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Multiple phenotypes and epigenetic profiles in a three-generation family history with GATA2 deficiency

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GATA2 is a master regulator of hematopoiesis. In humans, heterozygous germline GATA2 mutations lead to an autosomal dominant disorder known as GATA2 deficiency [1]. The clinical phenotype can vary significantly among GATA2 carriers, including in those of the same family. Patients may present with various hematological manifestations, including predisposition to myeloid neoplasms (most commonly myelodysplastic neoplasm (MDS), followed by acute myeloid leukemia and chronic myelomonocytic leukemia), bone marrow failure, immunodeficiency, neutropenia, and cytopenia, as well as non-hematological features like lymphedema, pulmonary and genitourinary alterations, deafness, and neurological disorders [1]. The median age for development of GATA2-related myeloid neoplasms is estimated around 20 years [2]. Myeloid malignant transformation is often accompanied by cytogenetic alterations, like monosomy 7 or trisomy 8, and acquired somatic mutations in SETBP1, ASXL1, and STAG2 [2, 3]. The clinical phenotype and cytogenetics of GATA2 deficiency are well-described, but penetrance and genotype-phenotype correlations remain unclear, particularly why some family members with the same GATA2 mutation remain asymptomatic [2]. Recently, it has been reported that epigenetic aberrant signatures, at early disease stage, could be potentially used as predictors of disease evolution [3]. Therefore, studies based on familial cohorts are crucial to understanding disease progression. Here we describe a familial case of GATA2 deficiency spanning three generations, involving four individuals carrying the c.1163T>C mutation (p.M388T) and exhibiting marked phenotypic variability (Fig. 1A). The p.M388T mutation in zinc finger domain 2 of GATA2 seems to impair protein function by reducing DNA-protein binding [4].

Patient #1 (P1) is a 75-year-old male without clinical features of GATA2 deficiency. Patient #2 (P2) is a 45-year-old male with deafness but no hematological manifestations. Patient #3 (P3) is a female diagnosed with pancytopenia at age 29 (Table S1). Her medical history includes Mycobacterium avium infection, thrombocytopenia, obstetric complications, and an arterial aneurysm. At age 31, she was diagnosed with MonoMac syndrome. Blood counts showed alterations in the myeloid (monocytosis, thrombocytopenia, and anemia) and lymphoid (inverted CD4:CD8 ratio, increased δγ lymphocytes, and lack of NK cells) lineages. Bone marrow (BM) analysis revealed MDS with multilineage dysplasia (MDS-MLD) and trisomy 8 (Table S1). The same year, she underwent allogeneic hematopoietic stem cell transplantation (HSCT) using an unrelated HLA-matched donor (10/10) after myeloablative conditioning. To date, she has maintained normal blood counts. Unfortunately, data on somatic mutations prior to transplantation are lacking. Finally, Patient #4 (P4) is a male who was identified as an asymptomatic GATA2 carrier at the age of 6, following the diagnosis of P3. At that time, P4 (P4.1) exhibited normocellular BM and normal blood counts (Table S1). At age of 8 (P4.2), BM analysis revealed hypocellularity with multilineage dysplasia affecting granulocytes, megakaryocytes, and erythrocytes, but without the presence of blasts; blood counts remained within normal parameters (Table S1). A follow-up 3 months later revealed peripheral blood (PB) neutropenia and thrombocytopenia (P4.3). He was subsequently diagnosed with myelodysplastic neoplasm with multilineage dysplasia (MDS-MLD), characterized by the acquisition of monosomy 7 and 0.3% blasts in the BM (Table S1). Whole-exome sequencing analysis on BM and PB cells of all P4 time-points, did not reveal additional somatic mutations. The same year, the patient underwent a successful allogenic HSCT (9/10). To date, at age 11, P4 exhibits 100% chimerism from an unrelated donor.

To identify potential epigenomic factors that could promote the progression of GATA2 deficiency in P4, we analyzed the genomic methylation profiles of PB mononuclear cells at three-time points for P4 and compared with P1, P2, P3, and three healthy donor (HD). This analysis was performed using the Infinium Human Methylation EPIC 850 K platform (Illumina). Principal component analysis (PCA) revealed that asymptomatic carrier P1 and deaf carrier P2 clustered with HD, correlating with the absence of hematological manifestations, while P3 formed a distinct cluster (Fig. 1B). Most interestingly all-time points of P4, from the

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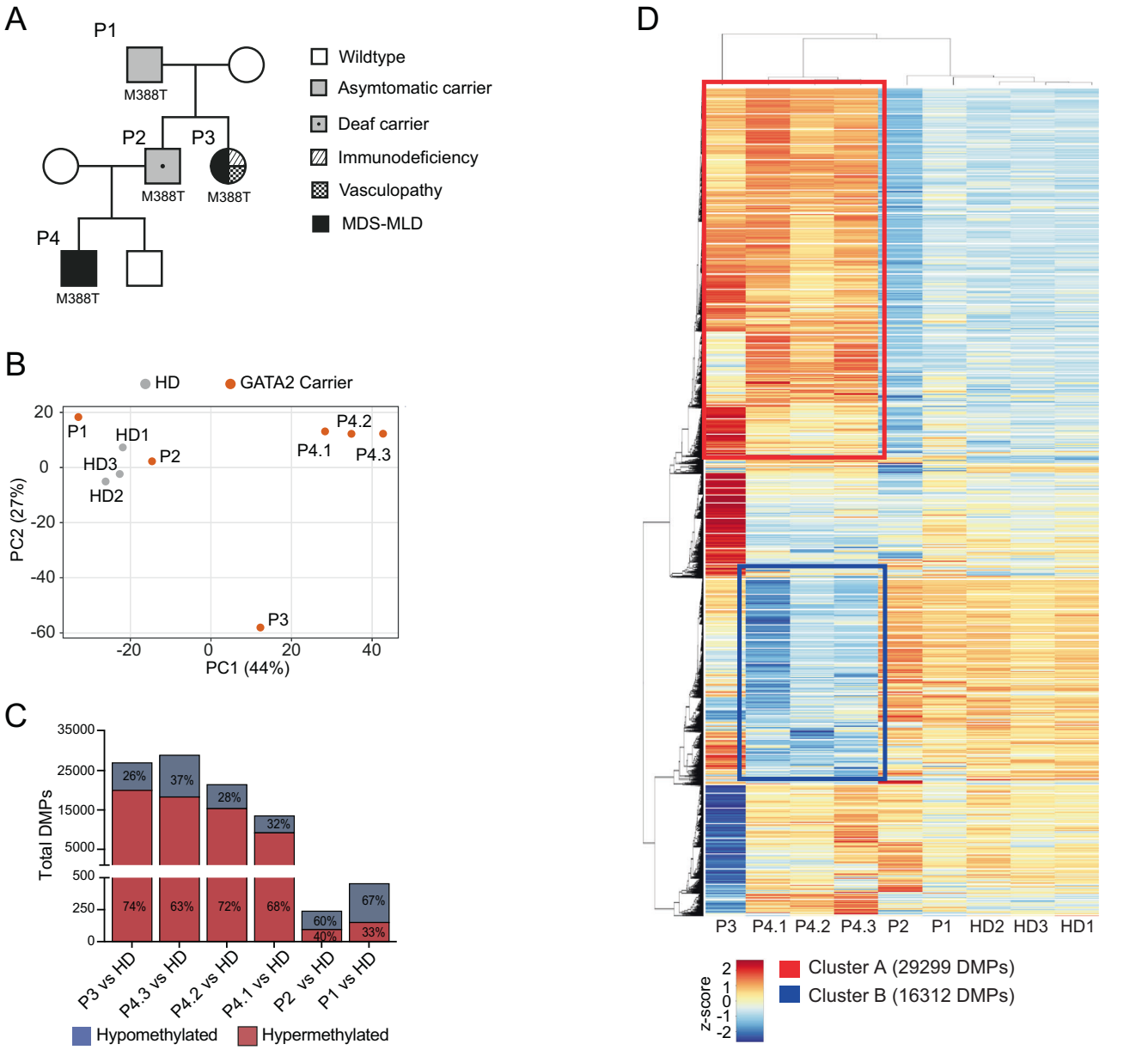
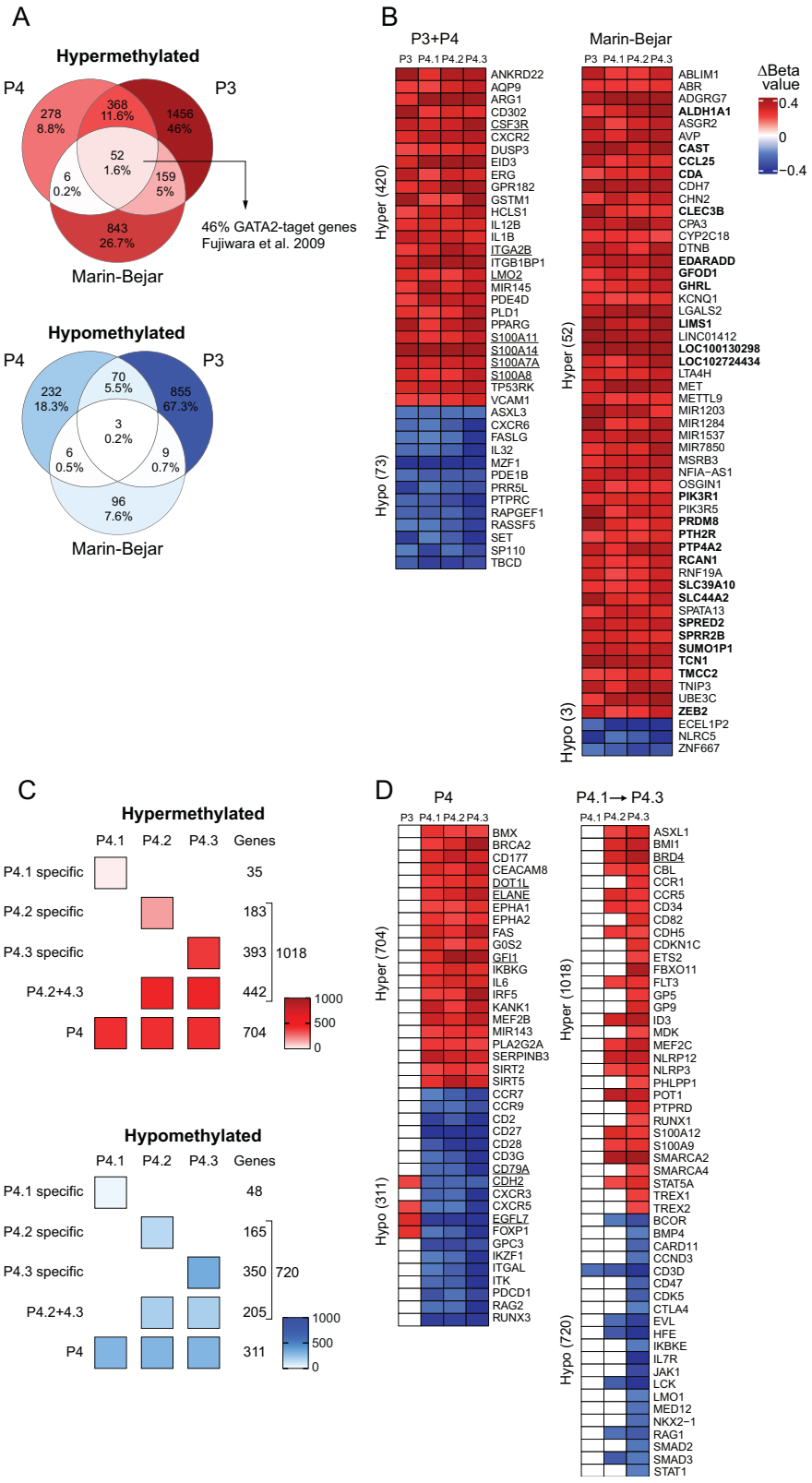


Fig. 1 Characterization of a GATA2-mutant pedigree with variable disease manifestations. A Genogram of the GATA2-mutated pedigree. Squares denote males and circles denote females. This three-generation GATA2 family presented four members with identical germline GATA2 mutations (p. Met388Thr; c.1163T>C) and variable clinical manifestations. **B** Principal Component Analysis (PCA) showing the distribution of GATA2 carriers (P#) and healthy donors (HD), based on DNA methylation profile of peripheral blood samples. **C** Number and percentage of hypomethylated and hypermethylated differentially methylated positions (DMPs) of each patient compared to the HD group. D) Heatmap of DMPs of peripheral blood. The hypermethylated DMP cluster in P3 and P4 samples is squared in red (Cluster A). The hypomethylated DMP cluster only in P4 samples is squared in blue (Cluster B). Scale β-values from -3 (blue/hypomethylated) to +3 (red/hypermethylated). Raw reads were processed using ShinyEpico (v1.14.0) package in R (v4.2.0). β-values are used to calculate the differences between groups. Δβ-value was considered significant when ≥0.225 and p-adj ≤ 0.05. *P*-value was calculated using the empirical Bayes moderated two-sided *t*-test. *P*-value is then adjusted using the Benjamini-Hochberg (FDR) method.

asymptomatic stage (P4.1) to MDS-MLD (P4.3) clustered together and separately from HD and P3 (Fig. 1B). We first identified a total of 64,618 differentially methylated positions (DMPs) associated to 18,283 genes by performing pairwise comparison of DNA methylation between all samples. Then we compared each patient individually to the HD group. Interestingly, P1 and P2 had less than 500 DMPs, with only 33–40% of them being hypermethylated. In contrast, P3 and P4 showed over 13,000 DMPs, revealing a hypermethylated profile when compared with HD (Fig. 1C). A descriptive analysis of the DMPs distribution was performed using as a reference the probe distribution of the Infinium MethylEPIC array from distal to proximal CpG island regions. The data revealed that the majority of DMPs were enriched in distal promoter regions, particularly in P3 and P4 (Fig. S1A). Furthermore, DMP distribution using the neighboring gene as reference showed an enrichment in intergenic and intronic regions with similar percentages in all patients (Fig. S1B).

Unsupervised analysis of DMPs revealed a shared DNA hypermethylation pattern (29,297 DMPs, cluster A) between P3 and all P4 time points, along with a unique subcluster of hypomethylated DMPs (16,310 DMPs, cluster B) in P4 (Fig. 1D). In contrast, P1 and P2 exhibited a DNA methylation profile similar



to that of HD (Fig. 1D). After DMP annotation, we identified 11,762 genes associated with 29,297 hypermethylated DMPs and 7942 genes linked to 16,310 hypomethylated DMPs. Enrichment analysis revealed that cluster A is enriched in inflammatory response and myeloid-related genes, while cluster B in T-cell-related genes (Fig. S1C and D). These data suggest a defect in the hematopoietic homeostasis in PB, characterized by dysregulation in the myeloid lineage, including reduced platelet formation and altered granulocyte production, as described in Table S1. Then, to assess whether GATA2 mutation might have a direct effect, we crossed the hyper- and hypomethylated genes with a publicly GATA2 chromatin immunoprecipitation sequencing (ChIP-seq)

Fig. 2 Epigenetic evolution highlights methylation changes in key hematopoietic genes. A Venn diagram of hypermethylated and hypomethylated genes of P3 and P4 from promoter associated differentially methylated positions (DMPs) compared to gene list described in Marin-Bejar et al. [3]. Full list can be found in Table S2. **B** Left, list of selected genes of intersection between P3 and P4 (420 hypermethylated and 73 hypomethylated) from Fig. 2A. Right, list of selected genes of intersection between P3, P4 and Marin-Bejar et al. [3] (52 hypermethylated and 3 hypomethylated) from Fig. 2A. Scale of $\Delta\beta$-values from -0.4 to 0.4 compared to healthy donors (HD). Underscore genes are mentioned in the manuscript. Gene in Bold are GATA2 targets. Full list can be found in Table S2. **C** Five stage-specific groups of hypermethylated (red) and hypomethylated (blue) genes from promoter associated DMPs across all P4 time points (P4.1, P4.2, and P4.3). The specific number of genes is indicated. Color range from 0 to 1000. **D** Left, list of selected genes that are in common across all P4 time points (704 hypermethylated and 311 hypomethylated) from Fig. 2C. Right, list of selected genes that are acquired during P4 disease evolution, P4.2 specific, P4.3 specific and P4.2 + P4.3 (1018 hypermethylated and 720 hypomethylated) from Fig. 2C. Scale of $\Delta\beta$-values from -0.4 to 0.4 compared to HD. Underscore genes are mentioned in the manuscript.

dataset [5]. Notably, 34% of the hypermethylated genes and 27% of the hypomethylated genes were GATA2 targets, highlighting the significant involvement of GATA2-regulated pathways (Fig. S1E). Furthermore, we extend our analysis comparing P3 and P4 profiles with already published methylome data of GATA2 patients [3] (Fig. 2A and B). Of note, 52 hypermethylated and 3 hypomethylated genes were common across all conditions [3]. Remarkably, 46% of the common hypermethylated genes were GATA2 targets (Fig. 2B). To identify potential epigenetic biomarkers, present already at asymptomatic stage, we performed a longitudinal analysis of the hyper- and hypomethylated genes across all P4 time points. As shown in Fig. 2C and D, a distinct epigenetic signature is already established at the P4.1 stage and is maintained in subsequent time points. Specifically, 704 hypermethylated and 311 hypomethylated genes are maintained across the transition from P4.1 to P4.3. Notably, among the 704 (420 shared with P3) hypermethylated genes we observed canonical genes critical for the normal hematopoiesis development such as ELANE [6], CSF3R [7], GFI1 [8], ITGA2B [9]; the S100A protein family with a prominent role in the regulation of the immune response [10] and LMO2 [11] (Fig. 2B and D). Among the 311 (73 shared with P3) hypomethylated genes some are oncogenes associated with hematological disorders, such as, CD79a [12], CDH2 [13] and EGFL7 [14] (Fig. 2B and D). Furthermore, the inactivation of DOTL1, which is a direct target of GATA2 [5] and a methyltransferase for H3K79, in P4 might dysregulate the expression of genes implicated in DNA damage response, cell cycle progression and erythropoiesis [15]. Of note DOT1L-KO in mice leads to pancytopenia and BMF, associated with a significant decrease of BRD4 expression [15]. Interestingly both genes are hypermethylated P4.3 (Fig. 2D). Although DOT1L became one of the most promising therapeutic targets to combat unfavorable gene expression in MLL-rearranged leukemia patients, its role as epigenetic regulator in MDS and AML is unknown. On the other hand, we observed specific epigenetic landscape of P4.2 and P4.3 (Fig. 2D), likely associated with disease progression.

Finally, Hypergeometric Optimization of Motif EnRichment (HOMER) (Fig. S1F) analysis revealed that the hypermethylated DMPs are enriched in TF motifs of the ETS family (ERG, FLI1, ETV2, and ETV1), consistent with our previous data and known associations with myeloid neoplasms [3]. Moreover, C/EBP and HLF genes are key TFs for myelopoiesis. On the other hand, hypomethylated DMPs are enriched in TF motifs of P53, HIF, RUNX1, and IRF3 among others, suggesting a collaboration of these TFs in the progression of the disease.

This study has several limitations. As it is a single-family study, the generalizability of the results may be limited and further validating studies with multiple GATA2 families are needed. Additionally, the lack of frozen primary cells at all P4 time-points hindered our ability to perform single-cell transcriptomic and genomic analyses, which could have provided further insight into the clonal evolution of the disease. Also, it is known that DNA methylation changes rapidly during childhood, therefore an age-matched HD for P4 would have been ideal.

Here we report an epigenetic study to unveil the complex phenotypic variability of a GATA2 familial case. We identify DMPs at early stage and maintained throughout the disease, which can potential be used as predictive epigenetic biomarkers for GATA2 deficiency progression. These findings encourage further longitudinal epigenetic studies to enhance understanding of DNA methylation role in predicting clinical outcomes in GATA2 patients.

DATA SHARING

All the data has already been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus with the following accession numbers: GSE281134 and GSE280776.

REFERENCES

1. Calvo KR, Hickstein DD. The spectrum of GATA2 deficiency syndrome. Blood. 2023;141:1524–32.
2. Wlodarski MW, Hirabayashi S, Pastor V, Stary J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127:1387–97.
3. Marin-Bejar O, Romero-Moya D, Rodriguez-Ubreva J, Distefano M, Lessi F, Aretini P, et al. Epigenome profiling reveals aberrant DNA methylation signature in GATA2 deficiency. Haematologica. 2023;108:2551–7.
4. Oleaga-Quintas C, de Oliveira-Junior EB, Rosain J, Rapaport F, Deswarte C, Guerin A, et al. Inherited GATA2 deficiency Is dominant by haploinsufficiency and displays incomplete clinical penetrance. J Clin Immunol. 2021;41:639–57.
5. Fujiwara T, O'Geen H, Keles S, Blahnik K, Linnemann AK, Kang YA, et al. Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. Mol Cell. 2009;36:667–81.
6. Horwitz MS, Corey SJ, Grimes HL, Tidwell T. ELANE mutations in cyclic and severe congenital neutropenia: genetics and pathophysiology. Hematol Oncol Clin North Am. 2013;27:19–41.
7. Sprenkeler EGG, Tool ATJ, Kreft IC, van Alphen FPJ, Seneviratne SL, et al. Loss-of-function mutations in CSF3R cause moderate neutropenia with fully mature neutrophils: two novel pedigrees. Br J Haematol. 2020;191:930–4.
8. Moroy T, Khandanpour C. Role of GFI1 in Epigenetic Regulation of MDS and AML pathogenesis: mechanisms and therapeutic implications. Front Oncol. 2019;9:824.
9. Dumon S, Walton DS, Volpe G, Wilson N, Dasse E, Del Pozzo W, et al. Itga2b regulation at the onset of definitive hematopoiesis and commitment to differentiation. PLoS One. 2012;7:e43300.
10. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol. 2003;170:3233–42.
11. Chambers J, Rabbitts TH. LMO2 at 25 years: a paradigm of chromosomal translocation proteins. Open Biol. 2015;5:150062.
12. Lenk L, Carlet M, Vogiatzi F, Spory L, Winterberg D, Cousins A, et al. CD79a promotes CNS-infiltration and leukemia engraftment in pediatric B-cell precursor acute lymphoblastic leukemia. Commun Biol. 2021;4:73.
13. Parker J, Hockney S, Blaschuk OW, Pal D. Targeting N-cadherin (CDH2) and the malignant bone marrow microenvironment in acute leukaemia. Expert Rev Mol Med. 2023;25:e16.
14. Cheng Z, Dai Y, Pang Y, Jiao Y, Liu Y, Cui L, et al. High EGFL7 expression may predict poor prognosis in acute myeloid leukemia patients undergoing allogeneic hematopoietic stem cell transplantation. Cancer Biol Ther. 2019;20:1314–8.
15. Kealy L, Runting J, Thiele D, Scheer S. An emerging maestro of immune regulation: how DOT1L orchestrates the harmonies of the immune system. Front Immunol. 2024;15:1385319.

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AUTHOR CONTRIBUTIONS

DR-M and AG designed the study and wrote the manuscript. DR-M, JP, OM-B, ET-S, JR-U, and AL performed the genomic studies and data analysis. LM-S, CDH, JM, and AC were involved in patient care, sample collection, testing and interpreting the clinical data. AG, JC, AC, and MWW supervised data analysis. All authors contributed to the manuscript and provided final approval.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All methods were performed in accordance with the relevant guidelines and regulations. This study was approved by institutional review boards at Bellvitge (PR097-20) and Sant Joan de Déu (PIC-141-20) Hospitals. All patients or their guardians signed informed consents for sample collection in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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