

Congenital disorders associated with platelet dysfunctions

Paquita Nurden, Alan T. Nurden

CRPP/PTIB, Hôpital Xavier Arnoz, Pessac, France

Summary

Genetic defects of the megakaryocyte lineage give rise to bleeding syndromes of varying severity. Blood platelets are unable to fulfill their hemostatic function of preventing blood loss on vessel injury. Spontaneous bleeding is mostly mucocutaneous in nature. Most studied are deficiencies of glycoprotein (GP) mediators of adhesion (Bernard-Soulier syndrome) and aggregation (Glanzmann thrombasthenia) which concern the GPIb-IX-V complex and the integrin α IIb β 3, respectively. Defects of primary receptors for stimuli include the P2Y₁₂ADP receptor pathology. Agonist-specific deficiencies in the platelet aggregation response and abnormalities of signaling pathways are common and lead to trauma-related bleeding. Inherited defects of secretion from storage organelles, of ATP production, and of the generation of procoagulant activity are also encountered. In some disorders, such as the Chediak-Higashi, Hermansky-Pudlak, Wis-

kott-Aldrich and Scott syndromes, the molecular lesion extends to other cells. In familial thrombocytopenia (FT), platelets are produced in insufficient numbers to assure haemostasis. Some of these disorders affect platelet morphology and give rise to the so-called 'giant platelet' syndromes (*MYH9*-related diseases) with changes in megakaryocyte maturation within the bone marrow and premature release of platelets. Diseases of platelet production may extend to other cells and in some cases interfere with development. Transfusion of platelets remains the most common treatment of severe bleeding, management with desmopressin is common for mild disorders. Substitute therapies are available including rFVIIa and the potential use of TPO analogues for FT. Stem cell or bone marrow transplantation is being used for severe diseases while gene therapy may be on the horizon.

Keywords

Platelets, inherited disorders, platelet function, platelet production, treatment

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Introduction

This review will detail the most common inherited disorders affecting platelet function and platelet production (1–3). They give rise to bleeding syndromes of varying intensity. Spontaneous bleeding is mostly mucocutaneous in nature; excessive trauma-related bleeding is a feature of milder forms. Studies on these disorders have provided essential information on the mechanisms of platelet function and platelet production. Figure 1 illustrates the principal disorders affecting platelet surface constituents, while Figure 2 shows those affecting intracellular components. Table 1 summarizes the major inherited thrombocytopenias. A final section of this review deals with treatment.

Defects of platelet adhesion

Bernard-Soulier syndrome (BSS)

BSS is a severe bleeding disorder characterized by thrombocytopenia, decreased platelet adhesion, abnormal prothrombin

consumption, reduced platelet survival and giant platelets (1, 3). Platelet counts can descend to 20,000/ μ l. Electron microscopy (EM) shows many cytoplasmic vacuoles and membrane complexes in the giant platelets, abnormalities that extend to megakaryocytes (MKs). The disease is characterized by an abnormal platelet attachment to subendothelial-bound von Willebrand factor (VWF) due to quantitative or qualitative defects of the GPIb-IX-V complex. GPIb α contains the VWF binding site and two thrombin-binding sites located within the N-terminal domain (4, 5). An additional absence of GPIb α -related binding sites for P-selectin, TSP1, factor XI, factor XII, α M β 2 and high-molecular-weight kininogen may also contribute to the disease phenotype (6). The products of four separate genes (*GPIBA*, *GPIBB*, *GP9* and *GP5*) assemble within the maturing MK in the bone marrow to form the GPIb-IX-V complex as present in the platelet membrane. Mutations within *GPIBA*, *GPIBB* and *GP9* mostly prevent constitution and/or trafficking of the complex through the Golgi apparatus and endoplasmic reticulum (ER) (7). In occasional rare variant forms of BSS, platelets express

Correspondence to:
Alan T. Nurden
CRPP/PTIB, Hôpital Xavier Arnoz
33604 Pessac, France
Tel.: +33 5 57 10 28 51, Fax: +33 5 57 10 28 64
E-mail: Alan.Nurden@cnrshl.u-bordeaux2.fr

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Table 1: Inherited thrombocytopenias: genetic mutations and associated phenotype.

Syndrome	Affected gene chromosomal location	Inheritance	Associated phenotype
<i>MYH9</i> -related diseases May-Hegglin anomaly, Fechtner, Epstein & Sebastian syndromes	<i>MYH9</i> 22q12–13	AD	Various combinations of leukocyte inclusions, deafness, nephritis, cataracts. Large platelets
Mediterranean macrothrombocytopenia	<i>GPIBA</i> , possibly others 17p13	AD	Large platelets
Bernard-Soulier syndrome	<i>GPIBA</i> , <i>GPIBB</i> , <i>GP9</i> 17p13, 22q11 and 3q21	AR	Giant platelets, platelet adhesion defect, abnormal aggregation to thrombin
Platelet-type VWD	<i>GPIBA</i> 17p13	AD	Enlarged platelets. Defective adhesion due to spontaneous binding of VWF to GPIb α and cleavage of VWF multimers
Familial platelet disorder/acute myelogenous leukemia	<i>RUNX1</i> (<i>CBFA2</i> , <i>AML1</i>) 21q22	AD	Myelodysplasia, propensity for leukemia. Platelet dysfunction
Chromosome 10/THC2	<i>FLJ14813</i> 10p11–12	AD	None
Paris-Trousseau / Jacobsen syndromes	Hemizygous deletion including <i>FLI1</i> 11q23	AD	Cardiac and facial defects, mental retardation Enlarged platelets and large granules
Gray platelet syndrome	Unknown	Mostly recessive	Myelofibrosis, Enlarged platelets with no α -granules, platelet dysfunction
Congenital amegakaryocytic thrombocytopenia (CAMT)	<i>c-MPL</i> 1p34	AR	Severe thrombocytopenia at birth. Progressive aplasia
Thrombocytopenia and absent radii (TAR)	Large deletion 1q21.1	AR	Shortened/absent radii bilaterally
Amegakaryocytic thrombocytopenia with radio-ulnar synostosis	<i>HOXA11</i> 7p15–14	AD	Fused radius, incomplete range of motion
Wiskott-Aldrich syndrome	<i>WAS</i> Xp11.23-p11.22	X-linked	Immunodeficiency, eczema, lymphoma, small platelets. Defective platelet and lymphocyte function
X-linked thrombocytopenia (XLT)	<i>WAS</i>	X-linked	Small platelets, no immune problems
GATA-1-related thrombocytopenia with dyserythropoiesis	<i>GATA1</i> Xp11.23	X-linked	Dyserythropoiesis \pm anemia, β -thalassemia in some patients (XLTT). Platelet dysfunction, large platelets

AR, autosomal recessive inheritance; AD, autosomal dominant inheritance. The DiGeorge/Velocardiofacial syndrome is occasionally familial but largely *de novo* in origin and is not included in the Table. Type 2B VWD is accompanied by a familial thrombocytopenia in some families.

VWD and be associated with a moderate thrombocytopenia. This clinical condition resembles type 2B VWD and its diagnosis requires care.

Type 2B VWD

Although not generally recognized, giant platelets and thrombocytopenia can occur in type 2B VWD (given by mutations within exon 28 of the *VWF* gene) (10). In rare families, severe macrothrombocytopenia is associated with the presence of circulating platelet aggregates. In one such family (with an R1308P VWF substitution), platelets showed signs of apoptosis with an altered expression of Ca²⁺-ATPases and signs of caspase-3-mediated poly (ADP-ribose) polymerase (PARP) hydrolysis (11). Interestingly, culture of CD34+ cells from the peripheral blood of a member of this family resulted in an unusual surface expression of the mutant VWF on mature MKs and intertwined proplatelets. Early association of neosynthesized VWF with GPIb may be responsible for this finding and the probable pro-

duction of platelets from immature MKs. The fact that increased platelet size occurs both in the absence of GPIb α (BSS) and when GPIb-VWF interactions are abnormal suggests that cross-talk between MKs and their environment is a key step for normal platelet production.

Inherited variants of agonist receptors and signaling pathways

Deficient collagen receptor functions

Integrin $\alpha 2\beta 1$ is a platelet surface collagen receptor that is shared with a wide variety of cell types. Although excessive bleeding linked to $\alpha 2\beta 1$ deficiency has been hinted at in the past, proof for a specific pathology is still lacking. Natural changes in receptor density are given by single nucleotide polymorphisms (SNPs) in both coding and noncoding regions of the $\alpha 2$ gene (12). Specific haplotypes can account for up to five-fold changes

in platelet surface $\alpha 2\beta 1$ expression and affect the collagen response. Some patients lacking a collagen-induced aggregation response have platelets deficient in GPVI, a member of the immunoglobulin superfamily of cell membrane receptors (13). Platelet expression of GPVI is also under the control of SNPs and epigenetic factors while acquired antibodies can induce shed-dase activity by members of the metalloproteinase-disintegrin (ADAM) family and loss of GPVI (14, 15). The platelet-collagen interaction in flowing blood occurs through a multistep process with an involvement of both $\alpha 2\beta 1$ and GPVI whose signaling capacity requires FcR γ -chain. The possibility that rare variant function haplotypes or acquired loss of receptors influence this reactivity has to be taken into account when examining disease states suspected to be given by mutations in the collagen receptor genes.

Pathologies of ADP and ATP receptors

Platelets possess two classes of purinergic receptor for ADP: P2Y₁ that mediates Ca²⁺-mobilization and shape change; and P2Y₁₂, responsible for macroscopic platelet aggregation. P2Y₁ and P2Y₁₂ belong to the seven transmembrane domain family of G-protein-linked receptors. Rare patients with an autosomal recessive hereditary disease linked to a much decreased and reversible platelet aggregation to ADP (despite a normal shape change and Ca²⁺-mobilisation) have been much studied. A specific receptor defect was confirmed when analysis of PCR products from the P2Y₁₂ coding region of genomic DNA of a French patient revealed a mutant allele at this locus (16). Mutations in other patients have since been described (17). In this pathology, platelets show identical functional changes to normal platelets treated with the anti-platelet drugs ticlopidine and clopidogrel for which P2Y₁₂ is the molecular target. So far, no human pathology of the P2Y₁ receptor has been reported. A synergistic role of ADP in the platelet response to low doses of other stimuli also means that platelets with dysfunctional P2Y₁₂ show a decreased sensitivity to agonists such as thromboxane A₂ (TXA₂), collagen and thrombin. P2X₁, a purinergic receptor for ATP, modulates platelet intracellular Ca²⁺ levels in response to other agonists (18) and is another potential pathologic target. A natural dominant negative P2X₁ receptor disorder due to a deletion of a single amino acid has been described in a young girl with a bleeding syndrome (19).

Altered function of other receptors for primary agonists

A defective platelet aggregation to TXA₂ in Japanese families was linked to an Arg₆₀ → Leu substitution in the TXA₂ receptor (20). This receptor is present in two isoforms in platelets, differing only in their carboxyl-terminal tails and in their capacity to activate adenylate cyclase. The mutated α -form results in impaired signal transmission. An absent platelet response to adrenaline is often seen in routine screening of platelet function although its contribution as a cause of excessive bleeding remains unknown. While congenital defects of the α_2 -adrenergic receptor associated with a decreased platelet response to adrenaline have been reported (21), such findings may reflect the complex haplotypes now known to exist within this gene (22). Similarly, while pathologies of receptors for platelet activating factor (PAF) and

for serotonin have been described in the context of inflammation, depression and even eating disorders (23, 24), their possible contribution to bleeding syndromes is unknown. All of these receptors belong to the seven transmembrane domain receptor family of which other major examples in human platelets are the thrombin receptors, PAR-1, and PAR-4. So far, human pathologies of the PAR receptors have not been described.

Defects that relate to abnormalities within intracellular signaling pathways

Platelet pathologies involving the signal transduction pathways into which surface receptors are locked mostly concern patients with mild bleeding disorders and defects of platelet aggregation which affect some stimuli more than others. Already described are patients with i) an impaired Ca²⁺ mobilisation, ii) defective inositol-1,4,5-triphosphate (InsP₃) production and a reduced phosphorylation of the protein, plekstrin, by protein kinase C, iii) a deficiency of the phospholipase C- $\gamma 2$ isoform, and iv) a specific decrease in platelet membrane G α_q and platelets that respond less well to several agonists including a decreased activation of $\alpha IIb\beta 3$ (reviewed in [25]). These initial reports of abnormalities in signalling pathways are the first of what may prove to be a long list of disorders of platelets. Recent surveys within our Reference Center have found that such patients may be much more common than was once assumed, possibly because of an increased awareness of their possible presence and of an improved diagnostic capacity. To highlight them, some examples of abnormal function testing in platelets from patients studied in our laboratory are shown in Figure 3. A major effort is underway to uncover the genetic defects responsible for these phenotypes.

Enzyme deficiencies

Patients with congenital deficiencies of cyclooxygenase-1, prostaglandin H synthetase-1, thromboxane synthetase, lipoxigenase, glycogen-6 synthetase and of ATP metabolism have all been reported and lead to platelet function abnormalities often resembling those seen in storage pool disease or after aspirin ingestion (see [25]).

Defects of secretion (storage pool disease, SPD)

This heterogeneous collection of inherited disorders contain some well-characterized examples of intracellular defects of platelets (Fig. 2); some disorders are due to defects in genes that encode a protein whose function extends to several cell types but where from a haemostasis point of view the defect mostly concerns secretion-dependent aggregation.

Defects of α -granules

These are the storage site for proteins that are either synthesized in MK or endocytosed from plasma. The organelle membranes contain a variety of glycoproteins (e.g. P-selectin and CD63) that are translocated to the plasma membrane during secretion. Specific deficiencies of α -granule-stored proteins may be associated with inherited deficiencies of the corresponding plasma proteins (e.g. factor V deficiency, fibrinogen in afibrinogenemia, VWF in type 3 VWD). Only disorders unique to the α -granule pool will be described here.

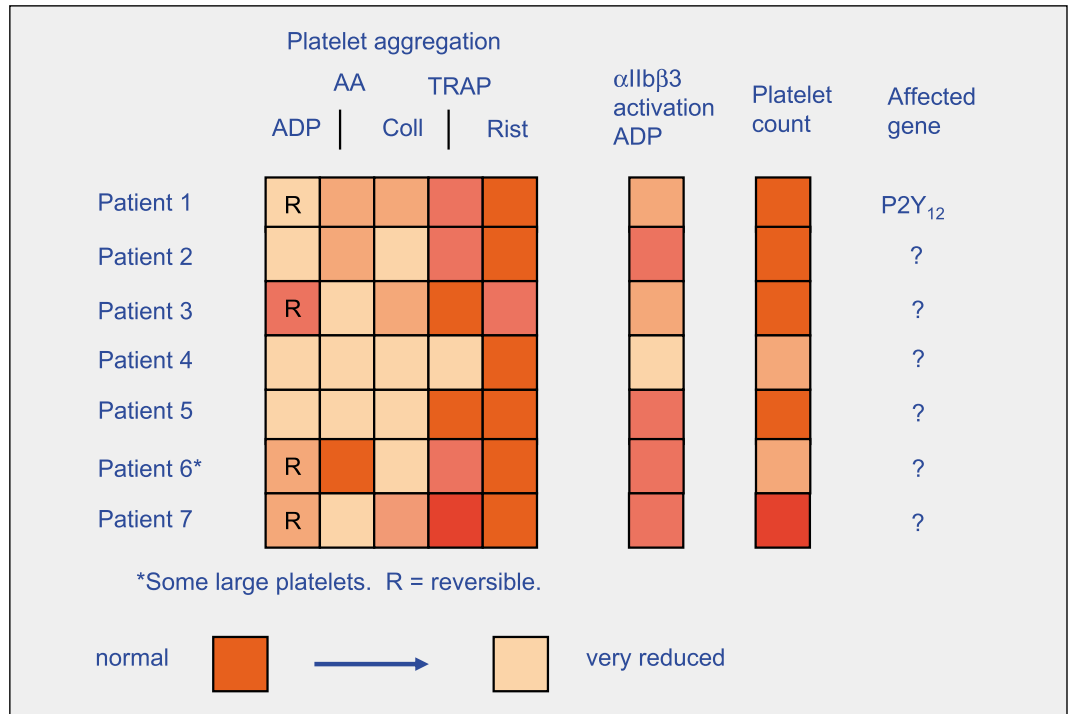


Figure 3: Checkerboard showing the differential aggregation responses of a series of patients with platelet aggregation abnormalities. Note the large diversity. Activation of α IIb β 3 was measured using PAC-1 binding by flow cytometry. Only for patient 1, with a mutation in the P2Y₁₂ gene (14), is the genetic defect known.

Gray platelet syndrome (GPS)

A mild bleeding disorder with mostly autosomal recessive inheritance, GPS is characterized by the absence of α -granules and their contents (26). The basic molecular defect appears to involve packaging or storage of proteins during α -granule biogenesis in MK. Another feature is the early onset of myelofibrosis, a finding attributed to the spontaneous release from MK of newly synthesised growth factors. GPS patients are often moderately thrombocytopenic and platelets somewhat enlarged (Table 1). A negative regulation of megakaryocytopoiesis by spontaneously released cytokines/chemokines and/or other proteins is likely. There is a tendency for secretion-dependent platelet aggregation to be abnormal, but while thrombin-induced platelet aggregation is particularly affected in some patients, in others it is the collagen response that is lacking. The latter is linked to a reduced platelet content of GPVI speculated to be due to an abnormal sheddase activity by members of the ADAM family (27).

The genetic defect responsible for GPS has yet to be described, a recent report of X-linked GPS due to a GATA1 Arg216Gln mutation is atypical (28). Other variant disorders affecting α -granules include the White platelet syndrome and the Medich giant platelet disorder where platelets also have scroll-like membranous inclusions (29).

Quebec platelet disorder

This autosomal dominant bleeding disorder was first described in two French-Canadian families. Platelets show protease-related degradation of many α -granule proteins (including P-selectin) even though α -granule ultrastructure is preserved (30, 31). Thrombocytopenia is sometimes observed. A platelet aggregation deficiency is most striking with epinephrine, the reason for this is unknown. The fact that bleeding responds to fibrino-

lytic inhibitors rather than platelet transfusions led to the discovery that platelets in this disorder possessed unusually large amounts of urokinase-type plasminogen activator, a protein that is released on platelet activation (32).

ARC syndrome

Mutations in *VPS33B*, encoding a regulator of SNARE-protein-dependent fusion have been described in the arthrogyryposis-renal dysfunction-cholestasis (ARC) syndrome. Mainly affecting young children, platelet dysfunction and low granule content are associated with a multisystem disorder featuring renal tubular and other dysfunctions. The defect extends to both stored and membrane components of α -granules (33).

Defects of dense (δ) granules

These are storage sites for serotonin and the nucleotides ADP and ATP. SPD affecting dense granules may be a common cause of defects of secretion-dependent aggregation. The granule deficiency may be severe or partial, in some patients it may also extend to α -granules ($\alpha\delta$ -storage pool deficiency) (25, 34). When platelet deficiencies of dense granules are associated with abnormalities of other lysosome-related organelles, they lead to clearly defined phenotypes. This is the case in the Hermansky-Pudlak, Chediak-Higashi and Griscelli syndromes where melanosomal defects cause a lack of pigmentation of the skin and hair.

Hermansky-Pudlak syndrome (HPS)

Here, oculocutaneous albinism is an additional feature as is ceroid-lipofuchsin storage in the reticulo-endothelial system; granulomatous colitis or fatal pulmonary fibrosis may occur in some cases (35). HPS is common on the island of Puerto Rico

where a 16-base duplication in exon 15 of the HPS-1 gene, encoding a 79 kDa protein with two membrane spanning domains, predominates and results in a frameshift. Defects in at least eight genes (HPS-1 through HPS-8) are now known to cause distinct HPS subtypes in man. The HPS proteins interact with each other in complexes called BLOCS; the genetic defects disrupt these, thereby affecting organelle biosynthesis and protein trafficking (36). In HPS-2, it is the beta3A subunit of the AP-3 adaptor complex that is abnormal. One manifestation is an increased routing of lysosomal membrane proteins such as CD63 to the plasma membrane. HPS-2 is associated with innate immunity defects (37).

Chediak-Higashi syndrome (CHS)

Here, a bleeding syndrome is associated with severe immunologic defects and progressive neurological dysfunction if the patient survives to adulthood (35). The immunodeficiency leads to the development of a lymphoproliferative syndrome and an accelerated phase in ~90% of patients. The hallmark of CHS is the presence of giant inclusion bodies in a variety of granule-containing cells including platelets. The CHS gene (*LYST*) has been cloned and a series of frameshift and nonsense mutations were initially described that resulted in a truncated CHS protein and a severe phenotype (38). Rare missense mutations may be associated with a milder form of the disease (39). *LYST* is a large protein with distinct structural domains including 'BEACH' and 'HEAT' suggestive of a function in membrane contact interactions and organelle protein trafficking.

GrisCELLI syndrome

Patients have partial albinism and silver hair. Different subtypes associate neurological defects and/or severe immunodeficiency with a defective cytotoxic activity of lymphocytes. The major clinical difficulty is a fatal hemophagocytic syndrome caused by inappropriate lymphoid-cell activation and cytokine release. GrisCELLI disease is given by mutations in the genes encoding myosin Va, *Rab27a* (a small GTPase), or melanophilin (35). Only mutations in *Rab27a* are associated with immune deficiency. Differential diagnosis with HPS type II can be difficult. This is shown by the case of a child with a heterozygous *Rab27a* mutation, but with bleeding and an impaired secretion-dependent platelet aggregation (40). While a novel homozygous *AP3B1* mutation was detected, the patient subsequently developed fulminant hemophagocytic lymphohistiocytosis that was resistant to therapy.

Wiskott-Aldrich syndrome (WAS)

This X-linked recessive disease combines thrombocytopenia and small platelets with eczema, recurrent infections due to immune deficiency and an increased risk for autoimmunity and malignancy. A milder form without the immune problems is known as hereditary X-linked thrombocytopenia. WAS platelets aggregate poorly and have a low granule number. T lymphocytes among other blood cells also show defective function. The *WAS* gene is composed of 12 exons and encodes a 502 amino acid signaling protein termed WASp. Genetic defects in *WAS* result either in the decreased expression of WASp or its absence. While mutations in exons 1 and 2 mostly give rise to hereditary

X-linked thrombocytopenia, this is probably due to the high prevalence of missense mutations and a partially expressed and functional protein (41, 42). WASp is a key regulator of actin polymerization in hematopoietic cells; it is involved in signal transduction with tyrosine phosphorylation sites and adapter protein function. WAS could be considered as a pathology of the cytoskeleton. Deficiency of WASp induces premature proplatelet formation in the marrow where a lack of actin-rich podosomes slows down MK migration to the vascular sinus (43). Mutations in *WAS* that lead to spontaneously activated WASp with increased actin polymerizing activity give rise to an X-linked form of neutropenia with an intrinsic failure of myelopoiesis through defective mitosis and cytokinesis (44).

Glanzmann thrombasthenia (GT)

Platelets from patients with GT fail to aggregate due to quantitative or qualitative defects of the integrin, α IIb β 3. In normal haemostasis, α IIb β 3 on activated platelets binds the adhesive proteins which form the protein bridges that link platelets during aggregation. The principal of these are fibrinogen (Fg) and, under conditions of high flow, VWF, but fibronectin, vitronectin and CD40L may all have a role. Although GT platelets attach to subendothelium after injury, platelet spreading on the exposed surface, a process that involves α IIb β 3, is defective in addition to thrombus build up (45). Clot retraction is often absent.

Analysing platelet surface glycoproteins by flow cytometry will identify α IIb β 3 deficiency; this can be followed by selective sequencing of the *ITGA2B* and *ITGB3* genes. *ITGA2B* spans 17 kb and is comprised of 30 exons, *ITGB3* spans 46 kb and has 15 exons; they colocalize to 17q21–23. Genetic defects occur along the length of both genes. Nonsense mutations and splice site mutations with frameshifts are common, as also are missense mutations giving rise to amino acid substitutions (a comprehensive list is to be found on the GT database: <http://sinaicentral.mssm.edu/intranet/research/glanzmann>). Certain mutations predominate in ethnic groups in Israel and the French gypsy population. But by and large, mutations are specific for each family; they either prevent subunit biosynthesis in MKs or inhibit transport of the precociously formed α IIb β 3 complexes from the ER to the Golgi apparatus and/or their export to the cell surface (46). Occasionally, sufficient mutated α IIb β 3 may be processed to allow at least a partial clot retraction and uptake and storage of plasma Fg into α -granules (another task of α IIb β 3). Analysis of GT is now quite advanced and population studies are underway (47).

The β 3 subunit is also a component of the vitronectin receptor (α v β 3) expressed on many cells, including endothelial cells, osteoclasts, fibroblasts, monocytes and activated B lymphocytes. It has but a minor presence in platelets. In GT, α v β 3 is absent if the genetic lesion affects β 3 production. However, it remains unclear as to whether patients with β 3 gene defects have a distinctive phenotype, for while α v β 3 has been implicated in angiogenesis, no evidence for abnormal vessel development, increased rates of abortion or of susceptibility to cancer has been forthcoming in the human disease. Certainly GT patients are not protected against atherosclerosis (48).

The first report of variant GT with expressed but nonfunctional integrin, described an Asp¹¹⁹->Tyr substitution in β 3, a

mutation which helped to identify an RGD-binding site (see [1]). Studies on other variants then revealed that the codon for Arg²¹⁴ of *ITGB3* is a mutational hotspot and that substitution within the MIDAS domain prevented the expression of the ligand-binding epitope on platelet stimulation. A Ser⁷⁵²->Pro substitution in the cytoplasmic domain of $\beta 3$, or a stop codon leading to a truncated protein containing only the first eight of the 47 aa normally present in the cytoplasmic domain, confirmed a role for integrin cytoplasmic domains in 'inside-out' signaling and activation of α IIb $\beta 3$ (see [1, 3]).

Patients with Cys⁵⁶⁰->Arg and Cys⁵⁹⁸->Tyr mutations in $\beta 3$ have platelets that express residual surface α IIb $\beta 3$ able to spontaneously bind Fg (49, 50). This situation recalls platelet-type VWD where normal vWF multimers spontaneously bind to a mutated GPIb α subunit and block its function. Not least, these results elegantly confirm how the mechanism responsible for activation and perhaps the change from a bent to a straightened conformation are influenced by long range constraints within the tertiary structure of 'inactivated' α IIb $\beta 3$ (51). A young Italian man with what was described as a 'thrombasthenia-like' syndrome associated an aggregation deficiency with mild thrombocytopenia and platelets with size heterogeneity suggesting that megakaryocytopoiesis was also affected. Total platelet α IIb $\beta 3$ content was about 50% of normal, but the surface pool was decreased to about 18%. A heterozygous Arg⁹⁹⁵->Gln substitution in the GFFKR region of the cytoplasmic domain of α IIb was shown in transfection experiments to account for the unusual phenotype (1, 52).

Also to be mentioned are the very rare patients with LAD-III/variant syndrome in which life-threatening bleeding is associated with necrotic nonpassing lesions and poor wound healing in early life. Infections range from bacterial pneumonia and early septicemia to fungal disease. The complex clinical features combine lymphocyte, neutrophil and platelet integrin dysfunction due to mutations in their CalDAG-GEFI gene which abolishes 'inside-out' integrin activation although allowing their expression (53).

GT is discussed in more detail in (1).

Scott syndrome

The Scott syndrome is a rare inherited disorder caused by defective scrambling of phospholipids on blood cells including platelets (54). Originally described by Weiss et al. (55), the disease is manifested by a decreased fibrin formation during shear-dependent adhesion of platelets to subendothelium. Scott platelets when activated are unable to translocate phosphatidylserine (PS) to the outer phospholipid leaflet of the membrane bilayer, with the result that factors Va and Xa fail to bind leading to a decreased capacity of the platelets to convert prothrombin into thrombin. This lack of thrombin generation is sufficient to induce a bleeding syndrome. Stimuli that induce this translocation under physiologic conditions include a thrombin and collagen mixture and complement C5b-9. Microvesiculation, a process that can be readily measured by flow cytometry using FITC-annexin V, accompanies PS expression and is also defective in Scott syndrome. Thus there is a lack of diffusion of procoagulant activity in the circulation. The detection in a Scott syndrome patient of

a heterozygous missense mutation in the adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1), implicated in the exofacial transport of PS, may offer clues on the basis of this disease (56).

Familial thrombocytopenias (FT)

Bleeding syndromes that arise through an inherited defect of platelet production constitute a heterogeneous group of diseases (Table 1) (2, 57). Some disorders included in Table 1 associate a low circulating platelet count with well characterized platelet functional and morphological abnormalities and have been dealt with in preceding sections. Often in FT, platelet dysfunction has not been shown or is considered to be secondary.

Defects in transcription factors

An altered megakaryocytopoiesis resulting from transcription factor defects is a major cause of FT. Abnormalities can also extend to other marrow cells and/or interfere with development. An example is the association of bone marrow failure and skeletal defects attributed to a HOXA11 mutation in two unrelated families. Homeobox genes encode regulatory proteins central to bone morphogenesis as well as haematopoietic cell differentiation (58). In the autosomal dominant Paris-Trousseau syndrome, a decreased platelet production and a mild haemorrhagic tendency are associated with a haplodeficiency of chromosome 11 (deletion at 11q23). Platelets are often enlarged and many have giant α -granules formed by fusion after MK maturation (59). The disease is caused by hemizygous loss of the *FLII* gene. This leads to some cells having pathologically low Fli1 protein levels during what, in the normal situation, is transient monoallelic *FLII* expression at an early stage of MK differentiation (60). The result is a subpopulation of immature cells that fail to reach the platelet production stage. The 11q23 deletion is also seen in the context of Jacobsen's syndrome where patients have congenital heart defects, trigonocephaly, facial dysmorphism, mental retardation and malfunctions of multiple organs. Pancytopenia and/or thrombocytopenia are seen in some but not all of these patients.

X-linked familial dyserythropoietic anaemia and thrombocytopenia occur through mutations in the *GATA-1* gene (2, 57). Platelets are often enlarged and aggregate poorly to collagen (61). *GATA-1* contains two zinc fingers, the C-terminal of which accounts for sequence-specific DNA binding and the N-terminal for both stabilization of DNA binding and for the interaction with FOG-1 (Friend of GATA). Phenotype may be influenced by whether mutations preferentially affect *GATA-1* interaction with DNA or FOG-1. X-linked thrombocytopenia (XLT) without anaemia may be given by *GATA-1* mutations that affect its interaction with FOG1 but which allow *GATA-1* binding to DNA (62). In contrast, a R216Q substitution in the N-terminal finger of *GATA-1* that destabilized binding to palindromic DNA sites but which did not affect its interaction with FOG-1, was associated with red cell abnormalities consistent with β -thalassaemia (XLTT) (63). A low transcription of the *GATA-1* target genes, GPIb α and GPIX is a characteristic of *GATA-1* pathologies and platelets from such patients also display few α -granules (see section on the GPS). Erythrocytes are abnormal in size and shape.

GATA-1 mutations are also associated with Down syndrome acute megakaryoblastic leukemia.

Monoallelic mutations in the gene encoding the hematopoietic transcription factor RUNX1 (CBFA2, AML1) give rise to FT with a predisposition to acute myelogenous leukemia (2, 57). Haplodeficiency, and missense or nonsense mutations interfering with DNA binding, lead to an arrest of MK maturation with an expanded population of progenitor cells (64). Mutated RUNX1 can also heterodimerize with normal protein promoting loss of function. RUNX1 may act as a tumor suppressor. The propensity to develop leukemia probably requires that patients have a higher tendency to develop a second mutation either in RUNX1 or a related gene, a process that may be facilitated by the expanded population of progenitor cells. Impaired platelet aggregation and secretion have been linked to a deficiency of protein kinase- θ and defective phosphorylation of plekstrin and myosin light chain (65). Platelet expression profiling revealed a decreased expression of a series of genes including that of myosin regulatory light chain polypeptide (MYL9) (66).

Defects in megakaryocyte production

In congenital amegakaryocytic thrombocytopenia (CAMT), severe thrombocytopenia at birth rapidly develops into a pancytopenia in most children. Patients have low numbers of MKs in their marrow; TPO is unable to fulfill its normal thrombopoietic role due to abnormalities in the *c-MPL* gene encoding the TPO receptor. Patients with an early development into aplasia are more likely to have frameshift or nonsense mutations and a complete loss of *c-MPL* (67). Missense mutations leading to residual *c-MPL* were said to be associated with a slower progression of the disease, a conclusion that has been challenged (68). Evidence was provided that elevated levels of the inhibitory cytokines, TNF- α and IFN- γ probably contribute to the pancytopenia. Activating mutations in *c-MPL* give rise to familial essential thrombocythemia (69).

Thrombocytopenia with absent radii (TAR syndrome) is a rare congenital defect associating CAMT-like thrombocytopenia and osteodysgenesis with shortened (or absent) forearms due to bilateral radial aplasia. Although other skeletal anomalies can be present, hands and fingers are unaffected. Serum TPO levels are elevated, and platelets of TAR patients fail to respond to recombinant TPO in combination with suboptimal concentrations of platelet activators. Screening the *c-MPL* gene has so far failed to show mutations; a defect in signal transduction has been proposed. A deletion at 1q21.1 may be associated with the disease (70).

Defects of the cytoskeleton and giant platelet syndromes

This group mainly concerns *MYH9*-related diseases affecting the nonmuscle myosin heavy-chain IIA (myosin-IIA) (Table 1). Platelets can be truly giant with distinct ultrastructural modifications that extend to MKs taken from marrow biopsies (see [2]). Phenotypic variations include the association of macrothrombocytopenia with variable combinations of Döhle-like bodies in leukocytes, nephritis, sensorineural hearing loss and cataracts. Distinctive immunofluorescence patterns for myosin-IIA in leukocytes have become a diagnostic test for *MYH9* defects (71).

Mutations often reoccur in unrelated families, and although haplotype analysis can identify common ancestors, a widespread geographical distribution often makes this unlikely (72). Strikingly, the same mutations can be associated with different phenotypes suggesting that the diseases are not truly monogenic. Amino acid substitutions in the head domain (with Ca^{2+} -ATPase activity) are more often associated with deafness and renal disease, while those affecting the rod (and myosin-IIA assembly) often only have a haematological consequence (73). Haploinsufficiency may have a role (71), but other genetic and/or environmental factors probably intervene to determine phenotype. Possibly, II-B and II-C myosin isoforms can compensate for the malfunction of defective II-A in some tissues (74). Decreased MLC phosphorylation and myosin-IIA function in MKs in *MYH9*-related disease could slow MK migration towards the sinusoids as well as blurring the signaling mechanism for proplatelet formation (75).

We have recently reported ultrastructural modifications of platelets including giant forms in patients with mutations in the *FLNA* gene encoding filamin A (76). These mutations are associated with abnormal neuronal migration resulting in periventricular nodular heterotopia (PNH), an X-linked dominant disease. Significantly, filamin A is thought to be the attachment site for GPIIb α in the cytoskeleton.

Intriguingly, macrothrombocytopenia is also associated with sitosterolaemia, a recessively inherited metabolic condition in which the absorption of both cholesterol and plant-derived cholesterol-like molecules in the gut is unrestricted and in excess (77). Mutations in ATP-binding cassette transporters ABCG5 and ABCG8 are at the basis of this disease.

Other causes

A major question concerns the true abundance of inherited disease with a low platelet count, often falsely diagnosed as immune thrombocytopenic purpura (ITP). A recent survey of patients with macrothrombocytopenia in our Reference Center in Bordeaux revealed that a high percentage did not fall into the categories covered by Table 1 suggesting that other molecular causes are frequent. Studies on an N-ethyl-N-nitrosourea mutagenesis screen in mice also point to a wider range of causes of thrombocytopenia. For example, mutations in the cofilin partner *Aip/Wdr1* cause autoinflammatory disease and macrothrombocytopenia (78). Mouse models have also shown that programmed anuclear cell death delimits platelet life span and suggests that pro-survival Bcl-xL is a candidate gene for mutations in patients with congenitally shortened platelet life span and normal sized platelets (79). Also highlighted are chaperone or enzyme mutations affecting platelet glycoprotein glycosylation and where thrombocytopenia can be associated with kidney disease (80).

Therapy and management

Patients with the above disorders are managed during severe bleeding episodes with the major goal of providing sufficient numbers of active platelets to assure a minimal haemostatic function (81, 82). Correct diagnosis is essential and especially so for FT in order to avoid confusion with PTI and inappropriate

treatment including splenectomy. Diagnostic algorithms are being designed but need to be both extended and improved (83). Disorders such as GT and BSS can be severe with life-threatening spontaneous bleeding. Platelet transfusions remain the mainstay of treatment, except for local bleeding where nasal packing or autologous fibrin glue can be used. A major problem in GT and BSS is isoimmunisation, with patients forming antibodies against glycoproteins missing from their own platelets (see [1]). Some isoantibodies recognize epitopes on active sites of the glycoproteins, so their presence will render transfused platelets refractory as well as leading to an accelerated destruction. Menorrhagia is often a problem and can be controlled by oral contraceptives. In the milder platelet function disorders, bleeding is less often spontaneous, but trauma-related bleeding can be a problem during childbirth or surgery. Desmopressin is often used preventively and by increasing VWF secretion from endothelial cells reduces the bleeding tendency, although it does not correct the platelet function defect (81). Recombinant factor VIIa (rFVIIa, NovoSeven) is frequently used to stop bleeding in GT and BSS and has mostly proved successful. It is particularly recommended in patients with inhibitors. Arguments can be made for its more general use in GT and BSS to reduce the risk of inhibitor formation. Factor VIIa helps to restore thrombin generation in GT with a resultant tighter fibrin mesh (84). Increased formation of fibrin around vascular lesions will also favorize

platelet accumulation, for platelets have a receptor for fibrin and this continues to be expressed in GT (85). It is our experience that elderly patients are more likely to experience angiodysplasia with gastrointestinal bleeding. In GT, this is difficult to control even with factor VIIa. The use of somatostatin (or derivatives such as octreotide) with or without oestrogen therapy as an alternative treatment for angiodysplasia has given encouraging results (86). In the Quebec syndrome, antifibrinolytic agents are advised (82). Stable TPO analogues are currently being tested to increase platelet production in ITP patients and would be interesting to try in severe congenital thrombocytopenias with a high bleeding risk (87).

Haematopoietic stem cell transplantation is recommended for children with severe diseases such as Chediak-Higashi syndrome, WAS, CAML and has been occasionally used in GT and BSS (reviewed in 81). Notwithstanding the success of this approach, long-term neurologic dysfunctions have been observed in adult Chediak-Higashi syndrome patients (88). Gene therapy may be on the horizon for certain of these disorders (89, 90).

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