Inherited thrombocytopenias

Alan T. Nurden, Paquita Nurden

Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, Pessac, France. E-mail: alan.nurden@cnrshl.u-bordeaux2.fr.

DOI: 10.3324/haematol.11256

Bleeding syndromes that arise through an inherited defect of platelet production constitute a heterogeneous group of rare platelet disorders of growing importance.[1,2] Some, including the Bernard-Soulier syndrome (BSS) and Wiskott-Aldrich syndrome (WAS), associate a low circulating platelet count with a deficiency in a known functional protein (Table 1). In others, platelet dysfunction has not been shown and the genetic cause lies in the inability of megakaryocytes (MK) to mature and to produce platelets in sufficient numbers. In congenital amegakaryocytic thrombocytopenia, there is an increased tendency towards the development of leukemia, while in others such as the Jacobsen syndrome, the defects extend outside megakaryocytopoiesis and interfere with the development and/or functioning of major organs. In many of these rare diseases, the low platelet count is accompanied by changes in platelet morphology including the presence of enlarged or giant platelets. The elucidation of the genetic basis of familial thrombocytopenias is providing basic knowledge of how MK develop from the pluripotent hematopoietic stem cell (HSC) under the influence of thrombopoietin (TPO) and other cytokines. This short review will mainly deal with the biology and genetics of inherited thrombocytopenias.

Mediterranean macrothrombocytopenia

Over 30 years ago, a series of 145 subjects from Italy and the Balkan peninsula were reported to have what was termed Mediterranean macrothrombocytopenia.[3] The diagnostic criteria included a moderately low platelet count (70,000-150,000/μL), increased mean platelet volume and mild bleeding. These patients mostly had autosomal dominant inheritance. A series of unrelated Italian families was subsequently studied by linkage analysis and mutation screening.[4] In six of them, a heterozygous A156V missense substitution was identified in GPIBα while in eight of ten patients GPIb-IX density on platelets was at levels reduced to those of BSS heterozygotes. This is somewhat enigmatic, for BSS is classically a disorder with autosomal recessive inheritance and an increased percentage of large platelets in obligate carriers is not an absolute rule.[5] It is possible that another as yet unidentified factor contributes to the Mediterranean macrothrombocytopenia phenotype.

DiGeorge or velocardiofacial syndrome

Although this disorder can show autosomal recessive inheritance, in most patients it is acquired. The phenotype is linked to a monoallelic chromosome 22q11.2 microdeletion. Phenotypic features include conotruncal cardiac abnormalities, learning disabilities, velopharyngeal insufficiency, immunodeficiency, facial dysmorphism and thymic hypoplasia. Studies on mutant mice suggest that a haploinsufficiency of a single gene, *TBX1* (encoding a T-box containing transcription factor), largely accounts for the phenotype.[6] Surveys of patients with DiGeorge syndrome suggest that mild thrombocytopenia and platelets of increased size affect about 20% of patients.[7] Adjacent to *TBX1* is the *GPIBB* gene, and its deletion can give rise to BSS when accompanied by a pathological mutation on the second allele.[8] Defining the factors that give rise to giant platelets

in DiGeorge syndrome would help clarify the origin of enlarged platelets in inherited thrombocytopenias. Bleeding is not a noted characteristic of DiGeorge syndrome patients.

MYH9-related thrombocytopenia syndromes

May-Hegglin anomaly (MHA), Fechtner syndrome, Sebastian platelet syndrome and Epstein syndrome constitute a group of related disorders with autosomal dominant inheritance and giant platelets.[1,2,9] Thrombocytopenia is often mild; bleeding is infrequent and rarely life-threatening. Myocardial infarction has been reported in elderly patients. Sometimes, however, thrombocytopenia can be very severe. Phenotypic variations depend on the association of giant platelets with variable combinations of Döhle-like bodies in leukocytes, nephritis, sensorineural hearing loss and cataracts. In Epstein syndrome, neither easily detectable leukocyte inclusions nor cataracts occur but giant platelets, kidney disease and deafness are all present.[9,10] Significantly, mature MK from bone marrow biopsies show distinct morphological abnormalities proving that the defect manifests prior to platelet production.[11]

Molecular studies have shown that defects in the *MYH9* gene encoding the non-muscle myosin heavy-chain IIA (myosin-IIA)[12,13] characterize all four syndromes. The same mutations often recur within unrelated families, and although haplotype analysis can identify common ancestors, a widespread geographical distribution makes this unlikely in many cases. Somatic mutations have already been reported. Myosin is a hexameric enzyme composed of two heavy chains and two pairs of light chains. The heavy chain aminoterminus forms a globular head that binds to actin and ATP, has ATPase activity, and is required for motor activity.[14] The C-terminal α-helical domain features a coiled-coil and a single rod-like tail that allows the molecules to polymerize into bipolar filaments. Myosin-IIA occurs in platelets, monocytes and granulocytes and in organs such as kidney, eye and ear.[10,15] Of importance is the fact that some mutations are repeated in different phenotypes suggesting that the diseases are not truly monogenic. An example is an exon 20 mutation (D1424N) in the coiled coil found first in May-Hegglin anomaly but then in Fechtner syndrome and also in Sebastian platelet syndrome.[13,16] Another is an exon 16 mutation (R702C) that has been located in Epstein syndrome and Fechtner syndrome as well as a variant of Alport syndrome.[16] Intriguingly, these mutations give rise to amino acid substitutions in, respectively, the rod and head domains of myosin-IIA, suggesting that *MYH9*-related disease is expressed independently of the site of the structural change. The question of the role of haploinsufficiency must therefore be asked.

Recent studies on mouse models imply that myosin-IIA complexes and their upstream signaling pathways regulate the timing of proplatelet formation.[17] The latter was premature in MK derived from *Myh9*$^{-/-}$ embryonic stem cells, while raised expression of myosin-IIA activity or constitutive phosphorylation of regulatory myosin light chain (MLC) led to reduced proplatelet formation. MLC phosphorylation in MK is controlled by Rho-associated kinase (ROCK) and ROCK inhibition was shown to promote proplatelet formation.[17,18] *In vivo*, this could mean that a lifting of the restraints imposed on proplatelet formation by the Rho-ROCK-MLC-myosinIIA pathway is the key trigger for proplatelet formation at the sinusoids. An extracellular stimulus for platelet production could be provided by stromal cell-derived factor (SDF-1 or CXCL12) a chemoattractant known to dampen Rho activity.

Pathologically decreased MLC phosphorylation in MKs in *MYH9*-related disease could slow MK migration towards the sinusoids as well as giving rise to premature proplatelet formation. Despite these advances, the reasons for phenotypic variability are still unclear. Do other genetic and/or environmental factors also intervene as suggested, for example, by studies on a large family with Fechtner syndrome in which five out of ten affected members showed no signs of renal lesions at the time of study.[19] One possible explanation is that additional II-B and II-C myosin isoforms can compensate for the malfunction of defective II-A isoform when expressed in the same tissues.[15] The potential role of fibulin-1, encoding an extracellular matrix protein as a disease modifier, has recently been underlined.[20]

Type 2B von Willebrand disease (Type 2B VWD)

In this disorder, bleeding occurs through the spontaneous binding of mutated plasma VWF to the platelet GPIb receptor, thereby preventing its adhesive function. Although reportedly not the general rule, both giant platelets and thrombocytopenia occur in type 2B VWD. In rare families, severe macrothrombocytopenia is associated with circulating platelet aggregates.[21] Studies on a French family with an R1308P VWF substitution have shown that such platelets appear to be produced prematurely and show signs of apoptosis with an altered expression of $Ca2^{+}$-ATPases and signs of caspase-3-mediated poly (ADP-ribose) polymerase (PARP) hydrolysis.[21] 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 MK and intertwined proplatelets suggesting that platelet production is perturbed. Early association of neosynthesized VWF with GPIb may be interfering with the normal maturation and/or migration of MK. A similar phenotype with circulating agglutinated platelets was previously reported in the Montreal platelet syndrome.[1,2]

Thrombocytopenia with platelets of normal size

In one large family, autosomal dominant and lifelong moderate to severe thrombocytopenia was associated with normal sized platelets and a late block in terminal dif-

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

Syndrome	*Gene mutation*	*Chromosomal location inheritance*	*Associated phenotype*
MYH9-related diseases May-Hegglin anomaly, Fechtner, Epstein & Sebastian syndromes	MYH9	22q11 Autosomal dominant	Various combinations of neutrophil inclusions, deafness, nephritis, cataracts. Enlarged platelets
Mediterranean macrothrombocytopenia	*GPIBA*, possibly others	17per-p12 Autosomal dominant	Enlarged platelets
Bernard-Soulier syndrome	*GPIBA, GP1BB, GP9*	17, 22 and 3 Autosomal recessive	Giant platelets, platelet adhesion defect
DiGeorge/Velocardiofacial syndrome	Hemizygous microdeletion including *GP1BB*	22q11 Autosomal dominant	Cardiac, facial, parathyroid, and thymus anomalies, cognitive defects
Familial platelet disorder/acute myelogenous leukemia	*RUNX1 (CBFA2, AML1)*	21q22.2 Autosomal dominant	Myelodysplasia, acute myeloid leukemia. Platelet dysfunction
Chromosome 10/THC2	*FLJ14813*	10p12-11.2 Autosomal dominant	Bleeding, normal sized platelets
Paris-Trousseau/Jacobsen syndromes	Hemizygous deletion including FLI1	11q23 Autosomal dominant	Psychomotor retardation, facial anomalies (Jacobsen syndrome). Enlarged platelets
Gray platelet syndrome	Unknown	Unknown Mostly recessive	Myelofibrosis, enlarged platelets, platelet dysfunction, absent α-granules
Congenital amegakaryocytic thrombocytopenia	*c-MPL*	1p34 Autosomal recessive	Severe thrombocytopenia at birth. Progressive pancytopenia
Thrombocytopenia and absent radii (TAR)	Unknown, c-Mpl signaling	Unknown Autosomal recessive	Shortened/absent radii bilaterally
Thrombocytopenia with radio-ulnar synostosis	*HOXA11*	7p15-p14.2 Autosomal dominant	Fused radius, incomplete range of motion
Wiskott-Aldrich syndrome	*WAS*	Xp11.23-p11.22 X-linked	Immunodeficiency, eczema, lymphoma, small platelets. Defective platelet and lymphocyte function
X-linked thrombocytopenia (XLT)	*WAS*	Xp11.23-p11.22	Small platelets, no immune problems
GATA-1-related thrombocytopenia with dyserythropoiesis	*GATA1*	Xp11.23 X-linked	Dyserythropoiesis ± anemia, thalassemia in some patients (XLTT). Platelet dysfunction, large platelets

ferentiation of MK.[22] Clinical manifestations include a propensity to easy bruising and increased bleeding at times of hemostatic stress. Myeloid or erythroid cells are not affected and there is no progression to aplastic anaemia. A novel putative kinase, FLJ14813, was identified as a candidate gene on chromosome 10 in this disorder.[23]

Familial thrombocytopenia with a predisposition to acute myelogenous leukemia

Studies on another large kindred linked a bleeding tendency to an autosomal dominant disorder of platelet production and function, and a propensity to develop myeloid leukemia.[24] Thrombocytopenia was moderate and platelet size normal. Linkage to markers on chromosome 21q identified an 880-kb interval containing the disease gene. Further analysis on the above and other families revealed nonsense mutations, missense mutations or intragenic deletion of one allele of the hematopoietic transcription factor *RUNX1* (*CBFA2, AML1*) gene, abnormalities that cosegregated with the disease in affected families.[25,26] *RUNX1* is thought to act as a tumor suppressor. The haplodeficiency and missense or nonsense mutations interfering with DNA binding appear to lead to decreased CFU-MK and insufficient production of platelets from birth. Mutated RUNX1 may heterodimerize with normal protein and lead to loss of function. The propensity to develop leukemia requires that patients have a higher tendency to develop a second mutation either in *CBFA2* or another gene.[26] This may be aided by the presence of an expanded population of undifferentiated HSC. When platelets of one such patient were examined, impaired platelet aggregation, secretion, protein phosphorylation of plekstrin and MLC, and GPIIb-IIIa activation were accompanied by a decreased platelet expression of myosin light chain regulatory polypeptide gene and other genes by platelet expression profiling.[27]

Gray platelet syndrome

A mild bleeding disorder for which both autosomal recessive and autosomal dominant inheritance have been described, gray platelet syndrome (GPS) is characterized by the platelet's inability to store α-granule proteins.[28] Platelets

are enlarged in this disorder but not giant. Thrombocytopenia is moderate, and the absence of α-granule contents gives the platelets a typical gray appearance on blood smears. It remains controversial whether the defect extends to neutrophils.[28] A feature of most patients is the early onset of myelofibrosis (which remains stable), a finding attributed to the inability of MK to store newly synthesized platelet-derived growth factors which, as a result, are released into the marrow. There is a tendency for secretion-dependent platelet aggregation to be abnormal. Collagen-induced platelet aggregation is particularly affected in a cohort of GPS patients with an acquired deficiency of the collagen receptor, GPVI.[29] This probably results from an aberrant metalloprotease activity. Electron microscopy has shown that the α-granules in gray platelets and megakaryocytes are small and almost empty rather than absent, many vacuoles are to be seen. Residual α-granule proteins can be detected in the surface-connected canalicular system (SCCS) and the basic defect appears to involve packaging or storage of the α-granule contents. Dense granules and their contents are normal. Emperipolesis (passage of other blood cells through megakaryocytes) has also been described in GPS perhaps linked to an abnormal surface expression of P-selectin.[30] Probably GPS is a heterogeneous disorder with more than one molecular cause. X-linked GPS due to a *GATA1* Arg216Gln mutation has been reported.[31] However this mutation has previously been associated with X-linked thrombocytopenia with thalassemia (XLTT) (*see later in the text*).

Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome (WAS), an X-linked recessive disease characterized by moderate to severe thrombocytopenia, is characterized by a predisposition to infection, and eczema due to immune deficiency.[1,2] A milder form without the immune problems is known as hereditary X-linked thrombocytopenia (XLT). WAS platelets are small and show a decreased aggregation response. The disease is not exclusive to platelets, since T lymphocytes also show defective function. The gene responsible for WAS has been cloned and encodes a 502 amino acid protein (p) termed WASp that is selectively expressed in HSC lineages. Mutations or other genetic defects localize throughout the *WASP* gene and result either in the decreased expression of WASp or its absence.[32] Mutations in exons 1, 2 and 3 were initially reported to results more likely in XLT. However, this may be due to a high prevalence of missense mutations in this region; a comprehensive phenotype-genotype study in Japan suggested that the clinical phenotype depended on the presence or absence of WASp.[33] WASp-negative patients show a much higher susceptibility to infection and eczema as well as having a greater tendency for malignancies.

WASp is a multifunctional protein involved in signal transduction, possessing tyrosine phosphorylation sites and adapter protein function. It has a subtle role in cytoskeleton formation, and stimulates actin assembly by the Arp2/3 complex.[34] Studies on a murine model have identified a critical role for WASp during murine platelet biogenesis.[35] The absence of WASp led to a defective MK interaction with collagen, a lack of formation of actin-rich podosomes and of SDF-1-induced MK migration. It was concluded that platelets were released prematurely ectopically into the marrow space. So, more evidence has been obtained pointing to an abnormal interaction of MK with their environment in the generation of familial thrombocytopenias. The finding that lentiviral vectors targeting WASp expression to hematopoietic cells efficiently transduce and correct cells from WAS patients, offers long-term hope for gene therapy in WAS.[36] Stem cell transplantation is currently performed.

Paris-Trousseau syndrome

The Paris-Trousseau syndrome is a rare disorder in which low platelet production and a mild hemorragic tendency are associated with a haplodeficiency of chromosome 11 (deletion at 11q23.3-24).[37] Thrombocytopenia can be chronic ($<50\times10^9$/L) despite normal platelet survival. The platelet aggregation response may be abnormal. Paris-Trousseau syndrome is often said to have autosomal dominant inheritance. Most reports concern children. Bone marrow dysmegakaryopoiesis is a constant feature; circulating platelets are often enlarged and some have characteristic giant α-granules. Morphologically distinct populations of MK are present; one composed of normal cells and the other of large numbers of small immature cells with arrested maturation. Occasionally, the platelet count may rapidly increase after birth and even normalize. The giant α-granules may represent granule fusion after platelet release. Paris-Trousseau syndrome is a variant of the much more frequently encountered Jacobsen syndrome in which a 11q.23 deletion can give rise to congenital heart defects, trigonocephaly, mental retardation, respiratory infections and malfunctions of multiple organs.[37,38] Pancytopenia and/or thrombocytopenia are also seen in some but not all patients with Jacobsen syndrome. A feature of the deletion that occurs within 11q23-q24 is that it affects but one allele. Of variable length, the deletion includes two ETS transcription factor genes, *ETS-1* and *FLI-1* that control, among other things, platelet membrane glycorotein expression.[39,40] Lentivirus-mediated overexpression of *FLI-1* in $CD34^+$ cells from a Paris-Trousseau syndrome patient restored megakaryocytopoiesis *in vitro*, thereby proving that *FLI-1* hemizygous deletion contributes to the hematopoietic defects.[41] The authors elegantly showed that *FLI-1* expression is transiently monoallelic in $CD41^+CD42^-$ progenitors from normal donors, while it is predominantly biallelic in the other stages of megakaryocytopoiesis. A half-reduction in *FLI-1* gene dosage generated a subpopulation of $CD41^+CD42^-$ cells completely lacking *FLI-1* transcription. The decreased *FLI-1* protein was suggested to prevent megakaryocyte differentiation, and to explain the presence of a subpopulation of micromegakaryocytes that fail to reach the platelet production stage.

Congenital amegakaryocytic thrombocytopenia (CAMT)

Here, severe thrombocytopenia at birth may develop into a pancytopenia. With autosomal recessive inheritance, affected patients have low numbers of MK in their marrow from birth, but show no physical abnormalities. The defects concern the incapacity of TPO to fulfill its normal thrombopoietic role as a result of abnormalities in the *c-MPL* gene that encodes the TPO receptor (c-MPL).[1,2] Ballmaier *et al.*[42] defined heterogeneity in congenital amegakaryocytic thrombocytopenia, distinguishing patients with a severe form and early development into pancytopenia, and a second group with mild thrombocytopenia in the first year of life but which then worsens with pancytopenia occurring in later life. Of eight point mutations detected in their study, frameshift or nonsense mutations predicted a complete loss of c-Mpl in five patients with severe disease. Missense mutations in three patients possessing residual c-Mpl were associated with a slower progression of the disease. The same authors have more recently extended their findings to a larger group of patients.[43] However, in this issue of the journal, Savoia *et al.*[44] have challenged this conclusion. The authors diagnosed five CAMT patients, but failed to find a correlation between the type of mutation and the clinical course. Furthermore, they provided evidence that elevated levels of the inhibitory cytokines, TNF-α and IFN-γ, probably contribute to the pancytopenia. Importantly, these results suggest that bone marrow transplantation should not be delayed in children with mutations that might predict residual activity of c-Mpl.

Amegakaryocytic thrombocytopenia with radio-ulnar synostosis

A small number of families have been reported in which family members have an association of bone marrow failure and radio-ulnar synostosis (and other skeletal abnormalities), with what appeared to be autosomal dominant inheritance.[45,46] In some children, symptomatic thrombocytopenia with bleeding required correction by bone marrow or umbilical cord stem-cell transplantation. Marrow studies showed few or no MK. Pancytopenia developed in some but not all individuals. A heterozygous mutation was found in exon 2 of the homeobox gene, *HOXA11*. This mutation was only found in affected individuals and occurred in a domain critical for DNA binding. More recent studies have shown that this mutation gives rise to a truncated HOXA11 protein with defective DNA-binding ability.[47] Furthermore, it appears that the mutation interferes with the early stages of MK differentiation. However, it is so far unclear whether both the skeletal and hematological problems relate to *HOXA11* gene deficiency although a predisposition to pancytopenia suggests an abnormality at the level of the HSC.

GATA-1-linked thrombocytopenia with dyserythropoiesis

A special category of familial thrombocytopenia segregates with dyserythropoiesis with or without anemia. Immunodeficiency and eczema are absent, platelets are often large.[1,2] Thrombocytopenia ranges from moderate to severe (10,000-40,000 platelets/μL); splenomegaly, reticulocytosis, and unbalanced hemoglobin chain synthesis resembling β-thalassemia minor may be present. Bleeding times are prolonged and platelet function moderately affected. The gene responsible for this combination of defects was first mapped to the X-chromosome (Xp11-12). The transcription factor *GATA-1* together with its cofactor FOG-1 (Friend of GATA) regulates erythropoiesis and megakaryocytopoiesis. The *GATA-1* gene is located at Xp11-12 and was found to be mutated in a series of families with XLT and dyserythropoiesis.[48–50] *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.

In general, MKs and platelets from these patients display few α-granules (*see Section on the GPS*) and an abormal membrane development. Erythrocytes are abnormal in size and shape. In one family two affected male half siblings were both severely anemic and thrombocytopenic at birth and thereafter.[49] A V205M substitution was found in GATA-1 that abrogated its interaction with FOG-1. Such findings underscored the importance of FOG-1:GATA-1 associations in both MK and erythroid development. Freson *et al.*[50] studied a family with XLT without anemia (but with some dyserythropoietic features). A D218G substitution in GATA-1 affected its interaction with FOG1 although *GATA-1* binding to DNA was normal. The bone marrow showed an increased number of large MK. Structurally abnormal and enlarged platelets and dysmorphic red cells were present. Semiquantitative RNA analysis revealed a low transcription of the GATA-1 target genes, GPIb, and GPIX; and a reduced transcription of late MK maturation markers. Yu *et al.*[51] reported a R216Q substitution in the N-terminal finger of GATA-1 in the family that originally led to the description of XLT, and in which red cell abnormalities were consistent with β-thalassemia (XLTT). Studies with recombinant GATA-1 showed that the mutation destabilized its binding to palindromic DNA sites but did not affect FOG-1 binding. Overall, a defective interaction of GATA-1 with either DNA or FOG-1 can disrupt hematopoiesis and give rise to disorders sharing common themes but with diverging phenotypes. Defects in *GATA-1* also extend to Down syndrome acute megakaryoblastic leukemia.[48] The role of *GATA-1* was confirmed by transgenic mouse models, with immature low ploidy megakaryocyte progenitors proliferating profusely *in vitro* as a result of a unique megakaryocyte differentiation arrest, a finding associat-

ed with dyserythropoietic anemia. Transgenic rescue of *GATA-1* deficient mice with GATA-1 lacking a FOG-1 association site (GATA-1V205G) phenocopied patients with a V205 mutation.[52]

Thrombocytopenia with absent radii

Thrombocytopenia with absent radii (TAR) syndrome is a rare congenital defect associating CAMT-like thrombocytopenia and osteodysgenesis, namely shortened (or absent) forearms due to bilateral radial aplasia.[53] Although other skeletal anomalies can be present, hands and fingers are unaffected. Inheritance appears to be autosomal recessive. Thrombocytopenia is very severe at birth, but platelet numbers increase during childhood and platelet count can be near normal in adulthood. Intracranial hemorrhage is a particular risk in early life when mortality is at its greatest. TPO levels are elevated in the serum, and platelets of TAR patients failed to respond to recombinant TPO as measured by testing TPO synergism to suboptimal concentrations of platelet activators.[54] Studies on *in vitro* MK differentiation and expression of the *c-MPL* gene in six patients showed a profound defect in MK progenitors.[55] This was associated with a blockage in MK differentiation with cells expressing GPIb without GPIIb-IIIa. Megakaryocyte differentiation was poorly stimulated by TPO or other cytokines and this was associated with a decrease in *c-MPL* transcripts and TPO receptor protein. However, screening the *c-MPL* gene has so far failed to show mutations and a defect in signal transduction has been proposed to explain the disorder.

Conclusions

We have briefly covered some of the major causes of inherited thrombocytopenias. However, an important question concerns the true abundance of inherited diseases with a low platelet count, so often falsely diagnosed as immune thrombocytopenic purpura. A recent survey of patients with macrothrombocytopenias in our Reference Center in Bordeaux revealed that in a cohort of 38 unrelated patients, 27 did not fall within the categories covered by this review, thereby suggesting other molecular causes. Australian studies on an N-ethyl-N-nitrosourea mutagenesis screen in mice also point to a wider range of causes of thrombocytopenia.[56-58] For example, the mouse models have shown that programmed anuclear cell death delimits platelet life span and suggests that pro-survival *Bcl-xL* is another candidate gene for mutations in patients with congenitally shortened platelet life span and normal sized platelets.[56] Also to be looked out for are chaperone or enzyme mutations affecting platelet glycoprotein glycosylation and where thrombocytopenia can be associated with kidney disease.[57] Finally, mutations in the cofilin partner Aip/Wdr1 cause autoinflammatory disease and macrothrombocytopenia.[58] These are exciting times and much work remains to be done.

References

1. Drachman JG. Inherited thrombocytopenia: when a low platelet count does not mean ITP. Blood 2004;103:390-8.
2. Geddis AE, Kaushansky K. Inherited thrombocytopenias: toward a molecular understanding of disorders of platelet production. Curr Opin Pediatr 2004; 16:15-22.
3. Behrens WE: Mediterranean macrothrombocytopenia. Blood 1975; 46:199-208.
4. Savoia A, Balduini CL, Savino M, Noris P, Del Vecchio M, Perrotta S, et al. Autosomal dominant macrothrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome. Blood 2001;97:1330-5.
5. Nurden AT, Nurden P. Inherited disorders of platelet function. In: Michelson AD, ed. Platelets (edition 2). Elsevier Science (USA), 2007;1025-50.
6. Kelly RG, Jerome-Majewska LA, Papaioannou VE. The del 22q11.2 candidate gene Tbx1 regulates branchiomeric myogenesis. Hum Mol Genet 2004; 13:2829-40.
7. Lawrence S, McDonald-McGinn DM, Zackai E, Sullivan KE. Thrombocytopenia in patients with chromosome 22q11.2 deletion syndrome. J Pediatr 2003; 143:277-8.
8. Kato T, Kosaka K, Kimura M, Imamura S, Yamada O, Iwai K, et al. Thrombocytopenia in patients with 22q11.2 deletion syndrome and its association with glycoprotein Ib-β. Genet Med 2003; 5:113-9.
9. Kunishima S, Saito H. Congenital macrothrombocytopenia. Blood Reviews 2006; 20:111-21.
10. Arrondel C, Vodovar N, Knebelmann B, Grunfeld JP, Gubler MC, Antignac C, et al. Expression of the nonmuscle myosin heavy chain IIA in the human kidney and screening for MYH9 mutations in Epstein and Fechtner syndromes. J Am Soc Nephrol 2002; 13:65-74.
11. Nurden P, Nurden A. Giant platelets, megakaryocytes and the expression of glycoprotein Ib-IX complexes. CR Acad Sci Paris 1996; 319:717-26.
12. Kelley MJ, Jawien W, Ortel TL, Korczak JF. Mutation of MYH9, encoding non-muscle myosin heavy chain A, in May-Hegglin anomaly. Nature Genet 2000; 26:106-8.
13. Heath KE, Campos-Barros A, Toren A, Rozenfeld-Granot G, Carlsson LE, Savige J, et al. Nonmuscle myosin heavy chain IIa mutations define a spectrum of autosomal dominant macrothrombocytopenias: May-Hegglin anomaly, and Fechtner, Sebastian, Epstein, and Alport-like syndromes. Am J Hum Genet 2001; 69:1033-45.
14. Hu A, Wang F, Sellers JR: Mutations in human nonmuscle myosin IIA found in patients with May Hegglin anomaly and Fechtner syndrome result in impaired enzymatic function. J Biol Chem 2002: 277;46512-7.
15. Marigo V, Nigro A, Pecci A, Montanaro D, Di Stazio M, Balduini CL, et al. Correlation between the clinical phenotype of MYH9-related disease and tissue distribution of class II nonmuscle myosin heavy chains. Genomics 2004;83:1125-33.
16. Kunishima S, Matsushita T, Kojima T, Amemiya N, Choi YM, Hosaka N, et al. Identification of six novel MYH9 mutations and genotype-phenotype relationships in autosomal dominant macrothrombocytopenia with leukocyte inclusions. J Hum Genet 2001; 46:722-9.
17 Chen Z, Naveiras O, Balduini AA, Mammoto A, Conti MA, Adelstein RS, et al. The May-Hegglin anomaly gene Myh9 is a negative regulator of platelet biogenesis modulated by the Rho-ROCK pathway. Blood 2007;110:171-9.
18. Chang Y, Aurade F, Larbret F, Zhang H, Le Couedic JP, Mormeux L, et al. Proplatelet formation is regulated by the Rho/ROCK pathway. Blood 2007;109:4229-36.
19. Ghiggarei GM, Caridi G, Magrini U, Sessa A, Savoia A, Seri M, et al. Genetics, clinical and pathological features of glomerulonephritis associated with mutations of nonmuscle myosin IIA (Fechtner syndrome). Am J Kidney Dis 2003; 41:95-104.
20. Toren A, Rozenfeld-Granot G, Heath KE, Amariglio N, Rocca B, Crosson J, et al. MYH9 spectrum of autosomal-dominant giant platelet syndromes: unexpected association with fibulin-1 variant-D inactivation. Am J Hematol 2003;74:254-62.
21. Nurden P, Debili N, Vainchenker W, Bobe R, Bredoux R, Corvazier E, et al. Impaired megakaryocytopoiesis in type 2B Willebrand disease with severe thrombocytopenia. Blood 2006;108:2587-95.

22. Bithell TC, Didisheim P, Cartwright GE, Winthrobe MM. Thrombocytopenia inherited as an autosomal dominant trait. Blood 1965;25:231-40.
23. Gandhi MJ, Cummings CL, Drachman JG. FLJ14813 missense mutation; a candidate for autosomal dominant thrombocytopenia on human chromosome 10. Hum Hered 2003;55:66-70.
24. Ho CY, Otterud B, Lagare RD, Varvil T, Saxena R, DeHart DB, et al. Linkage of a familial platelet disorder with a propensity to develop myeloid malignancies to human chromosome 21q22.1-22.2. Hum Mol Genet 1996; 4:763-6.
25. Song W-J, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nature Genet 1999;29:166-75.
26. Michaud J, Wu F, Osato M, Cottles GM, Yanagida M, Asou N, 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;99:1364-72.
27. Sun L, Gorospe JR, Hoffman EP, Rao AK. Decreased platelet expression of myosin regulatory light chain polypeptide (MYL9) and other genes with platelet dysfunction and CBFA2/RUNX1 mutation: insights from platelet expression profiling. J Thromb Haemost 2007;5:146-54.
28. Nurden AT, Nurden P. The Gray platelet syndrome: Clinical spectrum of the disease. Blood Reviews 2007;21:21-36.
29. Nurden P, Jandrot-Perrus M, Combrie R, Winckler J, Arocas V, Lecut C, et al. Severe deficiency of glycoprotein VI in a patient with gray platelet syndrome. Blood 2004;104:107-14.
30. Falik-Zaccai T, Anikster Y, Rivera CE, Horne MK, Schliamser L, Phornphutkul C et al. A new genetic isolate of gray platelet syndrome (GPS): Clinical, cellular and hematologic characteristics. Mol Genet Metab 2001;74:303-13.
31 Tubman VN, Levine JE, Campagna DR, Monahan-Earley R, Dvorak AM, Neufeld EJ, et al. X-linked gray platelet syndrome due to a GATA1 Arg216Gln mutation. Blood 2007; 109:3297-9.
32. Zhu QL, Watanabe C, Liu T, Hollenbaugh D, Blaese RM, Kanner SB et al. Wiskott-Aldrich syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression and phenotype. Blood 1997;90:2680-9.
33. Imai K, Morio T, Jin Y, Itoh S, Kajiwara M, Yata J, et al. Clinical course of patients with WASP gene mutations. Blood 2004;103:456-64.
34. Kim AS, Kakalis LT, Abdul-Manan N, Liu GA, Rosen MK: Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. Nature 2000;404:151-8.
35. Sabri S, Foudi A, Boukour S, Franc B, Charrier S, Jandrot-Perrus M, et al. Defiency in the Wiskott-Aldrich syndrome protein induces premature proplatelet formation and platelet production in the bone marrow compartment. Blood 2006; 108:134-40.
36. Charrier S, Dupré L, Scaramuzza S, Jeanson-Leh L, Blundell MP, Danos O, et al. Lentiviral vectors targeting WASp expression to hematopoietic cells, efficiently transduce and correct cells from WAS patients. Gene Ther 2007;14:415-28.
37. Favier R, Jondeau K, Boutard P, Grossfeld P, Reinert P, Jones C, et al. Paris-Trousseau syndrome: clinical, hematological, molecular data of ten new cases. Thromb Haemost 2003; 90:893-7.
38. Grossfeld PD, Mattina T, Lai Z, Favier R, Jones KL, Cotter F, Jones C. The 11q terminal deletion disorder: a prospective study of 110 cases. Am J Med Genet 2004;129:51-61.
39. Wang X, Crispino JD, Letting DL, Nakazawa M, Poncz M, Blobel GA. Control of megakaryocyte-specific gene expression by GATA-1 and FOG-1: role of Ets transcription factors. EMBO J 2002;21:5225-34.
40. Eisbacher M, Holmes ML, Newton A, Hogg PJ, Khachigian LM, Crossley M, Chong BH. Protein-protein interaction between Fli-1 and GATA-1 mediates synergistic expression of megakaryocyte-specific genes through cooperative DNA binding. Mol Cell Biol 2003;23:3427-41.
41. Raslova H, Komura E, Le Couédic JP, Larbret F, Debili N, Feunteun J, et al. FLI1 monoallelic expression combined with its hemizygous loss underlies Paris-Trousseau/Jacobsen thrombopenia. J Clin Invest 2004; 114:77-84.
42. Ballmaier M, Germeshausen M, Schulze H, Cherkaoui K, Lang S, Gaudig A, et al. c-Mpl mutations are the cause of congenital amegakaryocytic thrombocytopenia. Blood 2001; 97:139-46.
43. Germeshausen M, Ballmaier M, Weite K: MPL mutations in 23 patients suffering from congenital amegakaryocyte thrombocytopenia: the type of mutation predicts the course of the disease. Hum Mutat 2006;27:296.
44. Savoia A, Dufour C, Locatelli F, Noris P, Ambaglio C, Rosti V et al. Congenital amegakaryocytic thrombocytopenia: Clinical and biological consequences of five novel mutations. Haematologica 2007;92:1186-93.
45. Thompson AA, Nguyen LT. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with HOXA11 mutation. Nature Genet 2000;26:397-8.
46. Thompson AA, Woodruff K, Feig SA, Nguyen JT, Schanen NC. Congenital thrombocytopenia and radio-ulnar synostosis: a new familial syndrome. Br J Haematol 2001; 113:866-70.
47. Horvat-Switzer RD, Thompson AA. HOXA11 mutation in amegakaryocytic thrombocytopenia with radio-ulnar synostosis syndrome inhibits megakaryocytic differentiation in vitro. Blood Cells Mol Dis 2006;37:55-63.
48. Cantor AB. GATA transcription factors in hematologic disease. Int J Hematol 2005;81:378-84.
49. Nichols KE, Crispino JD, Poncz M, White JG, Orkin SH, Maris JM, et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat Genet 2000;24:266-70.
50. Freson K, Matthijs G, Thys C, Marien P, Hoylaerts MF, Vermylen J, 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;11:147-52.
51. Yu C, Niakan KK, Matsushita M, Stamatocyannopoulos 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;100:2040-5.
52. Shimiza R, Ohneda K, Engel JD, Trainor CD, Yamamoto M. Transgenic rescue of GATA-1-deficient mice with GATA-1 lacking a FOG-1 association site phenocopies patients with X-linked thrombocytopenia. Blood 2004;103:2560-7.
53. Geddis AE. Inherited thrombocytopenia: Congenital amegakaryocytic thrombocytopenia and thromboocytopenia with absent radii. Semin Hematol 2006;43:196-203.
54. Ballmaier M, Schulze H, Cremer M, Folman CC, Strauss G, Weite K. Defective c-Mpl signaling in the syndrome of thrombocytopenia with absent radii. Stem Cells 1998;16 Suppl2:177-84.
55. Letestu R, Vitrat N, Massé A, Le Couedic JP, Lazar V, Rameau P, et al. Existence of a differential blockage at the stage of amegakaryocyte precursor in the thrombocytopenia and absent radii (TAR) syndrome. Blood 2000;95:1633-41.
56. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, et al. Programmed anuclear cell death delimits platelet lifespan. Cell 2007;128:31173-86.
57. Alexander WS, Viney EM, Zhang J-G, Metcalf D, Kauppi M, Hyland CD, et al. Thrombocytopenia and kidney disease in mice with a mutation in the C1galt1 gene. Proc Natl Acad Sci USA 2006;103:16442-7.
58. Kile BT, Panapoulis AD, Stirzaker RA, Hacking DF, Tahtamouni LH, Willson TA, et al. Mutations in the cofilin partner Aip/Wdr1 cause autoinflammatory disease and macrothrombocytopenia. Blood 2007 May 21; [Epub ahead of print].