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Inherited Platelet Dysfunction and Hematopoietic Transcription Factor Mutations

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Abstract

Transcription factors (TF) are proteins that bind to specific DNA sequences and regulate expression of genes. The molecular and genetic mechanisms in most patients with inherited platelet dysfunction 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 include runt related transcription factor 1 (RUNX1), Fli-1 proto-oncogene, ETS transcription factor (FLI1), GATA-binding protein 1 (GATA1), and growth factor independent 1B transcriptional repressor (GFI1B). These TFs act in a combinatorial manner to bind sequence-specific DNA within a promoter region 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 transcription factors affect diverse aspects of megakaryocyte biology, and platelet production and function, culminating in thrombocytopenia, platelet dysfunction, and associated clinical features. Mutations in TFs may occur more frequently in patients with inherited platelet dysfunction than generally appreciated. This review focuses on the alterations in hematopoietic TFs in the pathobiology of inherited platelet dysfunction.

Keywords

Transcription factors; Platelet function disorders; RUNX1; GATA1; FLI1; GFI1B

INTRODUCTION

Transcription factors (TF) are proteins that bind to specific DNA sequences and regulate expression of genes. There is now increasing evidence that mutations in hematopoietic TFs are an important underlying cause for defects in platelet production, morphology, and function. The major hematopoietic TFs implicated in patients with impaired platelet function include runt related transcription factor 1 (RUNX1), Fli-1 proto-oncogene, ETS transcription

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DECLARATIONS OF INTEREST

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factor (FLI1), GATA-binding protein 1 (GATA1), and growth factor independent 1B transcriptional repressor (GFI1B). These TFs act in a combinatorial manner to bind sequence-specific DNA within a promoter region to regulate lineage-specific gene expression, either as activators or repressors.[1,2] TF mutations can induce numerous rippling downstream effects by simultaneously altering the expression of multiple genes. Each of the hematopoietic TFs are involved in megakaryocyte (MK) differentiation and platelet biogenesis by modulating vital processes such as endomitosis, cytoplasmic maturation, and proplatelet formation. Perturbations in these processes can result in defects in platelet production, morphology, and multiple aspects of platelet function.[3]

In 1969, Weiss and colleagues described a family with an autosomal dominant inherited platelet disorder due to decreased dense granule contents.[4] This family was subsequently found to have partial α-granule deficiency, reflecting a defect in both types of granules.[5] In 2002, this family was reported to have the Familial Platelet Disorder with Predisposition to Acute Myeloid Leukemia (FPD/AML)(Mendelian Inheritance in Man [MIM] 601399) with a heterozygous Y260X mutation in the *RUNX1* gene,[6] thereby providing a genetic mechanism for the inherited platelet defects and evidence for a mutation in a TF. Similarly, some patients previously reported with abnormal platelet function or granule deficiency (α or dense) have subsequently been shown to harbor TF mutations.[7–13] These studies that identified TF mutations in patients with platelet dysfunction have advanced a paradigm shift in the identification of underlying gene abnormalities associated with platelet dysfunction from mutations in candidate genes based on the altered platelet phenotype to mutations in TFs that regulate multiple platelet aspects at the same time.

Mutations in TFs may occur more frequently in patients with inherited platelet dysfunction than generally appreciated. Stockley and colleagues[10] performed next generation sequencing on 13 unrelated patients who were part of the UK Genotyping and Phenotyping Study of Platelets (UK-GAPP) cohort, with clinical bleeding and impaired platelet aggregation and dense granule secretion. Heterozygous *RUNX1* or *FLI1* mutations were uncovered in 6 of the 13 index patients, with the mutations also found in other family members. Four of the index patients had mild thrombocytopenia. Although further studies in platelets and MKs are needed to establish the link between the genetic mutations and the platelet defects, these studies focus attention on the need to consider hematopoietic TF mutations in the pathogenesis of inherited platelet dysfunction. Also of note, not all such patients have clear-cut thrombocytopenia and a normal platelet count may not exclude an underlying TF mutation. This review focuses on the hematopoietic TF mutations associated with inherited platelet function defects (Table 1). These include RUNX1, FLI1, GATA1, and GFI1B, with the latter two also being associated with concurrent defects in erythropoiesis. Although mutations in other TFs (e.g. ETV6[14]) have been associated with altered platelet production and thrombocytopenia, abnormalities in platelet function studies are not so well-documented. This is in part due to the limitations imposed by the decreased platelet count in performing the platelet function studies, especially in patients with severe thrombocytopenia, and the need to modify existing approaches that are routinely used in clinical laboratories to document abnormal platelet function.

RUNX1

RUNX1 - also known as core-binding factor subunit alpha-2 (CBFA2) and acute myeloid leukemia 1 (AML1) – is a member of the Runt family of TFs that associates with a co-factor, core-binding factor subunit beta (CBFB). It is encoded by the *RUNX1* gene located on chromosome 21 (21q22.3) and is frequently mutated in patients with AML and MDS.[3,15] RUNX1 is essential for definitive hematopoiesis; in murine models *RUNX1* deletion is associated with complete lack of hematopoiesis during embryogenesis and death *in utero* as a result of hemorrhage.[16] Of note, murine heterozygous *RUNX1* mutations do not recapitulate the human phenotype.[17]

In humans, *RUNX1* heterozygous mutations are associated with FPD/AML. Over 40 families have been described to date,[18] though the disorder is likely under-recognized. A number of distinct *RUNX1* mutations have been discovered, ranging from frameshift, nonsense, and missense mutations to deletions involving a portion of the *RUNX1* gene or more extensively involving chromosome 21q22.[6,19] Most mutations involve the conserved Runt domain near the N-terminus, and result in impaired binding of RUNX1 to its transcriptional target at the regulatory DNA sequences. A C-terminal transactivating domain mutation (Y260X) has also been reported.[3] Most described *RUNX1* mutations produce haplodeficiency, with approximately 50% of RUNX1 activity preserved, though some mutations may almost completely abrogate RUNX1 activity through a dominant negative effect, which has been postulated to markedly increase risk for leukemia development.[6,19,20] Patients with *RUNX1* mutation have a phenotype characterized by thrombocytopenia, several platelet function defects, and a substantially increased risk of AML or MDS (over 40% at a median age of 33 years).[7,18,19,21] Patients typically only have a mild to moderate bleeding tendency despite the platelet dysfunction and mild to moderate thrombocytopenia, with usually normal-sized platelets.[19,22] Notably, some patients may not have thrombocytopenia nor any bleeding symptoms.[19,22] This is particularly relevant in the setting of hematopoietic stem cell transplantation because recurrence of leukemia has been observed in the recipient following transplantation using an undiagnosed sibling with a *RUNX1* mutation as a donor.[22] Deletions in chromosome 21q22 can also occur and are thought to result from sporadic germline mutation.[19] These deletions can produce syndromic features such as dysmorphic facial features, mental retardation, with multiple organ abnormalities. Some affected individuals have been observed to develop AML or MDS at a much younger age than is typically seen in FPD/AML (three described cases ranging from 5 to 8 years).[19]

Abnormalities in several aspects of platelets and MKs have been documented in association with *RUNX1* haplodeficiency. In addition to thrombocytopenia, the platelet function abnormalities include impaired platelet aggregation and secretion upon activation, dense and/or α-granule storage pool deficiency, reduced protein phosphorylation of myosin light chain and pleckstrin, impaired αIIbβ3 activation, lower production of 12-hydroxyeicosatetranenoic acid (12-HETE, a product of 12-lipoxygenase) and decreased protein kinase C-θ (an enzyme that phosphorylates numerous proteins, including pleckstrin).[6,8,23–25] Platelet albumin and IgG – which are not synthesized by MKs – were

also reduced in one patient, suggesting defect in platelet uptake from plasma and incorporation into α-granule of proteins.[8]

Recent studies indicate that one of the mechanisms underlying the dense granule defect associated with *RUNX1* mutations is dysregulation of *PLDN,* which is a direct transcriptional target of RUNX1.[26] *PLDN* encodes for pallidin, a protein that is involved in biogenesis of dense granules and is decreased in the pallid mouse and the human Hermansky-Pudlak syndrome.[27–29] There is also evidence that patients with *RUNX1* mutations may have global defects that compromise agonist-stimulated secretion of contents of the α- and dense-granules, as well as from the acid hydrolase containing vesicles, which is unrelated to a deficiency in the granule contents.[10,30–32] Thus, these patients may have decreased secretion on platelet activation related to abnormalities at the granule level and due to abnormalities that compromise the secretory pathways.

In general, each transcription factor regulates the tissue specific expression of multiple genes.[3] The multiple defects in platelet responses and pathways likely arise due dysregulation of numerous MK/platelet genes that are transcriptional targets of RUNX1 and participate in diverse aspects of MK/platelet biology. In our platelet transcript profiling studies of a patient with *RUNX1* mutation numerous genes relevant to multiple platelet biological pathways were downregulated.[33] Several genes have been shown to be direct transcriptional targets of RUNX1, including 12-lipoxygenase (*ALOX12*),[25] platelet factor 4 (*PF4*),[34] platelet myosin light chain (*MYL9*),[35] protein kinase C-θ (*PRKCQ*),[36] palladin (*PLDN*),[26] and the thrombopoietin receptor (*c-MPL*).[37] These impact various aspects of MK biology, platelet production, structure, signaling, and responses. The gene that encodes TF NF-E2 has also been shown to be a direct transcriptional target of RUNX1.[18] NF-E2 has been implicated in platelet granule development and αIIbβ3 signaling.[18]

RUNX1 has a prominent role in megakaryopoiesis. Conditional *RUNX1* knockouts in mouse models show impaired MK maturation, with the presence of abnormal micromegakaryocytes and significant reduction in MK polyploidization.[38] In patients with *RUNX1* mutation, MKs cultured from stem cells also demonstrate defects in differentiation and polyploidization. This has been attributed to dysregulated non-muscle 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.[24,39] Detection of platelet MYH10 protein expression has been proposed as a marker genetic defects in *RUNX1* and *FLI1*.[40]

Studies of Connelly and colleagues[21] further strengthened the role of RUNX1 in megakaryopoiesis and platelet granule formation, and raise the exciting possibility of gene targeting therapy. The investigators created induced pluripotent stem cells (iPSCs) from 2 FPD/AML patients with Y260X mutation and showed that targeted *in vitro* correction of *RUNX1* can recover MK defects. They differentiated skin fibroblasts from the patients and showed impaired MK production and structure abnormalities, such as abundance of vacuoles and α- and dense-granule deficiency. Gene targeting corrected 2 of 7 cloned iPSCs. The corrected clones produced ~ 40–60% greater CD41+CD42+ MKs and exhibited recovery of

abnormal phenotypic features. The targeted expression of *RUNX1* was associated with the up-regulation of numerous MK genes and accounted for the phenotypic differences. This ability to resolve defective MK differentiation with *RUNX1* correction has recently been corroborated by Lizuka and colleagues.[41]

FLI1

FLI1 is member of the E-twenty-six (ETS) family of TFs and is encoded by the *FLI1* gene located on the distal end of the long arm of chromosome 11 (11q24.1-q24.3). FLI1 plays a major role in both early and late megakaryopoiesis.[3] Homozygous *FLI1* murine knockouts die from embryonic hemorrhaging, thought to be due to the substantial role of FLI1 in megakaryopoiesis as well as endothelium and hemangioblast specification.[2] Heterozygous *FLI1* deletion in mice do not have an abnormal hematologic phenotype.[42] FLI1 regulates several genes relevant to platelet number and function, including glycoprotein IIb (*ITGA2B*), glycoprotein 1bα chain (*GP1BA*), glycoprotein 9 (*GP9*), the thrombopoietin receptor (*c-MPL*), and platelet factor 4 (*CXCL4/PF4*).[2]

In humans, hemizygous defects in *FLI1* resulting from distal deletion of a variable part of the long arm of chromosome 11 are associated with the Jacobsen syndrome (MIM 147791, ~1/100,000 births), and the associated platelet disorder, the Paris-Trousseau syndrome (PTS) (MIM 188025). Patients with the Jacobsen syndrome may have an array of manifestations, including mental retardation, developmental delay, abnormal craniofacial appearance, and abnormalities in multiple organ systems, include cardiac, renal and genitourinary, neurologic, and the hematologic systems.[43,44] The majority of cases of Jacobsen syndrome are a result of *de novo* mutation in either the paternal or maternal gamete, with the remainder occurring from *de novo* translocations, unbalanced segregation of familial balanced translocation or chromosomal rearrangements such as a ring chromosome 11 (with loss of genetic material at distal end).[45] PTS is present in > 90% of individuals with Jacobsen syndrome, but can also occur in isolation without the Jacobsen syndrome phenotype, presumably due to a smaller deletion of chromosome 11. PTS is characterized by congenital macrothrombocytopenia, giant α-granules (1–2 μm) in 1–5% of platelets, bone marrow dysmegakaryopoiesis, and platelet function defects.[42] Bone marrow dysmegakaryopoiesis results in increased number of MKs, with the presence of a dimorphic MK population - one normal and the other consisting of immature micromegakaryocytes with reduced survival - owing to transient monoallelic *FLI1* expression in a single MK precursor.[46,47] Platelet survival is normal.[46] Interestingly, the moderate to severe congenital thrombocytopenia in children with Jacobsen syndrome/PTS improves over time, to even a normal range, over a period of months to years.[45] Patients with PTS typically have an increased bleeding tendency of variable severity.[10,42,48] Platelet function abnormalities in α- and dense secretion in response to thrombin have been observed.[10,46]

Although the platelet dysfunction has been ascribed to hemizygous deletions containing the *FLI1* locus, other genetic mechanisms have also been described. In patients with platelet dysfunction with impaired platelet aggregation and dense granule secretion, Stockley and colleagues uncovered *FLI1* alterations due to point mutations (rather than deletions) that were predicted to interfere with FLI1 binding to cis-regulatory DNA sequences by altering

the α-helix of the ETS domain.[10] Stevenson and colleagues also recently reported on 2 patients with the PTS platelet phenotype and significant history of bleeding symptoms with homozygous inheritance of hypomorphic *FLI1* alleles from unaffected consanguineous parents who had a heterozygous *FLI1* point mutation within the DNA binding domain.[42] This discovery extends the spectrum of hematopoietic TF mutations beyond the heterozygous state to the homozygous state. The normal platelet count and function of the parents with heterozygous *FLI1* mutation also suggests that the mechanisms by which *FLI1* alterations produce abnormalities in megakaryopoiesis and platelet function remain to be elucidated.[48]

GATA1

GATA1, a member of the GATA family, is a zinc finger hematopoietic TF. The *GATA1* gene is located on the short arm of the X-chromosome (Xp11.23).[3,44] GATA1 is highly expressed in megakaryocytes, erythroid cells, mast cells and eosinophils, and is indispensible for terminal differentiation of these lineages.[2,3,9,49] GATA1 is composed of two homologous zinc fingers, the N-terminal finger (N-finger) and C-terminal finger (C-finger). The GATA1 C-finger binds to specific GATA motif DNA sequences. The N-finger, through association with a critical nuclear co-factor protein called Friend of GATA1 (FOG-1), enhances the stability of GATA1 binding to especially complex or palindromic GATA1 DNA binding sites.[9,49] Alterations in GATA1 function can thus be through mutations that affect the *GATA1* DNA binding domain or its association with FOG-1, with adverse effects on both megakaryopoiesis and erythropoiesis.[9,49–53] *GATA1* null mutations in mice are lethal *in utero* at approximately day 10, from severe anemia rather than from bleeding.[2] Consistent with essential roles in erythropoiesis and megakaryopoiesis, a wide spectrum of both erythroid and MK lineage genes (e.g. *GP1BA, GP1BB, ITGA2B, GP9, PF4, c-MPL, NF-E2*) contain GATA1 binding motifs within their cis-regulatory elements.[49,51]

Several families with platelet disorders associated with distinct *GATA1* mutations have been described (Table 2). Given that *GATA1* resides on the X-chromosome, these disorders follow an X-linked mode of inheritance. The first reported pedigrees described families with X-linked syndromes consisting of macrothrombocytopenia and dyserythropoiesis with or without anemia (MIM 300367), with the severity of the latter modulated by the degree of disruption of GATA1 binding to FOG-1. In the first reported family two male members had severe fetal anemia requiring *in utero* red cell transfusion. The affected individuals also had significant thrombocytopenia, and required platelet transfusions to maintain platelet count. Both underwent hematopoietic stem cell transplant (HSCT) at a young age. Genetic analysis uncovered a *GATA1* V205M mutation that severely impaired GATA1 interaction with FOG-1.[50] Another family whose affected members also had severe dyserythropoietic anemia and thrombocytopenia was later described, with six affected boys dying before age 2. A *GATA1* D218Y mutation was found, which impairs GATA1/FOG-1 interaction to a similar degree as the V205M mutation.[49] This contrasts to mutations that produce less severe alterations in GATA1/FOG-1 affinity. In these cases, two pedigrees with distinct *GATA1* mutations (D218G, G208S) were reported.[51,52] Affected members had significant thrombocytopenia with substantial bleeding symptoms and mild to moderate evidence of dyserythropoiesis, but had no anemia. This suggests that megakaryopoiesis may be more

sensitive to a given degree of disruption in GATA1/FOG-1 interaction than erythropoiesis.[52] Of note, another *GATA1* mutation (G208R) has also been reported in a patient with dyserythropoietic anemia and significant thrombocytopenia, but anemia was not severe and bleeding symptoms improved with age.[54]

GATA1 mutations have also been implicated in X-linked thrombocytopenia with β-thalassemia (MIM 314050). Affected patients generally have moderate macrothrombocytopenia, platelet dysfunction, α-granule deficiency, imbalanced globin chain synthesis, red cell hemolysis, and evidence of dyserythropoiesis and dysmegakaryopoiesis.[9,53,55] *GATA1* R216Q mutations within the GATA1 N-finger have been identified in these cases, which is unique in that it impairs binding of the GATA1 N-finger to complex or palindromic DNA sites rather than through interruption of GATA1/FOG-1 interaction, as with previously described mutations.[9,53] Subsequently, a *GATA1* R216Q mutation was also identified in a family with the Gray Platelet Syndrome (GPS), with macrothrombocytopenia and α-granule deficiency.[56] One family member had a mild β-thalassemia-like phenotype. Some experts advocate classifying this family as X-linked thrombocytopenia with β-thalassemia rather than as GPS.[57]

Affected individuals with these *GATA1* mutations have generally demonstrated impaired platelet agglutination with ristocetin and reduced platelet aggregation with collagen, reflecting decreased expression of the GPIb-IX-V complex and glycoprotein VI, respectively.[51,58] In one study, there was also evidence of reduced platelet GSα expression, indicating incomplete maturation of megakaryocytes. Platelet life span appears to be normal in patients with *GATA1* mutations.[51] Overall, because of the significant bleeding manifestations in some individuals with *GATA1* mutations, hematopoietic stem cell transplant or genetic therapy has been proposed as a potential treatment.[52]

More recently, families with *GATA1* splice mutations have also been described. Affected individuals have more pronounced defects in erythropoiesis than megakaryopoiesis, though there is evidence of platelet function defects. A germline *GATA1* splice mutation (332G->C, V74L) within exon 2, leading to only production of short GATA1 isoforms (GATA1s), was uncovered in a family with macrocytic anemia, neutropenia, but normal platelet counts (X-linked anemia with or without neutropenia and/or platelet abnormalities (MIM300835)).[59] Despite normal platelet counts, affected males had impaired platelet aggregation upon activation with ADP, epinephrine, and collagen, as well as decreased α- and dense granules by electron microscopy.[59] Zucker and colleagues[60] have also reported a case of a child with dyserythropoietic anemia, megakaryocyte dysplasia, thrombocytosis, and a subclinical platelet function defect. Genetic analysis revealed a splice mutation in the *GATA1* 5'UTR that also leads to production of GATA1s. Platelet aggregation studies revealed subclinical mild defects in response to arachidonic acid. These *GATA1* splice mutations highlight diverse genetic mechanisms within hematopoietic TFs that can lead to platelet function abnormalities, even in patients without thrombocytopenia. From a different perspective, the Bernard-Soulier Syndrome has also been reported to occur due to a mutation in a GATA binding site of the *GPIbβ* promoter, highlighting a causative mechanism in the regulatory region of a gene rather than the coding region.[61]

GFI1B

GFI1B is a DNA binding zinc-finger TF with prominent roles in both erythropoiesis and megakaryopoiesis.[62] The *GFI1B* gene is located on the long arm of chromosome 9 (9q34.13). GFI1B is highly expressed in the megakaryocyte-erythrocyte progenitors and appears to promote differentiation of these lineages in part by regulation of TGFβ signaling.[63,64] Unique amongst the hematopoietic TFs discussed thus far, GFI1B functions predominantly as a transcriptional repressor, and influences target gene expression through epigenetic mechanisms to modulate chromatin structure. These mechanisms involve the recruitment of histone-modifying enzymes such as histone methyltransferases and histone deacetylases to target gene promoters and enhancers.[63] GFI1B consists of 3 domains – an N-terminal repressor "SNAG" (SNAIL/GFI1) domain where epigenetic modifiers are recruited, a less characterized middle region, and a C-terminal DNA binding domain containing 6 zinc fingers, with zinc fingers 3–5 binding to the consensus DNA binding site and the other zinc fingers partaking in interactions with other proteins.[12,63] *GFI1B* knockout mice die at approximately embryonic day 15 likely due to both defective erythropoiesis and megakaryopoiesis.[65] Interestingly, a conditional mouse knockout of *GFI1B* showed that in this setting GFI1B also contributes to hematopoietic stem cells remaining dormant, as GFI1B absence results in mobilization of hematopoietic stem cells to the peripheral blood.[66] Consistent with the role of GFI1B in erythrocyte and megakaryocyte differentiation and maturation, there was evidence of decreased peripheral red blood cells, with a more marked reduction in circulating platelets.[66] Known transcriptional targets of GFI1B include the genes *BCLXL, SOCS1, SOCS3, CDKN1A, GATA3, MEIS1*, and *RAG1/2*.[63] However, as compared with the hematopoietic TFs discussed earlier, less is known about the genetic targets of GFI1B as it relates to MK development, platelet structure, and function.

Despite the known prominent role for *GFI1B* in erythropoiesis and megakaryopoiesis, it was not until recently in 2013 that mutations in *GFI1B* were linked to human disease. Stevenson and colleagues studied a family, first reported in 1976, with an autosomal dominant disorder characterized by mild to moderate macrothrombocytopenia, red cell anisopoikilocytosis, α-granule deficiency, and platelet dysfunction.[11,67] They identified a single nucleotide insertion in exon 7 of *GFI1B* (c.880-881insC) that predicts a frameshift mutation within the fifth zinc-finger, part of the DNA-binding domain.[11] Affected members of the family had variable bleeding tendency. Platelet function studies showed impaired platelet aggregation responses to multiple agonists, with all 3 affected individuals exhibiting reduced platelet aggregation in response to collagen activation. Expression of platelet glycoproteins were also decreased, including glycoproteins IIIa and Ibα. Platelet P-selectin and fibrinogen were also markedly reduced, consistent with the α-granule deficiency observed on electron microscopy.

A second family with autosomal dominant *GFI1B* mutation with a GPS phenotype was reported shortly thereafter.[12] This family, originally described in 1968, has affected members with moderate macrothrombocytopenia and α-granule deficiency.[12,68] Affected individuals had moderate to severe bleeding tendency with markedly reduced platelet β-thromboglobulin and platelet factor 4 along with decreased α-granules on electron microscopy. Platelet expression of glycoprotein Ibα was reduced in almost all affected

patients. One affected family member also had a bone marrow biopsy, which showed stage I bone marrow fibrosis and evidence of emperipolesis, with the presence of intact neutrophils within megakaryocytes. Genetic analysis uncovered a nonsense mutation in exon 6 (c.859C->T, p. Gln287*), resulting in a truncated GFI1B protein lacking 44 carboxy-terminal amino acids, including 4 amino acids located within the fifth zinc-finger domain that is responsible for DNA binding to the core consensus sequence. Functional studies showed attenuated ability of truncated GFI1B to repress transcription. Co-transfection experiments with nonmutant and mutant (truncated) GFI1B also showed decreased transcriptional repression, indicating that mutant GFI1B exerts a dominant negative effect, possibly by interaction with proteins that would otherwise cooperate with nonmutant GFI1B to promote transcriptional repression. Introduction of the truncated *GFI1B* mutation in a murine model also showed differentiation of MKs with dysplastic features, including hypolobulated nuclei and irregular contours. MKs within the bone marrow aspirate and generated ex-vivo from two affected family members showed similar dysplastic features.[12] The identification of an autosomal dominant mechanism leading to GPS is notable, as inheritance in other described GPS pedigrees has been autosomal recessive, with biallelic mutations in *NBEAL2*, which encodes a BEACH protein involved in vesicular trafficking.[69–71] Although α-granule biogenesis and maintenance is not completely understood, it is clear that alterations in multiple genes may lead to α-granule deficiencies through diverse mechanisms, including *NBEAL2, VPS33B* (in the arthrogryposis, multiplex congenital, renal dysfunction, and cholestasis syndrome),[72,73] and mutations in *RUNX1, GATA1*, and *GFI1B*. With *NBEAL2* mutations, a murine knockout model produced proinflammatory MKs that led to α-granule loss after initial formation and drove other characteristic features of GPS such as myelofibrosis, emperipolesis, and splenomegaly.[74] Thus, GPS phenotype can manifest through multiple genotypic alterations.

Since the reports of the first two families with *GFI1B* mutation, genetic evaluation of patients enrolled in the international Biomedical Research Centres/Units Inherited Diseases Genetic Evaluation (BRIDGE) consortium (https://bridgestudy.medschl.cam.ac.uk/index.shtml), who are suspected of having a high likelihood of an inherited platelet disorder, has identified 8 additional cases of *GFI1B* mutation, with 7 of the mutations being distinct.[62] One of the *GFI1B* mutations was a biallelic mutation.[62] The findings further underscore that hematopoietic TF mutations in platelet disorders may be under-recognized. Future studies in MKs/platelets are likely to provide insights that further establish the causal link between the genetic defect and the heterogeneous platelet abnormalities.

CONCLUSION

There is increasing evidence that mutations in hematopoietic TFs represent an important underlying genetic basis for disorders of platelet function and number, and that these mutations may be more common than previously appreciated. Recognition of TF mutations is important, as it yields proper diagnosis, and can inform prognosis (e.g. risk of malignancy in *RUNX1* mutations) or direct treatment (e.g. HSCT in some cases of *GATA1* mutation). These patients constitute an untapped reservoir of information into the genetic and molecular mechanisms that govern platelet and MK biology.

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Table 1

Selected genes regulated by transcription factors whose mutations are associated with platelet dysfunction

Transcription Factor	Location	Selected Transcriptional Targets
RUNX1	Chromosome 21q22.3	*ALOX12, PF4, MYL9, PRKCQ, c-MPL, MYH9, MYH10, NF-E2, PLDN*
FLI1	Chromosome 11q24.1-24.3	*ITGA2B, GP1BA, GP9, c-MPL, PF4*
GATA1	Chromosome Xp11.23	*GP1BA, GP1BB, ITGA2B, GP9, PF4, c-MPL, NF-E2*
GFI1B	Chromosome 9q34.13	*BCLXL, SOCS1, SOCS3, CDKN1A, GATA3, MEIS1, RAG1/2*

Table 2

GATA1 Mutations and Associated Abnormalities

Phenotype	Mutation	Disruption in GATA1/FOG-1 Interaction	Anemia	Thrombocytopenia
Thrombocytopenia +/− Dyserythropoietic Anemia (MIM 300367)	V205M[50]	*+++*	*++*	*++/+++*
	D218Y[49]	*+++*	*++*	*+++*
	D218G[51]	*+*	*−*	*+/++/+++*
	G208S[52]	*+*	*−*	*++*
	G208R[54]	no information	*++*	*+++*
Thrombocytopenia with β-thalassemia (MIM 314050)	R216Q[9,53,55,75]	− (disrupts DNA binding)	+/−	+ and GPS
Macrocytic anemia; Neutropenia; Normal platelet count (MIM 300835)	Splice mutation 332G->C, V74L[59]	−	+/++/+++ (many cases also with neutropenia)	−
Dyserythropoietic Anemia; Megakaryocyte dysplasia; Thrombocytosis	Splice mutation in 5'UTR[60]	−	+++ (occasional neutropenia)	−

Anemia: + Hb ≥ 10 g/dL, ++ Hb 7 – < 10 g/dL, +++ Hb < 7 g/dL

Thrombocytopenia: + 70,000–90,000 × 10^9/L, ++ ≥ 20,000– < 70,000 × 10^9/L, +++ < 20,000 10^9/L

GPS, Gray platelet syndrome