Received: 14 March 2023 | Revised: 29 May 2023 | Accepted: 30 May 2023

DOI: 10.1002/gcc.23184

BRIEF REPORT

WILEY

The clinical phenotype of germline *RUNX1* mutations in relation to the accompanying somatic variants and *RUNX1* isoform expression

David Cabrerizo Granados[1] | Indira Barbosa[1] | Panagiotis Baliakas[2] | Eva Hellström-Lindberg[1] | Vanessa Lundin[1]

[1]Center for Hematology and Regenerative Medicine, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden

[2]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Correspondence
Vanessa Lundin, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden.
Email: vanessa.lundin@ki.se

Funding information
Vetenskapsrådet, Grant/Award Number: 2020-01902; Wenner-Gren Stiftelserna

Abstract

Germline *RUNX1* mutations lead to familial platelet disorder with associated myeloid malignancy (FPDMM), characterized by thrombocytopenia, abnormal bleeding, and an elevated risk of developing myelodysplastic neoplasia (MDS) and acute myeloid leukemia (AML) at young age. However, it is not known why or how germline carriers of *RUNX1* mutations have a particular propensity to develop myeloid hematologic malignancies, but the acquisition and composition of somatic mutations are believed to initiate and determine disease progression. We present a novel family pedigree that shares a common germline *RUNX1*^{R204*} variant and exhibits a spectrum of somatic mutations and related myeloid malignancies (MM). *RUNX1* mutations are associated with inferior clinical outcome; however, the proband of this family developed MDS with ring sideroblasts (MDS-RS), classified as a low-risk MDS subgroup. His relatively indolent clinical course is likely due to a specific somatic mutation in the *SF3B1* gene. While the three main RUNX1 isoforms have been ascribed various roles in normal hematopoiesis, they are now being increasingly recognized as involved in myeloid disease. We investigated the *RUNX1* transcript isoform patterns in the proband and his sister, who carries the same germline *RUNX1*^{R204*} variant, and has FPDMM but no MM. We demonstrate a *RUNX1a* increase in MDS-RS, as previously reported in MM. Interestingly, we identify a striking unbalance of *RUNX1b* and -*c* in FPDMM. In conclusion, this report reinforces the relevance of somatic variants on the clinical phenotypic heterogeneity in families with germline RUNX1 deficiency and investigates a potential new role for *RUNX1* isoform disequilibrium as a mechanism for development of MM.

KEYWORDS
FPDMM, germline mutations, MDS, myeloid malignancies, RUNX1, *RUNX1* isoforms

1 | INTRODUCTION

The runt-related transcription factor 1 (RUNX1) is expressed in three main isoforms (a, b, and c) and is a key regulator of definitive hematopoiesis and megakaryocyte differentiation.[1] Somatic *RUNX1* mutations have been detected in around 10% of myelodysplastic neoplasia (MDS) and acute myeloid leukemia (AML), and are being considered driver mutations, associated with high-risk disease and poor overall survival.[2–5] Heterozygous germline mutations in the *RUNX1* gene cause familial platelet disorder with associated myeloid malignancy (FPDMM), a rare autosomal dominant disease that encompasses thrombocytopenia, functional platelet defects and predisposition to hematologic malignancies, mainly MDS and AML, with a median penetrance of 44%.[3] Germline variants in the *RUNX1* gene are not sufficient to initiate disease, but require the acquisition of specific secondary somatic genomic aberrations.[6] Nevertheless, the effects of acquired variants on clinical outcome as well as the genetic and molecular basis of leukemic transformation due to germline RUNX1 deficiency are largely unknown. Moreover, the RUNX1 isoforms play differential roles in normal hematopoiesis, but their function in hematological disease is unclear.[7–10] Overexpression of *RUNX1a* has been identified in myeloid malignancy (MM) progression and, recently, in the pathogenesis of trisomy 21-associated myeloid leukemia.[11,12] However, *RUNX1* isoform expression patterns in FPDMM or a role in RUNX1 deficiency remain to be investigated.

We herein present a novel family pedigree that shares a germline *RUNX1*^{R204*} variant and displays a spectrum of somatic mutations with related MM. Interestingly, while *RUNX1* mutations are associated with inferior clinical outcome, the proband of this family developed MDS with ring sideroblasts (MDS-RS), classified as a low-risk MDS subgroup, and exhibited a rather indolent clinical course. We investigate the effect of this *RUNX1* mutation on isoform expression and identify distinct transcript levels of *RUNX1* isoforms, potentially in a disease stage-specific manner.

2 | MATERIALS AND METHODS

2.1 | Next-generation sequencing

Next-generation targeted sequencing was performed using the TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA), targeting 54 genes associated with MM, analyzing bone marrow and peripheral blood mononuclear cells (MNCs) from III-1 before allogenic stem cell transplantation; peripheral blood MNCs from II-1; and buccal swabs from III-1 and III-2. The Genomic Medicine Sweden Custom Panel (Twist Bioscience), analyzing 187 variants previously reported to be associated with MM, was performed on bone marrow MNCs from III-1 after transplantation and on peripheral blood MNCs from III-2. Clinical samples were obtained with written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the Ethics Research Committee at Karolinska Institutet (2017/1090-31/4 and 2011/1257-31/1).

2.2 | Transcript isoform expression assay

Bone marrow MNCs from III-1 at ages 53 and 57 and from III-2 at age 58 were analyzed as well as bone marrow MNCs from two healthy individuals, a 48-year-old male and a 60-year-old female, as controls. Total mRNA was extracted using RNeasy PLUS Mini Kit (Qiagen, Hilden, Germany) and cDNA synthesis was carried out using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. Gene expression of *RUNX1* exons 6-7a, 1-2, and 3a/b-4 was analyzed to calculate *RUNX1a*, *RUNX1c*, and *RUNX1a/b/c*, respectively. The following primers were used: *ACTB* F 5′-GCAAAGACCTGTACGCCAAC-3′, R 5′-AGTACTTGCGCTCAGGAGGA-3′; *RUNX1a* (exons 6-7a) F 5′-CTGAACCACTCCACTGCCTT-3′, R 5′-CTCCAGGGTGCTGTGTCTTC-3′; *RUNX1c* (exons 1–2) F 5′-TTTCCTTCGTACCCACAGTGC-3′, R 5′-CGTGGACGTCTCTAGAAGGATT-3′; *RUNX1a/b/c* (exons 3a/b-4) F 5′-CCAACTTCCTCTGCTCCGTG-3′, R 5′-CGTGGACGTCTCTAGAAGGATT-3′. Real-time PCR was performed using a PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Waltham, MA) on a CFX384 TouchTM Real-Time PCR Detection System. All samples were run in triplicate. The relative expression of *RUNX1* exons as well as the ratios of transcript isoforms *RUNX1a*, *-b* and, *-c* were calculated as previously described.[10] Briefly, expression of each exon boundary was calculated using the delta Ct-method with *ACTB* as a housekeeping gene. The expression of each exon boundary was normalized by dividing its relative expression by the relative expression of total *RUNX1a/b/c*. Since *RUNX1b* has no distinctive exon boundary, its relative expression was calculated by subtracting the relative expression of *RUNX1a* and *-c* from total *RUNX1a/b/c*. Each *RUNX1* transcript isoform ratio was obtained by dividing each isoform expression with the total *RUNX1* expression.

3 | RESULTS

3.1 | Clinical features

The proband (III-1; Figure 1A) was a 53-year-old male who had suffered from mild thrombocytopenia since youth. After experiencing increased tiredness and reduced physical condition, he was diagnosed with MDS-RS. Evaluation of his bone marrow identified 61% RS, phenotypic erythroid precursors with granules of excess iron. No RS were observed in the re-evaluation of his bone marrow samples from the age of 44. The patient did not respond to erythropoietin treatment and was transfusion dependent for 3 years until he successfully underwent allogenic stem cell transplantation in 2020. Genetic investigation of variants related to MM at the time of diagnosis identified a pathogenic *RUNX1* variant (NM_001754.5: c.610C>T, p.Arg204*) with a variant allele frequency (VAF) of 50%, later confirmed to be germline by buccal swab analysis (Figure 1, Table 1). Moreover, he carried pathogenic somatic variants in the *SF3B1* (NM_012433.3: c.1866G>T, p.Glu622Asp) and *CUX1* (NM_181552.3: c.2062+1G>T p.?; Figure 1B, Table 1) genes, while the karyotype was normal.

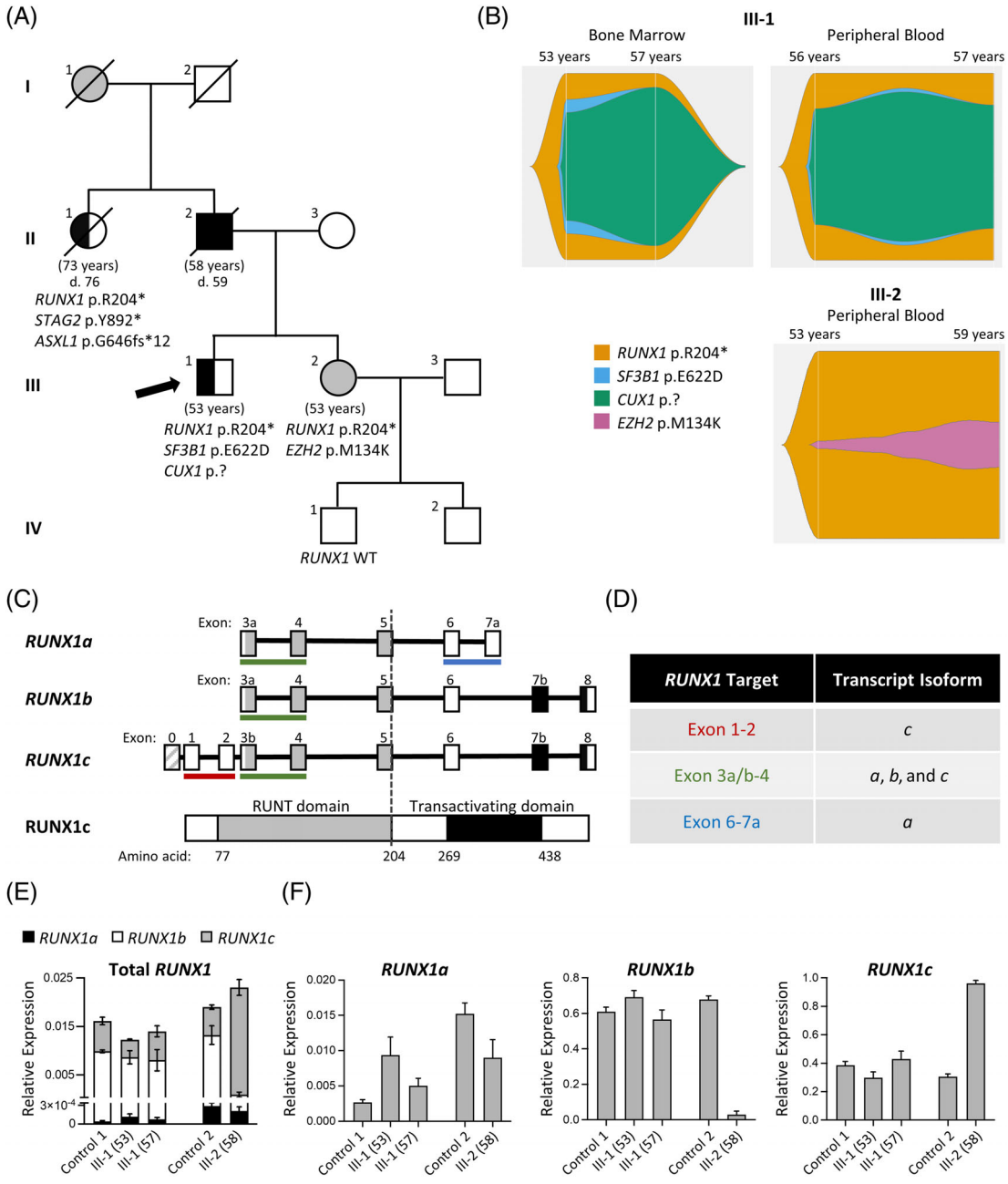

FIGURE 1 Mutational status and *RUNX1* isoform analysis of family pedigree. (A) Family pedigree of proband (III-1, indicated by arrow) showing segregation of MM and myeloid-related mutated genes. Squares represent males and circles females. White symbols represent unaffected individuals; gray symbols individuals with thrombocytopenia and no MM; black/white symbols individuals with MDS; black symbols individuals with AML. Age at diagnosis is provided in parenthesis for affected individuals. Age of death is shown preceded by d. WT (wild type) represents individuals without mutations in *RUNX1*. (B) Fish plot of *RUNX1*, *SF3B1*, and *CUX1* variant allele frequency in III-1 bone marrow and peripheral blood (upper). Fish plot of *RUNX1* and *EZH2* variant allele frequency in III-2 peripheral blood (lower). (C) *RUNX1* isoforms detailed by exons (upper) and RUNX1c showing protein domains and amino acid numbers (lower). Dashed line marks the location of the specific *RUNX1* mutation. Colored bars represent the exons targeted by respective primer listed in (D). (D) *RUNX1* exon boundary targets and the main transcript isoform detected by real-time PCR. (E) Isoform frequency and total *RUNX1* expression, by using exon boundary 3a/b-4 common for all three main *RUNX1* isoforms, with *ACTB* as reference gene. Age is provided in parenthesis. Mean of three technical replicates and standard deviation is shown. (F) *RUNX1* transcript isoform expression divided by total expression of *RUNX1*, calculated as exon boundary 3a/b-4, with standard deviation. *RUNX1b* transcript isoform ratio was calculated using the delta Ct-method with *ACTB* as reference gene, as previously described.[10] Controls 1 and 2 represent two healthy bone marrow donors, male and female, respectively.

TABLE 1 Genomic landscape and clinical management of patients in pedigree.

Family member	III-1	III-2	II-1*
Genomic landscape			
Karyotype	46, XY	46, XX	46, XX
Germline mutation (VAF)	*RUNX1* c.610C>T p.R204* (49%)	*RUNX1* c.610C>T p.R204* (52%)	*RUNX1* c.610C>T p.R204* (49%)
Other bone marrow (*blood) mutations (VAF)	*CUX1* c.2062+1G>T p.? (43%) *SF3B1* c.1866G>T p.E622D (42%)	*SAMD9L* c.910G>T p.V304F (49%) *EZH2* c.401T>A p.M134K (10%)	*TET2* c.5309C>T p.P1770L (50%) *STAG2* c.2676T>A p.Y892* (26%) *ASXL1* c.1934dupG p.G646fs*12 (15%)
Clinical management			
Diagnosis	MDS-RS (61% ring sideroblasts)	Thrombocytopenia	MDS-EB2
Age at diagnosis	53 years	53 years	73 years
Survival	Alive up to date	Alive up to date	76 years
Treatment	54 years: EPO start dose followed by full dose with no effect From 55 years: Chronic transfusion need 58 years: Allogenic stem cell transplantation 60 years: No relapse up to today	Annual check-ups	No records

Abbreviation: VAF, variant allele frequency.

Screening of the proband's sister (III-2; Figure 1A), who suffered from mild thrombocytopenia, revealed that she was a carrier of the same *RUNX1* variant (Figure 1B). She was therefore followed, according to the Nordic guidelines,[13] with regular complete blood counts and performance of next-generation sequencing of blood samples, which revealed a likely pathogenic variant in the *EZH2* gene (NM_004456.5: c.401T>A, p.Met134Lys), with an increase in VAF from 2% to 12% over the past 6 years (Figure 1B). Moreover, sequencing detected a variant of unknown significance (VUS) in the *SAMD9L* gene (NM_152703.5: c.910G>T, p.Val304Phe; Table 1). Until today, no sign of MM has been observed in III-2. Further, no *RUNX1* mutation or myeloid disorder has been reported for her progeny (IV-1 and -2; Figure 1A).

According to clinical records of the family, the father of the two siblings III-1 and -2 (II-2; Figure 1A) was diagnosed with AML at the age of 58 and died the following year. No genetic analysis of his leukemia was available. Moreover, their aunt (II-1; Figure 1A) was diagnosed with MDS with excess blasts (MDS-EB2) at the age of 73 and died 3 years later. Genomic profiling at the time of diagnosis showed that she carried the same *RUNX1* variant as proband III-1 and -2 with a 50% VAF. Moreover, she displayed pathogenic variants in the *STAG2* (NM_001282418.1: c.2676T>A, p.Tyr892*) and *ASXL1* (NM_015338.5: c.1934dupG, p.Gly646fs*12) genes with VAFs of 26% and 15%, respectively, plus a VUS in *TET2* (NM_001127208.2: c.5309C>T, p.Pro1770Leu) with a 50% VAF (Table 1). Finally, the grandmother of III-1 and -2 was reported to have abnormal blood counts but no further information was available (I-1; Figure 1A).

3.2 | Isoform analysis

Previous studies have uncovered a role for *RUNX1a* expression in myeloid neoplasia.[9–12] However, it is unknown how *RUNX1* mutations affect *RUNX1* isoform expression patterns. Therefore, *RUNX1* transcript isoform levels were assessed in the proband and his sister, both of whom carriers of *RUNX1*^{R204*} that causes a truncation of the RUNX1 transactivating domain present in isoforms RUNX1b and -c but absent in RUNX1a (Figure 1C). Gene expression analysis was performed on bone marrow MNCs: III-1 at the ages of 53 and 57, when the MDS-RS worsened, and III-2, at the age of 58. Bone marrow MNCs from one healthy male and female, aged 48 and 60, respectively, were included as controls. Total mRNA was extracted, and reverse transcription quantitative real-time PCR was performed to assess the expression of the three main *RUNX1* isoforms by their differential exon usage (Figure 1C,D). The relative expression of the transcript isoforms *RUNX1a/b/c* were calculated, as previously described.[10] We found that while the total amount of *RUNX1* expression relative to the housekeeping gene *ACTB* was comparable between the siblings and healthy controls, the proportions of transcript isoforms differed (Figure 1E). In III-1, the relative expression of *RUNX1b* and *-c* transcripts were similar to the controls but a slight increase of the *RUNX1a* isoform was observed at both time points (Figure 1E,F). On the contrary, III-2 showed reduced levels of *RUNX1a* but most interestingly, a strong disequilibrium in *RUNX1b:RUNX1c* transcript isoforms was observed, with *RUNX1b* being heavily suppressed and *RUNX1c* elevated compared to her brother and healthy controls (Figure 1E,F).

4 | DISCUSSION

In addition to predisposing germline *RUNX1* variants, acquired somatic mutations and their cooperation are presumed to affect penetrance and drive disease onset and progression.[14] In the family reported here, the same *RUNX1* mutation was accompanied by distinct somatic mutational profiles, resulting in clinical heterogeneity of hematological diseases. The father of the proband developed AML and the aunt high-risk MDS with somatic mutations related to poor prognosis, including *STAG2* and *ASXL1*.[5,6] On the other hand, the sister, III-2, has not developed any MM and remains stable under yearly surveillance. Notably, her $EZH2^{M134K}$ clone, harboring a mutation associated with poor prognosis in myeloid neoplasms, has increased in peripheral blood over the years and could suggest clonal hematopoiesis.[15] Prediction and preventative measures are crucial to reduce cancer risk. However, with the current knowledge in the field, it is still not feasible to foresee who among the carriers of germline RUNX1 deficiency will develop MDS/AML and when. Hence, identification and monitoring of germline mutation carriers is desirable.[3,4]

RUNX1 mutations, whether somatic or germline, are considered high-risk and are associated with adverse clinical outcomes. Nonetheless, the proband, III-1, developed MDS-RS, classified as a low-risk subtype of MDS by the World Health Organization.[16] Moreover, according to the recent Molecular International Prognostic Scoring System for MDS, co-mutation between *RUNX1* and *SF3B1* are defined as $SF3B1^{\beta}$ with adverse outcome.[17] While the genotype of the proband indicates a $SF3B1^{\beta}$ profile, his clinical phenotype is in accordance with the more favorable $SF3B1^{\alpha}$. Thus, while the *RUNX1* variant likely affected platelet function, we hypothesize that the inherited predisposition to leukemia in his case has been "sheltered" by the $SF3B1^{E622D}$ mutation, which is associated with indolent MDS.[18] Whether the adverse risk implicated in germline RUNX1 deficiency is alleviated by the acquisition of a less aggressive mutation, or whether pathogenesis is dictated by the order of mutations remains to be addressed. However, if the genotype to phenotype discrepancy observed here is a general feature, further discrimination of the $SF3B1^{\beta}$ classification may be relevant.

A mechanism for RUNX1 isoforms in the pathogenesis of MM is yet to be uncovered and isoform expression may depend on cell type, disease stage and specific mutational profile. Here, we investigated the expression pattern of the three main *RUNX1* isoforms as a consequence of $RUNX1^{R204^*}$ mutation, which causes a truncation of the transactivating domain, hampering the functionality of RUNX1b and -c, but not RUNX1a. III-1 showed an increase in *RUNX1a* expression, which may be related to his MDS since overexpression of *RUNX1a* has been observed in myeloid neoplasia.[11] RUNX1a has been considered a natural dominant negative isoform by competing with the other isoforms, affecting hematopoietic stem and progenitor cell proliferation and differentiation.[9] However, in the bone marrow MNCs analyzed here, the *RUNX1a* isoform constituted a minimal part of total *RUNX1* expression, which was mainly accounted for by *RUNX1b* and *-c* expression. Interestingly, his sister, an asymptomatic carrier, showed a substantial decrease in *RUNX1b* expression and elevated *RUNX1c* levels, compared to her brother and healthy control. RUNX1b and -c are full-length isoforms with similar structure but distinct expression: RUNX1b is highly expressed in megakaryocyte progenitors and decreases over differentiation in myeloid cells, whereas RUNX1c is expressed in committed blood cells.[7,8] The *RUNX1* isoform disequilibrium observed in our study is in line with previous reports and further argues for its potential role in the development of MM in the context of FPDMM, for instance, by expediting the differentiation of myeloid cells, or a prestage to a MM.[10] Recently, a *RUNX1a:RUNX1c* disequilibrium was shown to promote trisomy 21-associated leukemia, and restoration of the isoform balance had strong anti-leukemic effects, reinforcing a role for RUNX1 isoforms as potential targets in blood cancer.[12]

In summary, we provide support to the notion that somatic genetic aberrations impact the clinical phenotypic heterogeneity of individuals with germline *RUNX1* mutations and highlight a potential role for the different RUNX1 isoforms in the pathogenesis and evolution of the disease. Follow-up of the *RUNX1* isoform equilibrium of carriers of germline *RUNX1* mutations may uncover underlying development of the disease and serve as a potential marker of malignant transformation. Further investigation in larger series of patients is warranted to reach solid conclusions.

AUTHOR CONTRIBUTIONS

David Cabrerizo Granados and Panagiotis Baliakas designed the *RUNX1* isoform analysis, David Cabrerizo Granados performed RNA extraction and RT-qPCR analysis. Eva Hellström-Lindberg, Panagiotis Baliakas, and Indira Barbosa assisted with clinical information and scientific expertise. Eva Hellström-Lindberg and Indira Barbosa provided primary cell samples from patients and healthy donors. Vanessa Lundin supervised the collection of data. David Cabrerizo Granados and Vanessa Lundin wrote the manuscript. All authors read and approved the final manuscript.

FUNDING INFORMATION

This work was supported by the Wenner-Gren Foundation and Vetenskapsrådet, Grant No. 2020-01902.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Vanessa Lundin https://orcid.org/0000-0003-2335-3370

REFERENCES

1. Ichikawa M, Yoshimi A, Nakagawa M, Nishimoto N, Watanabe-Okochi N, Kurokawa M. A role for RUNX1 in hematopoiesis and myeloid leukemia. *Int J Hematol*. 2013;97(6):726-734.
2. He W, Zhao C, Hu H. Prognostic effect of RUNX1 mutations in myelodysplastic syndromes: a meta-analysis. *Hematology*. 2020;25(1):494-501.

3. Simon L, Spinella JF, Yao CY, et al. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. *Blood*. 2020;135(21):1882-1886.
4. Hellström-Lindberg E, Tobiasson M, Greenberg P. Myelodysplastic syndromes: moving towards personalized management. *Haematologica*. 2020;105(7):1765-1779.
5. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627. quiz 3699.
6. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. *J Clin Oncol*. 2011;29(5):504-515.
7. Draper JE, Sroczynska P, Tsoulaki O, et al. RUNX1B expression is highly heterogeneous and distinguishes megakaryocytic and erythroid lineage fate in adult mouse hematopoiesis. *PLoS Genet*. 2016;12(1):e1005814.
8. Menegatti S, Potts B, Garcia-Alegria E, et al. The RUNX1b isoform defines hemogenic competency in developing human endothelial cells. *Front Cell Dev Biol*. 2021;9:812639.
9. Tsuzuki S, Hong D, Gupta R, Matsuo K, Seto M, Enver T. Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1. *PLoS Med*. 2007;4(5):e172.
10. Engvall M, Karlsson Y, Kuchinskaya E, et al. Familial platelet disorder due to germline exonic deletions in RUNX1: a diagnostic challenge with distinct alterations of the transcript isoform equilibrium. *Leuk Lymphoma*. 2022;63(10):2311-2320.
11. Sakurai H, Harada Y, Ogata Y, et al. Overexpression of RUNX1 short isoform has an important role in the development of myelodysplastic/myeloproliferative neoplasms. *Blood Adv*. 2017;1(18):1382-1386.
12. Gialesaki S, Bräuer-Hartmann D, Issa H, et al. RUNX1 isoform disequilibrium promotes the development of trisomy 21 associated myeloid leukemia. *Blood*. 2022;141(10):1105-1118.
13. Baliakas P, Tesi B, Wartiovaara-Kautto U, et al. Nordic guidelines for germline predisposition to myeloid neoplasms in adults: recommendations for genetic diagnosis, clinical management and follow-up. *Hema*. 2019;3(6):e321.
14. Homan CC, King-Smith SL, Lawrence DM, et al. The RUNX1 database (RUNX1db): establishment of an expert curated RUNX1 registry and genomics database as a public resource for familial platelet disorder with myeloid malignancy. *Haematologica*. 2021;106(11):3004-3007.
15. Swerdlow SH, Campo E, Harris NL, et al. *International Agency for Research on C, World Health O. WHO classification of tumours of haematopoietic and lymphoid tissues*. International Agency for Research on Cancer; 2017.
16. Chase A, Score J, Lin F, et al. Mutational mechanisms of EZH2 inactivation in myeloid neoplasms. *Leukemia*. 2020;34(12):3206-3214.
17. Bernard E, Tuechler H, Greenberg PL, et al. Molecular international prognostic scoring system for myelodysplastic syndromes. *NEJM Evidence*. 2022;1(7):EVIDoa2200008.
18. Dalton WB, Helmenstine E, Pieterse L, et al. The K666N mutation in SF3B1 is associated with increased progression of MDS and distinct RNA splicing. *Blood Adv*. 2020;4(7):1192-1196.

How to cite this article: Cabrerizo Granados D, Barbosa I, Baliakas P, Hellström-Lindberg E, Lundin V. The clinical phenotype of germline *RUNX1* mutations in relation to the accompanying somatic variants and *RUNX1* isoform expression. *Genes Chromosomes Cancer*. 2023;62(11):672-677. doi:10.1002/gcc.23184