REVIEW ARTICLE

Inherited platelet-based bleeding disorders

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Summary. Inherited platelet-based bleeding disorders include abnormalities of platelet number and function, and are generally classified based on the abnormal functions or responses. However, a clear distinction is problematic, and in this review, the classification has been based on abnormalities of platelet components that share common characteristics.

Inherited thrombocytopenias are rare, but probably underdiagnosed. They are usually classified according to both platelet size and the presence or absence of clinical features other than those deriving from the platelet defect. Hereditary disorders of platelet function can be classified as resulting from: (i) abnormalities of the platelet receptors for adhesive proteins; (ii) abnormalities of the platelet receptors for soluble agonists; (iii) abnormalities of the platelet granules; (iv) abnormalities of the signal-transduction pathways; (v) abnormalities of the membrane phospholipids; and (vi) miscellaneous abnormalities of platelet function. The literature on these disorders is reviewed, and the underlying defects discussed.

Keywords: inherited bleeding disorders, inherited thrombocytopenias, platelet abnormalities, platelets.

Introduction

When a blood vessel is injured, platelets adhere to the exposed subendothelium (platelet adhesion), are activated (platelet activation) and secrete their granule contents (platelet secretion), including some platelet agonists (adenosine diphosphate (ADP), serotonin) which, by interacting with specific platelet receptors, contribute to the recruitment of additional platelets to form aggregates (platelet aggregation). In addition, platelets play a role in the coagulation mechanism, providing the necessary surface of procoagulant phospholipids (platelet procoagulant activity). Congenital or acquired abnormalities of platelet number or function are associated with a heightened risk for bleeding, proving that platelets play an important role in hemostasis. Typically, patients with platelet disorders have mucocutaneous bleedings of variable severity, and excessive hemorrhage after surgery or trauma. In this brief chapter, I shall review the main inherited platelet-based bleeding disorders. Abnormalities of platelet function due to defects of plasma proteins [e.g. von Willebrand disease (VWD), afibrinogenemia] will not be considered in this review. Due to space limitations, I shall focus on the more recently described and less well-known abnormalities of the platelet receptors for ADP, referring the interested reader to very good and recent reviews for more details on the remaining disorders [1–4].

Classification

Inherited platelet-based bleeding disorders include abnormalities of platelet number (inherited thrombocytopenias) and function (inherited disorders of platelet function) (Table 1). Some disorders are characterized by both thrombocytopenia and abnormalities of platelet function. Inherited disorders of platelet function are generally classified based on the functions or responses that are abnormal. However, since platelet functions are intimately related, a clear distinction between disorders of platelet adhesion, aggregation, activation, secretion and procoagulant activity is in many instances problematic. For example, platelets that are deficient in the glycoprotein (GP) complex Ib/IX/V, which is a receptor for von Willebrand factor (VWF), do not adhere normally to the subendothelium and for this reason are generally included in the group of abnormalities of platelet adhesion. However, they also do not undergo normal activation and aggregation at high shear, do not aggregate normally to thrombin, and display abnormal procoagulant responses. For this reason, I chose a classification of the inherited disorders of platelet function based on abnormalities of platelet components that share common characteristics: (i) platelet receptors for adhesive proteins; (ii) platelet receptors for soluble agonists; (iii) signal transduction pathways; and (iv) procoagulant phospholipids. Inherited disorders of platelet function that are less well characterized are grouped in a fifth category of miscellaneous disorders.

Inherited thrombocytopenias

Although inherited thrombocytopenias are rare, their frequency is probably underestimated because of diagnostic difficulties. Moreover, not all the existing forms have yet been identified, and some patients remain without a definite diagnosis

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Table 1 Inherited platelet-based bleeding disorders

Inherited thrombocytopenias[*]
With small platelets
    Wiskott–Aldrich syndrome
    X-linked thrombocytopenia
With normal-sized platelets
    Familial platelet disorder and predisposition to acute myelogenous leukemia
    Congenital amegakaryocytic thrombocytopenia
    Amegakaryocytic thrombocytopenia with radio-ulnar synostosis
    Thrombocytopenia with absent radii syndrome
    Autosomal dominant thrombocytopenia
With large platelets
    Bernard–Soulier syndrome
    Velocardiofacial syndrome
    Platelet-type von Willebrand disease
    Benign mediterranean macrothrombocytopenia
    Dyserythropoietic anemia with thrombocytopenia
    X-linked thrombocytopenia with thalassemia
    Paris–Trousseau –Jacobsen's syndrome
    MYH9-related disease (May–Haegglin anomaly, Sebastian syndrome, Fechtner syndrome, Epstein syndrome)
    Gray platelet syndrome
    Montreal platelet syndrome
    Macrothrombocytopenia with platelet expression of glycophorin A
Inherited disorders of platelet function
Abnormalities of the platelet receptors for adhesive proteins
    GP Ib-V-IX complex (Bernard–Soulier syndrome, platelet-type von Willebrand disease, Bolin–Jamieson syndrome)
    GP IIb/IIIa (αIIb/β3) (Glanzmann's thrombasthenia)
    GP Ia/IIa (α2/β1)
    GPVI
    GPIV
Abnormalities of the platelet receptors for soluble agonists
    Thromboxane A2 receptor
    α_2-adrenergic receptor
    P2Y12 receptor
Abnormalities of the platelet granules
    δ-granules (δ-storage pool deficiency, Hermansky–Pudlak ssyndrome, Chediak–Hygashi syndrome, thrombocytopenia with absent radii syndrome, Wiskott–Aldrich syndrome)
    α-granules (gray platelet syndrome, Quebec platelet disorder, Paris–Trousseau–Jacobsen syndrome)
    α- and δ-granules (α,δ-storage pool deficiency)
Abnormalities of the signal-transduction pathways
    Abnormalities of the arachidonate/thromboxane A2 pathway
    Gαq deficiency
    Partial selective PLC-β_2 isozyme deficiency
    Defects in pleckstrin phosphorylation
    Defective Ca^{2+} mobilization
    Hyperresponsiveness of platelet Gsα
Abnormalities of membrane phospholipids
    Scott syndrome
    Stormorken syndrome
Miscellaneous abnormalities of platelet function
    Primary secretion defects
    Other platelet abnormalities (Montreal platelet syndrome, osteogenesis imperfecta, Ehlers–Danlos syndrome, Marfan's syndrome, hexokinase deficiency, glucose-6-phosphate deficiency)

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[*]Syndromic forms are in *Italics* (for details, see reference [5]).

despite accurate investigation. The Italian Gruppo di Studio delle Piastrine has recently proposed an algorithm to assist clinicians in the diagnosis of inherited thrombocytopenias [5]. A correct diagnostic approach is essential, not only to avoid the use of potentially harmful treatments that are generally given to patients with acquired thrombocytopenias, but also to classify patients with known disorders and to identify families with uncharacterized forms. The study of these new entities by the coordinated efforts of physicians, biologists and geneticists will improve diagnostic skills and provide insights into the molecular basis of platelet production and function.

Inherited thrombocytopenias are usually classified according to both platelet size and the presence (syndromic) or the absence (non-syndromic) of clinical features other than those deriving from the platelet defect [Table 1]. For a detailed discussion of the clinical and biological features of hereditary thrombocytopenias see [6,7].

Hereditary disorders of platelet function

Abnormalities of the platelet receptors for adhesive proteins

Abnormalities of the GP Ib-V-IX complex

Bernard–Soulier syndrome (BSS)   BSS is caused by defects in the genes for GPIbα, GPIbβ or GPIX, while defects in the gene for GPV are not associated with BSS. The molecular defects that are responsible for BSS, including frame shifts, deletions, point mutations have recently been reviewed [2]. Characterized by autosomal recessive inheritance (only one case has been characterized by autosomal dominant inheritance), prolonged bleeding time, thrombocytopenia, giant platelets and decreased platelet survival, the syndrome is associated with quantitative or qualitative defects of the platelet glycoprotein complex GPIb/IX/V. The degree of thrombocytopenia may be overestimated when the platelet count is performed with automatic counters, because giant platelets, which may be as frequent as 70–80% in occasional patients, may reach the size of red blood cells and consequently are not recognized as platelets by the counters. Typically, Bernard–Soulier syndrome (BSS) platelets do not agglutinate with ristocetin and this defect is not corrected by the addition of normal plasma. The platelet response to physiological agonists is normal, with the exception of low concentrations of thrombin, because GPIbα (one of the two components of GPIb) plays a critical role in platelet aggregatory, secretory and procoagulant responses to thrombin [8–11]. *In vitro* studies have shown that the interaction of BSS platelets with the subendothelium is impaired at both high and low shear rates.

Bleeding events, which may be very severe, can be controlled by platelet transfusion. Most heterozygotes, with few exceptions, do not have a bleeding diathesis.

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Platelet-type, or pseudo, von Willebrand disease (VWD)   VWD is a disorder of primary hemostasis that is due to complete or partial defects of VWF, an adhesive protein that plays an essential role in platelet adhesion and aggregation under high shear rates [12]. Platelet-type (or pseudo) VWD is not due to defects of VWF, but to gain of the functional phenotype of the platelet GPIbα, which has an increased avidity for VWF, leading to the binding of the largest VWF multimers to resting platelets and to their clearance from the circulation. Since the high molecular weight VWF multimers are the most hemostatically active, their loss is associated with bleeding risk, as in type 2B VWD, which is caused by a gain of function abnormality of the VWF molecule. Platelet-type VWD is an autosomal dominant disease, which is associated with amino acid substitutions occurring within the disulfide-bonded double loop region of GPIbα (G233V and M239V) [13–15].

Bolin–Jamieson syndrome   This is a rare, autosomal dominant mild bleeding disorder associated with a larger form of GPIbα in one allele [16]. It has been proposed that it is associated with a large multimer form of the size polymorphism occurring in the mucin-like domain [17].

Abnormalities of GP IIb/IIIa (αIIb/β3), Glanzmann's thrombasthenia (GT) This is an autosomal recessive disease that is caused by lack of expression of or qualitative defects in one of the two glycoproteins forming the integrin αIIb/β3, which in activated platelets binds the adhesive glycoproteins (fibrinogen at low shear, VWF at high shear) that bridge adjacent platelets, securing platelet aggregation. The diagnostic hallmark of the disease is the lack, or severe impairment, of platelet aggregation induced by all agonists; severe forms are characterized by lack of fibrinogen in the platelet granules. Platelet clot retraction is defective. GT platelets normally bind to the subendothelium, but they fail to spread. The disease is associated with bleeding manifestations that are similar to those of patients with BSS, although of lower severity.

The defect is caused by mutations or deletions in the genes encoding for one of the two glycoproteins forming the αIIb/β3 integrin. In GT due to mutations in the β3, the levels of platelet vitronectin receptor (α_v/β_3) are also decreased, but the phenotype of these patients is no different from that of the other GT patients [2]. The molecular defects that are responsible for GT have been recently reviewed [2] and are available on an Internet database (http//med.mssn.edu/glanzmanndb).

Abnormalities of GP Ia/IIa (α_2/β_1) Two patients with mild bleeding disorders associated with deficient expression of the platelet receptor for collagen GPIa/IIa (α_2/β_1) and selective impairment of platelet responses to collagen have been described [18,19]. Their platelet defect spontaneously recovered after the menopause, suggesting that α_2/β_1 expression is under hormonal control.

Abnormalities of GPVI A selective defect of collagen-induced platelet aggregation was also described in another mild bleeding disorder, characterized by deficiency of platelet GPVI [20], a member of the immunoglobulin superfamily of receptors, which mediates platelet activation by collagen [21]. The molecular defects that are responsible for the platelet abnormality have not been characterized in the patients described so far. The possibility should be explored that the molecular abnormality lies in the gene encoding for the Fcγ receptor, which is the signaling subunit of GPVI [22].

Abnormalities of GPIV GPIV binds collagen, thrombospondin and probably other proteins. Its physiologic role is unclear, because its deficiency, which is common in healthy individuals from Japan and other East Asian populations, is not associated with an abnormal phenotype [2].

Abnormalities of the platelet receptors for soluble agonists

Thromboxane A2 (TXA$_2$) receptor In 1981, three reports of impaired platelet responses to TxA$_2$ in patients with bleeding disorders were published [23–25]. In one patient, the stable TxA$_2$ mimetic U46619 was tested and found to be unable to elicit normal platelet responses [26], providing convincing evidence that the platelets had a defect at the receptor level. In 1993, a similar patient with a mild bleeding disorder was described, whose platelets had normal number of TxA$_2$-binding sites and normal equilibrium dissociation rate constants [26]. Despite the normal number of TxA$_2$ receptors, TxA$_2$-induced IP3 formation, Ca^{2+} mobilization and GTPase activity were abnormal, suggesting that the abnormality of these platelets was impaired coupling between TxA$_2$ receptor, G protein and PLC. These last two patients were subsequently found to have an Arg60→Leu mutation in the first cytoplasmic loop of the TxA$_2$ receptor [27], affecting both isoforms of the receptor [28,29]. The mutation was found exclusively in the affected members of the two unrelated families and was inherited as an autosomal dominant trait.

α$_2$-adrenergic receptors Subjects with selective impairment of platelet response to epinephrine, decreased number of the platelet α$_2$-adrenergic receptors and mildly prolonged bleeding times have been described. However, the relationship between this defect and the bleeding manifestations still needs to be defined [3].

P2 receptors The P2 receptors interact with purine and pyrimidine nucleotides. Human platelets express at least three distinct P2 receptors stimulated by adenosine nucleotides: P2Y$_1$ [30], P2Y$_{12}$ [31,32], and P2X$_1$ [33–36].

Adenosine triphosphate (ATP) is the physiological agonist of the P2X$_1$ receptor, the role of which in platelet activation is controversial. The failure by many studies to show that α,β-methylene-ATP promotes platelet shape change or aggregation is attributable to rapid desensitization of the receptor by adenine nucleotides released during the preparation of platelet suspensions [37]. Recent studies showed that P2X$_1$ plays an important role in platelet aggregation and thrombus formation under high shear rates [38]. A dominant-negative mutation in the P2X$_1$ receptor gene has been described in a patient with a severe bleeding disorder, which, however, was associated with impaired platelet aggregation induced by adenosine diphosphate (ADP), suggesting that other defects were probably responsible for the bleeding diathesis in this patient [39].

Binding of ADP to P2Y$_1$ leads to the Gq mediated activation of β-isoforms of PLC, which leads to a transient increase in the concentration of intracellular calcium, platelet shape change and aggregation [40–42]. Stimulation with ADP of normal platelets in the presence of P2Y$_1$ antagonists [30,40–47] or of P2Y$_1^{-/-}$ murine platelets [48,49] does not induce shape change and normal aggregation, but inhibits adenylyl cyclase and elicits a slowly progressive and sustained platelet aggregation not accompanied by shape change. These platelet responses are mediated by the other platelet receptor for ADP, P2Y$_{12}$ [31]. Therefore it appears that, while P2Y$_1$ has a role in the initiation of platelet activation, P2Y$_{12}$ is essential for a sustained, full aggregation response to ADP. The concurrent activation of the G$_q$ and G$_i$ pathways is necessary for full platelet aggregation induced by ADP.

P2Y$_{12}$ also mediates the potentiation of platelet secretion by ADP [50,51] and the stabilization of thrombin-induced platelet aggregates [52,53].

Congenital defects of the platelet ADP receptors Only patients with congenital defects of the platelet P2Y$_{12}$ receptors have been described. The first patient was described in 1992 by Cattaneo *et al.* [54]. He had a lifelong history of excessive bleeding, prolonged bleeding time and abnormalities of platelet aggregation that are 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), except that the aggregation response to ADP was severely impaired. Other abnormalities of platelet function found in this patient were: (i) no inhibition by ADP of PGE$_1$-stimulated platelet adenylyl cyclase; (ii) normal shape change and normal (or mildly reduced) mobilization of cytoplasmic ionized calcium induced by ADP; (iii) presence of about 30% of the normal number of platelet-binding sites for ^{33}P-2MeSADP [55] or ^{3}H-ADP. After the identification and cloning of P2Y$_{12}$, it was possible to characterize this defect at a molecular level. The patient's P2Y$_{12}$ gene displayed a homozygous 2-bp deletion in the open-reading frame, located at bp294 from the start methionine, thus shifting the reading frame for 33 residues before introducing a stop codon, causing a premature truncation of the protein [3].

Three additional patients, one male (patient 2) [57] and two sisters (patients 3 & 4) [50] with very similar characteristics were later described. Similar to patient 1, patients 3 and 4 displayed a homozygous single bp deletion in P2Y$_{12}$ gene occurring just beyond the third transmembrane domain, thus shifting the reading frame for 38 residues before introducing a stop codon, causing a premature truncation of the protein [31]. In contrast, the molecular defect responsible for the abnormal phenotype of patient 2 is less well defined. The patient has one mutant and one wild-type allele. The mutant allele contains a deletion of 2 bp within the coding region, at position 240, thus shifting the reading frame for 28 residues before introducing a stop codon, causing a premature truncation of the protein [31]. As biochemical studies of patient 2's platelets indicated that he was completely defective for the G$_i$-linked receptor, it is likely that he has a second, as yet unidentified mutation that silenced his wild-type allele [31].

A new patient (5) with congenital bleeding disorder associated with abnormal P2Y$_{12}$-mediated platelet responses to ADP has more recently been characterized [58]. The platelet phenotype is very similar to that of other patients with P2Y$_{12}$ deficiency, except that the number and affinity of ^{33}P-2Me-SADP binding sites was normal. Analysis of the patient P2Y$_{12}$

gene revealed, in one allele, a G→A transition changing the codon for Arg256 in the sixth transmembrane domain to Gln and, in the other, a C→T transition changing the codon for Arg265 in the third extracellular loop to Trp. Neither mutation interfered with receptor surface expression but both altered function, since ADP inhibited the forskolin-induced increase of cAMP markedly less in Chinese hamster ovary (CHO) cells transfected with either mutant $P2Y_{12}$ type than with the wild-type receptor. In accordance with previous studies of the $P2Y_1$ receptor [59,60], these findings identify regions corresponding to the extracytoplasmic end of TM6 and EL3, whose structural integrity is necessary for normal functioning of a G protein-coupled receptor.

The study of the children of patient 2 and patient 5 allowed the characterization of a heterozygous $P2Y_{12}$ defect [50,58]. Their platelets underwent a normal first wave of aggregation after stimulation with ADP, but did not secrete normal amounts of ATP after stimulation with different agonists. This secretion defect was not caused by impaired production of thromboxane A_2 or low concentrations of platelet granule contents, and is therefore very similar to that described in patients with an ill-defined and probably heterogeneous group of congenital defects of platelet secretion, sometimes referred to with the general term primary secretion defect (PSD, see below), which is the most common congenital disorder of platelet function. The results of this study therefore confirm the hypothesis that (some) patients with PSD are heterozygous for the severe defect of $P2Y_{12}$ [51].

Based on the hypothesis that PSD is due to heterozygous $P2Y_{12}$ deficiency, it is likely that the severe defect is relatively common and that, due to its characteristics and to the fact that it is not yet well known, it is currently underdiagnosed, being confused with other platelet function abnormalities [61,62]. It is therefore important to emphasize that this condition should be suspected when ADP, even at relatively high concentrations (10 µM or higher), induces a slight and rapidly reversible aggregation that is preceded by normal shape change. Of the two possible confirmatory diagnostic tests, measurement of the platelet-binding sites for radiolabeled 2MeSADP and inhibition of stimulated adenylyl cyclase by ADP, the second is preferred because it is easier to perform, cheaper, more specific, and sensitive not only to quantitative abnormalities of the receptor but also to functional defects.

Abnormalities of the platelet granules

Abnormalities of the δ-granules (δ–storage pool deficiency) The term δ–storage pool deficiency (δ–SPD) defines a congenital abnormality of platelets characterized by deficiency of dense granules in megakaryocytes and platelets. It may present as an isolated platelet function defect, or be associated with a variety of congenital disorders. Between 10% and 18% of patients with congenital abnormalities of platelet function have SPD [63,64]. The inheritance is autosomal recessive in some families and autosomal dominant in others [1].

δ–SPD is characterized by a bleeding diathesis of variable degree, mildly to moderately prolonged skin bleeding time, abnormal platelet secretion induced by several platelet agonists, and impaired platelet aggregation. Typically δ–SPD platelets have decreased levels of δ-granule constituents: ATP and ADP [65,66], serotonin, calcium and pyrophosphate [67,68]. The bleeding time is usually prolonged, and the extent of its prolongation is inversely related to the amount of ADP or serotonin contained in the granules [69,70].

Normal aggregation responses to ADP or epinephrine have been observed in some patients [71], 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, which showed that about 25% of the patients had normal aggregation responses, while only 33% had aggregation tracings typical for a platelet secretion defect [63]. Lumiag-gregometry, which measures platelet aggregation and secretion simultaneously, may prove a more accurate technique than platelet aggregometry for diagnosing patients with δ-SPD and, more generally, with platelet secretion defects.

The Hermansky–Pudlak syndrome (HPS) and the Chediak–Hygashi syndrome (CHS) are rare syndromic forms of δ-SPD. HPS is an autosomal recessive disease of subcellular organelles of many tissues, involving abnormalities of melanosomes, platelet δ-granules and lysosomes [1]. It is characterized by tyrosinase-positive oculocutaneous albinism, a bleeding dia-thesis due to δ- SPD and ceroid–lypofuscin lysosomal storage disease. HPS can arise from mutations in different genetic loci [1,72–74]. CHS is also an autosomal recessive disorder, char-acterized by variable degrees of oculocutaneous albinism, very large peroxidase-positive cytoplasmic granules in a variety of hemopoietic (neutrophils) and non-hematopoietic cells, easy bruisability due to δ-SPD, and recurrent infections, associated with neutropenia, impaired chemotaxis and bactericidal activ-ity, and abnormal NK function [75]. The syndrome is lethal, leading to death usually in the first decade of life. The gene responsible for CHS is very large and several mutations have been described (reviewed in [1]).

Two types of hereditary thrombocytopenia: thrombocytope-nia and absent radii syndrome [76], and the Wiskott–Aldrich syndrome [77] may be associated with δ-SPD.

Abnormalities of the α-granules

Gray platelet syndrome The condition owes its name to the gray appearance of the patient platelets in peripheral blood smears caused by the scarcity of platelet granules. Since its first description [78], about 40 new cases have been reported in the literature, many belonging to a single family in Japan. The inheritance pattern seems to be autosomal recessive, although in one family it seemed to be autosomal dominant. 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 isolated reduction of the platelet α-granule content. Mild to moderate myelofibrosis has been described

in some patients and hypothetically ascribed to the action of cytokines that are released by the hypogranular platelets and megakaryocytes in the bone marrow [79,80]. The basic defect in GPS is probably defective targeting and packaging of endogenously synthesized proteins in platelet α-granules.

Quebec platelet disorder   The Quebec platelet disorder is an autosomal dominant qualitative platelet abnormality, characterized by severe post-traumatic bleeding complications unresponsive to platelet transfusion, abnormal proteolysis of α-granule proteins, severe deficiency of platelet factor V, deficiency of multimerin, reduced-to-normal platelet counts, and markedly decreased platelet aggregation induced by epinephrine [81,82]. Multimerin, one of the largest proteins found in the human body, is present in platelet α-granules and in endothelial cell Weibel–Palade bodies. It binds factor V and its activated form, factor Va. Its deficiency in patients with the Quebec platelet disorder is probably responsible for the defect in platelet factor V, which is likely to be degraded by abnormally regulated platelet proteases.

Jacobsen or Paris–Trousseau syndrome   This is a rare syndrome that is associated with a mild hemorragic diathesis and is characterized by congenital thrombocytopenia, normal platelet life span, and 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, which are unable to release their content upon platelet stimulation with thrombin. A deletion of the distal part of one chromosome 11 (del [11]q23.3qter) was found in the affected patients [83,84].

Abnormalities of the α– and δ– granules (α,δ– storage pool deficiency) α,δ-storage pool deficiency is characterized by deficiencies of both α- and δ-granules [85,86]. The clinical picture and the platelet aggregation abnormalities are similar to those of patients with δ-SPD.

Abnormalities of the signal-transduction pathways   Congenital abnormalities of the arachidonate/thromboxane A_2 pathway, involving the liberation of arachidonic acid from membrane phospholipids, defects of cyclooxygenase or thromboxane synthetase are associated with platelet function defects and mild bleeding (reviewed in [1]). Other congenital abnormalities of the platelet signal-transduction pathways that have been described involve G-proteins (Gαq deficiency) [87], the phos- phatidylinositol metabolism (partial selective PLC-α2 isozyme deficiency) [88], and defects in pleckstrin phosphorylation [89], which have been recently reviewed by Rao *et al.* [3].

Three patients were recently described, with a polymorphism of the gene encoding the extra-large stimulatory G-protein α-subunit (XLSα), associated with hyperresponsiveness of platelet, Gsα-enhanced intraplatelet cAMP generation and a bleeding syndrome [90]. The functional polymorphism in these patients involves the imprinted region of the XLSαgene, a phenomenon not described previously for platelet disorders but already known for defects expressing phenotypically in other tissues.

Abnormalities of membrane phospholipids

Scott syndrome   This is a rare bleeding disorder associated with the maintenance of the asymmetry of the lipid bilayer in the membranes of blood cells, including platelets [91], leading to reduced thrombin generation and defective wound healing. The cause of the defect is still unclear [4].

Stormorken syndrome   Resting platelets from patients with this syndrome display a full procoagulant activity [92]. Therefore, compared with the Scott syndrome, this condition represents the other side of the coin, yet surprisingly it is also associated with a bleeding tendency. Platelets respond normally to all agonists, with the exception of collagen.

Miscellaneous abnormalities 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 [93]. The term was later used to indicate the platelet secretion defects not associated with platelet granule deficiencies or abnormalities of the arachidonate pathway [50,51] or, generally, all the abnormalities of platelet function associated with defects of signal transduction [94]. With the progression of our knowledge in platelet pathophysiology, this heterogeneous group, which includes the majority of patients with congenital disorders of platelet function [94], will become progressively smaller, losing those patients with better defined biochemical abnormalities responsible for their platelet secretion defect. For example, patients with heterozygous P2Y$_{12}$ deficiency were once included in this group of disorders until their biochemical abnormality was identified [50,51,61].

Other platelet abnormalities

Spontaneous platelet aggregation and decreased responses to thrombin are observed in patients with Montreal platelet syndrome, a rare and poorly characterized congenital thrombocytopenia with large platelets [95].

Platelet function abnormalities have been reported in osteogenesis imperfecta, Ehlers–Danlos syndrome, Marfan's syndrome, hexokinase deficiency and glucose-6-phosphate deficiency [3].

Acknowledgements

References

1 Cattaneo M. Congenital disorders of platelet secretion. In: Gresele P, Page C, Fuster V, Vermylen J, eds. *Platelets in Thrombotic and Non-Thrombotic Disorders*. Cambridge: Cambridge University Press. 2002: 655–73.

2 Clemetson KJ, Clemetson JM. Platelet adhesive protein defect disorders. In: Gresele P, Page C, Fuster V, Vermylen J, eds. *Platelets in Thrombotic and Non-Thrombotic Disorders*. Cambridge: Cambridge University Press. 2002: 639–54.

3 Rao AK. Congenital platelet signal transduction defects. In: Gresele P, Page C, Fuster V, Vermylen J, eds. *Platelets in Thrombotic and Non-Thrombotic Disorders*. Cambridge: Cambridge University Press. 2002: 674–88.

4 Clemetson KJ, Clemetson JM. Congenital disorders of platelet function. *Haematologica* (Supplement 85: The Platelet ADP Receptors). 2000, 37–45.

5 Balduini CL, Cattaneo M, Fabris F, Gresele P, Iolascon A, Savoia A. Inherited thrombocytopenias: proposal of a diagnostic algorythm by the Italia 'Gruppo di Studio delle Piastrine'. *Haematologica* 2003; **88**: 582–92.

6 Balduini CL, Iolascon A, Savoia A. Inherited thrombocytopenias: from genes to therapy. *Haematologica* 2002; **87**: 860–80.

7 Van Geet C, Freson K, Devos R, Vermylen J. Hereditary thrombocytopenias. In: Gresele, P, Page, C, Fuster, V, Vermylen, J, eds. *Platelets in Thrombotic and Non-Thrombotic Disorders*. Cambridge: Cambridge University Press. 2002: 515–27.

8 De Marco L, Mazzucato M, Masotti A, Ruggeri ZM. Localization and characterization of an alpha-thrombin-binding site on platelet glycoprotein Ib alpha. *J Biol Chem* 1994; **269**: 6478–84.

9 Jamieson GA, Okumura T. Reduced thrombin binding and aggregation in Bernard–Soulier platelets. *J Clin Invest* 1978; **61**: 861–4.

10 De Candia E, Hall SW, Rutella S, Landolfi R, Andrews RK, De Cristofaro R. Binding of thrombin to glycoprotein Ib accelerates the hydrolysis of Par-1 on intact platelets. *J Biol Chem* 2001; **276**: 4692–8.

11 Dormann D, Clemetson KJ, Kehrel BE. The GPIb thrombin-binding site is essential for thrombin-induced platelet procoagulant activity. *Blood* 2000; **96**: 2469–78.

12 Ruggeri ZM. Structure of von Willebrand factor and its function in platelet adhesion and thrombus formation. *Best Pract Res Clin Haematol* 2001; **14**: 257–79.

13 Russell SD, Roth GJ. Pseudo-von Willebrand disease: a mutation in the platelet glycoprotein Ib alpha gene associated with a hyperactive surface receptor. *Blood* 1993; **81**: 1787–91.

14 Miller JL, Cunningham D, Lyle VA, Finch CN. Mutation in the gene encoding the alpha chain of platelet glycoprotein Ib in platelet-type von Willebrand disease. *Proc Natl Acad Sci USA* 1991; **88**: 4761–5.

15 Dong J, Schade AJ, Romo GM, Andrews RK, Gao S, McIntire LV, Lopez JA. Novel gain-of-function mutations of platelet glycoprotein IBalpha by valine mutagenesis in the Cys209-Cys248 disulfide loop. Functional analysis under statis and dynamic conditions. *J Biol Chem* 2000; **275**: 27663–70.

16 Bolin RB, Okumra T, Jamieson GA. New polymorphism of platelet membrane glycoproteins. *Nature* 1977; **269**: 69–70.

17 Meyer M, Schellenberg I. Platelet membrane glycoprotein Ib: genetic polymorphism detected in the intact molecule and in proteolytic fragments. *Thromb Res* 1990; **58**: 233–42.

18 Nieuwenhuis HK, Sakariassen KS, Houdijk WP, Nievelstein PF, Sixma JJ. Deficiency of platelet membrane glycoprotein Ia associated with a decreased platelet adhesion to subendothelium: a defect in platelet spreading. *Blood* 1986; **68**: 692–5.

19 Kehrel B, Balleisen L, Kokott R, Mesters R, Stenzinger W, Clemetson KJ, van de Loo J. Deficiency of intact thrombospondin and membrane glycoprotein Ia in platelets with defective collagen-induced aggregation and spontaneous loss of disorder. *Blood* 1988; **71**: 1074–8.

20 Moroi M, Jung SM, Okuma M, Shinmyozu K. A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. *J Clin Invest* 1989; **84**: 1440–5.

21 Jandrot-Perrus M, Busfield S, Lagrue AH, Xiong X, Debili N, Chickering T, Le Couedic JP, Goodearl A, Dussault B, Fraser C, Vainchenker W, Villeval JL. Cloning, characterization, and functional studies of human and mouse glycoprotein VI. a platelet-specific collagen receptor from the immunoglobulin superfamily. *Blood* 2000; **96**: 1798–807.

22 Tsuji M, Ezumi Y, Arai M, Takayama H. A novel association of Fc receptor gamma-chain with glycoprotein VI and their co-expression as a collagen receptor in human platelets. *J Biol Chem* 1997; **272**: 23528–31.

23 Lages B, Malmsten C, Weiss HJ, Samuelsson B. Impaired platelet response to thromboxane-A2 and defective calcium mobilization in a patient with a bleeding disorder. *Blood* 1981; **57**: 545–52.

24 Samama M, Lecrubier C, Conard J, Hotchen M, Breton-Gorius J, Vargaftig B, Chignard M, Lagarde M, Dechavanne M. Constitutional thrombocytopathy with subnormal response to thromboxane A2. *Br J Haematol* 1981; **48**: 293–303.

25 Wu KK, Le Breton GC, Tai HH, Chen YC. Abnormal platelet response to thromboxane A2. *J Clin Invest* 1981; **67**: 1801–4.

26 Fuse I, Mito M, Hattori A, Higuchi W, Shibata A, Ushikubi F, Okuma M, Yahata K. Defective signal transduction induced by thromboxane A2 in a patient with a mild bleeding disorder: impaired phospholipase C activation despite normal phospholipase A2 activation. *Blood* 1993; **81**: 994–1000.

27 Hirata T, Kakizuka A, Ushikubi F, Fuse I, Okuma M, Narumiya S. Arg60 to Leu mutation of the human thromboxane A2 receptor in a dominantly inherited bleeding disorder. *J Clin Invest* 1994; **94**: 1662–7.

28 Hirata T, Ushikubi F, Kakizuka A, Okuma M, Narumiya S. Two thromboxane A2 receptor isoforms in human platelets. Opposite coupling to adenylyl cyclase with different sensitivity to Arg60 to Leu mutation. *J Clin Invest* 1996; **97**: 949–56.

29 Okuma M, Hirata T, Ushikubi F, Kakizuka A, Narumiya S. Molecular characterization of a dominantly inherited bleeding disorder with impaired platelet responses to thromboxane A2. *Pol J Pharmacol* 1996; **48**: 77–82.

30 Leon C, Hechler B, Vial C, Leray C, Cazenave JP, Gachet C. The P2Y1 receptor is an ADP receptor antagonized by ATP and expressed in platelets and megakaryoblastic cells. *FEBS Lett* 1997; **403**: 26–30.

31 Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 2001; **409**: 202–7.

32 Zhang FL, Luo L, Gustafson E, Lachowicz J, Smith M, Qiao X, Liu YH, Chen G, Pramanik B, Laz TM, Palmer K, Bayne M, Monsma FJ Jr. ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. *J Biol Chem* 2001; **276**: 8608–15.

33 Sun B, Li J, Okahara K, Kambayashi J. P2X1 purinoceptor in human platelets. Molecular cloning and functional characterization after heterologous expression. *J Biol Chem* 1998; **273**: 11544–7.

34 Clifford EE, Parker K, Humphreys BD, Kertesy SB, Dubyak GR. The P2X1 receptor, an adenosine triphosphate-gated cation channel, is expressed in human platelets but not in human blood leukocytes. *Blood* 1998; **91**: 3172–81.

35 Vial C, Hechler B, Leon C, Cazenave JP, Gachet C. Presence of P2X1 purinoceptors in human platelets and megakaryoblastic cell lines. *Thromb Haemost* 1997; **78**: 1500–4.

36 Scase TJ, Heath MF, Allen JM, Sage SO, Evans RJ. Identification of a P2X1 purinoceptor expressed on human platelets. *Biochem Biophys Res Commun* 1998; **242** (3): 525–8.

37 Rolf MG, Brearley CA, Mahaut-Smith MP. Platelet shape change evoked by selective activation of P2X1 purinoceptors with alpha,beta-ta-methylene ATP. *Thromb Haemost* 2001; **85**: 303–8.

38 Cattaneo M, Marchese P, Jacobson KA, Ruggeri ZM. New insights into the role of P2X1 in platelet function. *Haematologica (Suppl 1)- Platelet ADP Receptors* 2002; **10**: 13–4.

39 Oury C, Toth-Zsamboki E, Van Geet C, Thys C, Wei L, Nilius B, Vermylen J, Hoylaerts MF. A natural dominant negative P2X1 receptor due to deletion of a single amino acid residue. *J Biol Chem* 2000; **275**: 22611–4.

40 Hechler B, Leon C, Vial C, Vigne P, Frelin C, Cazenave JP, Gachet C. The P2Y1 receptor is necessary for adenosine 5′-diphosphate-induced platelet aggregation. *Blood* 1998; **92**: 152–9.

41 Savi P, Beauverger P, Labouret C, Delfaud M, Salel V, Kaghad M, Herbert JM. Role of P2Y1 purinoceptor in ADP-induced platelet activation. *FEBS Lett* 1998; **422**: 291–5.

42 Jin J, Daniel JL, Kunapuli SP. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J Biol Chem* 1998; **273**: 2030–4.

43 Jarvis GE, Humphries RG, Robertson MJ, Leff P. ADP can induce aggregation of human platelets via both P2Y (1) and P (2T) receptors. *Br J Pharmacol* 2000; **129**: 275–82.

44 Geiger J, Honig-Liedl P, Schanzenbacher P, Walter U. Ligand specificity and ticlopidine effects distinguish three human platelet ADP receptors. *Eur J Pharmacol* 1998; **351**: 235–46.

45 Fagura MS, Dainty IA, McKay GD, Kirk IP, Humphries RG, Robertson MJ, Dougall IG, Leff P. P2Y1-receptors in human platelets which are pharmacologically distinct from P2Y (ADP)-receptors. *Br J Pharmacol* 1998; **124**: 157–64.

46 Daniel JL, Dangelmaier C, Jin J, Ashby B, Smith JB, Kunapuli SP. Molecular basis for ADP-induced platelet activation. I. Evidence for three distinct ADP receptors on human platelets. *J Biol Chem* 1998; **273**: 2024–9.

47 Jantzen HM, Gousset L, Bhaskar V, Vincent D, Tai A, Reynolds EE, Conley PB. Evidence for two distinct G-protein-coupled ADP receptors mediating platelet activation. *Thromb Haemost* 1999; **81**: 111–7.

48 Leon C, Hechler B, Freund M, Eckly A, Vial C, Ohlmann P, Dierich A, LeMeur M, Cazenave JP, Gachet C. Defective platelet aggregation and increased resistance to thrombosis in purinergic P2Y (1) receptor-null mice. *J Clin Invest* 1999; **104**: 1731–7.

49 Fabre JE, Nguyen M, Latour A, Keifer JA, Audoly LP, Coffman TM, Koller BH. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat Med* 1999; **5**: 1199–202.

50 Cattaneo M, Lecchi A, Lombardi R, Gachet C, Zighetti ML. Platelets from a patient heterozygous for the defect of P2CYC receptors for ADP have a secretion defect despite normal thromboxane A2 production and normal granule stores: further evidence that some cases of platelet 'primary secretion defect' are heterozygous for a defect of P2CYC receptors. *Arterioscler Thromb Vasc Biol* 2000; **20**: E101–E106.

51 Cattaneo M, Lombardi R, Zighetti ML, Gachet C, Ohlmann P, Cazenave JP, Mannucci PM. Deficiency of (33P), 2MeS–ADP binding sites on platelets with secretion defect, normal granule stores and normal thromboxane A2 production. Evidence that ADP potentiates platelet secretion independently of the formation of large platelet aggregates and thromboxane A2 production. *Thromb Haemost* 1997: **77**: 986–90.

52 Cattaneo M, Canciani MT, Lecchi A, Kinlough-Rathbone RL, Packham MA, Mannucci PM, Mustard JF. Released adenosine diphosphate stabilizes thrombin-induced human platelet aggregates. *Blood* 1990; **75**: 1081–6.

53 Trumel C, Payrastre B, Plantavid M, Hechler B, Viala C, Presek P, Martinson EA, Cazenave JP, Chap H, Gachet C. A key role of adenosine diphosphate in the irreversible platelet aggregation induced by the PAR1-activating peptide through the late activation of phosphoinositide 3-kinase. *Blood* 1999; **94**: 4156–65.

54 Cattaneo M, Lecchi A, Randi AM, McGregor JL, Mannucci PM. Identification of a new congenital defect of platelet function characterized by severe impairment of platelet responses to adenosine diphosphate. *Blood* 1992; **80**: 2787–96.

55 Gachet C, Cattaneo M, Ohlmann P, Hechler B, Lecchi A, Chevalier J, Cassel D, Mannucci PM, Cazenave JP. Purinoceptors on blood platelets: further pharmacological and clinical evidence to suggest the presence of two ADP receptors. *Br J Haematol* 1995; **91**: 434–44.

56 Conley PB, Jurek MM, Vincent D, Lecchi A, Cattaneo M. Unique mutations in the P2Y12 locus od patients with previously described defects in ADP-dependent aggregation. *Blood* 2001; **98**: 43b.

57 Nurden P, Savi P, Heilmann E, Bihour C, Herbert JM, Maffrand JP, Nurden A. An inherited bleeding disorder linked to a defective interaction between ADP and its receptor on platelets. Its influence on glycoprotein IIb-IIIa complex function. *J Clin Invest* 1995; **95**: 1612–22.

58 Cattaneo M, Zighetti ML, Lombardi R, Martinez C, Lecchi A, Conley PB, Ware J, Ruggeri ZM. Molecular bases of defective signal transduction in the platelet P2Y12 receptor of a patient with cangenital bleeding. *Proc Natl Acad Sci USA* 2003; **100**: 1978–83.

59 Moro S, Hoffmann C, Jacobson KA. Role of the extracellular loops of G protein-coupled receptors in ligand recognition: a molecular modeling study of the human P2Y1 receptor. *Biochemistry* 1999; **38**: 3498–507.

60 Moro S, Guo D, Camaioni E, Boyer JL, Harden TK, Jacobson KA. Human P2Y1 receptor: molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. *J Med Chem* 1998; **41**: 1456–66.

61 Cattaneo M, Gachet C. ADP receptors and clinical bleeding disorders. *Arterioscler Thromb Vasc Biol* 1999; **19**: 2281–5.

62 Cattaneo M, Lecchi A. Patients with congenital abnormality of platelet aggregation induced by Ca (2+) ionophores may have a defect of the platelet P2Y (12) receptor for ADP. *Br J Haematol* 2001; **115**: 485–7.

63 Nieuwenhuis HK, Akkerman JW, Sixma JJ. Patients with a prolonged bleeding time and normal aggregation tests may have storage pool deficiency: studies on one hundred six patients. *Blood* 1987; **70**: 620–3.

64 Rao AK. Congenital disorders of platelet secretion and signal transduction. In: Colman, RW, Hirsh, J, Marder, VJ, Clowes, AW, George, JN, eds. *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*. Philadelphia, PA: JB Lippincot, Williams & Wilkins. 2001:893–904.

65 Holmsen H, Weiss HJ. Hereditary defect in the platelet release reaction caused by a deficiency in the storage pool of platelet adenine nucleotides. *Br J Haematol* 1970; **19**: 643–9.

66 Holmsen H, Weiss HJ. Further evidence for a deficient storage pool of adenine nucleotides in platelets from some patients with thrombocytopathia – 'storage pool disease'. *Blood* 1972; **39**: 197–209.

67 Weiss HJ, Witte LD, Kaplan KL, Lages BA, Chernoff A, Nossel HL, Goodman DS, Baumgartner HR. Heterogeneity in storage pool deficiency: studies on granule-bound substances in 18 patients including variants deficient in alpha-granules, platelet factor 4, beta-thromboglobulin, and platelet-derived growth factor. *Blood* 1979; **54**: 1296–319.

68 Lages B, Scrutton MC, Holmsen H, Day HJ, Weiss HJ. Metal ion contents of gel-filtered platelets from patients with storage pool disease. *Blood* 1975; **46**: 119–30.

69 Akkerman JW, Nieuwenhuis HK, Mommersteeg-Leautaud ME, Gorter G, Sixma JJ. ATP-ADP compartmentation in storage pool deficient platelets: correlation between granule-bound ADP and the bleeding time. *Br J Haematol* 1983; **55**: 135–43.

70 Cattaneo M, Lecchi A, Agati B, Lombardi R, Zighetti ML. Evaluation of platelet function with the PFA-100 system in patients with congenital defects of platelet secretion. *Thromb Res* 1999; **96**: 213–7.

71 Lages B, Weiss HJ. Biphasic aggregation responses to ADP and epinephrine in some storage pool deficient platelets: relationship to the role of endogenous ADP in platelet aggregation and secretion. *Thromb Haemost* 1980; **43**: 147–53.

72 Hazelwood S, Shotelersuk V, Wildenberg SC, Chen D, Iwata F, Kaiser-Kupfer MI, White JG, King RA, Gahl WA. Evidence for locus heterogeneity in Puerto Ricans with Hermansky–Pudlak syndrome. *Am J Hum Genet* 1997; **61**: 1088–94.

73 Oh J, Ho L, Ala-Mello S, Amato D, Armstrong L, Bellucci S, Carakushansky G, Ellis JP, Fong CT, Green JS, Heon E, Legius E, Levin AV, Nieuwenhuis HK, Pinckers A, Tamura N, Whiteford ML, Yamasaki H, Spritz RA. Mutation analysis of patients with Hermansky–Pudlak syndrome: a frameshift hot spot in the HPS gene and apparent locus heterogeneity. *Am J Hum Genet* 1998; **62**: 593–8.

74 Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS. Altered trafficking of lysosomal proteins in Hermansky–Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol Cell* 1999; **3**: 11–21.

75 Introne W, Boissy RE, Gahl WA. Clinical, molecular, and cell biological aspects of Chediak–Higashi syndrome. *Mol Genet Metab* 1999; **68**: 283–303.

76 Day HJ, Holmsen H. Platelet adenine nucleotide 'storage pool deficiency' in thrombocytopenic absent radii syndrome. *JAMA* 1972; **221**: 1053–4.

77 Grottum KA, Hovig T, Holmsen H, Abrahamsen AF, Jeremic M, Seip M. Wiskott–Aldrich syndrome: qualitative platelet defects and short platelet survival. *Br J Haematol* 1969; **17**: 373–88.

78 Raccuglia G. Gray platelet syndrome. A variety of qualitative platelet disorder. *Am J Med* 1971; **51**: 818–28.

79 Jantunen E, Hanninen A, Naukkarinen A, Vornanen M, Lahtinen R. Gray platelet syndrome with splenomegaly and signs of extramedullary hematopoiesis: a case report with review of the literature. *Am J Hematol* 1994; **46**: 218–24.

80 Caen JP, Deschamps JF, Bodevin E, Bryckaert MC, Dupuy E, Wasteson A. Megakaryocytes and myelofibrosis in gray platelet syndrome. *Nouv Rev Fr Hematol* 1987; **29**: 109–14.

81 Tracy PB, Giles AR, Mann KG, Eide LL, Hoogendoorn H, Rivard GE. Factor V (Quebec): a bleeding diathesis associated with a qualitative platelet Factor V deficiency. *J Clin Invest* 1984; **74**: 1221–8.

82 Hayward CP, Rivard GE, Kane WH, Drouin J, Zheng S, Moore JC, Kelton JG. An autosomal dominant, qualitative platelet disorder associated with multimerin deficiency, abnormalities in platelet factor V, thrombospondin, von Willebrand factor, and fibrinogen and an epinephrine aggregation defect. *Blood* 1996; **87**: 4967–78.

83 Breton-Gorius J, Favier R, Guichard J, Cherif D, Berger R, Debili N, Vainchenker W, Douay L. A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha-granules and chromosome 11 deletion at 11q23. *Blood* 1995; **85**: 1805–14.

84 Hart A, Melet F, Grossfeld P, Chien K, Jones C, Tunnacliffe A, Favier R, Bernstein A. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. *Immunity* 2000; **13**: 167–77.

85 Weiss HJ, Lages B, Vicic W, Tsung LY, White JG. Heterogeneous abnormalities of platelet dense granule ultrastructure in 20 patients with congenital storage pool deficiency. *Br J Haematol* 1993; **83**: 282–95.

86 Weiss HJ, Witte LD, Kaplan KL, Lages BA, Chernoff A, Nossel HL, Goodman DS, Baumgartner HR. Heterogeneity in storage pool deficiency: studies on granule-bound substances in 18 patients including variants deficient in alpha-granules, platelet factor 4, beta-thromboglobulin, and platelet-derived growth factor. *Blood* 1979; **54**: 1296–319.

87 Gabbeta J, Yang X, Kowalska MA, Sun L, Dhanasekaran N, Rao AK. Platelet signal transduction defect with Galpha subunit dysfunction and diminished Galphaq in a patient with abnormal platelet responses. *Proc Natl Acad Sci USA* 1997; **94**: 8750–5.

88 Lee SB, Rao AK, Lee KH, Yang X, Bae YS, Rhee SG. Decreased expression of phospholipase C-beta 2 isozyme in human platelets with impaired function. *Blood* 1996; **88**: 1684–91.

89 Gabbeta J, Yang X, Sun L, McLane MA, Niewiarowski S, Rao AK. Abnormal inside-out signal transduction-dependent activation of glycoprotein IIb-IIIa in a patient with impaired pleckstrin phosphorylation. *Blood* 1996; **87**: 1368–76.

90 Freson K, Hoylaerts MF, Jaeken J, Eyssen M, Arnout J, Vermylen J, Van Geet C. Genetic variation of the extra-large stimulatory G protein alpha-subunit leads to Gs hyperfunction in platelets and is a risk factor for bleeding. *Thromb Haemost* 2001; **86**: 733–8.

91 Weiss HJ. Scott syndrome: a disorder of platelet coagulant activity. *Semin Hematol* 1994; **31**: 312–9.

92 Solum NO. Procoagulant expression in platelets and defects leading to clinical disorders. *Arterioscler Thromb Vasc Biol* 1999; **19**: 2841–6.

93 Weiss HJ. Congenital disorders of platelet function. *Semin Hematol* 1980; **17**: 228–41.

94 Rao AK, Gabbeta J. Congenital disorders of platelet signal transduction. *Arterioscler Thromb Vasc Biol* 2000; **20**: 285–9.

95 Milton JG, Frojmovic MM, Tang SS, White JG. Spontaneous platelet aggregation in a hereditary giant platelet syndrome (MPS). *Am J Pathol* 1984; **114**: 336–45.