Acquired Bernard-Soulier syndrome as the presenting feature of GATA2-related myeloid neoplasm in an adolescent: an insight into the mechanisms underlying the platelet defect

Pseudo-Bernard-Soulier syndrome is an acquired rare bleeding condition characterized by thrombocytopenia, large platelets lacking functional GPIb-IX and impaired response to ristocetin, mimicking classic Bernard-Soulier syndrome, which is caused by biallelic GPIb-IX mutations.[1] It may develop secondary to autoimmune and lymphoproliferative disorders, myelodysplastic syndrome and Gaucher disease.[1-4] GATA2 deficiency is a heterogeneous disorder associated with protean clinical manifestations, including monocyte, dendritic, B- and natural killer (NK)-cell deficiency, immunodeficiency, warts, lymphedema and predisposition to myeloid malignancies.[5] We report a girl with GATA2 deficiency who developed pseudo-Bernard-Soulier syndrome heralding the onset of a myeloid neoplasm consistent with atypical chronic myeloid leukemia (aCML) and investigate the mechanisms linking the acquired platelet defect to malignant transformation in this setting.

A 12-year-old girl was referred for severe bleeding. She had experienced epistaxis, bruising and hypermenorrhea since the age of 11 years, requiring blood transfusions, nasal packing and hormonal therapy. She had macrothrombocytopenia (platelet count 60x10^9/L) and anemia (hemoglobin 6.2 g/dL, MCV 105 fL, reticulocytes 0.9%). Leukocyte count (6.5x10^9/L), coagulation tests and von Willebrand factor were normal. Impedance aggregometry showed absent response to ristocetin with preserved response to other agonists. Large platelets were evident by microscopy and flow cytometry (Figure 1A), which revealed two platelet populations with different GPIb-IX levels, one with complete absence of this complex (34-37% of platelets) and another which expressed GPIb-IX, albeit at reduced levels, as revealed by decreased mean fluorescence intensity (Figure 1B), whereas GPIIb-IIIa was preserved or increased (*data not shown*). Whole exome seguencing (WES) followed by a virtual platelet disorders panel, including *GP1BA*, *GP1BB* and *GP9*, was negative. Recent onset macrothrombocytopenia together with defective ristocetin-induced aggregation and GPIb-IX expression in the absence of *GP1BA/GP1BB/GP9* mutations was consistent with pseudo-Bernard-Soulier syndrome. The Institutional Ethics Committee approved the study and informed consent was provided.

Six months later, the patient continued with severe bleeding and developed leukocytosis (15-30x10^9/L), persistent anemia (MCV 120 fL), increased fetal hemoglobin (5.6%) and worsening thrombocytopenia (platelet count 25-50x10^9/L). Differential count disclosed blasts (1-3%), myeloid progenitors (6-12%), bands (3-8%), neutrophilia (70-85%) with prominent hyposegmentation and pseudo-Pelger-Hüet forms and low monocyte counts (0.5-1%). Monocytopenia was confirmed by flow cytometry (*data not shown*). The bone marrow showed increased numbers of small megakaryocytes (MK), with predominant mono- and bi-nuclear forms, some displaying multinuclearity, myeloid hyperplasia, myeloid and erythroid dysplasia and 3% myeloid blasts (Figure 1C), with normal cytogenetics and absence of BCR::ABL fusion transcript. Spleen size was normal. Due to suspicion of a clonal disorder, WES obtained at first admission, aimed at excluding classical Bernard-Soulier, was re-evaluated, disclosing a novel *GATA2* mutation and additional *CSF3R*, *SETBP1* and *ASXL1* mutations, revealing the presence of clonal alterations in myeloid genes at initial presentation, before the development of overt leukocytosis and myeloid dysplasia. A targeted next-generation sequencing (NGS) myeloid panel[6] confirmed these variants (Table 1), except for *GATA2*, which was not covered, and revealed allelic imbalance (80/20) of SNP *CUX1* c.4350G>A (rs410825) at 7q22. Repeated myeloid panel at 15-month follow-up showed clonal evolution with increased *ASXL1*, *CSF3R* and *SETBP1* VAF, 95/5 allelic imbalance at *CUX1* and acquisition of *U2AF1* and *ETV6* mutations (Table 1). The subset of platelets lacking GPIb-IX rose to 66-69% (Figure 1D). Collectively, morphologic and genetic features were consistent with aCML.

Sanger sequencing of purified T cells disclosed the *GATA2*, but not *ASXL1*, *CSF3R* and *SETBP1* variants (Figure 1E). Fragment analysis revealed the 22-bp *GATA2* deletion in whole blood and T cells with approximately 50% VAF, which remained stable at follow-up (Table 1, Figure 1F), strongly suggesting its germline origin. Unfortunately, germline tissue was not available. The *GATA2* variant was absent in her unaffected parents, supporting *de novo* origin, consistent with the fact that most germline *GATA2* variants occur *de novo*.[7] The patient was treated with hydroxyurea with partial response. Stem cell transplantation (SCT) with a matched *GATA2*-negative sibling donor was planned. However, the patient developed severe COVID-19 during pre-transplant preparation followed by pulmonary aspergillosis and died of pulmonary hemorrhage at the age of 14.

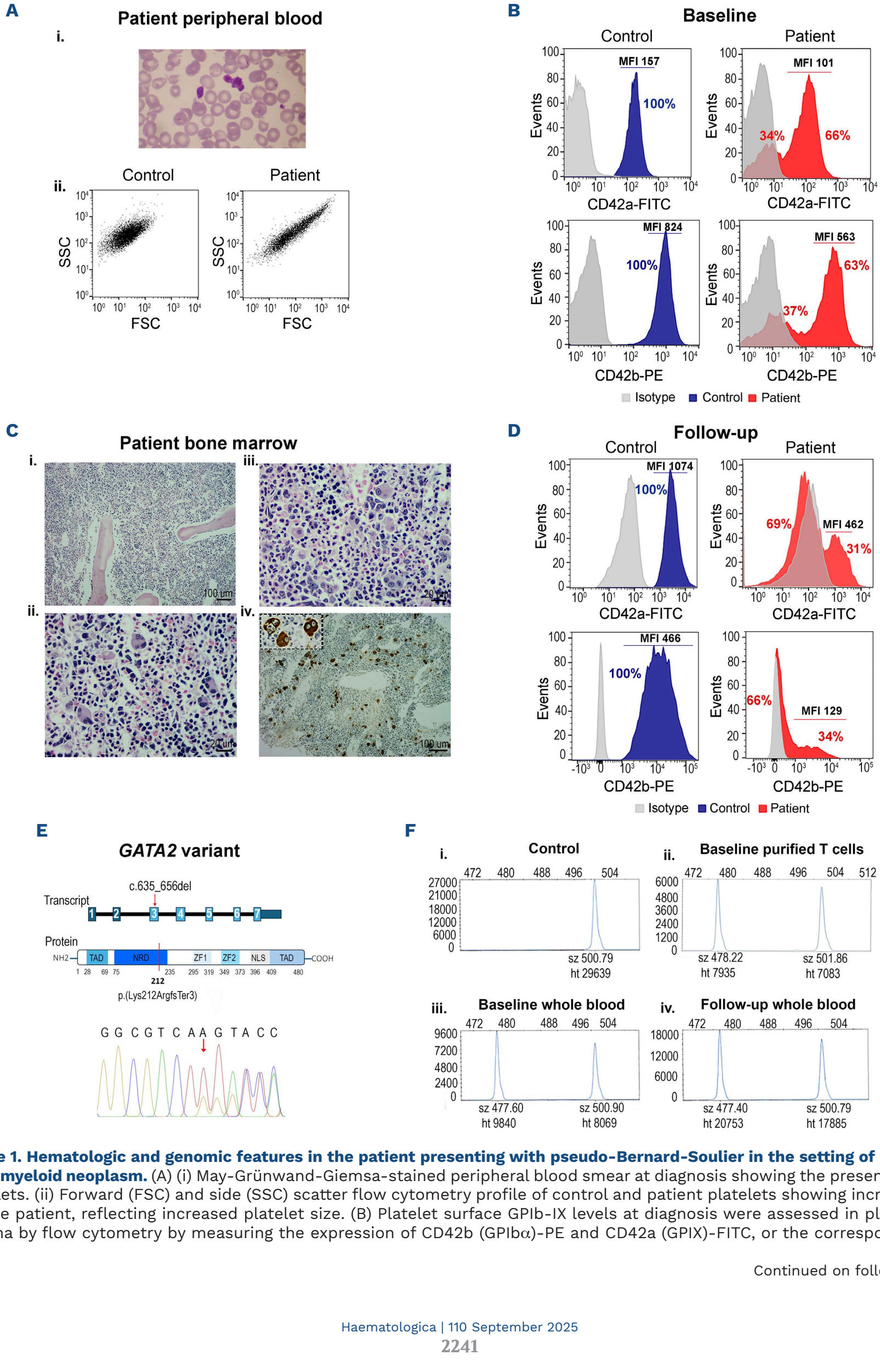

Figure 1. Hematologic and genomic features in the patient presenting with pseudo-Bernard-Soulier in the setting of GATA2-related myeloid neoplasm. (A) (i) May-Grünwand-Giemsa-stained peripheral blood smear at diagnosis showing the presence of large platelets. (ii) Forward (FSC) and side (SSC) scatter flow cytometry profile of control and patient platelets showing increased FSC for the patient, reflecting increased platelet size. (B) Platelet surface GPIb-IX levels at diagnosis were assessed in platelet-rich plasma by flow cytometry by measuring the expression of CD42b (GPIbα)-PE and CD42a (GPIX)-FITC, or the corresponding iso-

Continued on following page.

typic controls, after gating CD61 (GPIIIa)-FITC- or CD41 (GPIIb)-PE-positive events, respectively, on double-labeled tubes. Patient platelets (red histogram) and platelets from a healthy individual (control) assessed in parallel (blue histogram) are shown. The percentage of GPIbα- and GPIX-positive (63% and 66%, respectively) and -negative (37% and 34%, respectively) platelets and mean fluorescence intensity (MFI) of GPIbα-IX positive platelets is depicted. (C) Bone marrow histology showing: (i) hypercellularity with myeloid and megakaryocytic hyperplasia, (ii-iv) small megakaryocytes, with predominant mono- and bi-nuclear forms, some displaying multinuclearity and others with emperipolesis (inset). Hematoxylin and eosin staining (i-iii) and immunohistochemistry for CD61 (iv) images are shown at 4x (i), 10x (iv) or 40x (ii-iii) magnification; scale bars: 100 μm (i, iv) and 20 μm (ii, iii), respectively. (D) Platelet surface expression of GPIb-IX at 15-month follow-up. Patient platelets (red histogram) and platelets from healthy individual (control) assessed in parallel (blue histogram) are shown. The percentage of GPIbα- and GPIX-positive (34% and 31%, respectively) and -negative (66% and 69%, respectively) platelets and MFI of GPIbα-IX positive platelets is depicted. (E) Schematic representation of the GATA2 variant at the transcript and protein level (upper panel) and DNA sequencing chromatogram traces for the *GATA2* variant in purified T cells (bottom). Arrow indicates the frameshift generated by the 22-bp deletion. (F) Fragment length analysis of the *GATA2* deletion by capillary electrophoresis and GeneScanning of the fluorescent PCR product showing: (i) one peak in the control sample and (ii-iv) two peaks (mutated and wild-type alleles) in patient purified T-cell and whole blood DNA obtained at diagnosis and 15-month follow-up.

To investigate the mechanisms underlying the pseudo-Bernard Soulier phenotype, and considering that both GATA2 deficiency and aCML may be associated with autoimmune phenomena, we first assessed whether the decrease in GPIb-IX could be mediated by anti-GPIb-IX autoantibodies, as reported in other settings.[1,2] To this end, normal platelets were incubated with patient plasma and ristocetin-induced platelet agglutination was assessed by lumi-aggregometry. Patient plasma did not inhibit ristocetin-induced platelet agglutination (Figure 2A) and, furthermore, GPIb-IX expression was not substantially altered under these conditions (Figure 2B), indicating that the decrease in GPIb-IX was unlikely to be due to immune-mediated mechanisms. The fact that platelets lacking GPIb-IX were larger than GPIb-IX-positive ones (*data not shown*) suggested the decrease in GPIb-IX could originate at the MK level resulting in the production of large platelets. To assess this hypothesis, we cultured patient-derived MK, which showed a striking decrease in the output of $CD61^{+}CD42^{+}$ cells (Figure 2C), indicating decreased MK maturation or reduced GPIb-IX expression on mature MK. Finally, we measured *GP1BA*, *GP1BB* and *GP9* transcript levels in patient platelets, as a reflection of MK gene expression, and found reduced *GP1BB*, while *GP1BA* and *GP9* were preserved (Figure 2D), suggesting that low GPIbβ could lead to reduced expression of the whole complex at the cell surface.

GATA2 deficiency is a leading cause of myeloid neoplasms in children and adolescents, and *GATA2* mutations represent the most frequent germline defect underlying pediatric myelodysplastic syndrome (MDS).[8] In this patient, identification of a *GATA2* variant with a stable VAF of around 50%, which was also present in a non-myeloid specimen lacking the other clonal abnormalities, such as T cells, strongly supports its germline origin. There were no other signs of GATA2 deficiency except for monocytopenia, highlighting the marked clinical variability of this syndrome. Abnormal MK morphology and atypical nuclear features characteristic of GATA2 deficiency, such as multinuclearity, were evident. Patients with GATA2 deficiency are at high risk of malignant transformation, most developing MDS/AML, while a smaller subset present with *de novo* AML and chronic myelomonocytic leukemia.[8] This patient developed a myelodysplastic/myeloproliferative overlap neoplasm characterized by prominent neutrophilia and neutrophilic dysplasia with increasing proportions (>10%) of immature myeloid precursors in circulation, fulfilling the diagnostic criteria for aCML,[9] which is a very

Table 1. Pathogenic and likely pathogenic variants in myeloid genes.

Gene	c.DNA	Protein	Classification	Baseline VAF (%)	Follow-up VAF (%)	Purified T cells
				NGS panel		
SETBP1	c.2609G>A	p.Gly870Asp	Pathogenic	71	93	Wild-type
CSF3R	c.1853C>T	p.Thr618Ile	Pathogenic	34	49	Wild-type
ASXL1	c.1861_1871del	p.Ala621ProfsTer10	Pathogenic	12	22	Wild-type
U2AF1	c.470A>C	p.Gln157Pro	Pathogenic	-	17	NE
ETV6	c.691G>T	p.Glu213Ter	Likely pathogenic	-	3	NE
				Fragment analysis		
GATA2	c.635_656del	p.Lys212ArgfsTer3	Likely pathogenic	55	53	Mutant*

Likely pathogenic or pathogenic variants in myeloid genes identified in whole blood and T cells. Variant allele frequency (VAF) was assessed in whole blood at diagnosis (baseline) and at 15-month follow-up by a next-generation sequencing-based targeted myeloid gene panel for *SETBP1*, *CSF3R*, *ASXL1*, *U2AF1* and *ETV6* variants and by fragment analysis for the 22-bp *GATA2* deletion. Mutations were assessed in purified T cells by Sanger sequencing or by both Sanger sequencing and fragment analysis (for *GATA2*). *Fragment analysis of the *GATA2* variant in purified T cells disclosed 53% VAF. RefSeq transcripts for GATA2: NM_032638; SETBP1: NM_015559; CSF3R: NM_000760; ASXL1: NM_015338; U2AF1: NM_001025203; ETV6: NM_001987. NE: not evaluated.

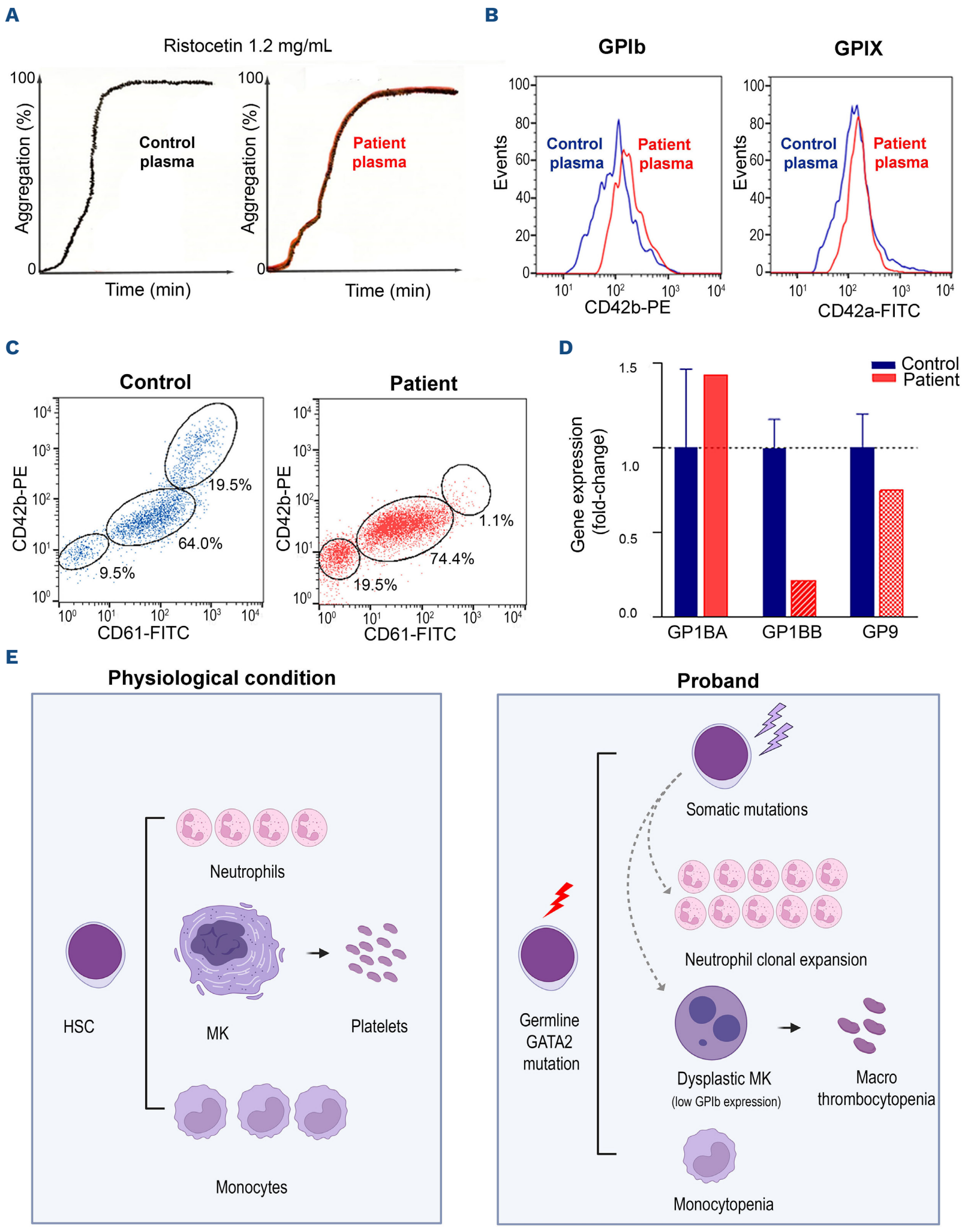

Figure 2. Mechanisms underlying the platelet defect. (A) Washed control platelets (200x10^9/L) were resuspended in either control (left) or patient (right) plasma and platelet aggregation in response to 1.2 mg/mL ristocetin was evaluated by light transmission aggregometry. (B) Washed control platelets were incubated with either control or patient plasma during 30 minutes (min) and then platelet surface GPIbα and GPIX expression was assessed by flow cytometry using CD42b-PE and CD42a-FITC, respectively. (C) Megakaryocytes (MK) were cultured from patient and control peripheral blood CD34$^+$ hematopoietic progenitors separated

Continued on following page.

by immunomagnetic methods in serum-free StemSpan medium supplemented with TPO 10 ng/mL, SCF 25 ng/mL and IL-6 10 ng/mL. On day 12 of culture, cells were analyzed by flow cytometry using CD61-FITC and CD42b-PE to assess megakaryocyte differentiation and maturation, respectively. The ellipse gates show the percentage of $CD61^{-}CD42b^{-}$, $CD61^{+}CD42b^{-}$ and $CD61^{+}CD42b^{+}$ (mature MK) cells. (D) *GP1BA*, *GP1BB* and *GP9* mRNA expression was assessed by qPCR relative to *ITGA2B* on patient and control (N=4) purified washed platelets. (E) Proposed mechanism of disease. The proband was born with a *de novo* germline *GATA2* mutation. Acquisition of additional somatic mutations in myeloid genes triggered evolution to a neutrophilic neoplasm consistent with atypical chronic myeloid leukemia. Dysplastic megakaryocytes yield low numbers of large platelets displaying reduced GPIbIX expression, leading to a pseudo-Bernard-Soulier phenotype. Monocytopenia, which represents a frequent feature of GATA2 germline deficiency, is also evident. HSC: hematopoietic stem cell; MK: megakaryocyte. Created with BioRender.

rare hematologic neoplasm in children. Development of aCML in the setting of germinal GATA2 deficiency has not, to our knowledge, been reported previously, expanding the list of neoplasms associated with this syndrome. The somatic mutational profile at disease onset included mutations in *ASXL1*, *SETBP1* and *CSFR3*. Clear signs of clonal progression were documented during follow-up with an increase in *ASXL1*, *CSF3R*, *SETBP1* VAF and acquisition of new mutations. In addition to somatic variants, co-operating events during malignant transformation in GATA2 deficiency patients involve cytogenetic abnormalities, such as monosomy 7 or partial 7q deletion.[7] Allelic imbalance was noted at locus *CUX1*, suggesting an interstitial deletion involving 7q22. Timely SCT is fundamental in this setting, but, unfortunately, the patient died due to an opportunistic infection following hospitalization for COVID-19.

Severe bleeding secondary to Bernard-Soulier-like defect heralded the overt manifestation of the myeloid neoplasm and was the presenting feature in this patient. The defect in platelet aggregation and GPIb-IX expression could not be reproduced when normal platelets were incubated with patient plasma, rendering the presence of an autoantibody against GPIb-IX unlikely. Instead, low *GP1BB* transcript levels were found, providing a plausible explanation leading to the reduction in GPIb-IX. Interestingly, GP1bβ has been shown to be down-regulated after shRNA-induced GATA2 silencing[10] and GATA2 binding to *GP1BB* regulatory regions was documented by ChIP-seq in primary human MK,[11] suggesting GATA2 regulates *GP1BB*. On this basis, we considered the possibility that GATA2 deficiency could contribute to reduced GPIbβ transcript levels, leading to altered GPIb-IX complex assembly and surface expression. However, defective GPIb-IX expression or platelet dysfunction have not been described in patients with GATA2 deficiency and thrombocytopenia is a rare finding before malignant transformation.[12] In addition, the degree of surface GPIb-IX reduction was not homogeneous, as would be expected for a germline abnormality, as two platelet populations were found, one negative for GPIb-IX and another which expressed GPIb-IX, although at reduced levels. Moreover, the GPIb-IX-negative subset increased during follow-up, in parallel with the increase in the VAF of somatic mutations, while *GATA2* VAF remained stable. Altogether, these features suggest that the underlying myeloid neoplasia itself, rather than the *GATA2* germline defect, was responsible for the platelet defect. Remarkably, previous reports have described the development of pseudo-Bernard-Soulier in 4 children with MDS,[3,13,14] 2 of them showing monosomy 7,[3,14] of whom one displayed two platelet populations with different degrees of GPIb-IX reduction,[3] similar to our case, suggesting that low GPIb-IX could represent a clonal abnormality in this background. In addition to reduced GPIb-IX, abnormal platelet formation by dysplastic MK, which were prominent in the patient's bone marrow, likely contributed to thrombocytopenia. In fact, platelet counts dropped progressively, paralleling MDS progression and clonal evolution. The proposed sequence of events is depicted in Figure 2E.

In conclusion, this report highlights that an initial hemorrhagic presentation due to acquired platelet dysfunction should prompt suspicion of an underlying myeloid neoplasm. Whether the presence of germline *GATA2* mutations may facilitate this hemorrhagic phenotype remains to be demonstrated and requires further evidence.

Authors

Laureano J. Kamiya,[1*] María D. Morell,[2*] Rosana F. Marta,[1,3] Marina Narbaitz,[4] Christophe Marzac,[5] Nora P. Goette,[1] Geraldine De Luca,[1,3] Paola R. Lev,[1,3] María F. Metrebian,[4] Daiana Ganiewich,[6] Andrea S. Llera,[6] Hana Raslova,[7] Paula G. Heller[1,3#] and Ana C. Glembotsky,[1,3#]

[1]Hematology Research Division, Institute for Medical Research Alfredo Lanari, School of Medicine, University of Buenos Aires (UBA), Buenos Aires, Argentina; [2]Hospital Infantil Municipal de Córdoba, Córdoba, Argentina; [3]Institute for Medical Research (IDIM), UBA-National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina; [4]Department of Pathology, Institute of Hematological Research, National Academy of Medicine, Buenos Aires, Argentina; [5]Laboratory of Hematology, Institut Gustave Roussy, Villejuif, France; [6]Laboratory of Molecular and Cellular Therapy, Leloir Institute-National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina and [7]Equipe labellisée Ligue Nationale contre le Cancer, INSERM, UMR 1287, Gustave Roussy, Université Paris Saclay, Villejuif France

**LJK and MDM contributed equally as first authors.*
#PGH and ACG contributed equally as senior authors.

Correspondence:
A.C. GLEMBOTSKY - anaglem@gmail.com

https://doi.org/10.3324/haematol.2025.287580

Received: February 13, 2025.
Accepted: May 26, 2025.
Early view: June 5, 2025.

Disclosures
No conflicts of interest to disclose.

Contributions
LJK performed research, investigated patient laboratory and genetic features, analyzed and interpreted the data, and wrote the manuscript. DM provided clinical features and patient follow-up, performed clinical studies and discussed results. MN performed histopathological analysis and discussed results. RFM, NPG, GDL and PRL investigated patient laboratory features, analyzed the data and discussed the results. DG, AL, CM and HR performed genomic studies, analyzed and interpreted the data, discussed results and contributed to the writing of the manuscript. PGH and ACG conceived the study, analyzed and interpreted the data, and wrote the manuscript.

Acknowledgments
The authors are grateful to the Fundacion Natali Dafne Flexer for continuous support.

Funding
This study was supported by grants from the National Agency for the Promotion of Science and Technology (ANCyT), Argentina (PICT 2018 01364), the Fondation Nelia et Amadeo Barletta and the Grupo Cooperativo Argentino de Hemostasia y Trombosis (CAHTalizar).

Data-sharing statement
The original data and protocols pertaining to this case are available from the corresponding author upon reasonable request.

References

1. Devine DV, Currie MS, Rosse WF, Greenberg CS. Pseudo-Bernard-Soulier syndrome: thrombocytopenia caused by autoantibody to platelet glycoprotein Ib. Blood. 1987;70(2):428-431.
2. Stricker RB, Wong D, Saks SR, Corash L, Shuman MA. Acquired Bernard-Soulier syndrome. Evidence for the role of a 210,000-molecular weight protein in the interaction of platelets with von Willebrand factor. J Clin Invest. 1985;76(3):1274-1278.
3. Berndt MC, Kabral A, Grimsley P, Watson N, Robertson TI, Bradstock KF. An acquired Bernard-Soulier-like platelet defect associated with juvenile myelodysplastic syndrome. Br J Haematol. 1988;68(1):97-101.
4. Kelsey H, Christopoulos C, Gray AA, Machin SJ. Acquired pseudo-pseudo Bernard-Soulier syndrome complicating Gaucher's disease. J Clin Pathol. 1994;47(2):162-165.
5. Calvo KR, Hickstein DD. The spectrum of GATA2 deficiency syndrome. Blood. 2023;141(13):1524-1532.
6. Glembotsky AC, Marin Oyarzún CP, De Luca G, et al. First description of revertant mosaicism in familial platelet disorder with predisposition to acute myelogenous leukemia: correlation with the clinical phenotype. Haematologica. 2020;105(10):e535.
7. Wlodarski MW, Hirabayashi S, Pastor V, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127(11):1387-1397.
8. Wlodarski MW, Collin M, Horwitz MS. GATA2 deficiency and related myeloid neoplasms. Semin Hematol. 2017;54(2):81-86.
9. Szuber N, Orazi A, Tefferi A. Chronic neutrophilic leukemia and atypical chronic myeloid leukemia: 2024 update on diagnosis, genetics, risk stratification, and management. Am J Hematol. 2024;99(7):1360-1387.
10. Huang Z, Dore LC, Li Z, et al. GATA-2 reinforces megakaryocyte development in the absence of GATA-1. Mol Cell Biol. 2009;29(18):5168-5180.
11. Tijssen MR, Cvejic A, Joshi A, et al. Genome-wide analysis of simultaneous GATA1/2, RUNX1, FLI1, and SCL binding in megakaryocytes identifies hematopoietic regulators. Dev Cell. 2011;20(5):597-609.
12. Donadieu J, Lamant M, Fieschi C, et al. Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. Haematologica. 2018;103(8):1278-1287.
13. Frigeni M, Galli M. Childhood myelodysplastic syndrome associated with an acquired Bernard-Soulier-like platelet dysfunction. Blood. 2014;124(16):2609.
14. Hiçsönmez G, Gümrük F, Cetin M, Ozbek N, Tuncer M, Gürsel T. Bernard-Soulier-like functional platelet defect in myelodysplastic syndrome and in acute myeloblastic leukemia associated with trilineage myelodysplasia. Turk J Pediatr. 1995;37(4):425-429.