preleukemic situation as virtually all CLL patients appear to have a preleukemic phase of monoclonal B-cell lymphocytosis.[20] Understanding which genes are involved in the transition of monoclonal B-cell lymphocytosis into overt CLL and investigating to what extent antigen stimulation and an inflammatory proactive microenvironment favor this transition may provide a clue to many unanswered questions.

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Bernard-Soulier syndrome

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(Related Original Article on page 417)

Bernard-Soulier syndrome (BSS) is an inherited, usually autosomal recessive, platelet bleeding abnormality, characterized by a prolonged bleeding time, large platelets and thrombocytopenia.[1] In 1975, Nurden and Caen reported that platelets from BSS patients lacked a major surface membrane glycoprotein complex,[2] subsequently demonstrated to be the component subunits of the glycoprotein (GP)Ib-IX-V complex.[3,4] In this issue of the journal, Savoia and colleagues describe 13 patients with BSS from ten unrelated families with causative mutations in GPIbα, GPIbβ and GPIX, and attempt to relate the severity of the bleeding phenotype with genotype.[5]

Structure and function of the GP Ib-IX-V complex

The GPIb-IX-V complex is a pivotal receptor complex in hemostasis and thrombosis. In binding von Willebrand Factor (VWF), it mediates the initial contact adhesion of platelets to exposed vascular subendothelium or ruptured plaque in damaged vessels at high shear flow rates

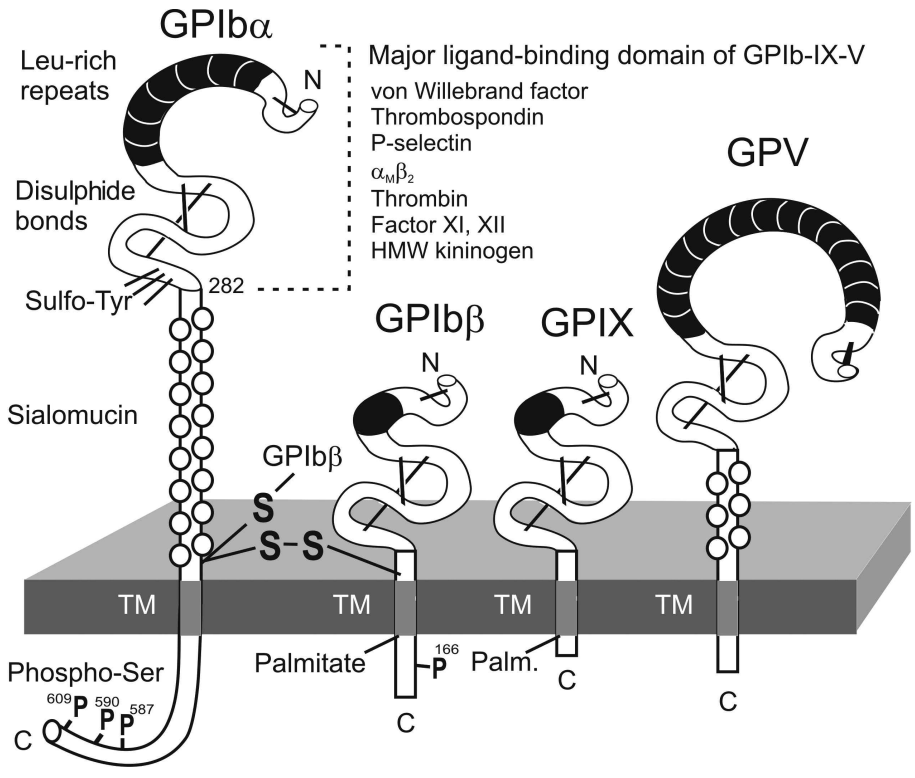

Figure 1. The GPIb-IX-V complex composed of GPIbα disulphide-linked to two GPIbβ subunits, and noncovalently associated with GPIX and GPV. Disulphide bonds within domains either side of leucine-rich repeat domains are depicted as solid black bars. The position of sulphated tyrosine residues (Sulfo-Tyr at 276, 278 and 279 of GPIbα), phosphorylated serine residues (Phospho-Ser) and palmitylated Cys residues of GPIbβ and GPIX are indicated. C, C-terminus; N, N-terminus; TM, transmembrane domain.

(>800^{s-1}).[6] GPIb-IX-V/VWF interaction is also a critical event in deep venous thrombosis.[7] The GPIb-IX-V complex consists of four subunits, GPIbα disulphide-linked to two GPIbαβ subunits, GPIX and GPV in a ratio of 2:4:2:1, respectively (Figure 1).[8] Each subunit contains one or more, ~24 amino acid, leucine-rich repeats, disulphide-looped N- and C-terminal capping sequences, a transmembrane sequence and a cytoplasmic domain. GPIbα also contains a mucin-like domain elevating the major ligand-binding domain located within the N-terminal 282 residues. In addition to its primary role in binding VWF, this N-terminal domain of GPIbα is a major binding site for multiple ligands mediating platelet interactions with matrix and other cell types in thrombosis and inflammation (Figure 1). Other adhesive ligands include P-selectin,[9] which is surface expressed on activated platelets and activated endothelial cells, and the leukocyte integrin, $\alpha_M\beta_2$ (also termed Mac-1 or CD11b/CD18).[10] These two interactions are fundamental to crosstalk between platelets and leukocytes, including those involving platelet- and leukocyte-derived microparticles, in both thrombosis and the co-associated inflammatory response.[11] The GPIb-IX-V complex is also a key receptor in mediating platelet-dependent coagulation, particularly with respect to the intrinsic pathway of coagulation, and has binding sites within the N-terminal domain of GPIbα for high molecular weight (HMW) kininogen, Factors XI and XII and α-thrombin.[6]

The GPIb-IX-V also plays a role in maintaining platelet shape by linking the platelet surface to a sub-membranous network of actin filaments, the platelet membrane skeleton. This involves the central portion of the cytoplasmic tail of GPIbα, particularly Phe568 and Trp570, which provides a binding site for the actin-associated protein, filamin A.[6] Other proteins known to bind to the cytoplasmic face of GPIb-IX-V either directly or indirectly through bound binding partners include calmodulin and the signaling assemblage protein, 14-3-3ζ, as well as other proteins potentially involved in propagating signals downstream of GPIb-IX-V/VWF engagement such as PI 3-kinase, TRAF4, Hic-5, the p47 subunit of NADPH oxidase, the Src family kinase, Lyn, and Syk.[6,12] Binding of VWF to the GPIb-IX-V complex initiates a signaling cascade leading to activation of the platelet integrin, $\alpha_{IIb}\beta_3$ (GPIIb-IIIa), and platelet aggregation. The most receptor-proximal signaling protein identified is the Src family kinase, Lyn.[13,14] VWF is considered a weak agonist, with full platelet activation requiring augmentation of signals through the thromboxane A2- and ADP-dependent signaling pathways.[15]

Bernard-Soulier syndrome: phenotype

Bernard-Soulier syndrome is characterized clinically by a history of epistaxis, gingival and cutaneous bleeding, and hemorrhage post trauma. In females it can also be

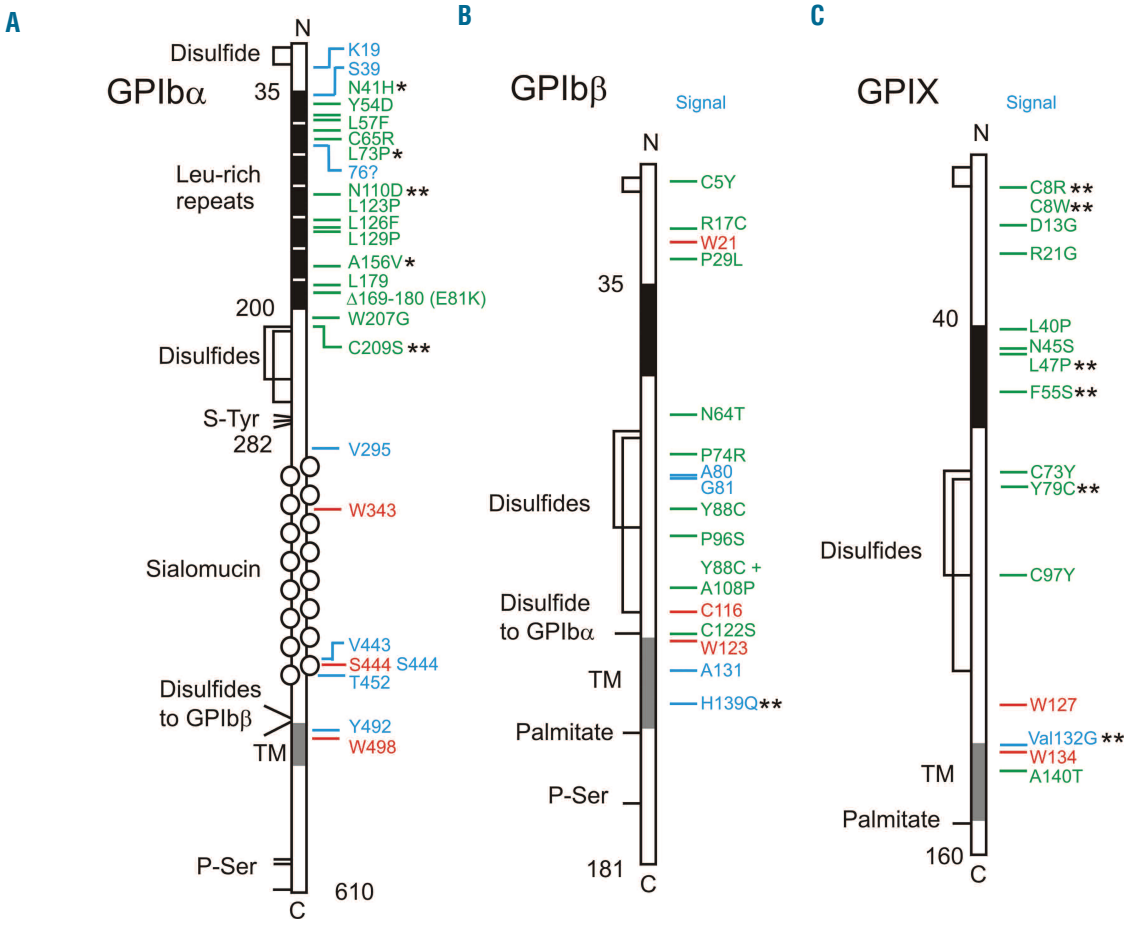

Figure 2. Mutations of (A) GPIbα, (B) GPIbβ and (C) GPIX associated with Bernard-Soulier syndrome, mapped to the mature protein structure, indicating missense mutations or short deletions (green), nonsense mutations leading to premature stop (red), or mutations causing a frameshift leading to stop (blue), based mainly on Lanza[16] and the Bernard-Soulier syndrome registry and website (*http://www.bernardsoulier.org/*) and references therein. Mutations also occur in the GPIbβ and GPIX signal sequences leading to BSS. There are no reported mutations in GPV, which is not essential for functional GPIb-IX expression.[6,17,18] The N-terminal 282 residues of GPIbα constitutes the major ligand-binding domain of GPIb-IX-V, with distinct or partially overlapping interactive sites for multiple ligands: VWF, thrombospondin, P-selectin, $\alpha_M\beta_2$ (Mac-1), thrombin, Factor XI, Factor XII and HMW kininogen. *autosomal dominant inheritance. **mutations detected by Savoia *et al.*[5]

associated with severe menorrhagia. Clinical presentation includes a prolonged skin bleeding time, thrombocytopenia, and large platelets on peripheral blood smear, and as such, cases of BSS are frequently misdiagnosed as idiopathic thrombocytopenic purpura (ITP) in the absence of further clinical investigation. The clinical profiles of the first fifty-five literature reports of BSS patients/families have been previously reported in detail.[1] BSS platelets are characterized by deficient ristocetin-dependent platelet agglutination as a clinical laboratory surrogate for assessment of GPIb-IX-V/VWF interaction. The component subunits of the GPIb-IX-V complex are present, except in very rare exceptions, at either very low levels or are undetectable by flow cytometry or by SDS-gel analysis and Western blotting.[1,5] One interesting exception is the Bolzano variant of BSS, involving an A156V mutation (Figure 2) in which the platelets express essentially normal levels of the GPIb-IX-V complex which is, however, dysfunctional and cannot bind VWF.[19] Thus either or both absent ristocetin-induced platelet aggregation or absent or near absent GPIb-IX-V content should ideally be employed to confirm the diagnosis of BSS.

In addition to these abnormalities, BSS platelets show additional functional defects including increased membrane deformability, poor aggregation response to low, but not high, doses of α-thrombin, and decreased capacity to support thrombin generation during platelet-dependent coagulation (less prothrombin is converted to thrombin).[1] Platelet aggregation to other platelet agonists such as collagen and ADP is normal relative to platelets from a normal individual at the same platelet count. The majority of these phenotypic differences in BSS platelets can be explained in terms of the known function of the GPIb-IX-V complex. The very poor or absent ristocetin-induced platelet agglutination is due to the absence of the GPIb-IX-V complex and hence the VWF binding site on GPIbα, whilst the prolonged skin bleeding time presumptively reflects a combination of this defect coupled with the low platelet count and decreased thrombin production. The large platelets and low platelet count in BSS are presump-

tively due to the absence of GPIbα and the filamin A binding site that links the GPIb-IX-V complex to the platelet membrane skeleton since the large platelet defect and low platelet count that also occurs in BSS mice (GPIbα knockout) are largely rescued by expression of an α-subunit of GPIb in which most of the extracytoplasmic sequence has been replaced by an isolated domain of the α-subunit of the human interleukin-4 receptor but in which the cytoplasmic sequence is normal.[20] The absence of the normal GPIbα interaction with filamin also appears to be the cause for the increased membrane deformability seen in BSS platelets.[21] The poor response of BSS platelets to α-thrombin is consistent with evidence that binding of α-thrombin to GPIbα enhances the capacity of α-thrombin to activate platelets through the platelet thrombin receptor PAR-1.[6,22] Finally, the decreased capacity of BSS platelets to support thrombin generation is consistent with a role for the GPIb-IX-V complex in facilitating activation of the intrinsic pathway of platelet activation by providing a platelet binding site for Factors XI and XII.[6]

Bernard-Soulier syndrome: genotype

A large number of mutations in GPIbα, GPIbβ and GPIX have now been described that are causative for Bernard-Soulier syndrome (Figure 2).[16] These include missense mutations, short deletions, nonsense mutations resulting in a premature stop codon, and mutations causing a frameshift that also lead to a premature translational stop codon. No mutations have been reported in GPV that are causative for BSS consistent with a lack of a requirement for GPV expression for expression of the other subunits of the GPIb-IX-V complex[6,17,18]

Does Bernard-Soulier syndrome genotype correlate with the severity of bleeding?

In this issue, Savoia and colleagues begin to address the intriguing question of whether BSS genotype correlates with the severity of bleeding.[5] Studies in mice frequently demonstrate that phenotype can vary dependent on the genetic background of the mouse in which the gene has been deleted and thus other genetic differences that affect hemostasis undoubtedly contribute to the marked variability seen in bleeding tendency amongst BSS patients.[1,5] What is less clear is whether the BSS genotype itself is also associated with the severity of bleeding phenotype. GPIbα is involved in binding of multiple ligands relevant to different aspects of hemostasis including VWF, thrombospondin, P-selectin, $\alpha_M\beta_2$ (Mac-1), thrombin, Factor XI, Factor XII and HMW kininogen and thus one would predict the potential for differences based on the degree of GPIbα expression *versus* its complete absence, or between low levels of normal GPIbα and similar low levels of GPIbα with functional mutations in the N-terminal GPIbα ligand-binding domain. In the Savoia paper,[5] it is not possible to assess an overall relationship between genotype and bleeding phenotype since most of the BSS patients in their study are a single example of a specific genotype. There are, however, 5 BSS patients in their study from three different families that involve mutation of GPIX Cys8 (either C8R or C8W) and all had a mild bleeding phenotype. In contrast, a previous study addressing genotype/phenotype in a large Swiss family found that 4 BSS patients homozygous for an N45S mutation in GPIX had variable bleeding risk.[23] Resolution of whether BSS genotype can indeed result in differences in the severity of the bleeding phenotype probably awaits more detailed genetic studies in mice with BSS and larger BSS patient cohort studies.

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