mechanisms of action. In so doing, we can look forward to entering the era of personalized medicine in FL.

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● ● ● PLATELETS AND THROMBOPOIESIS

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# Parsing the repertoire of GPIb-IX-V disorders

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In this issue of *Blood*, Sivapalaratnam et al[1] report an association between unique, rare monoallelic variants in *GP1BB*, which encodes the glycoprotein (GP)Ibβ subunit of the platelet GPIb-IX-V complex, and autosomal dominant macrothrombocytopenia.

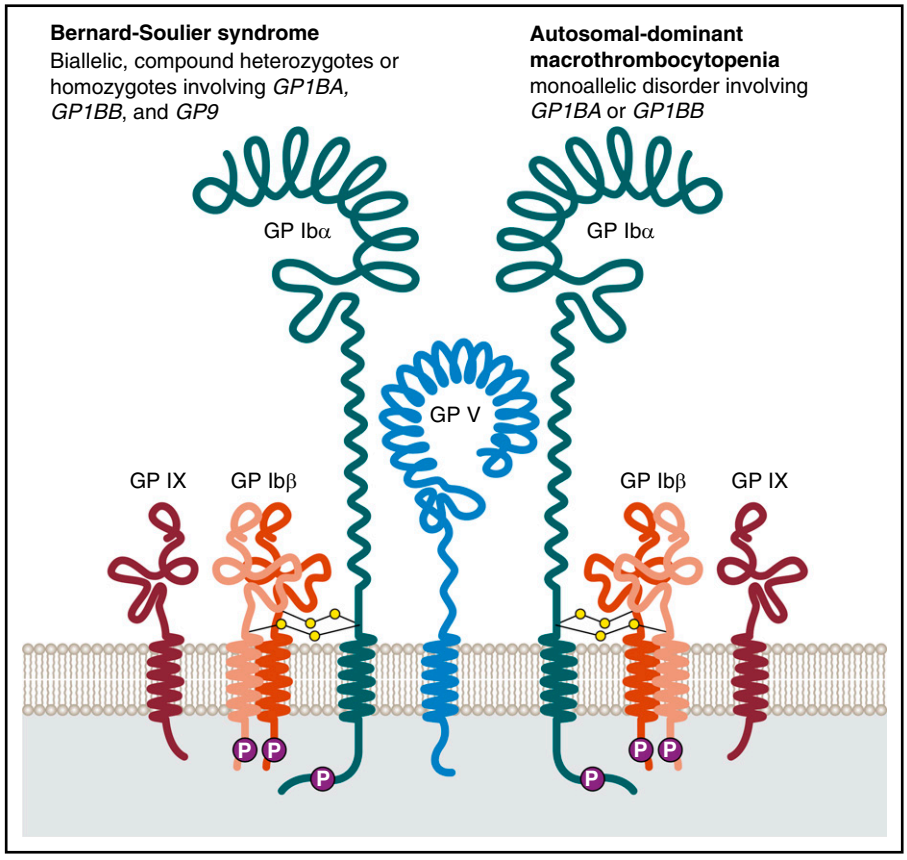
Platelet GPIb-IX-V is a major player in hemostasis, serving as the receptor for von Willebrand factor (VWF) and mediating the platelet–subendothelium interactions. Deficiencies of this multiprotein complex or the plasma VWF result in a bleeding diathesis. The GPIb-IX-V complex, expressed on megakaryocytes and platelets, contains 4 distinct transmembrane subunits, GPIbα, GPIbβ, GPIX, and GPV (see figure).[2] Each subunit is a distinct gene product.[2]

The best recognized inherited bleeding disorder involving the GPIb-IX-V complex is the Bernard-Soulier syndrome (BSS), an autosomal recessive disorder arising from homozygous or compound heterozygous variants of *GP1BA*, *GP1BB*, and *GP9*.[3] In 1948, 2 French physicians, Jean Bernard and Jean-Pierre Soulier, first reported a severe bleeding disorder associated with thrombocytopenia and giant platelets, later shown to be characterized by decreased ristocetin-induced platelet agglutination.[3,4] To date, 45, 39, and 28 variants in *GP1BA*, *GP1BB*, and *GP9*, respectively, have been identified in the BSS.[4] *GP5* variants have not been implicated in the BSS. In contrast to these loss-of-function variants, some *GP1BA* variants have conferred an increase in affinity of the platelet complex for VWF, resulting in the platelet-type vWD associated with a secondary decrease in plasma high molecular weight VWF multimers.[3]

More recently, an association between monoallelic variants in *GP1BA* and an autosomal dominant macrothrombocytopenia has emerged (see figure).[4-6] The most frequent of these is the *GP1BA* variant (p.Ala172Val), labeled as the "Bolzano" variant, associated with mild thrombocytopenia and increased platelet volume, with or without mild bleeding symptoms.[4-6] Three other monoallelic *GP1BA* variants have also been implicated.[4] Although *GP1BB* variants have been linked to autosomal dominant macrothrombocytopenia in 3 Japanese families,[1,4] this association is not well established.

Sivapalaratnam et al extend the repertoire of GPIb-V-IX complex-related disorders by documenting a statistical association between rare nonsynonymous monoallelic variants in *GP1BB* and autosomal dominant macrothrombocytopenia. Their approach used high-throughput whole-genome DNA sequencing coupled with human phenotype ontology coding of clinical and laboratory phenotypes. The study population consisted of 1 discovery and 2 validation cohorts. The discovery cohort comprised 1542 patients with a suspected inherited bleeding or platelet disorder with unknown molecular etiology, or their relatives, enrolled in the National Institute for Health Research BioResource. One validation cohort contained 75 patients with thrombocytopenia evaluated with the ThromboGenomics gene panel, whereas another contained 301 patients from a Japanese cohort with suspected inherited thrombocytopenia and their relatives. Within the discovery cohort, there was a strong association identified between rare (allele frequency $<1/10\,000$) nonsynonymous monoallelic *GP1BB* variants and macrothrombocytopenia, with a family history suggestive of autosomal dominant inheritance. Eight probands with *GP1BB* variants were identified from the discovery cohort; they did not have other genetic variants previously implicated in macrothrombocytopenia. In the validation cohorts, 10 further *GP1BB* variants were identified, for a total of 18 probands (27 total cases), with 9 distinct *GP1BB* variants across cohorts.

Patients with *GP1BB* variants had platelet counts ranging from 47 to 172 $\times$ $10^9$/L (mean, 108 $\times$ $10^9$/L) and increased platelet volumes (10.7-14.3 fL; mean, 12.74 fL). Macrothrombocytopenia was defined as a platelet count below 150 $\times$ $10^9$/L and mean platelet volume above 12 fL. Patients with platelet anisocytosis with a subset of abnormally large platelets were also considered




Schematic drawing of the GPIb-IX-V complex showing GP1bα, GPIbβ, GPIX, and GPV subunits that constitute the complex on platelet surface. Biallelic variants in *GP1BA*, *GP1BB*, and *GP9* result in Bernard-Soulier syndrome. Monoallelic variants in *GP1BA* and *GP1BB* are associated with isolated macrothrombocytopenia. Figure reproduced with modifications from Luo et al[2] with permission. P, phosphorylation sites. Professional illustration by Patrick Lane, ScEYEnce Studios.

to have macrothrombocytopenia, emphasizing the importance of recognizing the heterogeneity in platelet size. Bleeding symptoms were observed in 9 of 23 female patients, but not in any of the 8 male patients from whom information was available, indicating that bleeding diathesis is mild, if at all discernible. Although no information is presented regarding platelet function, 8 of 9 family members studied showed a reduction of at least 30% in platelet GPIbα by flow cytometry, a level of deficiency unlikely by itself to markedly impair platelet agglutination by ristocetin.

These studies extend the rapidly increasing list of genes implicated in inherited thrombocytopenias.[7,8] Specifically, they document a clinically detectable autosomal dominant macrothrombocytopenia phenotype in association with variant *GP1BB* genotype. Relevant to note, most obligate carriers of biallelic BSS do not appear to have deranged platelet size or number and are asymptomatic,[3,4] although this premise is ripe for a careful second look. The *GPIBB* variants identified by Sivapalaratnam et al may have a dominant negative effect, which is yet to be demonstrated.

From a clinical viewpoint, *GPIBB* variants need to be considered in the context of an unexplained inherited or chronic macrothrombocytopenia. Studies from the International Consortium focusing on 211 unrelated families with BSS reiterate[4] that even in patients with biallelic BSS, ~50% were initially misdiagnosed, mostly as having immune thrombocytopenia, with almost all receiving steroids or intravenous immunoglobulin, and several a splenectomy. Not all had severe bleeding symptoms. This is compounded by the compromised accuracy of both the platelet count and size rendered by automated counters in the presence of large platelets.[9] A mandatory quick look at the blood smear may suggest a macrothrombocytopenia, even if the automated rendition of the mean platelet volume is not striking.

From a biological perspective, although 1 functional phenotype of the abnormal platelet GPIb-IX-V expression (altered platelet interaction with VWF and agglutination with ristocetin) is easy to articulate, less is known regarding the effect on megakaryocyte biology that culminates in altered platelet structure, number, and production. The GPIb-IX-V complex is implicated in late megakaryopoiesis, proplatelet formation, and platelet production.[10] How does the interaction of GPIb-IX-V with VWF regulate platelet biogenesis? Studies with variants of *GPIBA* and *GPIBB* provide exciting opportunities.

These studies of Sivapalaratnam et al shed new light on the GPIbβ moiety and advance the parsing of the subunits of platelet GPIb-IX-V complex. Hopefully, similar studies will unravel the effects of monoallelic variants of GP9 and the mystery surrounding the remaining member, GPV, which appears silent in interactions with VWF.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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