

# Platelets

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## Inherited Platelet Disorders

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The inherited platelet disorders are a heterogeneous collection of rare diseases that are infrequently encountered in clinical practice. They are, however, fascinating abnormalities, which have taught us a great deal about normal platelet biochemistry and physiology. In this section of the presentation we will review disorders of the platelet membrane, platelet

granule packaging disorders, the hereditary macrothrombocytopenias, platelet signaling disorders and disorders of platelet coagulant function. The molecular basis of the disorders, the cardinal features of their clinical presentation and best methods to make their diagnosis and the latest information regarding therapy will be presented.

Blood platelets have several important functions. They adhere to sites of vascular injury, generate biological mediators, secrete their granule contents, form multicellular aggregates and serve as a nidus for plasma coagulation reactions. In order to carry out these tasks, the platelet undergoes dramatic structural rearrangements, utilizes multiple membrane receptors, which bind small molecule mediators, adhesive glycoproteins and constituents of the vascular subendothelium, and activates a network of complex signaling pathways. All of these events occur within seconds of vascular injury. Collectively, they help to maintain the integrity of the vascular system. It should not be surprising that mutations occasionally arise that perturb these complex reactions and lead, in some cases, to disordered hemostasis. While inherited disorders of platelet function are relatively rare, they have provided important information about normal platelet physiology. In addition, many of the key steps in platelet signaling pathways and several of the key platelet receptor proteins have been used as targets for the development of new antithrombotic agents.

In this paper, we will review the major heritable disorders of platelet function, describe the molecular pathogenesis and clinical manifestations of these disorders and recommend appropriate therapy. An outline of the major inherited platelet disorders is shown in **Table 1**.

### Platelet Membrane Disorders

#### *Glanzmann's thrombasthenia*

Glanzmann's thrombasthenia, literally translated as weak platelets, is a rare disorder in which platelets can carry out most biochemical reactions but fail to form aggregates. The platelet count is normal and the platelets are of normal size with normal morphology. They adhere normally to vascular subendothelium and can secrete granule contents and carry out the normal signaling reactions. Their failure to aggregate is due to the loss or dysfunction of the platelet integrin receptor complex CD41 more commonly known as  $\alpha_{IIb}\beta_3$  or GpIIb/IIIa. When platelets are activated, the complex binds the plasma glycoprotein fibrinogen. Since each fibrinogen molecule contains two GpIIb/IIIa binding sites, platelets are linked together into multi-cellular aggregates. There are approximately 50,000 GpIIb/IIIa binding sites on each platelet.

The inherited form of the disorder is an autosomal recessive trait and can arise from multiple mutations that perturb the biosynthesis and assembly of the multi-subunit GpIIb/IIIa complex.<sup>1-11</sup> The carrier state, in which there is a 50% reduction in the number of GpIIb/IIIa molecules on the platelets, is asymptomatic, and in the absence of consanguinity most patients with thrombasthenia are compound heterozygotes who carry two independent mutations. Techniques are available for the prenatal diagnosis of the disorder.<sup>7,12</sup>

Patients with Glanzmann's disease have lifelong mucosal bleeding and may require platelet transfusions for severe bleeding episodes. The efficacy of platelet transfu-

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**Table 1. Classification of inherited disorders of platelet function.**

1. Defects in platelet-vessel wall interaction (disorders of adhesion)
    - a. von Willebrand disease (deficiency or defect in plasma vWF)
    - b. Bernard-Soulier syndrome (deficiency or defect in GPIb)
  2. Defects in platelet-platelet interaction (disorders of aggregation)
    - a. Congenital afibrinogenemia (deficiency of plasma fibrinogen)
    - b. Glanzmann thrombasthenia (deficiency or defect in GPIIb-IIIa)
  3. Disorders of platelet secretion and abnormalities of granules
    - a. Storage pool deficiency
    - b. Quebec platelet disorder
  4. Disorders of platelet secretion and signal transduction (primary secretion defects)
    - a. Defects in platelet-agonist interaction (receptor defects)  
Receptor defects: thromboxane A<sub>2</sub>, collagen, ADP, epinephrine
    - b. Defects in G-protein activation  
G $\alpha$ q deficiency  
G $\alpha$ s abnormalities  
G $\alpha$ i1 deficiency
    - c. Defects in phosphatidylinositol metabolism Phospholipase C-2 deficiency
    - d. Defects in calcium mobilization
    - e. Defects in protein phosphorylation (pleckstrin) PKC- $\gamma$  deficiency
  - f. Abnormalities in arachidonic acid pathways and thromboxane A<sub>2</sub> synthesis  
Impaired liberation of arachidonic acid  
Cyclooxygenase deficiency  
Thromboxane synthase deficiency
5. Defects in cytoskeletal regulation
    - a. Wiskott-Aldrich syndrome
  6. Disorders of platelet coagulant-protein interaction (membrane phospholipid defects)
    - a. Scott syndrome
  7. Miscellaneous

(Modified from Rao AK. Inherited disorders of platelet signal transduction. *Am J Med Sci* 1998;316:69–77).

sion is limited as patients may become allo-immunized to platelets. In addition, some patients have developed antibodies against the GPIIa/IIIa complex, which makes transfusion therapy ineffective. There are now a number of clinical trials showing that patients with Glanzmann's who are refractory to platelet transfusions can be treated with recombinant factor VIIa, which can be lifesaving or can allow emergent surgery or delivery to proceed with minimal morbidity.<sup>13-19</sup>

Occasionally patients may develop an acquired form of thrombasthenia due to auto-antibodies which bind to epitopes on the GPIIb/IIIa complex that participate in the

binding of fibrinogen. This has been noted in patients with immune thrombocytopenia and in patients with normal platelet counts. Treatment with corticosteroids or other maneuvers that raise the platelet count may not improve platelet function in thrombocytopenic patients, and the risk of bleeding persists. The antibody can be demonstrated in platelet eluates from affected patients. In some of the non-thrombocytopenic cases the antibody may be transient and disappear over time. Aggressive immunotherapy with agents like rituximab may be indicated.

### **Bernard-Soulier syndrome**

The Bernard-Soulier syndrome (BSS) is a second rare autosomal recessive disorder caused by mutations in various polypeptides in the GPIb/IX/V complex, which is the principal receptor for the von Willebrand's protein (vWF). Estimates are that the disease occurs in 1 in 10<sup>6</sup> births. In a thorough review Lopez and colleagues summarized the clinical features of 55 independent reported cases of BSS.<sup>20</sup> In the absence of a functioning vWF receptor, platelets cannot adhere to vascular subendothelium under high shear stress. Patients with BSS also have giant platelets, sometimes approaching the size of lymphocytes, and mild to moderate thrombocytopenia. The large size is thought to arise from the lack of an interaction between actin-binding protein in the platelet cytoskeleton and the cytoplasmic domain of the GPIb $\alpha$  polypeptide. The 25,000 GPIb/IX/V sites on each platelet are the major locus for platelet sialic acid residues, and the lack of sialic acid in BSS may shorten platelet survival and lead to thrombocytopenia.

The majority of the known mutations prevent proper assembly of the complex. There are a few informative missense mutations in which a defective complex is assembled. For example, in the Bolzano variant, substitution of VAL for ALA at position 156 in the GPIb $\alpha$  polypeptide impairs vWF binding.<sup>21</sup> Despite the presence of a normal GPIb $\alpha$  cytoplasmic domain, the platelets are still large. There is a single report of an autosomal dominant form of BSS due to a Leu to Phe substitution at residue 57.<sup>22</sup> Both of these mutations occur in the leucine-rich repeat regions of the molecule. Presumably the Leu57Phe mutation prevents cooperative interactions with normal GPIb $\alpha$  subunits.

As noted for Glanzmann's disease, BSS is an autosomal recessive trait and most carriers are asymptomatic. Genetic analysis has revealed a high incidence of consanguinity in affected families and the many individuals with the disease who have been studied are homozygotes for the identical mutation. Treatment with platelet transfusions is effective until either allo-immunization or the development of anti-GPIb antibodies supervenes. Some patients may respond to DDAVP, and there is anecdotal evidence that fibrinolytic inhibitors like EACA can be of help. Based on already cited studies in patients with Glanzmann's disease, rVIIa should also be effective therapy for patients with BSS. Stem cell transplantation would provide definitive treatment for both Glanzmann's disease and BSS but is

too hazardous at present. It is encouraging to note that carriers of disease-causing genes, who have half the normal number of the respective receptors, are usually asymptomatic or only mildly affected, suggesting that induction of a chimeric state with only partial correction of the underlying defect might be effective.

### **Pseudo or platelet type von Willebrand disease**

Pseudo or platelet type von Willebrand disease arises from mutations in the GpIb $\alpha$  polypeptide that make the platelet hypersensitive to vWF.<sup>23</sup> Patients have mucosal bleeding, borderline thrombocytopenia and laboratory findings that resemble those of Type IIB vWD. Patients have heightened sensitivity to Ristocetin and loss of high  $M_r$  vWF multimers. Like IIB vWD, this is an autosomal dominant disorder. One diagnostic feature of platelet-type vWD is that patient platelets will agglutinate when challenged with asialo-vWF. An accurate diagnosis is important as the treatment of bleeding may require platelet transfusions rather than vWF concentrate. The platelets are of normal size.

The two well-characterized mutations causing this gain of function phenotype occur in short linear sequence, residues 233 to 239, located within a disulfide bonded loop formed by Cys209 and 248 of GpIb $\alpha$ . They are Gly233Val and Met239Val.<sup>24-27</sup> Predictions from molecular modeling are that these mutations introduce a conformational change in GpIb $\alpha$ , which permits binding to vWF without any intervening modulator like shear stress or vWF binding to vascular subendothelium. This raises the interesting possibility that “activation” involves both platelets and vWF or that certain platelet conformations can bind to “unactivated” vWF.

### **ADP receptor deficiency**

ADP receptor deficiency is a newly discovered autosomal recessive disorder of platelets.<sup>28-30</sup> The receptor involved,  $P_2Y_{12}$ , is a member of the seven-transmembrane-domain receptor family and signals through  $G_i$ , is one of two purinergic receptors expressed on the platelet surface.  $P_2Y_{12}$  deficiency induces a mild bleeding syndrome with posttraumatic and post-surgical blood loss. Platelet aggregation to ADP is reduced and rapidly reversible.  $P_2Y_{12}$  activation inhibits adenylate cyclase activity, while the second receptor  $P_2Y_1$  regulates platelet shape change and  $Ca^{2+}$  fluxes. While  $P_2Y_1$  initiates the platelet response to ADP,  $P_2Y_{12}$  is required for large aggregates to form and be sustained. ADP released from damaged tissues or activated platelets plays a role in platelet aggregation induced by most physiologic stimuli so  $P_2Y_{12}$  deficiency reduces the platelet response to low concentrations of collagen and thrombin. In flow chamber studies of  $P_2Y_{12}$  deficient platelets do not form large macroaggregates on a collagen substrate. Heterozygotes have mildly abnormal platelet function. The first patient to be characterized had one  $P_2Y_{12}$  allele that did not express receptor protein and a second allele with a 2-bp deletion within codon 240, which induced a 28 residue frame-shift

and a stop codon.<sup>3</sup> There was a second report of a homozygous 2-bp deletion in three Italian patients which produced a truncated protein that was not expressed. Finally, there is a recent report of a compound heterozygote patient with two missense mutations with normal expression of  $P_2Y_{12}$  but loss of function.<sup>31</sup>

Although bleeding seems to be mild in these patients, the only available treatment at present is platelet transfusion. The widely used anti-platelet drug clopidogrel produces the same phenotype as ADP receptor deficiency and presumably works by blocking  $P_2Y_{12}$  activity.  $P_2Y_{12}$  expression is restricted to platelets and glial cells although its role in neural function remains unknown.

### **Collagen receptor deficiency**

Collagen receptor deficiency has been described in several unrelated patients. It has become clear that there are two distinct receptors on the platelet surface that mediate adhesion to collagen. There is an integrin protein,  $\alpha_2\beta_1$  (also called GpIa/IIa), and a second non-integrin protein, GpVI. The order of two interactions and the relative contribution of each collagen binding receptor to adhesion and subsequent signaling events is under intense study. GpVI is linked to the platelet Fc gamma receptor and likely signals through that pathway. Studies with knockout mice suggest that both receptors are needed for optimal adhesion.

There were reports several decades ago of reduced adhesion, GpIa/IIa deficiency and mild bleeding in a Dutch kindred.<sup>32,33</sup> Patients have also been described with mild bleeding and GpVI deficiency.<sup>34</sup> Recently an interesting family with gray platelet syndrome ( $\alpha$  storage pool disease) and defective collagen adhesion has been described. Affected members also have a severe deficiency in GpVI providing further evidence for the important role of this protein in collagen-mediated platelet adhesion.<sup>35</sup>

### **Platelet Granule Disorders**

The term platelet storage pool disease (SPD) encompasses a range of disorders with variable reduction in the number and the contents of dense granules ( $\delta$ -granules) and  $\alpha$ -granules as well as combined defects.<sup>36</sup> The most common disorder is isolated dense granule deficiency ( $\delta$ -SPD). More rarely patients are encountered with  $\alpha/\delta$ -SPD, which includes patients with marked deficiencies of dense granules combined with variable reduction in platelet  $\alpha$ -granules. In addition some patients have  $\alpha$ -SPD, or the gray platelet syndrome, characterized by a severe reduction in  $\alpha$ -granule number and contents. SPD can be restricted to the platelet and cause a mild hemostatic defect or be part of systemic syndromes of defective granule assembly and packaging.

Although the disorders have been known for 35 years their molecular mechanism is not well understood. In 1969 Weiss et al described a kindred in which 10 members in 4 generations had a bleeding diathesis.<sup>37</sup> Six of the affected members were studied and had impaired release of platelet

adenosine diphosphate (ADP) and small platelets. In a subsequent analysis of the same family, Holmsen and Weiss postulated that they lacked the storage, or non-metabolic, pool of ADP.<sup>38,39</sup> By electron microscopy, Weiss showed a marked decrease in platelet dense bodies.<sup>36</sup> Since both serotonin and the storage pool of adenine nucleotides are deficient in these platelets, it was postulated that the dense bodies contain these mediators. Weiss then studied 7 patients with albinism (Hermansky-Pudlak syndrome) and 4 other unrelated patients who lacked dense granules and dense granule substances and termed this  $\delta$ -SPD. Seven other patients had variable deficiencies of  $\alpha$ -granules and of the platelet proteins platelet factor 4 (PF4),  $\beta$ -thromboglobulin, fibrinogen, and platelet-derived growth factor (PDGF) along with dense granule defects. The disorder in 1 patient with the most profound  $\alpha$ -granule defect and the partial  $\alpha$ -granule deficiency coupled with  $\delta$  granule deficiency observed in 6 members of 2 unrelated families were both designated  $\alpha/\delta$ -SPD.  $\delta$ -storage pool disease appears to be an autosomal recessive trait. Secretable acid hydrolases were normal in all these patients, consistent with their localization in  $\lambda$  granules (lysosomes), which are not affected in either disorder.<sup>40</sup>

Weiss pointed out that storage pool deficiency can also be an acquired disorder and that  $\delta$ -SPD is seen in some patients with myeloproliferative disorders, myelodysplasia and acute leukemia.<sup>41</sup> Several patients with systemic lupus erythematosus have been described who have acquired SPD secondary to premature release of granule contents induced by circulating immune complexes.

Lages demonstrated heterogeneity in SPD patients.<sup>42</sup> Some patients with severe  $\alpha/\delta$ -SPD had half normal amounts of the P-selectin, an  $\alpha$ -granule protein, while others had normal P-selectin content. In a previous study, both the content and the surface expression of P-selectin were normal platelets from 2 patients with  $\alpha$ -SPD.

It is of interest that the proband of a family with  $\delta$ -SPD reported by Caen died of primary pulmonary hypertension. Herve suggested that the pulmonary hypertension in this patient was due to an increased level of plasma 5-hydroxytryptamine (5-HT) following loss of the buffering usually provided by platelets which can take up and store plasma serotonin.<sup>43</sup> Administration of ketanserin, a 5-HT antagonist, substantially reduced pulmonary hypertension in the delta SPD patient.

### The Hereditary Macrothrombocytopenias

These are a heterogeneous group of disorders characterized by autosomal dominant inheritance of mild to moderate thrombocytopenia with large platelets and varying degrees of platelet dysfunction.<sup>44,45</sup> Some of the syndromes have, in addition, leukocyte inclusions, interstitial nephritis, sensorineural hearing loss and cataracts. The four most common defects—the May-Heggelin anomaly, the Fechtner syndrome, the Sebastian syndrome and Epstein syndrome—may all be variants of a single disorder as they are

all due to mutations in the heavy chain of a prominent non-muscle myosin MYH9.<sup>46-48</sup> The molecular pathology of several of the less common but phenotypically similar defects is not yet understood. The clinical characteristics of the major syndromes are summarized in **Table 2**.

May-Heggelin anomaly is the most prevalent disorder and the abnormalities are restricted to platelets and leukocytes. The platelets are large, almost the size of red cells and lymphocytes, and there are prominent leukocyte inclusions. In the Fechtner syndrome patients have nephritis, hearing loss and cataracts and distinct spherical granules in their leukocytes. The platelets are enlarged but less so than in May-Heggelin patients. Patients with the Sebastian syndrome have the same leukocyte and platelet abnormalities as Fechtner patients but not additional organ defects. Finally, patients with the Epstein syndrome have the nephritis and hearing loss along with defects in platelet adhesion and aggregation but no leukocyte inclusions. Patients with Eckstein syndrome have nephritis and deafness like Epstein patients but normal platelet function. Finally patients with the Enyeart syndrome have giant platelets and thrombocytopenia. In this case however, there are inclusions in the platelets but not in the leukocytes.

Several of the giant platelet syndromes have associated membrane protein defects. For example patients with defects in GpIb/IX/V as previously described have giant platelets and moderate thrombocytopenia. Patients with the velocardiofacial syndrome have conotruncal cardiac defects and learning defects along with defective platelet GpIb as they have a deletion on 22q11.2. There is another giant platelet syndrome associated with loss of the platelet collagen receptor (GpIa/IIa) that is accompanied by mitral insufficiency and a rare form of familial macrothrombocytopenia with a defect in GpIV, mild platelet dysfunction and no leukocyte inclusions.

Mediterranean macrothrombocytopenia, seen in Greek and Italian populations, is characterized by varying degrees of thrombocytopenia and the presence of large to giant platelets. Although the molecular explanation is incomplete, a subset of these patients are heterozygous carriers of the BSS. The molecular etiology of the remaining cases of Mediterranean macrothrombocytopenia is as yet unexplained. These patients, like other BSS carriers, are either asymptomatic or have a mild bleeding diathesis. A substantial number of the patients studied carried the Bolzano variant described above (Val156Ala) which may cause more symptoms in heterozygotes.<sup>49</sup>

### Platelet Signaling Disorders

Although not as well characterized as the defects described above, there is increasing evidence that specific defects in post-receptor signaling pathways can impair platelet function. These defects have been recently reviewed by Rao et al.<sup>50</sup> They describe defects in specific G protein subunits and in phospholipase C isoenzymes that impair platelet activation. The molecular basis for these defects is less clear

**Table 2. The hereditary macrothrombocytopenias.**

Disorder	Features
May-Heggelin anomaly	Autosomal dominant Very large platelets Normal platelet function Leukocyte inclusions MYH9 mutation
Sebastian syndrome	Autosomal dominant Near normal platelet function Granulocyte inclusions distinct from those in May-Heggelin MYH9 mutation
Fechtner Syndrome	Autosomal dominant Granulocyte inclusions like those in Sebastian syndrome Nephritis, deafness, cataracts MYH9 mutation
Epstein syndrome	Autosomal dominant Platelet dysfunction No granulocyte inclusions Nephritis and deafness MYH9 mutation
Eckstein syndrome	Like Epstein Syndrome but normal Platelet function MYH9 mutation
Bernard-Soulier syndrome	Autosomal recessive Absent GpIb/IX complex Severe platelet adhesion defect
Grey Platelet syndrome	Autosomal recessive inheritance Absence of platelet a granules Mild platelet dysfunction
Giant platelets with the velocardiofacial syndrome	Autosomal recessive inheritance Defective GpIb/IX Mutation/Deletion at 22q11.2 Conotruncal heart defects Severe learning disability
Giant platelets and mitral valve insufficiency	Autosomal recessive inheritance Mild bleeding Absent collagen receptor (GpIa/IIa) Decreased platelet agg to ADP, thrombin Arachidonate
Familial macrothrombocytopenia	Autosomal dominant and GpIV deficiency Defect/absence GpIV Reduced aggregation to ADP and epinephrine Mild bleeding No neutrophil inclusions
Montreal platelet syndrome	Autosomal dominant inheritance Spontaneous platelet aggregation Prolonged bleeding time
Enyeart syndrome	Autosomal recessive inheritance Mild to severe bleeding Small inclusions in platelets
Mediterranean macrothrombocytopenia	Pathogenesis unclear Restricted to Greeks and Italians Some are heterozygous Bernard-Soulier carriers Many have the Bolzano variant of GpIb $\alpha$ Val156Ala

(Modified from Rodriguez et al. Mayo Clin Proc.2003;78:1416-1421.)

as many of the enzymes are expressed in multiple tissues but seem to be selectively deficient in the platelet. Conversely, some mutant G proteins thought to play a role in hemostasis cause no discernible bleeding when they are knocked out in mice or absent because of endocrine or other genetic disorders in man. From a clinical point of view most of these patients have mild bleeding and rarely or infrequently require therapy. However, it is useful to be aware that these defects exist and perhaps refer patients to centers interested in studying them so the nature and the extent of the defects can be ascertained.

### Platelet Coagulant Function

In addition to its important role in primary hemostasis, i.e., formation of the platelet plug, platelets play an important role in secondary hemostasis or plasma coagulation. The major coagulation reactions, particularly the assembly of the prothrombinase complex and the production of thrombin, proceeds many times more rapidly on the platelet surface than in the fluid phase or on artificial lipid micelles. Prior to activation, the platelet membrane is quiescent and cannot support coagulation reactions. In this state phosphatidylserine (PS) and phosphatidylethanolamine (PE) are restricted to the inner membrane leaflet and phosphatidylcholine (PC) is expressed on the outer leaflet. There is an *aminophospholipid translocase* that maintains this lipid asymmetry.<sup>51</sup> Following platelet activation by a variety of stimuli, this membrane asymmetry is lost and PS and PE appear on the outer leaflet. This rearrangement is mediated by a calcium-dependent phospholipid *scramblase*.<sup>52</sup> The appearance of PS on the platelet outer membrane leaflet facilitates the assembly of the prothrombinase complex and provides a six log enhancement of thrombin generation.

To underscore the importance of this pathway, there is a rare platelet defect, Scott syndrome, in which the primary hemostatic function of platelets is intact but the platelets do not support prothrombinase assembly and thrombin generation.<sup>53</sup> Platelets from patients with Scott syndrome undergo normal adhesion, aggregation and secretion reactions. However, they do not support coagulation in a number of in vitro assays including the generation of platelet factor III activity or prothrombin consumption. Scott syndrome platelets do not express PS following exposure to platelet agonists. Although the specific mutations have not been defined, platelets from these patients lack requisite *scramblase* activity. Finally there may be an inverse condition, the Stormorken syndrome, in which platelets are constitutively activated and express PS without prior activation.<sup>54,55</sup> It has been postulated that these patients have a defective *aminophospholipid translocase*.

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