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ORIGINAL PAPER

Platelets, Thrombosis and Haemostasis

Insights into the clinical, platelet and genetic landscape of inherited thrombocytopenia with malignancy risk

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Summary

Inherited thrombocytopenia (IT) with germline variants in *RUNX1*, *ETV6* or *ANKRD26* carries a high risk (10%–45%) of developing haematological malignancy (IT-HM). We evaluated the clinical, platelet and molecular characteristics in 37 patients with RUNX1-related thrombocytopenia (RT), 9 with ETV6-RT and 20 with ANRKD26-RT. Genetic diagnosis was delayed by about 20 years from the identification of thrombocytopenia. Bleeding tendency was present in 25%–30% of RUNX1-RT and ANKRD26-RT patients. Platelet aggregation was impaired in 90% of all patients, while reduced activation and granule secretion were heterogeneous. Most RUNX1-RT patients had low glycoprotein Ia (GPIa) levels, which may be a useful disease biomarker. Sixteen distinct genetic variants in *RUNX1*, four in *ETV6* and four in *ANKRD26* were identified in patients. The clinical profile showed immune, skin, gastrointestinal and other comorbidities in many patients. One third of the cases developed a malignancy: This included eight RUNX1-RT patients with myelodysplastic syndrome (MDS), five with acute myeloid leukaemia (AML), and one with chronic myeloid leukaemia (CML) Ph+. One patient with ETV6-RT subsequently developed B-cell acute lymphoblastic leukaemia (B-ALL) during childhood. Three cases with ANKRD26-RT demonstrated a multifaceted clinical

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presentation, including B-ALL Ph+, MDS and breast cancer. The high incidence of HM development highlights the importance of early diagnosis in life.

KEYWORDS
ANKRD26, *ETV6*, inherited thrombocytopenia, IT, malignancy predisposition, *RUNX1*

INTRODUCTION

Inherited thrombocytopenias (ITs) are rare diseases caused by defects in ~40 genes involved in megakaryopoiesis and thrombopoiesis, often presenting with an increased bleeding risk.[1–3] Many ITs predispose to other congenital defects, haematological malignancies, bone marrow (BM) failure or non-haematological diseases.[4] The 2016 WHO classification identified germline monoallelic variants in *RUNX1*, *ETV6* and *ANKRD26* as drivers of the 'hereditary myeloid neoplasms with platelet disorders'.[5]

RUNX1-related thrombocytopenia (–RT), also known as familial platelet disorder with predisposition to myeloid leukaemia (RUNX1-RT, FPD/AML), is characterized by mild-to-moderate bleeding, variable thrombocytopenia with normal platelet size, platelet dysfunction and a 45% of early in life (median age: 33 years) development of acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS), with rare cases (1%–3%) of T-acute lymphoblastic leukaemia (ALL).[6,7]

Patients with ETV6-RT and ANKRD26-RT also present with mild bleeding, thrombocytopenia and platelet dysfunction. ETV6-RT patients have a 25% risk of B-cell acute lymphoblastic leukaemia (B-ALL) in childhood and a 5%–10% risk of AML or MDS, with rare cases of solid tumours.[8] ANKRD26-RT patients with 5′UTR variants have a 5%–10% chance of AML/MDS, with a median onset at 35 years.[9]

Germline variants in *RUNX1* and *ETV6* alone do not induce leukaemia, and additional mutations are required.[8,10] In RUNX1-RT-associated leukaemia, somatic mutations frequently involve the wild-type *RUNX1* allele, *PHF6*, *BCOR* and *TET2*.[11,12] Furthermore, ETV6-RT patients with leukaemia commonly harbour mutations in *BCOR*, *RUNX1* and *KRAS*, alongside fusion proteins.[8,13]

There are more than 400 reported cases of RUNX1-RT patients,[14,15] at least 96 cases of ETV6-RT[16] and above 300 patients with ANKRD26-RT.[17] The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/; accession on 29 May 2025) includes up to 301, 91 and 30 pathogenic (PV) or likely pathogenic (LPV) germline variants in *RUNX1*, *ETV6* and *ANKRD26*, respectively, and many more variants of uncertain significance (VUS).

This study evaluates the clinical, platelet and genetic profiles of a large Spanish cohort of IT with predisposition to haematological malignancies (IT-HM).

METHODS

Patient recruitment and clinical evaluation

This study comprised patients with IT-HM recruited at the Spanish Multicentre Project of Inherited Platelet Disorders (IPDs) (Grupo Español de Alteraciones Plaquetarias Congénitas, GEAPC). The study was approved by the Hospital Reina Sofía Ethics Committee (Murcia, Spain) and adhered to the Declaration of Helsinki. Written informed consent was obtained from all patients.

Medical history, physical examination, neoplasia follow-up, BM analysis and comorbidities assessment were conducted by haematologists and other specialists at collaborating centres and reviewed by the research team. Bleeding tendency was assessed using the ISTH-BAT.[18,19]

Platelet studies and molecular analysis

Venous blood was collected in K3 EDTA (7.5%) for blood counts, blood films and nucleic acid purification and in 0.105 M sodium citrate for platelet functional studies. Genomic deoxyribonucleic acid (DNA) was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany).

Platelet function was assessed using Platelet Function Analizer (PFA)-100 tests (collagen-Adenosine-5'-Diphosphate [ADP] and collagen-epinephrine cartridges) as well as flow cytometry evaluation of platelet membrane glycoproteins (GP), αIIbβ3 integrin activation (CD41/CD61 Monoclonal Antibody [PAC1] binding) and granule secretion (CD62, CD63).[20,21] Light transmission aggregometry (LTA) was performed using a TA-8V aggregometer (Stago, France) in the patient's platelet-rich plasma (PRP) with a platelet count of $\geq 50 \times 10^9$ platelets/L and in control PRP adjusted to the same platelet count. Platelet dense granule content was evaluated by whole-mount electron microscopy.[22]

To classify patients' platelet function status as normal or impaired, at least two healthy controls were analysed in parallel to reduce intra-individual variability. Impairment was defined as a ≥30% reduction in aggregation, activation or granule secretion in response to ≥2 agonists, or specifically collagen in RUNX1-RT patients, compared to controls.[23]

Patients' DNA was analysed by high-throughput sequencing (HTS) with a panel of 102 genes.[24] Variant analysis was performed with DIGEVAR (https://digevar2.imib.es/),[25,26] and variant pathogenicity was assessed according to the ACMG/AMP general criteria[27] or the ClinGen adapted ones in the case of RUNX1-RT patients.[28] Segregation of candidate variants in pedigrees was assessed by Sanger sequencing.

Statistical analysis

Spearman's r test was used to ascertain quantitative correlations (e.g. platelet count vs. ISTH-BAT) and Fisher's exact test for qualitative associations (e.g. mutation type vs. neoplasia). Analyses were conducted using GraphPad.

RESULTS

Patients' general description

Between 2008 and 2024, 66 patients from 31 families were diagnosed with IT-HM: 37 patients (20 families) with RUNX1-RT, 9 (5 families) with ETV6-RT and 20 (6 families) with ANRKD26-RT. The inclusion criteria are detailed in Table S1. Of note, this cohort accounted for 15.9% of IT cases in our Spanish cohort of IT (Figure S1).

Family history of thrombocytopenia was present in 60% of RUNX1-RT, 80% of ETV6-RT and 83% of ANRKD26-RT families (1–8 affected members per family) (Figure 1A). There was also a family background of neoplasia in 45%, 60% and 50% of the families, respectively (1–15 affected members), mainly haematological malignancies (66%) but also diverse solid tumours (Figure 1A; Table S2).

The median age at the time of initial thrombocytopenia report, which was available in 82% of patients, was 26 years for RUNX1-RT, 12 years for ETV6-RT and 16 years for ANRKD26-RT (range: 0–80 years) (Figure 1B). Remarkably, diagnosis occurred later in these patients, at median ages of 43, 30 and 37 years respectively (Figure 1C).

A complete blood count at diagnosis showed thrombocytopenia in 88.9% of RUNX1-RT cases and in all ETV6-RT and ANRKD26-RT patients, with most showing normal platelet size (Figure 1D,E; Table S3). Furthermore, common findings in RUNX1-RT and ETV6-RT cases, but not in ANKRD26-RT patients, included anaemia (30.6% and 33.3%), leucopenia (38.9% and 44.4%) and neutropenia (52.8% and 66.7%) (Figure 1F–H; Table S3).

Blood films suggested thrombocytopenia in 74.3% of RUNX1-RT patients, 88.9% of ETV6-RT cases (8 of 9) and all ANKRD26-RT patients (Figure 1I). Additional findings include, in some cases, a few large platelets, granulocytic or erythroid dysmorphia and myeloid blasts (>5%) (Table S4).

Clinical presentation of the patients

At the time of diagnosis, a mild bleeding tendency was observed in a quarter of RUNX1-RT (8 of 31) and ANKRD26-RT (6 of 20) cases, while in none of the ETV6-RT patients (Figure 1J; Table S3). Furthermore, 82.5% of the patients exhibited extra haematological comorbidities of the following type:

Allergy and immunology

Allergic or immunological disorders were diagnosed in 41.7% of the RUNX1-RT patients, with allergic asthma (6 cases) and allergic rhinoconjunctivitis (5 cases) being the most prevalent. The remaining cases included mite/pollen allergy (3 cases), drug allergy, eosinophilia, allergic sinusitis, multiple food allergies, immunoglobulin A (IgA) deficiency (2 cases of each type) and reactive urticaria, lichen planus and recurrent otitis (1 case each). No allergies were reported in ETV6-RT patients, while three of 20 ANKRD26-RT patients had allergic asthma and one was allergic to pollen.

Dermatology

Skin disorders were identified in 19 (52.8%) RUNX1-RT patients, with atopic dermatitis, melanocytic nevus and eczema (7, 3 and 2 cases respectively) being the most prevalent. The remaining cases exhibited one of the following conditions: chalazion, alopecia areata, seborrheic dermatitis, pityriasis versicolor, psoriasis, atopic cheilitis labialis, hyperpigmented spots and generalized warts.

Among ETV6-RT patients, one of seven had seborrheic keratosis, and another had epithelioid haemangioendothelioma. For ANKRD26-RT, 45% also had dermatological disorders, including seborrheic dermatitis, melanocytic nevus and atopic dermatitis (two cases of each). Single cases exhibited hyperkeratosis, generalized warts, psoriasis, diffuse alopecia and erythematous rash.

Gastroenterology

Gastrointestinal disorders were reported in 11 of 36 RUNX1-RT, 2 of 7 ETV6-RT and 7 of 20 ANKRD26-RT patients.

Among RUNX1-RT patients, three had haemorrhoids and individual cases had conditions such as appendicitis, eosinophilic esophagitis, chronic constipation, pancreatitis, peptic ulcer, recurrent cholangitis, hiatal hernia, diverticulosis, chronic gastritis, gastric infection and gastrooesophageal reflux. ETV6-RT patients had cholelithiasis or coeliac disease. Lastly, among ANKRD26-RT patients, two exhibited frequent constipation, and single cases had cholelithiasis, coeliac disease, ileitis, recurrent diarrhoea, dysphagia and faecal occult blood followed by colonoscopy.

Other pathologies

Table S5 shows other comorbidities observed in 58.3%, 28.6% and 45% of RUNX1-RT, ETV6-RT and ANKRD26-RT patients respectively.

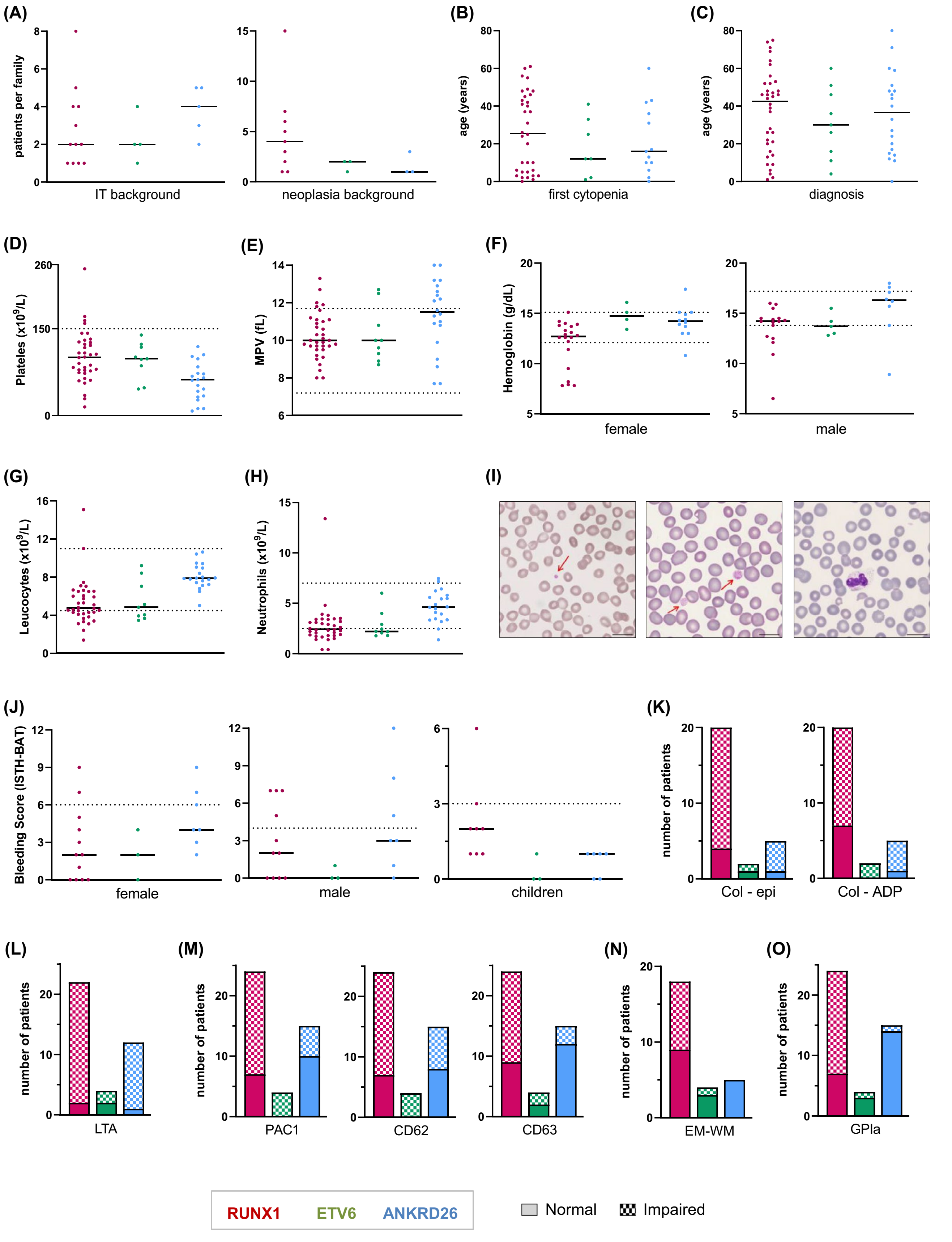
(A)
patients per family
IT background
neoplasia background
(B)
age (years)
first cytopenia
(C)
age (years)
diagnosis
(D)
Plateles (x10^9/L)
(E)
MPV (fL)
(F)
Hemoglobin (g/dL)
female
male
(G)
Leucocytes (x10^9/L)
(H)
Neutrophils (x10^9/L)
(I)
(J)
Bleeding Score (ISTH-BAT)
female
male
children
(K)
number of patients
Col - epi
Col - ADP
(L)
number of patients
LTA
(M)
number of patients
PAC1
CD62
CD63
(N)
number of patients
EM-WM
(O)
number of patients
GPIa
RUNX1
ETV6
ANKRD26
Normal
Impaired

FIGURE 1 Familial, haematological and platelet features in patients with RUNX1-RT, ETV6-RT or ANKRD26-RT. (A) Dot plot showing the number of affected members per family with a background of thrombocytopenia or neoplasia. (B) Age at first detection of thrombocytopenia. (C) Age at molecular diagnosis. (D–H) Laboratory findings and blood smears in patients: (D) Platelet counts, (E) Mean platelet volume (MPV), (F) haemoglobin levels, (G) leucocyte counts, (H) neutrophil counts. (I) Representative blood films in these patients showing (i) thrombocytopenia without morphological abnormalities, (ii) macrothrombocytopenia and (iii) granulocyte hyposegmentation. Bar: 5 μm. (J) Bleeding tendency assessed by the ISTH-BAT in women, men and children (<18 years) at diagnosis. (K–O) Bar charts showing the distribution of patients with normal versus abnormal values in different platelet function tests: (K) PFA-100 test using both collagen-ADP (col-ADP) and collagen-epinephrine (col-epi) cartridges. (L) Agonist-induced platelet aggregation (LTA). (M) Flow cytometry assessment of PAC-1 (αIIbβ3 activation), CD62 (α-granule secretion) or CD63 (δ-granule secretion) following agonist stimulation. (N) δ-granule content assessed by whole-mount electron microscopy (EM-WM). (O) Glycoprotein Ia (GPIa, collagen receptor), assessed by flow cytometry. Dotted lines indicate abnormal cut-off (platelets: $<150\times10^9$/L; MPV: 7.2–11.7 fL; female haemoglobin: 12.1–15.1 g/dL; male haemoglobin: 13.8–17.2 g/dL; leucocytes: $4.5–11\times10^9$/L; neutrophils: $2.5–7\times10^9$/L; BS females ≥6, BS males ≥4, BS children ≥3). Graphs were generated with GraphPad 9.

Malignancy progression

Eighteen patients (27.3%) developed malignancies: 14 in RUNX1-RT, 1 in ETV6-RT and 3 in ANKRD26-RT. The median age at neoplasm diagnosis was 48, 12 and 49 years respectively (range: 6–81) (Table 1; Figure S2A).

Eight of 14 RUNX1-RT patients (57.1%) developed different subtypes of MDS. Three had MDS with low blasts (MDS-LB), two developed MDS with increased blasts (MDS-IB1) while one had MDS-IB2 and the last two presented hypoplastic MDS (MDS-h) (Table 1). Five patients developed AML: two patients with acute myelomonocytic leukaemia (AMML); two patients with AML without maturation and the last patient with AML with maturation (Table 1). Finally, one patient (5.3%) developed Philadelphia-positive chronic myeloid leukaemia (CML Ph+) (Table 1).

One ETV6-RT patient was diagnosed with B-ALL in childhood, while the ANKRD26-RT patients developed B-ALL Ph+, MDS-LB and breast cancer (Table 1).

A total of 77.8% (14/18) achieved complete remission with negative minimal residual disease (MRD). The remaining 47 patients without malignancy have a median age of 35–37 years and have undergone regular follow-up for 3–6 years after diagnosis (Figure S2B).

BM evaluation

BM was assessed in 34 (51.5%) patients: 23 with RUNX1-RT, 5 with ETV6-RT and 6 with ANKRD26-RT, with a median age of 42, 16 and 37 years respectively (range: 0–81). Notably, BM analysis in non-malignant cases preceded that in patients who developed malignancy (Table 2; Figure S2C).

In non-malignant RUNX1-RT patients, BM showed normal or reduced cellularity and megakaryocyte (Mks) counts, with normal morphology, except for two cases with dysplastic, hypolobulated or small-sized features. Erythroid and/or granulocytic dysplasia and blasts were rare (Table 2). All AML and CML cases had hypercellular BM, whereas MDS cases had normocellular or hypocellular BM, with 50% showing megakaryocytic dysplasia and 35.7% erythroid or granulocytic dysplasia (Table 2).

Non-malignant ETV6-RT patients had normocellular BM without Mks dysplasia, although 75% had moderate dysgranulopoiesis. The childhood B-ALL case had hypercellular BM with lymphoblast infiltration (Table 2).

Of the four non-malignant ANKRD26-RT cases, one biopsy was inconclusive, one had normal BM and two had megakaryocytic dysplasia. The B-ALL Ph + patient had hyperplastic BM with reduced megakaryocytic, erythroid and granulocytic lineages and 80% lymphoblast infiltration, whereas the MDS patient had hypocellular BM without dysplasia (Table 2).

Platelet functional characterization

Platelet function analysis was performed in 24 RUNX1-RT patients, 4 ETV6-RT cases and 15 ANKRD26-RT patients, although not all tests could be performed in all cases due to sample availability or severe thrombocytopenia.

Sixteen (80%) and 13 (65%) of the 20 RUNX1-RT patients had a prolonged PFA-100 occlusion times using the col-epi and col-ADP cartridges respectively. In ETV6-RT, two patients showed prolonged closure times with col-ADP and one with col-epi. In ANKRD26-RT, 80% (4 of 5) had prolonged occlusion times with both cartridges (Figure 1K).

In LTA assays, an impaired aggregation response with multiple agonists (arachidonic acid, ADP, epinephrine or collagen) was found in 91.7%, 50% and 91.6% of RUNX1-RT, ETV6-RT and ANKRD26-RT patients respectively (Figure 1L).

Flow cytometry assays revealed reduced αIIbβ3 integrin activation (PAC-1 binding) and α-granule secretion (CD62) in 70.8% of RUNX1-RT patients. Of these, 10 and 12 cases had decreased PAC1 and CD62 binding, respectively, after stimulation with ADP, Thrombin Receptor Activator Peptide 6 (TRAP6) and Collagen Related Peptide (CRP). All ETV6-RT patients showed impaired activation and α-granule secretion, while five and seven of 15 ANKRD26-RT cases had reduced PAC1 binding and CD62 release, respectively, after TRAP6 or CRP stimulation (Figure 1M).

Dense granule secretion (CD63 release) was impaired in 62.5% of the RUNX1-RT patients, 50% of the ETV6-RT patients and 20% of the ANKRD26-RT patients. In addition, the whole-mount assay showed reduced δ-granule content in 50% of RUNX1-RT (9 of 18), one ETV6-RT patient (25%) and in none of the five ANKRD26-RT cases examined (Figure 1N; Figure S3A).

TABLE 1 Characteristics of patients with RUNX1-RT, ETV6-RT or ANKRD26-RT who developed haematological or solid malignancy.

	Sex	GD	Type of neoplasia	WHO classification 2022	Age at IT suspicion/ diagnosis/ neoplasia	GD before neoplasia	Molecular findings	Cytogenetic findings	Treatment	Response	Alive
P1	F	RUNX1 c.287delT p.Asn96Thrfs*26	HM	AML with maturation	2/4/6	Yes	Somatic mutation in *STAT3* and *JAK3*	46,XX,der(18);21[10],46 ,XX[10]	CT + allo-HSCT CT: mitoxantrona y Ara-C Allo-HSCT: RD 10/10	CR with negative MRD	Yes
P2	M	RUNX1 c.416G>A p.Arg166Gln	HM	AMML	31/39/42	Yes	Somatic mutation in the other allele of *RUNX1, NRAS, TET2* and FLT3 + ITD	46,XY,-22,+mar[6]/46,XY[19]	CT + allo-HSCT Induction: Idarubicin and cytarabine (3 + 7 regimen) and midastaurin Consolidation: cytarabine + midostaurin Allo-HSCT: URD 10/10	CR with negative MRD	Yes
P3	F	RUNX1 c.416G>A p.Arg166Gln	HM	MDS-LB	60/69/72	Yes	Somatic mutation in the other allele of *RUNX1, TET2* and *PHF6*	46,XX[20]	No	-	Yes
P4	M	RUNX1 c.416G>A p.Arg166Gln	HM	MDS-LB	48/48/49	Yes	No somatic variants detected. FISH negative for Chr5, Chr7, Chr8 and *TP53*	46,XY[20]	No	-	Yes
P5	F	RUNX1 c.416G>A p.Arg166Gln	HM	MDS-IB1	n/a/71/71	No	n/a	n/a	CT: 5-Azacytidine, without response Filgrastim	NR	No
P6	F	RUNX1 c.416G>A p.Arg166Gln	HM	AML without maturation	10/74/74	No	Somatic mutations in the other allele of *RUNX1* and *EZH2*	46,XX[20]	CT: Idarubicin and cytarabine (3 + 7 regimen)	Refractory	No
P7	F	RUNX1 c.416G>A p.Arg166Gln	HM	AML without maturation	46/46/47	Yes	Somatic mutations in *WT1* FISH: *MLL* rearrangement *EVI*	83–90,XXXX[10]/46,XX[20]	CT: Idarubicin and cytarabine (3 + 7 regimen) and quizartinib Allo-HSCT: n/a	CR with negative MRD	Yes
P8	M	RUNX1 c.416G>A p.Arg166Gln	HM	MDS-LB	46/46/46	No	Somatic mutations in *SRSF2* and *EZH2*	46,XY[20]	No	-	Yes
P9	F	RUNX1 c.586A>G p.Thr196Ala	HM	MDS-IB1	42/48/52	Yes	Somatic mutations in *IDH1*	46,XX[20]	No	-	Yes
P10	M	RUNX1 c.586A>G p.Thr196Ala	HM	MDS-h	n/a/ post-mortem/48	No	n/a	46,XY,Del(7q)[20]	Allo-HSCT: URD 10/10	CR with negative MRD	No
P11	F	RUNX1 c.610C>T p.Arg204*	HM	AMML	n/a/46/46	No	Somatic mutations in *FLT3* and *SF3B1*	47,XX,+6[2]/46,XX[15]	CT + allo-HSCT CT: Idarubicin and cytarabine (3 + 7 regimen) and midastaurin Allo-HSCT: URD 10/10	CR with negative MRD	Yes
P12	F	RUNX1 c.802C>T p.Gln268*	HM	MDS-IB2	24/26/32	Yes	No somatic variants detected. FISH negative for Chr5, Chr7, Chr8 and *TP53*	46,XX[20]	CT + allo-HSCT CT: 5-Azacytidine Allo-HSCT: RD 10/10	CR with negative MRD	Yes
P13	M	RUNX1 c.849G>C p.Gln283His	HM	MDS-h	55/62/70	Yes	Somatic mutations in *ASXL1*	46,XY[20]	No	-	Yes
P14	F	RUNX1 c.1076C>G p.Pro359Arg	HM	CML Ph positive	41/41/41	No	BCR/ABL traslocation	46,XX,t(9;22)[20]	Nilotinib	Major molecular response	Yes

TABLE 1 (Continued)

	Sex	GD	Type of neoplasia	WHO classification 2022	Age at IT suspicion/ diagnosis/ neoplasia	GD before neoplasia	Molecular findings	Cytogenetic findings	Treatment	Response	Alive
P15	F	ETV6 c.1103T>G p.Phe368Cys	HM	B-ALL NOS	12/26/12	No	n/a	46,XX[20]	CT: ALL-SEHOP-2005 protocol	CR with negative MRD	Yes
P16	M	ANKRD26 c.-118C>G	HM	B-ALL Ph positive	42/79/81	Yes	BCR/ABL p210+ traslocation	47,XYY,t(9;22)[20]	CT: ALL 07/OPH—PETHEMA old- fragile Maintenance with methotrexate, mercaptopurine and imatinib	Major molecular response	No
P17	M	ANKRD26 c.-118C>G	HM	MDS-LB	n/a/44/37	No	No somatic variants detected. FISH negative for Chr5, Chr7, Chr8 and *TP53*	46,XY[20]	No	-	Yes
P18	F	ANKRD26 c.-118C>G	Solid	Breast cancer	n/a/51/49	No	-	-	RT and Tamoxifen	-	Yes

Note: Age is shown in years.

Abbreviations: ALL, acute lymphoblastic leukaemia; allo-HSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; AMML, Acute myelomonocytic leukaemia; NOS, Not Otherwise Specified; B-ALL, acute lymphoblastic leukaemia type B; CML, chronic myeloid leukaemia; CR, complete response; CT, chemotherapy; FISH, Fluorescence in situ hybridization; GD, genetic diagnosis; HM, haematological malignancy; IT, inherited thrombocytopenia; MDS, myelodysplastic syndrome; MDS-h, Hypoplastic MDS; MDS-IB, MDS with increased blasts; MDS-LB, MDS with low blasts; MRD, minimal residual disease; n/a, not applicable; NR, no response; Ph, Philadelphia (chromosome); RD, related donor; RT, radiotherapy; URD, unrelated donor.

All patients had normal levels of GPIb-IX, αIIbβ3 (GPIIbIIIa) and GPVI, with no CD34+ expression. However, 70.8% of RUNX1-RT patients showed a 30%–70% reduction in GPIa levels (Figure 1O; Figure S3B).

Molecular diagnosis

Twenty-four different germline variants were identified: 16 in *RUNX1*, four in *ETV6* and four in *ANKRD26* (Figure 2; Table S6). No candidate variants were identified in any of the 20 thrombocytopenia-free relatives evaluated.

Eight of the *RUNX1* variants were missense, four in the Runt homology domain and four in the transactivation domain (TAD) domains of the protein. In addition, there were three nonsense variants (18.8%), two frameshifts (12.5%), two large deletions (12.5%), one affecting exons 1 and 2 and another deleting the entire RUNX1 gene (del21q22.3) and one splicing variant (6.2%) (Figure 2A). All *ETV6* variants were missense, affecting either the regulatory or the ETS domains (Figure 2B). *ANKRD26* variants were all located in the 5′UTR, three in the RUNX1-binding domain and one upstream (Figure 2C).

Multigenerational inheritance was observed in 55% of the RUNX1-RT families, while 40% of the cases had de novo or mosaic variants (Figure 2A, underlined variants). In one patient (5%), inheritance could not be determined, but the variant was confirmed in the patient's daughter. In ETV6-RT, 80% of families showed multigenerational inheritance, while one case (20%) was de novo (Figure 2B). In ANKRD26-RT, autosomal dominant inheritance was detected in 66.6%, while one case (16.7%) was de novo and another was not studied due to the unavailability of parental samples (Figure 2C).

Ten of the 16 *RUNX1* variants (62.5%) have been previously reported (Table S6), including two (p.Thr196Ala and p.Gln268) newly characterized by our group.[25,29] They are classified as LPV and PV in both ClinVar and ClinGen (Figure 2A). Six were known PV listed in ClinVar, ClinGen or the literature;[30–36] one was recently reclassified as VUS-LBV, while del21q22.3 is described in studies but not in databases (Table S6).[24,30] The remaining six *RUNX1* variants (43.8%) were new, with three classified as VUS and three not listed in ClinVar (Figure 2A; Table S6).

Three of the four *ETV6* variants (75%) were previously reported (Table S6),[37–39] two of which were classified as PV in ClinVar. The novel p.Ser323Cys was classified as LPV in ClinVar (Figure 2B).

Lastly, all *ANKRD26* variants had been previously described and were classified with conflicting pathogenicity in ClinVar (Figure 2C; Table S6).[40–43]

DISCUSSION

This study reports one of the largest series of IT-HM patients worldwide. Importantly, genetic diagnosis of our RUNX1-RT, ETV6-RT and ANKRD26-RT patients was

TABLE 2 Bone marrow histology in RUNX1-RT, ETV6-RT and ANKRD26-RT patients with or without haematological malignancies.

	RUNX1 no malignancy (n = 9)	RUNX1 malignancy (n = 14)	ETV6 no malignancy (n = 4)	ETV6 malignancy (n = 1)	ANKRD26 no malignancy (n = 4)	ANKRD26 malignancy (n = 2)
Age (y/o)	14 [0–54]	47 [6–74]	28 [2–46]	12	31 [25–36] (2 n/a)	59 [37–81]
Bone marrow cellularity						
Hypercellular	0	5 (35.7%)	0	1 (100%)	1 (33.3%)	1 (50%)
Normocellular	5 (55.6%)	6 (42.9%)	4 (100%)	0	2 (66.7%)	0
Hypocellular (including low for age cellularity)	4 (44.4%)	3 (21.4%)	0	0	0	1 (50%)
Megakaryocyte proportion						
Increased	0	1 (7.1%)	1 (25%)	0		0
Normal	7 (77.8%)	11 (78.6%)	3 (75%)	0	3 (100%)	1 (50%)
Decreased	2 (22.2%)	2 (14.3%)	0	1 (100%)	0	1 (50%)
Bone marrow dysplasia						
Megakaryocytic dysplasia (hypolobulated and/or small-sized, dysmegakaryopoiesis)	2 (22.2%)	7 (50%)	0	0	2 (66.7%)	0
Erythroid dysplasia (maturational asynchronism, changes in size)	0	5 (35.7%)	1 (25%)	0	0	0
Granulocytic dysplasia (hyposegmentation and/or hypogranulation, maturational asynchronism, dysgranulopoiesis, chromatin clumping)	1 (11.1%)	5 (35.7%)	3 (75%)	0	0	0
Blasts (>5%)	0	5 (35.7%)	0	1 (100%)	0	1 (50%)

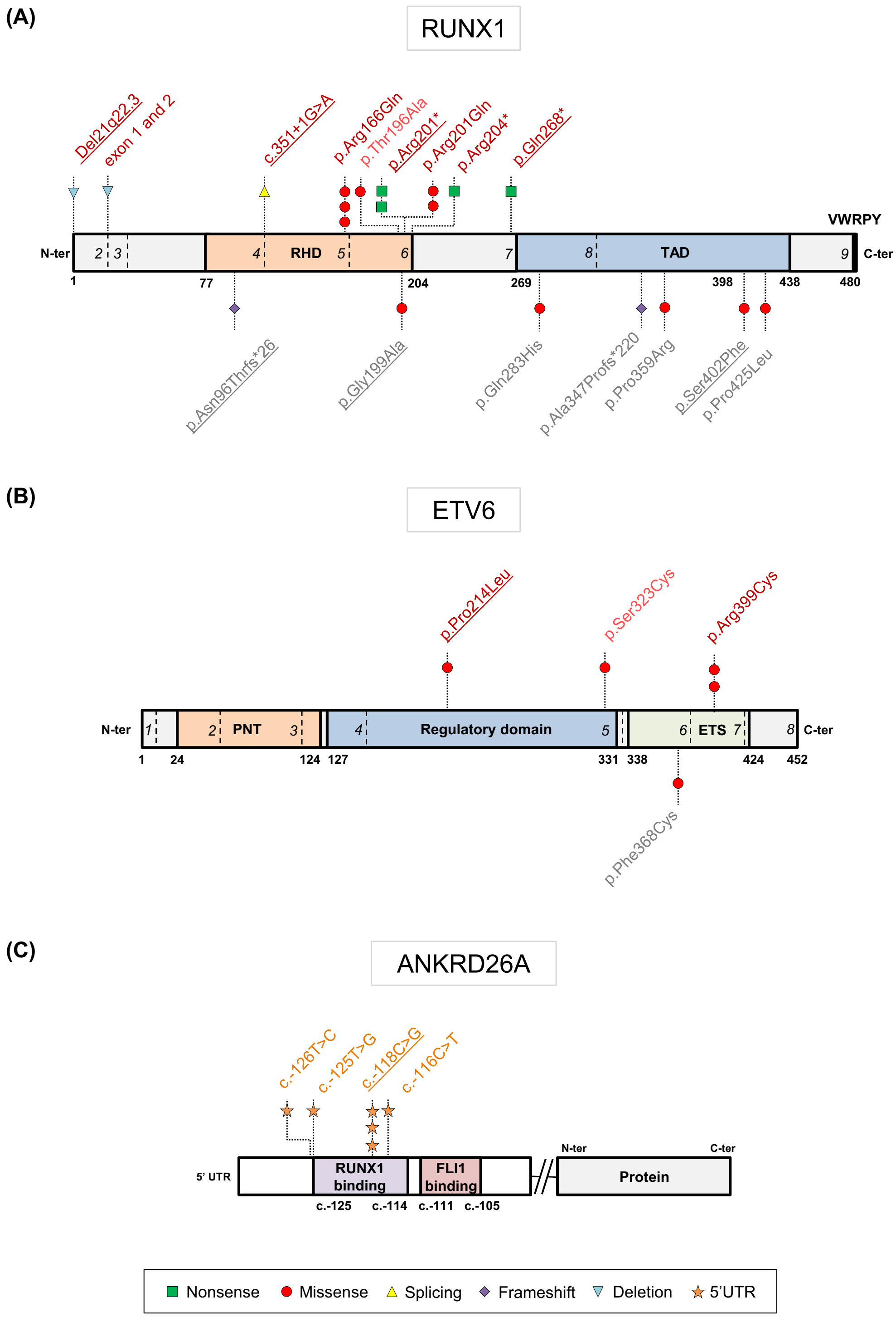

FIGURE 2 Genetic analysis in patients with suspected inherited thrombocytopenia identified germline variants in (A) *RUNX1*, (B) *ETV6* and (C) *ANKRD26*. The type of variant is indicated by shape and colour. Each shape represents one family. Dark red text denotes a pathogenic variant (PV) classification according to ClinVar (and ClinGen for RUNX1). Light red text indicates likely pathogenic (LP) variants, grey represents variants of uncertain significance (VUS) and orange denotes conflicting pathogenicity classifications. Underlined variants are de novo. The diagram displays the various domains, start and end amino acids and exons. ETS, a family of transcriptional activators or repressors; PNT, pointed domain; RHD, Runt homology domain; TAD, transactivation domain.

delayed by 17, 18 and 21 years, respectively, after the initial detection of thrombocytopenia.

This delay likely stems from the underestimation of asymptomatic thrombocytopenia, limited access to sequencing tools—mainly Sanger—and the perception that genetic diagnosis was not essential for clinical care. Fortunately, recent advances in the understanding of IPDs, the adoption of HTS and multicentre studies like the one led by GEAPC in Spain have improved diagnostic accuracy and shifted clinical perspectives.[24,44] As a result, asymptomatic cases are now more thoroughly investigated, often uncovering genetic causes relevant to personalized care. Nonetheless, genetic screening remains challenging due to the frequent identification of VUS, which require cautious interpretation to avoid unnecessary distress for patients and families.[45,46]

Mild to moderate thrombocytopenia was confirmed in the patients, except a few relatives in RUNX1-RT pedigrees, but pathological bleeding was exhibited by only 25%–30% of RUNX1-RT and ANKRD26-RT patients. In addition, bleeding exhibited a correlation with platelet count in ANKRD26-RT patients, but not in RUNX1-RT or ETV6-RT patients (Table S7; Figure S4A). A moderate clinical phenotype is detrimental to the early diagnosis of these patients.[45,47–49]

In agreement with findings in other cohorts,[11,50,51] platelet dysfunction was prevalent in this study, with variable PFA-100, aggregation and secretion defects in most patients. A notable finding in RUNX1-RT patients was that 70% exhibited reduced GPIa levels (30%–70%), which aligns with lower *ITGA2* expression levels that have been previously reported.[23,25] Despite the absence of a discernible correlation between bleeding and GPIa levels or platelet function (Table S7), a negative correlation was identified between lower platelet counts and decreased GPIa expression, granule secretion defects and impaired LTA in these patients (Table S7; Figure S4B). These findings suggest that GPIa quantification and granule secretion could serve as potential biomarkers for the diagnosis of RUNX1-RT. It is also interesting that a significant correlation exists between platelet count and LTA impairment in ETV6-RT patients (Table S7; Figure S4C), although this finding requires validation with a larger cohort.

The molecular analysis of RUNX1-RT patients revealed the presence of diverse candidate variants across the protein (Figure 2A). The most prevalent PV was c.497G>A (p.Arg166Gln), identified in 13 patients from three families and associated with a high neoplasia incidence (50%). Large genomic deletions were also identified, emphasizing the necessity for comprehensive genetic analysis, including copy number assessment.[50] However, the variant type did not correlate with bleeding, platelet count, function or GPIa levels (Table S7). Patients with PV tended to have lower platelet counts, impaired aggregation and decreased granule secretion compared to those with VUS (Table S7; Figure S4D). Furthermore, ETV6-RT patients exhibited exclusively missense variants within key protein domains, while ANKRD26-RT variants were confined to the 5′UTR. Among *ANKRD26* variants, c.-118C>G was the most prevalent, with its identification in 10 patients from three families (representing 50% of the cohort). It is noteworthy that 40% of RUNX1-RT and 20% of ETV6-RT and ANKRD26-RT patients exhibited de novo variants, a finding that is consistent with the observations reported in other IT, such as MYH9-RT, where de novo mutations occur in approximately 30% of cases.[52,53]

The malignancy rate found in this study is consistent with previous reports[4]: 37.8% of RUNX1-RT patients developed myeloid neoplasia, 11.1% of ETV6-RT patients had childhood B-ALL and 10% of ANKRD26-RT patients developed haematological malignancies. Notably, half of these patients were genetically diagnosed only after the neoplasia had developed (Table 1). This emphasizes the diagnostic challenge posed by mild or asymptomatic thrombocytopenia, which frequently remains unrecognized until a more severe phenotype manifests.

The AML treatment regimens included chemotherapy and allogeneic transplantation in patients younger than 70 years. Recent studies indicate that a significant subset of RUNX1-RT patients can achieve long-term survival with conventional AML treatment, with leukaemic progression linked to specific genetic events.[54] One ETV6-RT patient was initially diagnosed with B-ALL of somatic origin and treated with chemotherapy. Fourteen years later, a germline ETV6 variant was identified, highlighting the need for precise genetic diagnosis for optimal patient management. Furthermore, RUNX1-RT and ANKRD26-RT patients who developed haematological malignancies had significantly lower platelet counts, and a similar trend was observed in malignant ETV6-RT cases (Table S7; Figure S4E).

BM analysis was performed in 60% of RUNX1-RT and ETV6-RT cases but only 30% of ANKRD26-RT cases, reflecting a lack of consensus on when BM studies should be conducted.[55,56] Among non-malignant RUNX1-RT patients, 22.2% showed atypical Mks or dysmegakaryopoiesis. Importantly, BM analysis was conducted at multiple hospitals rather than centrally, but our findings align with previous studies.[57] A novel observation was the high incidence (75%) of granulocyte dysplasia in non-malignant ETV6-RT patients, which contrasts with previous reports describing primarily dyserythropoiesis, Mks hyperplasia and small, hypolobulated Mks.[16] This finding may suggest the benefit of longitudinal follow-up of these patients, which could reveal early clonal haematopoiesis.

A high incidence of immune disorders was observed in RUNX1-RT patients (40%), in agreement with previous studies' observations.[50] The pivotal role of RUNX1 in immune regulation may account for the high prevalence of allergies and autoimmune conditions.[58,59] It is noteworthy that many of our RUNX1-RT, ETV6-RT and ANKRD26-RT patients also exhibited dermatological changes, suggesting that immune dysregulation potentially leads to skin disease. Other studies have reported frequent eczema in RUNX1-RT patients.[50,60] The high rate of heterogeneous comorbidities in patients with these ITs strongly supports a multidisciplinary approach to their clinical assessment and follow-up.

In conclusion, this study provides a comprehensive characterization of the clinical phenotype, malignancy risk, genetic landscape and platelet function in patients with IT-HM. Our findings reveal significant heterogeneity and highlight critical diagnostic challenges, particularly in asymptomatic thrombocytopenia. Reduced GPIa levels and impaired collagen-induced aggregation may serve as diagnostic biomarkers of RUNX1-RT patients. The high incidence of haematological malignancies underscores the importance of early genetic diagnosis, which could enable closer monitoring and timely intervention to enhance outcomes, quality of life and overall survival of patients with IT-HM.

AUTHOR CONTRIBUTIONS

AM-Q, JMB, MLL and JR designed the research; AM-Q, AS-F, AZ-C, PLG-G, LD-A, RB and JR performed platelet function and molecular assays and data analysis. AS-F, AR-A, TS, NVB, NR, RC, PE, JE, NF-M, FF-M, LH, JH-A, AL, ML-D, MM-S, EL, TM, LM, MN, RO, IP-P, FR, ES, CS, CS-A, RV-L, JRG-P, MLL and JMB performed clinical assessment and management of the patients. AM-Q, AS-F, MLL, JMB and JR wrote the manuscript. All authors helped in data interpretation and critically reviewed the paper.

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CONFLICT OF INTEREST STATEMENT

The authors state that they have no conflict of interest in relation to this work.

DATA AVAILABILITY STATEMENT

All data from this study will be freely accessible upon request to the corresponding authors.

ETHICS STATEMENT

The project was approved by the Ethics Committee of the Hospital Reina Sofía (Murcia, Spain), and complied with the standards of the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all patients.

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REFERENCES

1. Nurden AT, Nurden P. Inherited thrombocytopenias: history, advances and perspectives. Haematologica. 2020;105(8):2004–19.
2. Pecci A, Balduini CL. Inherited thrombocytopenias: an updated guide for clinicians. Blood Rev. 2021;48:100784.
3. Palma-Barqueros V, Revilla N, Sanchez A, Zamora Cánovas A, Rodriguez-Alén A, Marín-Quílez A, et al. Inherited platelet disorders: an updated overview. Int J Mol Sci. 2021;22(9):4521.
4. Melazzini F, Zaninetti C, Balduini CL. Bleeding is not the main clinical issue in many patients with inherited thrombocytopaenias. Haemophilia. 2017;23(5):673–81.
5. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
6. Hayashi Y, Harada Y, Huang G, Harada H. Myeloid neoplasms with germ line RUNX1 mutation. Int J Hematol. 2017;106(2):183–8.
7. Schlegelberger B, Heller PG. RUNX1 deficiency (familial platelet disorder with predisposition to myeloid leukemia, FPDMM). Semin Hematol. 2017;54(2):75–80.
8. Feurstein S, Godley LA. Germline ETV6 mutations and predisposition to hematological malignancies. Int J Hematol. 2017;106(2):189–95.
9. Noris P, Perrotta S, Seri M, Pecci A, Gnan C, Loffredo G, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood. 2011;117(24):6673–80.
10. Ng IK, Lee J, Ng C, Kosmo B, Chiu L, Seah E, et al. Preleukemic and second-hit mutational events in an acute myeloid leukemia patient with a novel germline RUNX1 mutation. Biomark Res. 2018;6:16.
11. Homan CC, Scott HS, Brown AL. Hereditary platelet disorders associated with germ line variants in RUNX1, ETV6, and ANKRD26. Blood. 2023;141(13):1533–43.
12. Preudhomme C, Renneville A, Bourdon V, Philippe N, Roche-Lestienne C, Boissel N, et al. High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. Blood. 2009;113(22):5583–7.
13. Porter CC. Germ line mutations associated with leukemias. Hematology Am Soc Hematol Educ Program. 2016;2016(1):302–8.
14. Brown AL, Arts P, Carmichael CL, Babic M, Dobbins J, Chong CE, et al. RUNX1-mutated families show phenotype heterogeneity and a somatic mutation profile unique to germline predisposed AML. Blood Adv. 2020;4(6):1131–44.
15. Lawal RA, Deuitch NT, Bresciani E, Kajdic A, Chong S, Craft K, et al. Minority patients in the NIH RUNX1-FPDMM natural history study present mostly with large deletions affecting RUNX1. Blood. 2024;144(Suppl 1):798.
16. Di Paola J, Porter CC. ETV6-related thrombocytopenia and leukemia predisposition. Blood. 2019;134(8):663–7.
17. Rafei H, DiNardo CD. Hereditary myeloid malignancies. Best Pract Res Clin Haematol. 2019;32(2):163–76.
18. Elbatarny M, Mollah S, Grabell J, Bae S, Deforest M, Tuttle A, et al. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. Haemophilia. 2014;20(6):831–5.
19. Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, et al. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. J Thromb Haemost. 2010;8(9):2063–5.
20. Marin-Quilez A, Di Buduo CA, Diaz-Ajenjo L, Abbonante V, Vuelta E, Soprano PM, et al. Novel variants in GALE cause syndromic macrothrombocytopenia by disrupting glycosylation and thrombopoiesis. Blood. 2023;141(4):406–21.
21. Palma-Barqueros V, Revilla N, Zaninetti C, Galera AM, Sánchez-Fuentes A, Zámora-Cánovas A, et al. Src-related thrombocytopenia: a fine line between a megakaryocyte dysfunction and an immune-mediated disease. Blood Adv. 2022;6(17):5244–55.
22. Dupuis A, Bordet JC, Eckly A, Gachet C. Platelet delta-storage pool disease: an update. J Clin Med. 2020;9(8):2508.
23. Glembotsky AC, Sliwa D, Bluteau D, Balayn N, Oyarzún CP, Raimbault A, et al. Downregulation of TREM-like transcript-1 and collagen receptor alpha2 subunit, two novel RUNX1-targets, contributes to platelet dysfunction in familial platelet disorder with predisposition to acute myelogenous leukemia. Haematologica. 2019;104(6):1244–55.
24. Bastida JM, Lozano ML, Benito R, Janusz K, Palma-Barqueros V, del Rey M, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. Haematologica. 2018;103(1):148–62.
25. Palma-Barqueros V, Bastida JM, Lopez Andreo MJ, Zámora-Cánovas A, Zaninetti C, Ruiz-Pividal JF, et al. Platelet transcriptome analysis in patients with germline RUNX1 mutations. J Thromb Haemost. 2023;21:1352–65.
26. Palma-Barqueros V, Bury L, Kunishima S, Lozano ML, Rodríguez-Alen A, Revilla N, et al. Expanding the genetic spectrum of TUBB1-related thrombocytopenia. Blood Adv. 2021;5:5453–67.
27. Richards S, Aziz N, Bale S, Bick D, das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
28. Luo X, Feurstein S, Mohan S, Porter CC, Jackson SA, Keel S, et al. ClinGen myeloid malignancy variant curation expert panel recommendations for germline RUNX1 variants. Blood Adv. 2019;3(20):2962–79.
29. Lamolda M, Montes R, Simon I, Perales S, Martínez-Navajas G, Lopez-Onieva L, et al. GENYOi005-A: an induced pluripotent stem cells (iPSCs) line generated from a patient with familial platelet disorder with associated myeloid malignancy (FPDMM) carrying a p.Thr196Ala variant. Stem Cell Res. 2019;41:101603.
30. Hussein IRBR, Chaudhary A, Malki A, Al Qahtani M. Case report: interstitial deletion 21q22.13-Q22.3 in a male patient with developmental delay, holoprosencephaly, dysmorphic features, and multiple congenital anomalies. J Mol Biomark Diagn. 2015;6(2):1000222.
31. Owen CJ, Toze CL, Koochin A, Forrest DL, Smith CA, Stevens JM, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008;112(12):4639–45.
32. Rio-Machin A, Vulliamy T, Hug N, Walne A, Tawana K, Cardoso S, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Nat Commun. 2020;11(1):1044.
33. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999;23(2):166–75.
34. Tsai SC, Shih LY, Liang ST, Huang YJ, Kuo MC, Huang CF, et al. Biological activities of RUNX1 mutants predict secondary acute leukemia transformation from chronic myelomonocytic leukemia and myelodysplastic syndromes. Clin Cancer Res. 2015;21(15):3541–51.
35. Wu D, Luo X, Feurstein S, Kesserwan C, Mohan S, Pineda-Alvarez DE, et al. How I curate: applying American Society of Hematology-clinical genome resource myeloid malignancy variant curation expert panel rules for RUNX1 variant curation for germline predisposition to myeloid malignancies. Haematologica. 2020;105(4):870–87.

36. Zhao LJ, Wang YY, Li G, Ma LY, Xiong SM, Weng XQ, et al. Functional features of RUNX1 mutants in acute transformation of chronic myeloid leukemia and their contribution to inducing murine full-blown leukemia. Blood. 2012;119(12):2873–82.
37. Gilad O, Dgany O, Noy-Lotan S, Krasnov T, Yacobovich J, Rabinowicz R, et al. Syndromes predisposing to leukemia are a major cause of inherited cytopenias in children. Haematologica. 2022;107(9):2081–95.
38. Noetzli L, Lo RW, Lee-Sherick AB, Callaghan M, Noris P, Savoia A, 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.
39. Zhang MY, Churpek JE, Keel SB, Walsh T, Lee MK, Loeb KR, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015;47(2):180–5.
40. Balduini A, Raslova H, Di Buduo CA, Donada A, Ballmaier M, Germeshausen M, et al. Clinic, pathogenic mechanisms and drug testing of two inherited thrombocytopenias, ANKRD26-related thrombocytopenia and MYH9-related diseases. Eur J Med Genet. 2018;61(11):715–22.
41. Diep RT, Corey K, Arcasoy MO. A novel nucleotide substitution in the 5′ untranslated region of ANKRD26 gene is associated with inherited thrombocytopenia: a report of two new families. Ann Hematol. 2019;98(7):1789–91.
42. Greene D, Richardson S, Turro E. A fast association test for identifying pathogenic variants involved in rare diseases. Am J Hum Genet. 2017;101(1):104–14.
43. Marconi C, Canobbio I, Bozzi V, Pippucci T, Simonetti G, Melazzini F, et al. 5'UTR point substitutions and N-terminal truncating mutations of ANKRD26 in acute myeloid leukemia. J Hematol Oncol. 2017;10(1):18.
44. Bastida JM, Benito R, Lozano ML, Marín-Quilez A, Janusz K, Martín-Izquierdo M, et al. Molecular diagnosis of inherited coagulation and bleeding disorders. Semin Thromb Hemost. 2019;45:695–707.
45. Downes K, Borry P, Ericson K, Gomez K, Greinacher A, Lambert M, et al. Clinical management, ethics and informed consent related to multi-gene panel-based high throughput sequencing testing for platelet disorders: communication from the SSC of the ISTH. J Thromb Haemost. 2020;18(10):2751–8.
46. Greinacher A, Eekels JJM. Simplifying the diagnosis of inherited platelet disorders? The new tools do not make it any easier. Blood. 2019;133(23):2478–83.
47. Galera P, Dulau-Florea A, Calvo KR. Inherited thrombocytopenia and platelet disorders with germline predisposition to myeloid neoplasia. Int J Lab Hematol. 2019;41(Suppl 1):131–41.
48. Kirchmaier CM, Pillitteri D. Diagnosis and management of inherited platelet disorders. Transfus Med Hemother. 2010;37(5):237–46.
49. Perez Botero J, Di Paola J. Diagnostic approach to the patient with a suspected inherited platelet disorder: who and how to test. J Thromb Haemost. 2021;19(9):2127–36.
50. Cunningham L, Merguerian M, Calvo KR, Davis J, Deuitch NT, Dulau-Florea A, et al. Natural history study of patients with familial platelet disorder with associated myeloid malignancy. Blood. 2023;142(25):2146–58.
51. Ovsyannikova GS, Fedorova DV, Tesakov IP, Martyanov AA, Ignatova AA, Ponomarenko EA, et al. Platelet functional abnormalities and clinical presentation in pediatric patients with germline RUNX1, ANKRD26, and ETV6 mutations. Haematologica. 2022;107(10):2511–6.
52. Bury L, Megy K, Stephens JC, Grassi L, Greene D, Gleadall N, et al. Next-generation sequencing for the diagnosis of MYH9-RD: predicting pathogenic variants. Hum Mutat. 2020;41(1):277–90.
53. Fernandez-Prado R, Carriazo-Julio SM, Torra R, Ortiz A, Perez-Gomez MV. MYH9-related disease: it does exist, may be more frequent than you think and requires specific therapy. Clin Kidney J. 2019;12(4):488–93.
54. Ernst MPT, Versluis J, Valk PJM, Bierings M, Tamminga RYJ, Hooimeijer LH, et al. Disease characteristics and outcomes of acute myeloid leukemia in germline RUNX1 deficiency (familial platelet disorder with associated myeloid malignancy). Hema. 2025;9(1):e70057.
55. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;128(14):1800–13.
56. Godley LA, DiNardo CD, Bolton K. Germline predisposition in hematologic malignancies: testing, management, and implications. Am Soc Clin Oncol Educ Book. 2024;44(3):e432218.
57. Kanagal-Shamanna R, Loghavi S, DiNardo CD, Medeiros LJ, Garcia-Manero G, Jabbour E, et al. Bone marrow pathologic abnormalities in familial platelet disorder with propensity for myeloid malignancy and germline RUNX1 mutation. Haematologica. 2017;102(10):1661–70.
58. Bellissimo DC, Chen CH, Zhu Q, Bagga S, Lee CT, He B, et al. Runx1 negatively regulates inflammatory cytokine production by neutrophils in response to toll-like receptor signaling. Blood Adv. 2020;4(6):1145–58.
59. Seo W, Taniuchi I. The roles of RUNX family proteins in development of immune cells. Mol Cells. 2020;43(2):107–13.
60. Nitschke NJ, Almosailleakh M, Niu Y, Hansen JW, Raaschou-Jensen K, Jespersen JS, et al. Frequency and functional characterization of RUNX1 germline variants in myeloid neoplasms. Hum Mutat. 2023;2023(1):4738660.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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