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Case report

Two novel *RUNX*1 mutations in a patient with congenital thrombocytopenia that evolved into a high grade myelodysplastic syndrome

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ABSTRACT

Here we report two new *RUNX*1 mutations in one patient with congenital thrombocytopenia that transformed into a high grade myelodysplastic syndrome with myelomonocytic features. The first mutation was a nucleotide base substitution from guanine to adenine within exon 8, resulting in a nonsense mutation in the DNA-binding inhibitory domain of the Runx1 protein. This nonsense mutation is suspected a *de novo* germline mutation since both parents are negative for the mutation. The second mutation identified was an in-frame six nucleotide base pair insertion in exon 5 of the *RUNX*1 gene, which is predicted to result in an insertion in the DNA-binding runt homology domain (RHD). This mutation is believed to be a somatic mutation as it was mosaic before allogeneic hematopoietic cell transplantation and disappeared after transplant. As no other genetic mutation was found using genetic screening, it is speculated that the combined effect of these two *RUNX*1 mutations may have exerted a stronger dominant negative effect than either *RUNX*1 mutation alone, thus leading to a myeloid malignancy.

1. Introduction

Runx1 is an important transcription factor for myeloid development [1,2]. Impairments in Runx1 function lead to a block in myeloid differentiation and can drive leukemogenesis [3]. Clinically, germline mutations in the RUNX1 gene cause an autosomal dominant familial platelet disorder (FPD) with propensity to transform into myeloid malignancies such as myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML) [4–8]. Although rare, enhanced awareness of *RUNX*1 mutations has led to increased reports in recent literature, suggesting that the disease is actually more common than once thought.[9] Germline mutations in *RUNX*1 have important clinical implications and knowledge about such mutations is critical.

2. Case report

We report a case of an 18 year-old Caucasian male who was found to have thrombocytopenia at birth, with a platelet count of 58,000/mm^3. Bone marrow biopsy was first performed in 1993, at 13 months of age, and showed 70–80% cellular marrow with occasional megakaryocytes and no evidence of dysplastic changes or increased monocytes. There was no history of thrombocytopenia or bleeding tendency in his family. Throughout childhood, his platelet counts averaged between 90,000 and 120,000/mm^3. He had a lifelong history of easy bruising, however had no major bleeding episodes. He underwent several operations including spinal fusion surgery for scoliosis, later requiring revision, and a tonsillectomy with adenoidectomy. He received pre- and post-operative platelet transfusions as well as intravenous aminocaproic acid for his spinal operations without significant bleeding. He also had history of several infections, including lower extremity cellulitis, periorbital cellulitis, and a perirectal abscess. The patient was otherwise developmentally and phenotypically normal and was of normal intelligence.

In 2007, the patient was noted to have a mild normocytic anemia with hemoglobin and hematocrit of 10 g/dL and 30%. Four years later,

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at 18 years of age, the patient developed a mild leukopenia (3200/mm^3), as well as worsening of his baseline thrombocytopenia to around 60,000/mm^3. Bone marrow biopsy revealed 65–70% cellular marrow with 10% myeloblasts aberrantly expressing CD7, dysplastic erythroid progenitors and megakaryocytes, increased monocytes (25%), increased eosinophils, and no fibrosis. Cytogenetic analysis revealed normal male karyotype. FISH was negative for *AML*1-*ETO* (*RUNX*1-*RUNX*1*T*1) gene fusion and *CBFB* gene rearrangements. Molecular studies for *CEBPA*, *c-MPL*, *FLT*3, and *NPM*1 gene mutations were negative. The bone marrow pathology was interpreted as a high grade MDS (RAEB-1, Intermediate-2 IPSS risk). Although there were some features of CMML in the bone marrow, the patient did not have an elevated number of peripheral blood monocytes to meet criteria for this diagnosis.

The patient's pancytopenia worsened over the ensuing weeks and follow-up bone marrow biopsy revealed a marrow that was 75% cellular with 15% dysplastic myeloblasts aberrantly expressing CD7, 23% dysplastic monocytes, and 5% eosinophils. Cytogenetics showed normal male karyotype. FISH was negative for abnormalities in chromosomes 5, 7, 8 and 20. Additionally, FISH probes for *PDGFRA*, *PDGFRB*, *CBFB*, and *FGFR*1 detected no gene rearrangements. Genetic analysis found no mutations in *FLT*3 or *NPM*1. Lumbar puncture showed no evidence of central nervous system (CNS) involvement.

Because of his history of thrombocytopenia since birth evolving to MDS, DNA sequencing of *RUNX*1 was performed on both blood and buccal swab specimens. Two mutations were discovered: the first mutation, c.837G > A, was a nucleotide base substitution from guanine to adenine within exon 8 (Fig. 1), which is predicted to result in a nonsense mutation of amino acid tryptophan (W) to a premature stop codon (X) (p.W279X) in the DNA-binding inhibitory domain of the Runx1 protein. This mutation was found in 100% of cells analyzed. The second mutation identified, c.422_423insAAGGCC, was an in-frame six nucleotide base pair insertion in exon 5 of the *RUNX*1 gene, which is predicted to result in an insertion of an arginine (R) and proline (P) (p.S141_A142insRP) in the DNA-binding runt homology domain (RHD) of the Runx1 protein. This second mutation displayed a mosaic pattern.

Peripheral blood from both the mother and father showed wild type *RUNX*1 only and no evidence of mutations.

The patient was diagnosed with a *RUNX*1 double mutant, pre-leukemic MDS with myelomonocytic differentiation and administered induction chemotherapy with cytarabine and idarubicin. Prior to receiving induction chemotherapy, the patient elected for sperm banking to prepare for possible assisted reproductive technology in the future. The patient achieved an immediate complete remission (CR) following induction chemotherapy and then received once cycle of consolidation treatment with high dose cytarabine. Because of his underlying *RUNX*1 mutations and high risk for relapse disease [10], he immediately underwent an allogeneic hematopoietic cell transplant (allo-HCT) from a matched unrelated donor. Six months after transplant, oral mucosa cells harvested by buccal swab were analyzed for *RUNX*1 sequencing and revealed persistence of the exon 8 *RUNX*1 mutation with disappearance of the exon 5 insertion mutation. These results support the notion of a constitutional *RUNX*1 mutation in exon 8 and an accessory somatic *RUNX*1 mutation in exon 5.

Two years after allo-HCT the patient has normal blood counts, normal bone marrow morphology, no evidence of graft-versus-host disease, and is off immunosuppression.

3. Discussion

A familial platelet disorder with a propensity to myeloid malignancy (FPD/MM) was first reported in 1978 and since then approximately 30 pedigrees with *RUNX*1 germline mutations have been reported in the literature [9,11]. The *RUNX*1 gene is composed of 10 exons (1–6, 7A, 7B, 7C and 8). Distinct promoter

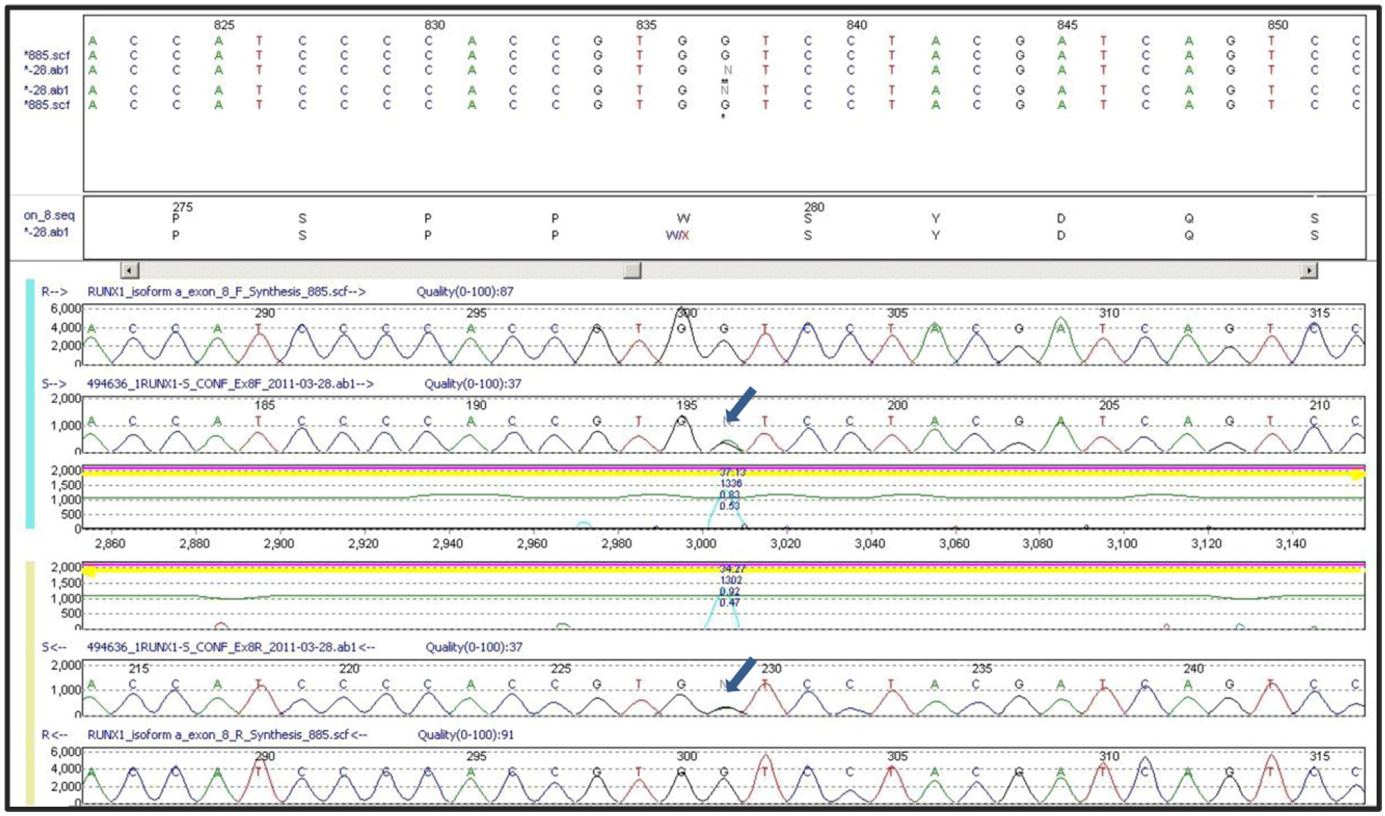

Fig. 1. Germline RUNX1 gene mutation in a patient with congenital thrombocytopenia that evolved into a high grade myelodysplastic syndrome. Arrows indicate RUNX1 c.837G > A (p.W279X) mutation using Sanger sequencing.