



Inherited platelet disorders including Glanzmann thrombasthenia and Bernard-Soulier syndrome

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Inherited platelet disorders (IPDs) are a heterogeneous group of diseases affecting platelet production, morphology, and function. The degree of thrombocytopenia and functional abnormality of platelets determines the clinical manifestations. Although severe deficiencies may cause excessive bleeding beginning in early childhood, most of IPDs have mild bleeding tendencies and therefore are not always easy to distinguish from acquired platelet disorders. The diagnosis of IPD may require extensive laboratory investigation, because current routine laboratory tests are not satisfactory for differential diagnosis in some cases, and most of the specific tests are not readily available in many countries. This review summarizes the classification and clinical and molecular characteristics of known IPDs, including Bernard-Soulier syndrome and Glanzmann thrombasthenia, with a focus on current challenges in the laboratory diagnosis and management of bleeding in these patients.

Introduction

Platelets, the smallest cells in the blood, are involved in hemostasis, inflammation, tissue remodeling, and wound healing. They play a crucial role in hemostasis: adhesion and aggregation of the platelets lead to the formation of a “hemostatic plug.” Subsequent activation of the coagulation system induces fibrin generation on the surface of activated platelets. Either acquired or inherited thrombocytopenia and/or platelet function defect will cause bleeding. In clinical practice, most of the platelet disorders are due to acquired problems including drugs and metabolic diseases. Since Dr Eduard Glanzmann’s description of “thrombasthenia” a century ago, several inherited platelet disorders (IPDs) have been identified. Numerous classifications have been proposed based on platelet count, size, function, or underlying genetic abnormality (Table 1). Although IPDs are rare, studies to elucidate their pathogenesis have contributed enormously to current knowledge. The identification of molecular pathology in patients with IPD has improved our understanding of normal megakaryocyte and platelet physiology, as well as the mechanisms of hemostasis and thrombosis. These investigations also gave very important insight into the development of platelet- and megakaryocyte-directed therapies in patients with thrombosis and thrombocytopenia.

Clinical findings, laboratory evaluation, and management of IPDs

The degree of thrombocytopenia and functional abnormalities of platelets determines the severity of bleeding symptoms. IPD patients usually present with mucocutaneous bleeding such as easy bruising, purpura, gingival bleeding, and epistaxis beginning in early childhood. Spontaneous life-threatening bleeding (intracranial hemorrhage, massive gastrointestinal or genitourinary bleeding) is rare.¹⁻⁴ Menorrhagia and bleeding during pregnancy and labor are of special concern in female patients.^{5,6} Unexpected excessive bleeding after trauma or surgery may be the first symptom in milder cases.¹⁻⁷ Rarely, IPD is a component of a complex of multisystem disorder such as Fanconi anemia, Chediak-Higashi syndrome, or May-Hegglin anomaly (Table 2). The presence of skeletal, facial, ocular, audiological, neurological, renal, cardiac, and immune

problems associated with bleeding is suggestive of the existence of such disorders.^{2,3}

IPD patients with isolated macrothrombocytopenia share common clinical and basic laboratory features of other acquired platelet disorders and are sometimes misdiagnosed as immune thrombocytopenia. It is important to distinguish patients with IPD from those with acquired platelet disorders to avoid unnecessary or potentially harmful treatments.² A blood smear is helpful for patients with myosin heavy chain 9 (MYH9)-related diseases (giant platelets and Döhle-like inclusion bodies within leukocytes) and Gray platelet syndrome (pale platelets); a smear also gives information about the platelet count because automated cell counters may not recognize giant platelets. Optical platelet counters or flow cytometric analysis may help with platelet counting.^{1,2,7} Bone marrow biopsy is required if the IPD patient has pancytopenia as in Fanconi anemia or severe thrombocytopenia as in congenital amegakaryocytic thrombocytopenia. Coagulation tests should be normal.^{1,7} Skin bleeding time (Ivy, template) is prolonged, but is not recommended as a screening test for investigation of patients with IPD because it is invasive and poorly reproducible.¹ A platelet function analyzer (PFA) assay simulates a damaged vessel wall using collagen + ADP- and collagen + epinephrine-embedded cartridges. Citrated whole blood passes through these cartridges at high shear stress and platelets bind to the membrane of the cartridge, blocking the system, and generating the “closure time.” PFA is very sensitive in detecting Bernard-Soulier syndrome (BSS), platelet-type von Willebrand disease (VWD), and Glanzmann thrombasthenia (GT), but may be normal in patients with storage pool deficiencies and platelet membrane phospholipid disorders.^{1,3,7} The basic investigation of IPD should include light transmission aggregometry (LTA) using ADP, collagen, ristocetin, epinephrine, and arachidonic acid at different concentrations.⁸ LTA shows characteristic patterns in patients with BSS, platelet-type VWD, and GT. Although LTA is accepted as the gold standard for diagnosing IPD, it is difficult to obtain platelet-rich plasma in pediatric cases and in patients with thrombocytopenia.^{7,8} LTA may be normal in some patients with storage pool diseases, so the measurement of platelet nucleotide

Table 1. Classification of inherited platelet disorders

1. Decreased production of platelets
 - a. CAMT
 - b. Congenital hypo/amegakaryocytic thrombocytopenia with skeletal abnormalities
 - i. TAR syndrome
 - ii. ATRUS
 - iii. Fanconi anemia
2. MYH9-related diseases:
 - a. May-Hegglin anomaly
 - b. Epstein syndrome
 - c. Fechtner syndrome
 - d. Sebastian syndrome
3. Platelet membrane phospholipid abnormalities:
 - a. Scott syndrome
 - b. Stormorken syndrome
4. Platelet granule deficiencies (storage pool disease)
 - a. α -granule defects:
 - i. Gray platelet syndrome
 - ii. Paris-Trousseau syndrome
 - iii. Quebec platelet syndrome
 - iv. ARC syndrome
 - b. Dense granule defects:
 - i. Hermansky-Pudlak syndrome
 - ii. Chediak-Higashi syndrome
 - iii. Griscelli syndrome
 - c. α - and dense granule defects
5. Disorders of platelet surface receptors
 - a. GP IIb-IX-V defects
 - i. Bernard-Soulier syndrome
 - ii. Platelet-type VWD
 - iii. Velocardiofacial syndrome
 - b. Integrin $\alpha_2\beta_3$ (GP IIb-IIIa) defects: Glanzmann thrombasthenia
 - c. GPVI defects
 - d. Integrin $\alpha_2\beta_1$ (GP Ia/IIa) defects
 - e. Integrin $\alpha_5\beta_1$ (VLA-5) defects
 - f. Integrin $\alpha_6\beta_1$ (VLA-6) defects
 - g. Integrin $\alpha_V\beta_3$ defects
6. Miscellaneous: GATA-1 related thrombocytopenia, Wiskott-Aldrich syndrome, Mediterranean macrothrombocytopenia, Montreal platelet syndrome, familial platelet disorder with propensity to myeloid malignancy (FDP/AML)

content and release is recommended.⁷ Flow cytometric analysis measures specific receptor densities of platelets and is very informative for BSS and GT, which are associated with glycoprotein (GP) deficiencies rather than dysfunction.^{7,9} Flow cytometry also gives information about platelet count and reticulated platelets. Further tests are only available at specialized centers. Electron microscopy describes the ultrastructural abnormalities as seen in storage pool diseases; Western blotting, ELISA, or radioimmunoassay can be used for qualitative and quantitative analysis of specific platelet proteins; and genetic analysis reveals the exact molecular pathology from IPD.^{1,2,7} Despite all of these complicated, expensive, and time-consuming platelet function tests, the results are usually inconclusive in nearly half of patients being evaluated for IPD.² The genetic analysis will demonstrate underlying molecular pathology in these patients, but difficulties arise due to the devastatingly large number of each candidate's genes. The development of next-generation sequencing techniques have not only improved the speed and cost of genetic investigations, but have also begun to accumulate very interesting data about the genetic causes of IPD in the last decade.^{10,11}

The majority of patients with IPD do not need a therapy on a regular basis, but require treatment after injury, during surgical procedures,

and during bleeding episodes. Education of patients about maintaining dental hygiene, avoiding contact sports and activities carrying bleeding risk, and avoiding the use of antiplatelet drugs are very important.^{1,12} Bleeding from minor cuts, nose, and gingiva can be stopped by applying pressure. Topical hemostatic agents such as gelatin sponges, fibrin sealants, and antifibrinolytic drugs such as tranexamic acid are used to decrease minor bleeding symptoms.¹

Desmopressin (DDAVP, 1-amino-8-D-arginine vasopressin) stimulates the release of VWF from endothelial cells and increases factor VIII levels in plasma. It is indicated for the prevention or treatment of bleeding episodes in patients with type-1 VWD and in patients with hemophilia A with factor VIII levels > 2% to 5%.^{1,13} The data on the efficacy and safety of desmopressin in the treatment of IPD patients are limited because the literature includes only small case series with different results.² Although desmopressin has no direct effect on platelets,¹⁴ the ultra-large von Willebrand Factor (VWF) released by desmopressin may facilitate platelet adhesion and decrease the bleeding time in some patients with IPD, such as those with BSS, storage pool diseases, and MYH9-related disorders. Desmopressin has no effect on bleeding in patients with GT.⁴ It can be given as IV infusion over 30 minutes, subcutaneous injection, or intranasal spray.¹ A major side effect of desmopressin is water retention and hyponatremia. Desmopressin also may bind to the vasopressin receptors of blood vessels and uterus. It should be used cautiously for pregnant women due to potential risk of preterm labor.¹⁵

Management of menorrhagia in patients with IPD involves both gynecologic and hematologic treatments. Oral contraceptives or hormonal intrauterine devices together with tranexamic acid may reduce bleeding during menses.^{1,4,12} Patients with severe bleeding after trauma, surgery, or delivery may require platelet and RBC transfusions. HLA-compatible platelet concentrates are recommended to avoid alloimmunization if possible.^{1,2,4} Recombinant factor VIIa can be used in patients with life-threatening bleeding who are unresponsive to platelet transfusions and in patients with alloantibodies.^{1,2,4,12} Thrombopoietin (TPO)-mimetic drugs have been shown to increase platelet counts in some patients with MYH9-related disorders,² but the efficacy and safety of these drugs should be carefully investigated. Hematopoietic stem cell transplantation is used only in IPD patients with BM failure or megakaryocytic aplasia (see below).

Decreased production of platelets

Megakaryocytic commitment of hematopoietic stem cells is the first step for platelet production. Several transcription factors, including GATA-1, FLI-1, and FOG-1, are involved in this process. The differentiation of the megakaryoblast to megakaryocyte and production of platelets is primarily regulated by TPO. TPO binds to the c-Mpl receptor and mediates the growth and maturation of megakaryocytes. TPO/cMPL signaling has been shown to be crucial, not only for normal thrombopoiesis, but also for the maintenance of stem cells. Several mutations on TPO, cMPL, and some other cytokines have been reported in patients with inherited thrombocytopenia and BM failure.¹⁶

Congenital amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare, autosomal-recessive disorder presented at birth with isolated severe thrombocytopenia (platelet counts usually < 50 × 10⁹/L). Although no other skeletal or mental abnormalities are expected in

Table 2. Storage pool diseases

	Inheritance, locus, and gene(s)	Platelets	Bleeding diathesis	Other features
Alpha (α) storage pool diseases				
Gray platelet syndrome	AR, AD? 3p21.31 <i>NBEAL2</i>	Mild to moderate thrombocytopenia Increased MPV Absent or decreased α -granules	Mild to moderate Prolonged bleeding time Variable aggregation responses	Splenomegaly Bone marrow fibrosis
Paris-Trousseau syndrome	AD 11q23 <i>FLI1</i>	Mild thrombocytopenia Abnormal and giant α -granules	Mild bleeding tendency	Dysmegakaryopoiesis Facial, cardiac, mental abnormalities
Quebec platelet disorder	AD 10q22.2 <i>PLAU</i>	Normal count or mild thrombocytopenia Decreased α -granule proteins Over-expression of urokinase-type plasminogen activator	Mild to moderate Delayed bleeding Decreased aggregation with epinephrine	
Arthrogyposis, renal dysfunction and cholestasis (ARC) syndrome	AR	Normal count or mild thrombocytopenia	Mild bleeding tendency	Joint contractions
	15q26.1	Large and pale platelets	Decreased aggregation with ADP and arachidonic acid	Facial abnormalities
	<i>VPS33B, VIPAR</i>	Decreased α -granule proteins		Cholestasis Renal tubular acidosis
Dense (δ)-granule storage pool diseases				
Hermansky-Pudlak syndrome	AR <i>Multiple</i>	Platelet count normal Decreased δ -granule proteins	Mild to severe bleeding Prolonged bleeding time	Oculocutaneous albinism, nystagmus Lysosomal deposition of ceroid lipofuscin Granulomatous colitis Pulmonary fibrosis Neutropenia
Chediak-Higashi syndrome	AR 1q42.3 <i>CHS1/LYST</i>	Platelet count normal Giant inclusions in platelets and leukocytes	Mild to moderate bleeding tendency	Oculocutaneous albinism Severe immunodeficiency, pancytopenia, organomegaly Neurological abnormalities
Griscelli syndrome (type 1-3)	AR 15q21.2, 15q21.3, 2q37.3 <i>MYO5A, RAB27A, MLPH</i>	Normal count or mild thrombocytopenia	Mild to moderate bleeding Impaired secretion-dependent platelet aggregation	Albinism with silvery gray hair Immunodeficiency Neurological abnormalities



Figure 1. Arthrogryposis (persistent joint contractures), renal dysfunction, and cholestasis (ARC) syndrome is a very rare multisystem disorder. The degeneration of the anterior motor neuron cells causes multiple joint contractures, muscle weakness, and fibrosis. Decreased alpha granule proteins in ARC syndrome leads to platelet aggregation defects and bleeding tendency.

patients with CAMT, the bleeding tendency may cause complications in the development of an affected child, such as intracranial hemorrhage.^{17,18} Platelet size and morphology are normal on the blood smear. TPO levels are high (usually 10- to 50-fold increased) because of the decreased platelet and megakaryocyte counts. A BM biopsy shows an absence or decreased number of megakaryocytes at the time of diagnosis. Several mutations on the c-MPL gene, which cause either disrupted receptor structure (type I mutations) or impaired signaling (type II mutations), were described in patients with CAMT. Although CAMT starts with isolated megakaryocytic hypoplasia earlier in life, almost all patients will eventually progress to aplastic anemia. The risk of myelodysplastic syndrome and leukemia is also increased in these patients. Hematopoietic stem cell transplantation is the only curative treatment option.^{1,17,18}

Thrombocytopenia with absent radii syndrome

Thrombocytopenia with absent radii (TAR) syndrome is characterized by thrombocytopenia with the absence of a radius in each forearm (Figure 1). Other skeletal abnormalities are bone defects in the lower and upper extremities, short stature, and facial abnormalities. Renal and cardiac abnormalities may also be present. Inheritance of TAR syndrome is complex: an autosomal-recessive pattern is described in most cases. Affected children born with severe thrombocytopenia usually require platelet transfusions. Allergic reactions to cow's milk may be seen in half of the patients and this may worsen the thrombocytopenia. The platelet counts recover during childhood and may even be normal in adult patients with TAR syndrome.^{19,20} BM biopsy shows isolated megakaryocytic hypoplasia with normal myeloid and erythroid progenitors. Serum TPO level is high and TPO signaling is found to be defective.¹⁹ The genetic background of TAR syndrome is unknown. Investigations showed no abnormality on the cMPL gene. A microdeletion on chromosome 1q21.1 has been identified in patients with TAR syndrome with unknown significance and has been also found in their unaffected relatives.¹⁹ Recently, Albers et al demonstrated that TAR syndrome is caused by compound inheritance of a low-frequency noncoding single nucleotide polymorphism and a rare null mutation in RNA binding motif protein 8a gene (*RBM8a*). Proximal deletions of 1q21.1 are found to be associated with TAR

syndrome, whereas distal deletions or duplications are found to be associated with skeletal and neuropsychiatric abnormalities.²⁰ Another interesting feature of TAR syndrome is the increasing platelet count as the patient ages.¹⁹ The investigation of cytokine profiles in TAR patients of different ages should provide important information about the differences in megakaryopoiesis/thrombopoiesis between neonates and adults.

Amegakaryocytic thrombocytopenia with radioulnar synostosis

Amegakaryocytic thrombocytopenia with radioulnar synostosis (ATRUS) is a very rare cause of inherited thrombocytopenia characterized by fusion of the radius and ulna near the elbow, resulting in limited pronation and supination of the forearm. So far, fewer than 10 families with ATRUS are reported in the existing literature. Recently, a homeobox transcription factor (*HOXA11*) gene mutation was described in patients with ATRUS.²¹

Fanconi anemia

Fanconi anemia is an autosomal-recessive disorder characterized by progressive BM failure, multiple congenital abnormalities, and predisposition to malignancy. Fanconi anemia cells have chromosomal instability and hypersensitivity to DNA interstrand cross-linking agents such as mitomycin C and diepoxybutane. Although abnormalities concerning the radius may be associated with Fanconi anemia, hypoplasia or aplasia of thumbs with development of BM failure distinguishes it from TAR syndrome.²²

MYH9-related diseases

The MYH9 gene encodes nonmuscle myosin IIA heavy chain, a protein involved in cell motility and maintenance of cell shape. Mutations of the MYH9 gene were found to be associated with macrothrombocytopenia, nephritis, hearing loss, and inclusion bodies in leukocytes (Döhle-like bodies) and are classified as "MYH9-related diseases."^{2,23,24} The localization of the MYH9 gene mutation determines the clinical phenotype: mutations in the motor domain are associated with more severe defects such as lower platelet counts and early onset of hearing loss and nephritis compared with the milder phenotype seen in tail-domain mutations.²⁴ The molecular mechanisms causing hematological, renal, and ophthalmological abnormalities are unknown. The majority of patients with MYH9-related disorders have mild to moderate bleeding tendencies. BM megakaryocyte number and morphology is normal. Platelet counts are variable, but lifespan of platelets is normal. Döhle-like inclusion bodies, which are clusters of ribosomes and nonmuscle myosin IIA heavy chain microfilaments, may be seen within granulocytes, eosinophils, and monocytes on a peripheral blood smear or with immunostaining using specific antibodies.^{23,24} Platelet aggregation studies reveal minor abnormalities such as absence of a shape change curve in the aggregation slope and an impaired response to collagen.^{1,4,7} Periodic evaluation of renal functions and hearing tests are recommended in these patients.^{23,24}

Platelet membrane phospholipid abnormalities

In resting platelets, the outer layer of the platelet membrane contains phosphatidylcholine, which carries a neutral charge, whereas the inner layer is rich in negatively charged phospholipids such as phosphatidylserine. After activation of the platelets, negatively charged phospholipids are exposed on the outer layer and provide a surface for activation of coagulation factors. Externalization of inner phospholipids also mediates apoptosis, cell clearance, and



Figure 2. Thrombocytopenia-absent radii syndrome. The bilateral absence of radii in the forearm cause radial deviation of the hands. Reduced platelet count leads to bleeding diathesis.

complement binding.²⁵ Scott syndrome is an inherited bleeding disorder characterized by the loss of the capacity of platelets to externalize phosphatidylserine. Platelet count, size, adhesion, and aggregation activities are normal. The impaired binding of activated factor V and factor VIII results in diminished thrombin generation. Mutations of a transmembrane protein (TMEM)-16F were described in 2 patients with Scott syndrome.^{26,27} Stormorken syndrome is defined as “inverse Scott syndrome,” characterized by increased externalization of phosphatidylserine. Although there is increased platelet procoagulant activity, patients have mild to moderate bleeding tendencies with unknown reasons. Asplenia, neurological symptoms, and ichthyosis were also reported in patients with Stormorken syndrome.²⁸

Platelet granule deficiencies (storage pool diseases)

Three types of granules are present in platelets. Dense (δ) granules contain nonprotein molecules such as ADP, ATP, calcium, magnesium, and serotonin. α -granules store proteins either produced by megakaryocytes or acquired from the blood by endocytosis. These proteins include VWF, fibrinogen, thrombospondin, factor V, fibronectin, and platelet-derived growth factor. Lysosomes contain hydrolases able to eliminate circulating platelet aggregates. Deficiencies of α - and/or dense granules (storage pool diseases) may cause bleeding diathesis²⁷⁻³⁰ (Table 2). α -granule disorders (Gray platelet syndrome, Paris-Trousseau syndrome, Quebec platelet disorder, ARC-arthrogryposis, renal dysfunction, and cholestasis syndrome) usually present with mild to moderate bleeding. Other components of these disorders are prominent, such as facial and skeletal abnormalities in Paris-Trousseau syndrome and ARC syndrome.^{1,2,29} (Figure 2) Quebec platelet disorder is an autosomal-dominant disorder associated with overexpression of urokinase-type plasminogen activator in platelets that degrades α -granule proteins. The release of excessive urokinase-type plasminogen activator during clot formation also leads to increased fibrinolysis. Delayed onset of bleeding after trauma is an important feature of Quebec platelet disorder.³¹ Dense granule disor-

ders (Hermansky-Pudlak syndrome, Chediak-Higashi syndrome, Griscelli syndrome) are associated with moderate to severe bleeding tendency and may present with other abnormalities, including oculocutaneous albinism, pulmonary fibrosis, immune deficiency, and neurological abnormalities^{29,30} (Table 2).

Disorders of platelet surface receptors

Platelets express different molecules on their surface, including leucine-rich-repeat receptors such as GP Ib-IX-V complex; integrins such as $\alpha_{IIb}\beta_3$ (GP IIb-IIIa); proteins of the Ig superfamily such as GP VI; G-protein-coupled receptors such as PAR1, PAR4, and ADP receptors; and C-type lectin receptors such as P-selectin. Expression and activity of these receptors are dependent on the activation status of platelets. Platelet surface receptors and their signaling processes are very important for hemostasis. Two inherited platelet surface receptor disorders, BSS and GT, clearly show that even genetic deficiency of a single platelet receptor may cause bleeding tendency.

Bernard Soulier syndrome

The GP Ib-IX-V complex is expressed on platelets and megakaryocytes. The receptor consists of 4 distinct polypeptide subunits that are encoded by different genes: GPIb α , GPIb β , GPIX, and GPV. The major component of this complex, GPIb α , binds to VWF and several other molecules involved in hemostasis, thrombosis, and inflammation, such as thrombin, P-selectin, factor XI, factor XII, high-molecular-weight kininogen, thrombospondin, and β -2 GPI.^{1,2,12,32} After endothelial damage, GPIb α binds to collagen-bound VWF, which expresses a normally cryptic binding site for GPIb α . GPIb α also may bind VWF in plasma under high shear conditions or when defective VWF cleavage occurs, such as in thrombotic microangiopathies.^{12,32,33}

BSS is an inherited bleeding disorder characterized by macrothrombocytopenia and an absence of ristocetin-induced platelet aggregation. The prevalence of the syndrome is estimated at less than 1 in 1 000 000.³³ The majority of BSS cases are inherited in an autosomal-recessive pattern. Mutations responsible for BSS are heterogeneous: nonsense and missense mutations and frameshift insertions or deletions may be found in genes encoding GP Ib α , GP Ib β , or GP IX. These mutations inhibit expression of the GP Ib-IX-V receptor on the surface of platelets. There is no reported case of an isolated GPV gene mutation.^{1,12,32,33} Rarely, mutation may only impair VWF binding with normal expression of the receptor (Bolzano variant).^{1,12}

As in other IPDs, BSS manifests itself by a bleeding tendency in early childhood. Easy bruising, purpura, epistaxis, gingival bleeding, menorrhagia, and excessive bleeding after surgery or trauma are common signs. Although the severity of bleeding is associated with a genetic defect affecting receptor functions and platelet counts, it is quite variable in patients who have the same mutations.^{32,33} Other genetic differences and acquired conditions affecting hemostasis may influence bleeding severity in these patients. Heterozygotes may not have any bleeding symptoms, but giant platelets may be seen on a peripheral blood smear.³³

Platelet counts are variable mostly due to the presence of giant platelets and usually range from 20 to 140 $\times 10^9$ in BSS patients.³³ The association between macrothrombocytopenia and GP Ib-IX-V mutations is not known. Decreased platelet lifespan, impaired megakaryopoiesis, and defective interaction of GP Ib-IX-V complex with cytoskeleton have been hypothesized.^{12,33} Leukocyte

counts and morphology should be examined carefully for the differential diagnosis of other giant platelet syndromes. Skin bleeding time and PFA-100 closure time are prolonged. Routine coagulation tests should be normal. Prothrombin consumption and thrombin generation tests are markedly decreased because of the defective binding of FXI and thrombin.³⁴ Results of platelet aggregation studies are pathognomonic for BSS: normal aggregation responses with ADP, arachidonic acid, collagen, and epinephrine with the absence of an aggregation curve with ristocetin. Impaired aggregation response may be seen at low concentrations of thrombin.^{1,7} Flow cytometric analysis of platelets is also characteristic for BSS: normal binding with CD41 (GP IIb) and CD61 (GPIIIa) antibodies, but defective binding with CD42a (GPIX), CD42b (GP Ib α), CD42c (GP Ib β), and CD42d (GPV) antibodies suggest BSS.⁷ Immunoblotting after separating components of the GP Ib-IX-V complex with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) may describe the defective fragments but needs specialized interpretation.^{1,2,9}

Platelet-type (or pseudo) VWD

Platelet-type (or pseudo) VWD is an autosomal-dominant disorder characterized by mild thrombocytopenia, large platelets, and a mild-to-moderate bleeding diathesis. GP Ib α gene mutations (a 27-bp deletion and 4 point mutations) that cause increased binding of GP Ib α to VWF have been found in patients with platelet-type VWD.³⁵ Abnormally increased affinity of GP Ib α for larger VWF multimers results in clearance of both VWF and platelets. A very similar pattern may be seen in patients with type 2B VWD, which is caused by VWF mutations with increasing affinity to GP Ib α . Both disorders are characterized by reduction of larger VWF multimers in plasma, increased response to ristocetin-induced aggregation assay, decreased ristocetin cofactor activity of the plasma, and normal or mildly decreased VWF antigen and FVIII levels. Discrimination between platelet-type VWD and type 2B VWD needs more detailed analysis such as ristocetin-induced aggregation mixing assays and genetic analysis of involved molecules.³⁶ Bleeding episodes are treated with platelet transfusions in patients with platelet-type VWD, whereas VWF concentrates are chosen for patients with type 2B VWD.³⁵

Velocardiofacial syndrome

Velocardiofacial syndrome (VCFS) is an inherited disorder characterized by abnormal pharyngeal arch development. VCFS can present in numerous ways, as more than 180 clinical features have been described. Palatal abnormalities, craniofacial defects, cardiac abnormalities, hypotonia, defective thymic development, and immune deficiency are common features of the syndrome.³⁷ VCFS is caused by a microdeletion located on chromosome 22q11.2. The platelet receptor GPIIb β gene is located in the same chromosome and microdeletion may also cause GPIIb β deficiency. These patients may have macrothrombocytopenia and decreased aggregation with ristocetin, as seen in heterozygous BSS carriers. Children with VCFS may require major surgery for their anomalies and this carrier state may increase the bleeding risk.³⁷

Glanzmann thrombasthenia

GT is an autosomal-recessive bleeding disorder characterized by a defective platelet integrin $\alpha_{IIb}\beta_3$ receptor. The integrin $\alpha_{IIb}\beta_3$ (GP IIb-IIIa) receptor is abundantly expressed on platelets: ~ 80 000 copies are found on the surface of each platelet. The receptor is a heterodimer consisting of α_{IIb} and β_3 subunits found in an inactive state in resting platelets. After platelet activation, inside-out signal-

ing triggers the conformational change in the transmembrane and extracellular domains, which allows binding with ligands. The activated integrin $\alpha_{IIb}\beta_3$ binds to fibrinogen and VWF, as well as some other molecules such as vitronectin and fibronectin. A major function of the receptor is to mediate platelet aggregation by binding bivalent fibrinogen or multivalent VWF molecules; it also mediates spreading and firm adhesion of platelets to the subendothelial matrix.³⁸⁻⁴⁰ The α_{IIb} and β_3 subunits of the receptor are encoded by different genes located on the same chromosome (chromosome 17). Homozygous or compound heterozygous mutations of either the α_{IIb} (*ITGA2B*) or β_3 (*ITGB3*) gene are responsible for quantitative or qualitative defects seen in GT.^{10,41,42} Three types of GT are described: patients with severe $\alpha_{IIb}\beta_3$ deficiency (expression levels < 5%) are classified as having type I GT, patients with moderate deficiency (10%-20% expression) are classified as having type II GT, and patients with higher expression (> 20%) but with a dysfunctional $\alpha_{IIb}\beta_3$ are classified as having the variant form of GT. Mutations responsible for type I and type II disease are distributed in both *ITGA2B* and *ITGB3* genes. The majority of patients with variant form have mutations located on the *ITGB3* gene.^{41,42}

Although the exact prevalence of the GT is unknown, it is estimated to be ~1 in 1 000 000.⁴¹ A slight female predominance (58% vs 42%) is reported. Similar to other IPDs, mucocutaneous bleeding starting in childhood is the major clinical finding. Bleeding severity is quite variable in patients with GT.^{2,41} The presence of thrombophilic mutations such as the FV Leiden mutation and prothrombin gene mutation may change bleeding patterns in these patients.⁴¹ An increased risk of malignancies, osteosclerosis, and enhanced angiogenesis were described in mice genetically deficient in β_3 , but have not been reported in humans lacking the β_3 subunit.⁴³

Platelet counts are normal in GT except for the variant forms with activating mutations of $\alpha_{IIb}\beta_3$.⁴¹ Skin bleeding time and PFA-100 closure times are both prolonged. Aggregation studies with LTA show no platelet aggregation in response to collagen, ADP, epinephrine, and arachidonic acid. The aggregation response to high-dose ristocetin is usually normal, but may be reversible in some cases.⁷ In flow cytometric analysis, absence or greatly decreased levels of CD41 and CD61 and normal levels of CD42 are consistent with a diagnosis of GT.²³ Flow cytometry, however, may not recognize variant cases expressing functionally abnormal $\alpha_{IIb}\beta_3$. Platelet aggregation studies and genetic analysis are preferred for these patients.^{1,42}

Other platelet surface receptor deficiencies are very rare, and majority of these defects have no significant hemostasis in humans.¹² GPVI (collagen receptor) deficiency is reported only 6 patients with bleeding diathesis (2 with compound heterozygous mutations, 4 with homozygous mutations).⁴⁴

Miscellaneous

GATA-1 related thrombocytopenia. The *GATA-1* gene is located on the X-chromosome and encodes GATA-1 protein, which belongs to the GATA family of transcription factors. GATA-1 plays an important role in the development erythroid and megakaryocytic cells. Several mutations have been described in *GATA-1*, resulting in platelet abnormalities (dysmegakaryopoiesis, thrombocytopenia, large or small platelets, α -granule deficiency) and dyserythropoietic anemia with different clinical severity. Increased hemoglobin A2, persistence of hemoglobin F, and unbalanced production of α - and β -globulin synthesis may cause a "beta-thalassemia-like phenotype" in some patients.⁴⁵ The same phenotype was also described in

a child with congenital erythropoietic porphyria caused by a R216W mutation of the *GATA-1* gene.⁴⁶ GATA-1s is a shorter isoform of GATA-1 with loss of the N-terminal transcription activation domain. It has been shown to be associated with the development of acute megakaryoblastic leukemia and transient myeloproliferative disorder in patients with Down syndrome.⁴⁷

Familial platelet disorder with propensity to myeloid malignancy. Familial platelet disorder with propensity to myeloid malignancy is an autosomal-dominant disorder characterized by thrombocytopenia and a genetic predisposition to the myeloid malignancies. Germline *RUNX1* mutations have been described in these families. Hematopoietic stem cell transplantation using a sibling known to be negative for *RUNX1* mutations is the only curative option.⁴⁸

Wiskott-Aldrich syndrome. Wiskott-Aldrich syndrome (WAS) is an X-linked recessive immunodeficiency syndrome caused by WAS protein gene (*WASp*) mutations. WAS protein regulates actin filament reorganization in hematopoietic cells and regulates lymphocyte and platelet functions. The different *WASp* mutations generate different phenotypes: WAS, X-linked thrombocytopenia, and X-linked neutropenia. The classic or severe WAS is characterized by microthrombocytopenia, eczema, and susceptibilities to infections, autoimmune diseases, and malignancies.⁴⁹ These patients have a very poor prognosis. Currently, the only curative therapy is the allogeneic hematopoietic stem cell transplantation if a matched donor is available. Genetically modified autologous hematopoietic stem cell transplantation may represent an alternative form of therapy in these patients.⁵⁰

Disclosures

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