antibodies and flow cytometry. The fibrinogen binding study on activated platelets using FITC-tagged anti-fibrinogen antibody showed normal fibrinogen binding. In spite of the normal levels of GPIIb-IIIa and normal fibrinogen binding the platelets showed complete absence of aggregation in response to ADP (6 μM) and collagen (4 μg/mL). Thus the fibrinogen-binding site in our patient's GPIIb-IIIa complex does not seem to be affected by the mutation; it is post-fibrinogen binding changes and subsequent platelet aggregation that seem to be affected. This may be due to the substituted proline residue, which gives rigidity to the protein structure not allowing it to undergo the necessary conformational changes required for platelet aggregation. The mutation in exon 3 of GPIIIa involving a Serine123Proline substitution, has not been reported in literature so far

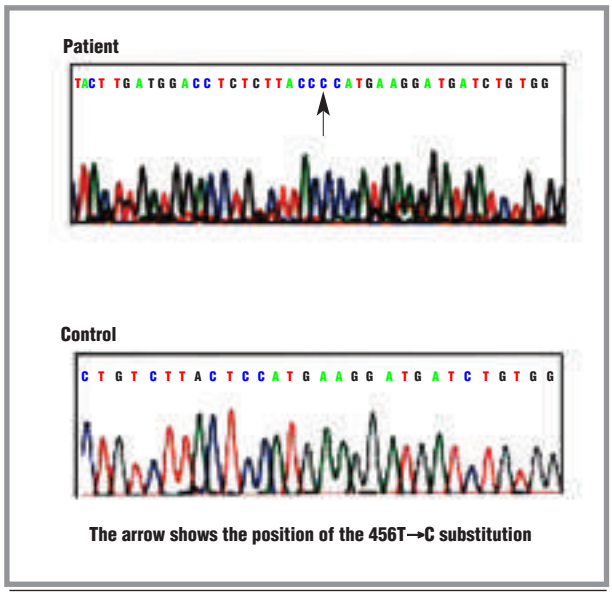

Figure 2. Electropherogram of exon 3 of the GPIIIa gene from the patient and from a control.

although it has been reported that Ser121Ala[10] affected neither αIIβ3 expression nor the ability of the heterodimer to interact with the complex specific monoclonal antibody. Although we could not do expression studies of the mutated integrin on CHO cells, the presence of this mutation in the parents of the patient and the plausibility of it interfering with platelet function makes it a genuine candidate for this important abnormality. In conclusion the patient appeared to carry a homozygous mutation in the GPIIIa gene, which may be responsible for her variant GT-like phenotype. The mutation described here will help further understanding of the GPIIb-IIIa structure and functions and GPIIb-IIIa.

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Stem Cell Transplantation

Basal CD34+ cell count predicts peripheral blood progenitor cell mobilization and collection in healthy donors after administration of granulocyte colony-stimulating factor

We analyzed factors predicting CD34+ cell mobilization and collection after granulocyte colony-stimulating factor (G-CSF) administration in 47 healthy donors. Basal CD34+ cell count and sex were the two variables that significantly predicted a better CD34+ cell mobilization, and greater age was the only variable associated with lower CD34+ cell yields.

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Despite the extensive use of granulocyte colony-stimulating factor (G-CSF) in healthy donors for peripheral blood progenitor cell (PBPC) mobilization, there is still a lack of consistent pre-mobilization variables that accurately predict a donor's response to this cytokine.[1-4] Among such variables, the number of CD34+ cells circulating in steady state, i.e., before G-CSF administration, has only rarely been reported.[5] The purpose of this study was to try to identify clinically significant factors that could influence the effectiveness of CD34+ cell mobilization and collection with special focus on the value of the basal CD34+ cell count in 47 healthy first-time donors from 12 centers undergoing PBPC mobilization and collection. Donors received G-CSF subcutaneously at a median (range) dose of 12 (10-22) μg/kg per day in two separated doses. PBPC collections were started on day five, i.e., after four days of filgrastim in every donor. The median blood volume processed was three times the donor's total blood volume and 20 donors (43%) underwent large volume leukapheresis (LVL) (Table 1). Three different determinations of CD34+ cells were done in each donor: first, baseline CD34+ cell count (before G-CSF administration); second, enumeration of CD34+ cells in peripheral blood on the morning of collection (after G-CSF); finally the number of CD34+ cells in the apheresis bag (CD34+ cells collected). Enumeration of CD34+ hematopoietic cells was performed by a single platform method based on the ISHAGE protocol.[6] The total number of CD34+ cells/mL in peripheral blood on the first day of apheresis (after G-CSF administration) was used to evaluate the effectiveness of mobilization. This variable was examined separately by linear regression analysis against independent variables (sex, age, weight, dose of G-CSF, baseline white cell count, and baseline CD34+ cell count). A p level <0.05 was considered sta-