Inherited Platelet Disorders: A Short Introduction

Barbara Zieger[1] Doris Boeckelmann[1]

[1] Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center, University of Freiburg, Freiburg, Germany

Hamostaseologie 2023;43:52–59.

Address for correspondence Barbara Zieger, MD, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, University Medical Center Freiburg, Mathildenstr. 1, 79106 Freiburg, Germany (e-mail: barbara.zieger@uniklinik-freiburg.de).

Abstract

Platelets play an important role regarding coagulation by contributing to thrombus formation by platelet adhesion, aggregation, and α-/δ-granule secretion. Inherited platelet disorders (IPDs) are a very heterogeneous group of disorders that are phenotypically and biochemically diverse. Platelet dysfunction (thrombocytopathy) can be accompanied by a reduction in the number of thrombocytes (thrombocytopenia). The extent of the bleeding tendency can vary greatly. Symptoms comprise mucocutaneous bleeding (petechiae, gastrointestinal bleeding and/or menorrhagia, epistaxis) and increased hematoma tendency. Life-threatening bleeding can occur after trauma or surgery. In the last years, next-generation sequencing had a great impact on unrevealing the underlying genetic cause of individual IPDs. Because IPDs are so diverse, a comprehensive analysis of platelet function and genetic testing is indispensable.

Keywords

- inherited platelet disorders
- next-generation sequencing
- Hermansky-Pudlak syndrome
- *GNE*

Introduction

Congenital thrombocytopenias/-pathies are divided into defects of platelet receptors, of the cytoskeleton, granule secretion (storage pool disorders), signal transduction, of membrane phospholipids, megakaryopoiesis, and enhanced platelet clearance. However, the differentiation criteria can overlap so that a classification can change depending on the focus; for example, defects in megakaryopoiesis are often associated with secretion disorders. The underlying causes for inherited thrombocytopenia are also manifold. Around 60 different inherited platelet disorders (IPDs) have been described[1] and the number is growing due to next-generation sequencing (NGS) for genetic analysis. IPDs are quite rare, but vary in prevalence. A precise specification of the prevalence is still difficult because patients with mild or moderate disorders may be unnoticed or not investigated.

Comprehensive investigations are necessary to characterize a congenital platelet defect. Determination of the platelet count and assessment of the peripheral blood smear are essential. For platelet function analysis, platelet aggregometry according to Born (light transmission aggregometry) is the gold standard. Advanced diagnostic includes analysis of the expression and function of platelet receptors and platelet α- and δ-granule secretion using flow cytometry (fluorescence activated cell sorting). Immunofluorescence microscopy can also be performed to analyze platelet defects.[2] Furthermore, electron microscopic analysis of platelets is used for elucidation of structural defects such as loss of platelet granules. Time-consuming candidate gene sequencing using direct sequencing has been replaced by NGS (NGS-panel, whole exome sequencing [WES]) during the last years, providing fast growing knowledge about the genetic background of certain platelet disorders.[3] NGS-panel analysis performed early can contribute to an immediate diagnosis, especially when clearly pathogenic or likely pathogenic variants have been identified and there is a match in genotype/phenotype correlation. However, numerous genes are involved in the complex control of platelet formation and function, and because the different NGS panels do not cover all these genes, genetic analysis cannot identify all the genetic defects in platelet disorders so far. In addition, variants of uncertain significance that are frequently identified often require further investigation to clarify their effects on platelet phenotype. Furthermore, it seems that WES

received
October 31, 2022
accepted after revision
November 23, 2022

DOI https://doi.org/10.1055/a-1987-3310.
ISSN 0720-9355.

analysis is more successful if TRIO sequencing has been performed. In addition, if the platelet phenotype is well-defined, NGS data can more successfully be assessed. Nonetheless, NGS-panel and WES have helped unravel the underlying defect in many IPDs and make the diagnosis more specific. Therefore, the bleeding risk can be better estimated and precautions can be made before surgery. In addition, the correct diagnosis is also important because defects in certain genes that cause platelet disorders are associated with syndromic disorders or with an increased risk to develop an oncological disease.[4] In this short introduction, we provide an insight into the complexity and heterogeneity of this group of defects by presenting selected IPDs.

Platelet Receptor Defects with or without Thrombocytopenia

Regarding the classic receptor defects such as Bernard-Soulier syndrome (BSS, OMIM#231200, GPIb/V/IX complex), Glanzmann thrombasthenia (GT, OMIM#273800, integrin $\alpha_{IIb}\beta_3$), or P2Y12 (ADP)-receptor (OMIM#609821, P2YR12) defect, biallelic genetic alterations are normally causing the disease. In patients with GT, the platelets fail to aggregate due to a defect in platelet-to-platelet attachment resulting in a moderate to severe bleeding diathesis. Normally, $\alpha_{IIb}\beta_3$ integrin is expressed at high density on the platelet surface and activated $\alpha_{IIb}\beta_3$ acts as a receptor for fibrinogen and other adhesive proteins. The receptor is a key element in platelet activation and aggregation.

Besides this autosomal-recessive (AR) mode of inheritance, monoallelic alterations can lead to a milder phenotype (e.g., in autosomal-dominant [AD] transmitted Bernard Soulier syndrome[5] or to a milder P2Y12 receptor defect).[6] A heterozygous variant in one of the two GT-related genes (*ITGA2B* and *ITGB3*) affecting the salt bridge between the two receptor subunits leads to macrothrombocytopenia and enlarged α-granules[7,8] and clearly differs from the findings of classical GT (reduced integrin $\alpha_{IIb}\beta_3$ expression and/or activation).[9]

In contrary, platelet type von Willebrand disease (PTvWD, OMIM #177820) is caused by an autosomal dominant "gain-of-function" mutation in the *GP1BA* gene, which encodes the GPIbα (vWF-binding site) subunit of the GPIb/V/IX complex. The mutation leads to increased platelet interaction with the vWF and spontaneous aggregation of the patient's platelets and thus to thrombocytopenia.[10]

Other platelet receptor defects are collagen receptor defects and thrombin receptor defects (►Table 1).

Defects of Signaling Transduction

The shape change of the activated platelets requires a reorganization of the actin cytoskeleton. After binding of fibrinogen, the conformation of the $\alpha_{IIb}\beta_3$ integrin receptor changes and an intracellular signaling network is activated which controls the organization of the actin cytoskeleton. Defects of $\alpha_{IIb}\beta_3$ activation have been demonstrated for patients with autosomal recessive CalDAG-GEFI deficiency (*RASGRP2*, OMIM#615888)[11,12] or a Kindlin3 defect (*FERMT3*, OMIM#612840).[13]

CalDAG-GEFI (calcium and diacylglycerol-regulated guanine nucleotide exchange factor I) activates Rap1, which initiates "inside-out" signaling for $\alpha_{IIb}\beta_3$. Binding of talin shifts the integrin into its high-affinity state.[14] Since the first report in 2014 by Canault et al,[11] more than 29 mutations in *RASGRP2* (which codes for CalDAG-GEFI) are listed in the Human Gene Mutation Database (access 09/2022). Bleeding manifestations start early in life and can be severe.

Biallelic *FERMT3* genetic alterations lead to leukocyte adhesion deficiency III (LAD-III) syndrome. Characteristically, patients suffer from severe bacterial infections and bleeding disorders. They present with leukocytosis and a platelet disorder (impaired fibrinogen binding, although the $\alpha_{IIb}\beta_3$ receptor is normally expressed). Because the encoded protein Kindlin3 activates β1, β2, and β3 integrins, the defect affects not only platelets but also leukocytes and combines severe immunodeficiency and bleeding syndrome.[15]

Signaling from the integrin to the cytoskeleton is initiated by the protein kinase Src. Src regulates the signaling pathway through its activation state. Besides its role in platelet signaling, Src seems to play an important role in platelet formation.[16] Patients with a "gain of function" variant in the Src gene show thrombocytopenia, myelofibrosis, severe bleeding diathesis, and bone pathologies. In the platelets, a reduction in α-granules has been demonstrated[17] (►Table 2).

Storage Pool Disorders

Platelets comprise three groups of intracellular secretory organelles: α-granules, δ-granules, and lysosomes. The α-granules contain membrane-bound proteins which can be expressed on the platelet 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). In δ-granules, serotonin, histamine, nucleotides (ATP, ADP), and ions (Ca^{2+}, Mg^{2+}, pyrophosphate) are stored. They are members of the lysosome-related organelles (LROs). LROs also include melanosomes and cytotoxic T cell granules. Typical markers for δ-granules and their secretion are serotonin and the membrane protein granulophysin (CD63).

Classical α-Granule Defect

Gray platelet syndrome (*NBEAL2*; OMIM#139090) is inherited autosomal recessive. The *NBEAL2* gene codes for NBEAL2 a member of the BEACH domain-containing proteins, which is involved in the regulation of membrane dynamics and intracellular vesicle transport in platelets.[18] Patients have enlarged platelets lacking α-granules and therefore, platelets appear gray on blood smear. Bleeding symptoms are variable. Other classical α-granule defects are the ARC syndrome and the Quebec platelet disorder (►Table 3).

Table 1 Platelet receptor defects

Disease	Receptor	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
BSS	GPIb/V/IX	*GP1BA*, *GP1BB*, *GP9*/AR, AD	Moderate to severe MTP. LTA: absence of platelet agglutination after ristocetin stimulation. FC: absence or severe dysfunction of VWF-receptor GPIb/IX. Moderate to severe bleeding symptoms. In monoallelic BSS milder TP and bleeding
PTvWD	GPIb/V/IX (specific: $GPIb_{\alpha}$/vWF-binding)	*GP1BA*/AD ("gain of function")	Mild to severe TP. LTA: hyperaggregation with low-dose ristocetin. Absent to mild bleeding tendency
GT	$\alpha_{IIb}\beta_3$ (GPIIa/IIIb)	*ITGA2B*, *ITGB3*/AR	Normal platelet count, LTA: absent or severely reduced aggregation with most agonists (ADP, TxA2, collagen, thrombin). Max. agglutination with ristocetin normal and/or 2nd wave reduced. FC: Absence or decreased $\alpha_{IIb}\beta_3$ expression, nonfunctional $\alpha_{IIb}\beta_3$. Severe bleeding tendency
Autosomal dominant macrothrombocytopenia	$\alpha_{IIb}\beta_3$ (GPIIa/IIIb)	*ITGA2B*, *ITGB3*/AD	Mild to moderate MTP. FC: impaired αIIbβ3 activation. Moderate bleeding tendency
Defect of the collagen receptor $\alpha_2\beta_1$ (platelet adhesion)	$\alpha_2\beta_1$ (GPIa/IIa)	*ITGA2*/AD	Normal platelet count. LTA: impaired or absent response to collagen and GPVI agonists (convulxin). Mild bleeding tendency
Defect of the collagen receptor GPVI (platelet activation)	GPVI	*GP6*/AR	
Defect of the thrombin receptor	PAR1	*F2R*	Normal platelet count. LTA: impaired or absent response to thrombin
ADP receptor defect, BDPT 8	P2YR12	*P2RY12*/AR, AD	Normal platelet count. LTA: impaired with ADP (almost all concentrations). Impaired VASP analysis. Mild to severe bleeding

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; BDPT, bleeding disorder platelet-type; BSS, Bernard-Soulier syndrome; FC, flow cytometry; GT, Glanzmann thrombasthenia; LTA, light transmission aggregometry; MTP, macrothrombocytopenia; PTvWD, platelet-type von Willebrand disease; TP, thrombocytopenia; VASP, vasodilator-stimulated phosphoprotein; VWF, von Willebrand factor.

Classical δ-Granule Defects

Patients with Hermansky-Pudlak syndrome (HPS) usually present with oculocutaneous albinism (disorder of the melanosomes) and bleeding symptoms (platelet function defect). The platelet function disorder is caused by a platelet δ-granule secretion defect. Platelets show a significantly reduced CD63 expression after stimulation with thrombin (in flow cytometry) and a lack of δ-granules (in whole mount transmission electron microscopy). HPS is autosomal recessively inherited and comprises a heterogeneous group of syndromic diseases characterized by defective LROs. Pathogenic variants in 11 HPS genes encoding subunits of three BLOC complexes (BLOC-1 to BLOC-3) or the AP-3 complex are associated with different types of HPS (HPS-1 to HPS-11).[19,20] Patients with HPS-2 or HPS-10 suffer[21] additionally from an immune deficiency. Neurological abnormalities have also been described in HPS-10.[22] Patients with HPS-1, HPS-4 (both affected genes encode for BLOC-3 subunits), and HPS-2 (AP-3 complex) have been described to develop pulmonary fibrosis or granulomatous colitis during the course of their disease. Patients with mutations in BLOC-2 subunits (HPS-3, HPS-5, and HPS-6) seem to have milder phenotypes with variable degree of hypopigmentation. Panel sequencing helps identify even rare types of HPS (e.g., HPS-7 and HPS-11).[23,24]

Table 2 Defects of signal transduction

Disease	Affected protein	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
CalDAG-GEFI defect	Guanine exchange factor-1 (CalDAG-GEFI)	*RASGRP2/AR*	Normal platelet count. LTA: severely impaired with different agonist (ADP, epinephrine) and with low-dose collagen and TRAP6. FC: impaired $\alpha_{IIb}\beta_3$ integrin activation. Moderate to severe bleeding
Leukocyte adhesion deficiency, type III	Kindlin-3	*FERMT3/AR*	Normal platelet count, severe leukocytosis. FC: $\alpha_{IIb}\beta_3$ integrin activation defect. Severe bleeding tendency. LADIII phenotype: recurrent bacterial infections
Src defect	Tyrosine kinase Src	*SRC/AD*	Only one pedigree described so far (see above)

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; FC, flow cytometry; LTA, light transmission aggregometry.

Patients with the Chediak-Higashi syndrome (CHS) (OMIM #214500) suffer from an immunodeficiency as the most important clinical feature. In addition, they present with oculocutaneous albinism, neurological abnormalities, and a mild tendency to bleed due to alterations in the *LYST* (lysosomal trafficking regulator/CHS1) gene.[25]

Meanwhile three types of Griscelli syndrome (GS1–3) have been described with a varying phenotype spectrum depending on the gene affected. The encoded proteins are part of a tripartite complex important for intracellular melanosome transport.[26] Patients usually present with partial albinism including pigmentary dilution of the skin and hair, the presence of large clumps of pigment in hair shafts, and an accumulation of melanosomes in melanocytes. Other syndromic features comprise neurologic impairment (mostly GS1) and immunodeficiency with a predisposition to develop hemophagocytic syndrome (mostly GS2).[27,28]

Defects of the Cytoskeleton

Cytoskeletal defects are usually associated with thrombocytopenia (30–100 $\times$ 10^9/L). The occurrence of either micro- or macrothrombocytopenia is possible. The affected proteins are involved in the reorganization of the actin cytoskeleton (►Table 4).

MYH9-related platelet defects (OMIM#155100) are characterized by macrothrombocytopenia. The *MYH9* gene encodes the non-muscle myosin type IIA heavy chain (NMMHC-IIA) which is also expressed in cochlea and kidney. Platelets and neutrophils are affected because they express MYH9 as the only myosin II isoform. The aggregation of the altered protein presumably leads to the formation of the typical Döhle bodies in granulocytes which can be identified on blood smears.[29,30] The location of the pathogenic variant in the *MYH9* gene determines the extent of the disease. Progressive deafness, cataracts, or nephropathy have been observed when the N-terminal region of the protein is affected. The syndromic features historically led to the description of the Fechner syndrome, May-Hegglin anomaly, Sebastian syndrome, and Epstein syndrome, which are now comprised as MYH9-related disease.

Defects of Megakaryopoiesis

Germline pathogenic variants in genes encoding transcriptional factors or repressors (e.g., in *RUNX1*, *ETV6*, and *ANKRD26*) regulating megakaryopoiesis may be associated with an increased risk to develop myeloid malignancies.[4,31,32] The transcription factor GATA1 plays a role in the normal development of erythroid cells and megakaryocytes and defects may be associated with anemia. There is an increasing evidence that the phenotype of GATA1 alterations is highly variable and depends on the affected site in the transcription factor or also the amino acid exchanged.[33] The phenotype ranges from severe to mild thrombocytopenia or even normal platelet count with or without anemia and myelofibrosis.[34] However, a platelet granule secretion defect is reported even in cases with mild thrombocytopenia or normal platelet counts and GATA1 variants.[35,36] Other defects affecting the megakaryopoiesis are summarized in ►Table 5.

Deficient Glycosylation Leads to Platelet Clearance

Congenital thrombocytopenia can be caused not only by a reduced production but also by an accelerated degradation of platelets. Sialic acid binds to platelet surface glycoproteins and is known to protect platelets from degradation via the Ashwell–Morell receptor.[37,38] The *GNE* gene encodes an enzyme that initiates and regulates the biosynthesis of N-acetylneuraminic acid, a precursor of sialic acids. *GNE* pathogenic variants are autosomal recessive associated with adult-onset progressive GNE myopathy (with or without

Table 3 Classical α- and δ-granule secretion defects

Disease	Affected protein	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
α-Granule defect			
GPS	Neurobeachin-like 2	*NBEAL2*/AR	Moderate to severe TP, typically gray platelets (blood smear). LTA: impaired with several agonists. FC: reduction or absence of α-granules. Moderate to severe bleeding tendency. Elevated vitamin B12 serum level, sometimes myelofibrosis
ARC syndrome	VPS33B VPS33B-interacting protein	*VPS33B*/AR *VIPAS39*/AR	For VPS33B described: Platelet count: normal to decreased. FC: reduction or absence of α-granules. Frequent episodes of severe bleeding. Severely syndromic features: arthrogryposis, cholestatic liver disease, and renal dysfunction
Quebec platelet disorder (isolated)	Urinary plasminogen activator	*PLAU* (duplication)/AD	Moderate TP. LTA: absent or reduced with epinephrine. FC: α-granule protein defect (P-selectin). Increased fibrinolytic activity. Often postsurgery bleeding
δ-Granule defect			
HPS types 1–11	Proteins of vesicle formation and trafficking	*HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, BLOC1S6, HPS10, BLOC1S5*/AR	Normal platelet count. LTA: reduced or absence of second wave with weak/low-dose agonists (ADP, epinephrine, collagen). FC: CD63 release defect, impaired uptake of serotonin or mepacrine. EM: δ-granule defect. Mild to moderate bleeding tendency. Syndromic features depending on the HPS type: oculocutaneous albinism, accumulation of lipofuscin-like ceroid material in cells of the phagocytic mononuclear system, immunodeficiency with neutropenia, pulmonary fibrosis, granulomatous colitis
CHS	Lysosomal-trafficking-regulator	*LYST*/AR	Normal platelet count. LTA: reduced or absence of second wave with weak/low-dose agonists (ADP, epinephrine, collagen). FC: CD63 release defect, impaired uptake of serotonin or mepacrine. EM: δ-granule defect Mild to moderate bleeding tendency. Syndromic features: oculocutaneous albinism, immunodeficiency with predisposition to recurrent infections. High probability for the development of hemophagocytic lymphohistiocytosis with an accelerated phase
Griscelli syndrome type 1–3	Myosin VA protein	Type 1: *MYO5A*/AR	Normal platelet count. δ-granule defect as in HPS and CHS. Syndromic features according to GS type: albinism, silver hair, neurological defects, lymphohistiocytosis, variable neutropenia, decreased cytotoxic function of NK cells, and T-lymphocytes
	GTPase Rab27a	Type 2: *RAB27A*/AR	
	Melanophilin	Type 3: *MLPH*/AR	

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ARC, arthrogryposis, renal dysfunction, and cholestasis; CHS, Chediak-Higashi syndrome; EM, electron microscopy; FC, flow cytometry; GPS, gray platelet syndrome; HPS, Hermansky-Pudlak syndrome; LTA, light transmission aggregometry.

Table 4 Cytoskeleton defects

Disease	Affected protein	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
MYH9-related MTP, nonsyndromic and syndromic	Non-muscle myosin type IIA (NMMHC-IIA)	*MYH9*/AD	MTP with or without basophilic neutrophilic inclusions (Döhle bodies) in blood smear. Absent to mild bleeding tendency. Strong genotype–phenotype relationship regarding syndromic features: hearing loss, kidney disease, cataracts
Filamin A defect, syndromic MTP	Filamin A (actin binding protein 280)	*FLNA*/XLR	Moderate MTP. Mild bleeding tendency. Syndromic features: nodular periventricular heterotopia; skeletal malformations, mental retardation, heart valve dystrophy, intestinal obstruction, bone dysplasia
α-Actinin 1 defect	α-Actinin 1	*ACTN1*/AD	Mild to moderate MTP. Absent to mild bleeding
β1-Tubulin defect	β1-Tubulin	*TUBB1*/AD	Platelet count: normal to mild/moderate MTP. Absent to mild bleeding tendency
Wiskott-Aldrich syndrome	WASP	Type 1: *WAS*/XLR	Moderate to severe microthrombocytopenia or TP. Severe bleeding tendency. Syndromic features: immunodeficiency, eczema, lymphoproliferative and autoimmune disorders
	WIPF1 (stabilizes WASP)	Type 2: *WIPF1*/AR	

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; MTP, macrothrombocytopenia; TP, thrombocytopenia; XLR, X-linked recessive.

Table 5 Defects of megakaryopoiesis

Disease	Affected protein	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
Defect of early megakaryopoiesis			
CAMT	TPO receptor	*MPL/AR*	Severe neonatal TP, hypomegakaryocytic. Severe bleeding tendency. Progression to generalized bone marrow aplasia
Syndromic with skeletal defects			
Radioulnar synostosis with amegakaryocytic thrombocytopenia	Onkoprotein EVI1 Homeobox A11	*MECOM*/AD *HOXA11*/AD	Amegakaryocytic TP. Bilateral radio-ulnar synostosis
TAR syndrome	RNA-binding motif protein 8A	*RBM8A/AR*	Moderate to severe neonatal TP. Severe bleeding tendency. Syndromic features: bilateral absence of radius with or without other skeletal abnormalities, potential kidney, cardiac or central nervous system anomalies
Defects of transcription factors with predisposition to myeloid malignancies			
Familial platelet disorder with associated myeloid malignancy	Runt-related transcription factor	*RUNX1*/AD	Mild to moderate neonatal TP. FC: platelet granule deficiency. Absent to moderate bleeding tendency. Risk (~40%) of acute myeloblastic leukemia or myelodysplastic syndrome
ETV6-related thrombocytopenia	ETS family transcriptional repressor	*ETV6*/AD	Mild to moderate TP. Platelets may show elongated α-granules.

(*Continued*)

Table 5 (*Continued*)

Disease	Affected protein	Gene/Mode of inheritance	Platelet count, abnormalities in platelet function, bleeding phenotype, additional features
			Absent to mild bleeding tendency. Predisposition (~25%) to acquired lymphoid, myeloid, and myeloproliferative syndromes
ANKRD26-related mild to moderate thrombocytopenia	Ankyrin repeat domain-containing protein 26	*ANKRD26*/AD	Mild to moderate neonatal TP. Absent to mild bleeding tendency. Approximately 8% of patients acquire myeloid neoplasms
Defects of transcription factors			
X-linked thrombocytopenia with or without dyserythropoietic anemia	GATA1 (zinc finger DNA-binding transcription factor)	*GATA1*/XLR	Mild to severe MTP. Platelets granule deficiency and functional defect. Mild to severe bleeding tendency. Can associate with dyserythropoiesis with or without anemia, β-thalassemia, neutropenia, splenomegaly or congenital erythropoietic porphyria; dysplastic megakaryocytes
GFI1B-related thrombocytopenia	GFI1B (transcriptional repressor important for hematopoiesis and megakaryopoiesis)	*GFI1B*/AD, AR	Mild to severe MTP. Platelets with α/δ- granule deficiency and aggregation defect. Absent to severe bleeding tendency. Red blood cells with anisopoikilocytosis, dysplastic megakaryocytes
FLI1-related bleeding disorder (type 21) Paris-Trousseau and Jacobsen syndrome	Friend leukemia integration 1 transcription factor	*FLI1*/AD, AR Del11q23 *(including FLI1)*/AD	Mild to moderate MTP. Impaired platelet granules. Absent to moderate bleeding tendency

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CAMT, congenital amegakaryocytic thrombocytopenia; MTP, macrothrombocytopenia; TAR, thrombocytopenia-absent radius; TP, thrombocytopenia; TPO, thrombopoietin; XLR, X-linked recessive.

thrombocytopenia) and autosomal dominant with sialuria. Interestingly, so far only a few children with biallelic *GNE* variants leading to isolated thrombocytopenia have been described.[39–41] Recently, we identified compound heterozygous *GNE* variants in a young girl suffering from severe congenital thrombocytopenia. We showed decreased α2,3 sialic acid and increased terminal galactose and α2,6 sialic acid moieties of the girl's platelets.[42] There is growing evidence for a mutational hot spot in the ADP/substrate domain of the GNE enzyme underlying isolated thrombocytopenia.[43] However, it has to be monitored if these young patients will develop GNE myopathy in their adult life.

Conclusion

Based on impaired platelet aggregometry, the diagnosis of IPD seems to be simple; however, it is still a major workflow necessary to further clarify the individual IPD. NGS has accelerated genetic analysis, but extensive studies are needed to further analyze platelet phenotypes (flow cytometry, immunofluorescence microscopy, electron microscopy, lectin array) and novel variants identified. Genetic analysis may help better assess the risk of developing additional features in syndromic diseases (e.g., lung fibrosis in some types of HPS) or the predisposition to myeloid malignancies (e.g., RUNX1). Finally, many IPD especially δ-granule secretion defects are still unsolved. In familial IPD, WES should be performed to extend the genetic analysis and to identify possible new genes involved in platelet physiology.

Conflict of Interest

Research funding from CSL Behring and Takeda (to B.Z.).

References

1 Bastida JM, Benito R, Lozano ML, et al. Molecular diagnosis of inherited coagulation and bleeding disorders. Semin Thromb Hemost 2019;45(07):695–707
2 Zaninetti C, Greinacher A. Diagnosis of inherited platelet disorders on a blood smear. J Clin Med 2020;9(02):539
3 Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. Haematologica 2018;103(01):148–162
4 Daly ME. Transcription factor defects causing platelet disorders. Blood Rev 2016
5 Savoia A, Balduini CL, Savino M, et al. Autosomal dominant macrothrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome. Blood 2001;97(05):1330–1335

6 Lecchi A, Femia EA, Paoletta S, et al. Inherited dysfunctional platelet $P2Y_{12}$ receptor mutations associated with bleeding disorders. Hamostaseologie 2016;36(04):279–283
7 Favier M, Bordet JC, Favier R, et al. Mutations of the integrin αIIb/β3 intracytoplasmic salt bridge cause macrothrombocytopenia and enlarged platelet α-granules. Am J Hematol 2018;93(02): 195–204
8 Akuta K, Kiyomizu K, Kashiwagi H, et al. Knock-in mice bearing constitutively active αIIb (R990W) mutation develop macrothrombocytopenia with severe platelet dysfunction. J Thromb Haemost 2020;18(02):497–509
9 Sandrock-Lang K, Oldenburg J, Wiegering V, et al. Characterisation of patients with Glanzmann thrombasthenia and identification of 17 novel mutations. Thromb Haemost 2015;113(04): 782–791
10 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 U S A 1991;88 (11):4761–4765
11 Canault M, Ghalloussi D, Grosdidier C, et al. Human CalDAG-GEFI gene (RASGRP2) mutation affects platelet function and causes severe bleeding. J Exp Med 2014;211(07):1349–1362
12 Canault M, Alessi MC. RasGRP2 structure, function and genetic variants in platelet pathophysiology. Int J Mol Sci 2020;21(03): 1075
13 Jurk K, Schulz AS, Kehrel BE, et al. Novel integrin-dependent platelet malfunction in siblings with leukocyte adhesion deficiency-III (LAD-III) caused by a point mutation in FERMT3. Thromb Haemost 2010;103(05):1053–1064
14 Stefanini L, Bergmeier W. RAP GTPases and platelet integrin signaling. Platelets 2019;30(01):41–47
15 Mory A, Feigelson SW, Yarali N, et al. Kindlin-3: a new gene involved in the pathogenesis of LAD-III. Blood 2008;112(06):2591
16 De Kock L, Freson K. The (patho)biology of SRC kinase in platelets and megakaryocytes. Medicina (Kaunas) 2020;56(12):633
17 Turro E, Greene D, Wijgaerts A, et al; BRIDGE-BPD Consortium. A dominant gain-of-function mutation in universal tyrosine kinase SRC causes thrombocytopenia, myelofibrosis, bleeding, and bone pathologies. Sci Transl Med 2016;8(328):328ra30
18 Kahr WH, Hinckley J, Li L, et al. Mutations in NBEAL2, encoding a BEACH protein, cause gray platelet syndrome. Nat Genet 2011;43 (08):738–740
19 Huizing M, Malicdan MCV, Wang JA, et al. Hermansky-Pudlak syndrome: mutation update. Hum Mutat 2020;41(03):543–580
20 Pennamen P, Le L, Tingaud-Sequeira A, et al. BLOC1S5 pathogenic variants cause a new type of Hermansky-Pudlak syndrome. Genet Med 2020;22(10):1613–1622
21 Huizing M, Helip-Wooley A, Westbroek W, Gunay-Aygun M, Gahl WA. Disorders of lysosome-related organelle biogenesis: clinical and molecular genetics. Annu Rev Genomics Hum Genet 2008; 9:359–386
22 Ammann S, Schulz A, Krägeloh-Mann I, et al. Mutations in AP3D1 associated with immunodeficiency and seizures define a new type of Hermansky-Pudlak syndrome. Blood 2016;127(08): 997–1006
23 Boeckelmann D, Wolter M, Käsmann-Kellner B, Koehler U, Schieber-Nakamura L, Zieger B. A novel likely pathogenic variant in the *BLOC1S5* gene associated with Hermansky-Pudlak syndrome type 11 and an overview of human BLOC-1 deficiencies. Cells 2021;10(10):2630
24 Boeckelmann D, Wolter M, Neubauer K, et al. Hermansky-Pudlak syndrome: identification of *novel* variants in the genes *HPS3*, *HPS5*, and *DTNBP1* (HPS-7). Front Pharmacol 2022;12:786937
25 Kaplan J, De Domenico I, Ward DM. Chediak-Higashi syndrome. Curr Opin Hematol 2008;15(01):22–29
26 Van Gele M, Dynoodt P, Lambert J. Griscelli syndrome: a model system to study vesicular trafficking. Pigment Cell Melanoma Res 2009;22(03):268–282
27 Ménasché G, Pastural E, Feldmann J, et al. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 2000;25(02):173–176
28 Thomas ER, Walker LJ, Pullaperuma S, et al. Griscelli syndrome type 1: a report of two cases and review of the literature. Clin Dysmorphol 2009;18(03):145–148
29 Fernandez-Prado R, Carriazo-Julio SM, Torra R, Ortiz A, Perez-Gomez MV. MYH9-related disease: it does exist, may be more frequent than you think and requires specific therapy. Clin Kidney J 2019;12(04):488–493
30 Kunishima S, Matsushita T, Kojima T, et al. Immunofluorescence analysis of neutrophil nonmuscle myosin heavy chain-A in MYH9 disorders: association of subcellular localization with MYH9 mutations. Lab Invest 2003;83(01):115–122
31 Hayashi Y, Harada Y, Huang G, Harada H. Myeloid neoplasms with germ line RUNX1 mutation. Int J Hematol 2017;106(02):183–188
32 Topka S, Vijai J, Walsh MF, et al. Germline ETV6 mutations confer susceptibility to acute lymphoblastic leukemia and thrombocytopenia. PLoS Genet 2015;11(06):e1005262
33 Freson K, Matthijs G, Thys C, et al. Different substitutions at residue D218 of the X-linked transcription factor GATA1 lead to altered clinical severity of macrothrombocytopenia and anemia and are associated with variable skewed X inactivation. Hum Mol Genet 2002;11(02):147–152
34 Freson K, Wijgaerts A, Van Geet C. GATA1 gene variants associated with thrombocytopenia and anemia. Platelets 2017;28(07):731–734
35 Bastida JM, Malvestiti S, Boeckelmann D, et al. A novel GATA1 variant in the C-terminal Zink finger compared with the platelet phenotype of patients with a likely pathogenic variant in the N-terminal Zink finger. Cells 2022;11(20):3223
36 Jurk K, Adenaeuer A, Sollfrank S, et al. Novel *GATA1* variant causing a bleeding phenotype associated with combined platelet α-/δ-storage pool deficiency and mild dyserythropoiesis modified by a *SLC4A1* variant. Cells 2022;11(19):3071
37 Hoffmeister KM, Falet H. Platelet clearance by the hepatic Ashwell-Morrell receptor: mechanisms and biological significance. Thromb Res 2016;141(Suppl 2, Suppl 2):S68–S72
38 Sørensen AL, Rumjantseva V, Nayeb-Hashemi S, et al. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. Blood 2009;114(08):1645–1654
39 Futterer J, Dalby A, Lowe GC, et al; UK GAPP Study Group. Mutation in *GNE* is associated with severe congenital thrombocytopenia. Blood 2018;132(17):1855–1858
40 Li X, Li Y, Lei M, et al. Congenital thrombocytopenia associated with GNE mutations in twin sisters: a case report and literature review. BMC Med Genet 2020;21(01):224
41 Revel-Vilk S, Shai E, Turro E, et al. *GNE* variants causing autosomal recessive macrothrombocytopenia without associated muscle wasting. Blood 2018;132(17):1851–1854
42 Zieger B, Boeckelmann D, Anani W, et al. Novel GNE gene variants associated with severe congenital thrombocytopenia and platelet sialylation defect. Thromb Haemost 2022;122(07):1139–1146
43 Bottega R, Marzollo A, Marinoni M, et al. GNE-related thrombocytopenia: evidence for a mutational hotspot in the ADP/substrate domain of the GNE bifunctional enzyme. Haematologica 2022;107(03):750–754