Pathogenic Aspects of Inherited Platelet Disorders

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Abstract

Inherited platelet disorders (IPDs) constitute a large heterogeneous group of rare bleeding disorders. These are classified into: (1) quantitative defects, (2) qualitative disorders, or (3) altered platelet production rate disorders or increased platelet turnover. Classically, IPD diagnostic is based on clinical phenotype characterization, comprehensive laboratory analyses (platelet function analysis), and, in former times, candidate gene sequencing. Today, molecular genetic analysis is performed using next-generation sequencing, mostly by targeting enrichment of a gene panel or by whole-exome sequencing. Still, the biochemical and molecular genetic characterization of patients with congenital thrombocytopathias/thrombocytopenia is essential, since postoperative or posttraumatic bleeding often occurs due to undiagnosed platelet defects. Depending upon the kind of surgery or trauma, this bleeding may be life-threatening, e.g., after tonsillectomy or in brain surgery. Undiagnosed platelet defects may lead to additional surgery, hysterectomy, pulmonary bleeding, and even resuscitation. In addition, these increased bleeding symptoms can lead to wound healing problems. Only specialized laboratories can perform the special platelet function analyses (aggregometry, flow cytometry, or immunofluorescent microscopy of the platelets); therefore, many IPDs are still undetected.

Keywords

- inherited platelet disorders
- bleeding symptoms
- primary hemostasis

Introduction

Platelets are elementary for primary hemostasis. Therefore, patients with hereditary platelet disorders often suffer from mucocutaneous bleeding (petechiae, hematoma, epistaxis, menorrhagia) and can develop peri- and postoperative bleeding, especially in surgery in mucous membrane areas (i.e., tooth extraction, circumcision).[1,2] Inherited platelet disorders (IPDs) often remain undetected until the onset of bleeding symptoms, especially in patients with a platelet count above 100×10^9/L. The diseases are often difficult to diagnose, since analyses are very time-intensive. Platelet morphology analyses by light and immunofluorescence microscopy contribute to the differentiation from immune thrombocytopenia or even help to diagnose the underlying platelet defect.[3,4]

After vascular injury, platelets adhere via their glycoprotein (GP) Ib/V/IX receptor to the von Willebrand factor (VWF). In addition, platelet integrin αIIbβ3 receptors are activated, which bind fibrinogen and contribute to platelet aggregation and further stabilization of the thrombus. Thrombin is released and in turn activates further platelets via the thrombin receptor.

Depending on the kind of platelet defect, IPDs are divided into defects of: the receptors, the cytoskeleton, secretion of granules, megakaryocyte maturation, signal transduction pathways, or the membrane phospholipids. Several IPDs are syndromic diseases with additional

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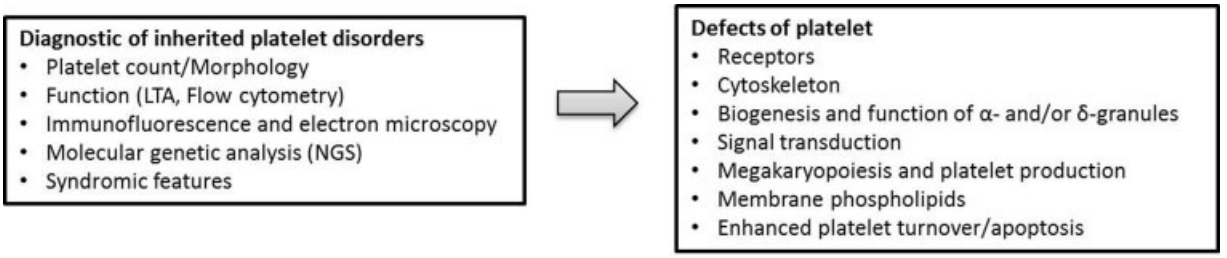

Fig. 1 Overview diagnostic and defects of inherited platelet disorders. LTA, light transmission aggregometry; NGS, next-generation sequencing.

clinical and pathological findings. Recent genetic analysis using high-throughput or next-generation sequencing (NGS) brought new insights in the variety of IPDs. New genes have been discovered to be associated with IPDs; among them are genes linked to increased platelet turnover (e.g., *GNE*, *GALE*) and apoptosis (e.g., *CYCS*).[5–7] Some patients with mild thrombocytopenia/thrombocytopathy and often a mild bleeding phenotype may have germline transcription factor defects involved in megakaryopoiesis. These patients have a higher risk of developing myeloid or lymphoid malignancies. Regarding the growing number of genes associated with IPDs, NGS analysis will help to gather further novel insights regarding inherited platelet defects. Diagnostic approaches and platelet defects are summarized in ►Fig. 1.

Platelet Receptor Defects

A very well-characterized platelet receptor defect is the **Bernard–Soulier syndrome** (BSS) caused by a quantitative or qualitative GPIb/V/IX receptor complex disorder. This receptor mediates the adhesion of VWF to the platelets.[8] Pathogenic variants in *GP1BA*, *GP1BB*, or *GP9*, which encode for the subunits GPIbα, GPIbβ, and GPIX, respectively, are responsible for the disease.[9] To date, no pathogenic variants in *GP5* (coding for the subunit GPV) has been described in patients with BSS. The platelet count is often below 100×10^9/L, but it can also be normal. Giant platelets and impaired platelet agglutination after stimulation with ristocetin are typical for BSS. In the flow cytometry of the platelets, the expression of GPIb/V/IX is reduced and/or the function of this receptor is impaired (reduced VWF-binding). In all three affected genes, founder mutations are known with higher frequency in certain populations.[9] The European founder mutation in *GP9* c.182A> G (p.Asn61Ser) is the most frequently described pathogenic variant for BSS[10] and seems to be associated with a milder phenotype.[11] Other pathogenic variants in *GP9* are frequent in the Japanese population (p.Cys89Tyr or p.Trp143*)[10,12] or in patients from India or Pakistan (p.Cys24Arg).[13–15] BSS is inherited as either autosomal recessive or (more rarely) autosomal dominant.[16] Monoallelic pathogenic variants in *GP1BA* and *GP1BB* are responsible for the autosomal dominant trait.[17,18] The Bolzano variant in GPIbα (p.Ala156Val in the mature subunit) is frequent in Italian patients and associated with a mild form of BSS with mild thrombocytopenia and bleeding tendency.[19] Using whole-exome sequencing for seven family members, Trizuljak et al identified another monoallelic variant in *GP1BA* (c.176T > G, p.Leu59Arg) segregating with macrothrombocytopenia in the family.[20] Overall, monoallelic BSS is associated with mild macrothrombocytopenia and a lack of bleeding diathesis, whereas biallelic BSS is usually more severe and patients may exhibit serious bleeding.

Another platelet receptor defect is **Glanzmann's thrombasthenia** (GT), a disorder of the fibrinogen receptor integrin αIIbβ3 (GPIIb/IIIa). GT is caused by pathogenic variants in *ITGA2B* or *ITGB3*, which encode the subunits αIIb (GPIIb) or β3 (GPIIIa).[21,22] Bleeding severity varies widely and may be modified by additional defects in other genes.[23,24] In patients with BSS and GT, hemorrhagic symptoms are observed already in the neonatal age and in early childhood. Type 1 GT is defined by complete absence or expression less than 5% of the integrin αIIbβ3 and combined with a severe bleeding phenotype.[25,26] Other patients with GT and a milder bleeding phenotype may exhibit only slightly decreased expression of the receptor, however, impaired fibrinogen binding.

Platelets comprise two primary collagen receptors, the integrin α2β1 and the platelet-specific receptor GPVI. Signal transduction seems to be coupled with the engagement of both GPVI and, to a lesser extent, α2β1.[27] Like the α2β1 integrin, the collagen receptor GPVI mediates platelet binding to collagen or GPVI-specific agonists, e.g., the snake venom convulxin. In patients with a slight tendency to bleed, a defect in the collagen receptor GPVI (encoded by *GP6*) has been demonstrated.[28] α2β1, like the other integrins, is in a low-affinity state on resting platelets and requires inside-out signals to efficiently bind to collagen. It is now established that the initial platelet contact with collagen and the subsequent initiation of integrin activation are strictly dependent on functional GPVI.[29] Whether α2β1 deficiency may lead to a bleeding phenotype remains elusive. Adenosine diphosphate (ADP) intensifies the platelet response induced by other platelet agonists (change in shape, aggregation, granule secretion) and therefore stabilizes platelet aggregates. ADP mediates its effect via two G protein–coupled receptors: **$P2Y_{12}$** and **$P2Y_1$**. $P2Y_{12}$ (Gi protein coupled) plays a role primarily in platelet activation, while the $P2Y_1$ receptor (Gq protein coupled) is primarily responsible for changing the shape of platelets. Clopidogrel, prasugrel, and ticagrelor irreversibly inhibit the $P2Y_{12}$ receptor. Mutations in the *P2Y12* gene lead to a reduced and reversible aggregation after stimulation with ADP (aggregometry analysis). A reduced ligand binding or ligand binding affinity of the $P2Y_{12}$

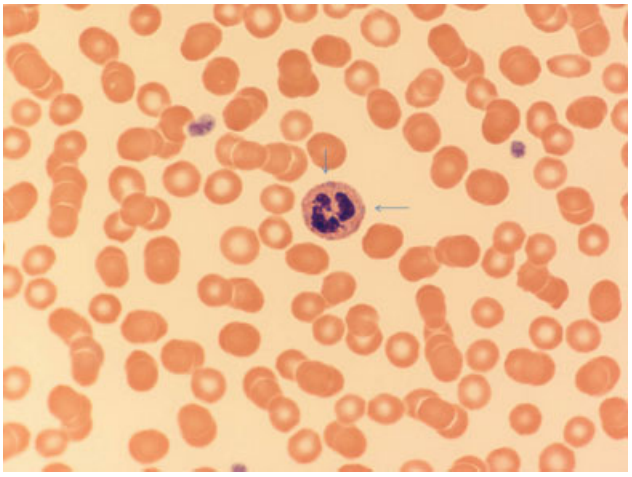

Fig. 2 Light microscopy: Döhle bodies (*arrows*) in leukocytes and giant platelets in a patient with MYH9-RD (Giemsa staining).

receptor and a disruption of the signal transduction pathway have been described.[30] Besides autosomal recessive inheritance, autosomal dominant variants have also been reported.[31,32]

Defects of the Cytoskeleton

Defects of structural proteins involved in the platelet cytoskeleton are often associated with macro- or microthrombocytopenia (30–100 × 10^9/L). Macrothrombocytopenia has been observed in defects of MYH9, filamin A, α-actinin, and β1-tubulin.

Pathogenic variants in the ***MYH9*** (**myosin heavy chain 9**) gene, which encodes the heavy chain of the nonmuscular myosin IIA (myosin 9, NMMHC-IIA), are associated with MYH9-associated macrothrombocytopenia (MYH9-related disease, MYH9-RD). As MYH9 is expressed in platelets, granulocytes, the cochlea, and the kidney, patients with an *MYH9*-associated platelet defect may suffer additionally from loss of hearing, cataract, or nephropathy.[33,34] Depending on the localization of the pathogenic variant within the gene, different phenotypes can develop in MYH9-RD.[35] Severe thrombocytopenia, early onset of end-stage renal failure, and deafness occur if the pathogenic variants are located in the globular head domain of MYH9 (5′-end). Blood smear often shows typical Döhle bodies in leukocytes (►**Fig. 2**) presumably because of the aggregation of the modified protein.[34]

Filamin A connects the cell membrane with the cytoskeleton and can interact with more than 80 different partners, including cell surface receptors (GPIbα and αIIbβ3) or signal proteins (Syk and STIM1). Filamin is essential to regulate cell morphology and motility.[36,37] Mutations in the X-linked filamin A gene (*FLNA*) can be associated with a wide range of symptoms, including macrothrombocytopenia and, due to a neuronal migration defect, periventricular nodular heterotopy.[38]

The protein **α-actinin 1** is involved in the organization of the cytoskeleton and interacts with various cytoskeletal proteins and receptors (e.g., β-integrin). Mutations in the coding gene (*ACTN1*) lead to macrothrombocytopenia and disorganization of the actin filaments because the formation of platelet precursor cells is disturbed.[39]

β1-tubulin, encoded by the *TUBB1* gene, is a major component of microtubules, which are abundant especially in megakaryocytes. Autosomal dominant mutations lead to macrothrombocytopenia. In patients with a β1-tubulin defect, platelet aggregometry and flow cytometry do not show obvious alterations; however, immunohistochemical studies show that the typical ring shape of β1-tubulin is missing.[40]

Microthrombocytopenia is seen in patients with X-linked **Wiskott–Aldrich syndrome** (WAS). Besides the microthrombocytopenia, patients with WAS often suffer from eczema and immunodeficiency. The symptoms of this disease are very variable and range from severe combined forms to thrombocytopenia without immunodeficiency, probably depending on the location of the pathogenic variants in the gene.[41] The WAS protein (WASP) regulates actin filament organization in the cytoskeleton. Disturbances in the formation and structure of the cytoskeleton and an increased breakdown rate of platelets are probably the causes of thrombocytopenia.[42] The number of **δ**-granules is markedly reduced. In patients with the severe form of WAS, human stem cell transplantation has successfully been performed.

Platelet Secretion Defects

Platelets comprise three groups of intracellular secretory organelles: α-granules, **δ**-granules, and lysosomes. The most common granules are the α-granules containing membrane-bound proteins that can be expressed on the platelets surface after platelet activation (e.g., the adhesion molecule P-selectin) and soluble proteins that are excreted to the extracellular space (e.g., VWF, thrombospondin, factor V, and fibrinogen). **δ**-granules ("dense bodies") are smaller and rarer in platelets than α-granules. In **δ**-granules, serotonin, histamine, nucleotides (ATP, ADP), and ions (Ca^{2+}, Mg^{2+}, pyrophosphate) are stored. Typical markers for **δ**-granules and their secretion are serotonin and the membrane protein granulophysin (CD63, LAMP3). **δ**-granules are members of the lysosome-related organelles (LROs). LROs also comprise melanosomes and cytotoxic T-cell granules.

After activation of platelets, the actin cytoskeleton dissolves its cross-links to enable new interactions and to induce granule secretion. The α-granules fuse with each other in a process termed homotypic fusion. In addition, α-granule membrane fuses with the platelet's plasma membrane to increase platelet surface area and to secrete their substances into the extracellular space. Secretion defects of the granules are called storage pool disease (SPD). SPD can include only α-granules or only **δ**-granules or both types of granules. The reduction of **δ**-granules in megakaryocytes and platelets is supposed to be the most common inherited cause of thrombocytopathy (10–18% of patients with thrombocytopathy). Their genetic causes are mostly still unknown.[43]

Classic α-Granule Defects

Gray platelet syndrome (GPS), an autosomal recessive disease, is associated with a secretion disorder of α-granules.

The causes for GPS are mutations in the *NBEAL2* gene (neurobeachin-like 2). The product of this gene is a member of the BEACH domain-containing proteins, which is involved in the regulation of membrane dynamics and intracellular vesicle transport in platelets.[44] Patients have enlarged platelets lacking α-granules and therefore platelets appear gray on blood smear. Bleeding symptoms are variable. Due to secretion of α-granules within the bone marrow, the risk of developing myelofibrosis is enhanced. Vitamin B_{12} and soluble FASL (marker for activated T-cells and natural killer cells) have been shown to be increased in GPS patients.[45]

A syndromic disorder is the **arthrogryposis–renal dysfunction–cholestasis (ARC) syndrome**. Children with ARC syndrome often present with arthrogryposis, renal tubulopathy, and cholestasis in combination with bleeding diathesis due to a platelet defect. Platelets of these patients appear pale because of the reduced number of α-granules or sometimes even total loss of α-granules. ARC syndrome is a severe disease; most of the affected children die within the first 2 years of life. ARC syndrome is caused due to defects in membrane-associated VPS33B proteins (*VPS33B* gene) that regulate SNARE proteins and are involved in granule development or due to defects in the VPS33B interacting protein (*VIPAS39* gene).[46,47]

In the **Quebec platelet disorder**, the urokinase-plasmin activator within the α-granules is being upregulated. Thus, proteins contained within the α-granules are depleted by plasmin. This defect originates in a duplication within the *PLAU* (urokinase-plasminogen activator) gene.[48]

Classic δ-Granule Defects

Typically, patients with **Hermansky–Pudlak syndrome** (HPS) present with oculocutaneous albinism and increased bleeding symptoms due to platelet function deficiency. HPS is typically characterized by a defect of the melanosomes, of platelet δ-granules (►Fig. 3), and of lysosomes. This disease is caused by mutations in one of the 11 HPS genes (*HPS1–11*).[49,50] The encoded proteins are involved in the biogenesis of four ubiquitously expressed multi-subunits complexes: BLOC (biogenesis of lysosome-related organelles complex)-1, BLOC-2, BLOC-3, and AP-3 (adaptor protein-3). Interestingly, patients with BLOC-2 deficiency (HPS3, HPS5, and HPS6) often show a milder phenotype regarding the albinism. Patients with HPS1 and HPS2 (*AP3B1* gene) may develop pulmonary fibrosis.[51,52] Patients with HPS2 or HPS10 (*AP3D1* gene) additionally suffer from an immune defect.[53]

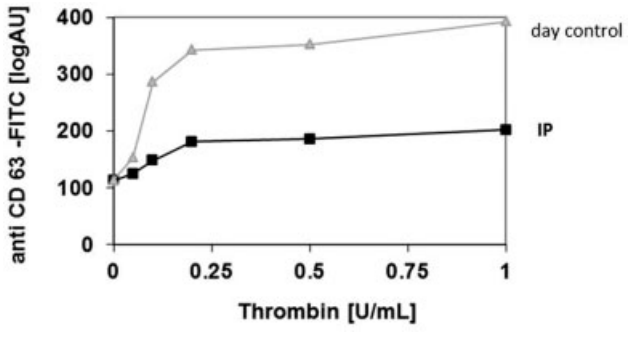

Fig. 3 Flow cytometry: reduced platelet CD63 expression after stimulation with thrombin hinting to a platelet δ-granule secretion defect in a patient with HPS1 (IP) compared with healthy control. Data are expressed as log arbitrary units (logAU) of anti-CD63-stained unstimulated and thrombin-stimulated platelets from the patient and a healthy control. The patient is a homozygous carrier of a pathogenic variant in *HPS1* (NM_000195.4:c.972dup, p.Met325Hisfs*128).

Patients with an autosomal recessive **Chédiak–Higashi syndrome** (CHS) harbor mutations in the *LYST* gene (lysosomal trafficking regulator/CHS1), which lead to immunodeficiency, oculocutaneous albinism, neurological anomalies, and a mild platelet disorder.[54] The platelet aggregometry after stimulation with the agonists collagen, ADP, and epinephrine is impaired. The typical peroxidase-positive inclusion bodies within neutrophils can be identified in the blood smear. Many of the patients develop hemophagocytic lymphohistiocytosis (HLH) during the disease.[55]

Griscelli syndrome type 2, caused by pathogenic variants in the *RAB27A* gene, is primarily associated with an uncontrolled T lymphocyte and macrophage activation syndrome, often associated with HLH.[56] As RAB27A protein is responsible for transport of granules, mutations within this gene lead to intracellular disruption of transportation and a platelet δ-granule defect.

Another δ-granules defect is related to alterations in the ***SLFN14*** gene (Schlafen family member 14). Patients harboring a dominant pathogenic variant in *SLFN14* suffer from thrombocytopenia with a significant reduction of δ-granules and ATP secretion. Patients show impaired aggregometry after stimulation with thrombin receptor activating peptide (TRAP), ADP, and collagen. SLFN14 is involved in megakaryopoiesis and platelet maturation.[57]

Combined α-/δ-Granule Secretion Defects

Familial hemophagocytic lymphohistiocytosis (FHL) comprises rare genetically heterogeneous disorders of lymphocyte cytotoxicity (autosomal recessive inherited). FHL-2 is caused by mutations in the perforin encoding gene *PRF1*. Other FHL types are due to mutations in genes coding for proteins important for intracellular trafficking/exocytosis of perforin-containing lytic granules: FHL-3 (*UNC13D*), FHL-4 (*STX11*), FHL-5 (*STXBP2*). A granule secretion defect has been reported in FHL-3[58] and FHL-5.[59] UNC13D protein (former Munc13–4) is responsible for merging the lysosomal granule with the cell membrane. Thus, granule secretion within the synapsis between cytotoxic T-cells and the infected target cell is impaired.

Defects Signal Transduction Pathways

Abnormalities in platelet receptor–specific signaling pathways may lead to a bleeding disorder as shown for the **Src defect**. Src is a nonreceptor tyrosine kinase, which is particularly abundant in platelets, where Src plays a major role triggering essential signal transduction pathways. Shape change of activated platelets requires reorganization of the actin cytoskeleton. Upon fibrinogen binding during platelet activation, conformation of the fibrinogen receptor αIIbβ3

changes and an intracellular signal network is activated that controls the organization of the actin cytoskeleton. αIIbβ3-mediated "outside-in" signaling is initiated by activation of Src.[60] Patients with a "gain of function" variant in the *SRC* gene present with thrombocytopenia, myelofibrosis, severe bleeding diathesis, and bone defects.[61] In patients with Src defect, the number of α-granules in platelets is reduced.

Another rare congenital disease caused by defective integrin activation of leukocytes and platelets is **leukocyte adhesion deficiency-III** (LAD-III), which is autosomal recessively inherited. LAD-III results from mutations in the *FERMT3* gene, which codes for the kindlin-3 protein.[62] Kindlin-3 is expressed in hematopoietic cells, interacts with the fibrinogen receptor αIIbβ3, and is significantly involved in the αIIbβ3 receptor activation. LAD-III is characterized by integrin signaling dysfunction in leukocytes and platelets (concerning β1, β2, and β3 integrin subunits), while integrin expression is normal. Platelets of LAD-III patients fail to aggregate because of an impaired activation of the αIIbβ3 receptor.[63] This explains the combined immunological and platelet defect, i.e., the patients suffer from very severe bacterial infections and an increased bleeding tendency.

CalDAG-GEFI deficiency (calcium and diacylglycerol-regulated guanine nucleotide exchange factor I) also affects platelet function and may cause severe bleeding. Mutations in the *RASGRP2* gene, which codes for CalDAG-GEFI, lead to a disruption of integrin αIIbβ3 activation and aggregation despite normal αIIbβ3 levels in homozygous patients.[64] This signaling pathway plays a role in the "inside-out" activation of the integrin αIIbβ3. Rap1b activation is triggered by CalDAG-GEFI, a Ca^{2+} and DAG-regulated guanine nucleotide exchange factor. In activated platelets, Rap1b interacts with the reorganized actin-based cytoskeleton.

Defects of Megakaryopoiesis

Megakaryopoiesis is a differentiation process that depends on a large number of genes. Defects in megakaryopoiesis caused by mutations in genes coding for hematopoietic transcription factors can lead to thrombocytopenia with or without an associated functional platelet defect. Cell type–specific transcription factors such as ***RUNX1***, ***ETV6***, ***FLI1***, ***GFI1B***, and ***GATA1*** play an essential role in megakaryopoiesis.[65,66] Germline mutations in genes coding for transcription factors are often associated with the risk of developing myeloid or lymphoid malignancies.[67,68]

RUNX1 (runt-related transcription factor 1) regulates, among others, the expression of MYH10 (myosin heavy chain 10 or nonmuscle myosin IIB). MYH10 expression is essential to switch from mitosis to endomitosis in megakaryocyte differentiation. The reduction of MYH10 leads to immature megakaryocytes.[69] Mutations in the *RUNX1* gene are inherited in an autosomal dominant manner and often lead to mild thrombocytopenia and thrombocytopathy (impaired α- and δ-granule secretion).[70,71] In RUNX1-related thrombocytopenia (RUNX1-RT), there is an increased predisposition for myelodysplastic syndrome (MDS) or acute myeloid leukemia.[72,73]

ETV6 (ETS variant 6), a member of the ETS (E26 transformation-specific) family, acts as a transcriptional repressor that requires homodimerization to exert repression of other ETS domain containing transcription factors, such as FLI1.[74] Autosomal dominant germline mutations in the *ETV6* gene lead to mild-to-moderate thrombocytopenia, platelet dysfunction, and increased levels of circulating CD34+ hematopoietic progenitors.[75] Patients have an increased risk of developing acute leukemia, MDS, or solid tumors.[76]

The ***FLI1*** gene (friend leukemia virus integration 1) encodes an ETS transcription factor family member. Deletions on chromosome 12q23 that include *FLI1* cause two syndromes: Paris-Trousseau syndrome and Jacobsen syndrome.[77–79] Patients show thrombocytopenia with enlarged α-granules and a secretion defect after stimulation with thrombin.[80] Many patients suffer from a congenital heart disease, facial dysmorphia, and psychomotor retardation. FLI1 targets, among others, the genes *MPL*, *ITGA2*, and *GP1BA*. Pathogenic FLI1 variants can cause thrombocytopenia, disaggregation after stimulation with low-dose ADP, collagen, and TRAP, and granule defects (giant α-granules and/or missing δ-granules).[81,82]

In patients with mutations in the ***GFI1B*** gene, platelets also appear gray in the blood smear and show an α-/δ-granule secretion defect in flow cytometry analysis. *GFI1B* encodes the transcription factor GFI1B (growth factor-independent 1B), which plays an important role in erythropoiesis and megakaryocyte development.[83] This disease is autosomal dominantly inherited. For both GFI1B-RT and RUNX1-RT, it has been shown that platelets still express the stem cell antigen CD34.[84]

GATA1-associated thrombocytopenia is inherited in an X-linked manner. Because GATA1 regulates NBEAL2, α-granules are decreased.[85] GATA1 is required for the terminal differentiation of definitive erythroid and megakaryocytic cells; therefore, patients present additionally with anemia.[86] Depending on the degree of impairment of the GATA1 protein function, there is great clinical variability regarding the severity of the disease.

The **ANKRD26** (ankyrin repeat domain-containing protein 26)-related thrombocytopenia (ANKRD-RT) is a mostly mild thrombocytopenia that is inherited dominantly and due to mutations in the 5′ untranslated region of *ANKRD26*.[87] The platelet size and appearance are normal. Patients are at an increased risk of developing leukemia.[88,89] GATA1-related disease (GATA1-RD), GFI1B-RT, and ANKRD26-RT are defects of megakaryocyte maturation.[90] The autosomal recessive inherited **congenital amegakaryocytic thrombocytopenia** (CAMT) is due to a deficiency of megakaryocytes in the bone marrow. The megakaryocyte deficiency is caused by mutations in the thrombopoietin receptor encoded by the ***MPL*** gene.[91] In the course of the disease, aplastic anemia and pancytopenia often occur.[92] In CAMT with radioulnar synostosis, there is a combination of a skeletal anomaly of the forearm and hypomegakaryocytic thrombocytopenia. Genetic alterations in the homeobox gene ***HOXA11*** or in the gene ***MECOM*** (coding for the oncoprotein EVI1) were identified as cause in these patients.[93,94]

Hypomegakaryocytic thrombocytopenia and bilateral lack of the radius is typical for the **TAR syndrome** (thrombocytopenia with radial aplasia). In TAR syndrome, the patient's thumbs are formed, which is an important feature to distinguish it from other syndromes with radial aplasia. The TAR syndrome is autosomal recessive inherited and is caused by genetic alterations in the *RBM8A* gene.[95]

The ***FYB1*** gene codes for the "adhesion and degranulation-promoting adapter protein" (ADAP). ADAP is a hematopoietic-specific protein that is, among others, involved in platelet activation and integrin-mediated cell adhesion. Homozygous mutations in the *FYB1* gene lead to mild microthrombocytopenia. The platelets showed an increased basal P-selectin and PAC-1 expression. Using a cone and platelet analyzer (Impact-R), a reduced adhesion of platelets to fibrinogen and VWF was reported.[96]

Defects of Membrane Phospholipids

Upon platelet activation, anionic phospholipids, mainly phosphatidylserine (PS), translocate from the inner to the outer leaflet of the platelet membrane so that thrombin can be generated on the surface. Exposure of PS at the surface of platelets and platelet-derived microparticles is a major component of normal hemostasis because it supports formation of enzyme complexes that are involved in the conversion of prothrombin to thrombin. Defects of membrane phospholipids are associated with impaired platelet procoagulant activity and thrombin formation. In patients with autosomal recessive inherited **Scott syndrome**, mechanism for translocating PS (membrane lipid scrambling) to the platelet membrane is defective. Patients' platelets show impaired annexin binding or a significantly reduced thrombin generation potential caused by mutations in the ***ANO6*** gene, which codes for the transmembrane protein 16F (TMEM16F).[97] TMEM16F is a critical component of the calcium-dependent phospholipid scrambling activity. Ca^{2+}-dependent phospholipid scramblases transport phospholipids bidirectionally.[98]

Stormorken syndrome is caused by autosomal dominant mutations in the *STIM1* gene, which encodes a Ca^{2+} sensor of the endoplasmic reticulum. As a result, permanent Ca^{2+} influx through the Orai canal provokes premature platelet activation. Patients' platelets present with high exposure of aminophospholipids on the outer surface of the plasma membrane. Unstimulated platelets have already increased levels of annexin V, PAC-1, P-selectin, and CD63 on their surface.[99] The phenotype of this extremely rare disease is very complex, comprising bleeding diathesis, myopathies, hyposplenism, hypocalcemia, miosis, and intellectual disorders.[100]

Other Causes of IPDs

Macrothrombocytopenia has also been observed in defects of the protein kinase A. Patients with a homozygous mutation in the *PRKACG* gene, which codes for cyclic adenosine monophosphate (cAMP)-dependent protein kinase catalytic subunit gamma, also have macrothrombocytopenia. This mutation leads to increased filamin A degradation, disruption of platelet secretion, Ca^{2+} mobilization, and the cytoskeleton organization.[101]

Conclusion

IPDs are a very heterogeneous group of diseases affecting platelet number and function. Specialized analyses such as flow cytometry, immunofluorescence analyses, and molecular genetic analyses using NGS improved the identification of individual IPDs. However, in some cases, these investigations remain without finding and the diagnostic is still challenging. Whole-exome sequencing can identify new genes associated with a platelet phenotype and therefore contribute to expand knowledge about IPDs. Defects in megakaryopoiesis can be associated with an increased risk of developing an oncological disease. These patients should be carefully monitored in specialized centers.

Conflict of Interest

The authors declare that they have no conflict of interest.

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