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MEGAKARYOPOIESIS AND PLATELET PRODUCTION

Genetics of inherited thrombocytopenias

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The inherited thrombocytopenia syndromes are a group of disorders characterized primarily by quantitative defects in platelet number, though with a variety demonstrating qualitative defects and/or extrahematopoietic findings. Through collaborative international efforts applying next-generation sequencing approaches, the list of genetic syndromes that cause thrombocytopenia has expanded significantly in recent years, now with over 40 genes implicated. In this review, we focus on what is known about the genetic etiology of inherited thrombocytopenia syndromes and how the field has worked to validate new genetic discoveries. We highlight the important role for the clinician in identifying a germline genetic diagnosis and strategies for identifying novel causes through research-based endeavors.

Introduction

Megakaryopoiesis and thrombopoiesis are tightly regulated components of hematopoiesis that result in the production and release of up to 10^{11} platelets daily to maintain a normal concentration of 150 to 400 $\times$ 10^9/L circulating platelets. These cells are required for adequate hemostasis through the formation of a stable clot at sites of blood vessel injury. Thrombocytopenia, traditionally defined as a platelet count of $<150 \times 10^9$/L, has many causes including immune destruction, medication-induced aplastic anemia, or as a manifestation of an inherited bone marrow failure syndrome. In this review, we focus on the diagnosis and pathogenesis of inherited thrombocytopenias, with a special emphasis on genetics. These diseases represent a growing collection of germline variant–associated thrombocytopenias whose primary manifestation is inadequate circulating platelet numbers. Many of these syndromes have extrahematopoietic manifestations, and even within the hematopoietic compartment, there is increasing evidence that genes previously thought to be platelet-restricted in their effects may actually have a broader impact on overall blood cell formation. Although many of the inherited thrombocytopenia syndromes are rare, dissecting their genetic underpinning has greatly contributed to our understanding of basic megakaryocyte and platelet biology.

Megakaryopoiesis/thrombopoiesis and the inherited thrombocytopenias

Bone marrow–resident hematopoietic stem cells (HSCs) are multipotent cells with self-renewal capacity able to generate all mature blood lineage cells in a process termed hematopoiesis. Traditional models portray a hierarchy of differentiation beginning with a bifurcation between common lymphoid progenitors and common myeloid progenitors, the latter which ultimately give rise to megakaryocyte-erythroid progenitors and subsequently megakaryocytes (MKs).[1-4] Over the last decade, this binary model has been challenged by new findings that suggest that there is a subset of HSCs that express von Willebrand factor (VWF) and have a strong MK bias and limited lymphoid potential. More importantly, the VWF^+ HSCs can give rise to VWF^- HSCs, whereas the opposite is not true, indicating that these cells are high in the hematopoietic hierarchy.[5] As MKs differentiate and mature in the bone marrow, they develop polyploidy, increase the numbers of specialized granules and their cytoplasmic volume, extend cytoplasmic extensions, and develop a complex demarcation membrane system.[6] The steps governing commitment of megakaryocyte-erythroid progenitors and MK progenitors toward final stages of MK differentiation are highly regulated. This process initially requires thrombopoietin (TPO) engaging its receptor MPL, leading to downstream JAK2-mediated signaling.[7,8] Although most steps in MK maturation involve TPO, it is not essential for final MK maturation and subsequent thrombopoiesis, the process by which proplatelets and ultimately platelets are formed.[9] Several transcription factors have been identified as crucial in megakaryopoiesis/thrombopoiesis. Additionally, the formation of proplatelets at the demarcation membrane system occurs only after mature megakaryocytes migrate to and extend protrusions through the vascular sinusoidal space. Under sheer stress from vascular blood flow, platelets are released.[6,10] This migration and the dynamic, reversible growth and extension of proplatelet processes requires extensive cytoskeletal reorganization[11] and dynamic remodeling, which involves a variety of molecules including CDC42, PAK2, ADF/COFILIN, β1-tubulin, WASP, and many others.[12] The average platelet lifespan is ~7 to 10 days[13] with peripheral clearance in part mediated by programmed anuclear cell death and loss of sialic acid with subsequent clearance by Kupffer cells in liver sinusoids.[14-16] Therefore, variants in genes involved in every step of these complex processes can cause inherited thrombocytopenia (Figure 1).

The inherited thrombocytopenias are an expanding group of disorders[17-20] characterized by familial thrombocytopenia and bleeding tendency of various severity with either small, normal, or large sized platelets (Table 1). In some disorders, the defect may be quantitative only, whereas in others, there may be qualitative/functional defects as well. Patients may be identified in

the newborn period with easy bruising, petechiae, mucosal bleeding, and thrombocytopenia. However, this constellation of symptoms can also be observed in the much more common allo- and autoimmune thrombocytopenia disorders and in infection- or drug-induced thrombocytopenia. Some of the more common inherited thrombocytopenias are associated with enlarged platelet size (macrothrombocytopenia) or notably small platelet size (microthrombocytopenia); however, as the list of inherited thrombocytopenias expands, so too does the recognition of disorders with platelets that would appear normal in size and morphology on routine peripheral blood smear. Obvious family history of thrombocytopenia should prompt the clinician to consider an underlying genetic etiology, though de novo variants, especially in autosomal dominant syndromes, and the lack of symptomatic relatives as often occurs in recessive disorders means that an absence of family history should not exclude consideration of these entities. Finally, persistent thrombocytopenia, extrahematopoietic abnormalities, bleeding or bruising out of proportion to the degree of thrombocytopenia, refractoriness to medical treatments typically used in immune-mediated thrombocytopenia, and nonresponse to splenectomy should prompt evaluation for an underlying genetic cause.

Diagnostic evaluations

The need to correctly identify patients with inherited thrombocytopenias is pressing. First, many of these patients have extrahematopoietic phenotypes that themselves may require additional medical treatment. Second, identification can help guide proper management. In some individuals, their inherited thrombocytopenia may be mistaken for immune thrombocytopenia, prompting unnecessary splenectomy. Not only will the patient experience persistent thrombocytopenia but would now be at risk for postoperative complications including life-long increased risk for infection. Additional clinical implications are the ability to provide perioperative guidance for necessary surgeries, including the use of antifibrinolytics, and understanding the role and timing of HSC transplant or gene therapy. Although most of the inherited thrombocytopenias do not have a specific treatment, the importance of a precise diagnosis in some of them is critical. For example, the diagnosis of congenital amegakaryocytic thrombocytopenia due to deleterious variants in *MPL* or *THPO* will prompt referral for a bone marrow transplant as the only curative option for these patients. Finally, some inherited thrombocytopenia syndromes are associated with an increased risk of hematopoietic malignancy. Although prospective data validating the ability of screening to impact hematopoietic malignancy outcomes in these populations are lacking, a germline diagnosis can help clinicians provide genetic counseling regarding personal risk and family planning.

Diagnostic evaluation of the thrombocytopenic patient with suspected inherited disorder should begin with a thorough history and physical examination. Key history elements include duration of thrombocytopenia, response to previous therapies, other medical history (especially of hearing or vision abnormalities and kidney, heart, or neurologic disease), review of growth curves, and family history including of hematopoietic malignancy. Physical exam should include thorough review of systems involved in specific syndromes including careful examination of the skin and musculoskeletal, cardiac, and neurologic systems (Figure 2). Initial laboratory evaluation starts with a complete blood count using an electronic counter to calculate platelet count and size and to determine red cell indices. However, caution must be used in patients with platelet macrocytosis as cell type can be incorrectly assigned, leading to inaccurate estimate of both platelet number and volume.[21] The peripheral smear should be reviewed under routine light microscopy by an experienced hematologist. Special attention should be paid to size, shape, number, and granule appearance. Review should not be limited to the platelet compartment as several inherited thrombocytopenias may demonstrate abnormalities in other blood cell types. For example, in X-linked thrombocytopenia with thalassemia due to variants in *GATA1*, patients can have microcytosis and reticulocytosis evidenced by polychromasia and anisocytosis.[22] Giant platelets, those larger than the size of a normocytic red blood cell, can be observed in Bernard-Soulier syndrome[23] and *MYH9*-related disease, the latter of which can also display leukocyte cytoplasmic inclusions termed Döhle-like body inclusions.[24] A paucity of α granules, as can be observed in gray platelet syndrome,[25-27] *GFI1b*-related thrombocytopenia,[28,29] or *SRC*-related thrombocytopenia,[30,31] can give platelets a "pale" appearance in addition to platelet macrocytosis. In *FLI1*-associated thrombocytopenia[32-34] or thrombocytopenia caused by deletions in 11q23,[35,36] some patients' platelets demonstrate a single, condensed-appearing granule. Small platelets may be observed in Wiskott-Aldrich syndrome, X-linked thrombocytopenia, *ARPC1B*-related thrombocytopenia, or *FYB*-related thrombocytopenia. Platelet aggregation can further suggest specific etiologies, for example demonstrating an increased response to ristocetin in platelet-type von Willebrand disease; however, there can be heterogeneity in platelet aggregation findings, and these studies may be most useful to support the suspicion of an inherited thrombocytopenia over a diagnosis of ITP.[37] Additional functional assays may have more limited availability given requirements for specialized laboratory testing. This includes platelet glycoprotein expression by flow cytometry, which is mostly used to confirm the diagnosis of Bernard-Soulier syndrome in patients with macrothrombocytopenia and Glanzmann thrombasthenia in patients with absent aggregation and normal platelet count, and whole-mount transmission electron microscopy (TEM) to evaluate dense granule deficiency, as well as thin section TEM for α granule evaluation and other platelet structural abnormalities. Although whole-mount TEM usually confirms the diagnosis of Hermansky-Pudlak syndrome and other dense granule deficiencies,[38] thin section TEM provides useful information for the diagnosis of *NBEAL2*-, *GFI1B*-, *FLI1*-, and *STIM1*-related thrombocytopenias.

Some specific extrahematopoietic findings may also allow the clinician to further narrow the diagnostic possibilities. For example, several inherited thrombocytopenia–associated genes comprise a syndrome that includes radioulnar dysostosis (Table 1). Interestingly, this includes many transcription factors important for megakaryopoiesis including *FLI1*, *HOXA11*, *MECOM*, and *RBM8A*. A family history of hematopoietic malignancy should prompt consideration of thrombocytopenia related to variants in *RUNX1*, *ETV6*, or *ANKRD26*. Sensorineural hearing loss is a feature of *MYH9*-, *DIAPH1*-, and *CDC42*-associated thrombocytopenia, all of which are implicated in cytoskeletal function. Interestingly, germline variants in several genes have been implicated in promoting increased platelet clearance including *FYB*, *GP1BA*-gain-of-function, *STIM1*, *GNE*, and *WAS*. Indeed, splenectomy has been shown to improve platelet counts in patients

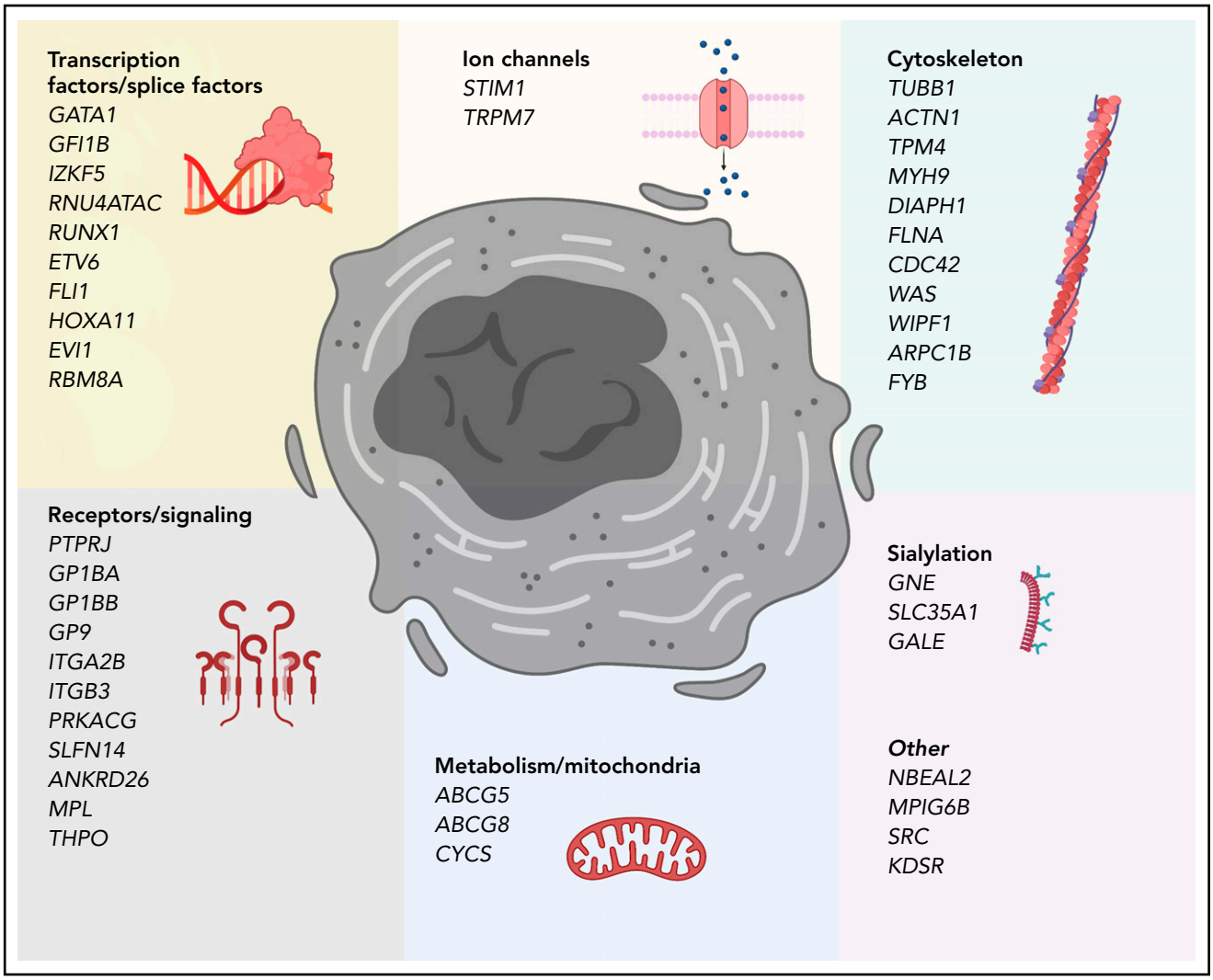

Figure 1. Genes that cause inherited thrombocytopenias grouped by established and potential cellular mechanisms involved in megakaryocyte biology. These genes also correspond to the tier 1 and tier 2 gene lists curated by the International Society of Thrombosis and Hemostasis Genomics in Hemostasis Subcommittee.

with Wiskott-Aldrich syndrome presumably by eliminating the main site for platelet clearance.[39] Lastly, patients with 22q11 deletion syndrome may experience thrombocytopenia of varying severity together with other canonical features such as facial dysmorphism, hypocalcemia, athymia, congenital heart disease, and recurrent infections.[40,41] Although the deletion typically encompasses the critical platelet signaling receptor *GP1BB*, the role of hemizygosity at this region for thrombocytopenia and bleeding has recently been called into question.[42] Furthermore, immune cytopenias responsive to immunomodulation have been previously documented in this patient population,[43,44] confounding the ontogeny of their platelet phenotypes.

In a recent excellent review, Pecci and Balduini provide relative frequencies of the genetic etiology behind identifiable inherited thrombocytopenias in a large 335-family collection from Italy.[45] First, they note that in their estimation, ~50% of patients with high suspicion for an inherited thrombocytopenia will not have a known, identifiable underlying cause. Of those families where a more precise genetic diagnosis is made, the combination of features discussed above plus medical history and physical examination could provide high diagnostic support for an inherited thrombocytopenia in over half of patients. However, ultimately, the majority of pathogenic genetic variants may present as isolated thrombocytopenia (Table 1).

Genetic diagnosis, cost, and ethical considerations

Since the application of next-generation sequencing (NGS) to patients with inherited thrombocytopenias, there has been an explosion of newly identified causative genes. Indeed, in the 5-year period from 2015-2019, at least 20 distinct genetic entities causing inherited thrombocytopenia were identified. Several recently identified thrombocytopenia genes may have normal sized platelets, no distinctive morphology on review of peripheral smear, and are not associated with other hematopoietic or extrahematopoietic organ system involvement such as with *IZKF5*-,[46] *ETV6*-,[47-50] and *THPO*-related thrombocytopenia. These examples highlight how NGS can provide diagnostic clarity that may change management, for example advising on and screening for malignancy risk in *ETV6*, *RUNX1*, and *ANKRD26* patients and their families. Indeed, NGS is now being used in

Table 1. Comprehensive list of inherited thrombocytopenias

Mechanism	Gene	Inheritance	Syndrome	Platelet size	Bleeding	Extrahematopoietic manifestations	Reference
Transcription/ splice factors	*GATA1*	X-linked	*GATA1*-associated thrombocytopenia	Large or normal	Severe	Dyserythropoietic anemiaImbalanced globin chain synthesis with normal red cell morphology, splenomegaly	71,72 64,102
	GFI1B	AR/AD	*GFI1B*-related thrombocytopenia	Large	Severe	—	28,29
	IZKF5	AD	*IZKF5*-related thrombocytopenia	Normal		—	46
	RNU4ATAC	AR	Roifman syndrome	Normal		Growth retardation, skeletal dysplasia, intellectual delay, hypogammaglobulinemia, dysmorphic facial features, retinal dystrophy. Decreased B-cell numbers	97,103
	RUNX1	AD	FPD-PMM	Small, normal, or slightly enlarged		Predisposition to leukemia	104–107
	ETV6	AD	—	Normal	Moderate	Predisposition to leukemia	47–50,108
	FLI1/ del(11q23)	AR/AD	Paris-Trousseau, Jacobsen	Large		Abnormal facial features, cardiac abnormalities, intellectual disability, skin abnormalities.*	32–36
	HOXA11	AD	Radioulnar dysostosis with amegakaryoctic thrombocytopenia	Normal	Severe	Radio-ulnar dysostosis	109
	MECOM (EVI1)	AD	Radioulnar dysostosis with amegakaryoctic thrombocytopenia	Normal	—	Radio-ulnar dysostosis, some patients with sensorineural hearing loss, intellectual disability. Some patients without radio-ulnar dysostosis	110–112
	RBM8A/ del(1q21.1)	AR	TAR	Normal	—	Skeletal abnormalities (absence of radii, up to absence of upper limbs, can have lower-limb defects)	113,114
Cytoskeletal	*TUBB1*	AD	*TUBB1*-related thrombocytopenia	Large	—	—	115
	ACTN1	AD	*ACTN1*-related thrombocytopenia	Large	—	—	116
	TPM4	AD	*TPM4*-related thrombocytopenia	Large	—	—	117
	MYH9	AD	*MYH9*-Related disease	Giant	—	Sensorineural hearing loss, kidney disease, cataracts	24
	DIAPH1	AD	*DIAPH1*-related thrombocytopenia	Large	—	Sensorineural hearing loss	118,119
	FLNA	X-linked	*FLNA*-related thrombocytopenia	Large	—	Variable brain white matter changes, skeletal dysplasia, intellectual disability	120,121

bBSS, biallelic Bernard-Soulier syndrome; CAMT, congenital amegakaryocytic thrombocytopenia; CARST, congenital autosomal recessive small-platelet thrombocytopenia; FPD-PMM, familial platelet disorder with propensity for myeloid malignancy; GPS, gray platelet syndrome; mBSS, monoallelic Bernard-Soulier syndrome; N/A, information not available; PTVWD, platelet-type von Willebrand disease; TAR, thrombocytopenia absent radii; TKS, Takenouchi-Kosaki syndrome; WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia; XLTT, X-linked thrombocytopenia with thalassemia.

**FLI1* homozygous missense variant causes thrombocytopenia without the extrahematopoietic features of 11q23 deletion syndrome.[33]

†Variants distinct from those causing classic or variant Glanzmann thrombasthenia.

Table 1. (continued)

Mechanism	Gene	Inheritance	Syndrome	Platelet size	Bleeding	Extrahematopoietic manifestations	Reference
	CDC42	AD	TKS	Large	—	Cardiac defect, developmental delay, dysmorphic facial features, sensorineural hearing loss	122–124
	WAS	X-linked	WAS	Small	—	Immunodeficiency, recurrent infections, eczema	125,126
	WIPF1	AR	WAS-like	Normal	—	Immunodeficiency, recurrent infections, eczema	127
	ARPC1B	AR	ARCP1B-related thrombocytopenia	Small	—	Poor growth, eosinophilia/inflammatory disease, small vessel vasculitis	100
	FYB	AR	CARST	Small	—	Eczema	128
Signaling	*PTPRJ*	AR	*PTPRJ*-related thrombocytopenia	Small	—	—	84
	GP1BA	AR/AD	bBSS/mBSS/PTvWD	Giant/large/normal	—	—	129–132
	GP1BB	AR/AD	bBSS/mBSS	Giant/large	—	—	133,134
	GP9	AR	BSS	Giant	—	—	135
	ITGA2B†	AD	*ITGA2B*-related thrombocytopenia	Large	—	—	136,137
	ITGB3†	AD	*ITGB3*-related thrombocytopenia	Large	—	—	138–140
	PRKACG	AR	*PRKACG*-related thrombocytopenia	Large	Severe	—	141
	SLFN14	AD	*SLFN14*-related thrombocytopenia	Large	Severe	—	142
	ANKRD26	AD	*ANKRD26-related thrombocytopenia*	Normal	—	Predisposition to leukemia	143,144
	MPL	AR	CAMT	Normal	—	Progressive bone marrow failure (pancytopenia)	145–147
	THPO	AD	*THPO*-related thrombocytopenia	Normal/slightly enlarged	—	—	148
Ion channel	*STIM1*	AD	Stormorken syndrome	N/A	—	Congenital miosis, tubular myopathy with proximal muscle weakness, ichthyosis	149
	TRPM7	AD	*TRPM7*-related thrombocytopenia	Large	—	—	150
Metabolism/ mitochondrial	*ABCG5*	AR	Sisterolemia	Large	—	Elevated sterols, xanthomas, splenomegaly, stomatocytosis with hemolytic anemia	151–153
	ABCG8	AR	Sisterolemia	Large	—	—	151,153
	CYCS	AD	*CYSC*-related thrombocytopenia	Normal/small	—	—	154

bBSS, biallelic Bernard-Soulier syndrome; CAMT, congenital amegakaryocytic thrombocytopenia; CARST, congenital autosomal recessive small-platelet thrombocytopenia; FPD-PMM, familial platelet disorder with propensity for myeloid malignancy; GPS, gray platelet syndrome; mBSS, monoallelic Bernard-Soulier syndrome; N/A, information not available; PTVWD, platelet-type von Willebrand disease; TAR, thrombocytopenia absent radii; TKS, Takenouchi-Kosaki syndrome; WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia; XLTT, X-linked thrombocytopenia with thalassemia.

**FLI1* homozygous missense variant causes thrombocytopenia without the extrahematopoietic features of 11q23 deletion syndrome.[33]

†Variants distinct from those causing classic or variant Glanzmann thrombasthenia.

Table 1. (continued)

Mechanism	Gene	Inheritance	Syndrome	Platelet size	Bleeding	Extrahematopoietic manifestations	Reference
Sialylation	*GNE*	AR	*GNE*-related thrombocytopenia	Large	—	—	155,156
	SLC35A1	AR	*SLC35A1*-related thrombocytopenia	Large	—	—	157
	GALE	AR	*GALE*-related thrombocytopenia	Large	Severe	Mild anemia or febrile neutropenia present in some	158
Other	*NBEAL2*	AR	GPS	Large	—	Myelofibrosis	25–27
	MPIG6B	AR	Congenital macrothrombocytopenia with focal myelofibrosis	Large	—	Myelofibrosis, anemia	159
	SRC	AD	*SRC*-related thrombocytopenia	Large	—	Myelofibrosis, splenomegaly, edentulism, osteoporosis, dysmorphic facial features	30,31
	KDSR	AR	*KDSR*-related thrombocytopenia	Large	—	Keratoderma, ichthyosis	160,161

bBSS, biallelic Bernard-Soulier syndrome; CAMT, congenital amegakaryocytic thrombocytopenia; CARST, congenital autosomal recessive small-platelet thrombocytopenia; FPD-PMM, familial platelet disorder with propensity for myeloid malignancy; GPS, gray platelet syndrome; mBSS, monoallelic Bernard-Soulier syndrome; N/A, information not available; PTWD, platelet-type von Willebrand disease; TAR, thrombocytopenia absent radii; TKS, Takenouchi-Kosaki syndrome; WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia; XLTT, X-linked thrombocytopenia with thalassemia.

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many centers as an up-front component in the evaluation of inherited thrombocytopenias. In the United States, several NGS-targeted panels are available (including Versiti, 23-gene panel; Prevention Genetics, 30-gene panel; Blueprint Genetics, 37-gene panel; and several academic hospital–based clinical laboratories). Of note, when ordering panel-based testing, it is important to ensure that known noncoding pathogenic variants are covered, for example 5′ UTR variants in *ANKRD26*. Additionally, although NGS can detect small deletions, larger structural variants are missed; therefore, if those are suspected, other techniques such as Multiplex ligation-dependent probe amplification or array comparative genomic hybridization should be used. It is also important to mention that most commercial panels use software-automated reporting, therefore potentially missing small insertions and deletions. As highlighted in Table 1, none of these targeted panels incorporate all known causes of inherited thrombocytopenia, creating an inherent risk for false reassurance in the setting of "negative" genetic testing. Furthermore, it has been shown that the diagnostic yield of these genetic panels is much lower than expected.[51] Recently, Downes et al showed that in a large cohort of well phenotyped patients with disorders of bleeding and thrombosis, the diagnostic rate for patients with thrombocytopenia or a known disorder of platelet function was almost 50% and 26%, respectively, findings that were supported in another report by Bastida et al, underscoring the importance of adequate clinical and laboratory phenotyping.[52]

The genes that populate these panels are selected based on previous discoveries; however, as more patients are sequenced, new variants will be reported for whom it is difficult to assign pathogenicity. Indeed, there is a roughly linear relationship between the number of genes on an NGS-based panel and the number of variants of uncertain significance reported per patient.[53] And despite guidelines for variant classification outlined in the critical American College of Medical Genetics/Association for Molecular Pathology standards,[54] there are disease contexts for which rarity, benign variation, phenotypic variability, and other potential confounders have generated interlaboratory discordance in variant interpretation. In these contexts, including in the familial platelet disorders, there has been a clear need for expert review of variants. The goal is that systematic curation of these variants can allow for the determination of strong variant-disease associations. Recently, 2 international expert panels, the ClinGen Platelet Gene Curation Expert Panel[55] and the International Society on Thrombosis and Haemostasis Subcommittee on Genomics in Thrombosis and Hemostasis,[56] reported their initial curation efforts with the ultimate goal of sharing the information publicly and in real time for use by clinicians that work with patients with inherited disorders of hemostasis including inherited thrombocytopenias.

One challenge in the United States is the highly variable access to insurance coverage for NGS-based testing. For example, the Center for Medicare and Medicaid Service supports NGS testing nationally only for the indications of assessing germline breast and ovarian cancer risk.[57] Other germline indications are treated as "nationally noncovered." Current estimates are that 80% of insured individuals have coverage for targeted sequencing panels, which drops to only 56% for Medicaid enrollees.[58] Several aspects of genetic testing for inherited thrombocytopenia syndromes raise ethical questions beyond cost. For patients without

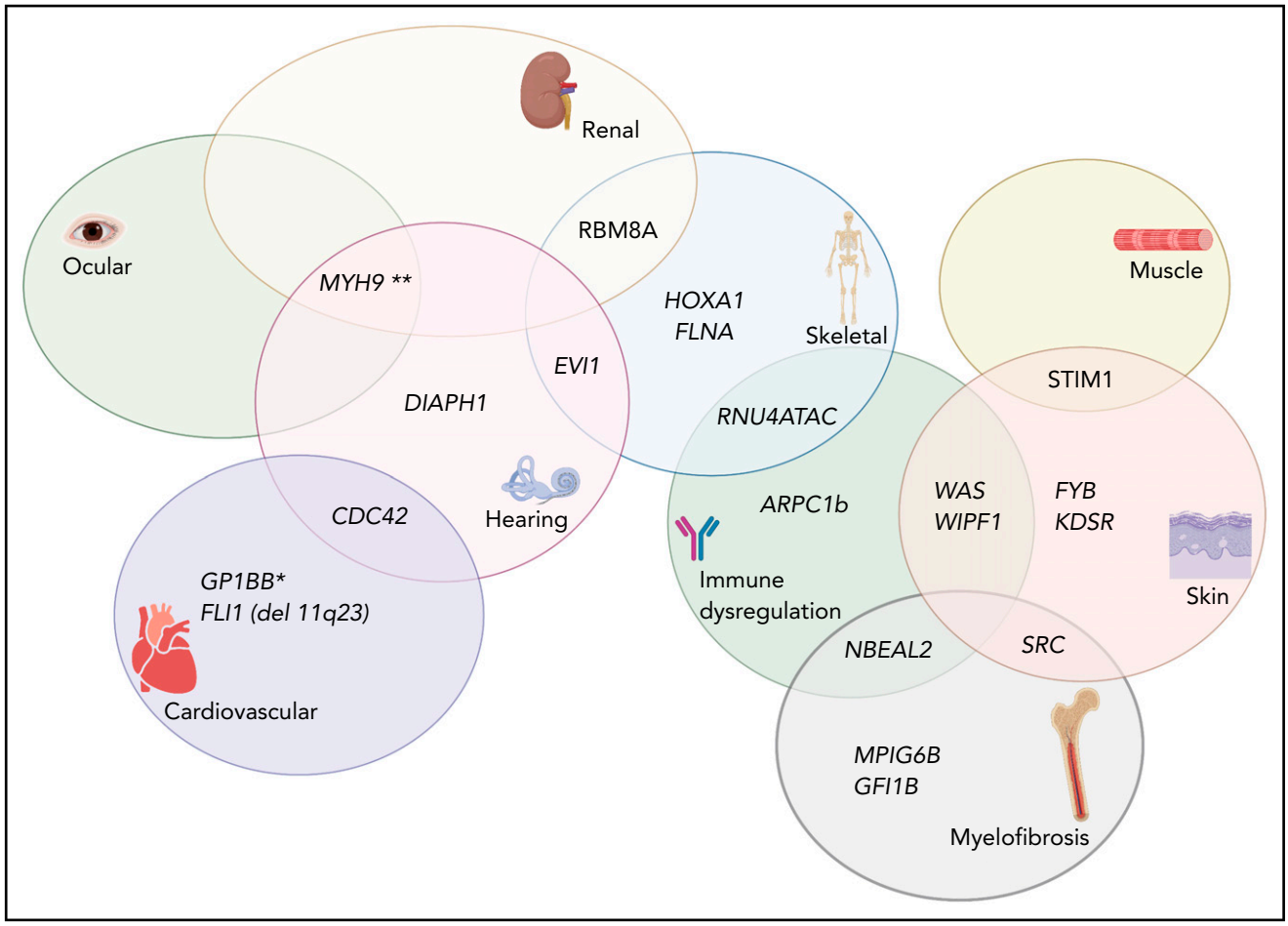

Figure 2. Complex Venn diagram showing the extrahematopoietic manifestations of select germline inherited thrombocytopenia syndromes. *The role of GP1BB in the thrombocytopenia associated with 22q11 del has been recently disputed (see Zwifelhofer et al[42]).

a family history of hematopoietic malignancy, it is crucial to explain the potential to identify variants in leukemia-associated genes such as *RUNX1*, *ETV6*, or *ANKRD26*. Understanding the purpose of testing for a minor is also critically important: does the testing serve a critical diagnostic function and therefore can be performed after acquiring informed consent, or does the testing serve a predictive purpose that will not change clinical management during the pediatric age range and therefore should be deferred?[59] Finally, clinicians should be experienced in the interpretation and counseling of results, including variants of uncertain significant or variants with implications for disease carrier status. For a further review of this topic, please see the recent guideline for ethical considerations of genetic testing in inherited platelet disorders.[60]

Variant interpretation

Exome sequencing has played a major role in the identification of pathogenic variants in inherited thrombocytopenias. However, as mentioned before, variant interpretation, especially for novel variants, requires careful curation and, if available, access to additional family members to facilitate the correct diagnosis. Additionally, insurance coverage for exome sequencing is estimated at only 63% of insured persons, dropping to 39% for patients with Medicaid. Although research-based exome or whole-genome sequencing (WGS) has led to an explosion in newly identified variants, there are several important considerations that can guide clinical and discovery-based interpretation including phenotyping and functional validation of variants.

Consider the discovery of *GATA1* variants and their role in thrombocytopenia syndromes (Table 2). One of 6 members of the GATA transcription factor family, *GATA1* contains 2 N-terminal transcriptional activation domains and C-terminal zinc fingers responsible for binding its consensus (A/T)GATA(A/G) sequence (reviewed in Crispino et al[61]). Although constitutive loss of *GATA1* in mice is embryonic lethal,[62] an inducible model highlighted its requirement for normal and stress erythropoiesis and demonstrated profound thrombocytopenia.[63] Germline variants in *GATA1* are linked to a variety of human diseases with aberrant hematopoiesis. An X-linked form of thrombocytopenia with globin chain imbalance resembling β-thalassemia was first described in 1977,[22] with the causative pathogenic variant confirmed over 25 years later. This study linked the original pedigree to a pathogenic GATA1 change p.R216Q impacting the ability of GATA1 transcription factor to bind DNA.[64] These patients demonstrate bone marrow dyserythropoiesis but only mild anemia. Although a second pedigree with the same variant phenocopied the original mild thrombocytopenia, β-thalassemia–like imbalance in globin synthesis, and dyserythropoiesis,[65] this latter study also commented on a severe

Table 2. Phenotypic variability in *GATA1*-related thrombocytopenia syndromes

Mechanism	Variant	Thrombocytopenia	Dyserythropoiesis/ erythroid hypoplasia	Anemia	Congenital porphyria	Globin synthesis imbalance	Neutropenia	Ref
Impaired binding to FOG1 cofactor	p.V205L	Severe	Dyserythropoiesis	Transfusion dependent	NR	NR	Absent	69
	p.V205M	Severe	Dyserythropoiesis	Severe (fetal hydrops), anemia improved over time	NR	NR	NR	72
	p.G208S	Severe	Dyserythropoiesis	Absent	NR	NR	Absent	71
	p.G208R	Severe	Dyserythropoiesis	Transfusion dependent, 1 proband improved with time	NR	NR	Absent	68,70
	p.D218Y	Severe	Dyserythropoiesis	Transfusion dependent	NR	NR	NR	73
	p.D218N	Moderate	Absent	Absent	NR	NR	Absent	75
	p.D218G	Severe	Absent	Absent	NR	NR	Absent	74
Impaired binding to DNA	p.R216Q*	Mild-moderate	Dyserythropoiesis	Mild	NR	Present	NR	22,64–66
	p.R216W	Moderate	Dyserythropoiesis	Mild	Present	Present	Absent	67
N-TAD truncation variants‡	c.220G>C†	Moderate	Diagnosed with Diamond-Blackfan anemia: bone marrow erythroid hypoplasia, no other BM abnormalities	Transfusion dependent, robust but transient response to corticosteroids; low reticulocyte counts, modest increase in HgbF	NR	NR	Moderate	80
Retained intron C-ZF	c.7230 C>T	Intermittent mild-moderate macrothrom-bocytopenia	Dyserythropoiesis	Severe but improved with time	NR	NR	Absent	76

N-TAD, N-terminal transactivation domain; C-ZF, C-terminal zinc finger; NR, not reported.

*Tubman et al[66] refer to the syndrome as "X-linked gray platelet syndrome."

†Two additional studies report patients with the c.220G>C variant as having a phenotype of dysertyrhopoietic anemia, but without thrombocytopenia.[77,78]

‡The c.2T>C,[79,81] c.-21A>G,[162] and c.220delG[80] variants also lead to N-TAD truncation variants; patients have DBA-like phenotype but do not have thrombocytopenia.

reduction of α-granules as detected on electron microscopy, a finding confirmed in an additional pedigree whose authors elected to term the syndrome "X-linked gray platelet syndrome."[66] Interestingly, a variant affecting the same residue but with a different substitution, p.R216W, has overlapping clinical features with the additional finding of congenital erythropoietic porphyria characterized by cutaneous photosensitivity, hirsutism, and red urine.[67] Similarly, although most variants that impact the ability of GATA1 to bind its cofactor *FOG1* are characterized by thrombocytopenia with dyserythropoiesis and transfusion-dependent anemia[68-72] including the variant p.D218Y,[73] overlapping variants p.D218N and p.D218G patients have thrombocytopenia alone without erythrocyte abnormalities.[74,75] Truncating *GATA1* variants caused by splice site alterations lead to exon 2 skipping and production of an expressed protein that lacks the N-terminal transactivating domain. These patients present with dyserythropoietic, steroid-responsive anemia with clinical features overlapping Diamond-Blackfan anemia (DBA), though only 2 of 5 pedigrees demonstrate elevated erythrocyte adenosine deaminase.[76-80] Interestingly, although the various exon 2 bordering splice variants were shown to produce the same short form of GATA1, only 1 pedigree was affected by thrombocytopenia,[80] whereas 2 separate families with the identical variant did not.[77,78] Across several families, confirmed heterozygous *GATA1* females were either asymptomatic or demonstrated mild thrombocytopenia and imbalanced globin chain synthesis reflecting skewed X-inactivation.

This example highlights the challenges that phenotypic variability can pose to the clinician and researcher. However, understanding the phenotypic overlap with DBA has led to further mechanistic insight into DBA pathogenesis. In an elegant study from the Sankaran laboratory, primary hematopoietic cells from patients with DBA and variants in the classic DBA gene *RPS19* were shown through polysome profiling to have a specific decrease in *GATA1* messenger RNA translation and a decreased amplitude in the *GATA1* target gene transcriptional signature.[81] Ineffective erythropoiesis in cultured primary cells could be partially rescued by *GATA1* overexpression, suggesting potential therapeutic implications.

Platelet phenotype variability as well as incomplete penetrance can also confound novel variant identification. Whole-exome or genome-based NGS strategies to identify novel, rare pathogenic variants can derive increased power by incorporating multiple family members, both affected and unaffected. Especially in families with an apparent autosomal dominant transmission pattern, filtering out variants that are absent from unaffected individuals can be an effective step to narrow variants of interest. Adding to this complexity, in some *GATA1* pedigrees, affected individuals can have improvement in their thrombocytopenia with age. Thrombocytopenia can be mild and potentially unrecognized in some family members, for example as seen across several large pedigrees of *RUNX1*-mutated familial platelet disorder. In the recently described syndrome of *IKZF5*-related thrombocytopenia,[46] rare missense variants from WGS of 105 thrombocytopenic patients were identified through a Bayesian inference framework using over 10 000 unaffected individuals. However, in one pedigree with 6 family members that ultimately underwent analysis for the variant in question, 1 family member carried the proposed pathogenic p.G134E yet had a normal platelet count of 184. The variant otherwise segregated with disease, which was characterized by a mild thrombocytopenia with normal platelet size and mild bleeding symptoms. This is one example of how in whole-exome sequencing/WGS studies of large pedigrees, mildly affected individuals may be phenotyped as "unaffected" and the variant filtered out as not segregating with disease. This emphasizes the need for complete phenotyping on individuals both affected and unaffected, with attention to not only absolute platelet number but also morphology and, when available, functional testing.

Validation of variants

Validation of potential novel variants should be accompanied by functional testing when possible. For decades, investigators have used molecular biology, cell biology and imaging and biochemistry techniques to successfully validate specific genetic variants in megakaryocytes, platelets, and other cell models, including western blots for protein detection, confocal microscopy for cellular defects associated with cytoskeleton genes, and reporter assays for transcription factors. Additionally, animal models of inherited thrombocytopenias have been generated over the years with mixed results. For example, whereas mouse knock-out models for *Myh9*, *Nbeal2*, and *Gp1bb* appear to replicate the phenotype observed in humans, others such as *Runx1*, *Was*, and *Mpl* only replicate certain features of the human disease[82,83] but not all. More recently zebrafish models have been successfully used to validate variants in *PTPRJ*[84] and SRC[31] and to establish a model of congenital amegakaryocytic thrombocytopenia (*MPL*).[85] Optimally, primary bone marrow hematopoietic tissue from affected patients and normal controls could be compared with assess markers of megakaryocyte maturation and morphology, granule production, and platelet function using platelet aggregation studies, flow cytometry, and other functional methods. However, it is also increasingly recognized that in vitro culture of megakaryocytes has limitations, although some new technologies have allowed investigators to culture megakaryocytes and study their function from minimal volumes of bone marrow aspirates.[86]

Recently, investigators were able to use induced pluripotent stem cells from patients with *ETV6* and *RUNX1* variants, as well as introducing the same gene variants by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 in an isogenic induced pluripotent stem cell line and studied their effect on hematopoietic progenitor and megakaryocyte maturation.[87] In the absence of primary patient samples, current technology also allows for the in vitro manipulation of hematopoietic stem and progenitor cells either through overexpression of variant alleles or through genetic deletion via CRISPR/CRISPR-associated protein 9. These cells can be differentiated with TPO along the megakaryocytic pathway to form proplatelet-forming megakaryocyte-like cells, allowing investigators to study the effect of these genetic defects on megakaryocyte differentiation and maturation.[88]

Structural modeling can be useful in predicting the impacts of variants on protein function; however, limitations exist, including the availability of a 3-dimension model and translation to functional impact. For example, a recent study looking at the impact of a large number of *ETV6* variants identified through sequencing of an acute lymphoblastic leukemia cohort developed a reporter system of transcriptional repressor activity.[89] Variants

predicted to be pathogenic and those that emerged from studies of individual thrombocytopenic/familial leukemia pedigrees do not strictly occur in the region at the *ETV6*-DNA interface. Emerging technologies such as base editor screens will allow for more rapid variant screening in relevant cellular models.[90] It is important to mention that although exome and short-read genome sequencing may allow for discovery of novel missense or small insertion/deletion variants, other genomic technologies are required to detect larger copy number or structural alterations. For example, long-read genomic sequencing facilitated the recent discovery of a structural variant causing a paired-duplication inversion leading to pathogenic gain-of-function *WAC-ANKRD26* fusion.[91] These variants will not be discernable by currently available clinical testing but may also be overlooked by most research-based sequencing efforts. As costs for long-read genome sequencing and additional advanced genomic techniques come down, the availability of these newer platforms will likely facilitate additional complex structural genomic abnormalities associated with inherited thrombocytopenia syndromes.

Lastly, there is an increasing appreciation for the effects of genes initially identified as being most important for megakaryopoiesis or thrombopoiesis on nonplatelet hematopoietic cells. A clear example is TPO, originally described as a cytokine that stimulates megakaryopoiesis[92,93] but later identified as playing an important role in HSC maintenance,[94] especially in promoting HSC quiescence.[95,96] Patients with Roifman syndrome due to biallelic germline variants in *RNU4ATAC* also display abnormal differentiation of B cells with associated hypogammaglobulinemia and recurrent viral infections.[97] Beyond factors implicated in differentiation, factors associated with platelet differentiation and granule formation such as *NBEAL2* have also been shown to impact mast cell differentiation and granule generation[98] as well as demonstrating clinical phenotype consistent with broader immune dysregulation.[99] This immune dysregulation also occurs in patients with variants in actin cytoskeletal organization genes (Table 1), for example patients with germline variants in *ARPC1B* who demonstrate megakaryocyte differentiation defects[100] but also disruption of T-cell lineage development.[101] As additional genes are added to the growing list of thrombocytopenia disorders, careful phenotyping will allow researchers to extend this new knowledge to other hematopoietic compartments.

Conclusions

Over the last 2 decades, the advancement in genomic techniques and decreased cost of sequencing have allowed for the elucidation of the genetic basis of many inherited thrombocytopenias. For most of these newly discovered genes, this was the result of international collaborative efforts that included clinicians, scientists, and statistical geneticists, underscoring the importance of collaboration and team science. Although the sequencing of patients and families that led to these discoveries paved the way for better understanding of megakaryocyte and platelet biology, challenges remain on the validation of genetic variants in available models. Ultimately, this "bedside to bench" approach that moved the field forward will hopefully make its way back to better inform patients about their disease.

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Footnote

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REFERENCES

1. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*. 2000;404(6774):193-197.
2. Nakorn TN, Miyamoto T, Weissman IL. Characterization of mouse clonogenic megakaryocyte progenitors. *Proc Natl Acad Sci USA*. 2003;100(1):205-210.
3. Nishikii H, Kanazawa Y, Umemoto T, et al. Unipotent megakaryopoietic pathway bridging hematopoietic stem cells and mature megakaryocytes. *Stem Cells*. 2015;33(7):2196-2207.
4. Pronk CJ, Rossi DJ, Månsson R, et al. Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell*. 2007;1(4):428-442.
5. Sanjuan-Pla A, Macaulay IC, Jensen CT, et al. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature*. 2013;502(7470):232-236.
6. Machlus KR, Italiano JE Jr. The incredible journey: from megakaryocyte development to platelet formation. *J Cell Biol*. 2013;201(6):785-796.
7. Kaushansky K. Thrombopoietin. *N Engl J Med*. 1998;339(11):746-754.
8. Kaushansky K. Lineage-specific hematopoietic growth factors. *N Engl J Med*. 2006;354(19):2034-2045.
9. Nishimura S, Nagasaki M, Kunishima S, et al. IL-1α induces thrombopoiesis through megakaryocyte rupture in response to acute platelet needs. *J Cell Biol*. 2015;209(3):453-466.
10. Junt T, Schulze H, Chen Z, et al. Dynamic visualization of thrombopoiesis within bone marrow. *Science*. 2007;317(5845):1767-1770.
11. Italiano JE Jr, Lecine P, Shivdasani RA, Hartwig JH. Blood platelets are assembled principally at the ends of proplatelet processes produced by differentiated megakaryocytes. *J Cell Biol*. 1999;147(6):1299-1312.
12. Ghalloussi D, Dhenge A, Bergmeier W. New insights into cytoskeletal remodeling during platelet production. *J Thromb Haemost*. 2019;17(9):1430-1439.
13. Leeksma CH, Cohen JA. Determination of the life of human blood platelets using labelled diisopropylfluorophosphanate. *Nature*. 1955;175(4456):552-553.
14. Mason KD, Carpinelli MR, Fletcher JI, et al. Programmed anuclear cell death delimits

platelet life span. *Cell*. 2007;128(6):1173-1186.

15. Deppermann C, Kratofil RM, Peiseler M, et al. Macrophage galactose lectin is critical for Kupffer cells to clear aged platelets. *J Exp Med*. 2020;217(4):e20190723.

16. Li Y, Fu J, Ling Y, et al. Sialylation on O-glycans protects platelets from clearance by liver Kupffer cells. *Proc Natl Acad Sci USA*. 2017;114(31):8360-8365.

17. Perez Botero J, Di Paola J. Diagnostic approach to the patient with a suspected inherited platelet disorder: who and how to test. *J Thromb Haemost*. 2021;19(9):2127-2136.

18. Bury L, Falcinelli E, Gresele P. Learning the ropes of platelet count regulation: inherited thrombocytopenias. *J Clin Med*. 2021;10(3):533.

19. Nurden AT, Nurden P. Inherited thrombocytopenias: history, advances and perspectives. *Haematologica*. 2020;105(8):2004-2019.

20. Palma-Barqueros V, Revilla N, Sánchez A, et al. Inherited platelet disorders: an updated overview. *Int J Mol Sci*. 2021;22(9):4521.

21. Noris P, Spedini P, Belletti S, Magrini U, Balduini CL. Thrombocytopenia, giant platelets, and leukocyte inclusion bodies (May-Hegglin anomaly): clinical and laboratory findings. *Am J Med*. 1998;104(4):355-360.

22. Thompson AR, Wood WG, Stamatoyannopoulos G. X-linked syndrome of platelet dysfunction, thrombocytopenia, and imbalanced globin chain synthesis with hemolysis. *Blood*. 1977;50(2):303-316.

23. Berndt MC, Andrews RK. Bernard-Soulier syndrome. *Haematologica*. 2011;96(3):355-359.

24. Seri M, Cusano R, Gangarossa S, et al; The May-Hegglin/Fechtner Syndrome Consortium. Mutations in MYH9 result in the May-Hegglin anomaly, and Fechtner and Sebastian syndromes. *Nat Genet*. 2000;26(1):103-105.

25. Gunay-Aygun M, Falik-Zaccai TC, Vilboux T, et al. NBEAL2 is mutated in gray platelet syndrome and is required for biogenesis of platelet α-granules. *Nat Genet*. 2011;43(8):732-734.

26. Gunay-Aygun M, Zivony-Elboum Y, Gumruk F, et al. Gray platelet syndrome: natural history of a large patient cohort and locus assignment to chromosome 3p. *Blood*. 2010;116(23):4990-5001.

27. Kahr WH, Hinckley J, Li L, et al. Mutations in NBEAL2, encoding a BEACH protein, cause gray platelet syndrome. *Nat Genet*. 2011;43(8):738-740.

28. Schulze H, Schlagenhauf A, Manukjan G, et al. Recessive grey platelet-like syndrome with unaffected erythropoiesis in the absence of the splice isoform GFI1B-p37. *Haematologica*. 2017;102(9):e375-e378.

29. Stevenson WS, Morel-Kopp MC, Chen Q, et al. GFI1B mutation causes a bleeding disorder with abnormal platelet function. *J Thromb Haemost*. 2013;11(11):2039-2047.

30. De Kock L, Thys C, Downes K, et al. De novo variant in tyrosine kinase SRC causes thrombocytopenia: case report of a second family. *Platelets*. 2019;30(7):931-934.

31. 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.

32. Saultier P, Vidal L, Canault M, et al. Macrothrombocytopenia and dense granule deficiency associated with FLI1 variants: ultrastructural and pathogenic features. *Haematologica*. 2017;102(6):1006-1016.

33. Stevenson WS, Rabbolini DJ, Beutler L, et al. Paris-Trousseau thrombocytopenia is phenocopied by the autosomal recessive inheritance of a DNA-binding domain mutation in FLI1. *Blood*. 2015;126(17):2027-2030.

34. Stockley J, Morgan NV, Bem D, et al; UK Genotyping and Phenotyping of Platelets Study Group. Enrichment of FLI1 and RUNX1 mutations in families with excessive bleeding and platelet dense granule secretion defects. *Blood*. 2013;122(25):4090-4093.

35. Breton-Gorius J, Favier R, Guichard J, et al. A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha-granules and chromosome 11 deletion at 11q23. *Blood*. 1995;85(7):1805-1814.

36. Favier R, Jondeau K, Boutard P, et al. Paris-Trousseau syndrome: clinical, hematological, molecular data of ten new cases. *Thromb Haemost*. 2003;90(5):893-897.

37. Rabbolini D, Connor D, Morel-Kopp MC, et al; Sydney Platelet Group. An integrated approach to inherited platelet disorders: results from a research collaborative, the Sydney Platelet Group. *Pathology*. 2020;52(2):243-255.

38. Badin MS, Graf L, Iyer JK, Moffat KA, Seecharan JL, Hayward CP. Variability in platelet dense granule adenosine triphosphate release findings amongst patients tested multiple times as part of an assessment for a bleeding disorder. *Int J Lab Hematol*. 2016;38(6):648-657.

39. Mullen CA, Anderson KD, Blaese RM. Splenectomy and/or bone marrow transplantation in the management of the Wiskott-Aldrich syndrome: long-term follow-up of 62 cases. *Blood*. 1993;82(10):2961-2966.

40. Nissan E, Katz U, Levy-Shraga Y, et al. Clinical features in a large cohort of patients with 22q11.2 deletion syndrome. *J Pediatr*. 2021;238:215-220.e5.

41. Ryan AK, Goodship JA, Wilson DI, et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J Med Genet*. 1997;34(10):798-804.

42. Zwifelhofer NMJ, Bercovitz RS, Weik LA, et al. Hemizygosity for the gene encoding glycoprotein Ibβ is not responsible for macrothrombocytopenia and bleeding in patients with 22q11 deletion syndrome. *J Thromb Haemost*. 2019;17(2):295-305.

43. Crowley TB, Campbell IM, Liebling EJ, et al. Distinct immune trajectories in patients with chromosome 22q11.2 deletion syndrome and immune-mediated diseases. *J Allergy Clin Immunol*. 2022;149(1):445-450.

44. Montin D, Marolda A, Licciardi F, et al. Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 deletion syndrome. *J Allergy Clin Immunol Pract*. 2019;7(7):2369-2376.

45. Pecci A, Balduini CL. Inherited thrombocytopenias: an updated guide for clinicians. *Blood Rev*. 2021;48:100784.

46. Lentaigne C, Greene D, Sivapalaratnam S, et al; NIHR BioResource. Germline mutations in the transcription factor IKZF5 cause thrombocytopenia. *Blood*. 2019;134(23):2070-2081.

47. Melazzini F, Palombo F, Balduini A, et al. Clinical and pathogenic features of ETV6-related thrombocytopenia with predisposition to acute lymphoblastic leukemia. *Haematologica*. 2016;101(11):1333-1342.

48. Moriyama T, Metzger ML, Wu G, et al. Germline genetic variation in ETV6 and risk of childhood acute lymphoblastic leukaemia: a systematic genetic study. *Lancet Oncol*. 2015;16(16):1659-1666.

49. Noetzli L, Lo RW, Lee-Sherick AB, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. *Nat Genet*. 2015;47(5):535-538.

50. Topka S, Vijai J, Walsh MF, et al. Germline ETV6 mutations confer susceptibility to acute lymphoblastic leukemia and thrombocytopenia. *PLoS Genet*. 2015;11(6):e1005262.

51. Greinacher A, Eekels JJM. Simplifying the diagnosis of inherited platelet disorders? The new tools do not make it any easier. *Blood*. 2019;133(23):2478-2483.

52. Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. *Haematologica*. 2018;103(1):148-162.

53. Shirts BH, Pritchard CC, Walsh T. Family-Specific variants and the limits of human genetics. *Trends Mol Med*. 2016;22(11):925-934.

54. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the

American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015; 17(5):405-424.

55. Ross JE, Zhang BM, Lee K, et al. Specifications of the variant curation guidelines for ITGA2B/ITGB3: ClinGen Platelet Disorder Variant Curation Panel. *Blood Adv.* 2021;5(2):414-431.

56. Megy K, Downes K, Morel-Kopp MC, et al. GoldVariants, a resource for sharing rare genetic variants detected in bleeding, thrombotic, and platelet disorders: communication from the ISTH SSC Subcommittee on Genomics in Thrombosis and Hemostasis. *J Thromb Haemost.* 2021; 19(10):2612-2617.

57. United States Center for Medicare and Medicaid Services. National Coverage Determination, Next Generation Sequencing (NGS) for Medicare Beneficiaries with Germline (Inherited) Cancer. https://www.cms.gov/medicare-coverage-database/view/ncd.aspx?NCDId=372. Accessed 1 October 2021.

58. Phillips KA, Douglas MP, Marshall DA. Expanding use of clinical genome sequencing and the need for more data on implementation. *JAMA.* 2020;324(20): 2029-2030.

59. Ross LF, Saal HM, David KL, Anderson RRAmerican Academy of Pediatrics; American College of Medical Genetics and Genomics. Technical report: ethical and policy issues in genetic testing and screening of children [published correction appears in *Genet Med.* 2013;15(4):321]. *Genet Med.* 2013;15(3):234-245.

60. Downes K, Borry P, Ericson K, et al; Subcommittee on Genomics in Thrombosis, Hemostasis. Clinical management, ethics and informed consent related to multi-gene panel-based high throughput sequencing testing for platelet disorders: communication from the SSC of the ISTH. *J Thromb Haemost.* 2020;18(10): 2751-2758.

61. Crispino JD, Horwitz MS. GATA factor mutations in hematologic disease. *Blood.* 2017;129(15):2103-2110.

62. Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. *Proc Natl Acad Sci USA.* 1996; 93(22):12355-12358.

63. Gutiérrez L, Tsukamoto S, Suzuki M, et al. Ablation of Gata1 in adult mice results in aplastic crisis, revealing its essential role in steady-state and stress erythropoiesis. *Blood.* 2008;111(8):4375-4385.

64. Yu C, Niakan KK, Matsushita M, Stamatoyannopoulos G, Orkin SH, Raskind WH. X-linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA-1 affecting DNA binding rather than FOG-1 interaction. *Blood.* 2002; 100(6):2040-2045.

65. Balduini CL, Pecci A, Loffredo G, et al. Effects of the R216Q mutation of GATA-1 on erythropoiesis and megakaryocytopoiesis. *Thromb Haemost.* 2004;91(1):129-140.

66. Tubman VN, Levine JE, Campagna DR, et al. X-linked gray platelet syndrome due to a GATA1 Arg216Gln mutation. *Blood.* 2007;109(8):3297-3299.

67. Phillips JD, Steensma DP, Pulsipher MA, Spangrude GJ, Kushner JP. Congenital erythropoietic porphyria due to a mutation in GATA1: the first trans-acting mutation causative for a human porphyria. *Blood.* 2007;109(6):2618-2621.

68. Del Vecchio GC, Giordani L, De Santis A, De Mattia D. Dyserythropoietic anemia and thrombocytopenia due to a novel mutation in GATA-1. *Acta Haematol.* 2005;114(2): 113-116.

69. Jamwal M, Aggarwal A, Sharma P, Bansal D, Maitra A, Das R. Phenotypic and genetic heterogeneity arising from a novel substitution at amino acid position Val205 in GATA1 related X-linked thrombocytopenia with dyserythropoietic anemia. *Blood Cells Mol Dis.* 2020;81:102391.

70. Kratz CP, Niemeyer CM, Karow A, Volz-Fleckenstein M, Schmitt-Gräff A, Strahm B. Congenital transfusion-dependent anemia and thrombocytopenia with myelodysplasia due to a recurrent GATA1(G208R) germline mutation. *Leukemia.* 2008;22(2):432-434.

71. Mehaffey MG, Newton AL, Gandhi MJ, Crossley M, Drachman JG. X-linked thrombocytopenia caused by a novel mutation of GATA-1. *Blood.* 2001;98(9): 2681-2688.

72. Nichols KE, Crispino JD, Poncz M, et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. *Nat Genet.* 2000;24(3): 266-270.

73. 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(2):147-152.

74. Freson K, Devriendt K, Matthijs G, et al. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. *Blood.* 2001; 98(1):85-92.

75. Hermans C, De Waele L, Van Geet C, Freson K. Novel GATA1 mutation in residue D218 leads to macrothrombocytopenia and clinical bleeding problems. *Platelets.* 2014;25(4):305-307.

76. Abdulhay NJ, Fiorini C, Verboon JM, et al. Impaired human hematopoiesis due to a cryptic intronic *GATA1* splicing mutation. *J Exp Med.* 2019;216(5):1050-1060.

77. Hollanda LM, Lima CS, Cunha AF, et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. *Nat Genet.* 2006;38(7):807-812.

78. Klar J, Khalfallah A, Arzoo PS, Gazda HT, Dahl N. Recurrent GATA1 mutations in Diamond-Blackfan anaemia. *Br J Haematol.* 2014;166(6):949-951.

79. Parrella S, Aspesi A, Quarello P, et al. Loss of GATA-1 full length as a cause of Diamond-Blackfan anemia phenotype. *Pediatr Blood Cancer.* 2014;61(7): 1319-1321.

80. Sankaran VG, Ghazvinian R, Do R, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. *J Clin Invest.* 2012;122(7): 2439-2443.

81. Ludwig LS, Gazda HT, Eng JC, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. *Nat Med.* 2014;20(7): 748-753.

82. Léon C, Dupuis A, Gachet C, Lanza F. The contribution of mouse models to the understanding of constitutional thrombocytopenia. *Haematologica.* 2016; 101(8):896-908.

83. Pecci A, Balduini CL. Lessons in platelet production from inherited thrombocytopenias. *Br J Haematol.* 2014;165(2): 179-192.

84. Marconi C, Di Buduo CA, LeVine K, et al. Loss-of-function mutations in *PTPRJ* cause a new form of inherited thrombocytopenia. *Blood.* 2019;133(12):1346-1357.

85. Lin Q, Zhang Y, Zhou R, et al. Establishment of a congenital amegakaryocytic thrombocytopenia model and a thrombocyte-specific reporter line in zebrafish. *Leukemia.* 2017;31(5):1206-1216.

86. Butov KR, Osipova EY, Mikhalkin NB, Trubina NM, Panteleev MA, Machlus KR. In vitro megakaryocyte culture from human bone marrow aspirates as a research and diagnostic tool. *Platelets.* 2021;32(7): 928-935.

87. Borst S, Nations CC, Klein JG, et al. Study of inherited thrombocytopenia resulting from mutations in ETV6 or RUNX1 using a human pluripotent stem cell model. *Stem Cell Reports.* 2021;16(6):1458-1467.

88. Fisher MH, Kirkpatrick GD, Stevens B, et al. ETV6 germline mutations cause HDAC3/NCOR2 mislocalization and upregulation of interferon response genes. *JCI Insight.* 2020;5(18):e140332.

89. Nishii R, Baskin-Doerfler R, Yang W, et al. Molecular basis of ETV6-mediated predisposition to childhood acute lymphoblastic leukemia. *Blood.* 2021;137(3):364-373.

90. Hanna RE, Hegde M, Fagre CR, et al. Massively parallel assessment of human variants with base editor screens. *Cell.* 2021;184(4):1064-1080.e20.

91. Wahlster L, Verboon JM, Ludwig LS, et al. Familial thrombocytopenia due to a complex structural variant resulting in a WAC-ANKRD26 fusion transcript. *J Exp Med.* 2021;218(6):e20210444.

92. de Sauvage FJ, Hass PE, Spencer SD, et al. Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. *Nature.* 1994;369(6481):533-538.

93. Kaushansky K, Lok S, Holly RD, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature*. 1994; 369(6481):568-571.

94. Kimura S, Roberts AW, Metcalf D, Alexander WS. Hematopoietic stem cell deficiencies in mice lacking c-Mpl, the receptor for thrombopoietin. *Proc Natl Acad Sci USA*. 1998;95(3):1195-1200.

95. Qian H, Buza-Vidas N, Hyland CD, et al. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell*. 2007;1(6): 671-684.

96. Yoshihara H, Arai F, Hosokawa K, et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell*. 2007;1(6):685-697.

97. Heremans J, Garcia-Perez JE, Turro E, et al; National Institute for Health Research BioResource. Abnormal differentiation of B cells and megakaryocytes in patients with Roifman syndrome. *J Allergy Clin Immunol*. 2018;142(2):630-646.

98. Drube S, Grimlowski R, Deppermann C, et al. The neurobeachin-like 2 protein regulates mast cell homeostasis. *J Immunol*. 2017;199(8):2948-2957.

99. Sims MC, Mayer L, Collins JH, et al; NIHR BioResource. Novel manifestations of immune dysregulation and granule defects in gray platelet syndrome. *Blood*. 2020; 136(17):1956-1967.

100. Kahr WH, Pluthero FG, Elkadri A, et al. Loss of the Arp2/3 complex component ARPC1B causes platelet abnormalities and predisposes to inflammatory disease. *Nat Commun*. 2017;8(1):14816.

101. Somech R, Lev A, Lee YN, et al. Disruption of thrombocyte and T lymphocyte development by a mutation in *ARPC1B*. *J Immunol*. 2017;199(12):4036-4045.

102. Raskind WH, Niakan KK, Wolff J, et al. Mapping of a syndrome of X-linked thrombocytopenia with Thalassemia to band Xp11-12: further evidence of genetic heterogeneity of X-linked thrombocytopenia. *Blood*. 2000;95(7):2262-2268.

103. Merico D, Roifman M, Braunschweig U, et al. Compound heterozygous mutations in the noncoding RNU4ATAC cause Roifman Syndrome by disrupting minor intron splicing. *Nat Commun*. 2015; 6(1):8718.

104. Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet*. 1999;23(2):166-175.

105. Michaud J, Wu F, Osato M, et al. In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. *Blood*. 2002; 99(4):1364-1372.

106. Ganly P, Walker LC, Morris CM. Familial mutations of the transcription factor RUNX1 (AML1, CBFA2) predispose to acute myeloid leukemia. *Leuk Lymphoma*. 2004; 45(1):1-10.

107. Tang C, Rabbolini DJ, Morel-Kopp MC, et al. The clinical heterogeneity of *RUNX1* associated familial platelet disorder with predisposition to myeloid malignancy - a case series and review of the literature. *Res Pract Thromb Haemost*. 2019;4(1):106-110.

108. Zhang MY, Churpek JE, Keel SB, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. *Nat Genet*. 2015;47(2): 180-185.

109. Thompson AA, Nguyen LT. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with HOXA11 mutation. *Nat Genet*. 2000;26(4):397-398.

110. Niihori T, Ouchi-Uchiyama M, Sasahara Y, et al. Mutations in MECOM, encoding oncoprotein EVI1, cause radioulnar synostosis with amegakaryocytic thrombocytopenia. *Am J Hum Genet*. 2015;97(6):848-854.

111. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood*. 2018;131(7):717-732.

112. Germeshausen M, Ancliff P, Estrada J, et al. MECOM-associated syndrome: a heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia. *Blood Adv*. 2018;2(6): 586-596.

113. Albers CA, Paul DS, Schulze H, et al. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. *Nat Genet*. 2012; 44(4):435-439, S1-2.

114. Klopocki E, Schulze H, Strauss G, et al. Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet*. 2007; 80(2):232-240.

115. Kunishima S, Kobayashi R, Itoh TJ, Hamaguchi M, Saito H. Mutation of the beta1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. *Blood*. 2009;113(2): 458-461.

116. Kunishima S, Okuno Y, Yoshida K, et al. ACTN1 mutations cause congenital macrothrombocytopenia. *Am J Hum Genet*. 2013;92(3):431-438.

117. Pleines I, Woods J, Chappaz S, et al. Mutations in tropomyosin 4 underlie a rare form of human macrothrombocytopenia. *J Clin Invest*. 2017;127(3):814-829.

118. Stritt S, Nurden P, Turro E, et al; BRIDGE-BPD Consortium. A gain-of-function variant in DIAPH1 causes dominant macrothrombocytopenia and hearing loss. *Blood*. 2016;127(23):2903-2914.

119. Westbury SK, Downes K, Burney C, et al; NIHR BioResource–Rare Diseases. Phenotype description and response to thrombopoietin receptor agonist in *DIAPH1*-related disorder. *Blood Adv*. 2018; 2(18): 2341-2346.

120. Parrini E, Ramazzotti A, Dobyns WB, et al. Periventricular heterotopia: phenotypic heterogeneity and correlation with Filamin A mutations. *Brain*. 2006;129(Pt 7): 1892-1906.

121. Nurden P, Debili N, Coupry I, et al. Thrombocytopenia resulting from mutations in filamin A can be expressed as an isolated syndrome. *Blood*. 2011;118(22): 5928-5937.

122. Martinelli S, Krumbach OHF, Pantaleoni F, et al; University of Washington Center for Mendelian Genomics. Functional dysregulation of CDC42 causes diverse developmental phenotypes. *Am J Hum Genet*. 2018;102(2):309-320.

123. Takenouchi T, Okamoto N, Ida S, Uehara T, Kosaki K. Further evidence of a mutation in CDC42 as a cause of a recognizable syndromic form of thrombocytopenia. *Am J Med Genet A*. 2016;170A(4):852-855.

124. Takenouchi T, Kosaki R, Niizuma T, Hata K, Kosaki K. Macrothrombocytopenia and developmental delay with a de novo CDC42 mutation: yet another locus for thrombocytopenia and developmental delay. *Am J Med Genet A*. 2015;167A(11): 2822-2825.

125. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. *Cell*. 1994;78(4):635-644.

126. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott-Aldrich syndrome. *J Pediatr*. 1994;125(6 Pt 1):876-885.

127. Lanzi G, Moratto D, Vairo D, et al. A novel primary human immunodeficiency due to deficiency in the WASP-interacting protein WIP. *J Exp Med*. 2012;209(1):29-34.

128. Levin C, Koren A, Pretorius E, et al. Deleterious mutation in the FYB gene is associated with congenital autosomal recessive small-platelet thrombocytopenia. *J Thromb Haemost*. 2015;13(7):1285-1292.

129. Miller JL, Lyle VA, Cunningham D. Mutation of leucine-57 to phenylalanine in a platelet glycoprotein Ib alpha leucine tandem repeat occurring in patients with an autosomal dominant variant of Bernard-Soulier disease. *Blood*. 1992;79(2):439-446.

130. Noris P, Perrotta S, Bottega R, et al. Clinical and laboratory features of 103 patients from 42 Italian families with inherited thrombocytopenia derived from the monoallelic Ala156Val mutation of GPIbα (Bolzano mutation). *Haematologica*. 2012;97(1):82-88.

131. Ware J, Russell SR, Vicente V, et al. Nonsense mutation in the glycoprotein Ib alpha coding sequence associated with Bernard-Soulier syndrome. *Proc Natl Acad Sci USA*. 1990;87(5):2026-2030.

132. Othman M, Notley C, Lavender FL, et al. Identification and functional

characterization of a novel 27-bp deletion in the macroglycopeptide-coding region of the GPIBA gene resulting in platelet-type von Willebrand disease. *Blood*. 2005; 105(11):4330-4336.

133. Kunishima S, Lopez JA, Kobayashi S, et al. Missense mutations of the glycoprotein (GP) Ib beta gene impairing the GPIb alpha/beta disulfide linkage in a family with giant platelet disorder. *Blood*. 1997;89(7): 2404-2412.

134. Sivapalaratnam S, Westbury SK, Stephens JC, et al; NIHR BioResource. Rare variants in GP1BB are responsible for autosomal dominant macrothrombocytopenia. *Blood*. 2017;129(4):520-524.

135. Noda M, Fujimura K, Takafuta T, et al. Heterogeneous expression of glycoprotein Ib, IX and V in platelets from two patients with Bernard-Soulier syndrome caused by different genetic abnormalities. *Thromb Haemost*. 1995;74(6):1411-1415.

136. Kunishima S, Kashiwagi H, Otsu M, et al. Heterozygous ITGA2B R995W mutation inducing constitutive activation of the αIIbβ3 receptor affects proplatelet formation and causes congenital macrothrombocytopenia. *Blood*. 2011; 117(20):5479-5484.

137. Peyruchaud O, Nurden AT, Milet S, et al. R to Q amino acid substitution in the GFFKR sequence of the cytoplasmic domain of the integrin IIb subunit in a patient with a Glanzmann's thrombasthenia-like syndrome. *Blood*. 1998;92(11):4178-4187.

138. Ghevaert C, Salsmann A, Watkins NA, et al. A nonsynonymous SNP in the ITGB3 gene disrupts the conserved membrane-proximal cytoplasmic salt bridge in the alphaIIbbeta3 integrin and cosegregates dominantly with abnormal proplatelet formation and macrothrombocytopenia. *Blood*. 2008;111(7): 3407-3414.

139. Gresele P, Falcinelli E, Giannini S, et al. Dominant inheritance of a novel integrin beta3 mutation associated with a hereditary macrothrombocytopenia and platelet dysfunction in two Italian families. *Haematologica*. 2009;94(5):663-669.

140. Jayo A, Conde I, Lastres P, et al. L718P mutation in the membrane-proximal cytoplasmic tail of beta 3 promotes abnormal alpha IIb beta 3 clustering and lipid microdomain coalescence, and associates with a thrombasthenia-like phenotype. *Haematologica*. 2010;95(7):1158-1166.

141. Manchev VT, Hilpert M, Berrou E, et al. A new form of macrothrombocytopenia induced by a germ-line mutation in the PRKACG gene. *Blood*. 2014;124(16): 2554-2563.

142. Fletcher SJ, Johnson B, Lowe GC, et al; UK Genotyping and Phenotyping of Platelets study group. SLFN14 mutations underlie thrombocytopenia with excessive bleeding and platelet secretion defects. *J Clin Invest*. 2015;125(9):3600-3605.

143. Noris P, Perrotta S, Seri M, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. *Blood*. 2011;117(24):6673-6680.

144. Pippucci T, Savoia A, Perrotta S, et al. Mutations in the 5′ UTR of ANKRD26, the ankirin repeat domain 26 gene, cause an autosomal-dominant form of inherited thrombocytopenia, THC2. *Am J Hum Genet*. 2011;88(1):115-120.

145. Ballmaier M, Germeshausen M, Schulze H, et al. c-mpl mutations are the cause of congenital amegakaryocytic thrombocytopenia. *Blood*. 2001;97(1): 139-146.

146. Germeshausen M, Ballmaier M. CAMT-MPL: congenital amegakaryocytic thrombocytopenia caused by MPL mutations - heterogeneity of a monogenic disorder - comprehensive analysis of 56 patients. *Haematologica*. 2021;106(9): 2439-2448.

147. Germeshausen M, Ballmaier M, Welte K. MPL mutations in 23 patients suffering from congenital amegakaryocytic thrombocytopenia: the type of mutation predicts the course of the disease. *Hum Mutat*. 2006;27(3):296.

148. Noris P, Marconi C, De Rocco D, et al. A new form of inherited thrombocytopenia due to monoallelic loss of function mutation in the thrombopoietin gene. *Br J Haematol*. 2018;181(5):698-701.

149. Nesin V, Wiley G, Kousi M, et al. Activating mutations in STIM1 and ORAI1 cause overlapping syndromes of tubular myopathy and congenital miosis. *Proc Natl Acad Sci USA*. 2014;111(11):4197-4202.

150. Stritt S, Nurden P, Favier R, et al. Defects in TRPM7 channel function deregulate thrombopoiesis through altered cellular Mg(2+) homeostasis and cytoskeletal architecture. *Nat Commun*. 2016;7(1): 11097.

151. Rees DC, Iolascon A, Carella M, et al. Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. *Br J Haematol*. 2005; 130(2):297-309.

152. Su Y, Wang Z, Yang H, et al. Clinical and molecular genetic analysis of a family with sitosterolemia and co-existing erythrocyte and platelet abnormalities. *Haematologica*. 2006;91(10):1392-1395.

153. Wang Z, Cao L, Su Y, et al. Specific macrothrombocytopenia/hemolytic anemia associated with sitosterolemia. *Am J Hematol*. 2014;89(3):320-324.

154. Morison IM, Cramer Bordé EM, Cheesman EJ, et al. A mutation of human cytochrome c enhances the intrinsic apoptotic pathway but causes only thrombocytopenia. *Nat Genet*. 2008;40(4):387-389.

155. 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.

156. 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.

157. Kauskot A, Pascreau T, Adam F, et al. A mutation in the gene coding for the sialic acid transporter SLC35A1 is required for platelet life span but not proplatelet formation. *Haematologica*. 2018;103(12): e613-e617.

158. Seo A, Gulsuner S, Pierce S, et al. Inherited thrombocytopenia associated with mutation of UDP-galactose-4-epimerase (GALE). *Hum Mol Genet*. 2019;28(1): 133-142.

159. Hofmann I, Geer MJ, Vögtle T, et al. Congenital macrothrombocytopenia with focal myelofibrosis due to mutations in human G6b-B is rescued in humanized mice. *Blood*. 2018;132(13):1399-1412.

160. Bariana TK, Labarque V, Heremans J, et al. Sphingolipid dysregulation due to lack of functional KDSR impairs proplatelet formation causing thrombocytopenia. *Haematologica*. 2019;104(5):1036-1045.

161. Takeichi T, Torrelo A, Lee JYW, et al. Biallelic mutations in KDSR disrupt ceramide synthesis and result in a spectrum of keratinization disorders associated with thrombocytopenia. *J Invest Dermatol*. 2017;137(11):2344-2353.

162. Zucker J, Temm C, Czader M, Nalepa G. A child with dyserythropoietic anemia and megakaryocyte dysplasia due to a novel 5'UTR GATA1s splice mutation. *Pediatr Blood Cancer*. 2016;63(5):917-921.