Review Series

blood

CLINICAL PLATELET DISORDERS

Hematopoietic transcription factor mutations: important players in inherited platelet defects

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Transcription factors (TFs) are proteins that bind to specific DNA sequences and regulate expression of genes. The molecular and genetic mechanisms in most patients with inherited platelet defects are unknown. There is now increasing evidence that mutations in hematopoietic TFs are an important underlying cause for defects in platelet production, morphology, and function. The hematopoietic TFs implicated in patients with impaired platelet function and number include runt-related transcription factor 1, Fli-1 proto-oncogene, E-twenty-six (ETS) transcription factor (friend leukemia integration 1), GATA-binding protein 1, growth factor independent 1B transcriptional repressor, ETS variant 6, ecotropic viral integration site 1, and homeobox A11. These TFs act in a combinatorial manner to bind sequence-specific DNA within promoter regions to regulate lineage-specific gene expression, either as activators or repressors. TF mutations induce rippling downstream effects by simultaneously altering the expression of multiple genes. Mutations involving these TFs affect diverse aspects of megakaryocyte biology, and platelet production and function, culminating in thrombocytopenia and platelet dysfunction. Some are associated with predisposition to hematologic malignancies. These TF variants may occur more frequently in patients with inherited platelet defects than generally appreciated. This review focuses on alterations in hematopoietic TFs in the pathobiology of inherited platelet defects. (*Blood*. 2017;129(21):2873-2881)

Introduction

The complex process of platelet production starts with hematopoietic stem cell (HSC) differentiation and megakaryocyte (MK) lineage commitment, followed by MK maturation and eventual platelet release.[1] These processes are temporally and spatially regulated by hematopoietic transcription factors (TFs) and stimulated by thrombopoietin[1] (TPO) (Figure 1). TFs are proteins that bind to specific DNA sequences and regulate expression of genes. They function in a combinatorial manner as activators and repressors.[2,3] There is increasing evidence that mutations in hematopoietic TFs are an important underlying cause of inherited defects in platelet production, structure, and function. TF mutations can induce multiple effects by concurrently altering expression of numerous target genes. Several hematopoietic TFs have been implicated in platelet disorders and include RUNX1, FLI1, GATA1, GFI1B, ETV6, EVI1, and HOXA11 (Table 1). These are described in this review. They regulate processes involved in MK lineage commitment and maturation and platelet biogenesis.[4,5]

Some patients previously described with platelet defects, such as storage pool deficiency and other abnormalities, have more recently turned out to have mutations in specific TFs.[6-10] In 1969, Weiss et al described a family with an autosomal dominant inherited platelet disorder due to decreased dense granule (DG) contents.[11] This family was subsequently found to also have partial α-granule (AG) deficiency,[12] and in 2002 reported to have a heterozygous Y260X mutation in *RUNX1*.[6] Similarly, an underlying TF variant has been found in other patients.[7,9,10,13] These studies advance a paradigm shift regarding the genetic basis of inherited platelet defects from mutations in candidate genes, based on the altered platelet phenotype to mutations in TFs that concurrently dysregulate diverse genes and pathways to impact platelet number and function. Moreover, the same platelet phenotype may result from mutations in more than one TF or mechanisms. For example, AG deficiencies are associated with mutations in *RUNX1*,[6,10,14] *GATA1*,[13] and *GFI1B*,[7,9] in addition to *NBEAL2*.[15]

The advent of state-of-the art approaches, such as the next-generation sequencing (NGS), has augmented the discovery of TF mutations.[8,10,16] It is emerging that TF mutations may be the genetic basis of platelet defects more commonly than generally considered. For example, Stockley et al[8] found *RUNX1* or *FLI1* mutations in 6 of 13 index patients with clinical bleeding and impaired platelet aggregation and DG secretion studied with NGS; this has also been observed in other studies.[7,10] Some TF mutations are associated with concurrent defects in erythropoiesis (eg, *GATA1*) or an increased risk of hematologic malignancy (*RUNX1*, *ETV6*) with implications for prognosis and management.[3,17-20] Germ line alterations in hematopoietic TFs therefore need to be considered in the pathogenesis of inherited platelet defects.

RUNX1

RUNX1, also known as core-binding factor subunit α-2 (CBFA2) or AML1, is encoded by the *RUNX1* gene on chromosome 21q22.3. It is a member of the Runt family of three TFs that share a 128 amino acid (aa) conserved runt homology domain near the *N*-terminus; this domain associates with its co-factor, CBFβ and binds to sequence-specific DNA to regulate gene expression.[21] RUNX1 is indispensible for definitive hematopoiesis. *RUNX1* knockout mice lack primary

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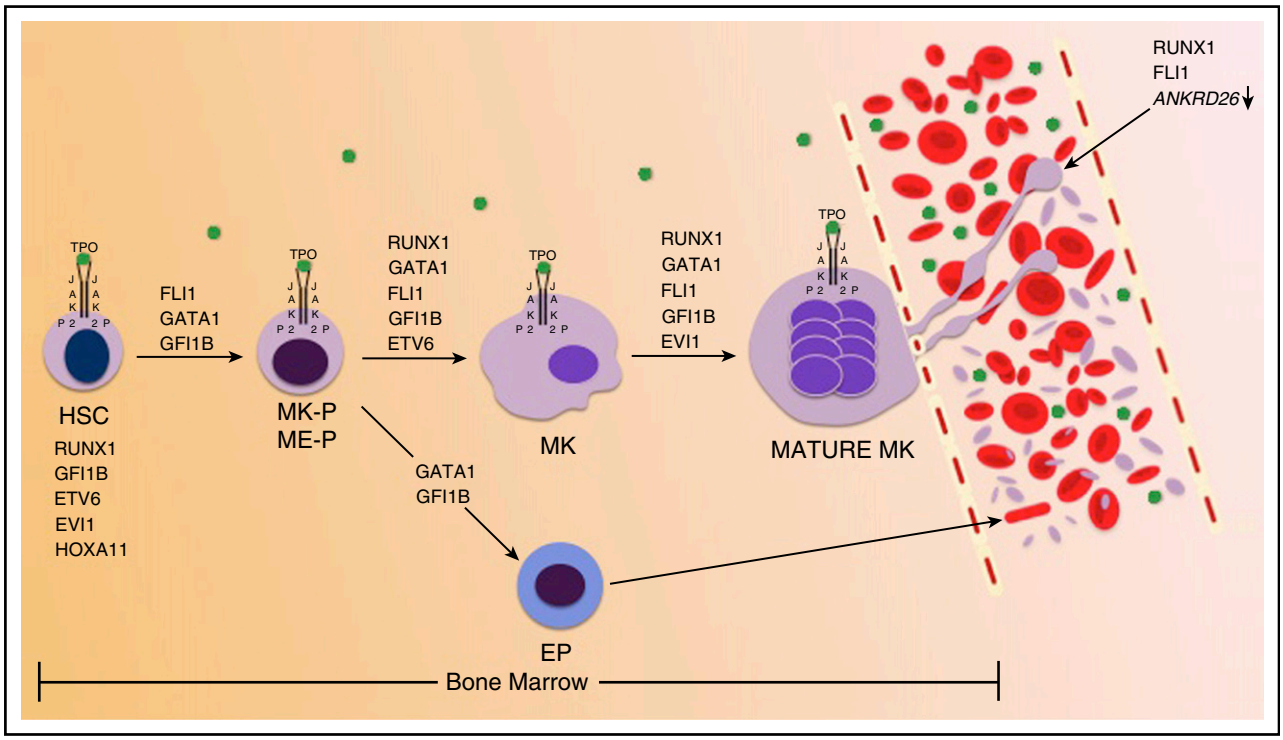

Figure 1. Schematic representation of hematopoietic TFs involved in normal platelet genesis. RUNX1, GFI1B, ETV6, EVI1, and HOXA11 are expressed in HSCs. As denoted above black arrows, various hematopoietic TFs, in combination with TPO stimulation, function to promote HSC differentiation, MK lineage commitment and maturation, and proplatelet formation and platelet release. Proplatelet formation and platelet release are also driven by RUNX1 and FLI1 silencing of *ANKRD26*. TPO is shown by green dots. EP, erythroid progenitor; ETS, E-twenty-six; ETV6, ETS variant 6; EVI1, ecotropic viral integration site 1; FLI1, Fli-1 proto-oncogene, ETS transcription factor; GATA1, GATA-binding protein 1; GF1IB, growth factor independent 1B transcriptional repressor; HOXA11, homeobox A11; ME-P, MK-erythroid progenitor; MK-P, MK progenitor; P, phosphorylated; RUNX1, runt-related transcription factor 1.

hematopoiesis during embryogenesis and die in utero because of bleeding.[22] Conditional *RUNX1* knockouts in mouse models show impaired MK maturation, with presence of abnormal micro-MKs and significant reduction in MK polyploidization.[23] In patients with *RUNX1* mutations, MKs cultured from stem cells also demonstrate defects in differentiation and polyploidization.[17,24] This has been associated with dysregulated nonmuscle myosin IIA (MYH9) and IIB (MYH10) expression, with impaired *MYH10* silencing and reduced *MYL9* and *MYH9* expression.[24] Reconstitution of *MYH10* silencing promotes MK polyploidization by allowing progression from mitosis to endomitosis (chromosome replication without cell division).[24,25]

Detection of platelet MYH10 protein expression has been proposed as a marker for genetic defect in *RUNX1* and *FLI1*.[26] RUNX1 regulates expression of numerous genes and pathways in MK/platelet genes.[27,28] Platelet transcript profiling of a patient with heterozygous *RUNX1* mutation showed downregulation of MK/platelet genes involved in diverse aspects of platelet production, structure, signaling, and function.[28]

Mutations in *RUNX1* are associated with thrombocytopenia, impaired platelet function, and a predisposition to acute leukemia. In 1985, Dowton et al[29] described a kindred of 22 members with a bleeding tendency, autosomal dominant thrombocytopenia and

Table 1. Hematopoietic TF mutations and platelet defects

TF	Chromosome location	Selected gene targets	Platelet dysfunction*	Associated hematologic abnormalities
RUNX1	21q22.3	*ALOX12*, *PF4*, *MYL9*, *PRKCQ*, *MPL*, *MYH9*, *MYH10*, *NFE2*, *PCTP*, *PLDN*, *ANKRD26*	Yes	Increased risk of MDS/AML
FLI1	11q24.1-24.3	*ITGA2B*, *GP1BA*, *GP6*, *GP9*, *MPL*, *PF4*, *NFE2*, *RAB27B*, *ANKRD26*	Yes	Unknown
GATA1	Xp11.23	*GP1BA*, *GP1BB*, *ITGA2B*, *GP9*, *PF4*, *MPL*, *NFE2*	Yes	Dyserythropoiesis
GFI1B	9q34.13	*BCLXL*, *SOCS1*, *SOCS3*, *CDKN1A*, *GATA3*, *MEIS1*, *RAG1/2*	Yes	Red cell aniso/poikilocytosis
ETV6	12p13	*PF4*	Unknown	Dyserythropoiesis; Increased risk of ALL
EVI1	3q26.12	*RBM8A*, *MPL*, *ITGA2B*, *ITGB3*	Unknown	BM failure
HOXA11	7p15.2		Unknown	BM failure

ALL, acute lymphoblastic leukemia; *ALOX12*, 12-lipoxygenase; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; *MYL9*, platelet myosin light chain; *PCTP*, phosphatidylcholine transfer protein; *PF4*, platelet factor 4; *PLDN*, pallidin; *PRKCQ*, protein kinase C-θ.

*All of the TF mutations have been associated with variable thrombocytopenia. Platelet count may be normal, as seen in some patients with mutations in *RUNX1* or *FLI1*. Thrombocytopenia may be particularly severe in patients with mutations in *GATA1*, *ETV6*, *EVI1*, and *HOXA11* (platelet count $<20 \times 10^9$/L). Abnormalities in platelet morphology, especially in AGs and DGs, and in aggregation and secretion responses on platelet activation have been described in association with mutations in *RUNX1*, *FLI1*, *GATA1*, and *GFI1B*. These abnormalities are described in the text and, for *RUNX1*, shown in Figure 2.

impaired platelet aggregation responses; 6 family members developed hematologic malignancies, including leukemia in 4 patients. Through this and other reports,[29-31] this entity came to be referred to as familial platelet disorder with predisposition to AML (Mendelian Inheritance of Man [MIM] 601399). Linkage analysis of patients with familial platelet disorder/AML mapped a potential locus to the long arm of chromosome 21q22.[31,32] In 1999, Song et al[17] identified heterozygous mutations in *RUNX1* (formerly CBFA2) in affected members of 6 families to establish the causal link, subsequently shown in several other families.[6,33]

Several families with *RUNX1* mutations have been reported and this disorder is likely under-recognized in patients with platelet defects. Most mutations are within the conserved runt domain with decreased RUNX1 binding to the target DNA,[6] although a mutation in the transactivating domain near the C-terminus has also been reported.[6] The mutations usually result in haplodeficiency, but in some patients there is markedly decreased RUNX1 activity due to a dominant negative effect.[6,34] Notably, the human phenotype is not recapitulated in murine heterozygous *RUNX1* mutations.[35] Patients with *RUNX1* mutations typically have a mild-to-moderate bleeding tendency due to the platelet dysfunction and thrombocytopenia, with normal-sized platelets; some patients may not have thrombocytopenia or bleeding symptoms.[8,36,37]

Platelet function abnormalities described in *RUNX1* haplodeficiency include impaired platelet aggregation and secretion upon activation, DG and/or AG deficiency, and decreased phosphorylation of myosin light chain (MLC) and pleckstrin, activation of αIIbβ3, production of 12-hydroxyeicosatetraenoic acid (a product of *ALOX12*), and protein kinase C-θ (which phosphorylates pleckstrin and other proteins) (Figure 2).[6,15,24,38,39] Deficiencies of DG or AG or of both granules have been reported, although not all studies examined both granule subsets. Platelet albumin and immunoglobulin G, two AG proteins not synthesized by MKs, were also reduced in 1 patient, suggesting a defect in platelet uptake and incorporation into granules.[38] Decreased expression of platelet *PLDN* has been observed in *RUNX1* haplodeficiency and *PLDN* is a direct transcriptional target of RUNX1.[40] This provides a potential mechanism for the DG deficiency because *PLDN* is involved in DG biogenesis, as observed in the pallid mouse and human Hermansky-Pudlak syndrome.[41,42] Moreover, patients with *RUNX1* mutations have defects in mechanisms that regulate agonist-stimulated secretion from AGs and DGs, as well as of the acid hydrolase containing vesicles, unrelated to a deficiency of granule contents.[8,43-45] Overall, the impaired secretion is related to abnormalities in granule biogenesis, as well as in secretory mechanisms (Figure 2).

A relatively small number of TFs in the genome regulate tissue-specific expression of a large number of genes.[5] Thus, multiple defects observed in MKs and platelets arise due to dysregulation of numerous RUNX1 target genes. Our platelet transcript profiling studies of a patient with *RUNX1* haplodeficiency showed that genes regulating several platelet pathways were downregulated.[28] Some have been shown to be direct transcriptional targets of RUNX1, including *ALOX12*,[39] *PF4*,[14] *MYL9*,[46] *PRKCQ*,[47] *PLDN*,[40] and the TPO receptor (*MPL*).[48] Another gene downregulated and a target of RUNX1 is *PCTP*,[49] which encodes for a protein implicated in platelet responses mediated by PAR4 agonist activation.[50] TF NF-E2 (*NFE2*) is also a transcriptional target of RUNX1.[51] NF-E2 has been implicated in platelet granule development and αIIbβ3 signaling.[51] These alterations impact various aspects of MK and platelet biology.

Recently, Connelly et al[52] have validated the causative role of *RUNX1* mutations and advanced the potential for gene therapy. Using induced pluripotent stem cells from skin fibroblasts of 2 patients with *RUNX1* Y260X mutation, they demonstrated impaired MK production and abnormalities including the presence of vacuoles and reduction in AGs and DGs. Targeted in vitro mutation correction rescued the defects in MK production and phenotype, with upregulation of relevant MK genes.

RUNX1 haplodeficiency is associated with increased risk of AML or MDS, with a >40% risk at a median age of 33 years.[36] The malignant transformation is heralded by the development of additional somatic mutations in genes, such as *CDC25C*, *GATA2*, *TET2*, *MLL2*, and *RB1*.[53,54] The level of RUNX1 activity appears to be important in predisposing to leukemic predisposition.[34] Relevant to note, acquired *RUNX1* mutations are common in patients with MDS and myeloid malignancies.[55] Sporadic germ line deletions involving chromosome 21q22 that include the *RUNX1* gene have also been reported, and may result in syndromic features (dysmorphic facies, mental retardation, and organ abnormalities) and predisposition to hematologic malignancies.[36]

Recognition of *RUNX1* and other mutations is important to prevent unnecessary therapies, such as based on an erroneous diagnosis of immune thrombocytopenic purpura. In patients with *RUNX1* mutations who develop MDS or leukemia, use of an undiagnosed *RUNX1* haplodeficient sibling donor has been associated with recurrence of leukemia.[37] It has been suggested that patients undergo surveillance for MDS and leukemia with clinical examination and blood counts every 6 to 12 months.[56]

FLI1

The *FLI1* gene is located on the distal end of the long-arm of chromosome 11 (11q24.1-q24.3) and is a member of the ETS family of TFs.[57,58] It has 452 aas, and shares with other ETS family members a winged helix-turn-helix ETS domain of 85 aas that recognizes DNA consensus sequence 5′-GGAA/T-3′.[58] FLI1 plays critical roles in megakaryopoiesis and angiogenesis.[3] *FLI1* knockout mice die of embryonic hemorrhage, attributed to the FLI1 role in both megakaryopoiesis and endothelium, and hemangioblast specification.[3] Murine heterozygous *FLI1* mutation does not recapitulate the human hematologic phenotype.[59] FLI1 transcriptionally binds and regulates a number of MK/platelet relevant genes, including for *ITGA2B*, *GP1BA*, *GP9*, *THPO*, and *PF4*.[3]

Hemizygous *FLI1* defects occur due to distal deletion of part of the long-arm of chromosome 11 and are associated with Jacobsen syndrome (MIM 147791), and the associated platelet disorder, Paris-Trousseau syndrome (PTS) (MIM 188025). Most cases of Jacobsen syndrome occur from de novo mutation in a parental gamete, with the rest from translocations or chromosomal rearrangement abnormalities that lead to loss of distal chromosome 11 material.[60] Patients with Jacobsen syndrome may have dysmorphic facies, developmental delay, and multiple organ abnormalities (eg, cardiac, renal and genitourinary, neurologic, and hematologic).[61] More than 90% of patients with Jacobsen syndrome also have PTS, attributed to deletion of *FLI1*. Such patients have variable bleeding tendency, congenital macrothrombocytopenia, and platelet dysfunction with impaired AG and DG secretion in response to thrombin.[8,59,62] A characteristic feature is the presence of giant AGs (1 to 2 μm) in 1% to 5% of platelets.[59,62] There is dysmegakaryopoiesis with increased MKs in the bone marrow (BM).[59] The MK population is dimorphic, one normal and one with small immature MKs, due to transient monoallelic *FLI1* expression in progenitors.[62,63] Interestingly, the platelet count in children with Jacobsen syndrome/PTS may spontaneously improve over months to years.[60]

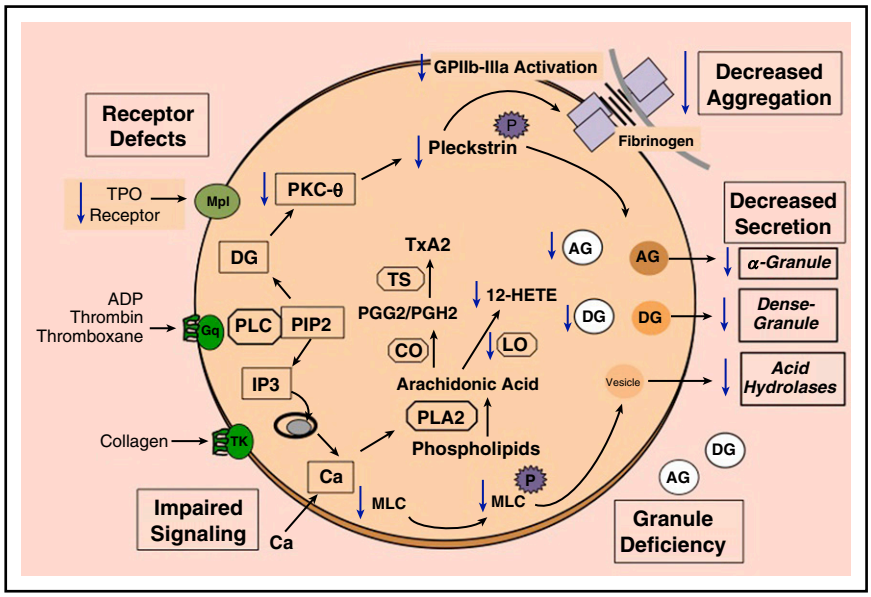

Figure 2. Schematic representation of selected platelet responses to activation and platelet function abnormalities associated with *RUNX1* mutations. Platelet receptor activation results in the formation of intracellular mediators that regulate the end responses, such as aggregation and secretion from AGs and DGs, and from vesicles bearing acid hydrolases. Receptor activation leads to hydrolysis of PIP_2 by phospholipase C to form diacylglycerol, which activates protein kinase C, and IP_3, which mediates the rise in cytoplasmic Ca^{2+} levels. Protein kinase C phosphorylates numerous proteins including pleckstrin. The increase in Ca^{2+} levels leads to other responses, such as activation of MYC kinase to phosphorylate MLC and activation of PLA_2, which mediates the release of free arachidonic acid from phospholipids. Arachidonic acid is converted by CO and TS to thromboxane A_2. Numerous defects in platelet function have been described in platelets with *RUNX1* haplodeficiency. These are shown with downward arrows (blue). Included below in this legend in parenthesis are some of the relevant genes that are RUNX1 targets and downregulated in *RUNX1* haplodeficiency. The abnormalities include reduction in the surface receptors for TPO (*MPL*); defects in signaling mechanisms, including impaired pleckstrin and MLC phosphorylation, and decreased *PRKCQ* and MLC (*MYL9*); decreased *ALOX12* and 12-HETE production; impaired activation of GPIIb-IIIa and aggregation on platelet activation; DG (*PLDN*) and AG (*PF4*) deficiency; and impaired secretion of AG and DG contents and from vesicles containing acid hydrolases. Other genes shown to be downregulated and not shown in the figure include *PCTP* and *NFE2*. 12-HETE, 12-hydroxyeicosatetraenoic acid; ADP, adenosine 5′-diphosphate; CO, cyclooxygenase; IP_3, inositoltrisphosphate; LO, lipoxygenase; PLA_2, phospholipase A_2; PGG2, prostaglandin G2; PGH2, prostaglandin H2; PIP_2, phosphatidylinositol bisphosphate; PLC, phospholipase C; TS, thromboxane synthase; TxA2, thromboxane A2.

Recent studies using NGS have uncovered novel *FLI1* variants in patients with platelet dysfunction. Stockley et al[8] identified *FLI1* mutations (3 missense and 1 4-bp deletion) in 3 of 13 unrelated index patients with excessive bleeding, and decreased platelet aggregation and reduced DG secretion. Additionally, Stevenson et al[59] reported on 2 patients with a PTS phenotype due to homozygous *FLI1* missense mutations inherited from consanguineous parents and predicted to alter the ETS DNA binding domain. The parents had normal a platelet count and function, indicating that the mechanisms by which *FLI1* variants lead to platelet defects remain to be fully elucidated.

GATA1

GATA1 is a zinc finger TF encoded by the *GATA1* gene located on the short-arm of the X-chromosome (Xp11.23). It is member of a family of TFs that bind to the GATA motif of DNA.[5] GATA1 has 2 homologous zinc fingers: the N-terminal finger, which associates with a nuclear co-factor protein called friend of GATA1 (FOG1) to enhance stability of GATA1 binding to complex or palindromic DNA binding sites, and the C-terminal zinc finger that mediates binding to the GATA DNA motif.[64,65] GATA1 is critical in both megakaryopoiesis and erythropoiesis.[3,5] It is highly expressed in MKs and erythroid cells, as well as mast cells and eosinophils, and is indispensable for terminal differentiation of these lineages.[3,5,64,65] *GATA1* knockout mice die in utero at approximately day 10 from severe anemia.[3] A wide spectrum of MK genes contain GATA1 motifs within their *cis*-regulatory domain, including *GP1BA*, *GP1BB*, *ITGA2B*, *GP9*, *PF4*, *THPO*, and *NFE2*.[65,66]

Several families have been described with X-linked platelet and red cell disorders associated with distinct *GATA1* variants (Table 2). The initial reports described families with macrothrombocytopenia and dyserythropoiesis with or without anemia (MIM 300367), with severity of anemia dependent on the extent of disruption of GATA1 interaction with FOG1. In one pedigree,[67] 2 boys had severe fetal anemia and thrombocytopenia requiring in utero red cell and platelet transfusions. Both children underwent HSC transplant (HSCT). Genetic analysis revealed *GATA1* V205M mutation that markedly impaired GATA1 interaction with FOG1. In another family[65] with severe dyserythropoietic anemia and thrombocytopenia, 6 affected boys died before age 2. The family had *GATA1* D218Y mutation, which conferred a similar degree of impairment in GATA1/FOG1 interaction as with *GATA1* V205M mutation. Mutations with less severe alterations in GATA1/FOG1 affinity (*GATA1* D218G, G208S, G208R) also manifest with thrombocytopenia, but are only mildly affected by red cell abnormalities, with dyserythropoiesis, but without significant anemia.[66,68,69]

GATA1 mutations have also been implicated in X-linked thrombocytopenia with β-thalassemia (MIM 314050), as first described in 1977.[70] These patients had moderate macrothrombocytopenia, platelet dysfunction, AG deficiency, imbalanced globin chain synthesis, red cell hemolysis, and evidence of dyserythropoiesis and dysmegakaryopoiesis.[64,70,71] *GATA1* R216Q mutation identified in these patients is unique in that, unlike other previously described *GATA1* mutations, GATA1/FOG1 interaction is not disrupted; rather, there is impaired binding of the GATA1 *N*-finger to complex or palindromic DNA sequences.[64,71] Subsequently, a family with the GPS was also found to have *GATA1* R216Q mutation; one member displayed a mild β-thalassemia–like phenotype.[13]

Table 2. Platelet disorders associated with *GATA1* mutations

Clinical disorder	Genetic mutation	Extent of disruption of GATA1/FOG1 interaction	Degree of anemia	Degree of thrombocytopenia
Thrombocytopenia ± dyserythropoietic anemia (MIM 300367)	V205M	Severe	++	++/+++
	D218Y	Severe	++	+++
	D218G	Mild	−	+/++/+++
	G208S	Mild	−	++
	G208R	Unknown	++	+++
Thrombocytopenia with β-thalassemia (MIM 314050)	R216Q	− (disrupts DNA binding)	±	+ and GPS
Macrocytic anemia; neutropenia; normal platelet count (MIM 300835)	Splice mutation 332G->C, V74L	−	+/++/+++ (many cases also with neutropenia)	−
Dyserythropoietic anemia; MK dysplasia; thrombocytosis	Splice mutation in 5′-untranslated region	−	+++ (occasional neutropenia)	−

Anemia: hemoglobin (Hb) ≥10 g/dL (+); Hb 7 to <10 g/dL (++); and Hb <7 g/dL (+++).
Thrombocytopenia: 70 000 to 90 000 × 10^9/L (+); ≥20 000 to <70 000 × 10^9/L (++); and <20 000 × 10^9/L (+++).
GPS, Gray platelet syndrome.

Individuals with *GATA1* mutations have shown reduced platelet aggregation in response to collagen and decreased agglutination with ristocetin, consistent with reduced expression of GPVI and GPIb-IX-V complex, respectively.[66,72] One study showed decreased platelet guanosine 5′-triphosphate binding protein GαS (*GNAS*) expression.[66] Platelet life span in these patients appears to be normal.[66] In patients with significant bleeding from thrombocytopenia and/or platelet dysfunction, HSCT or genetic therapy has been proposed.[68]

More recently, families with *GATA1* splice mutations have been described.[73,74] Affected individuals had normal or elevated platelet counts with evidence of platelet dysfunction. For example, a family[73] with macrocytic anemia and neutropenia (X-linked anemia with or without neutropenia and/or platelet abnormalities [MIM 300835]) was found to have a germ line *GATA1* splice mutation (332G- > C,V74L), which leads to production of only short GATA1 protein isoforms. Platelet studies demonstrated impaired platelet aggregation upon activation with adenosine 5′-diphosphate, epinephrine, and collagen; AGs and DGs were decreased. Another report[74] described a child with dyserythropoietic anemia, MK dysplasia, and thrombocytosis, with a splice mutation in the *GATA1* 5′-untranslated region that also resulted in short GATA1 protein isoforms. Platelet aggregation in response to arachidonic acid was decreased, although the child did not have bleeding symptoms.

GFI1B

GFI1B, a zinc finger TF encoded by the *GFI1B* gene, is located on the long-arm of chromosome 9 (9q34.13) and has 3 domains: an N-terminal repressor "SNAG" (SNAIL/GFI1) domain where epigenetic modifiers are recruited, a less well characterized middle domain, and a C-terminal DNA binding domain. The C-terminal domain contains 6 zinc fingers; zinc fingers 3 to 5 mediate DNA binding, whereas the remaining interact with other proteins.[9] GFI1B functions as a transcriptional repressor and utilizes epigenetic processes to modulate the chromatin structure of the target gene.[75]

GFI1B is essential for both megakaryopoiesis and erythropoiesis and is expressed in HSCs, ME-Ps, and during maturation of erythroid cells and MKs.[75] Regulation of transforming growth factor β signaling by GFI1B controls lineage-specific differentiation from ME-Ps.[75-77] *GFI1B* knockout mice die at approximately embryonic day 15, likely due to both defective megakaryopoiesis and erythropoiesis.[78] Known GFI1B transcriptional targets include *BCLXL*, *SOCS1*, *SOCS3*, *CDKN1A*, *GATA3*, *MEIS1*, and *RAG1/2*.[75]

GFI1B has been linked to platelet disorders relatively recently. In 2013, a family initially described in 1976[79] with autosomal dominant mild-to-moderate macrothrombocytopenia, red cell anisopoikilocytosis, AG deficiency, and platelet dysfunction, was shown to have a single nucleotide insertion in exon 7 of *GFI1B* (c.880-881insC).[7] The variant predicted a frameshift affecting the fifth zinc finger of GFI1B, in the DNA binding domain.[7] A second family with GPS phenotype and autosomal dominant *GFI1B* mutation was described shortly after.[9] This family, initially described in 1968,[80] was found to have a nonsense mutation in exon 6 of *GFI1B* (c.859C → T, p. Gln287*), which produces a truncated GFI1B protein without 44 C-terminal amino acids), including 4 aas within the fifth zinc finger DNA-binding domain.[9] Functional studies were consistent with a dominant negative effect exerted by mutant GFI1B protein.[9] Subsequently, 8 additional patients with *GFI1B* mutations (7 distinct variants) have been identified, with mutations being biallelic in 1 patient.[76] These patients were identified by NGS during evaluation for inherited platelet dysfunction. Another family has been reported[81] with a *GFI1B* mutation and phenotype similar to the first two described families.

Patients with G*FI1B* mutation have mild-to-moderate macrothrombocytopenia with variable bleeding tendency.[7,9] Studies in one family showed impaired platelet aggregation in response to multiple agonists; response to collagen was impaired in all.[7] Affected patients had reduced platelet expression of GPIbα and GPIIIa.[7,9] Platelet AG contents were reduced.[7,9] BM biopsy of an affected patient showed fibrosis and emperipolesis, with neutrophils within MKs.[9] MKs generated ex vivo from 2 patients displayed dysplastic features.[9] *GFI1B* mutation is associated with abnormal proplatelet formation and increased MK/platelet CD34 expression.[9,81]

ETV6

ETV6, also known as translocation-Ets leukemia is encoded by the *ETV6* gene located on chromosome 12p13, and is a member of the ETS family. ETV6 has 452 aas that form a 57-KDa protein with 3 functional

domains that include the pointed N-terminal, central regulatory, and C-terminal DNA binding domains.[82] The C-terminal DNA binding domain contains the highly conserved ETS domain that recognizes the consensus DNA sequence 5-GGAA/T-3 within a 9- to 10-bp sequence.[82] ETV6 functions as a transcriptional repressor and its activity is modulated through self-association and autoregulation, with the former occurring at the pointed N-terminal domain. ETV6 polymerization appears to increase the DNA binding affinity of the C-terminal domain.[82] The central regulatory domain is important for ETV6 to fully exert transcriptional repression.[18] ETV6 interacts with numerous binding partners, including FLI1, whereby it inhibits FLI1 transcriptional activity.[83]

ETV6 plays critical roles in hematopoiesis and vascular network maintenance.[84,85] In mice, complete *ETV6* knockout leads to embryonic lethality at day 10.5 to 11.5 with evidence of impaired yolk sac angiogenesis, and apoptosis of neural and mesenchymal cells.[84] ETV6 is required for maintenance of hematopoiesis in the BM through supporting survival of HSCs, but is not strictly required for earlier hematopoiesis in the yolk sac or liver.[84,85] ETV6 is also important in terminal maturation of MKs. Conditional *ETV6* homozygous knockout mice had an ~50% reduction in platelet count compared with heterozygous mutant mice, with an approximate fivefold compensatory elevation in MK-Ps.[85]

Heterozygous *ETV6* germ line mutations have been identified in several families with inherited thrombocytopenia, variable red cell macrocytosis, and multiple members with hematologic malignancies, primarily B-cell ALL.[18-20] Functional studies demonstrated impaired ETV6 nuclear localization, decreased transcriptional repression through a dominant negative mechanism, and defects in MK maturation.[18-20] Germ line *ETV6* mutations have been implicated in ~1% of childhood ALL patients.[86]

Patients with heterozygous germ line *ETV6* mutations have variable thrombocytopenia, with platelet count ranging from 8 to 132 000 $\times$ 10^9/L.[18,20] Platelet ultrastructure and mean volume are normal with the presence of a few elongated AGs.[18] It is unknown if platelet function is impaired in these patients. BM studies show the presence of hypolobulated MKs and mild dyserythropoiesis,[18]

EVI1

EVI1 is encoded by the MDS1 and EVI1 complex locus (*MECOM*) gene located on chromosome 3 (3q26.2).[5,87] EVI1 is a zinc finger TF; the N-terminal domain has 5 zinc fingers that recognize a GATA-like motif and a C-terminal domain that consists of 3 zinc fingers that recognize an ETS-like motif.[87] Through the C-terminal eighth zinc finger, EVI1 interacts with itself and other TFs, including RUNX1 and GATA1 (as well as SPI1, SMAD3, and FOS).[87] EVI1 is expressed in HSCs, MKs, and platelets[5] and is essential for HSC self renewal.[88] *EVI1* knockout mice die in utero at approximately day 10.5, with evidence of bleeding, hypocellularity, and paraxial mesenchyme disruption.[89] In conditional *EVI1* knockout mice, there is loss of HSC renewal, although HSC differentiation is preserved.[89] In cell lines, *EVI1* knockdown has been demonstrated to impair MK differentiation with reduction in *ITGA2B* and *ITGB3* expression.[5] EVI1 downregulates the TPO receptor (*MPL*).[90]

Mutations in *MECOM* with alteration of the EVI1 protein have been implicated in the congenital disorder radioulnar synostosis and amegakaryocytic thrombocytopenia (RUSAT).[87] Earlier candidate gene analysis in 2 unrelated families with RUSAT[91,92] associated heterozygous mutations in *HOXA11*, which encodes a TF implicated in bone morphogenesis and MK differentiation. However, several individuals have been reported with RUSAT but without a *HOXA11* mutation.[87] Three such individuals were found to carry missense variants in *MECOM*.[87] The mutations are located within the eighth zinc finger of EVI1, and postulated to attenuate target DNA binding and/or decrease EVI1 interaction with self or other regulatory proteins.[87]

Patients with RUSAT are characterized by congenital fusion of the proximal radius, and ulna and severe thrombocytopenia ($<10 \times 10^9$/L).[87,91,93] MKs are markedly decreased or absent in the BM.[93] Affected patients often develop BM failure, and several have been treated with HSCT. Interestingly, 3 individuals with RUSAT had sensorineural hearing impairment (1 with *HOXA11* and 2 with *MECOM* mutations).[87] Congenital deletion of part of chromosome 3q26 has also been reported; affected infants had thrombocytopenia, with 1 child developing aplastic anemia.[94,95]

EVI1 has also been implicated in the pathogenesis of another congenital platelet disorder, the thrombocytopenia with absent radii (TAR) syndrome. The thrombocytopenia may be transient and improve over time.[93] Most TAR syndrome patients are thought to be from compound inheritance, with mutation of the *RBM8A* gene on one allele and mutation of an EVI1 regulatory region of the *RBM8A* gene on the other allele.[96] The latter results in decreased *RBM8A* transcription and production of the gene product, Y14. The risk of familial malignancy with *MECOM* mutations is unknown, although individuals with severe thrombocytopenia and/or BM failure often undergo HSCT at a young age. High expression of *MECOM* is implicated in 5% to 10% of sporadic AML patients and is an adverse prognostic marker.[87] In leukemia cells, EVI1 disruption attenuates proliferation.[89]

Mutations in regulatory TF-binding sites

Apart from mutations in the genes encoding the TF, mutations in the regulatory TF-binding sites of transcriptional targets may also produce a clinical platelet disorder.[97,98] One example is the heterozygous variants in the 5′-untranslated region of *ANKRD26* associated with autosomal dominant thrombocytopenia.[99,100] *ANKRD26* encodes for an inner cell membrane protein that interacts with signaling proteins.[97] The *ANKRD26* variants abrogate binding of RUNX1 and FLI1, resulting in the loss of *ANKRD26* silencing, hyperactivation of the mitogen-activated protein kinases pathway, and impaired proplatelet formation,[97,99,100] Another example is a heterozygous missense mutation involving the GATA1-binding site in the promoter of *GP1BB*.[98] The patient had velocardiofacial syndrome with already partial deletion of one chromosome 22 (including *GP1BB*) and decreased platelet GPIbβ expression.

Conclusion

There is growing evidence that mutations in hematopoietic TFs represent an important genetic mechanism for inherited platelet disorders. These may be more common than previously appreciated in such patients. This shifts the emphasis from searching for causal gene variants based on phenotype and candidate genes, to the perspective that the driving mutation may be in a TF that regulates multiple genes expressed in MKs/platelets. This recognition is important also because of additional implications associated with TF mutations, such as the risk of malignancy. Overall, these patients are a unique source of

information into the genetic and molecular mechanisms governing MK and platelet biology.

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