

THROMBOCYTOPENIA CAUSED BY INHERITED HAEMATOPOIETIC TRANSCRIPTION FACTOR MUTATION: CLINICAL PHENOTYPES AND DIAGNOSTIC CONSIDERATIONS

David J. Rabbolini, Christopher M. Ward, *William S. Stevenson

*Department of Haematology and Transfusion Medicine, Royal North Shore Hospital;
Northern Blood Research Centre, Kolling Institute of Medical Research,
University of Sydney, Sydney, Australia*

**Correspondence to william.stevenson@sydney.edu.au*

Disclosure: The authors have declared no conflicts of interest.

Received: 10.03.16 **Accepted:** 24.06.16

Citation: EMJ Hematol. 2016;4[1]:100-109.

ABSTRACT

Inherited thrombocytopenias comprise a heterogeneous group of blood disorders with abnormalities in genes related to glycoproteins and adhesion molecules, signalling pathways, cytoskeletal components, granule formation, and transcription factor complexes. Recent improvements in sequencing technology have increased the number of transcription factor mutations that have been implicated as causative for these platelet disorders. Mutations in *RUNX1*, *GATA1*, *GFI1B*, *FLI1*, and *ETV6* share common features, including a variable bleeding history often associated with abnormal but non-specific changes in platelet morphology and platelet function testing. The phenotype of the underlying platelet disorder is often variable despite mutations in the same transcription factor, suggesting that the site of mutation and the protein domain that is perturbed is an important determinant of the clinical syndrome. Importantly, some of these transcription factor mutations are associated with other physical abnormalities, including an increased risk of acute leukaemia as well as solid organ malignancies. Genetic diagnosis of these disorders allows rational medical management to prevent bleeding, as well as providing an opportunity for family screening in order to reduce disease burden.

Keywords: Transcription factors, inherited thrombocytopenia, megakaryopoiesis, next-generation sequencing.

INTRODUCTION

Platelets mediate primary haemostasis after tissue trauma. Abnormalities in the number or function of platelets may predispose to spontaneous mucosal bleeding, or may precipitate problematic bleeding at the time of surgery. The correct diagnosis of platelet disorders provides an opportunity to prevent bleeding episodes and allows optimisation of haemostatic function for surgery. Isolated thrombocytopenia is a relatively common abnormality detected on full blood count analysis with most cases resulting from an increase in consumption of platelets in the circulation or a failure of adequate production from the bone marrow. Common causes include autoimmune or drug-

induced consumption, sepsis, immunodeficiency syndromes, or bone marrow suppression from malignancy. Inherited causes of thrombocytopenia are caused by a range of genetic defects and are important to correctly identify; isolated thrombocytopenia has been misdiagnosed as immune thrombocytopenia in a number of clinical cohort studies with subsequent inappropriate treatment using corticosteroid therapy and splenectomy.^{1,2} Traditional laboratory investigation of inherited causes of thrombocytopenia includes assessment of blood film morphology, functional aggregation studies, and flow cytometry.³ In recent years, an increasing use of genetic technologies with DNA sequencing has allowed better molecular characterisation of platelet disorders and provided

a platform for the discovery of new genetic defects that are causative of platelet-related bleeding.

THE ROLE OF TRANSCRIPTION FACTORS IN MEGAKARYOPOIESIS AND PLATELET PRODUCTION

Megakaryopoiesis and platelet production occur through a series of co-ordinated differentiation and maturation events that are orchestrated by cytokines and tightly regulated by networks of haematopoietic transcription factors.^{4,5} Different transcription factors have been identified to act at various stages of haematopoiesis,⁴

and in the megakaryocyte lineage a complex of key transcription factors (*GATA2*, *LYL1*, *TAL1*, *ERG*, *FLI1*, *RUNX1*, *LMO2*) expressed by haematopoietic stem cells is considered to initiate the development programme.⁵ These 'prime' sets of lineage-specific genes^{4,5} such as *GP1BA* and *GP1BB*, are uniquely expressed in platelets and provide haemostatic potential. The principal components of this complex are the GATA-binding transcription factors (*GATA1* and *GATA2*), E twenty-six (ETS) factors (*FLI1* and *GABP- α*), and the transcription activator-like helix-loop-helix factors that regulate target genes and interact with other factors.⁴

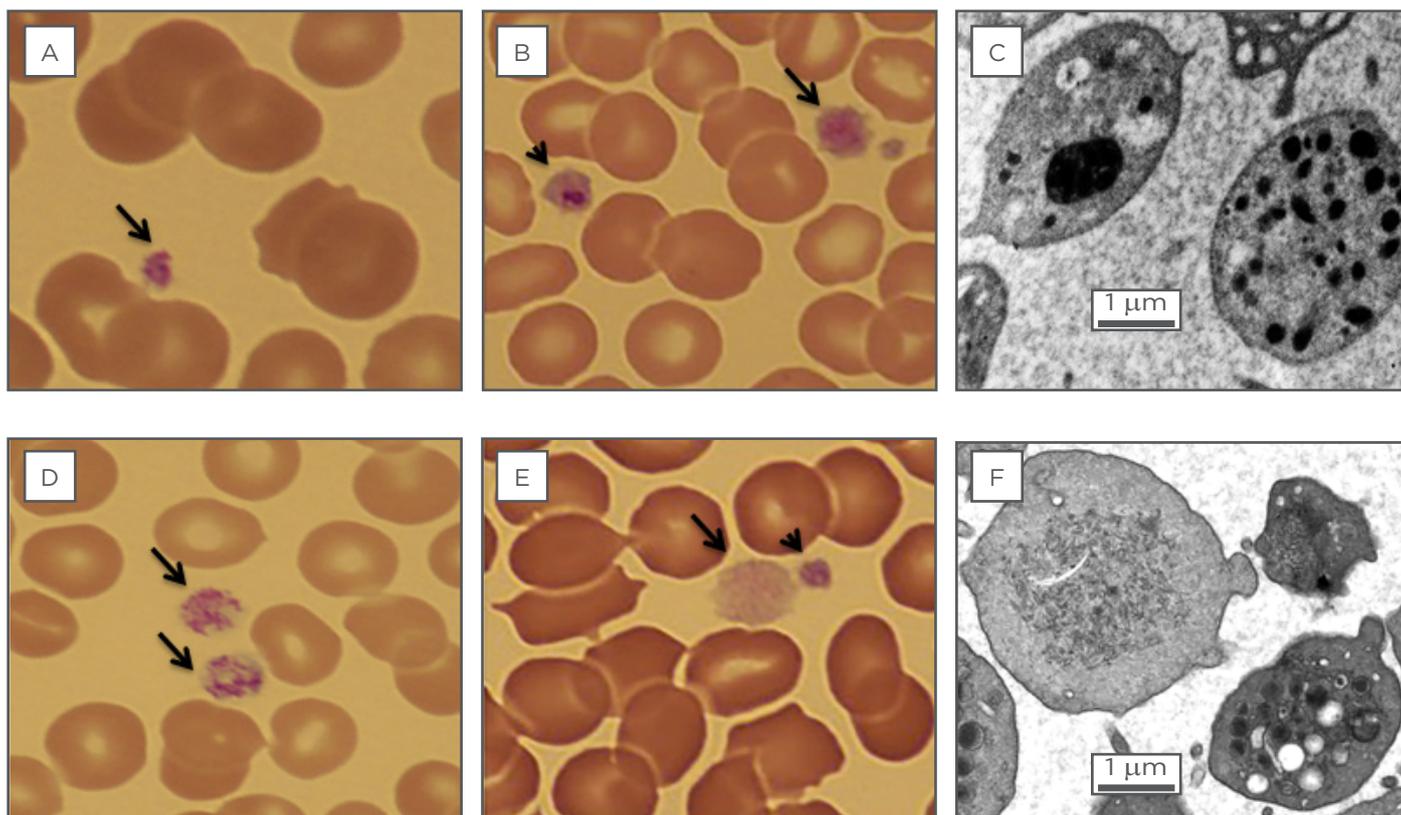


Figure 1: Platelet abnormalities associated with haematopoietic transcription factor mutation.

A) Photomicrograph of a blood film (100x) from an individual with a *RUNX1* C-terminal transactivation domain mutation. A normal sized platelet with normal granulation is seen (arrow).

B) Photomicrograph of a blood film (100x) from an individual with the *FLI1* *R324W* mutation. Two large abnormal platelets are seen (arrow and arrow head). One of these platelets (arrow head) appears to contain a giant granule.

C) Transmission electron micrograph from an individual with the *FLI1* *R324W* mutation showing a platelet with a giant fused α -granule and a platelet with normal α -granule distribution.

D) Photomicrograph of a blood film (100x) from an individual with the *GFI1B* *C168F* mutation. Two large platelets with normal granulation are seen (arrows).

E) Photomicrograph of a blood film (100x) from an individual with the *GFI1B* *H294fs* mutation. A Large abnormal hypogranular platelet is present (arrow) as well as a normogranular platelet (arrow head).

F) Transmission electron micrograph from an individual with the *GFI1B* *H294fs* mutation showing both an agranular platelet and a platelet with normal α -granule content.

Runt-Related Transcription Factor 1

The core binding factor (CBF) is a haematopoietic transcription complex that is composed of α (CBF- α) and β (CBF- β) subunits. RUNX1 is one of three possible α subunits (RUNX1, RUNX2, or RUNX3) that binds to DNA via a conserved 128-amino-acid runt homology domain (RHD).

CBF- β does not bind DNA directly but binds the RHD and stabilises the complex.⁶ RUNX1 acts on numerous target genes and proteins that are important in megakaryocytic maturation (for example *MYH10*), and platelet formation (*MPL*, *MYL9*, *MYH9*, *TUBB1/2*) and function (protein kinase C- θ and integrin α IIb β 3).⁷⁻¹⁰

Table 1: Genetic and clinical features of transcription factor mutations.

Gene references	Platelet disorder OMIM entry	Inheritance	Target megakaryocytic and platelet genes	Characterised mutations	Platelet phenotype	Associated features
<i>RUNX1</i> 11, 12, 13, 15, 16, 18, 19, 20	Familial platelet disorder and predisposition to acute myeloid leukaemia (601399).	AD	<i>MYH10</i> , <i>MPL</i> , <i>MYL9</i> , <i>TUBB1/2</i>	R201Q, K83E, R135fs, Y260X, A107P, T148fs, A129E	Symptoms: Mild-to-moderate bleeding usually present from childhood (variable). Blood film: Mild-to-moderate thrombocytopenia. (normal sized platelets) LTA: Reduced response to several platelet agonists (ADP, epinephrine, AA, collagen, TRAP). Flow cytometry: Deficiency of platelet dense granules with reduced uptake and release of mepacrine.	Increased risk of MDS/AML.
<i>GATA1 (XLT)</i> 27, 28, 29, 30, 31	X-linked thrombocytopenia with or without dyserythropoietic anaemia (300367)	X-linked recessive	<i>GPIBA</i> , <i>GPIBB</i> , <i>PF4</i> , <i>MPL</i> , <i>NFE2</i>	V205M, G208R, G208S, D218G, D218Y	Symptoms: Moderate-to-severe bleeding from infancy. Blood film: Moderate-to-severe macrothrombocytopenia. (pale platelets: reduced platelet α -granules)	Red cell anisopoikilocytosis and anaemia. Bilateral cryptochordism (V205M and G208R).
<i>GATA1 (XLTT)</i> ³³	X-linked thrombocytopenia with thalassaemia (314050).	X-linked recessive		R216Q	Symptoms: Mild-to-moderate bleeding tendency (from childhood) Blood film: Mild-to-moderate macrothrombocytopenia. (paleplatelets: reduced platelet α -granules) LTA: Absent platelet shape change in response to collagen. Normal platelet aggregation in response to collagen and ADP. Normal agglutination with ristocetin. Other: Abnormal clot retraction.	Unbalanced α : β haemoglobin chain production resembling mild β -thalassaemia. Mild haemolytic anaemia. Splenomegaly
<i>GFI1B</i> 41, 42, 43	GFI1B-related thrombocytopenia /Bleeding disorder, platelet-type, 17 (187900).	AD	<i>GFI1B</i> , <i>TGFBR3</i>	G272fs H294fs Q287*	Symptoms: Moderate bleeding severity (variable). Blood film: Mild-to-moderate macrothrombocytopenia. (heterogeneous reduction of platelet α -granules) LTA: Variably reduced response to numerous platelet agonists (ADP, AA, epinephrine, TRAP), most marked to collagen. Other: CD34 expression by megakaryocytes and platelets.	Red cell anisocytosis. Bone marrow: dysplastic megakaryocytes, emperipolesis, mild myelofibrosis.

Gene references	Platelet disorder OMIM entry	Inheritance	Target megakaryocytic and platelet genes	Characterised mutations	Platelet phenotype	Associated features
<i>FLII</i> 46, 47, 48	Paris-Trousseau thrombocytopenia (188025/600588).	AD	<i>MYH10</i> , <i>GP6</i> , <i>GP9</i> , <i>ITGA2B</i>	N331fs R337W Y343C	Blood film: Variable mild thrombocytopenia. LTA: Platelet dense granule secretion defect.	Alopecia
		AR		R324W	Symptoms: Moderate bleeding. Blood film: Moderate thrombocytopenia. LTA: Absent collagen induced platelet aggregation. Electron microscopy: Giant α -granules in 1-5% of platelets.	
		<i>De novo</i> ^a			Blood film: Moderate-to-severe neonatal macrothrombocytopenia. Counts may improve over months to years. Other: Platelet dysfunction with prolonged bleeding times that persist after normalisation of platelet counts. Electron microscopy: Platelets contain giant fused α -granules and/or are deficient in dense granules.	Intellectual disability, altered facial structure, heart defects.
<i>ETV6</i> 54, 55, 56	Thrombocytopenia 5 (616216).	AD	<i>PF4</i> , <i>MMP3</i>	P214L, L349P, R369Q, N385fs, R399C, R418G	Blood film: Moderate thrombocytopenia. LTA: Reduced responses to platelet agonists ADP and AA. Flow cytometry: Normal platelet glycoprotein expression.	Increased MCV. Predisposition to solid tumours and haematological malignancy, particularly ALL.
<i>EVII</i> 57, 58, 59, 60	Radioulnar synostosis with amegakaryocytic thrombocytopenia 2 (616738).	Uncertain	<i>AP-1</i> , <i>TGF-β</i>	T756A, H751R, R750W	Blood film: Moderate-to-severe thrombocytopenia.	Anaemia. Fusion of the radius and ulnar bones. Progressive bone marrow failure.

AA: arachidonic acid; AD: autosomal dominant; ALL: acute lymphoblastic leukaemia; AR: autosomal recessive; LTA: light transmission aggregometry; MCV: mean cell volume; MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia; OMIM: Online Mendelian Inheritance in Man; TRAP: thrombin activating peptide; ADP: adenosine diphosphate.

^aFamilial cases are rare with most individuals having *de novo* isolated 7-16 Mb deletions. Occasional individuals with AD inheritance have been reported.⁴⁹

¶ Mutations of *RUNX1* selected from OMIM. Over 27 mutations in 36 pedigrees have been identified.⁶⁵

Germ-line mutation of *RUNX1* on chromosome 21q22.12 is associated with an autosomal dominant disorder called familial platelet disorder with propensity to acute myeloid leukaemia (FPD/AML).¹¹⁻¹³ FPD/AML is characterised by dysmegakaryopoiesis, thrombocytopenia with normal sized platelets (Figure 1), a platelet function defect, and a propensity to develop

myelodysplasia or acute leukaemia.^{8,9,11-16} Mutations causing FPD/AML commonly affect the RHD and less commonly the C-terminal transactivation domain.¹⁷ Affected individuals have a variable mucosal bleeding tendency from childhood ranging from asymptomatic to moderate.¹⁷⁻¹⁹ Most individuals with FPD/AML have mild-to-moderate thrombocytopenia, however normal platelet

counts have been documented in a number of family pedigrees and do not exclude the presence of a significant *RUNX1* mutation.^{18,19} Similarly, the platelet functional defect detected by light transmission aggregometry may be variable and has included reduced responses to collagen, adenosine diphosphate (ADP), arachidonic acid (AA), and epinephrine (Table 1). These changes are attributed to a storage pool deficiency and in some cases resemble an 'aspirin' like defect.^{8,11-13,15,16,20} A recent review of 41 carriers with *RUNX1* alterations in nine unrelated French families described platelet dense granule release defects in 10 patients, regardless of the type of *RUNX1* mutation, suggesting a link between this defect and FPD/AML.¹⁸ Further qualitative platelet dysfunction is caused by impaired integrin $\alpha\text{IIb}\beta\text{3}$ activation.^{8,20}

The evolution to leukaemia appears partly determined by the functional impact of the specific *RUNX1* mutation.^{14,16} Mutations of either the RHD or C-terminal domain that alter the ability of *RUNX1* to bind DNA but retain an ability to heterodimerise with CBF- β , generate a dominant negative phenotype that interferes with the function of the wild-type allele.²¹ These dominant negative acting mutations are associated with a higher risk of leukaemic transformation (over 40% in some cases) compared with mutations that induce haploinsufficiency by reducing levels of wild-type *RUNX1* with resultant deregulation of key target genes, causing genomic instability and the potential to acquire additional somatic mutations, sometimes affecting the second *RUNX1* allele.^{14,17} An ability to predict the phenotype based on the mechanism of action of the *RUNX1* variant is therefore desirable and not possible without utilising diagnostic sequencing platforms.

Moreover, although the detection of a dense granule release defect in the presence of an autosomal dominant thrombocytopenia with normal platelet size may raise the suspicion of FPD/AML, the variable bleeding tendency, mild thrombocytopenia, and absence of morphological platelet abnormalities on the peripheral blood film (Figure 1) make the diagnosis of *RUNX1* mutations (solely by phenotypic testing) very difficult without a high index of clinical suspicion based on family history. Optimal management of affected individuals and their families is largely based on expert opinion.²² Recommendations include: referral to a specialist team consisting of a physician and genetic counsellor, initial blood tests comprising full blood count and full human-

leukocyte antigen typing of the patient and their first degree relatives (in the event that a bone marrow transplant is required in the future), bone marrow biopsy (to detect occult malignancy), and subsequent regular full blood count assessment and biannual haematological review.²²

Globin Transcription Factor 1

The GATA1 transcription factor is encoded by *GATA1* on chromosome Xp11.²³ and is expressed in erythroid cells, megakaryocytes, mast cells, and eosinophils.²³ During megakaryopoiesis, GATA1 assists in lineage commitment of 'primed' genes and, in concert with GATA2 and other transcription factors, including FLI1 and GABPA, regulates the activation of megakaryocyte and platelet specific genes such as *GPIBA*, *GPIBB*, *PF4*, *MPL*, and *NFE2*.^{4,24}

GATA1 has two important functional domains, the N terminal domain that mediates transcriptional activation and the C terminal zinc finger domain. The zinc finger domain is required for an interaction with its transcriptional co-factor, the friend of GATA1 (FOG1), and also influences GATA1 binding affinity to DNA at specific genomic sites.^{25,26} The location of GATA1 mutations within these protein domains has been correlated to clinical phenotypes. To date, all known mutations resulting in GATA1-related cytopenias affect the amino terminal zinc finger domain of GATA1. Five variants (*V205M*, *G208R*, *G208S*, *D218G*, and *D218Y*) have been reported to affect the FOG1 binding face of the amino terminal zinc finger.²⁷⁻³¹ These decrease the GATA1 to FOG1 interaction but preserve GATA1 to DNA binding ability and cause X-linked thrombocytopenia (XLT).²⁵ The severity of the clinical phenotype is dependent on the degree of the GATA to FOG1 interaction disruption caused by the mutation.³¹ Individuals with *V205M*, *G208R*, and *D218Y* have the most severe phenotype characterised by mucocutaneous bleeding from infancy, macrothrombocytopenia (some platelets displaying reduced granulation on the blood film), red cell anisopoikilocytosis, and severe anaemia requiring transfusions (Table 1). Interestingly, two male half siblings and an unrelated male child with *V205M* and *G208R*, respectively, had cryptorchidism.^{27,28} A milder phenotype was described in men from two unrelated families carrying *G208S* and *D218G* mutations.^{29,30} In these cases, the phenotype was characterised by profound bleeding, macrothrombocytopenia, and mild dyserythropoiesis without anaemia. X-linked thrombocytopenia with thalassaemia (XLTT) is

caused by mutations affecting the DNA binding face of the amino terminal zinc finger of *GATA1* resulting in decreased *GATA1* binding to DNA.³² A single variant, R216Q, has been reported in multiple families causing XLTT. This mutation causes milder anaemia and macrothrombocytopenia than those individuals with XLT.³³ In addition, a distinguishing feature is unbalanced $\alpha:\beta$ globin chain synthesis with the production of abnormal $\alpha 3:\beta 1$ and $\alpha 4$ haemoglobin molecules, creating a phenotype resembling mild β -thalassaemia.^{25,33} Notably, platelets in XLTT have reduced α granules, giving the appearance of grey platelets on the blood film (Table 1).³³

Growth Factor-Independent 1B

Growth factor-independent 1B (GFI1B) is a member of the GFI zinc-finger transcriptional repressor family. Its expression in differentiated cells is restricted to erythroid and megakaryocytic cells where it plays an essential role in development.^{34,35} GFI1B contains an N-terminal repressor Snail/Gfi1 (SNAG) domain, which binds protein complexes that mediate transcriptional change, and six C-terminal zinc fingers that demonstrate sequence specific DNA binding.³⁶ It is predicted that zinc fingers 3, 4, and 5 directly bind to DNA, while the functions of zinc fingers 1, 2, and 6 are not known.³⁷ GFI1B regulates megakaryocytic genes controlled by other lineage-restricted regulators, such as GATA1, FLI1, SCL1, TEL/ETV6, RUNX1, MKL2, and SRF, and binds to its own promoter, auto-regulating its expression.^{38,39}

Ten inherited mutations of GFI1B associated with thrombocytopenia have been documented.⁴⁰⁻⁴² Seven patients with non-synonymous single-nucleotide polymorphisms were detected by analysis of the *GFI1B* locus in 529 exome-sequenced cases enrolled by the BRIDGE consortium.⁴⁰ The best characterised mutations, G272fs, H294fs, and Q287*, disrupt the fifth DNA-binding zinc finger and produce a bleeding disorder with an autosomal dominant pattern of inheritance (Table 1).⁴¹⁻⁴³ Mild bleeding symptoms were reported in the proband who carried the G272fs mutation.⁴³ Individuals with the H294fs mutation experience variable bleeding; some experience significant bleeding including urological bleeding (requiring nephrectomy) and intracranial bleeding, while other members of the family report only bleeding following surgery.⁴¹ Laboratory features for both families include macrothrombocytopenia and platelets with

heterogeneous granulation with some platelets appearing relatively agranular (Figure 1).^{41,43,44} Notably, a platelet function defect was demonstrated by light transmission aggregometry in the three individuals tested carrying the H294fs mutation. All three individuals had absent responses to collagen and variably reduced responses to platelet agonists ADP, AA, epinephrine, and thrombin receptor-activating peptide. Similarly, individuals with the Q287* mutation have moderate thrombocytopenia (range: 42-116x10⁹/L) with moderate-to-severe bleeding symptomatology on bleeding scores.⁴² Platelets were agranular on blood film examination and pleomorphic megakaryocytes were found on bone marrow examination in association with mild myelofibrosis.⁴² Functionally, the G272fs, H294fs, and Q287* mutations de-repress transcription of GFI1B-related target genes and inhibit wild-type GFI1B transcriptional activity in a dominant-negative manner.⁴¹⁻⁴³ These transcriptional changes cause deficiency of key haemostatic proteins with marked reductions in platelet fibrinogen and P-selectin, and an increase in CD34 expression.^{42,43} Preliminary data from a mutation predicted to disrupt the first non-DNA binding zinc finger of GFI1B demonstrate phenotypic variability for abnormalities in this transcription factor. The C168F mutation has been reported by two groups^{40,45} and alters the conformation of the first zinc finger but does not disrupt transcription to the same degree as mutations altering the fifth zinc finger. The affected individuals appear to have a mild clinical phenotype with moderate thrombocytopenia (range: 78-121x10⁹/L) but no bleeding history, and platelets with normal granulation.⁴⁵ It is likely these individuals would not be diagnosed with traditional diagnostic platelet studies, but correct classification may be important as their mild phenotype may predict for reduced medical intervention at the time of surgery.

Friend Leukaemia Integration 1

Friend leukaemia integration 1 (FLI1) is a member of the ETS family of transcription factors and is predominantly expressed in vascular and haematopoietic tissue.⁴⁶ Point mutation and small deletion in the ETS DNA-binding domain of FLI1 are reported as causative of a bleeding disorder with abnormal platelet function (Table 1). Three families with autosomal dominant inheritance of a bleeding disorder associated with FLI1 mutation were identified in the UK GAPP study.⁴⁶ These individuals had a mild bleeding disorder with evidence

of defective secretion of dense granules on laboratory testing.⁴⁷ A further two siblings were reported in an Australian cohort⁴⁸ that had a bleeding disorder with a recessive pattern of inheritance. These two siblings had moderate mucosal bleeding symptomatology, moderate macrothrombocytopenia, and prominent abnormalities in collagen induced platelet aggregation (Figure 1) (Table 1). All reported point mutations appear to perturb transcription at the recognised FLI1 target *GP6* gene with subtle differences in phenotype, probably reflecting changes in the site of the described mutations in the ETS DNA-binding domain. Protein modelling predicts the autosomal dominant mutations alter the third α -helix that directly binds DNA, whereas the recessive mutation, *R324W*, is positioned outside the third α -helix and may alter the protein conformation of FLI1.⁴⁸

Hemizygous deletion of the entire FLI1 locus is also suggested to be responsible for the platelet defect associated with Jacobsen syndrome. Jacobsen syndrome is a multisystem disease characterised by developmental delay, short stature, congenital heart disease, and dysmorphic facial features.⁴⁸ It is due to a variably-sized deletion of the terminal end of chromosome 11q. Approximately 90% of individuals with Jacobsen syndrome have a platelet-related bleeding disorder called Paris-Trousseau thrombocytopenia.⁴⁹ Patients with Paris-Trousseau thrombocytopenia typically have neonatal thrombocytopenia that may improve with age, however platelet function remains abnormal throughout life. Paris-Trousseau platelets are variable in size and 15% of circulating platelets contain abnormal, large fused α -granules that are characteristic of this disorder.⁵⁰ Various genes in the deleted region have been proposed to be causative of the various organ pathologies with three lines of evidence, implicating FLI1 as responsible for the platelet defect. Firstly, small terminal deletions of 11q, not involving the FLI1 locus, are rarely reported to be associated with a partial Jacobsen syndrome and these individuals do not exhibit platelet pathology.⁵¹ *In vitro* studies of megakaryocytes derived from individuals with Paris-Trousseau thrombocytopenia suggest overexpression of FLI1 may rescue some features of the defect⁵² and finally, the characteristic giant fused α -granules present in Paris-Trousseau thrombocytopenia have also been demonstrated in the two individuals with the *R324W* mutation of the DNA-binding domain of FLI1 (Figure 1).⁴⁸

A mild bleeding disorder with thrombocytopenia and decreased α -granules is caused by decreased binding of FLI1 and RUNX1 to the 5' untranslated region of their transcriptional target gene, *ANKRD26*. This transcription factor binding defect leads to a failure of normal transcriptional silencing of the transcript during megakaryocyte development with subsequent abnormal cytokine signalling, causing thrombocytopenia and an observed predisposition to leukaemia.⁵³

ETS Variant 6

The transcriptional repressor, ETS variant 6 (ETV6), is also a member of the ETS family of transcription factors. Somatic mutations in ETV6 are recognised in haematological malignancy and deletion of the transcription factor in mice causes failure of haematopoiesis within the bone marrow.⁵⁴ Individuals with point mutations and small deletions in the ETS DNA-binding domain and the adjacent central domain of ETV6 have moderate thrombocytopenia with normal platelet volume that is inherited in an autosomal dominant manner.⁵⁵⁻⁵⁷ Some of these family members have bleeding symptomatology and at least one family had features of altered α -granule production with morphological evidence of granule elongation.⁵⁵⁻⁵⁷ Other haematological parameters are unaffected with the exception of an elevated mean corpuscular volume reported in some families.⁵⁵ A non-specific platelet functional defect with reduced responses to agonists ADP and AA was seen in one family harbouring a *P214L* mutation, but was not observed in another family with the *R418G* mutation (Table 1).⁵⁵ These described mutations act in a dominant-negative fashion to decrease transcriptional repression at the promoters of target genes *PF4* and *MMP3* as measured by luciferase reporter assays.⁵⁵ The described mutant ETV6 proteins also appear to dimerise with wild-type protein and promote aberrant cellular localisation of the transcription factor to the cytoplasm. Importantly, individuals in many of these families demonstrate an increased incidence of haematological malignancies and solid tumours, including colorectal cancer. There was a marked predominance of acute lymphoblastic leukaemia described with 10 cases reported in the eight unrelated families identified with germline ETV6 mutation.⁵⁵⁻⁵⁷

Ecotropic Viral Integration Site 1

The *MECOM* gene encodes EVI1 and MDS-EVI1 proteins from different transcription start sites. EVI1 is a transcriptional regulator with multiple zinc fingers that is normally expressed in haemopoietic tissue and is associated with a poor prognosis when over-expressed or mutated in myeloid leukaemia. Three distinct missense mutations predicted to disrupt the DNA binding function of the 8th zinc finger domain of EVI1 have been described in three unrelated children.⁵⁸ These children were born with moderate-to-severe thrombocytopenia associated with fusion of the radius and ulnar. Two of the children displayed concomitant anaemia and developed progressive bone marrow failure that required haematopoietic stem cell transplantation. Deletion of the entire *MECOM* locus and mutations in the homeodomain transcription factor, *HOXA11*, have been associated with very similar phenotypes.⁵⁹⁻⁶¹

DIAGNOSTIC STRATEGIES

Diagnosis of inherited thrombocytopenia and platelet function disorders are hindered by lack of consensus regarding a standardised approach to testing, as well as diagnostic criteria for the conditions themselves.⁶² This has prompted international groups and societies to draft

guidelines^{63,64} suggesting a logical step-wise approach using clinical symptoms and signs together with combinations of predominantly phenotypic screening and discriminatory assays to diagnose these conditions.⁶² Genetic testing in these algorithms is generally recommended as a third-tier investigation.⁶⁴ Transcription factors interact with each other and multiple target genes in a combinatorial manner,^{4,5} therefore mutation of a single transcription factor may disrupt the expression of multiple genes that are important in megakaryocyte maturation. The result, in many cases, is a complex platelet phenotype that is further influenced by the site of the mutation. Hence, it is not surprising that, despite multiple phenotypic tests that interrogate only isolated structural elements and/or functional pathways in platelets, a diagnosis is not achieved. An argument could be made that DNA-based diagnosis be prioritised in the diagnostic algorithm aiming for a 'one-stop one-step' diagnosis for patients. Sequencing in this context may provide a definitive diagnosis as well as indicate the likely impact of the mutation, based on the location of the mutation within a specific protein domain. Accurate diagnosis of these disorders may avoid the misdiagnosis of immune thrombocytopenia as well as allowing other members of the family to be screened to minimise bleeding events and identify inherited risk of oncogenesis.

Acknowledgements

Transmission electron microscopy was performed with the assistance of the scientific staff at the University of New South Wales and the University of Sydney Centres for Microscopy and Microanalysis.

REFERENCES

1. Bader-Meunier B et al. Misdiagnosis of chronic thrombocytopenia in childhood. *J Pediatr Hematol Oncol.* 2003;25(7):548-52.
2. Gohda F et al. Identification of inherited macrothrombocytopenias based on mean platelet volume among patients diagnosed with idiopathic thrombocytopenia. *Thromb Res.* 2007;119(6):741-6.
3. Harrison P et al. Guidelines for the laboratory investigation of heritable disorders of platelet function. *Br J Haematol.* 2011;155(1):30-44.
4. Pimkin M et al. Divergent functions of hematopoietic transcription factors in lineage priming and differentiation during erythro-megakaryopoiesis. *Genome Res.* 2014;24(12):1932-44.
5. Wilson NK et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. *Cell Stem Cell.* 2010;7(4):532-44.
6. Cohen MM Jr. Perspectives on RUNX genes: an update. *Am J Med Genet A.* 2009;149a(12):2629-46.
7. Heller PG et al. Low Mpl receptor expression in a pedigree with familial platelet disorder with predisposition to acute myelogenous leukemia and a novel AML1 mutation. *Blood.* 2005;105(12):4664-70.
8. Sun L et al. Association of CBFA2 mutation with decreased platelet PKC-theta and impaired receptor-mediated activation of GPIIb-IIIa and pleckstrin phosphorylation: proteins regulated by CBFA2 play a role in GPIIb-IIIa activation. *Blood.* 2004;103(3):948-54.
9. Bluteau D et al. Dysmegakaryopoiesis of FPD/AML pedigrees with constitutional RUNX1 mutations is linked to myosin II deregulated expression. *Blood.* 2012;120(13):2708-18.
10. Antony-Debre I et al. MYH10 protein expression in platelets as a biomarker of RUNX1 and FLI1 alterations. *Blood.* 2012;120(13):2719-22.
11. Ho CY et al. Linkage of a familial platelet disorder with a propensity to

- develop myeloid malignancies to human chromosome 21q22.1-22.2. *Blood*. 1996; 87(12):5218-24.
12. Downton SB et al. Studies of a familial platelet disorder. *Blood*. 1985;65(3): 557-63.
13. Song WJ et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet*. 1999; 23(2):166-75.
14. Antony-Debre I et al. Level of RUNX1 activity is critical for leukemic predisposition but not for thrombocytopenia. *Blood*. 2015;125(6): 930-40.
15. Buijs A et al. A novel CBFA2 single-nucleotide mutation in familial platelet disorder with propensity to develop myeloid malignancies. *Blood*. 2001;98(9): 2856-8.
16. Beri-Dexheimer M et al. Clinical phenotype of germline RUNX1 haploinsufficiency: from point mutations to large genomic deletions. *Eur J Hum Genet*. 2008;16(8):1014-8.
17. Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. *Haematologica*. 2011;96(10):1536-42.
18. Latger-Cannard V et al. Haematological spectrum and genotype-phenotype correlations in nine unrelated families with RUNX1 mutations from the French network on inherited platelet disorders. *Orphanet J Rare Dis*. 2016;11:49.
19. Owen CJ et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood*. 2008; 112(12):4639-45.
20. Gerrard JM, McNicol A. Platelet storage pool deficiency, leukemia, and myelodysplastic syndromes. *Leuk Lymphoma*. 1992;8(4-5):277-81.
21. Michaud J 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(4): 1364-72.
22. Churpek JE et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. *Leuk Lymphoma*. 2013;54(1):28-35.
23. Harigae H. GATA transcription factors and hematological diseases. *Tohoku J Exp Med*. 2006;210(1):1-9.
24. Vyas P et al. Consequences of GATA-1 deficiency in megakaryocytes and platelets. *Blood*. 1999;93(9):2867-75.
25. Ciovacco WA et al. Human phenotypes associated with GATA-1 mutations. *Gene*. 2008;427(1-2):1-6.
26. Tsang AP et al. FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell*. 1997; 90(1):109-19.
27. Nichols KE et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. *Nat Genet*. 2000;24(3): 266-70.
28. Del Vecchio GC et al. Dyserythropoietic anemia and thrombocytopenia due to a novel mutation in GATA-1. *Acta Haematol*. 2005;114(2):113-6.
29. Mehaffey MG et al. X-linked thrombocytopenia caused by a novel mutation of GATA-1. *Blood*. 2001;98(9): 2681-8.
30. Freson K. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. *Blood*. 2001;98(1): 85-92.
31. Freson K 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(2):147-52.
32. Yu C et al. 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(6):2040-5.
33. Balduini CL et al. Effects of the R216Q mutation of GATA-1 on erythropoiesis and megakaryocytopoiesis. *Thromb Haemost*. 2004;91(1):129-40.
34. Doerks T et al. Systematic identification of novel protein domain families associated with nuclear functions. *Genome Res*. 2002;12(1):47-56.
35. Vassen L et al. Gfi1b:green fluorescent protein knock-in mice reveal a dynamic expression pattern of Gfi1b during hematopoiesis that is largely complementary to Gfi1. *Blood*. 2007;109(6): 2356-64.
36. Grimes HL et al. The Gfi-1 proto-oncoprotein contains a novel transcriptional repressor domain, SNAG, and inhibits G1 arrest induced by interleukin-2 withdrawal. *Mol Cell Biol*. 1996; 16(11):6263-72.
37. Zweidler-Mckay PA et al. Gfi-1 encodes a nuclear zinc finger protein that binds DNA and functions as a transcriptional repressor. *Mol Cell Biol*. 1996;16(8): 4024-34.
38. Laurent B et al. Gfi-1B promoter remains associated with active chromatin marks throughout erythroid differentiation of human primary progenitor cells. *Stem Cells*. 2009;27(9):2153-62.
39. Foudi A et al. Distinct, strict requirements for Gfi-1b in adult bone marrow red cell and platelet generation. *J Exp Med*. 2014;211(5):909-27.
40. Chen L et al. Transcriptional diversity during lineage commitment of human blood progenitors. *Science*. 2014;345(6204): 1251033.
41. Stevenson WS et al. GF11B mutation causes a bleeding disorder with abnormal platelet function. *J Thromb Haemost*. 2013; 11(11):2039-47.
42. Monteferrario D et al. A dominant-negative GF11B mutation in the gray platelet syndrome. *N Engl J Med*. 2014;370(3): 245-53.
43. Kitamura K et al. Functional characterization of a novel GF11B mutation causing congenital macrothrombocytopenia. *J Thromb Haemost*. 2016. [Epub ahead of print].
44. Stevenson WS et al. Platelets are not all gray in GF11B disease. *Clin Genet*. 2015; 87(3):299.
45. Rabbolini DJ et al. The GF11B c.503G>T mutation in the first zinc finger domain predicts a milder bleeding phenotype than the GF11B c.880-881insC mutation. *J Thromb Haemost*. 2015;13416-7.
46. Hart A et al. Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. *Immunity*. 2000;13(2):167-77.
47. Stockley J et al. Enrichment of FLI1 and RUNX1 mutations in families with excessive bleeding and platelet dense granule secretion defects. *Blood*. 2013;122(25): 4090-3.
48. Stevenson WS et al. Paris-Trousseau thrombocytopenia is phenocopied by the autosomal recessive inheritance of a DNA-binding domain mutation in FLI1. *Blood*. 2015;126(17):2027-30.
49. Favier R et al. Jacobsen syndrome: Advances in our knowledge of phenotype and genotype. *Am J Med Genet C Semin Med Genet*. 2015;169(3):239-50.
50. Grossfeld PD et al. The 11q terminal deletion disorder: a prospective study of 110 cases. *Am J Med Genet A*. 2004;129A(1): 51-61.
51. Breton-Gorius J et al. A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet alpha-granules and chromosome 11 deletion at 11q23. *Blood*. 1995;85(7): 1805-14.
52. Bernaciak J et al. Clinical and molecular-cytogenetic evaluation of a family with partial Jacobsen syndrome without thrombocytopenia caused by an approximately 5 Mb deletion del(11)(q24.3). *Am J Med Genet A*. 2008;146A(19): 2449-54.
53. Bluteau D et al. Thrombocytopenia-associated mutations in the

- ANKRD26 regulatory region induce MAPK hyperactivation. *J Clin Invest.* 2014;124(2):580-91.
54. Wang LC et al. The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. *Genes Dev.* 1998;12(15):2392-402.
55. Noetzi L et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. *Nat Genet.* 2015;47(5):535-8.
56. Zhang MY et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. *Nat Genet.* 2015;47(2):180-5.
57. Topka S et al. Germline ETV6 Mutations Confer Susceptibility to Acute Lymphoblastic Leukemia and Thrombocytopenia. *PLoS Genet.* 2015;11(6):e1005262.
58. Niihori T et al. Mutations in MECOM, Encoding Oncoprotein EVI1, Cause Radioulnar Synostosis with Amegakaryocytic Thrombocytopenia. *Am J Hum Genet.* 2015;97(6):848-54.
59. Thompson AA, Nguyen LT. Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with HOXA11 mutation. *Nature genetics.* 2000;26(4):397-8.
60. Thompson AA et al. Congenital thrombocytopenia and radio-ulnar synostosis: a new familial syndrome. *Br J Haematol.* 2001;113(4):866-70.
61. Nielsen M et al. Deletion of the 3q26 region including the EVI1 and MDS1 genes in a neonate with congenital thrombocytopenia and subsequent aplastic anaemia. *J Med Genet.* 2012;49(9):598-600.
62. Gresele P et al. Diagnosis of suspected inherited platelet function disorders: results of a worldwide survey. *J Thromb Haemost.* 2014;12(9):1562-9.
63. Balduini CL et al. Inherited thrombocytopenias: a proposed diagnostic algorithm from the Italian Gruppo di Studio delle Piastrine. *Haematologica.* 2003;88(5):582-92.
64. Gresele P; Subcommittee on Platelet Physiology of the International Society on Thrombosis and Hemostasis. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. *J Thromb Haemost.* 2015;13(2):314-22.
65. Heller PG, Glembotzky AC. Familial platelet disorder with predisposition to acute myelogenous leukemia. *Atlas Genet Cytogenet Oncol Haematol.* 2013;17(2):138-43.