CORRESPONDENCE OPEN

Check for updates

CALR^{ins5}-mediated clonal hematopoiesis causes severe hemolytic anemia in a female *PGK1*^{Ser320Asn} carrier

© The Author(s) 2025, modified publication 2026

Blood Cancer Journal (2025)15:8; https://doi.org/10.1038/s41408-025-01216-w

INTRODUCTION

Hereditary hemolytic anemias (HHA) are rare inherited red blood cell (RBC) disorders caused by genetic abnormalities (hemoglobino/membrano/enzymopathies), characterized by anemia due to premature RBC destruction and intrinsic RBC defects. One of the rarer enzymopathies involves a deficiency in phosphoglycerate kinase 1 (PGK1), an X-linked gene critical for ATP production via glycolysis [1]. *PGK1* mutations causing deficiency (OMIM#300653) [2] follow an X-linked recessive inheritance pattern, affecting hemizygous males, while female carriers are generally asymptomatic or may have mild symptoms -consistent with mosaicism for PGK1 activity-[2].

Despite *PGK1* is ubiquitously expressed, its deficiency mainly affects the blood, central nervous system (CNS), and skeletal muscle, resulting in chronic hemolysis -with or without anemia-, neurological disorders, or myopathies [1]. Likely explained by the causative *PGK1* mutation [3], hemolytic anemias (HA) tends to co-occur with CNS defects, whereas myopathies are almost exclusively observed [3, 4].

To date, approximately 40 patients harboring 30 different mutations have been reported [5]. This report presents the first case of severe HA in a female *PGK1* mutation heterozygous carrier [6], which became apparent coinciding with the diagnosis of essential thrombocythemia (ET); and elucidates the mechanism by which a clonal disorder transforms a germline recessive disease into a tissue-specific dominant condition.

METHODS

Written informed consent was obtained from the participants. Clinical and laboratory data were extracted from electronic records. Details of collection and processing of peripheral blood (PB) samples, histological studies, enzymatic activity, sequencing, gene expression, X-chromosome inactivation (XCI), methylation variant calling, and phasing, can be found in the Supplementary Material.

RESULTS

Case presentation

In June 2021, a 32-year-old woman was admitted to the Emergency Department with a two-week history of asthenia and jaundice without choluria, recent infections, or medications. Laboratory tests identified mild regenerative macrocytic anemia (hemoglobin 10.4 g/dL; mean corpuscular volume (MCV) 105 fL [normal range (NR): 79–99 fL]; reticulocytes count, 204000/μL [NR: 15000–80000/μL]) associated with hemolysis pattern (total and indirect bilirubin (6.4 and 6.1 mg/dL, respectively) [NR: 0.30–1.20 and 0.10–0.30 mg/dL, respectively], LDH 674 U/L [NR: 208–378 U/L], undetectable haptoglobin), thrombocytosis (platelets 814000/μL), along with iron overload (ferritin 176 ng/mL [NR:10–120 ng/mL]) and hypergammaglobulinemia (1.9 g/dL) [NR: 0.8–1.4 g/dL]. Chronic liver disease, monoclonal gammopathy, hereditary spherocytosis, β-thalassemia, and autoimmune origin were excluded. Abdominal ultrasound did not show hepatosplenomegaly.

A PB smear showed anisocytosis, polychromasia, and thrombocytosis with anisothrombia (Fig. S1). Glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) levels were within reference limits.

Throughout recent years (2017–2019), compensated hemolysis was evidenced by normal hemoglobin levels but elevated bilirubin and LDH. In fact, due to recurrent hepatic colic, she underwent cholecystectomy in 2018. Notably, the onset of anemia coincided with the appearance of thrombocytosis (June 2019, Fig. 1A). Her pediatric history indicated she was born after two first-trimester miscarriages and was the oldest of three siblings (Fig. 1B). She suffered from neonatal sepsis caused by coagulase-positive staphylococci, followed by anemia and hyperbilirubinemia requiring RBC transfusion. Despite these early complications, she showed compensated hemolysis without signs of other hematological abnormalities.

The patient's family history revealed that whereas her youngest brother was healthy, her middle brother suffered severe HA from the neonatal period, with blood transfusions every 2–3 weeks, and spastic quadriplegia. Despite splenectomy was performed, he died in 2000 (at 7-year-old) of severe encephalopathy and HA. A *post-mortem* familial genetic study revealed the first case of *PGK*-Murcia mutation (*PGK1*^{Ser320Asn}) [6]. Then, his mother and sister (our patient at 16 year-old) were identified as asymptomatic female carriers [6].

Following clinical protocols, a customized sequencing panel for HHA confirmed the only presence of the *PGK1*^{Ser320Asn} mutation (Table S1), while other potential causes of jaundice were discarded. Evaluation of persistent thrombocytosis by bone marrow (BM) aspirate (Fig. 1C) and biopsy (Fig. S2) revealed marked myeloid hyperplasia of the erythroid and megakaryocytic lineages (with enlarged, mature, and hyperlobulated megakaryocytes). After ruling out *JAK2* mutations, *BCR::ABL* transcripts, or cytogenetic abnormalities, sequencing studies for myeloid malignancies were performed revealing a *CALR*^{ins5} mutation (type II) with a variant allele frequency (VAF) of 28.1%. Altogether, the propositus was diagnosed with *CALR*-mutated ET and HHA due to PGK1 deficiency, which is currently transfusion-dependent.

Biological and molecular studies

The activity quantification of enzymopathies-associated enzymes showed normality for all tested enzymes in the mother, while G6PD, hexokinase (HK), and PK activities were elevated in the

Received: 8 September 2024 Revised: 3 January 2025 Accepted: 23 January 2025
Published online: 01 February 2025

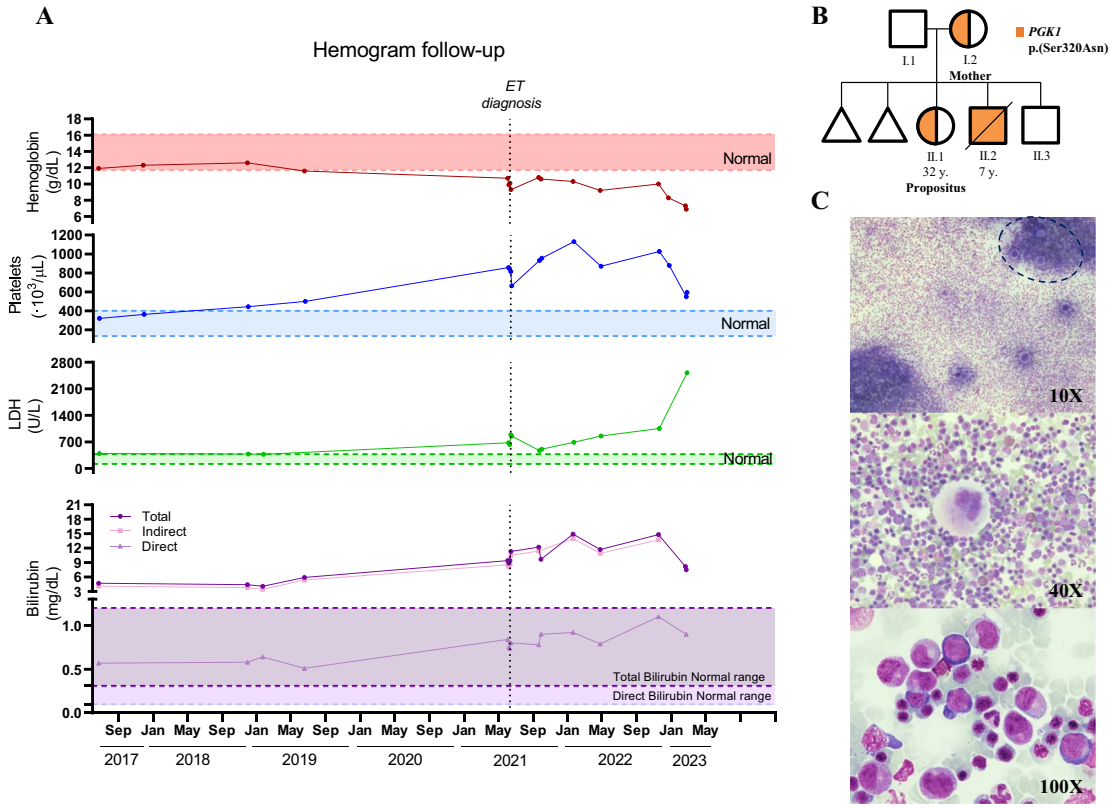


Fig. 1 Evolution of hemogram parameters, family history, and bone marrow characteristics of the propositus. A Hemoglobin (g/dL), platelets (10^3/µL), LDH (U/L), and bilirubin (mg/dL) levels of the propositus are shown over a 6-year period (June/2017–February/2023). The time of diagnosis of essential thrombocythemia (ET) is indicated. **B** Pedigree showing the presence of the *PGK1* p.Ser320Asn variant in family members. **C** Bone marrow aspirate of the propositus (II.1) at admission showing marked hypercellularity with hyperplasia of megakaryocytes (top), characteristically enlarged, mature, with hyperlobulated nuclei (middle), along with erythroid hyperplasia (bottom). The dashed line indicates an enriched zone in megakaryocytes within the cell clump.

propositus (Fig. 2A). Contrarily, PGK1 activity, near the lower limit of the normal range, together with severe reticulocytosis (480000/µL), supported a PGK1 deficiency. Additionally, erythrocyte deformability, measured by the laser-assisted optical rotation cell analyzer (LoRRca) Osmoscan module, showed a normal Osmotic Gradient Ektacytometry curve in the mother but slightly right-shifted curve in the patient, suggesting moderate overhydration likely due to metabolic impairment (Fig. 2B). Similarly to other erythroenzymopathies, the results of the rheological parameters were within the normal range (Table S2).

Next, we evaluated whether the somatic *CALR* mutation expanded hematopoietic progenitors with *PGK1*^{Ser320Asn}. As expected, the *CALR*^{ins5} mutation was identified exclusively in CD15+ (but not in CD3+ cells or in hair follicles) from the propositus (Fig. 2C). Then, *PGK1* mutated (*PGK1*^{mut}) and wild-type (*PGK1*^{wt}) mRNA levels were evaluated in both female carriers. Whereas in the mother the *PGK1*^{mut}:*PGK1*^{wt} ratio was <0.6 in CD3+ and CD15+ cells, supporting the predominant expression of the *PGK1*^{wt} form; in the propositus, the ratios were greater than 1 for both CD3+ and CD15+ cells, being even notably higher in the myeloid population (3.35 *vs* 19), confirming the predominant expression of the mutated form particularly in the myeloid line (Fig. 2C).

After discarding mutations in the *Xist* gene, we performed the HUMARA assay in CD3+ and CD15+ cells from both carriers. Consistent with previous results, the XCI allele ratios (269:279) showed moderate skewing in CD3+ and CD15+ cells from the mother (20:80 and 19:81, respectively) (Fig. 1D). In the propositus, however, while the ratio was normal for CD3+ cells (60:40), it was moderately skewed for CD15+ cells, but in the opposite direction to that observed in her mother (80:20), supporting that the most inactivated allele in the maternal myeloid cells was the most activated in the propositus' CD15+ cells.

Finally, nanopore sequencing of unmanipulated genomic DNA from maternal and propositus CD15+ cells allowed assessing in a single approach the methylation status of the CpG island of the *PGK1* promoter and the entire gene sequence for each allele. Consistent with mRNA analysis, the mother had methylated the *PGK1*^{mut} promoter, while the *PGK1*^{wt} was predominantly unmethylated (Fig. 2E). Conversely, in the propositus, the *PGK1*^{mut} allele was predominantly unmethylated, while the *PGK1*^{wt} allele was methylated.

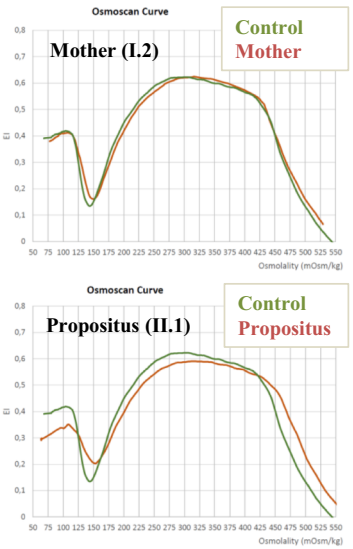
DISCUSSION

Epigenetics has revolutionized the classical concepts of dominant and recessive X-linked inheritance [7]. In 1961, Mary Lyon proposed the random and maintained inactivation of one of the two X-chromosomes in female cells during early development [7]. Today, it is known that up to 20–30% of healthy women may have highly skewed inactivation ratios, affecting the clinical expression of X-linked diseases in female carriers, ranging from normal expression to complete expression of the defect [7–9]. It has been proposed that this phenomenon could be a protective mechanism against X-linked disorders [7], as in an *F8* and *PGK1* mutated carrier [10], or in the propositus' mother (with moderate XCI and normal PGK1 activity). Our results showed that skewed inactivation can also be driven by an acquired oncogenic event, which expands an epigenetic signature in a single tissue with severe clinical consequences. Thus, the propositus and her mother were carriers for the *PGK1*-Murcia variant, which caused severe HA and encephalopathy in her younger brother [6]. When the family

A

Enzyme	Mother (UI/gHb)	Propositus (UI/gHb)	Reference (UI/gHb)
Phosphoglycerate kinase 1 (PGK1)	329	325	317.30 – 405.50
Glucose 6 phosphate dehydrogenase (G6PD)	9.60	12.90	6.12 – 10.20
Hexokinase (HK)	1.10	2.10	0.62 – 1.26
Pyruvate kinase (PK)	10.20	16.34	8.40 – 15.20
PK/HK index	9.37	7.70	7.20 – 15.60
Reticulocytes	49,200/µL	480,000/µL	15,000 – 80,000/µL

B



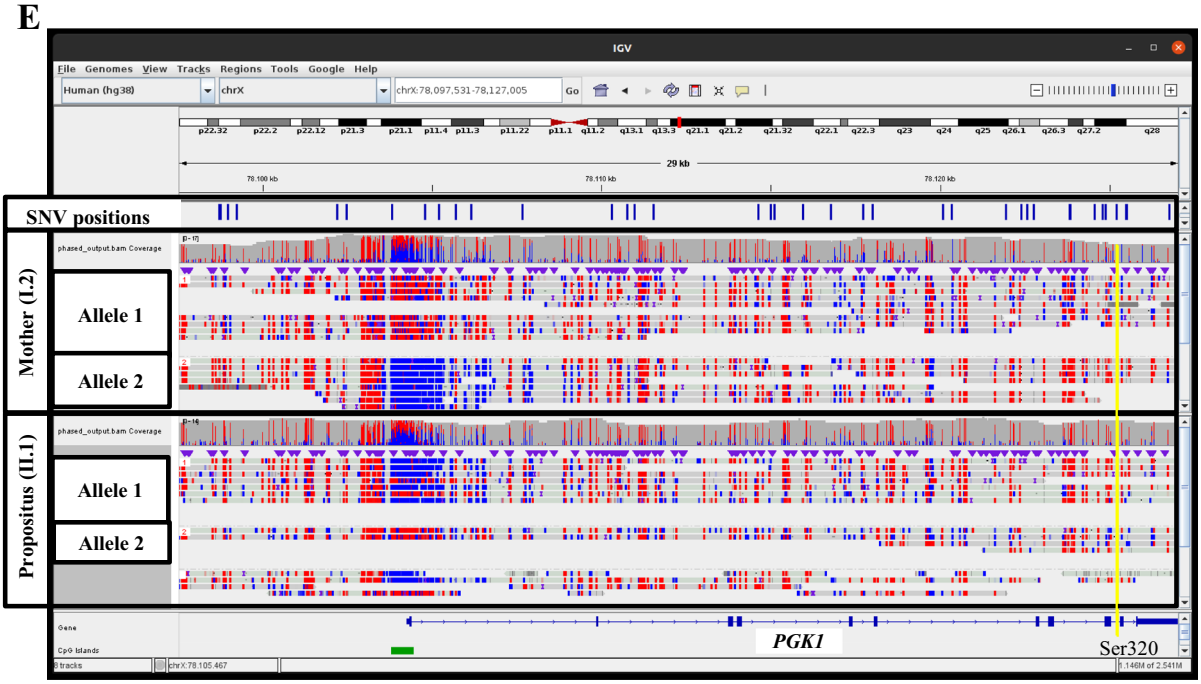
C

		CD3+ (Lymphoid lineage)	CD15+ (Myeloid lineage)
Mother (I.2)	*CALR*^{ins5}	Absent	Absent
	PGK1^{mut}	34%	29%
	PGK1^{wt}	66%	71%
	PGK1^{mut} : *PGK1*^{wt}	0.52	0.41
Propositus (II.1)	*CALR*^{ins5} (28.1%)	Absent	Present
	PGK1^{mut}	77%	95%
	PGK1^{wt}	23%	5%
	PGK1^{mut} : *PGK1*^{wt}	3.35	19

D

X-chromosome inactivation ratio	CD3+ (Lymphoid lineage)	CD15+ (Myeloid lineage)
Mother (I.2)	Moderate deviation (20:80)	Moderate deviation (19:81)
Propositus (II.1)	Normal (60:40)	Moderate deviation (80:20)

E



was firstly described, both heterozygous females showed normal PGK1 activity [6]. Over time, however, the propositus developed compensated chronic hemolysis without anemia, but, in adulthood, coinciding with the onset of progressive thrombocytosis, she manifested anemia. Despite high reticulocyte counts, PGK1 activity decreased, and erythrocytes showed an altered Osmoscan profile, similar to that observed in other enzymopathies [11]. However, since this is the first time that an Osmoscan profile of PGK deficiency has been evaluated, further studies are needed to confirm the specificity of this associated profile.

A relevance finding is the dissociation of HA from neurological impairment in the propositus, unlike her brother [6], likely

Fig. 2 Functional and genetic characterization of peripheral blood samples from the two female carriers of the *PGK1* mutation. **A** Enzymopathies-related enzyme activity levels of phosphoglycerate kinase 1 (PGK1), glucose-6-phosphate dehydrogenase (G6PD), hexokinase (HK), and pyruvate kinase (PK) were quantified. This table also shows the number of reticulocytes in the propositus (II.1) and her mother (I.2). Note that the PGK1 activity in the propositus (near the lower limit of the normal range), together with the severe reticulocytosis, supports a PGK1 deficiency. **B** Osmoscan profiles in the mother (I.2) and the propositus (II.1) using a Laser-Assisted Optical Rotation Cell Analyzer (LoRRca). The osmotic gradient ektacytometry (OGE) reflects the deformability of erythrocytes measured in a suspension medium with constant shear stress and a variable osmolality. Informative parameters of this curve are shown in Table S2. **C** *CALR*^{ins5} identification, quantification of VAF and mRNA levels of mutated and wild-type *PGK1* alleles in the CD3+ and CD15 + PB population of the mother (I.2) and the propositus (II.1). **D** X-chromosome inactivation ratio (269:279) by HUMARA assay in CD3+ and CD15 + PB maternal (I.2) and propositus (II.1) populations. The following reference limits were established: normal, <80:20; moderate deviation, 80:20–90:10; and marked deviation, >90:10. **E** Methylation studies of unmanipulated genomic DNA from myeloid progenitors. The CpG island of the *PGK1* promoter is shown in green. Methylated bases are shown in red, while unmethylated bases are shown in blue. Single nucleotide variant (SNV) positions are marked in blue. The yellow line indicates the position of the *PGK1* p.Ser320Asn variant. The two alleles differentiated by phasing are indicated. *PGK1* p.Ser320Asn variant is detected in allele 1.

explained by the different degrees of XCI in specific tissues [7–9]. Accordingly, the HUMARA assay revealed moderately skewed inactivation in the propositus' myeloid (but not lymphoid) progenitors. In line with our results, skewed XCI in the *substantia nigra* has been linked to parkinsonism in *PGK1*^{mut} carriers [2].

Nanopore sequencing confirmed the allele-specific differential methylation of the *PGK1* promoter associated with the presence of the mutated *CALR* clone, supplying the strengths of nanopore sequencing in both germline and oncogenic disorders. The methylation profiles led to different expression levels of *PGK1*^{mut} and *PGK1*^{wt}, explaining the dramatic clinical differences between both female carriers, despite their shared genotype.

Unfortunately, beyond the potential benefits of splenectomy or allogeneic BM transplantation [6], there is no available therapy for PGK1 deficiency. Our findings support that targeted therapies [12], as the potential use of CRISPR/Cas9 demethylation methods [13], could be promising. Particularly, therapies efficiently reducing the *CALR*^{ins5} allele burden, such as peginterferon, bomedemstat or the new anti-CALR monoclonal antibodies [14, 15], could ameliorate anemia symptoms.

In conclusion, our study demonstrated how a late somatic oncogenic event (*CALR*^{ins5}) [16], expanded the epigenetic signature of the oncogenic CD15+ clone (unmethylated promoter for *PGK1*^{Ser320Asn} allele), transforming a recessive X-linked disorder into a dominant one in a specific tissue, causing anemia without the expected neurological impairment [6].

M. L. Morales[1,2,8], H. Cano[1,8], B. de la Morena-Barrio[1,3], J. L. Vives-Corrons[4], E. J. Cuenca-Zamora[1,2,3], P. Garrido-Rodríguez[1,3], C. Bento[5], J. Pereira[5], J. Martínez Nieto[6], T. H. Chen-Liang[1], J. L. Fuster[7], S. Caracena[1], M. L. Lozano[1,3], R. Teruel-Montoya[1,2,3], J. Corral[1,3] and F. Ferrer-Marín[1,2,3]✉

[1]*Servicio de Hematología, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Instituto Murciano de Investigación Sanitaria (IMIB)-Pascual Parrilla, Universidad de Murcia, Murcia, Spain.* [2]*Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain.* [3]*CIBERER-ISCIII, Murcia, Spain.* [4]*Red cell Pathology and Hematopoietic Disorders, Institute for Leukaemia Research Josep Carreras, Badalona, Spain.* [5]*Unidade Funcional de Hematologia Molecular, Serviço Hematologia Clínica, Hospital Universitário de Coimbra, Coimbra, Portugal.* [6]*Instituto de Investigación Sanitaria del Hospital Clínico San Carlos, Madrid, Spain.* [7]*Sección de Oncohematología Pediátrica, Hospital Clínico Universitario Virgen de la Arrixaca. Instituto Murciano de Investigación Sanitaria (IMIB)-Pascual Parrilla, Murcia, Spain.* [8]*These authors contributed equally: M. L. Morales, H. Cano.* ✉*email: fferrer@ucam.edu*

DATA AVAILABILITY

All data from this study will be available upon request to the corresponding author.

REFERENCES

1. Beutler E. PGK deficiency. Br J Haematol. 2007;136:3–11.
2. Sakaue S, Kasai T, Mizuta I, Suematsu M, Osone S, Azuma Y, et al. Early-onset parkinsonism in a pedigree with phosphoglycerate kinase deficiency and a heterozygous carrier: do PGK-1 mutations contribute to vulnerability to parkinsonism? NPJ Parkinsons Dis. 2017;3:13.
3. Chiarelli LR, Morera SM, Bianchi P, Fermo E, Zanella A, Galizzi A, et al. Molecular insights on pathogenic effects of mutations causing phosphoglycerate kinase deficiency. PLoS ONE. 2012;7:e32065.
4. Svaasand EK, Aasly J, Landsem VM, Klungland H. Altered expression of PGK1 in a family with phosphoglycerate kinase deficiency. Muscle Nerve. 2007;36:679–84.
5. Gutierrez E, Bayes MG, Mallick J, Dell'osso L, Lyapichev KA, Muthukumar A. Recognition of a novel variant of phosphoglycerate kinase 1 deficiency PGK1 Galveston (c.472G > C) in a child with hemolytic anemia, neurologic dysfunction and myopathy. Pediatr Hematol Oncol. 2023;40:76–85.
6. Noel N, Flanagan JM, Ramirez Bajo MJ, Kalko SG, Mañú MdelM, Garcia Fuster JL, et al. Two new phosphoglycerate kinase mutations associated with chronic haemolytic anaemia and neurological dysfunction in two patients from Spain. Br J Haematol. 2006;132:523–9.
7. Carrel L, Brown CJ. When the Lyon(ized chromosome) roars: ongoing expression from an inactive X chromosome. Philos Trans R Soc Lond B Biol Sci. 2017;372:20160355.
8. Amos-Landgraf JM, Cottle A, Plenge RM, Friez M, Schwartz CE, Longshore J, et al. X chromosome-inactivation patterns of 1005 phenotypically unaffected females. Am J Hum Genet. 2006;79:493–9.
9. Wong GK, Barmettler S, Heather JM, Millar D, Penny SA, Huissoon A, et al. Aberrant X chromosome skewing and acquired clonal hematopoiesis in adult-onset common variable immunodeficiency. JCI Insight. 2019;4:e127614, 127614.
10. Dardik R, Avishai E, Lalezari S, Barg AA, Levy-Mendelovich S, Budnik I, et al. Molecular mechanisms of skewed X-chromosome inactivation in female hemophilia patients—lessons from wide genome analyses. Int J Mol Sci. 2021;22:9074.
11. Zaninoni A, Fermo E, Vercellati C, Consonni D, Marcello AP, Zanella A, et al. Use of laser assisted optical rotational cell analyzer (LoRRca MaxSis) in the diagnosis of RBC membrane disorders, enzyme defects, and congenital dyserythropoietic anemias: a monocentric study on 202 patients. Front Physiol. 2018;9:451.
12. Valentini G, Maggi M, Pey AL. Protein stability, folding and misfolding in human PGK1 deficiency. Biomolecules. 2013;3:1030–52.
13. Yang B, Tang H, Wang N, Gu J, Wang Q. Targeted DNA demethylation of the ZNF334 promoter inhibits colorectal cancer growth. Cell Death Dis. 2023;14:210.
14. Reis E, Buonpane R, Celik H, Marty C, Lei A, Jobe F, et al. Selective targeting of mutated calreticulin by the monoclonal antibody INCA033989 inhibits oncogenic function of MPN. Blood. 2024;144:2336–48.
15. Ferrer-Marín F, Hernández-Boluda JC, Alvarez-Larrán A. Essential thrombocythaemia: a contemporary approach with new drugs on the horizon. Br J Haematol. 2024;204:1605–16.
16. Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. Blood. 2023;141:1909–21.

AUTHOR CONTRIBUTIONS

MLM, HC, and FF-M wrote the first draft of the manuscript. JMN performed the HHA-related customized sequencing panel. JLV-C carried out enzymes activity and erythrocyte deformability studies. CB and JP performed HUMARA analysis and *Xist* evaluation. BMB, PG-R, and JC performed nanopore sequencing and analysis. MLM, EJC-Z, and RT-M carried out the rest of the experiments. SC obtained the histological images of the propositus. JLF provided propositus' familiar history. THC-L helped in clinical data collection. ML critically reviewed the first draft of the manuscript. RT-M,

JC, and FF-M conceived the experiments and overall supervision of the study. FF-M provided funding. All authors approved the final version of the manuscript.

FUNDING

This study has been funded by Instituto de Salud Carlos III (ISCIII) (grant "PI21/00347") and co-funded by the European Union. EJC-Z is supported by the training of the university teachers' program (grant FPU18/03189). MLM is supported by Next Generation EU (grant PMP21/00052).

COMPETING INTERESTS

BMB is a co-founder of Longseq Applications S.L. but declares that the research was conducted in the absence of any potential conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was reviewed and approved by the Clinical Research Ethics Committee of the Hospital Universitario Morales Meseguer (EST: 11/21). The patient and her family provided written informed consent in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41408-025-01216-w.

Correspondence and requests for materials should be addressed to F. Ferrer-Marín.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025, modified publication 2026