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.

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 GPIb–Ilia on platelets). The analysis of the molecular defects has led to an improved understanding of the biogenesis, expression and function GPIb–Ilia 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 elsewhere3 using FITC-tagged antibodies (Dako, Glostrup City, Denmark) to detect the abnormal glycoproteins. Denaturing gradient gel electrophoresis (DGGE) analysis and DNA sequencing were done to detect molecular abnormalities.4 Flow cytometric analysis showed the presence of normal levels of GPIb, GPIa/IIa, GPIb–Ilia and GPIb–IX on the patient’s platelet surface. The fibrinogen binding study on activated platelets showed normal levels of fibrinogen binding5 (Figure 1). Western blot analysis showed the presence of GPIb 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 GPIb 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.,6 β-subunit mutations affecting the Asp residue at 119 in integrin 3 should inhibit ligand binding function in other integrins as would mutations affecting 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 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 GPIb molecule on activation; furthermore, the hydroxyl group of the serine residue is known to be involved in many enzymatic reactions (serine protease).7 However, our patient showed normal levels of GPIb–Ilia complex, GPIb 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 GPIb–Ilia 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 on our patient’s GPIb–Ilia 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.
affected nei-

...circulating in steady state, i.e., cells/mL in peripher-
...cells in peripheral blood cells collect-
...cell count). A
...floor, New Bldg., New;

...cells in the apheresis bag (CD34 
...hematopoietic cells was per-
...cell mobilization and collection with special focus
...cell count predicts peripheral blood

...in the GPIIIa gene, which may be responsible for her variant GT-like phe-
...the parents of the patient and the plausibility of it inter-
...ed integrin on CHO cells, the presence of this mutation in
...interact with the complex specific monoclonal antibody. Although we could not do expression studies of the mutat-
...buffered saline and ddH2O (Sigma) in the ratios of 1:1:1 (v:v:v) and
...inventor of platelet function makes it a genuine candidate
...for this important abnormality. In conclusion the patient
...the GPIIIa structure and functions and GPI-

...appear to carry a homozygous mutation in the GPIIIIa
...the arrow shows the position of the 456T→C substitution

...although it has been reported that Ser121Ala affected nei-
...Ser121Ala depicted in the electropherogram. The translec-

...discussed elsewhere.19 For our electrophysiological stud-
...of channel current in the CHO cells, the presence of this mutation in
...in the GPIIIa gene, which may be responsible for her variant GT-like phe-

...The purpose of this study was to try to identify clinically sig-
...only rarely been reported.5 Among such variables, the number of CD34+
...administration, has only rarely been reported.5 Despite the extensive use of granulocyte colony-stimu-

...The arrow shows the position of the 456T→C substitution

...GPIIb-IIIa structure and functions and GPI-

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...Sona Nair, Kanjaksha Ghosh, Shrimati Shetty, Dipika Mohanty 

...and mobility. Integral role of oxygenated residues in cdllb3 (GPIlb-IIIa) 

...protein IIb-IIIa, mutation, homozygous.

...Glanzmann’s thrombasthenia, glycoprotein IIb-IIIa, 

...Keywords: Glanzmann’s thrombasthenia, glycoprotein IIb-IIIa, 

...Kulkarni S, Nair S, Ghosh K, Mohanty D, Walvekar V, et al. Functional and fibrogen receptor studies in platelets in pre-
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...and from a control.

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