Inherited Thrombocytopenias (IT)

Subjects: Hematology

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Inherited thrombocytopenias (IT) are a group of hereditary disorders characterized by a reduced platelet count sometimes associated with abnormal platelet function, which can lead to bleeding but also to syndromic manifestations and predispositions to other disorders.

inherited thrombocytopenias platelets bleeding

1. Introduction

Platelets, or thrombocytes, are small and anuclear blood cells with discoid shape and a size of 1.5–3.0 µm which play a crucial function in primary hemostasis. Their normal life span is 9–10 days and total circulating mass 10^{12}, thus about 10^{11} platelets are released each day from their bone marrow precursors, megakaryocytes, to maintain a normal circulating platelet count of 1.5 to 4 × 10^{9}/L.

Inherited thrombocytopenias (ITs) are a heterogeneous group of congenital disorders characterized by a reduction of platelet number, a widely-variable bleeding diathesis, sometimes aggravated by associated impairment of platelet function, and frequently associated with additional defects, which may heavily impact patient lives.

ITs are rare diseases, with an estimated prevalence of 2.7 in 100,000 [1] although this figure is probably underestimated because they are often misdiagnosed as immune thrombocytopenia (ITP). A recent study on the assessment of the frequency of naturally occurring loss-of-function variants in genes associated with platelet disorders (52% of which were associated with ITs) from a large genome aggregation database showed that 0.329% of subjects in the general population have a clinically meaningful loss-of-function variant in a platelet-associated gene [2].

The first IT, Bernard Soulier syndrome, was described in 1948 and subsequently only few additional forms were reported until Sanger sequencing first, and next generation sequencing later became widely applied rapidly bringing the known ITs from less than a dozen to currently at least 41 disorders caused by mutations in 42 different genes [3][4].

2. Hereditary Disorders of Platelet Number

Given the wide heterogeneity of IT, there is no consensus on their classification, and several criteria have been proposed, such as on clinical features (e.g., age at presentation, severity, associated developmental

abnormalities), platelet size or inheritance pattern (e.g., autosomal dominant, autosomal recessive and X-linked) [4][5][6].

Here we have grouped them according to the pathogenic mechanisms of thrombocytopenia.

ITs are primarily caused by mutations in genes involved in megakaryocyte differentiation, maturation and platelet production [7] (Table 1, Figure 1).

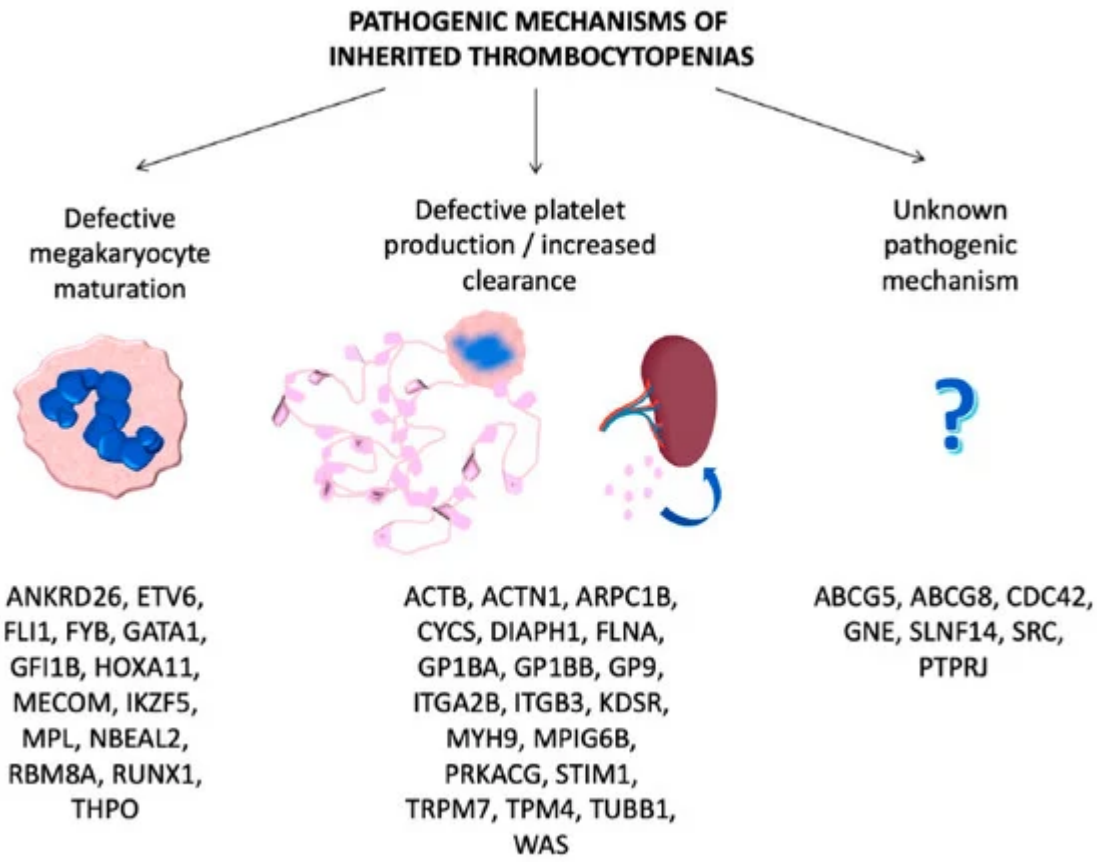

Figure 1. Genes involved in inherited thrombocytopenias classified according to the pathogenic mechanisms.

Table 1. IT classified based on the defective step of platelet count regulation involved.

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
Defective megakaryocyte maturation	ANKRD26	ANKRD26-related thrombocytopenia	Loss of ANKRD26 silencing during the last phases of megakaryocytopoiesis causes ERK1/2 phosphorylation that interferes with	Predisposition to hematological malignancies

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			megakaryocyte maturation [8]	
	ETV6	ETV6-related thrombocytopenia	ETV6 is a transcriptional repressor that promotes the late phases of megakaryopoiesis. Mutations in ETV6 cause defective megakaryocyte maturation and impaired proplatelet formation [9]	Predisposition to hematological malignancies
	FLI1	FLI1-related thrombocytopenia	FLI1 is a transcription factor regulating many genes associated with megakaryocyte development. Therefore, FLI1 mutations promote defective megakaryocyte maturation [10]	Not reported
	FLI1 deletion	Paris-Trousseau syndrome/Jacobsen syndrome		Abnormalities of heart and face, intellectual disabilities
	FYB	FYB-related thrombocytopenia	ADAP is a protein involved in the remodeling of cytoskeleton. Mutations in ADAP cause defective maturation of megakaryocytes and clearance of platelets [11]	Mild iron deficiency anemia
	GATA1	GATA1-relate disease	GATA1 is a transcription factor regulating many genes associated with megakaryocyte development therefore GATA1 defects cause alterations of	Dyserythropoietic anemia, beta-thalassemia, congenital erythropoietic porphyria, splenomegaly

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			megakaryocyte maturation [12]	
	GFI1B	GFI1B-related thrombocytopenia	GFI1B is a transcription factor involved in homeostasis of hematopoietic stem cells and development of megakaryocytes therefore GFI1B defects cause alterations of megakaryocyte maturation [13]	Mild myelofibrosis
	HOXA11	Amegakaryocytic thrombocytopenia with radio-ulnar synostosis	HOXA11 is a transcription factor involved in the regulation of early hematopoiesis, its defect causes reduced number of megakaryocytes [14]	Bilateral radioulnar synostosis, severe bone marrow failure culminating in aplastic anemia in majority of cases, cardiac and renal malformations, hearing loss, clinodactyly, skeletal abnormalities, pancytopenia
	MECOM		MECOM is a transcription factor involved in the regulation of early hematopoiesis, its defect causes reduced number of megakaryocytes [15]	
	IKZF5	IKZF5-related thrombocytopenia	IKZF5 is a previously unknown transcriptional regulator of megakaryopoiesis [16]	Not reported
	MPL	Congenital amegakaryocytic thrombocytopenia	MPL is the receptor for thrombopoietin. MPL defects cause impaired thrombopoietin binding and thus impaired	Acquired bone marrow aplasia

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			megakaryocyte maturation [17]	
	NBEAL2	Gray platelet syndrome	Mutations in NBEAL2 cause impaired megakaryocyte maturation however its role in megakaryocytopoiesis is not clear [18]	Myelofibrosis, immune dysregulation (autoimmune diseases, positive autoantibodies, reduced leukocyte counts), proinflammatory profile
	RBM8A	Thrombocytopenia-absent radius	RBM8A is a protein of the exon-junction complex involved in RNA processing. It has been hypothesized that RBM8A defects cause wrong mRNA processing of unknown components of the TPO-MPL pathway impairing megakaryocyte maturation [19]	Bilateral radial aplasia, anemia, skeletal, urogenital, kidney, heart defects
	RUNX1	Familial platelet disorder with predisposition to hematological malignancies	RUNX1 is a transcription factor regulating many genes associated with megakaryocyte development therefore RUNX1 mutations promote defective megakaryocyte maturation [20]	Predisposition to hematological malignancies
	THPO	THPO-related disease	THPO is the gene for thrombopoietin, essential for hematopoietic stem cell survival and megakaryocyte maturation [21]	Bone marrow aplasia

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
Defective platelet production/increased clearance	ACTB	Baraitser–Winter syndrome 1 with macrothrombocytopenia	Mutations in β-cytoplasmic actin inhibit the final stages of platelet maturation by compromising microtubule organization [22]	Microcephaly, facial anomalies, mild intellectual disability, developmental delay
	ACTN1	ACTN1-related thrombocytopenia	ACTN-1 is involved in cytoskeletal remodeling, defects in ACTN-1 cause defective proplatelet formation [23]	Not reported
	ARPC1B	Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease	The actin-related protein 2/3 complex (Arp2/3) is a regulator of the actin cytoskeleton and its mutation causes impaired proplatelet formation [24]	Immunodeficiency, systemic inflammation, vasculitis, inflammatory colitis, eosinophilia, eczema, lymphadenomegaly, hepato-splenomegaly, growth failure
	CYCS	CYCS-related thrombocytopenia	CYCS is a mitochondrial protein with a role in respiration and apoptosis. Mutations in CYCS cause ectopic premature proplatelet formation with an unknown mechanism [25]	Not reported
	DIAPH1	DIAPH1-related thrombocytopenia	DIAPH1 is involved in cytoskeletal remodeling, defects in DIAPH1 cause defective proplatelet formation [26]	Hearing loss
	FLNA	FLNA-related thrombocytopenia	Filamin A is involved in cytoskeletal remodeling, defects in	Periventricular nodular heterotopia and otopalatodigital

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			FLNA cause defective proplatelet formation [27]	syndrome spectrum of disorders
	GP1BA, GP1BB, GP9 (loss of function)	Bernard–Soulier syndrome monoallelic	The intracellular portion of the GPIb/IX/V complex links the receptor to the cytoskeleton. Disruption of this link causes impaired proplatelet formation [28]	Not reported
		Bernard–Soulier syndrome biallelic		
	GP1BA (gain of function)	Platelet-type von Willebrand disease	The extracellular portion of the GPIb/IX/V complex binds VWF. Constitutive binding of VWF to its receptor triggers the Src kinases pathway causing impaired proplatelet formation, ectopic platelet production and increased platelet clearance [29]	Not reported
	ITGA2B, ITGB3	ITGA2B/ITGB3-related thrombocytopenia	Constitutive activation of $\alpha_{IIb}\beta_3$ causes cytoskeletal perturbation leading to impaired proplatelet formation [30][31]	Not reported
	KDSR	Thrombocytopenia and erythrokeraderma	KDSR is an essential enzyme for de novo sphingolipid synthesis, this suggests an important role for sphingolipids as regulators of cytoskeletal organization during megakaryopoiesis and proplatelet formation [32]	Dermatologic involvement ranging from hyperkeratosis/ erythema to ichthyosis. One family with no or very mild skin lesions but associated anemia has been reported

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
	MYH9	MYH9-related disorder	MYH9 regulates cytoskeleton remodeling and mediates signal transduction pathways involved in proplatelet formation. Abnormalities of MYH9 cause hyperactivation of the Rho/ROCK pathway causing ectopic platelet formation [33]	Kidney disease, cataract, deafness, elevated liver enzymes
	MPIG6B	Thrombocytopenia, anemia and myelofibrosis	G6b-B is a transmembrane receptor with an ITIM motif with a not well defined role in proplatelet formation [34]	Microcitic anemia, myelofibrosis, leukocytosis may be present
	PRKACG	PRKACG-related thrombocytopenia	PKA activates many proteins involved in megakaryocyte and platelet function, among them FLNa and GPIbβ therefore its dysfunction causes impaired proplatelet formation [35]	Not reported
	STIM1	Stormorken syndrome	STIM1 mutations cause a constitutively active store operated Ca^{2+} release-activated Ca^{2+} (CRAC) channel which triggers Ca^{2+} entry with consequent increased clearance of activated platelets [36]	Tubular myopathy and congenital myosis. Severe immune dysfunction
	TRPM7	TRPM7-related thrombocytopenia	Defects of the Mg^{2+} channel TRPM7, a regulator of embryonic	Atrial fibrillation

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			development and cell survival, cause cytoskeletal alterations resulting in impaired proplatelet formation [37]	
	TPM4	TPM4-related thrombocytopenia	Tropomyosin 4 is an actin cytoskeletal regulator. Insufficient TPM4 expression in human and mouse megakaryocytes resulted in a defect in the terminal stages of platelet production [38]	Not reported
	TUBB1	TUBB1-related thrombocytopenia	Tubulin beta1 is a major component of microtubules therefore defects in TUBB1 cause impaired proplatelet formation [39]	Not reported
	WAS	Wiskott–Aldrich syndrome	The WASP protein is a regulator of the actin cytoskeleton and its defect causes ectopic platelet formation and increased platelet clearance [40]	Immunodeficiency, hematopoietic malignancies, eczema, autoimmune hemolytic anemia.
		X-linked thrombocytopenia		Not reported
Other/unknown pathogenic mechanism	ABCG5, ABCG8	Thrombocytopenia associated with sitosterolemia	ABCG5 and ABCG8 regulate plant sterol and cholesterol absorption. It is supposed that sterol-enriched platelets are more rapidly cleared [41]	Xanthomas and pre-mature coronary atherosclerosis due to hypercholesterolemia
	CDC42	Takenouchi-Kosaki syndrome with macrothrombocytopenia	CDC42 is a critical molecule in various biological processes	Defective growth and psychomotor development,

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expression of numerous genes, therefore these disorders are characterized by the concurrent alterations of multiple steps in MK and platelet development. For instance, RUNX1 transactivates transcription factors involved in MK maturation, proteins of the MK cytoskeleton (MYH9, MYL9, MYH10) or implicated in α and dense granule

Defective Step of Thrombopoiesis	Affected Gene [12]	Disorder	Pathogenic Mechanism (Reference) [47]	Additional Features (e.g., Syndromic Manifestations, Predisposition)
		[16]	including the cell cycle, cell division, and the formation of the actin cytoskeleton [42] [48]	intellectual disability, facial abnormalities, brain malformation, muscle tone abnormalities, immunodeficiency, eczema, hearing/visual disability, lymphedema, cardiac, genitourinary, and/or skeletal malformations
	GNE	GNE-related thrombocytopenia [8]	GNE encodes an enzyme involved in the sialic acid biosynthesis pathway and it is known that thrombocytopenia is associated with increased platelet desialylation [11][43]	Some patients presented myopathy with rimmed vacuoles with onset in early adulthood [18]
	SLNF14	SLNF14-related thrombocytopenia	SLNF14 is an endoribonuclease and its role in the generation of thrombocytopenia is unknown [44]	Not reported
	SRC	SRC-related thrombocytopenia	Src-family kinase regulates multiple signaling pathways, its role in the generation of thrombocytopenia is unknown [45]	Myelofibrosis, bone pathologies, bone marrow dysplasia, splenomegaly, congenital facial dysmorphism
[30][49][50]	PTPRJ	PTPRJ-related thrombocytopenia	PTPRJ is a protein tyrosine phosphatase expressed abundantly in platelets and megakaryocytes, its role in the generation	None

downstream of activated GPIbα [29].

Defective Step of Thrombopoiesis	Affected Gene	Disorder	Pathogenic Mechanism (Reference)	Additional Features (e.g., Syndromic Manifestations, Predisposition)
			of thrombocytopenia is unknown [46]	

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ectopic proplatelet formation (PPF) within the bone marrow and impaired CDC42 driven MK migration to the vascular niche [51]. The observation that splenectomy enhances the platelet count in WAS and XLT patients however, suggests that increased platelet clearance is also an important mechanism of thrombocytopenia in these disorders.

An additional group of IT belonging to those caused by impaired platelet production is due to variants in genes not directly involved in proplatelet formation, such as *CYCS*-RT, caused by dysfunction of a mitochondrial protein that causes thrombocytopenia by enhancing an apoptotic pathway [25], or *PRKACG*-RT, leading to dysfunction of PKA, which activates many proteins involved in megakaryocyte and platelet function such as FLNa and GPIbβ [35]. The Stormorken syndrome is due to gain of function mutations of STIM1 [52]. In these patients platelets circulate in an activated state due to a constitutively active store operated Ca^{2+} release-activated Ca^{2+} (CRAC) channel which triggers Ca^{2+} entry with consequent increased clearance of activated platelets by the spleen which causes a reduction in the number of circulating platelets [53].

Ectopic proplatelet formation in bone marrow is a peculiar mechanism causing thrombocytopenia in *FYB*, *GP1BA* (gain-of-function variants) and *MYH9*.

2.3. ITs Caused by Unknown Pathogenic Mechanisms

One last group of ITs is caused by variants in genes not known to be involved in megakaryocyte maturation or platelet production, and that cause thrombocytopenia by still unknown mechanisms.

An interesting IT is thrombocytopenia associated with sitosterolemia, a rare autosomal recessive disorder caused by mutations in two adjacent ATP-binding cassette transport genes (*ABCG5* and *ABCG8*) encoding proteins (sterolins-1 and -2) that pump sterols out of cells [54]. Among the manifestations of this complex disorder due to the accumulation of sterols in plasma and cell membranes are haematological abnormalities, including thrombocytopenia, provoked by the increased stiffness of sterol-enriched membranes with possible enhanced susceptibility to lysis and rupture [55].

Another recently discovered gene causing IT is *SLFN14*, an endoribonuclease degrading mRNA [44][56][57]. Alongside reduced platelet number, these patients show increased platelet clearance and platelet dysfunction. However, the mechanism through which mutations in *SLFN14* induce enhanced platelet turnover and abnormal platelet function is unknown. Similarly, the pathogenic mechanisms of one of the most recently reported causative genes of IT, GNE, are unknown. Mutations of *GNE*, the gene encoding Glucosamine (UDP-NAcetyl)-2-Epimerase/N-Acetylmannosamine kinase, cause sialuria and hereditary inclusion body myopathy [58] but are also

associated with severe thrombocytopenia characterized by shortened platelet lifespan, but the exact mechanisms have not been clarified [43].

3. Diagnostic Approach

3.1. Introduction

Patients referred for investigation of bleeding symptoms should undergo preliminary laboratory investigations including full blood count, prothrombin time, activated partial thromboplastin time and von Willebrand factor (VWF) screening tests (VWF antigen, ristocetin cofactor activity and factor VIII coagulant activity). If from full blood count thrombocytopenia is identified, a diagnostic work-up for IT should be pursued. If these are normal the presence of an inherited platelet function disorder (IPFD) should be explored. IPFD are listed under Table 2.

Table 2. Inherited platelet function disorders: disorders in which platelet dysfunction is the dominant phenotypic feature independent of platelet count.

Disease	Inheritance	Gene	Bleeding Diathesis
Arthrogryposis, renal dysfunction and cholestasis	AR	*VPS33B* *VIPAS39*	Severe
CalDAG-GEFI related platelet disorder	AR	*RASGRP2*	Moderate-severe
Cediak-Higashi Syndrome	AR	*CHS1*	Moderate-severe
Combined alpha-delta granule deficiency	AR/AD	Unknown	Mild-moderate
COX-1 deficiency	AR/AD	PTGSA	Moderate-severe
Delta granule deficiency	AR/AD	Unknown	Mild-moderate
Glanzmann thrombasthenia	AR	*ITGA2B*, *ITGB3*	Moderate-severe
Glycoprotein IV (GPIV) deficiency	AR	*GP4*	Mild

Disease	Inheritance	Gene	Bleeding Diathesis
Glycoprotein VI (GPVI) deficiency	AR	*GP6*	Mild
G_s platelet defect	AD (if paternally inherited)	*GNAS*	Mild
Hermansky–Pudlak syndrome	AR	*HPS1*, *ADTB3A*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1*, *BLOC1S3*, *AP3D1*, *BLOC1S6*	Moderate-severe
Leukocyte adhesion deficiency, type III	AR	*FERMT3*	Moderate-severe
P2Y12 deficiency	AR	*P2RY12*	Moderate-severe
Phospholipase A_2 ($cPLA_2$) deficiency	not determined	*PLA2G4A*	Moderate-severe
PKCδ deficiency	AR	*PRKCD*	Absent
Primary secretion defect	AR/AD	Unknown	Mild-moderate
Quebec platelet disorder	AD	*PLAU*	Moderate-severe
Scott syndrome	AR	*TMEM16F*	Mild-moderate
Thromboxane A2 receptor defect	AD	*TBXA2R*	Mild
T_x synthase deficiency	AD/AR	*TBXAS1*	Moderate

[59][60]

nature of acquired l history agnostic algorithm for inherited thrombocytopenias was proposed several years ago and it is still valid to orient towards specific disorders [61][62]. History and clinical examination are crucial for patients with syndromic forms, whereas cell counting and the examination of peripheral blood films may guide diagnosis in non-syndromic forms [63]. However, in most cases genetic studies are required to confirm the diagnostic suspicion [3][64]. Here we propose a diagnostic flow chart for diagnosis of IT.

3.2. Clinical Examination

The first step for IT diagnosis is a careful clinical evaluation of the proband, including the personal and family bleeding history. Treatment with drugs (continuous or intermittent), recent infection, previously diagnosed haematologic disease, nonhaematologic diseases known to decrease platelet counts (e.g., eclampsia, sepsis, DIC, anaphylactic shock, hypothermia, massive transfusions), recent live virus vaccination, poor nutritional status, pregnancy, recent organ transplantation from a donor sensitized to platelet alloantigens and recent transfusion of a

platelet-containing product in an allosensitized recipient should be excluded. Thrombocytopenia and/or bleeding history in other family members support the hypothesis of an IT, however a negative family history does not exclude it because some forms are recessive or derive from de novo mutations.

The most severe ITs, such as congenital amegakaryocytic thrombocytopenia or biallelic BSS, are typically identified early in infancy because of bleeding diathesis, while for several ITs spontaneous bleeding is absent or very mild explaining why they are often recognized in adult life.

Besides hemorrhagic manifestations, physical examination should also explore other organs/systems abnormalities for syndromic ITs.

In most syndromic forms the associated manifestations are present since the first months of life, such as in CAMT, Jacobsen and Wiskott–Aldrich syndrome and thrombocytopenia with absent radii, while in others they may become apparent later in life, such as renal failure in MYH9-RD, and in the latter case their genetic origin may be missed.

3.3. Laboratory Tests

At the first identification of thrombocytopenia, “pseudothrombocytopenia”, a relatively common artifactual phenomenon caused by platelet clumping in the test tube due to the presence of EDTA (ethylenediaminetetraacetic acid) as anticoagulant accounting for 0.07% to 0.27% of all cases of isolated thrombocytopenia, should be excluded [65].

Evaluation of peripheral blood smears can guide the diagnostic workup because 29 of the 41 forms that have been identified so far display morphological abnormalities of platelets, granulocytes, and/or erythrocytes [63].

When platelet size is reduced, X-linked thrombocytopenia (XLT), WAS and ITs associated with variants in FYB and PTPRJ should be considered [66]. When platelet size is enhanced MYH9-RD, BSS, GPS, thrombocytopenia linked to DIAPH1, FLNA, GATA-1, GNE, TUBB-1, GFI1b, PRKACG, SLF14, TRPM7, TPM4 and ACTN1, Paris-Trousseau thrombocytopenia, PT-VWD, ITGA2B/ITGB3-RT or thrombocytopenia associated with sitosterolemia should be considered. Among these, giant platelets characterize MYH9-RD, bBSS and TUBB1-RT. ITs associated with a normal platelet size instead are ATRUS, SRC-RT, TAR, thrombocytopenia and erythrokeraderma, CYCS-RT, FLI1-RT, IKZF5-RT, THPO-RT, ANKRD26-RT, CAMT, ETV6-RT and FPD/AML.

Abnormality of platelet granules may be observed in some ITs, with reduced or absent granules with enlarged platelets in GPS and GFI1b-RT and with reduced granules with normal-sized platelets in ANKRD26-RT [8][18][67].

Immunofluorescence performed on blood smears has recently been proposed as a method to identify defective membrane protein expression, disturbed distribution of cytoskeletal proteins, and reduction of α or delta granules, however this method requires interlaboratory validation [63].

Classic tests of platelet function, such as aggregometry (light transmission or impedance aggregometry), flow cytometry, secretion assays, electron microscopy and western blotting, may help for some ITs as subsequent steps in the diagnostic algorithm (Table 3) [13][68][69][70].

Table 3. Main features of inherited thrombocytopenias.

Form	Disease	Inheritance	Degree of Thrombocytopenia	Key Laboratory Features	References
Syndromic	Amegakaryocytic thrombocytopenia with radio-ulnar synostosis (ATRUS)	AD	severe	Normal platelet size and morphology	[14][15]
	Baraitser–Winter syndrome 1 with macrothrombocytopenia	AD	absent	Macrothrombocytopenia; leukocytosis with eosinophilia, leukopenia	[22]
	FLNA-related thrombocytopenia	XL	moderate	Macrothrombocytopenia; impaired platelet aggregation GPVI-triggered; heterogeneous α-granules, occasionally giant; abnormal distribution of FLNa	[27]
	GATA-1-related disease	XL	severe	Macrothrombocytopenia; reduced platelet aggregation by collagen and ristocetin; reduced α-granule content and release	[12]
	GNE-related thrombocytopenia	AR	from mild to severe	Macrothrombocytopenia	[43]
	Gray platelet syndrome	AR	moderate/severe	Macrothrombocytopenia; grey or pale platelets; dyserytropoiesis; absence of α-granules; defective TRAP-induced platelet aggregation	[18]
	Paris-Trousseau thrombocytopenia, Jacobsen syndrome	AD	severe	Macrothrombocytopenia; defective platelet aggregation by thrombin; giant α-granules	[10]

Form	Disease	Inheritance	Degree of Thrombocytopenia	Key Laboratory Features	References
	Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease	AR	moderate	Small platelets; eosinophilia; reduced platelet spreading; decreased platelet dense granules	[24]
	PTPRJ-related thrombocytopenia	AR	moderate/severe	Microthrombocytopenia; impaired activation by the GPVI-specific agonist convulxin and the thrombin receptor-activating peptide but normal response to ADP	[46]
	SRC-related thrombocytopenia	AD	moderate/severe	Platelets deficient in granules and rich in vacuoles	[45]
	Stormorken syndrome	AD	moderate/severe	Howell-Jolly bodies in red blood cells; enhanced annexin V binding, defective GPIIb/IIIa activation (PAC-1)	[36]
	Takenouchi-Kosaki syndrome with macrothrombocytopenia	AD	absent	Macrothrombocytopenia, abnormal platelet spreading and filopodia formation	[42]
	Thrombocytopenia-absent radius syndrome (TAR)	AR	severe	Normal platelet size and morphology, thrombocytopenia	[19]
	Thrombocytopenia and erythrokeraderma	AR	moderate	Thrombocytopenia and presence of 3-keto-dihydrosphingosine in plasma	[32]
	Thrombocytopenia, anemia and myelofibrosis	AR	mild/moderate	Macrothrombocytopenia, anemia	[34]
	Wiskott–Aldrich syndrome	XL	severe	Microthrombocytopenia; Reduced α/δ granules release	[40]
	X-linked thrombocytopenia	XL	mild/moderate	Microthrombocytopenia; Reduced α/δ granules	[40]

Form	Disease	Inheritance	Degree of Thrombocytopenia	Key Laboratory Features	References
				release	
Non-syndromic	ACTN1-related thrombocytopenia	AD	mild	Macrothrombocytopenia	[23]
	Bernard Soulier syndrome monoallelic biallelic	AD AR	mild moderate/severe	Macrothrombocytopenia; lack of platelet agglutination to ristocetin with normal aggregation to other agonists; severe reduction or complete lack of GPIb/IX/V	[28]
	CYCS-related thrombocytopenia	AD	mild	Normal platelet size and morphology	[25]
	FLI1-related thrombocytopenia	AD/AR	moderate	Reduced platelet aggregation in response to collagen and PAR-1 agonists; δ-granule deficiency	[10]
	FYB-related thrombocytopenia	AR	moderate/severe	Microthrombocytopenia; increased expression of P-selectin and PAC-1 by resting platelets but impaired upon stimulation with ADP	[11]
	GFI1b-related thrombocytopenia	AD/AR	mild/moderate	Macrothrombocytopenia; dyserytropoiesis; reduced α-granule content and release; diminished expression of GPIbα, red cell anisocytosis	[13]
	IKZF5-related thrombocytopenia	AD	absent	Thrombocytopenia; deficiency of platelet alpha granules.	[16]
	ITGA2B/ITGB3-related thrombocytopenia	AD	mild/moderate	Macrothrombocytopenia; reduced GPIIb/IIIa; defective GPIIb/IIIa activation (PAC-1)	[30][31][49]
	PT-VWD	AD	mild/moderate	Macrothrombocytopenia; increased response to ristocetin and decreased	[31][71][72]

Form	Disease	Inheritance	Degree of Thrombocytopenia	Key Laboratory Features	References
				VWF-ristocetin cofactor activity (VWF:RCo) Mixing tests discriminate the plasmatic (VWD type2B) from platelet (PT-VWD) origin of hyperreactivity to ristocetin	
	PRKACG-related thrombocytopenia	AR	severe	Macrothrombocytopenia; defective platelet $\alpha_{IIb}\beta_3$ activation and P-selectin exposure in response to TRAP6; defective Ca^{2+} mobilization in response to thrombin	[35]
	THPO-related thrombocytopenia	AD	mild	Normal or slightly increased platelet size	[21]
	TRPM7-related thrombocytopenia	AD	mild/moderate	Macrothrombocytopenia; aberrant distribution of granules	[37]
	Tropomyosin 4 (TPM)-related thrombocytopenia	AD	mild	Macrothrombocytopenia	[38]
	TUBB-1-related thrombocytopenia	AD	mild	Macrothrombocytopenia; platelet anisocytosis	[39]
	SLFN14-related thrombocytopenia	AD	mild/moderate	Macrothrombocytopenia; δ-granule deficiency with decreased ATP secretion in response to ADP, collagen and TRAP-6	[44]
Forms predisposing to additional diseases	ANKRD26-related thrombocytopenia	AD	mild/moderate	Reduced α-granules in some patients	[8]
	Congenital amegakaryocytic thrombocytopenia (CAMT)	AR	severe	Elevated serum levels of TPO	[17]
	DIAPH1-related thrombocytopenia	AD	mild/severe	Macrothrombocytopenia	[26]

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of platelet glycoproteins by flow cytometry, using a well-defined set of antibodies, is the gold standard for the diagnosis of biallelic and monoallelic BSS, *ITGA2B/ITGB3*-RT and *GFI1B*-RT.

The measurement of platelet granule content and secretion can reveal alterations, e.g., in WAS and thrombocytopenia with absent radii (TAR) a reduced number of dense-granules has been reported, GPS is characterized by absent or reduced α-granules [73], Paris-Trousseau (PTS) and Jacobsen syndromes show

Form	Disease	Inheritance [10][74]	Degree of Thrombocytopenia	Key Laboratory Features	References
[13]	ETV6-related thrombocytopenia	AD	mild/moderate	Decreased ability of platelets to spread on fibrinogen covered surfaces; abnormal clot retraction	[9]
	Familial platelet disorder with predisposition to hematological malignancies (FPD/AML) [27]	AD	moderate	Abnormal aggregation in response to multiple agonists; δ (occasionally α)-granule deficiency	[20]
	MYH9-related disease	AD	mild/severe	Macrothrombocytopenia; Döhl-like body cytoplasmic leukocyte inclusions	[33]
	Thrombocytopenia associated with sitosterolemia		moderate/severe	Macrothrombocytopenia; hyperactivatable platelets with constitutive binding of fibrinogen to $\alpha_{IIb}\beta_3$ integrin; shedding of GPIbα; impaired platelet adhesion to von Willebrand factor [75]	[41]

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Until a few years ago, in fact, when the inherited nature of thrombocytopenia was suspected, a series of laboratory tests (e.g., flow cytometry for platelet surface GPs, examination of peripheral blood smear and immunofluorescence assay for MYH9 protein aggregates in neutrophils, platelet aggregometry) were performed to orient towards the candidate gene/genes to be sequenced by Sanger sequencing [61]. The application of high throughput sequencing (HTS) techniques to platelet disorders has allowed for the discovery of several novel genes associated with IT in a few years and has opened the possibility of approaching IT diagnosis by a single-step strategy. In fact, the simultaneous screening of several genes by targeted sequencing platforms, whole exome sequencing (WES) or whole genome sequencing (WGS) has been shown to provide diagnosis in 30% to 50% of patients with suspected IT [76][77][78]. Indeed, HTS is being proposed as a first line diagnostic investigation by an increasing number of authors [77][78][79][80]. However, the interpretation of genetic variants is challenging and requires a careful expert team evaluation in light of a well characterized patient phenotype [79] and when new variants in diagnostic-grade (TIER1) genes are found by targeted sequencing, WES or WGS or new genes are identified by WES or WGS it is essential that rigorous guidelines (i.e., the ACMG guidelines [81]) are applied to confirm their pathogenicity [79]. No guidelines are available yet regarding which suspected IT patients should undergo genetic testing. Some ITs with pathognonomic laboratory or clinical features, such as BSS, TAR, *GATA1*-RD, ATRUS, Stormorken syndrome and WAS, can be clearly diagnosed without the need of genetic testing. For other ITs, for which a strong genotype–phenotype correlation has been described, e.g., *MYH9*-RD, genotyping may be advisable for prognostic evaluation and possible preventive intervention. Other forms that do not have any

specific diagnostic, clinical or laboratory features would require genetic testing for definite diagnosis. However, for some of these, e.g., *ACTN1*-RT or *TUBB1*-RT, a genetic diagnosis does not have any significant impact on patient management, while for others it may inform patients monitoring and treatment. Among these there are thrombocytopenias with normal platelet volume, including forms like FPD/AML, *ANKRD26*-RT and *ETV6*-RT which are predisposed to hematological malignancies (Figure 2). There are ethical implications of detecting variants in these genes and other unexpected genetic defects, such as a carrier status of a recessive gene. It is thus recommended to strictly follow an informed consent protocol ensuring that patients comprehend the possible implications of unsolicited genetic findings [80].

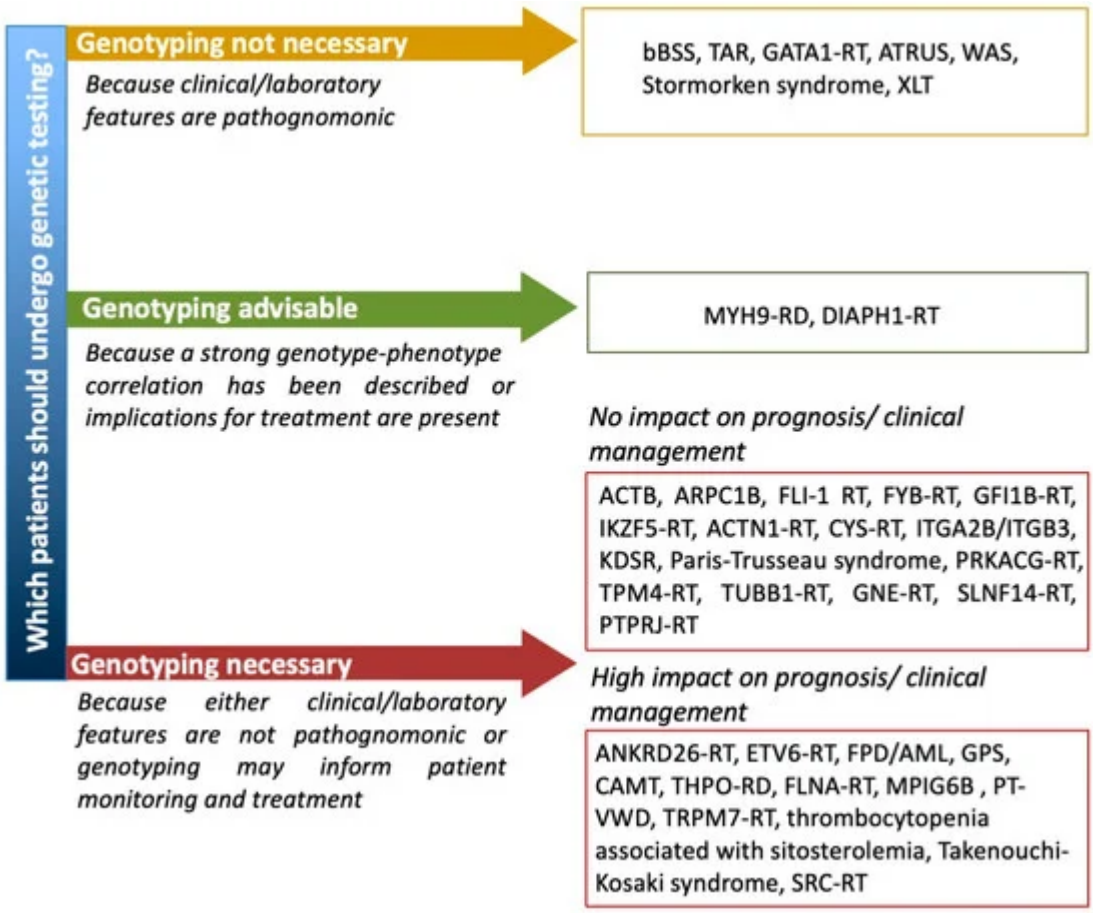

Figure 2. Proposal of a flow chart guiding the use of genetic testing for patients with suspected IT. ACTB = Baraitser–Winter syndrome 1 with macrothrombocytopenia, ARPC1B = Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease, ATRUS = amegakaryocytic thrombocytopenia with radio-ulnar synostosis, bBSS = biallelic Bernard Soulier syndrome, CAMT = congenital amegakaryocytic thrombocytopenia, MPIG6B = thrombocytopenia, anemia and myelofibrosis, PT-VWD = platelet-type von Willebrand disease, RD = related disorder, RT = related thrombocytopenia, TAR = thrombocytopenia with absent radii, XLT = X-linked thrombocytopenia, WAS = Wiskott–Aldrich syndrome.

In summary, the optimal diagnostic approach to ITs is still being debated and a combination of clinical/traditional laboratory approach with advanced gene sequencing techniques may provide the highest rate of diagnostic success [64], and the best patient management.

3.5. Undefined Aspects and Possible Future Research Lines

A consensus on the classification of ITs has not been reached yet, but it would be highly advisable to avoid, for example, ambiguity on disease nomenclature.

A guidance flow chart about which suspected IT patients should undergo genetic testing is not yet available and the generation of consensus documents promoted by the relevant international scientific societies (ISTH, EHA, ASH) is highly warranted.

Moreover, development of guidelines on informed consent documents, reporting of new variants in variant databases to improve variant classification, development of user-friendly interpretation softwares of HTS results, promotion of research for discovery of new genes causing IT and development of advanced cell-based models to study platelet formation and function are valuable future perspectives.

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