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## Case report

## Two novel *RUNX1* 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 *RUNX1* 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 *RUNX1* 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 *RUNX1* mutations may have exerted a stronger dominant negative effect than either *RUNX1* mutation alone, thus leading to a myeloid malignancy.

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### 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 *RUNX1* mutations has led to increased reports in recent literature, suggesting that the disease is actually more common than once thought.[9] Germline mutations in *RUNX1* 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<sup>3</sup>. 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<sup>3</sup>. 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<sup>3</sup>), as well as worsening of his baseline thrombocytopenia to around 60,000/mm<sup>3</sup>. 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 *AML1-ETO* (*RUNX1-RUNX1T1*) gene fusion and *CBFB* gene rearrangements. Molecular studies for *CEBPA*, *c-MPL*, *FLT3*, and *NPM1* 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 *FGFR1* detected no gene rearrangements. Genetic analysis found no mutations in *FLT3* or *NPM1*. 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 *RUNX1* 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 *RUNX1* 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.

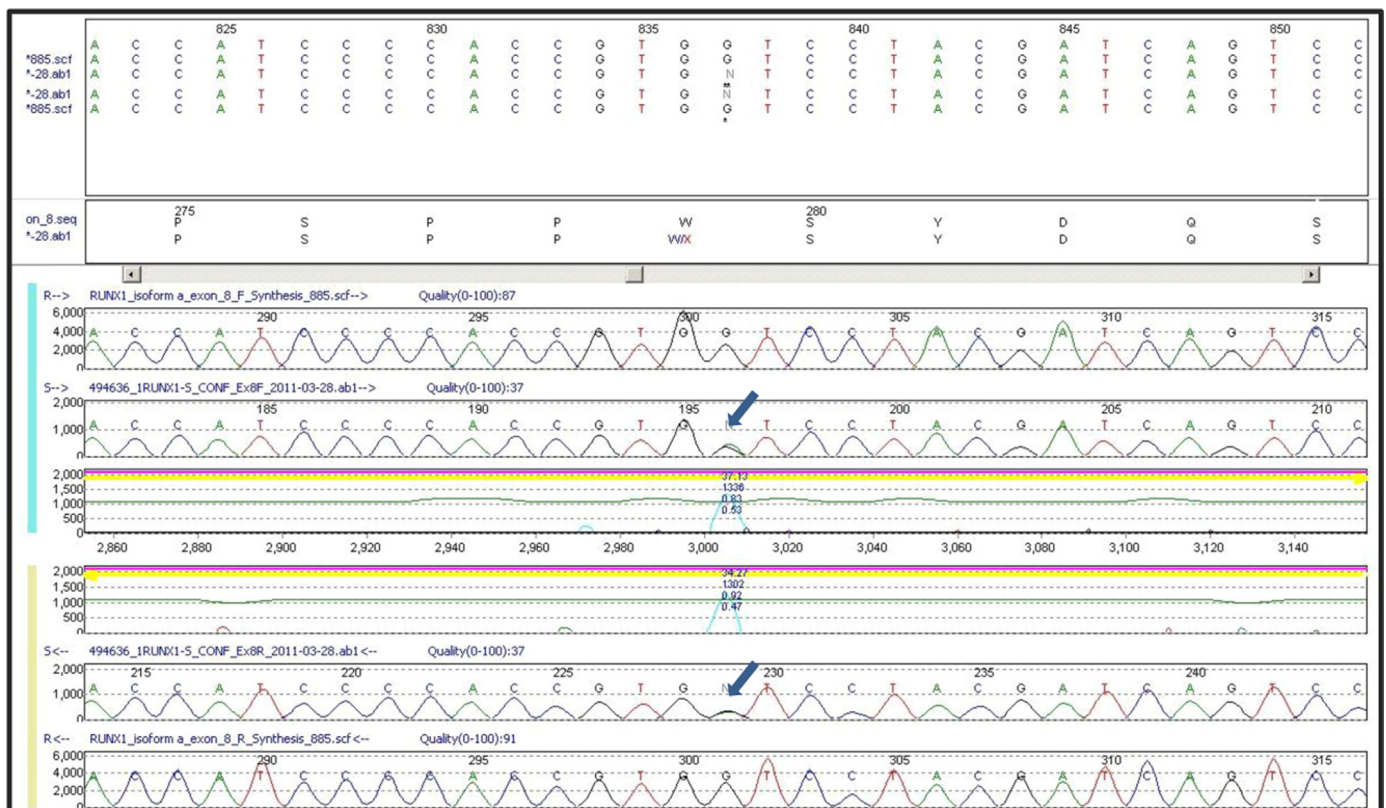
Peripheral blood from both the mother and father showed wild type *RUNX1* only and no evidence of mutations.

The patient was diagnosed with a *RUNX1* 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 *RUNX1* 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 *RUNX1* sequencing and revealed persistence of the exon 8 *RUNX1* mutation with disappearance of the exon 5 insertion mutation. These results support the notion of a constitutional *RUNX1* mutation in exon 8 and an accessory somatic *RUNX1* 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 *RUNX1* germline mutations have been reported in the literature [9,11]. The *RUNX1* 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.

regions and variable splicing leads to transcription of different isoforms, the longest being 480 amino acids in length. The N-terminal region encodes for the runt homology domain (RHD), which mediates both DNA binding and heterodimerization with its partner, core binding factor (CBF) beta. This region is coded on exons 3 through 5, which correlates to amino acids 50–177, and includes three specific DNA binding sites. The C-terminal region regulates gene transcription and encodes for the transcription activation domain on exons 7B and 8 (amino acids 243–371). Within this domain, there is a specific region known as the DNA-binding inhibitory domain (amino acids 184–291), which is important in down-regulating gene expression and a key site for gene mutations.

The Runx1 protein acts by binding with CBF-beta to form a transcription factor complex that is expressed in hematopoietic stem cells and regulates expression of several important hematopoietic genes. Runx1 also plays a role in the transcription of many genes involved in normal platelet function, which explains the mild thrombocytopenia and platelet dysfunction seen in FPD/MM [12,13]. Runx1 is also important in regulating transcription of many tumor suppressor genes [14,15], and has been found to regulate DNA damage repair [16].

Aberrations of the *RUNX1* gene are commonly seen in sporadic forms of AML and MDS, with chromosomal translocations more common in AML and point mutations in MDS [17–20]. In FPD/MM, *RUNX1* mutations are very heterogeneous and are often specific to individual pedigrees. The most common mutation site involves the RHD domain located near the N-terminus of Runx1, within exons three to five of *RUNX1* [9]. The RHD domain is especially prone to mutations as it contains easily mutable primary sequences that are prone to transition-type mutations via methylation of a cytidine residue [17]. Some mutations are more damaging than others and confer a greater risk of transformation to malignancy. Mutations that cause haploinsufficiency are most common; however, mutations that act in a “dominant negative” fashion are particularly high risk and act by competing for DNA binding sites or by preferentially binding to CBF-beta, thereby reducing wildtype Runx1 activity below 50%. As compared to mutations that cause haploinsufficiency, dominant negative mutations have a higher incidence of progression to MDS/AML [7,21]. Likewise, Runx1 mutations at the C-terminus have enhanced capacity to bind DNA due to loss of the DNA-binding inhibitory domain, leading to preferential expression of the mutant allele.

When mutated, Runx1 enhances myeloid cell proliferation, blocks cell differentiation, and leads to genomic instability, resulting in leukemogenesis [22–24]. Although *RUNX1* mutations are very important in promoting leukemic transformation, they are insufficient to initiate disease by themselves. Secondary mutations are required before patients develop malignancy [25]. An important second hit is that of the otherwise normal wildtype *RUNX1* gene, resulting in biallelic mutations in *RUNX1* [26]. This has led some to refer to *RUNX1* as the “gatekeeper gene” in the pathogenesis of *RUNX1*-associated acute leukemias [27].

The case presented above is an example of a spontaneous *RUNX1* germline mutation associated with a congenital thrombocytopenia that evolved into a myelomonocytic malignancy likely due to a secondary *RUNX1* somatic mutation. Reported here for the first time is a c.837G > A nonsense mutation in exon 8 resulting premature stop codon within the DNA-binding inhibitory domain, an important self-regulator of *RUNX1* transcription. Truncated Runx1 proteins are a common phenotype resulting in malignant transformation, and mutations in the DNA-binding inhibitory region are known to have a dominant negative effect [20]. Also reported here is a novel second mutation that is an in-frame insertion of six nucleotides within exon 5 of the *RUNX1* gene. This second mutation involved the DNA-binding RHD, where mutations

resulting in CMML have been reported before [28]. The second mutation is believed to be a secondary somatic mutation because it was mosaic prior to transplant and disappeared after transplant. Also, because of the absence of other genetic mutations (e.g., *CEBPA*, *FLT3*, *NPM1*, *PDGFR*), it is believed that these two *RUNX1* mutations may have exerted a stronger dominant negative effect than either *RUNX1* mutation alone, thus leading to malignancy with myelomonocytic differentiation. These hypotheses bear experimental study.

## Authorship

WBS, JRW, RAB, YL, and CRC evaluated and treated the patient. CRC and JMS designed the research report, analyzed the data, and wrote the paper. MML conducted the sequencing and analyzed the data. DT, RAH, JRW, RAB, YL, MML, and WBS wrote the paper.

## Conflict of interest

The authors declare no conflicts of interest.

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