# Congenital Disorders of Platelet Function

*a report by*
**Gian Marco Podda**, **Mariateresa Pugliano** *and* **Marco Cattaneo**

*San Paolo Hospital, Department of Medicine, Surgery and Dentistry, University of Milan*

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When a blood vessel is injured, platelets adhere to the exposed subendothelium (platelet adhesion). The platelets are activated (platelet activation) and secrete their granule contents (platelet secretion). The granule contents include platelet agonists (adenosine diphosphate [ADP] and serotonin) that, by interacting with specific platelet receptors, contribute to the recruitment of additional platelets to form aggregates (platelet aggregation). Platelets also play a role in coagulation, providing the necessary surface of procoagulant phospholipids (platelet procoagulant activity). Congenital or acquired abnormalities of platelet numbers or functions are associated with a heightened risk of bleeding, proving that platelets play an important role in haemostasis. Patients with platelet disorders typically have mucocutaneous bleedings of variable severity and excessive haemorrhage after surgery or trauma.

## Classification of the Congenital Disorders of Platelet Function

Inherited disorders of platelet function are generally classified based on the type of abnormal function. Platelet functions are intimately related and a clear distinction between the disorders of platelet adhesion, aggregation, activation, secretion and procoagulant activity may be problematic. We propose a classification of the inherited disorders of platelet function based on the shared common characteristic abnormalities of platelet components:

- platelet receptors for adhesive proteins;
- platelet receptors for soluble agonists;
- platelet granules;
- signal transduction pathways; and
- procoagulant phospholipids.

Those inherited disorders of platelet function that are less well characterised will be placed in a separate category of miscellaneous disorders.

## Abnormalities of the Platelet Receptors for Adhesive Proteins

### *Abnormalities of the Glycoprotein Ib–V–IX Complex*

#### *Bernard-Soulier Syndrome*

Bernard-Soulier syndrome (BSS) is associated with both quantitative and qualitative defects of the platelet glycoprotein complex GPIb–IX–V. The complex is formed of four glycoproteins. GPIb consists of two subunits, GPIb-α and GPIb-β. Characterised by an autosomal recessive inheritance (only one case has been characterised by autosomal dominant inheritance), BSS also exhibits prolonged bleeding times, variable degrees of thrombocytopenia, giant platelets, decreased platelet adhesion and abnormal prothrombin consumption. Electron microscopy shows cytoplasmic vacuoles and membrane complexes in the giant platelets. These abnormalities extend to megakaryocytes (MK). With an estimated prevalence of 1/1,000,000 cases,[1] BSS is a relatively severe bleeding disorder. Typical bleeding manifestations of the disorder include epistaxis, gum bleeding and both post-surgical and post-traumatic bleeding. Most heterozygotes have intermediate amounts of the GP complex and may have some giant platelets without a bleeding diathesis.[2–5]

In primary haemostasis initial platelet adherence and recruitment depends on GPIb-α binding to immobilised von Willebrand factor (VWF).[6] BBS platelets are characterised by a significantly reduced ability to adhere to the subendothelium. The disease phenotype is primarily due to the inability of VWF to bind to GPIb-α. The absence of GPIb-α-related binding sites for thrombin, P-selectin, thrombospondin-1 (TPS-1), factor XI, factor XII, α-Mβ-2 and high-molecular-weight kininogen may play an additional role in the impairment of haemostasis. BSS platelets do not agglutinate *in vitro* when exposed to the antibiotic ristocetin or to the snake venom protein botrocetin. This defect is not corrected by the addition of normal plasma. In this instance the platelet responses to physiological agonists are normal, with the exception of low concentrations of thrombin. Diagnosis of BSS is based on the demonstration of a GPIb–IX–V deficiency by either flow-cytometry or immunoblotting. BSS is associated with genetic defects in GPIb-α, GPIb-β and GPIX, preventing the constitution and trafficking of the receptor through the both the Golgi apparatus and the endoplasmic reticulum. Mutations within GPV do not lead to BSS. The molecular defects responsible for BBS include frame shifts, deletions and point mutations.[3–5,7]

#### *Platelet-type or Pseudo-von Willebrand Disease*

Platelet-type von Willebrand disease (VWD) or pseudo-VWD is an autosomal-dominant disease associated with amino acid substitutions that

Gian Marco Podda is a Fellow in the Thrombosis and Haematology Laboratory at San Paolo Hospital, University of Milan. Prior to this he was a Research Associate of Molecular and Experimental Medicine at the Scripps Research Institute in La Jolla. He received awards for his work from the Italian Society of Haemostasis and Thrombosis (SISET) and has submitted numerous papers on the subject of coagulation disorders. Dr Podda received his medical degree in 1997 from the University of Milan.

Mariateresa Pugliano is a Research Fellow within the Haematology and Thrombosis Unit at the San Paolo Hospital, University of Milan. Prior to this, she attended a specialisation course in Haaematology at the IRCCS Policlinico at the Mangiagalli and Regina Elena Foundation within the Maggiore Hospital in Milan. Dr Pudliano received her medical degree in 2004 from the University of Milan.

Marco Cattaneo is a Full Professor and Director of Internal Medicine in the Department of Medicine, Surgery and Dentistry at the San Paolo Hospital, University of Milan. He is Past President of the Italian Society for the Study of Thrombosis and Haemostasis (SISET) and has authored more than 170 original articles published in peer-reviewed scientific journals. Dr Cattaneo recieved his medical degree in 1975 from the University of Milan.

occur within the disulphide-bonded double loop region of GPIbα (Gly-233-Val and Met-239-Val).[8,9] Platelet-type VWD is not due to defects of VWF, but rather to a gain in function of the phenotype platelet GPIbα. This has an increased avidity for VWF, leading to the binding of the largest VWF multimers to resting platelets and their clearance from circulation.[3]

### *Abnormalities of Glycoprotein II-b and Glycoprotein III-a ($\alpha_{IIb}/\beta_3$)*

#### *Glanzmann's Thrombasthaenia*

Glanzmann's thrombasthenia (GT) is an autosomal recessive disease caused by a lack of expression or qualitative defects in one of the two GPs forming the integrin $\alpha_{IIb}/\beta_3$. In activated platelets the integrin $\alpha_{IIb}/\beta_3$ binds the adhesive glycoprotein (fibrinogen at low shear, VWF at high shear) that bridges adjacent platelets and secures platelet aggregation. The diagnostic hallmark of the disease is the lack (or severe impairment) of platelet aggregation induced by all agonists. Severe forms (GT-type-I) are characterised by a lack of fibrinogen in the platelet α-granules. GT patients display a phenotype that is similar to that of BSS patients, albeit less severe. Heterozygotes do not have a bleeding diathesis.[2–4] Diagnosis of GT is based on the presence of typical abnormalities in platelet function and on the demonstration that GPIIb/IIIa is absent or severely reduced on the platelet membrane. Flow cytometry is used as a screening test and clot retraction is often absent. Genetic defects can occur along the length of both genes. In the GPIIb ($\alpha_{IIb}$) subunit, splice site mutations and non-sense mutations, involving frame-shifts and giving rise to truncated proteins, are usually associated with severe forms of GT (type-I GT, according to early nomenclature).[3,4] Missense mutations may give rise to a less severe deficiency of the complex or to dysfunctional proteins.[3,4] Deletions, splice mutations and inversions in GPIII-a ($\beta_3$) involving frame-shifts and giving rise to truncated proteins, are usually associated with severe forms of GT.[3,4] A comprehensive list of mutations can be found in the GT database at sinaicentral.mssm.edu/intranet/research/glanzmann.

## Abnormalities of the Platelet Receptors for Soluble Agonists

### *Abnormalities of the Platelet Adenosine Diphosphate Receptor P2Y$_{12}$*

The P2Y$_{12}$ is one of the two G-protein-linked purinergic receptors that mediate the platelet responses to ADP. The first patient with a severe P2Y$_{12}$ deficiency was described in 1992.[10] He had a life-long history of excessive bleeding, prolonged bleeding time (15–20 minutes) and abnormalities of platelet aggregation similar to those observed in patients with defects of platelet secretion (reversible aggregation in response to weak agonists and impaired aggregation in response to low concentrations of collagen or thrombin). The aggregation response to ADP was severely impaired even at very high ADP concentrations (>10μM). P2Y$_{12}$ defects should be suspected when ADP, even at relatively high concentrations (10μM or higher), induces a slight and rapidly reversible aggregation preceded by a normal shape change. Measurement of the inhibition of stimulated adenylyl cyclase by ADP (which can be tested by measuring the platelet levels of cAMP or ovasodilator-stimulated phosphoprotein [VASP] phosphorylation) is the most accurate confirmatory test.

### *Defects of the Platelet Thromboxane A$_2$ Receptor*

Thromboxane-A$_2$ (Tx-A$_2$) formation on platelet activation is due to the action of phospholipase-A$_2$. It releases arachidonic acid (AA) from membrane phospholipids, as well as cyclo-oxygenase-1 (COX-1), which transforms arachidonic acid into endoperoxides, metabolised to Tx-A$_2$ by thromboxane synthase (TxA$_2$S). Released TxA$_2$ binds to its Gq-coupled TxA$_2$R.[15] Several homozygous and heterozygous patients suffering from lifelong mucosal bleeding and easy bruising have been found to have an Arg60 Leu mutation in the first cytoplasmic loop of the TxA$_2$R[16] affecting both receptor isoforms.[17,18] The mutation was inherited as an autosomal-dominant trait and the heterozygous patients did not differ from the homozygous patients in terms of aggregation and secretion responses of platelets to TxA$_2$.

## Defects of the Platelet Granules

Defects of the platelet granules comprise a heterogeneous group of disorders, including deficiencies of the delta and/or alpha granules, or their constituents (delta- and alpha-storage pool deficiency) and other less common defects of the alpha granules.

### *Defects of the Delta Granules*

#### *Delta-storage Pool Deficiency*

The term delta-storage pool deficiency, or delta-storage pool disease (δ-SPD), defines a congenital abnormality of platelets characterised by a deficiency of dense granules in megakaryocytes and platelets. Between 10 and 18% of patients with congenital abnormalities of platelet function have SPD.[19,20] The inheritance is autosomal recessive in some families and dominant in others. Patients with δ-SPD have mild to moderate bleeding diathesis characterised by mucocutaneous bleedings such as epistaxis, menorrhagia and easy bruising. Patients with the most severe forms may also experience post-surgical haemorrhagic complications, especially after tooth extraction and tonsillectomy. One case of intracranial bleeding has been reported.[19] SPD is characterised by a mild to moderate prolonged bleeding time, abnormal platelet secretion induced by several platelet agonists, impaired platelet aggregation and decreased platelet content of dense granules.[19,21,22]

In citrated platelet-rich plasma, primary aggregation induced by ADP or epinephrine and the agglutination response to ristocetin are normal. The second wave of aggregation and the aggregation in response to collagen are generally absent or greatly reduced.[23,24] The production of arachidonate metabolites can be defective after stimulation with epinephrine or collagen but normal with arachidonate.[25] The aggregation induced by sodium arachidonate or prostaglandin endoperoxides may be normal or decreased,[24,25] depending on the severity of ADP deficiency in platelet granules.[25] Normal responses to ADP or epinephrine have been observed in some patients,[26] indicating that there is a large variability in platelet aggregation in patients with δ-SPD. This has been well documented in a large study of 106 patients with δ-SPD.[20] Platelets from patients with isolated platelet δ-SPD had normal amounts of the δgranule membrane protein granulophysin, suggesting a qualitative rather than a quantitative type of δ-granule defect.[19]

Lumiaggregometry, which measures platelet aggregation and secretion simultaneously, may prove a more accurate technique than platelet aggregometry for diagnosing patients with δ-SPD and platelet secretion defects. The diagnosis of δ-SPD is essentially based on the finding of defective platelet secretion induced by several agonists, decreased platelet content of total ADP and adenosine triphosphate (ATP), an increase in the ATP/ADP ratio of >2.5–3.1[27] and a normal serum concentration of the stable

$TxA_2$ metabolite $TxB_2$. Methods involving the identification of mepacrine-loaded platelets by flow cytometry[28] may also prove to be useful for the diagnosis of this disorder.

### *The Hermansky-Pudlak Syndrome and the Chediak-Hygashi Syndrome*

The Hermansky-Pudlak syndrome (HPS) and the Chediak-Hygashi syndrome (CHS) are rare syndromic forms of δ-SPD.[19] HPS is an autosomal recessive disease of the sub-cellular organelles of many tissues involving abnormalities of melanosomes, platelet δ-granules and lysosomes.[19] It is characterised by tyrosinase-positive oculocutaneous albinism, a bleeding diathesis due to δ-SPD and ceroid-lypofuscin lysosomal storage disease. HPS can arise from mutations in different genetic loci.[19,29–31] CHS is also an autosomal recessive disorder characterised by variable degrees of oculocutaneous albinism, large peroxidase-positive cytoplasmic granules in a variety of haemopoietic (neutrophils) and non-haematopoietic cells, easy bruising due to δ-SPD, recurrent infections associated with neutropenia, impaired chemotaxis, bactericidal activity and abnormal NK function.[32] The syndrome is lethal, usually leading to death in the first decade.

### *Hereditary Thrombocytopenias*

Two types of hereditary thrombocytopenia may be associated with δ-SPD. They are the thrombocytopenia and absent radii syndrome (TAR) and the Wiskott-Aldrich syndrome (WAS).[33,34]

TAR is a developmental disorder characterised by thrombocytopenia and the bilateral absence of the radii. Platelet counts are usually in the range of 15,000–30,000µl in infancy and increase with age. TAR can be autosomal, recessive or dominant. Poor responses to collagen and absent secondary waves of aggregation in response to ADP or epinephrine, which are typical of defects of δ-granules, have been described in these patients.[35] WAS is an X-linked recessive disease characterised by micro-thrombocytopenia, immunodeficiency and eczema. It is caused by mutations in the WASP gene. The WASP protein regulates signal-mediated actin cytoskeleton rearrangement. Bleeding manifestations may be mild or severe. WAS patients have a marked reduction in dense granules and, more rarely, in alpha granules.[35]

## *Defects of the Alpha Granules*

### *Alpha-Storage Pool Deficiency – Grey Platelet Syndrome*

This condition owes its name to the grey appearance of the platelets in peripheral blood smears as a consequence of the rarity of platelet granules. The inheritance pattern seems to be autosomal recessive, although it seemed to be autosomal-dominant in some families[36,38] and X-linked in one.[39] Affected patients have a lifelong history of mucocutaneous bleeding, which may vary from mild to moderate in severity, prolonged bleeding time, mild thrombocytopenia, abnormally large platelets and an isolated reduction of the platelet α-granule content. Occasionally, patients may have more severe bleeding symptoms, including intracranial haemorrhage and post-surgical bleeding.[19] Splenomegaly may be present,[40,41] and splenectomy may be followed by a normalisation of the platelet count, but not by an amelioration of the bleeding diathesis.[41]

Grey platelets are severely and selectively deficient in soluble proteins contained in the α-granule: platelet factor 4, β-thromboglobulin, VWF, thrombospondin, fibrinogen, fibronectin, immunoglobulins and albumin. In contrast to soluble proteins, the α-granule membrane proteins are normal in GPS,[42–45] consistent with the demonstration of the presence of empty α-granules in the GPS platelets[46] and the normal production of precursors of α-granules in GPS megakaryocytes.[47] A decrease in secondary granules and secretory vesicles in neutrophils was recently described in some GPS patients.[48,49] Circulating platelets are reduced in number, relatively large and vacuolated, and contain normal numbers of mitochondria, δ-granules, peroxisomes and lysosomes. They specifically lack α-granules.[50] The degree of thrombocytopenia is usually mild, although cases with platelet counts as low as 20,000/µl have been described. Platelet aggregation studies show variable results in GPS patients. Platelet aggregation induced by ADP and adrenaline in citrated plasma was usually normal. Impaired aggregation responses induced by ADP or low concentrations of thrombin or collagen have been described in some patients.[41,51–54]

### *Quebec Platelet Disorder*

Quebec platelet disorder (QPD) is an autosomal dominant qualitative platelet abnormality characterised by the abnormal proteolysis of α-granule proteins, normal platelet counts and a markedly decreased platelet aggregation induced by epinephrine.[55,56] Patients with QPD experience severe post-traumatic and post-surgical bleeding complications, joint bleeds and large bruises that are unresponsive to platelet transfusion but are well controlled by the administration of antifibrinolytic agents.[57] Multimerin, one of the largest proteins found in the human body, is present in platelet α-granules and in endothelial cell Weibel-Palade bodies.[58–60] It binds with factor V and its activated form, factor Va. Its deficiency in patients with the QPD is probably responsible for the defect in platelet factor V. This is likely to be degraded by abnormally regulated platelet proteases.

### *Paris-Trousseau Syndrome Thrombocytopenia and the Jacobsen Syndrome – 11q Terminal Deletion Disorder*

Paris trousseau syndrome (PTS) and Jacobsen syndrome (JS; now termed 11-q terminal deletion disorder) are related disorders presenting a mild haemorrhagic diathesis. They are characterised by congenital thrombocytopenia, a normal platelet life span and an increased number of marrow megakaryocytes, many of which present with signs of abnormal maturation and intramedullary lysis. A fraction of the circulating platelets have giant α-granules that are unable to release their content upon platelet stimulation with thrombin. While the platelet defect is predominant in PTS, JS has a more severe phenotype, which includes congenital heart defects, mental retardation, gross and fine motor delays, trigonocephaly, facial dysmorphism and ophthalmological, gastrointestinal and genito-urinary problems.[61]

## *Defects of the Alpha and Delta Granules*

### *Alpha- and Delta-storage Pool Deficiency*

Alpha- and delta-storage pool deficiency is a heterogeneous congenital disorder of platelet secretion characterised by deficiencies of both α- and δ-granules.[62,63] It is important to note that blood samples should be collected in sodium citrate for measurement of platelet granule content as platelets from some individuals may undergo degranulation *in vitro* when blood is collected in ethylenediaminetetraacetic acid (EDTA), resembling α,δ-SPD.[19] Approximately 80% of platelets from the patient with severe α,δ-SPD expressed little or no P-selectin after stimulation. The remaining 20% expressed normal amounts. Compared with δ-SPD platelets, which have a normal density, α,δ-SPD platelets show a shift to the left of the

density distribution, suggesting that α-granules are a major determinant of platelet density.[64] The clinical picture and the platelet aggregation abnormalities are similar to those of patients with GPS or δ-SPD.

### Abnormalities of the Signal–Transduction Pathways

Congenital abnormalities of the arachidonate thromboxane $A_2$ pathway raise an impaired liberation of arachidonic acid from membrane phospholipids. In these patients $TxB_2$ production, after stimulation with ADP or thrombin, was impaired. It was normal with arachidonic acid stimulation.[65] Patients with congenital abnormalities in cyclo-oxygenase have been also been identified.[19] Platelets from these patients have the same functional defect as normal platelets treated with aspirin: impaired aggregation and secretion induced by ADP, epinephrine, collagen or arachidonic acid, normal responses to $TxA_2$ endoperoxides analogues and absent platelet $TxA_2$ production.

### Abnormalities of Membrane Phospholipids

#### *Scott Syndrome*

Scott syndrome is a very rare bleeding disorder associated with the maintenance of the asymmetry of the lipid bi-layer in the membranes of blood cells, including platelets.[66] It leads to reduced thrombin generation and defective wound healing. The cause of the defect is still not clearly understood.[3]

### Miscellaneous Disorders of Platelet Function

#### *Primary Secretion Defects*

The term 'primary secretion defect' was probably used for the first time by Weiss to indicate all those ill-defined abnormalities of platelet secretion not associated with platelet granule deficiencies.[67] It was later broadened to include the platelet secretion defects not associated with platelet granule deficiencies and abnormalities of the arachidonate pathway[11,68] or, more generally, all of the abnormalities of platelet function associated with defects of signal transduction.[69] With the progression of our knowledge in platelet pathophysiology, this heterogeneous group of patients with congenital disorders of platelet function will become progressively smaller. Patients with better-defined biochemical abnormalities responsible for their platelet secretion defect will be classified correctly. As an example, patients with heterozygous $P2Y_{12}$ deficiency were included in this group of disorders until their biochemical abnormality was identified.[70] ■


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## Editor's Recommendations

### Platelet $P_2$ Receptors – Old and New Targets for Antithrombotic Drugs

**Cattaneo M**

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Platelets possess three $P_2$ receptors for adenine nucleotides: $P2Y_1$ and $P2Y_{12}$, which interact with ADP, and $P2X_1$, which interacts with ATP. The interaction of adenine nucleotides with their platelet receptors plays an important role in thrombogenesis. The thienopyridine ticlopidine, an antagonist of the platelet $P2Y_{12}$ ADP receptor, reduces the incidence of vascular events in patients at risk, but it also has some important drawbacks: a relatively high incidence of toxic effects, delayed onset of action and high inter-individual variability in response. Another thienopyridine, clopidogrel, has superseded ticlopidine because it is an efficacious antithrombotic drug and is less toxic than ticlopidine. However, the high inter-patient variability in response still remains an important issue. These drawbacks justify the continuing search for agents that can further improve the clinical outcome of patients with atherosclerosis through greater efficacy and/or safety. A new thienopyridyl compound, prasugrel, which is characterised by higher potency and faster onset of action compared with clopidogrel, is currently under clinical evaluation. Two direct and reversible $P2Y_{12}$ antagonists, cangrelor and AZD6140, have very rapid onset and reversal of platelet inhibition, which makes them attractive alternatives to thienopyridines, especially when rapid inhibition of platelet aggregation or its quick reversal is required. Along with new $P2Y_{12}$ antagonists, inhibitors of the other platelet receptor for ADP, $P2Y_1$, and of the receptor for ATP, $P2X_1$, are under development and may prove to be effective antithrombotic agents. ■

### Inherited Traits Affecting Platelet Function

**Salles II, et al.**

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Inherited platelet disorders constitute a large group of diseases involving a wide range of genetic defects that can lead to bleeding symptoms of varying severity. They are associated with defects in surface membrane glycoproteins, resulting in, for example, Bernard-Soulier syndrome and Glanzmann thrombasthenia, causing defects in platelet adhesion and aggregation, respectively, as well as in receptors for agonists ($P2Y_{12}$, $TXA_2$) disrupting platelet signalling. Defects affecting platelet granules can be characterised by abnormalities of alpha-granules as in the grey platelet syndrome or dense granules as in Hermansky-Pudlak and Chediak-Higashi syndromes, the latter two also altering other cytoplasmic organelles such as melanosomes and therefore not restricted to platelets. Finally, defects in proteins essential to signalling pathways (in Wiskott-Aldrich syndrome) or in platelet-derived procoagulant activity (Scott and Stormorken syndromes) also impair platelet function. For most of the above disorders, mouse knockout models have been generated, which allowed the genotype–phenotype relationship to be confirmed, and also further unravelling the molecular causes of the disease and the mechanisms underlying primary haemostasis. More recently, interest has been growing in the effects of the more common polymorphisms that are found in the platelet glycoproteins as possible risk factors for thrombotic disorders. The new era of platelet genomics and proteomics will increase our knowledge of platelet disorders, which will improve their diagnosis and also provide a basis for new antithrombotic therapies. ■