Received: 18 June 2019 | Revised: 17 September 2019 | Accepted: 16 October 2019

DOI: 10.1002/rth2.12282

CASE REPORT

The clinical heterogeneity of *RUNX1* associated familial platelet disorder with predisposition to myeloid malignancy – A case series and review of the literature

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Handling Editor: Dr Neil Zakai

Abstract

Germline mutations of runt-related transcription factor-1 (RUNX1) cause familial platelet disorder with predisposition to myeloid malignancy (FPDMM), most commonly associated with thrombocytopenia and propensity to develop myeloid neoplasms. A key clinical question is which patients with a family history of thrombocytopenia should undergo genetic testing for *RUNX1* mutations. Typically, molecular diagnosis by genetic sequencing is performed when the clinical phenotype is suggestive of this diagnosis; however, our understanding of the spectrum of associated features suggestive of this diagnosis continues to evolve. Herein, we report a case series of 3 unrelated families with *RUNX1*-associated FPDMM and clinical phenotypes not typically reported with this condition. These cases expand our understanding of FPDMM and highlight the complexity of transcriptional regulation of hematopoiesis and its potentially diverse phenotypes. We describe our approach to diagnosis and management of these individuals and the importance of long-term surveillance in these cases.

KEYWORDS

familial platelet disorder, familial platelet disorder with predisposition to myeloid malignancy, hereditary myeloid malignancy, inherited thrombocytopenia, RUNX1

Essentials

- There is wide clinical heterogeneity of RUNX1-associated familial platelet disorder with predisposition to myeloid malignancy (FPDMM).
- Platelet size can be variable; we describe novel microthrombocytopenic presentation.
- Intragenic deletions are increasingly recognized and may otherwise be missed by conventional sequencing techniques.
- Family members of patients with germline RUNX1 mutation should be screened regardless of platelet count.

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1 | DESCRIPTION OF *RUNX1* KINDREDS

1.1 | Family A - RUNX1 deletion associated with T- lymphoblastic lymphoma

A 22-year-old man, patient A, proband for Family A (Genogram A, IV.1) presented with cervical lymphadenopathy. He had a background of chronic thrombocytopenia without an associated bleeding phenotype, International Society of Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT) score of 1.[1] On initial assessment, he had a hemoglobin of 157 g/L, white cell count of 9.5 × 10^9/L, and platelet count of 126 × 10^9/L with a normal mean platelet volume (MPV) of 9.2 fL (Table 1). Lymph node core biopsy showed diffuse proliferation of lymphoblasts with fine chromatin, positive on immunohistochemistry for pan-T-cell antigens CD3, CD4, and CD5 and the blastic marker terminal deoxynucleotidyl transferase (TdT) but not for CD34. Bone marrow biopsy was normocellular with mild dysplasia present in megakaryocytes but no evidence of acute lymphoblastic leukemia (ALL) infiltration. Tissue culture of the lymph node tissue failed to yield metaphases, but cytogenetic analysis of bone marrow demonstrated a normal 46,XY karyotype. Taken together, these features were consistent with a diagnosis of T-lymphoblastic lymphoma.

Patient A's mother (Genogram A, III.2) also had chronic thrombocytopenia (baseline platelet count, 70 × 10^9/L) and ALL (French-American-British Classification, L1; unspecified lineage, normal karyotype) at age 29, with subsequent development of a therapy-related myelodysplastic syndrome (MDS) decades after her initial chemotherapy exposure, necessitating matched unrelated donor (MUD) allograft at age 60. Her paternal cousin (Genogram A, III.3) had died of acute myeloid leukemia (AML) in his first decade of life, and multiple family members had previously received a diagnosis of chronic thrombocytopenia. Whole exome sequencing studies were performed on the index case, and the mother did not identify a pathogenic variant responsible for the presentation. Diagnosis of FPDMM in Patient A and his mother was then achieved by single-nucleotide polymorphism array that demonstrated a 0.81-Mb deletion on chromosome 21 (21q22.12), which included exons 1-3 of *RUNX1* (see Appendix S1). A constitutional deletion was confirmed with absence of the 5′probe of the *RUNX1* fluorescence in situ hybridization (FISH) assay using patient-derived cultured fibroblasts and was supported by increased *MYH10* expression in platelet lysates by western blot.[2] Limiting diagnostic consideration of underlying FPDMM to patients with thrombocytopenia and family history of myeloid malignancy alone may potentially miss families with less frequently reported lymphoid presentations.

1.2 | Family B - RUNX1 variant associated with eosinophilic leukemia

Patient B, the proband for Family B (Genogram B, III.2) is a young woman with a history of thrombocytopenia (baseline platelet count, 10-30 × 10^9/L) and ISTH-BAT score of 1. A family history of thrombocytopenia was present, and Patient B's maternal grandfather (Genogram B, I.1) had succumbed to myelofibrosis in the fifth decade of life. At age 30, Patient B underwent bone marrow biopsy, which demonstrated mild dysmegakaryopoiesis (predominantly of small hypolobated forms, and some with separated lobes were also noted), without other significant abnormalities. Subsequently, during a routine visit at age 43, Patient B presented with blood film abnormalities that included marked eosinophilia, abnormal hypogranular neutrophils accompanied by rapidly progressive splenomegaly consistent with chronic eosinophilic leukemia. Genetic studies demonstrated a clonal KIT-D816V mutation associated with a normal bone marrow karyotype, with no evidence of FIP1L1-PDGFRA or BCR-ABL1 on FISH and no evidence of mutation in JAK2, CALR MPL, SF3B1, CSF3R and ASXL1 by sequencing. Underlying FPDMM was subsequently confirmed by sequencing that demonstrated a constitutional *RUNX1* missense variant (c.C884G, p.S295X; see Appendix S1). She underwent induction chemotherapy and MUD allogeneic stem cell transplant and currently remains in remission. Patient B's mother (Genogram B, II.2) also developed acute myelomonocytic leukemia at age 72, with normal cytogenetic studies, and passed away within 12 months of induction chemotherapy. This case highlights the utility of surveillance in patients with potential *RUNX1* mutations, given the lag time to development of malignancy, and the importance of including older generations in familial testing once identified.

1.3 | Family C - RUNX1 variant and microthrombocytopenia

Patient C, the proband of Family C (Genogram C, III.3) was a 44-year-old man previously diagnosed with thrombocytopenia in

TABLE 1 Baseline platelet counts, mean platelet volume, and mean platelet diameters for proband patients in each family

	Baseline platelet count (×10^9/L)[a]	Mean platelet volume (fL)[b]	Mean platelet diameter (μm)[c]
Patient A	110-130	9.2	2.20
Patient B	10-30	9.9	2.94
Patient C	100-120	5.6	1.74

[a]Normal range = 150-400 × 10^9/L.

[b]Normal range = 9.4-12.8fl.

[c]Control mean platelet diameter 2.13 (95% confidence interval, 2.04-2.11), ranges as derived from Fixter et al.[18]

his 20s. His baseline platelet count was 100-120 × 10^9/L, despite which he reported abnormal mucocutaneous bleeding (ISTH-BAT score, 5). These bleeding symptoms segregated with thrombocytopenia in an autosomal dominant manner and a history of myeloid neoplasm occurring in the sixth decade (myelodysplasia and AML) was reported in 2 individuals (Genogram C, II.2 and II.3 respectively). Both Patient C and his 5-year-old son (Genogram C, IV.2) had small platelets on the peripheral blood film, with reduced MPV of 5.6 fL and mean platelet diameter of 1.74 (Table 1). Western blotting of platelet lysates demonstrated increased expression of MYH10 and next-generation sequencing (NGS) of both Patient A and his son demonstrated a hitherto unreported likely pathogenic *RUNX1* frameshift mutation (exon 1, c.187delG, p.V63fs; Appendix S1). Given the increased risk of developing MDS/AML in 30% to 40% of FPDMM patients,[3] close hematologic surveillance along with further genetic testing of kindred was recommended.

2 | DISCUSSION

Our case series of atypical presentations further confirms the significant clinical heterogeneity in FPDMM.[3–5] Various mutations have been described causing *RUNX1*-associated FPDMM and commonly include point mutations[6] (Family C), but can also be due to intragenic deletions (Family A), which have been reported in only 6 FPDMM cases to date.[7,8] The latter necessitate techniques beyond conventional short-read NGS and Sanger sequencing for detection. Germline *RUNX1* mutation alone is insufficient for development of malignancy, and additional secondary mutations with subsequent clonal evolution likely underlie the path to leukemogenesis.[9] Subsequent secondary mutations presumably alter the presenting phenotype of the disease – here, in Patient B, we describe KIT D618V that is typically associated with mast cell excess, but there is limited murine evidence that altered RUNX1 function may also alter lineage differentiation.[10]

There is wide variability in the degree of thrombocytopenia in FPDMM, and normal platelet counts have also been reported.[4] Like other myeloid neoplasms with germline predisposition and preexisting platelet disorders (*ETV6* and *ANKRD26*), platelet size is typically normal to slightly enlarged.[11] To our knowledge, Family C is the first reported family with FPDMM and reduced platelet size. Despite the presence of microthrombocytopenia, differentials of Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia were considered unlikely, given the autosomal dominant pattern of thrombocytopenia seen in Family C (Figure 1). *WAS*-, *FYB*-, and *CYCS*-related thrombocytopenia, which are thrombocytopenias associated with small platelets,[11] as well as, *ANKRD26/ETV6* were excluded through our NGS panel and/or subsequent Sanger sequencing. However, as whole genome sequencing was not performed, other potentially contributing variants have not been excluded.

RUNX1 is a key hematopoietic transcription factor that silences the expression of *MYH10* during maturation of megakaryocytes,[12] and increased platelet MYH10 expression has been identified as a marker of *RUNX1* mutation.[2,13] Increased platelet MYH10 expression was demonstrated in Families A and C, reinforcing the likelihood of the described RUNX1 variants being pathogenic in these individuals. Prior clinicopathologic studies have demonstrated dysplastic megkaryopoiesis in the absence of MDS/AML at baseline in patients with FPDMM.[8] Accordingly, dysmegakaryopoiesis in the setting of *RUNX1* alterations should be considered a preleukaemic lesion, as in Patient B's case where marrow evidence of dysmegakaryopoiesis preceded development of overt leukemia by over a decade. Lifetime risk of leukemic transformation is estimated to be 30% to 40%, with a wide variation in age of leukemia diagnosis (from 6 to 77 years).[14] As seen in Family B, the diagnosis of MDS/AML in younger generations may predate that of older generations, highlighting the need to screen widely across the family once a diagnosis of FPDMM is made.

There are emerging guidelines to direct the testing of patients at risk of inherited myeloid malignancy [15], though these remain broad and not specific to FPDMM. As illustrated in the above cases, we are still only beginning to understand the breadth of phenotype of *RUNX1*-associated FPDMM. As such, we propose that all patients with chronic thrombocytopenia and a family history of any hematologic MDS/malignancy be considered for testing. Furthermore, in our experience, there may be marked variability in platelet numbers/size such that all family members should be screened regardless of platelet count in the setting of known hematologic malignancy and a familial history of thrombocytopenia. In patients being considered for allogeneic stem cell transplant, we recommend *RUNX1* mutation testing via nonhematopoietic germline tissue such as skin fibroblasts. In patients found to have *RUNX1* mutations, genetic counseling should be offered, and kindred should be tested given therapeutic implications of affected family members becoming allogeneic stem cell donors.

Detection of *RUNX1*, whose kindred have yet to manifest with any MDS/AML poses a dilemma. Evolving knowledge regarding roles of cooperating "second-hit" mutations and the effects of RUNX1 dosage in clonal evolution[2,9] may assist in predicting risk of malignancy in such individuals. Given the potential long latency period between diagnosis of *RUNX1*-associated thrombocytopenia to development of malignancy and its variable phenotypic manifestations, establishing the frequency and nature of monitoring investigations remains an ongoing challenge.

In patients found to have *RUNX1* mutations, no consensus guidelines have been developed for surveillance to date. In line with others,[16,17] we recommend close surveillance with 6 to 12 monthly full blood counts and annual clinical review, with consideration for bone marrow examination with any concerning changes in hematologic parameters. Ultimately, with increasing recognition of this disorder and availability of technologies to detect both driver and secondary mutations in leukemogenesis, further structured management approaches may be achieved.

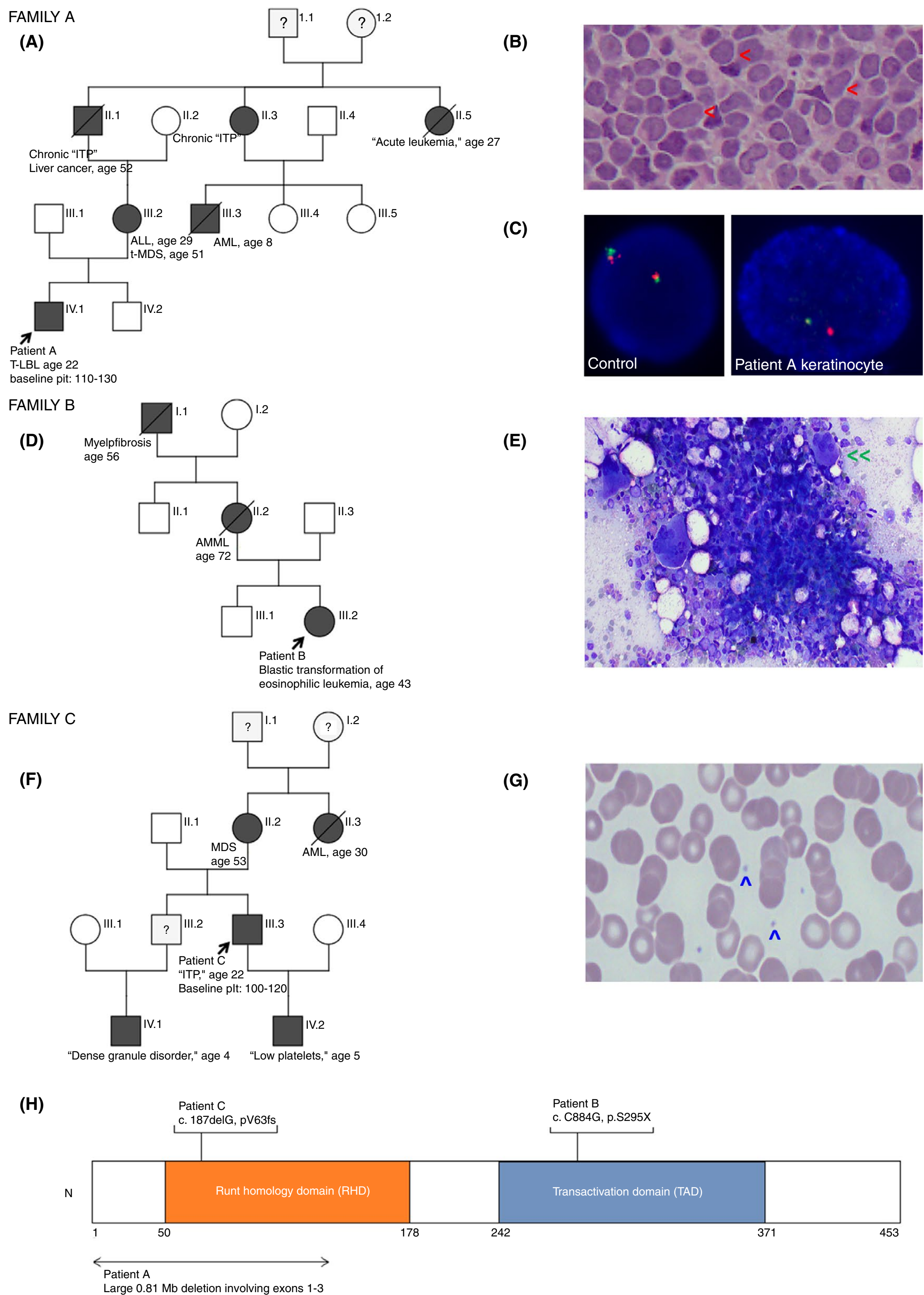

FIGURE 1 (A) Genogram A. (B) Core biopsy of thymus showing a diffuse infiltrate of abnormal large immature lymphoblasts (red arrowhead), hematoxylin and eosin stain at 40 × magnification. (C) Fluorescence in situ hybridization using a dual-coloured *RUNX1* break-apart probe set. On the left, a normal signal is detected in a haematopoietic cell (control); on the right, an abnormal break-apart signal is seen in a keratinocyte from Patient A. (D) Genogram B. (E) Patient B bone marrow aspirate. This is hypercellular and demonstrates prominent eosinophilia and dysplastic megakaryocytes (green double arrowhead), May-Grunwald-Giemsa stain at 20 × magnification. (F) Genogram C. (G) Representative image of the peripheral blood film from Patient C at diagnosis Wright-Giemsa stain at 40 × magnification, demonstrating unusually small platelets (blue arrowheads). (H) Schematic representation of RUNX1 showing the Runt homology domain (orange) and the transactivation domain (blue). The location of the RUNX1 variants identified in genograms A, B, and C are shown.

ACKNOWLEDGMENTS

The authors thank Nisha Singh for keratinocyte FISH analysis and Robyn Lukeis for performance of single-nucleotide polymorphism array.

RELATIONSHIP DISCLOSURE

The authors report nothing to disclose.

AUTHOR CONTRIBUTIONS

DR, WS, CW, M-C MK, and PC provided biological samples and/or analyzed clinical data. M-C MK performed MYH10 western blot analysis. WS, M-C MK, DR performed and analyzed next-generation sequencing data. DC performed mean platelet diameter measurements. CT, DR, M-C MK, CW, and WS wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tang C, Rabbolini DJ, Morel-Kopp M-C, et al. The clinical heterogeneity of *RUNX1* associated familial platelet disorder with predisposition to myeloid malignancy – A case series and review of the literature. *Res Pract Thromb Haemost*. 2020;4:106–110. https://doi.org/10.1002/rth2.12282