Novel Germline RUNX1 Mutation Associated with Familial Thrombocytopenia as well as B-Acute Lymphoblastic Leukemia: A Case Report and Review of the Literature

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Abstract
Germline RUNX1 mutations lead to a rare form of autosomal-dominant familial thrombocytopenia with a predisposition for myeloid malignancies and are classified as distinct entities by the WHO. We report a case of B lymphoblastic leukemia developing in a patient with a familial RUNX1 mutation, which is a first in the literature. An FLT3-ITD mutation as well as a balanced chromosomal translocation t(1;7) was present at the time of diagnosis of leukemia, favoring the theory that additional hits or mutations are necessary for malignant transformation in patients with a germline RUNX1 mutation. The transformed disease runs an aggressive course compared to the same malignancy associated with a somatic RUNX1 mutation. Additionally, family members should be screened for the mutation, followed up clinically if they carry the mutation, and should not be used as stem cell donors to treat the affected relatives.

Introduction
RUNX1 (runt-related transcription factor 1) is a master regulator of hematopoiesis and is transcribed by the \textit{RUNX1} gene, located in chromosome 21q. Germline mutations in \textit{RUNX1} cause autosomal-dominant familial platelet disorder (FPD) with a disposition to myeloid malignancies like myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), and rarely T lymphoblastic leukemia (T-ALL). These mutations are often unique to each family, with about 70 families described so far. FPD mani-
fests as mild-moderate thrombocytopenia with normal-sized platelets and functional platelet defects leading to prolonged bleeding. Approximately half of affected individuals develop hematologic malignancies in adulthood, mostly of myeloid origin. A few cases of T-ALL and 1 case of childhood B-ALL have been described so far in the literature in association with a germline RUNX1 mutation. We describe a novel RUNX1 mutation in an adult female with FPD evolving into B-ALL [1].

**Case Presentation**

A 44-year-old Caucasian female was seen due to a long history excessive bleeding. The patient had significant bleeding at menarche refractory to combined oral contraceptive pills, vaginal delivery at the age of 22 years with prolonged bleeding, menometrorrhagia requiring hysterectomy aged 27 years, and excessive bleeding with appendectomy and wisdom teeth removal. She had been treated with platelet transfusions and desmopressin. She reported increased bruising but denied bleeding from the gastrointestinal tract, gums, and epistaxis.

There were multiple family members across three generations of either sex with similar bleeding issues, all of whom were on her maternal side. The bleeding phenotype ranged from milder bleeding issues to fatal bleeding complications. The patient’s mother had a male cousin with “adult-onset leukemia,” and his son had died of “childhood leukemia” at the age of 10 years as well. The full pedigree is shown below (Fig. 1).

The laboratory findings showed a low normal platelet number (approx. 150,000/μL). The white cell and red cell lineage including morphology was unremarkable without any dysplastic forms. Platelet functional studies showed an abnormal platelet function assay (PFA). PFA collagen/epinephrine was >300 s (normal: 109–183 s), but PFA collagen/adenosine diphosphate (ADP) was 100 s (normal: 75–110 s). A decreased secondary response to ADP/collagen was noted on platelet aggregation studies. A normal response was seen to ristocetin. The von Willebrand factor antigen level was normal. Flow cytometry of platelet glycoprotein expression revealed a normal expression of GP1b and GPIIb/IIIa, ruling out Bernard-Soulier and Glanzmann thrombasthenia.
The patient’s brother was found to have a c159del (S53Rfs*9) RUNX1 mutation (frameshift in RUNX1). This RUNX1 mutation (c159 deletion) was confirmed in several family members, including the patient (Fig. 2). Based on the variant allele frequency of 47.38%, it was a heterozygous variant. This finding established the diagnosis of FPD related to the RUNX1 mutation. A year later, the patient developed fatigue, headaches, and palpitations with minimal activity and was found to have profound pancytopenia. A bone marrow biopsy showed 70% blasts (Fig. 3).

The blasts were positive for TdT (variable), CD34 (strong, granular cytoplasmic), CD20 (20% blasts), CD79a (20% blasts), PAX5 (variable), and CD19 (dim). They were negative for MPO, CD68, other monocytic markers, factor VIII, spectrin, E-cadherin, CD117, cCD3, sCD3, and CD10. Although a strong expression of CD19 and CD10 were not noted, the overall features were most consistent with B-ALL.

An FLT3-ITD mutation was detected, and karyotyping revealed 46,XX,der(7)t(1;7) (q12; q31)[9]/46,XX[11]. Her cerebrospinal fluid was negative for leukemic involvement. The patient was started on a hyperCVAD protocol and received a hyperCVAD cycle 1A as an inpatient. She received intrathecal methotrexate and intrathecal cytarabine for spinal prophylaxis. On day 14, a bone marrow biopsy showed morphologic remission with normal cytogenetic/FISH studies. The patient received the next cycle (1B) of hyperCVAD and was evaluated for an allogenic stem cell transplant. Subsequently, she underwent a matched unrelated donor allogenic stem cell transplant with 5.39 × 10⁶ CD34 cells/kg 3 months after the diagnosis of leukemia. Myeloablative conditioning with busulfan, cyclophosphamide, and antithymocyte globulin was utilized. Engraftment was achieved 2 weeks (day 15) after stem cell infusion. On post-transplant day 100, a bone marrow biopsy confirmed morphologic remission with no evidence of minimal residual disease. The treatment course was complicated by mucositis, neutropenic fever, infection of sinuses and scalp, gastrointestinal graft versus host disease (GVHD), and platelet refractoriness.

Sirolimus was used for GVHD prophylaxis and was stopped after 3 months due to thrombotic microangiopathy. Five months after her allogenic stem cell transplant, the patient developed deep venous thrombosis of the left lower extremity, which was successfully treated with low-molecular-weight heparin. Nine months after the stem cell transplant, the patient was profoundly neutropenic and presented with right arm cellulitis and right submandibular sialadenitis. A bone marrow biopsy was without morphologic evidence of leukemia, but was significant for hypocellularity with reduced erythropoiesis and granulopoiesis. The patient was thought to have developed aplastic anemia. Chimerism studies showed 100% donor myeloid cells, 96% donor T cells, and 100% donor B cells. Unfortunately, the patient succumbed to death from neutropenic sepsis shortly thereafter, while waiting to receive eltrombopag or romiplostim.

Fig. 2. Frameshift mutation in RHD: c.159delC causing p.Ser53ArgfsTer19.
Discussion

RUNX1 (also known as AML1, CBFA2, or PEPB2A) is a critical transcription factor for definite embryonic hematopoiesis [2]. RUNX1 genes encode a DNA-binding subunit that contains a highly conserved runt-homology domain (RHD) for sequence-specific DNA binding. The RUNX1 protein complexes with core-binding transcription factor to form a heterodimeric core-binding factor that regulates many genes important in hematopoiesis. More than 40 mutations have been described so far, mostly missense or deletion [3]. Most of the mutations causing FPD/AML are located in RHD, leading to disruption of DNA binding ability. Heterozygous germline mutations in the RUNX1 gene lead to FPD with propensity to myeloid malignancies [4].
FPD was first described in 1969, and the first family with FPD with a propensity to myeloid malignancies was reported in 1978 [5, 6]. The chromosome loci for \textit{RUNX1}, 21q, was identified in 1996 by linkage analysis [7]. Haploinsufficiency of \textit{RUNX1} due to nonsense or missense mutations of one allele was shown to be responsible for FPD with a propensity to myeloid malignancies in a landmark study published in Nature [4]. \textit{RUNX1} is expressed in hematopoietic stem cells and differentiating myeloid and lymphoid cells [8]. Haploinsufficiency of \textit{RUNX1} might constitutively activate \textit{MYH10} expression and affect megakaryocyte polyploidization [9]. A possible other theory is that downregulation of \textit{MYL9} due to \textit{RUNX1} haploinsufficiency could affect abnormal platelet production and function [10]. Additional events are necessary in malignant transformation, such as \textit{FLT3}-ITD [11].

Table 1. Reported cases of germline RUNX1 transformation to acute lymphoblastic leukemia

| Study               | Age, years | Sex | ALL type | Mutation type         | Additional hit(s) | Complete remission after induction chemotherapy | Post-remission course |
|---------------------|------------|-----|----------|-----------------------|-------------------|-----------------------------------------------|-----------------------|
| Nishimoto et al. [13] | 20         | Male | T-ALL    | R174X (non-sense)    | t(1;7)            | Yes                                           | Allogenic stem cell transplant from unrelated donor |
| Manchev et al. [14]  | 42         | Male | T-ALL    | R174Q (missense)     | TET2              | Yes (required 2 induction courses)             | Developed AML later   |
| Prebet et al. [15]   | 42         | Male | T-ALL    | R174Q (missense)     | ASLX1             | Yes (required 2 induction courses)             | Refused transplant    |
| Linden et al. [16]a | 3          | Female | B-ALL | c.568G>A (missense) | Trisomy 12p, trisomy/ tetrasomy 21q | Yes                                           | Alive after 2 years   |
| This study          | 47         | Female | B-ALL | c.159del (frameshift) | FLT3-ITD, t(1;7) | Yes                                           | Developed aplastic anemia after allogenic stem cell transplant |

*a Linden et al. described childhood B-ALL and thrombocytopenia due to \textit{RUNX1} mutation in a 3-year-old girl; however, the authors were not able to prove the germline nature of the mutation.
to be associated with FPD with a propensity to myeloid malignancies. This mutation is unique and was present in multiple family members. An association between the type of \textit{RUNX1} mutation, nature of secondary mutations, or type of leukemia has not been studied. \textit{FLT3-ITD} mutations are common in AML, and t(1;7) has been seen in T-ALL, in contrast to our case, which had features most compatible with B-ALL. However, it is likely that the \textit{FLT3-ITD} mutation and t(1;7) represent additional hits that predisposed FPD to acute leukemia. Importantly, family members should be screened for this mutation prior to consideration for stem cell donation for allogenic transplantation. It seems prudent that carriers of germline \textit{RUNX1} mutation be followed more closely.

Although core-binding leukemia comprise a favorable class of leukemia, the outcomes are poor for FPD with a propensity to myeloid malignancies when compared to those with a somatic \textit{RUNX1} mutation. Allogenic stem cell transplantation is pursued for eligible patients. However, it is important to screen family members for \textit{RUNX1} mutations, and \textit{RUNX1}-positive family members should not be used. Our patient developed aplastic anemia after successful matched unrelated donor allogenic stem cell transplant and succumbed to an infectious complication while retaining excellent donor chimerism. This course is not typical of \textit{RUNX1} germline status and remains unexplained.

**Conclusion**

FPD with propensity to myeloid malignancies is caused by inherited mutations in the \textit{RUNX1} gene. Thrombocytopenia is a common finding; clinicians must monitor these patients closely for the development of hematologic malignances. About half will develop hematologic malignances aged in their 30s or 40s. Although myeloid malignancies are commonly known to occur, lymphoid malignances are now also being reported. Here, we described the first confirmed B-ALL and a novel \textit{RUNX1} germline mutation in an adult.

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**Statement of Ethics**

Written informed consent was obtained for the publication of this case report from the patient’s next of kin.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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Author Contributions

N.K. wrote the case report and performed the literature search. A.K. treated the patient. A.K. and N.S. reviewed and edited the manuscript. N.S. performed and interpreted all the bone marrow studies.

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