A novel *RUNX1* mutation in familial platelet disorder with propensity to develop myeloid malignancies

We describe a Japanese family with familial platelet disorder with propensity to develop myeloid malignancies (FPD/MM). Among the three affected individuals, two members developed myeloid malignancies. Sequence studies demonstrate that all affected individuals of the pedigree display a heterozygous single nucleotide deletion in exon 8 of the *RUNX1* gene.

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RUNX1 (also known as CBFA2 or AML1) is a transcription factor that, together with its partner CBF, regulates a number of important genes essential for hematopoiesis.[1] RUNX1 also plays a critical role in neoplastic hematopoiesis. Several genetic rearrangements involving the RUNX1 gene on chromosome 21q22.3 have been identified in patients with acute leukemia, including the t(8;21), t(3;21) and t(12;21) translocations.[1] In addition to balanced translocations, point mutations of the gene also contribute to the pathogenesis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).[2-4] A mutation of the RUNX1 gene is also responsible for a hereditary disease, familial platelet disorder with a propensity to develop myeloid malignancies (FPD/MM).[5] FPD/MM is a rare disorder. So far, 13 pedigrees have been reported, mainly in European countries.[5-10] Clinically, the disorder is characterized by moderate thrombocytopenia from birth, impaired platelet function and a propensity to develop myeloid malignancies. Genetic and molecular studies reveal that all reported pedigrees of FPD/MM display a mutation in the *RUNX1* gene or LOH at the locus.[5-10] In this report, we present a Japanese family with FPD/MM and a novel RUNX1 mutation.

A pedigree map of the family under study is presented in Figure 1. A 37 year-old man (I-2), the proband of this pedigree, was admitted to hospital with a diagnosis of refractory anemia with excess blasts in August 2003, 10 years after thrombocytopenia was first noted incidentally. At that time, the white blood cell count was 3.6×10^9/L the hemoglobin level was 12.7g/dL and platelet count 68×10^9/L. Chromosomal analysis revealed the karyotype 46,XY, idic (7)(q11)[11/20], 46,XY,t(7;8)(q34;q11) [3/20] and 46,XY [6/20]. Platelet aggregation tests revealed impaired responses to collagen and epinephrine but a normal response to ristocetin. Stem cell transplantation from a family donor was considered. During the pretransplantation evaluation it was noticed that two additional family members (II-1 and II-2) displayed mild thrombocytopenia (platelet counts were 103×10^9/L and 86×10^9/L respectively). In April 2006, family member II-1 (17-year-old girl) developed pancytopenia, three years after her first documentation of thrombocytopenia. A bone marrow biopsy revealed acute panmyelosis with myelofibrosis with karyotype 47,XX, +8 [20/20]. The platelet counts of the brother of the proband (I-1) and the mother of the second patient (I-3) were normal. Based on these clinical findings, a tentative diagnosis of FPD/MM was made and the *RUNX1* gene was studied in all readily accessible members of the family. The study was approved by the Institutional Review Board of the University of Yamanashi, and written informed consent was obtained from patients and other family members. DNA was extracted

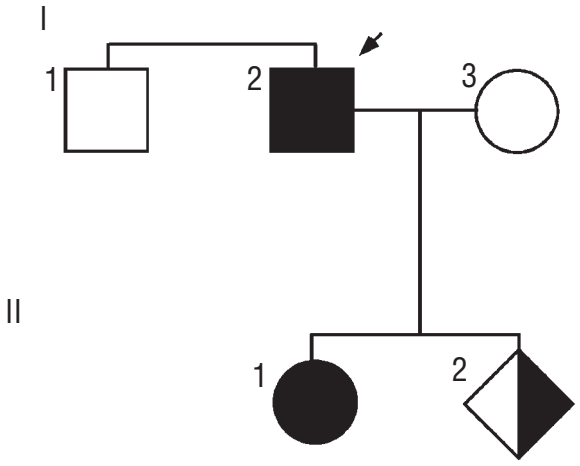

Figure 1. The family pedigree is shown. Half-filled symbol represents an individual who displays thrombocytopenia and completely filled symbols represent individuals with thrombocytopenia who developed myeloid malignancies.

from peripheral blood mononuclear cells by standard procedures. We amplified exons 1 to 8 of *RUNX1* gene from genomic DNA of the index case using intronic primers flanking each exon and direct DNA sequencing of PCR products. Initially, we identified a G to C substitution in exon 1, corresponding to position 102 of *AML1c* transcripts (NCBI accession number D43969).[11] However, we identified the same single nucleotide change in 6 out of 100 healthy Japanese volunteers and concluded that this is a moderately frequent single nucleotide polymorphism. We then identified a single nucleotide deletion of G at position 2484 within exon 8 (Figure 2A) corresponding to *AML1b* transcripts (NCBI accession number D43968). This mutation was also detected in buccal mucosa cells, indicating that this is a germline mutation. To confirm whether other affected individuals display this mutation we amplified exon 8 of each family member and digested the cDNA product with Eco57I, as the guanine deletion creates a new digestion site for the enzyme. As shown in Figure 2B, all affected members display this single nucleotide deletion (Figure 2B) but unaffected individuals do not. It results in a frame-shift following amino acid 303, and terminating following amino acid 565 (Figure 2C). This novel mutant form of RUNX1 encodes the Runt DNA binding domain, but obliterates the transactivation and inhibitory domains of the protein.

Although a mutation of *RUNX1* is found in a variety of de novo myeloid malignancies, including AML and MDS, the position of the mutations are clustered within the Runt homology domain in more than 80% of such cases.[12] Furthermore, the runt domain is the site of mutation in 10 of the 13 reported FPD/AML families who have an altered *RUNX1* gene. By contrast, Harada *et al*. reported that 9 out of 110 patients with secondary MDS or AML following MDS had a C-terminal *RUNX1* mutation.[3] Christiansen *et al*. also found C-terminal mutations in 10 out of 140 patients with therapy-related MDS or AML.[2] Interestingly, C-terminal mutations preferentially cause frame-shifts, whereas many of the Runt domain mutants are missense and nonsense mutations. As a result, most of the RUNX1 C-terminal mutants have an intact DNA binding domain but lack the transactivation domain.

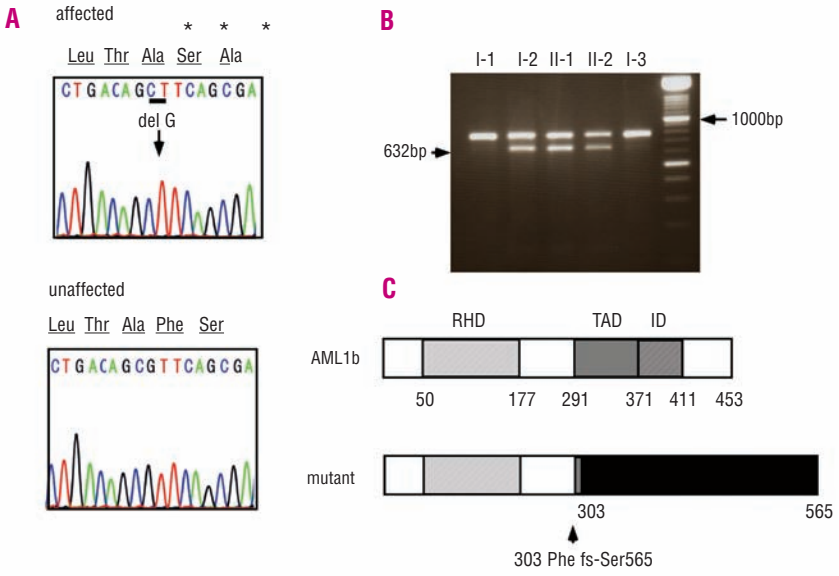

Figure 2. Mutation analysis of *RUNX1* gene in the pedigree. A. Sequence analysis of affected patient I-2 (upper) and an unaffected family member (lower) are shown. The arrow indicates the position of deletion of G shown in the patient. * indicates amino acid residues from wrong reading frame. B. PCR products of exon 8 of family members were digested with Eco57I and separated on 2% agarose gel. The product size is 772bp from wild type allele and 771 bp from mutated allele respectively. Deletion of G creates a new restriction site within the PCR products leading to generation of 632bp and 139bp fragments. C. Schematic structure of the wild type AML1b and the mutated *RUNX1* protein. RHD; Runt homology domain, TAD; transactivation domain, ID; transcription inhibition domain. Black bars indicate additional amino acid sequences originating from wrong reading frame.

There is increasing evidence to suggest that the RUNX1 C-terminal mutants function as dominant negative forms of the protein, inhibiting the function of the remaining normal wild type RUNX1.[3] The RUNX1 mutant presented in this study also lacks a functional C-terminal region due to a frame shift mutation. This suggests that the mutant form found in this family with FPD/MM also functions as a dominant negative form of RUNX1.

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